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Distraction-induced intestinal enterogenesis: Preservation of intestinal function and lengthening after re-implantation into normal jejunum

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Abstract

Background—Significant bowel lengthening can occur in an isolated intestinal segment with the use for linearly directed distractive forces; resulting in increased surface area and epithelial cell proliferation. We hypothesized that re-implantation of this lengthened intestine into normal jejunum would preserve this gain in intestinal length and function similar to normal jejunum.

Methods—An intestinal lengthening device was inserted into isolated jejunal segments in pigs, and fully expanded over 8 days. Lengthened segment were then re-implanted into normal intestinal continuity. Pigs were studied after another 28days. Function was assessed by motility, mucosal enzyme activity, barrier function and intestinal ion transport.

Results—Lengthened segments were significantly longer than control segments, and had nearly 2-fold greater surface area. Bowel lengthening was maintained 4 weeks after re-implantation. Motility after re-implantation was similar to non-operated pigs. Barrier function, mucosal disaccharidase levels and electrophysiologic measures declined immediately after lengthening, but returned to nearly normal levels 28 days after re-implantation.

Conclusion—Bowel lengthening results in a transient decline in mucosal absorptive function and smooth muscle contractility. However, function approaches that of normal bowel after re-implantation into enteric flow. These data may support the use of this technique as a potential new option for the treatment of patients with short bowel syndrome.

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INTRODUCTION

Short bowel syndrome (SBS) is a significant problem with extensive morbidity and mortality in clinical medicine¹. The loss of a significant amount of small intestine results in an inadequate absorption of enteral nutrients². Treatment of SBS typically involves supportive medical therapy with total or supplemental parenteral nutrition (TPN or PN). Although TPN has improved survival in patients with SBS, it is associated with complications including catheter-related morbidity, metabolic derangements and reduced quality of life^{1,3}. Various surgical methods aim to lengthen the remnant small bowel with procedures such as the Bianchi procedure⁴ and serial transverse enteroplasty⁵ have been applied to such patients. Although these procedures may be successful, they are limited to dilated areas of bowel and postoperative length gained is usually less than 2-fold⁶. These procedures are also associated with potential injury to the mesenteric vasculature and anastomotic leakage. Small bowel transplantation is a viable option for SBS patients who fail TPN. However, long-term outcomes have not appreciably improved over the past decade, with rejection and graft failure rates approximating 60% at 5 years⁷. Thus, despite a number of therapeutic options for SBS, other alternative treatments are needed.

Studies have shown successful elongation of the small intestine using mechanical force^{8,9}. However these reports were unlikely to be feasible in clinical setting, as these models were utilized an externally mounted device on a rodent's abdominal wall. To address this, we have previously shown that linearly-directed mechanical distractive forces resulted in over a doubling of the intestinal length. This utilized an implantable hydraulically-controlled device in large animal model^{10,11}. In the fact, our results have showed that mechanical forces induced not only elongation but also actual proliferation and function of the small intestine. However, in this study, the intestinal segment was taken out of the normal small bowel continuity. In a rat model, other investigators have suggested that re-implantation of a lengthened segment retained intestinal length using a rat model¹²; however, a full understanding of the functionality of these implants has not been done, nor its functionality after re-implantation. To better address this, we hypothesized that a re-implantation of such a lengthened segment of small bowel into the normal continuity of jejunal enteric flow in a pig would result not only in preserved small bowel lengthening, but retained absorptive and motile function.

METHODS

Animals

Specific pathogen free, 20–25kg female, young Yorkshire pigs (N=8) were used and maintained in a 12-h day-night rhythm at 23°C and a relative humidity of 40–60%. All experiments were approved by the University Committee on Use and Care of Animals at the University of Michigan.

Experimental design

We created two isolated vascularized segments of jejunum *in vivo*, with the remaining bowel replaced into continuity. In one segment, a device was inserted intraluminally into the isolated segment (Lengthened group), and the intestinal segment was closed surgically. One week postoperatively, the device was activated and began to elongate incrementally, as previously described¹³. An additional segment of bowel had a device implanted; however, no lengthening of this segment was performed. After 8 days of elongation (1.0mL per day of infused saline, and 1.2cm of length gain/day), the pigs underwent a repeat laparotomy. The Lengthened segment was carefully isolated with preservation of the vasculature, and re-implanted into normal intestinal continuity. The control segment was measured, and then

surgically removed. As an additional control, at the time of second laparotomy (re-implantation), a segment of native jejunum *in situ*, adjacent to the isolated Lengthened segment, was marked with 2 seromuscular non-absorbable sutures placed on the antimesenteric border 12cm apart. This served as a control to measure changes in jejunal length over this 28 day period.

After 28 days from the time of re-implantation the pigs were re-anesthetized, bowel segments isolated. The previously placed control sutures, and length of the lengthened bowel were identified, and the distance between them re-measured to assess the changes in length of native jejunum attributable to baseline growth rate, as well as the experimental segment. The re-implanted bowel was studied *in situ* (motility), and the pigs were subsequently killed.

Description of hydraulic-lengthening device—A hydraulic-driven concentric piston was designed as previously described was used¹³. Briefly, this device consisted of a series of telescoping syringes. The piston chambers of the syringes were connected internally. Fluid injected into the device produced incremental elongation; both ends of the device were coated with soft silicon bumpers to avoid bowel injury.

Anesthesia—Pigs were fasted the night before surgery and weighed preoperatively. The animals were given intramuscular induction agents (xylazine (2.2mg/kg) and tiletamine / zolazepam (6–8mg/kg)), and endotracheal intubation was performed. Surgical plane of anesthesia was maintained with continuous inhaled isoflurane (2–2.5%). Glycopyrrolate (0.02mg/kg) and cefazolin (50mg/kg) were administered at the start of surgical procedure. During the operation, lactated Ringer's solution was infused at physiologic rates.

Lengthening procedure—The lengthening procedure was performed as previously described¹³. Briefly, a midline laparotomy was performed and the bowel exposed. Two sections of mid-jejunum, located approximately 100cm from the ligament of Treitz, were isolated while retaining its vascular supply. The remaining bowel was placed back into continuity with end-to-end anastomosis, using a single layer 4-0 running polyglactin suture. A lengthening device was inserted intraluminally into one of the isolated segments (Lengthened segment). The ends of segment were sutured shut. The fluid injection line for device lengthening exited the bowel segment through a purse-string suture at one end. Similarly, a drain catheter was placed to prevent accumulation of mucosal secretions. The device fluid line and the drain were tunneled subcutaneously and exited the skin between scapulae. This also secured the isolated segment up against the parietal peritoneal surface to prevent volvulus. A separate section of jejunum contained a lengthening device and drain, but did not undergo lengthening. The abdomen was then closed in standard fashion.

Re-implanted procedure—The pig was re-anesthetized on postoperative day (POD) 14 (7 days of rest followed by 7 days of progressive bowel lengthening), and a second laparotomy was performed. The Lengthened segment was exposed with its vascular pedicles (Figure 1). The Lengthened segment was put back into normal jejunal enteric flow, using a single layer 4-0 running polyglactin suture, and then the intestinal continuity was restored. This segment (Re-implant segment) was labeled with a non-absorbable suture put at both ends of the antimesenteric border for future measurements of growth. In addition, a third portion of jejunum, not excised but simply left *in situ*, was marked with sutures in similar fashion over a 12 cm length. These non-absorbable sutures served as the growth rate of the bowel after re-implantation surgery. The control, non-lengthened segment was measured, and then surgically removed.

Gastrointestinal transit—Gastrointestinal transit activity was assessed using two methods, radiological contrast study and passage of a charcoal meal, performed at 5 and 7 days prior to the final harvesting experiment, respectively. Pigs were studied while awake without any sedation in both studies. Food was removed 12h prior to the experiment, but animal had free access to water until the beginning of the experiment.

Radiological contrast study: This study was performed by adding 50% barium sulfate (EZ-EM, New York) to the food. A small intestinal transit time was defined as an interval from the time of passage to the first portion of the small bowel (Figure 1) to the point where the barium was detected in the cecum. Abdominal radiographs were taken every 10 to 15 minutes until the cecum was identified, and defined as the small intestinal transit time (stomach-to-cecum transit).

Charcoal meal study: Pigs were fed 1 gram of enteric charcoal. Passage into the stool was observed every hour until detection, and was defined as total bowel transit time (oral-anus transit).

Mannometry—After 28 days post-re-implantation, *in vivo* measurements of motility were performed. Intraluminal pressure and contractility of the re-implanted intestinal segment, and another non-surgically manipulated segment, were measured with the use of a specially designed rubber balloon inserted into the intestine lumen using previously described methods¹⁴. Pressure was recorded by a pressure transducer (World Precision Instruments, Sarasota, FL), which was connected to a transducer amplifier (TBM4M, World Precision Instruments). After induction of general anesthesia, and prior to harvesting of tissue these studies were performed. The intraluminal balloon was secured into the intestinal segment with a suture to avoid movement. The pressure transducers were connected to balloon catheters, and the balloon was filled with water at 37°C (4.0–5.0ml). This volume was determined to be the level necessary to induce an intraluminal pressure of 10 cmH₂O. Baseline intraluminal pressure was determined and recorded after a 20 minute equilibration period. Body temperature was maintained at 37°C with a heating pad. To determine if the re-implanted segment had a normal smooth muscle response to cholinergic stimulation, intestinal pressure response to bethanechol (20 µg·kg⁻¹, Sigma, St Louis, MO) and charbachol (1 mg·kg⁻¹; Sigma, St Louis, MO) were given intravenously, with a minimum of a 20 min washout period, and return to baseline pressures before the next chemical was given. Pressures were recorded and analyzed by using a computer-assisted software Spike2 and data acquisition system for online analysis were both from Cambridge Electronic Design, Cambridge, England.

Morphology of intestinal segments

crypt-villus measurements: Formalin-fixed specimens were stained with hematoxylin and eosin. Villus height and crypt depth were measured with an optical micrometer. Specimens were oriented meticulously at right angles to the long axis of the bowel, such that a true longitudinal view of crypts and villi was obtained, and other crypt/villus complexes were excluded. Measurements were performed by an observer blinded to the identity of tissue specimens. A minimum of 20 crypt/villus complexes were measured per slide.

Epithelial proliferation—Proliferative rates of epithelial cells were assessed with the use of bromodeoxyuridine (BrdU) labeling as described previously¹³. Briefly, a single dose of 5-BrdU (50mg/kg, IM, Sigma Aldrich, St Louis, MO) was administered 4 hours before harvest of the intestinal segments. Histologic samples were stained with BrdU In-Situ Detection kit according to the manufacturer's instructions (BD PharMingen, San Diego, CA). The mean

number of proliferating cells in 16 crypts per histologic section was measured. Epithelial cell proliferation was expressed as the percentage of cells incorporating BrdU.

Baseline intestinal ion transport—Modified Ussing chambers (Physiologic Instruments, San Diego, CA) were used to measure ion transport and epithelial barrier function as described previously^{13, 15}. Briefly, mucosal and serosal compartments were filled with preheated 37°C 5mL of Krebs buffer. The Krebs buffer contained 110.0mM NaCl, 3.0 mM CaCl₂, 5.5mM KCl, 1.4 mM KH₂PO₄, 29.0mM NaHCO₃, and 1.2 mM MgCl₂ and was adjusted to a pH of 7.4. Each chamber was oxygenated continuously with O₂/CO₂ (95%/5%). The serosal buffer included 10 mM glucose as an energy source and was balanced osmotically with 10mM mannitol on the mucosal side. The spontaneous potential difference across the intestinal membrane was maintained at 5mV by an automated voltage clamp, and the injected short circuit current (Isc) was monitored continuously as an indication of net active ion transport. Baseline Isc was determined as an indication of the ion transport state of tissue and recorded after a 20-minute equilibration period.

Stimulated ion transport—Glucose uptake into enterocytes is linked to serosal sodium absorption, thus leading to a change in Isc with glucose absorption. To assess glucose-stimulated sodium absorption, 5 mL of 10 mM D-glucose was added to the mucosal chamber. Change in Isc was measured by subtracting the basal current from the peak current after addition of D-glucose. After returning to baseline, carbachol (10 μ M, a secretory Cl⁻ agonist) was used to measure chloride ion transport. Changes in Isc are as described above.

Epithelial permeability—Mucosal permeability was assessed by 2 methods: transepithelial resistance (TER) and the transepithelial passage of a tracer molecule, ³H-mannitol. TER in $\Omega\cdot\text{cm}^3$ was determined by using Ohm's law. After a 20 minute equilibration period, ³H-mannitol (3 μ Ci/mL, Sigma-Aldrich, St. Louis, MO) was added to the mucosal compartment. One-milliliter samples were taken from the serosal compartment every 10 minutes for 90 minutes and were analyzed for ³H in a scintillation counter. Results are expressed as the percentage of transepithelial passage of ³H-mannitol after 90minutes¹⁶.

Smooth muscle cell contractility—Jejunal smooth muscle cells were isolated as described previously¹⁷. Briefly, isolated smooth muscle from control, Lengthened and Re-implanted segments were cultured to confluence. Cells were then scraped off and allowed to float freely for 48h in standing flask with occasional shaking to prevent further settling and sticking to the bottom of the flask. Aliquots of cultured cell suspension (2.5×10^4 cells/0.5ml) were stimulated with acetylcholine (10^{-7} M, Sigma, St Louis, MO) for 30sec. The reaction was allowed to proceed for 30sec and stopped by the reaction of 0.1ml of acrolein at a final concentration of 1% (vol/vol). Individual length of cells in the control state or after addition of test agents was measured by computerized image micrometry and obtained from 50 cells encountered randomly in successive microscopic fields.

Disaccharidase activity—The activities of intestinal disaccharidases of harvested segments were measured as described by Dahlqvist¹⁸, for evaluation of the jejuna mucosa of the control, Lengthened and Re-implanted groups. Intestinal enzymes assayed included lactase, sucrase, isomaltase and maltase (Sigma-Aldrich, St Louis, MO). Briefly, mucosa was scraped off the underlying muscularis propria and immediately frozen in liquid nitrogen until analysis. Mucosal tissue was homogenized in distilled water and incubated with the appropriate disaccharide substrate-buffer at 37°C. Enzyme hydrolysis resulted in the formation of glucose. A quantitative measurement of glucose was then performed. All enzyme specific activities were expressed as units (U), defined as moles of glucose released per minute per microgram of mucosal protein.

Immunoblot analysis—Protein concentration determination and immunoblot assays were performed as previously described¹⁹. Targeted proteins used the following specific antibodies: rabbit anti ZO-1 (1;1000: Zymed Laboratories, San Francisco, CA), rabbit anti Occludin (1;2,000: Zymed), or rabbit anti Claudin-1 (1;2,000: Zymed). Detection of β -actin was performed in the same fashion by re-probing membranes with purified mouse anti β -actin (1:5,000, Sigma). Results were expressed as the ratio to β -actin densitometry expression.

Immunofluorescent staining—To further examine the distribution and density of tight junction proteins, immunofluorescent staining of small bowel from each study group was performed using previously described techniques²⁰. Primary antibody to ZO-1 (Invitrogen) was used at a 1:50 dilution, followed by goat anti-rabbit IgG-Texas Red (Santa Cruz) at 1:400. Primary antibody to occludin (Invitrogen) was used at 1:100, and secondary antibody was goat anti-mouse (IgG-FITC; Santa Cruz) at 1:400.

Statistical analysis

Data are reported as mean $\pm$ standard deviation (SD). Results were analyzed using *t* test for comparison of two means and a one-way analysis of variance (ANOVA) for comparison of multiple groups (with the post-hoc Bonferroni test to assess statistical difference between groups; Prism software; GraphPad Software, Inc., San Diego, CA). A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

General Outcome

Six of eight pigs in the study underwent successful completion of intestinal lengthening and re-implantation surgery. The remaining 2 were technical failures, and these pigs required euthanasia before completion of the experiment because of the development of small bowel obstructions before the re-implantation surgery. No pigs died prematurely after the re-implantation surgery.

Growth rate re-implanted intestine

Bowel lengthened at a rate of 1.2 cm/day during the lengthening period. At the time of initial harvest after 10 days, length increased from a mean of 10 cm to 20.3 cm. A moderate portion of this lengthened bowel was used for physiologic and histologic studies, and the ends of the bowel were discarded due to the fact that they were initially suture closed. Thus, the mean length used for the reimplantation was 10.8 cm. Bowel length in the re-implanted segment actually slightly increased after 4 weeks, going from a mean of 10.8 cm (range 10.5 to 11 cm) at the time of re-implantation to 12.3 cm (range 12 to 12.5 cm) after 4 weeks (~ 0.43 cm/week). This amount of growth was similar to that seen in an adjacent non-operated segment of control bowel (increased ~ 0.45 cm/week; 10.5 cm to 12.25 cm, pre- and post re-implant, respectively). Thus, the gain in length achieved with the initial lengthening is maintained after the re-implantation.

Gastrointestinal –transit time

To evaluate if the re-implantation surgery and lengthening process interfered with motility, we initially examined intestinal transit times. Activated charcoal was first detected in the stool of pigs that had a re-implantation of lengthened bowel between 17 and 20 hours (mean 18.2 ± 1.8 h). This compared nearly identically to a mean transit time of 17.0 ± 1.9 hours in non-operated pigs. Second, passage of radiographic contrast material through the small intestine and into the cecum was noted in all cases between 7 and 8 hours (mean 7.3 ± 0.4 h;

for both controls and lengthened groups). There were no significant differences between non-surgery pigs (normal pigs, base line) and re-implanted pigs for these studies. Importantly, no delay in transit was noted around the re-implanted segment, and no evidence of dilation of the small bowel was observed.

Manometry

Baseline intraluminal pressures in normal and re-implanted bowel were 10.7 ± 1.2 mmHg and 11.8 ± 1.5 mmHg, respectively ($P > 0.05$; Figure 2). The rate of spontaneous clustered contractions were noted at a rate of 7 to 9 /minute (mean 7.6/min) in the native jejunum, and were slightly less in the re-implanted segments (mean 6.5/min, range 5 to 7; $P = 0.21$). Peak pressure changes (post-bethanechol compared to mean baseline) were: 18.2 ± 2.5 mmHg and 16.0 ± 1.8 mmHg in normal and re-implanted bowel segments, respectively; and changes were not statistically significant between these two groups. The mean area under the curve (AUC, as measured from baseline till new baseline was achieved) was similar between native and re-implantation segments (83.5 ± 8.7 vs. 77.5 ± 9.3 , given at units of mmHg/40sec; $p = 0.47$). Peak pressure change after carbachol instillation was 14.5 ± 1.6 mmHg and 12.2 ± 1.2 mmHg in normal and re-implanted bowel segments, respectively; and changes were also not significantly different ($P = 0.39$). Duration of contractions were less than bethanechol, with the mean AUC being 32.5 ± 4.5 vs. 28.3 ± 6.3 for control and re-implanted segments, respectively; $P = 0.57$).

Smooth muscle cell contractility

To evaluate whether mechanical lengthening altered smooth muscle contractile properties, *in vitro* stimulation of isolated smooth muscle cells using acetylcholine was performed. Isolated smooth muscle cells from normal jejunum exhibited a sustained contraction in response to acetylcholine of $50.2 \pm 1.0\%$ decrease in cell length at 30sec; Figure 3. Lengthened segments showed a significantly decreased level of smooth muscle cell contractility ($24.5 \pm 0.5\%$ decrease in cell length at 30sec, $p < 0.001$). However, cell contractility after re-implantation significantly recovered compared to the lengthened segment ($36.3 \pm 0.8\%$ decrease in cell length at 30sec, $p < 0.001$); although these levels were still significantly lower than normal jejunum.

Crypt-villus morphology

A summary of histo-morphologic changes are shown in Table 1. Microscopic examination showed that villus height was reduced in the Lengthened group compared with normal, native jejunum. In contrast, crypt depth increased markedly in the Lengthened group compared with normal jejunum. This increased crypt depth in the Lengthened group most likely relates to the marked increase in epithelial cell proliferation (see below). However both villus height and crypt depth in the Re-implanted group were not significantly different from normal jejunum values. It was also interesting to note that in both the Lengthened and Re-implanted segments there was a markedly increased thickness of the muscularis propria. This suggests that the full-thickness of the intestinal wall underwent hypertrophic changes during enterogenesis.

Epithelial cell proliferation

Intestinal epithelial cell proliferation in crypts was assessed with BrdU nuclear staining. Mechanical lengthening resulted in a significant increase in proliferation the Lengthened group compared to normal jejunum ($24.0 \pm 6.6\%$ versus $15.5 \pm 4.8\%$, $P < 0.01$). Re-implantation into normal enteric flow, and 4 weeks after distractive forces were terminated, showed a markedly diminished level of epithelial cell proliferation; and levels were similar

to normal jejunum level ($15.9 \pm 4.7\%$, $P < .01$ compared to Lengthened group; Figure 4A and 4B).

Disaccharidase activity

Functionality of the gastrointestinal tract was assessed by measuring the level of disaccharidase activity. A significant loss of disaccharidase activity was noted in the Lengthened group compared with normal jejunum (Table 2). However, the disaccharidase activity in the Re-implanted group increased, and reached levels not significantly different from normal jejunum. This trend held for most disaccharidases, except for maltase which did not significantly change between groups.

Epithelial barrier function

Intestinal permeability was examined with ^3H -mannitol and changes in transepithelial resistance (TER). Lengthened segments demonstrated intact barrier function in comparison with normal jejunum (cumulative of transepithelial passage of ^3H -mannitol after 90 minutes (Figure 5A). Calculating permeability coefficient values for this passage (Papp, expressed at $10^{-6} \times \text{cm} \cdot \text{sec}^{-1}$; ¹⁵). Papp values were not significantly different between groups: 12.2 ± 4.3 vs. 11.8 ± 2.2 vs. 12.0 ± 2.5 , for control, Lengthened and Re-implanted groups, respectively; $P > 0.05$. Re-implanted segments also maintained intact barrier function as determined by TER measurements. TER values for jejunum from each group remained relatively stable over the 90 min incubation period (Figure 5B). Baseline TER ($\Omega \cdot \text{cm}^2$) at 0 mins after initial mounting for normal jejunum was $108.4 \pm 11.8 \Omega \cdot \text{cm}^2$, mechanical lengthening led to a significant decline in TER ($92.3 \pm 16.3 \Omega \cdot \text{cm}^2$, $P < 0.001$). However, this significantly improved to levels not significantly different from normal jejunum ($P > 0.05$) in the Re-implanted group ($102.7 \pm 11.3 \Omega \cdot \text{cm}^2$).

Epithelial ion transport

Baseline transepithelial current (I_{sc}) is an indicator of active ion transport, and directly correlates to ion shifts with nutrient transport (e.g., glucose/sodium), or due to non-specific stimulation of ion transport with carbachol (cholinergic response)¹⁵. Baseline I_{sc} was significantly decreased in the Lengthened group vs. normal jejunum ($8.8 \pm 2.2 \mu\text{A}/\text{cm}^2$ vs. $11.0 \pm 1.6 \mu\text{A}/\text{cm}^2$, respectively; $P < .05$). The baseline I_{sc} in the Re-implanted group increased and approached normal jejunum levels; however, no significant differences were noted between the Lengthened and Re-implanted groups ($9.8 \pm 1.8 \mu\text{A}/\text{cm}^2$). Although differences between groups were not significant, changes with regard to glucose and carbachol similarly showed a decline in the Lengthened group and a partial recovery in the Re-implanted group (Figure 6).

Tight junction protein expression

Because tight junction proteins are known to be a major contributor to epithelial barrier function, the abundance of these factors was examined using western immunoblotting. Mechanical lengthening resulted in a significant decrease in ZO-1 and claudin-1 expression (Figure 7). These levels returned to normal jejunal levels for these two junctional proteins. Occludin expression slightly increased compared to normal jejunum in the Lengthened group; however, there were no significant differences between the 3 study groups.

As another way to quantify changes in the tight junction, immunofluorescent imaging was also performed. Immunofluorescent imaging of ZO-1 and occludin showed a loss of immunofluorescent intensity and actual breaks in the integrity of the tight junction in the Lengthened group, with a near complete recovery in the Re-implanted group (Figure 8).

DISCUSSION

We have previously shown that the application of longitudinal, distractive mechanical force can induce increased length and growth in the jejunum of pigs while maintaining function-epithelial architecture¹³. These findings are in close agreement with previous studies performed in a rat model^{8,9}. In this present study, we confirmed the ability to induce significant bowel lengthening in relatively short periods of time. An approach taken by all of these experiments is taking the intestine out of the normal enteric flow. As such, a critical question is whether this lengthened bowel will retain the gain in bowel length. Chang, et al initially addressed this question in a rat model. These authors demonstrated retention of bowel length¹² 3 weeks after removing mechanical forces; however, the bowel was kept out of intestinal continuity. In the present study we also examined such retention in a large animal model, in hopes of translating these findings to the clinical setting. In addition, the study reimplanted these lengthened segments into the normal enteric flow and examined several functional aspects of this re-implanted bowel, including motility, barrier function and transport and absorption.

Our findings demonstrated that lengthened bowel retained, and actually continued to grow after re-implantation at similar rates observed with native jejunum. The lengthened bowel demonstrated loss of absorptive function, and some decline in barrier function when out of continuity from normal enteric flow. Aside from the loss of nutrient exposure, the rapid increase in bowel growth may have also led to a more immature intestinal epithelium, which has not fully differentiated. It is possible, that the additional 4 weeks after re-implantation, allows this bowel to regain these functions.

While the present study demonstrated several functions and a histo-morphology similar to normal jejunum, some functional features were different. This includes a decline in smooth muscle contractility, slight decline in barrier function and isoelectric current changes in response to carbachol. Further the rate of spontaneous clustered contractions, while similar to that previously reported for jejunum of large animals²¹. Although contraction rate was slightly lower in the re-implanted segment, the differences were not significant. Whether these more minor changes would result in adverse consequences remains to be determined. Our finding of preservation of intestinal transit and manometry after lengthening corroborated these findings in a rat model of enterogenesis when studied *in vitro*²². The fact that more clinically relevant functional findings were normal (including intestinal transit time, change in *Isc* with glucose and disaccharidase activity) also suggests that these other differences will not be significant.

It was also interesting to note that not only did the epithelium demonstrate proliferation, but the muscularis propria of the wall increased in thickness. A similar finding of muscular hypertrophy has recently been observed in mice undergoing adaptation in a model of short bowel syndrome²³. While this study did not investigate growth in other portions of the bowel, including the mesentery, our observations during surgical harvest suggested that the vasculature also expanded during the lengthening. This increase in muscularis propria hypertrophy may also account for the mechanisms responsible for enterogenesis, as an increase in insulin-like growth factor was identified in the muscularis propria of rats undergoing mechanical distraction²⁴.

Further studies will be needed to determine the mechanisms of action responsible for this rapid growth. As well, it is possible that additional growth may be gained by the use of exogenous administration of growth factors during the process of enterogenesis. Future modifications of this lengthening device have been explored to allow the process of distraction-induced enterogenesis to take place within the intestinal lumen, without taking

the bowel out of continuity – making this a much more appealing approach for clinical applications.

In conclusion, this study demonstrated that mechanical lengthening is retained long-term, and the gain in length is associated with retention in intestinal function. With future device modifications, the use of distraction-induced enterogenesis may provide for a safe and effective treatment option for patients with short bowel syndrome.

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Figure 1.
Schematic diagram of the operative technique and post-operative radiographic image.

- A.** Appearance of bowel at the beginning of the lengthening procedure. Note the single catheter shown is actually two, one drain and one infusion catheter.
- B.** Appearance of bowel at time of harvesting the lengthening segment during the re-implantation surgery.
- C.** Appearance of bowel after re-implantation. Note sutures on both ends are tagged with non-absorbable sutures for ease of identification.
- D.** Radiographic image of a small bowel follow through. Note arrows represent the start of the small bowel, and was used to time the transit through small bowel.

Figure 2.

Representative manometric tracing from a pig. Note 3 phases are shown: baseline prior to infusion of any substances; bethanechol; and carbachol, with one balloon within the mid-section of the lengthened bowel segment 4 weeks after re-implantation and one balloon in a non-operated area of jejunum. Note the degree of contractility and duration of this elevated contractile phase are similar between re-implanted and control segments of bowel. Time is shown in seconds, and at least 10 mins of level pressure readings was given prior to moving to the next phase. Pressure is given in cm of water.

Figure 3.

Summary of isolated smooth muscle cell contraction studies comparing non-surgically manipulated cells from jejunum, immediately after lengthening and at the time of harvesting post-re-implantation. Results are given as the mean percent ($\pm$ SD) contraction after stimulation by acetylcholine.

Jejunum

Lengthened

Re-Implanted

Figure 4.
Epithelial cell proliferation

- A.** Epithelial cell proliferation is expressed as the mean percent of BrdU positive cells per crypt ($\pm$ SD). BrdU was given 4 hours prior to harvesting of intestinal segments. Results are mean of at least 16 crypts per pig.
- B.** Representative histologic images of jejuna mucosa with BrdU staining. Magnification 40X.

Figure 5.
Assessment of epithelial barrier function

- A. Passage of ^3H -mannitol is shown as the percent of permeation of a tracer molecule from the mucosal to serosal surfaces. Results are cumulative and collected over a 90 min period. Note preserved barrier function between groups, no significant differences were detected.
- B. Transepithelial resistance (TER) is shown as the Ω/cm^2 for all 3 study groups over a 90 minute incubation period in Ussing chambers. Note a significant loss of TER in the Lengthened group. Whereas, the re-implanted group regained TER levels similar to native jejunum.

Figure 6.

Changes in the isoelectric current (ISC) are shown (based on change from steady state baseline and given as $\mu\text{A}/\text{cm}^2$) for the 3 study groups in response to glucose (A) and carbachol (B). Note a loss of responsiveness to glucose and carbachol in the Lengthened group, and a return to native jejunum levels in the Re-implanted group for glucose, but not carbachol. Values are mean $\pm$ SD, and not significantly different between the groups.

Figure 7.

Tight junction molecules, ZO-1, claudin-1 and occludin, abundance in each group shown as mean $\pm$ SD, and representative immunoblots for each group. Note a significant loss of ZO-1 and claudin-1 in the Lengthened group, and a partial recovery in the re-implanted group. Note no significant changes for occludin.

Figure 8.

Representative figures of tight junction staining (occludin and ZO-1) for each study group. Original magnification was 20X, staining was performed on freshly frozen tissue and imaging intensity was matched between samples. Note the loss of intensity and actual absence between some cells in the Lengthened group compared to the other two groups.

Table 1

	Villus height (μm)	Crypt depth (μm)	Total mucosal thickness (μm)	Villus width (μm)	Muscularis propria (μm)
Jejunum	528.8 $\pm$ 88.0	330.3 $\pm$ 69.9	914.4 $\pm$ 117.6	120.1 $\pm$ 19.4	678.0 $\pm$ 61.8
Lengthened	372.1 $\pm$ 81.2 *	428.1 $\pm$ 89.2 *	766.1 $\pm$ 95.4 *	134.0 $\pm$ 17.9 *	1018.7 $\pm$ 160.6 *
Re-implanted	473.5 $\pm$ 77.8 [#]	341.7 $\pm$ 91.7 [†]	861.0 $\pm$ 134.2 [‡]	148.7 $\pm$ 24.9 [#]	981.7 $\pm$ 169.4 [‡]

* $P < 0.05$ (ANOVA), Lengthened versus normal jejunum segment;

[†] $P < 0.05$ (ANOVA), Lengthened versus Re-implanted segment;

[‡] $P < 0.05$ (ANOVA), normal jejunum versus Re-implanted segment.

Data are mean $\pm$ SD.

Table 2

	Lactase	Sucrase	Isomaltase	Maltase
Jejunum	34.6±13.6	40.8±10.5	38.4±9.8	150.2±35.6
Lengthened	13.6±4.7*	21.8±6.7*	17.7±8.2*	106.5±38.2
Re-implanted	30.8±6.2 [†]	41.3±18.5 [†]	42.0±12.2 [†]	114.0±4.5

* $P < 0.05$ (ANOVA), Lengthened versus normal jejunum segment;

[†] $P < 0.05$ (ANOVA), Lengthened versus Re-implanted segment.

Data are mean ± SD. Enzyme specific activities were expressed as units (U), defined as moles of glucose released per minute per microgram of mucosal protein