Effect of Bowel Preparation and a Fiber-Free Liquid Diet on Expression of Transforming Growth Factor and Procollagen in Colonic Tissue Preoperatively and Postoperatively

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PURPOSE: Dehiscence of colonic anastomoses is prevalent and potentially fatal. In an attempt to reduce the likelihood of anastomotic dehiscence, the colon is cleansed before surgery and fiber-free diets are prescribed postoperatively. However, fiber-free diets induce colonic atrophy and impair healing. This study was designed to investigate the effect of bowel preparation and postoperative fiber-free diet on the local gene expression of transforming growth factor-beta 1 and procollagen type I. METHODS: Four Sprague-Dawley rats underwent bowel preparation with a fiber-free liquid diet and polyethylene glycol in a balanced electrolyte solution for two days (fiber-free preoperative diet group), whereas four rats received standard chow with fiber (preoperative diet with fiber group). On the third day tissue was obtained from the descending colon of each rat to assess the effect of bowel preparation. Forty additional rats had their bowels prepared and underwent transection of the descending colon and anastomosis. These rats were then randomly assigned to continue on the liquid diet (fiber-free postoperative diet group) or rat chow (postoperative diet with fiber group). On postoperative days 3, 5, 6, 7, and 14, colonic tissue was obtained from the anastomosis and analyzed with the use of semiquantitative reverse transcriptase-polymerase chain reaction to examine the relative expression of transforming growth factor-beta 1 and procollagen type I genes normalized to that of a constitutive gene. RESULTS: There was a decrease in the expression of the transforming growth factor-beta 1 and the procollagen type I genes in the fiber-free preoperative diet group compared with the preoperative diet with fiber group; however, this difference only reached statistical significance for procollagen type I. Postoperatively, significant increases in the expression of the transforming growth factor-beta 1 and procollagen type I genes over baseline levels were observed around postoperative day 7 in both groups, which temporally correlates with active phases of collagen deposition in the wounded colon. Expression of the procollagen type I gene, however, was significantly decreased at this time in the fiber-free postoperative diet group compared with the postoperative diet with fiber group. CONCLUSION: Although necessary to reduce septic complications, preoperative bowel preparation has a detrimental effect on the expression of transforming growth factor-beta 1 and procollagen type I. A postoperative fiber-free liquid diet also may be detrimental to the expression of these transcripts in the bowel. Alternative methods for delivery of colonic fuels are needed to create a better environment for colonic healing while eliminating bacteria and bulk. [Key words: Colonic healing; Bowel preparation; Postoperative diets; Elemental diets; Peptide growth factors; Transforming growth factor-beta 1; Procollagen I]

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Dehiscence is more common in colonic anastomoses than in small-bowel anastomoses.[1] Although significant advances have occurred in preoperative preparation and surgical techniques, leakage of colonic anastomoses continues to be a significant cause of perioperative morbidity and mortality in patients undergoing colonic surgery.[2] The reported incidence of anastomotic leakage ranges from as low as 0 percent to as high as 35 percent.[3]

Many factors have been proposed to explain this propensity of colonic anastomoses to develop dehiscence and leakage.[4-9] Two of these factors are the high bacterial counts present and the high intraluminal pressure created by the colonic contents. To reduce bacteria and intraluminal pressures, patients undergoing colonic surgery undergo a bowel preparation that consists of mechanical cleansing, fiber-free liquid diets, and antibiotics. These mea-

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sures, however, are associated with bowel atrophy.[10–13]

We demonstrated that the addition of a fiber source to an elemental diet improves the healing of colonic anastomoses,[14] whereas diversion of the fecal stream impairs colonic healing.[15] The mechanical strength of healing colonic anastomoses largely depends on submucosal collagen metabolism, which is regulated by expression of local growth factor. Accordingly, we demonstrated that during the healing of colonic anastomoses there is a temporal association between the gene expression of transforming growth factor-beta 1 (TGF-β_1) and of procollagen type I (PROC I).[16, 17] Therefore, the purpose of this study was to determine the effect of preoperative bowel preparation and of fiber-free liquid diet given postoperatively on the local anastomotic expression of TGF-β_1 and PROC I mRNAs.

METHODS

Forty-eight male Sprague-Dawley rats weighing 250 to 275 g were obtained from the Charles River Laboratories (Bridgeport, NJ) and acclimated to laboratory conditions in individual metabolic cages. All rats were housed in wire-bottom cages to prevent copraphagia and were randomly assigned to treatment groups. All conditions and procedures were approved by the Philadelphia Veterans Administration Medical Center Subcommittee on Animal Studies and met standards of the National Research Committee's guide on animal studies.

Analysis of Preoperative Bowel Preparation

Four rats were started on a preoperative bowel preparation consisting of a fiber-free liquid diet (FF_{preop} group), Vivonex® TEN (Sandoz Nutrition Corporation, Minneapolis, MN), and the osmotic purgative polyethylene glycol in a balanced electrolytic solution, GoLYTELY® (Braintree Laboratories Inc., Braintree, MA), for two days. FF_{preop} rats received 60 ml of liquid diet per day, which provided all nutritional requirements. Four additional rats were given an equivalent amount of nutrients for two days with fiber (WF_{preop} group), in the form of standard rat chow.

On the third day, all rats were anesthetized with intraperitoneal ketamine (90 mg/kg) and xylazine (1 mg/kg) and underwent a midline laparotomy. A standard site in the distal colon, 2.5 cm proximal to the pelvic brim, was chosen to perform our studies. Intact colonic specimens from each of the FF_{preop} and WF_{preop} rats were harvested 5 mm proximal and 5 mm distal from that site, and the rats were killed. Tissue specimens were snap frozen in liquid nitrogen and stored at −80°C until molecular analysis.

Analysis of Postoperative Diet

Forty rats received a two-day preoperative bowel preparation as described above. On the third day, these rats then underwent transection of the colon at the standard site, and in each rat a single-layer, end-to-end anastomosis was constructed with eight interrupted silk sutures placed in an inverting manner. The laparotomy was then closed, and the animals were returned to their cages and allowed to awaken. These 40 rats were randomly assigned either to remain on the fiber-free liquid diet in the postoperative period (FF_{postop} group; n = 20) or to begin receiving standard rat chow with fiber (WF_{postop} group; n = 20).

The colonic wall, 5 mm proximal and 5 mm distal to the anastomosis, was harvested from four FF_{postop} and four WF_{postop} rats on postoperative days (PODs) 3, 5, 6, 7, and 14. Rats with any visual evidence of dehiscence (abscess, fistula formation, or fecal contamination within the peritoneum) were noted and replaced for tissue analysis. Samples of colonic tissue were snap frozen in liquid nitrogen and then stored at −80°C until molecular analysis.

Molecular Analysis

Total RNA was isolated from colonic specimens with the use of RNAzol B™ (Tel-Test, Inc., Friendswood, TX), a modification of single-step guanidinium thiocyanate-phenol-chloroform extraction.[18] Specimens were homogenized and RNA extracted at 4°C with the addition of 0.2 volumes of chloroform. RNA was then precipitated at 4°C in one volume of isopropanol and washed in 75 percent ethanol. Finally, RNA was resuspended in diethylpyrocarbonate-treated water and stored at −80°C until the reverse transcription step. Concentration and purity of the RNA was determined spectrophotometrically and integrity was assessed by electrophoresis of total RNA through a denaturing formaldehyde-agarose gel.

Reverse transcription of total RNA samples was accomplished with the use of Superscript II™ reverse transcriptase (Gibco BRL, Gaithersburg, MD) according to the manufacturer's instructions. One μg of RNA was denatured at 70°C for ten minutes with 0.5 μg of oligo dT primer® (Invitrogen, San

Diego, CA). First-strand cDNA was synthesized by adding (final concentrations) first-strand buffer (50 mM Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$; Gibco BRL), dithiothreitol (10 mM; Gibco BRL) and a deoxynucleotide triphosphate mixture (0.5 mM each of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate; Promega, Madison, WI), and incubating with 1 μl (200 units) of Superscript II™ at 37°C for one hour. The reaction was terminated by incubating at 70°C for 15 minutes, and the cDNA diluted to a final volume of 100 μl with sterile distilled H_2O.

The expression of mRNA in all specimens was analyzed by amplification of the respective cDNA samples. Expression of the L7 ribosomal subunit mRNA was analyzed as an internal reaction standard. For the amplification reaction, 20 μl of cDNA was added to 79.4 μl of the master mix containing (final concentrations) polymerase chain reaction buffer (50 mM KCl, 10 mM Tris-HCl, 0.1 percent Triton X-100; Promega), $MgCl_2$ (2.5 mM; Promega), deoxynucleotide triphosphate mix (1.0 mM; Promega; including 2.5 μCi of ^{32}P-labeled deoxycytidine triphosphate; NEN), and forward and reverse gene-specific primers (0.4 μM; Gibco BRL) and denatured at 99°C for five minutes. Amplification was started by adding 0.6 μl (3 units) of *Taq* polymerase (Promega) and incubating in a thermal cycler (MJ Research, Watertown, MA). All samples were amplified for 35 cycles, each cycle consisting of denaturation at 94°C for one minute, primer annealing at 60°C for 30 seconds and extension at 72°C for 50 seconds. Radiolabeled amplification products then were subjected to electrophoresis on a 1.3 percent agarose gel, blotted onto a nitrocellulose membrane (Amersham, Arlington Heights, IL), and visualized on a phosphorimager. Gel images were quantitated with the use of a computer-based image analysis system (ImageQuant™ software, Molecular Dynamics, Sunnyvale, CA).

Statistical Analysis

Levels of TGF-β_1 and PROC I expression were normalized by calculating them as a percentage of the expression of the constitutive L7 ribosomal subunit gene. We previously demonstrated that the expression of this transcript is not altered with the progress of wound healing in this model.[16] Expression levels determined from all animals within a survival group were pooled and the mean level of expression calculated and reported with standard error of the mean. Data from preoperative groups were analyzed with the use of a one-way analysis of variance (ANOVA), with diet as the independent variable and gene expression as the dependent variable, to determine the overall significant differences across groups. Data from postoperative groups were analyzed with the use of a two-way ANOVA, with diet and survival time as the independent variables and gene expression as the dependent variable, to determine the overall significant differences across groups. Planned multiple comparisons were then conducted to determine differences between groups. For comparison purposes, the FF_{preop} group was used as a baseline control (POD 0) for both the FF_{postop} and WF_{postop} groups, because this group received the same preoperative bowel preparation as both of the postoperative treatment groups. The magnitude of treatment effects was calculated with the use of estimated omega squared (ϖ^2).

RESULTS

Effect of Preoperative Bowel Preparation on TGF-β_1 and PROC I Expression

There was no significant difference in the average weight between the FF_{preop} and the WF_{preop} groups (Fig. 1; POD 0). The relative expression of TGF-β_1 (mean ± standard error of the mean) was slightly lower in the FF_{preop} group (3.72 ± 1.22) compared with the WF_{preop} group (4.77 ± 1.17), although this difference was not statistically significant (Fig. 2). The magnitude of the effect of diet on TGF-β_1 expression was small ($\varpi^2 = 0.0$). PROC I expression in the FF_{preop} group (3.35 ± 0.40), however, was significantly decreased ($P = 0.04$) compared with that in the WF_{preop} group (5.26 ± 0.62; Fig. 2). The magnitude of the effect of diet on PROC I expression was large ($\varpi^2 = 0.42$).

Effect of Postoperative Diet on TGF-β_1 and PROC I Expression

Although both groups demonstrated weight gain throughout the course of the experiment, there were no significant differences in body weights between them at any time point examined (Fig. 1). Morphological inspection of anastomoses in the FF_{postop} (n = 20) and the WF_{postop} (n = 20) groups demonstrated two (10 percent) dehiscences, both occurring in the WF_{postop} group at POD 3. There were also higher

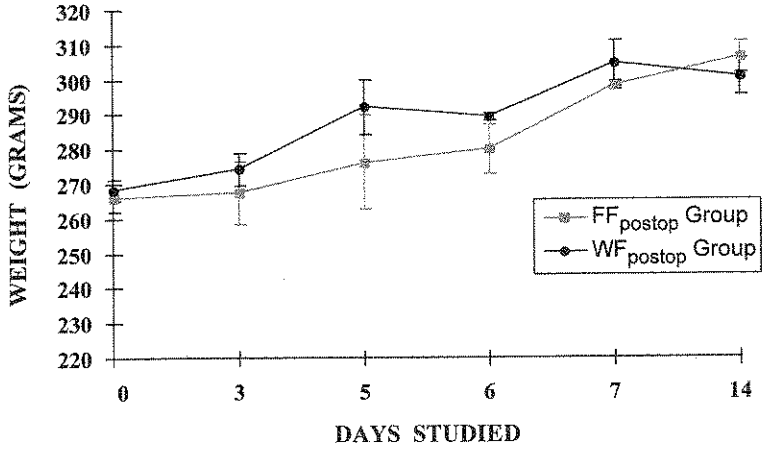

Figure 1. Mean body weights of the fiber-free postoperative diet (FF_{postop}) and postoperative diet with fiber (WF_{postop}) groups across the time course studied. Weight gain was not significantly different between these groups. Weights of the fiber-free preoperative diet *vs.* the preoperative diet with fiber groups are represented at day 0.

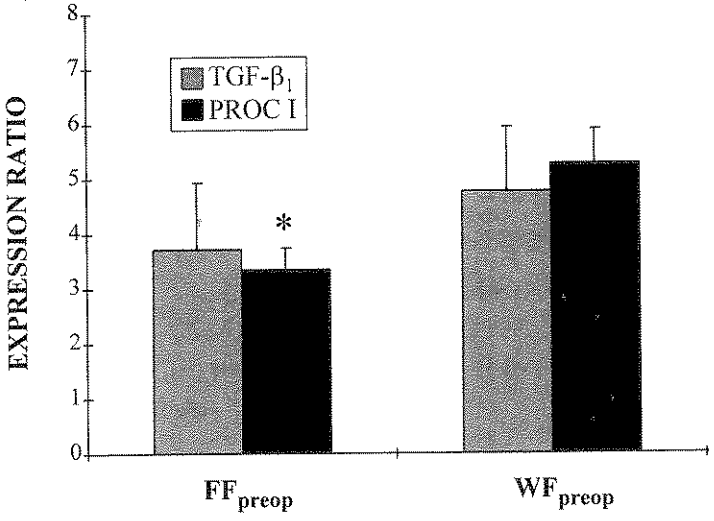

Figure 2. Mean expression of transforming growth factor-beta 1 (TGF-β_1) and procollagen type I (PROC I) in the fiber-free preoperative diet (FF_{preop}) and preoperative diet with fiber (WF_{preop}) groups, normalized to that of the L7 ribosomal subunit. Expression of PROC I in the FF_{preop} group was significantly decreased compared with that in the WF_{preop} group (*$P < 0.05$).

respective incidences of adhesions (15 *vs.* 10 percent) and fistula formation (5 *vs.* 0 percent) noted in the WF_{postop} group compared with the FF_{postop} group. None of these reached statistical significance, however.

Overall, both the FF_{postop} and the WF_{postop} groups demonstrated progressive increases in the expression of the TGF-β_1 transcript through POD 7 (Fig. 3). The FF_{postop} group showed significant increases in TGF-β_1 expression over baseline levels (POD 0 = 3.72 ± 1.22) only on PODs 6 (12.10 ± 3.15; $P = 0.03$) and 7 (16.45 ± 3.52; $P = 0.002$), whereas the WF_{postop} group demonstrated significant increases on PODs 5 (13.10 ± 3.67; $P = 0.02$), 6 (14.58 ± 3.59; $P = 0.007$), and 7 (21.35 ± 4.26; $P = 0.0001$). Although TGF-β_1 expression seemed to be consistently lower in the FF_{postop} group than in the WF_{postop} group at each time point examined, none of the differences between the two diet groups were statistically significant (Fig. 3). The magnitude of the effects of diet and survival time on TGF-β_1 expression were large ($\varpi^2 = 0.21$ and 0.60, respectively).

Both the FF_{postop} and the WF_{postop} groups also seemed to demonstrate progressive increases in the expression of the PROC I transcript through POD 7 (Fig. 4). Expression in the FF_{postop} group, however, was not statistically elevated over baseline levels (POD 0 = 3.35 ± 0.40) at any time point examined,

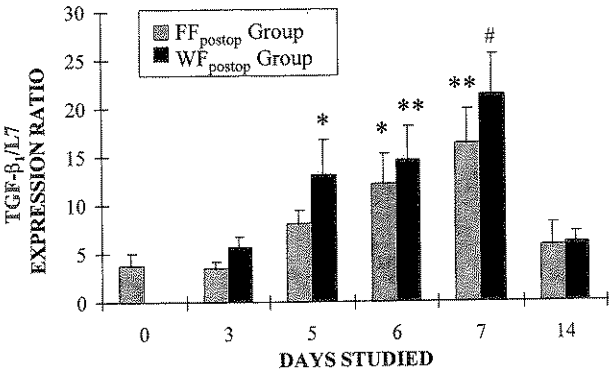

Figure 3. Mean expression of transforming growth factor-beta 1 (TGF-β_1) in the fiber-free postoperative diet (FF_{postop}) and postoperative diet with fiber (WF_{postop}) groups, normalized to that of the L7 ribosomal subunit, across the time course studied. Expression in the FF_{postop} group was significantly increased over baseline levels (day 0) on days 6 ($^*P < 0.05$) and 7 ($^{**}P < 0.01$), as was expression in the WF_{postop} group on days 5 ($^*P < 0.05$), 6 ($^{**}P < 0.01$) and 7 ($^{\#}P < 0.001$).

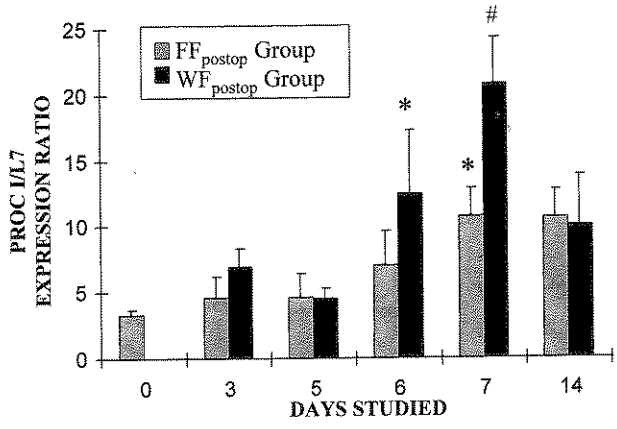

Figure 4. Mean expression of procollagen type I (PROC I) in the fiber-free postoperative diet (FF_{postop}) and postoperative diet with fiber (WF_{postop}) groups, normalized to that of the L7 ribosomal subunit, across the time course studied. Expression in the FF_{postop} group was not significantly increased over baseline levels (day 0) at any time point examined, but it was in the WF_{postop} group on days 6 ($^*P < 0.05$) and 7 ($^{\#}P < 0.001$). Additionally, expression in the FF_{postop} group on day 7 was significantly decreased compared with that in the WF_{postop} group at the same time ($^*P < 0.05$).

but it was elevated in the WF_{postop} group on PODs 6 (12.44 ± 4.82; $P = 0.02$) and 7 (20.80 ± 3.46; $P = 0.0001$). Throughout the time course PROC I expression was also slightly lower in the FF_{postop} group than in the WF_{postop} group, but it was significantly decreased on POD 7 ($P = 0.01$; Fig. 4). The magnitude of the effects of diet and survival time on PROC I expression were large ($\varpi^2 = 0.30$ and 0.46, respectively).

DISCUSSION

Our results show that in an anastomotic colon, the TGF-β_1 and PROC I genes are significantly upregulated in the postoperative period. This finding agrees with our previous studies and may indicate that endogenous TGF-β_1 and PROC I contribute to colonic anastomotic healing. In addition, administration of a fiber-free liquid diet is associated with diminished expression of the PROC I gene.

It is a common practice of surgeons to feed patients with fiber-free liquid diets (clear liquids or elemental diets) after the creation of a colonic anastomosis. The theory behind this practice is that fecal bulk passing through the newly formed anastomosis, held together only by sutures, would increase the incidence of complications. Our morphological data, although not reaching statistical significance, support this theory. We found that the rat chow diet group had a higher incidence of dehiscence, adhesions, and fistula formation. Other investigators reported similar findings.[19] Although liquid elemental diets are devoid of fiber, which decreases fecal bulk in the colonic lumen, these diets have been shown to influence the motility, secretions, and microflora of the gastrointestinal tract. One study, specific to the colon, showed that liquid elemental diets induce mucosal atrophy and hypothesized that this probably was mediated by a reduction in the proliferative activity of the stem cells of the colon.[10]

The effect of low-residue diets on collagen content in both unoperated and postoperative colonic tissue was examined by other investigators.[19–24] These studies showed that a low-residue diet is detrimental to the deposition of collagen. Although there is agreement on the diminished gain in collagen content in the low-residue diet, there is some controversy as to whether that translates into decreased anastomotic strength. Several studies[20, 21] demonstrated decreased anastomotic strength in animals given low-residue diets, but others did not corroborate these results.[19]

In this study we chose to use the initiating event of collagen synthesis, procollagen gene expression, as a means of investigating the effect of a low-residue diet on collagen synthesis in postoperative colonic tissue. Our results show that by POD 7 there is a significant decrease in the anastomotic expression of the PROC I

gene in animals fed a liquid diet. This finding is particularly interesting because, in this time course, it was also by POD 7 that both TGF-β_1 and PROC I demonstrated maximum expression as compared with baseline. This may be a critical time for anastomotic healing. A previous study showed that in the first four days after the creation of a colonic anastomosis, collagenolysis predominates over collagen synthesis. By the seventh postoperative day, collagen synthesis surpasses collagen degradation, resulting in a net gain in collagen content.[6]

Peptide growth factors are important regulators of wound healing in all tissues. Specifically, growth factor expression has been correlated with collagen expression and production.[25–29] Furthermore, TGF-β_1 may directly regulate the expression of the procollagen transcript.[30, 31] We chose to study the TGF-β_1 gene because previous studies conducted in our laboratory[17] showed a significant correlation between the upregulation of TGF-β_1 expression and of the PROC I gene in healing colonic anastomoses.

Our results show that a two-day bowel preparation decreases the colonic expression of the TGF-β_1 gene, although not enough to reach statistical significance. The administration of a liquid diet in the postoperative period also decreases the expression of this gene in anastomotic tissue. Although both the FF_{postop} and WF_{postop} groups, individually, showed significant increases in the expression of the TGF-β_1 gene during the study, the earlier increase in the expression of this transcript in the WF_{postop} group (POD 5) as compared with the FF_{postop} group (POD 6) and the consistent decreases in PROC I expression in the FF_{postop} group may indicate that the administration of fiber, or residue, in the postoperative period has a stimulatory effect on the expression of growth factors that support colonic healing.

Dietary carbohydrates are chemically and functionally diverse,[32, 33] but include various forms of starch (alpha-glucans), plant storage polysaccharides, and nonstarch polysaccharides (non-alpha-glucans), which comprise the plant cell wall. Depending on factors such as physical form and preparation, starch may be readily digestible or resistant to digestion in the small bowel. Both resistant forms of starch and nonstarch polysaccharides pass through the small bowel and are fermented to various degrees by anaerobic bacteria in the colon. Fermentation of these nondigestible polysaccharides contributes both to fecal bulking in the colon and to the production of the short-chain fatty acids (SCFA) acetate, proprionate, and *n*-butyrate. These events have contrasting effects on the healing of colonic anastomoses. As described above, fecal bulk may contribute to anastomotic complications. Clinical studies suggested that inadequate preoperative mechanical preparation of the colon is a significant factor in the pathogenesis of disruption of colonic anastomoses.[4] Conversely, all SCFA stimulate trophism in the gastrointestinal tract, and n-butyrate seems to be the preferred fuel of colonocytes. These facts previously led us to study the effects of fiber and SCFA in the colon and the small bowel. The addition of pectin, a fermentable fiber source, to a liquid diet administered *via* gastrostomy increased the mechanical strength of colonic anastomoses.[14] Furthermore, we showed that the luminal infusion of SCFA[15] and the intravenous administration of *n*-butyrate[34] also increases the mechanical strength of colonic anastomoses.

Successful healing of a colonic anastomosis depends on many factors. Although the most important of these may be good surgical technique, restoration of bowel continuity cannot occur without endogenous healing processes such as re-epithelialization and collagen deposition and crosslinking.[35] Unfortunately, under the conditions that necessitate many bowel anastomoses, these endogenous processes may be impaired (*e.g.*, malnourishment and chemotherapy).[6, 35] In these cases, dehiscence may occur despite the creation of a technically good anastomosis. This, therefore, warrants consideration of factors that may stimulate natural healing processes, such as fermentation and production of SCFA. Although some postoperative diets may include starch, which is a good source of butyrate, only a fraction of this may be fermentable. Clinically, any reduction in SCFA production (*e.g.*, elimination of nonstarch polysaccharides) could be detrimental under conditions of impaired healing, because there is evidence that the ameliorative properties of SCFA are dose-dependent.[33]

Dependence of the colon on SCFA trophism creates a unique challenge to the surgeon. Because colonic fuels (*i.e.*, fiber) and the bacteria that process them need to be removed from the colon before surgery and in the immediate postoperative period, alternative methods to supply fuels to the operated colon will have to be developed. Such alternatives could include the exogenous administration of growth factors that support the healing process, but have a diminished expression under low-residue conditions.

CONCLUSIONS

Although preoperative bowel preparation is necessary to reduce septic complicatons, it has detrimental effects on local gene expression of TGF-β_1 and on PROC I. A postoperative fiber-free liquid diet is also detrimental to the colonic expression of these transcripts. Alternative methods for delivery of colonic fuels are needed to create a better environment for colonic healing while eliminating bacteria and bulk.

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