IGF-I treatment facilitates transition from parenteral to enteral nutrition in rats with short bowel syndrome

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Gillingham, Melanie B., Elizabeth M. Dahly, Sangita G. Murali, and Denise M. Ney. IGF-I treatment facilitates transition from parenteral to enteral nutrition in rats with short bowel syndrome. Am J Physiol Regul Integr Comp Physiol 284: R363–R371, 2003. First published September 27, 2002; 10.1152/ajpregu.00247.2002.—The goal of growth factor treatment in patients with short bowel syndrome (SBS) is to facilitate transition from parenteral to enteral feedings. Ideal use of growth factors would be acute treatment that produces sustained effects. We investigated the ability of acute insulin-like growth factor I (IGF-I) treatment to facilitate weaning from total parenteral nutrition (TPN) to enteral feeding in a rat model of SBS. After a 60% jejunoileal resection + cecectomy, rats treated with IGF-I or vehicle were maintained exclusively with TPN for 4 days and transitioned to oral feeding. TPN and IGF-I were stopped 7 days after resection, and rats were maintained with oral feeding for 10 more days. In IGF-I-treated rats, serum concentration of IGF-I and final body weight were significantly greater because of a proportionate increase in carcass lean body mass than in vehicle-treated rats. Acute IGF-I treatment induced sustained jejunal hyperplasia on the basis of significantly greater concentrations of jejunal mucosal protein and DNA without a change in histology or sucrase activity. These results demonstrate that acute IGF-I facilitates weaning from parenteral to enteral nutrition in association with maintenance of a greater body weight and serum IGF-I concentration in rats with SBS.

intestinal adaptation; distal small bowel resection; body composition

The primary treatment for patients with short bowel syndrome (SBS) is long-term parenteral nutrition supplementation to maintain their nutritional status (1, 27). Although lifesaving, this therapy is expensive and associated with several serious complications, such as catheter sepsis and liver failure (9). Small bowel transplants have been used with limited success, so alternative treatment options are needed for this patient population (12).

Growth factor treatment to induce adaptation of residual intestine is under investigation in humans and animals. Growth hormone alone or in combination with a high-carbohydrate diet and glutamine has been used in humans with SBS with controversial results (2, 24, 28). Recently, short-term glucagon-like peptide-2 (GLP-2) treatment was tested in a small group of patients with SBS (10). GLP-2 treatment improved the intestinal absorption of energy and wet weight, which resulted in an increase in body weight and lean body and bone mass in seven of eight patients. Numerous growth factors, including insulin-like growth factor I (IGF-I), GLP-2, and epidermal growth factor, have been shown to enhance intestinal adaptation in enterally fed animals subjected to intestinal resection (8, 17, 25). We recently demonstrated that IGF-I can enhance intestinal adaptation in a parenterally fed rat model of human SBS (6).

The ultimate goal of growth factor treatment is to facilitate weaning from parenteral nutrition and to establish oral nutrition autonomy. Ideally, this would be accomplished with acute growth factor treatment that resulted in sustained effects for the patient and minimized the need for ongoing hormone therapy. Although numerous studies have examined the effects of short-term treatment with intestinotrophic growth factors in animal models, few have investigated whether the treatment has a sustained effect or the ability of the growth factor to facilitate transition from parenteral to enteral nutrition after resection. We previously described a rat model of SBS requiring total parenteral nutrition (TPN) that, similar to the human condition, has no jejunal adaptation after resection and is dependent on parenteral nutrition (6). IGF-I treatment in this animal model increased body weight gain and induced jejunal adaptation. The goal of this study was to investigate the ability of IGF-I to facilitate transition from parenteral to enteral nutrition and produce sustained effects on body weight and intestinal adaptation after the cessation of growth factor treatment in rats with SBS.

METHODS

Animals and experimental design. The animal facilities and protocols were approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Harlan, Madison, WI) weighing 225–250 g were housed in individual stainless steel cages

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with free access to water in a room maintained at 22°C on a 12:12-h light-dark cycle and allowed to acclimate to the facility. Three days before surgery, the animals were fed a fiber-free, semielemental, liquid diet ad libitum as a bowel preparation (Vital, donated by Ross Laboratories, Columbus, OH). Animals were randomized into three TPN groups: gut resection (R), gut resection + IGF-I (R + I), and transection control (T). Orally fed, nonsurgical, age-matched controls (oral) were included as a normal comparison. This group was allowed ad libitum access to a nutritionally complete semipurified diet with a macronutrient composition similar to the TPN solution.

The surgical procedure has been previously described (6). After anesthesia, resected animals were subjected to removal of bowel 40 cm distal to the ligament of Treitz to 1 cm distal to the cecum, and bowel continuity was reestablished with an end-to-side jejunal anastomosis. In transected animals, bowel 40 cm distal to the ligament of Treitz and 1 cm distal to the cecum was cut, and bowel continuity was restored. A two-layer closure of the incision included suturing of the peritoneum and muscle layers followed by closure of the outer skin. A catheter was placed in the superior vena cava via the external jugular vein for the delivery of TPN solution (15). Thirty-seven animals underwent resection or transection surgery, and there was a 95% survival rate from the surgical procedure. Of those that survived surgery, 94% had patent catheters at the end of postoperative day 7 and are included in the study results.

After surgery (day 0), infusion of TPN solution was initiated and water was provided ad libitum. All animals received oxyormorph HCl for pain management and prophylactic ampicillin for 48 h after surgery (6). IGF-I-treated animals received recombinant human IGF-I (rhIGF-I, 3.0 mg·kg body wt$^{-1}·day^{-1}$; courtesy of Genentech, South San Francisco, CA) for 6 days after surgery (days 1–6) concurrent with the continuous infusion of TPN. The amount of TPN solution was gradually increased from 20 g on day 0 to 40 g on day 1 and 60 g on days 2–4. The TPN solution contained (in g/l) 45 amino acids, 180 dextrose, and 28 lipid, providing 32% non-protein energy from fat and 68% non-protein energy from dextrose, similar to our previous report (6). Animals were allowed free access to the same preoperative liquid diet and continuing until the end of the experiment. TPN infusion was gradually decreased to 40 g on day 5 and to 20 g on day 6, and both TPN and IGF-I treatments were stopped on day 7. Thus, on days 7–17, animals were given free access to the liquid diet and water ad libitum but did not receive any parenteral nutrition or IGF-I. Body weight, the amount of TPN solution infused, and the volume of liquid diet consumed were measured and recorded daily. After 7 days of TPN and 10 days of oral feedings, rats were anesthetized by an injection of ketamine and xylazine (75 and 8 mg/kg body wt, respectively) and then killed by exsanguination.

**Jejunal and colonic tissue.** The remaining small and large intestines were removed and flushed with ice-cold saline. The first 10-cm section of jejunum distal to the ligament of Treitz was frozen for RNA extraction. The next 10-cm section of jejunum was used to measure mucosal wet weight, protein, (bicinchoninic acid protein assay; Pierce Chemical, Rockford, IL) and DNA (23) content, and sucrase activity (3). Jejunal segments used for mucosal analysis were slit along the mesenteric border, and mucosa was scraped from the muscularis with a glass slide. In the colon, the first 3-cm section distal to the anastomosis was discarded. The next 3-cm section was used to determine full-thickness wet weight and protein and DNA content. The next 1-cm section was used for histology and the remaining colon was frozen for RNA extraction.

**Histology.** Sections (1 cm) of jejunum and colon were fixed in a 10% buffered paraformaldehyde-methanol solution (Histochoice, Amresco, Solon, OH) for morphometric analysis. Fixed tissue was embedded in paraffin, cut into 5-µm sections, stained with hematoxylin and eosin, and examined for histomorphometry (6). Jejunal villus height and crypt depth were measured on ≥10 villus-crypt axes per animal using SigmaScan software (Jandel Scientific, San Rafael, CA). Col- lon crypt depth was measured similarly.

**Immunoactive IGF-I.** Intact jejunum, colon, and liver samples were homogenized in ammonium formate and spun at 14,000 g for 15 min, and the supernatant was applied to a C-2 bond elute column (Varian, Harbor City, CA), as previously described (7). Immunoactive IGF-I was extracted in 45% acetonitrile-3% trifluoroacetic acid. Total serum IGF-I as well as liver, jejunal, and colon immunoactive IGF-I concentrations were determined by RIA (7, 22).

**Western ligand blot.** Two microliters of serum were diluted in 20 µl of nonreducing Laemmli sample buffer and heated to 100°C for 10 min. Samples were then separated by SDSPAGE. Proteins were transferred to a polyvinylidene difluoride membrane and probed for IGF-binding proteins (IGFBPs) with biotinylated IGF-I (40 ng/ml) and then with streptavidin-horseradish peroxidase (1:500 dilution). The signal was visualized using enhanced chemiluminescence (Amersham Biosciences). A prestained protein standard (Bio-Rad, Hercules, CA) was used to determine molecular weights. The band intensities of 38,000–43,000 and 30,000–34,000 were quantified by OptiQuant and expressed as density light units relative to the transection control group (7).

**Body composition.** The concentration and percent composition of water, protein, and fat were determined on eviscerated rat carcasses, as previously described (31). Briefly, carcasses were freeze-dried to determine total carcass water, and dried carcass was homogenized in liquid nitrogen. Aliquots of freeze-dried carcass homogenate were assayed in duplicate for nitrogen content by micro-Kjeldahl analysis and for fat content by ether extraction. Carcass residue was calculated by difference.

**Jejunal and colonic IGF-I mRNA.** Total RNA was isolated from frozen jejunum and colon tissue using TRIzol reagent (GIBCO BRL, Gaithersburg, MD). IGF-I mRNA was measured by RNase protection assay (RPA) (13). A 464-bp cDNA fragment of rat IGF-I exons 2, 3, and 4 in a pGEM-4Z vector (GIBCO BRL, Gaithersburg, MD). IGF-I mRNA was measured by RNase protection assay (RPA) (13). A 464-bp cDNA fragment of rat IGF-I exons 2, 3, and 4 in a pGEM-4Z vector was linearized with EcoRI. T7 RNA polymerase was used to synthesize an antisense IGF-I RNA probe. Ribosomal 18S mRNA antisense template (Ambion, Austin, TX) was transcribed and cohybridized with the IGF-I probe and tissue RNA samples as a control. Single-strand RNA was removed by RNase digestion using the HybSpeed kit (Ambion) according to the manufacturer’s instructions, and protected bands were separated on acrylamide-urea gels. Gels were dried and exposed to phosphorimagery screens. Each sample was analyzed at least twice in separate RPAs. Protected bands were observed at 238 nt corresponding to the IGF-1 mRNA transcript from the exon 1 promoter and at 80 nt corresponding to 18S (13). The IGF-I vector was kindly provided by Dr. M. L. Adamo (San Antonio, TX). Protected bands were quantified by phosphorimaging (Packard Instrument, Meridian, CT). Relative band intensities were calculated by dividing the IGF-I band intensity by the 18S band intensity in each sample and then expressed as fold difference relative to transection controls.

**Statistics.** Groups were compared using one-way ANOVA and the protected least significant difference technique to
determine individual group differences (SAS Institute, Cary, NC). Significance levels for colon immunoreactive IGF-I and jejunal protein concentrations are based on logarithmically transformed data, because residual plots of the data indicated unequal variance between treatment groups. P ≤ 0.05 was considered statistically significant.

RESULTS

Body weight and composition. Mean daily body weights are illustrated in Fig. 1. There was no significant difference in body weight between groups before surgery (day −4 to day −1). After surgery, resected animals lost significantly more weight than transection controls because of the removal of gut tissue (6–9 g) during surgery (day 0). By day 7, the weight of the non-IGF-I-treated resected animals was ~10 g less than their preoperative body weight. IGF-I treatment improved weight gain after surgery, such that IGF-I-treated resected animals weighed significantly more than non-IGF-I-treated resected animals, and their body weight was not significantly different from that of transection controls at day 7. When TPN and IGF-I were stopped (day 7), both groups of resected animals maintained their body weights with oral feedings, while transection controls continued to gain weight. At the end of the experiment, IGF-I-treated resected animals weighed significantly more (13%) than vehicle-treated resected animals. Transection controls weighed significantly more than resected animals. Oral controls steadily gained weight throughout the experiment, and their final body weight was significantly greater than that of the other three surgical TPN treatment groups.

The observed changes in body weight occurred despite equal nutrient delivery and intake across TPN treatment groups. There was no significant difference in the amount of TPN infused between groups (300 ± 5, 290 ± 6, and 291 ± 6 g/7 days for T, R, and R + I, respectively). In addition, there was no significant diff-

Table 1. Body composition of animals transitioned from parenteral to enteral feedings after resection and/or IGF-I

<table>
<thead>
<tr>
<th>Group</th>
<th>Carcass Weight, g</th>
<th>Carcass Water g</th>
<th>Carcass Water %</th>
<th>Carcass Fat g</th>
<th>Carcass Fat %</th>
<th>Carcass Protein g</th>
<th>Carcass Protein %</th>
<th>Carcass Residue g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>292 ± 6*</td>
<td>185 ± 3*</td>
<td>63.1 ± 0.6*</td>
<td>38.1 ± 3.3*</td>
<td>13.0 ± 1.0*</td>
<td>66.2 ± 2.1*</td>
<td>22.6 ± 0.5</td>
<td>3.9 ± 2.3</td>
</tr>
<tr>
<td>T</td>
<td>262 ± 6†</td>
<td>174 ± 3†</td>
<td>66.2 ± 0.4†</td>
<td>25.9 ± 2.3†</td>
<td>8.8 ± 0.7†</td>
<td>57.2 ± 1.2†</td>
<td>21.9 ± 0.5</td>
<td>5.7 ± 1.3</td>
</tr>
<tr>
<td>R</td>
<td>290 ± 6§</td>
<td>157 ± 4§</td>
<td>68.7 ± 0.3§</td>
<td>12.6 ± 1.4§</td>
<td>6.2 ± 0.6§</td>
<td>44.9 ± 1.8§</td>
<td>22.4 ± 0.5</td>
<td>5.2 ± 1.1</td>
</tr>
<tr>
<td>R + I</td>
<td>225 ± 4‡</td>
<td>153 ± 3‡</td>
<td>67.9 ± 0.4‡</td>
<td>16.0 ± 1.6‡</td>
<td>7.0 ± 0.6‡</td>
<td>49.8 ± 1.6‡</td>
<td>22.1 ± 0.5</td>
<td>6.6 ± 1.7</td>
</tr>
<tr>
<td>P</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0002</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.77</td>
<td>0.70</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 8–10/group). T, transection control; R, resected; R + I, resected and treated with insulin-like growth factor I (IGF-I). Values in the same column with different symbols (*, †, ‡, §) are significantly different (1-way ANOVA, least significant difference).
ference in total liquid diet consumed from day 4 to the end of the experiment between groups (810 ± 37, 770 ± 32, and 809 ± 34 ml/13 days for T, R, and R + I, respectively). Thus smaller animals ingested more nutrients per kilogram body weight (244 ± 8, 287 ± 12, and 267 ± 11 average kcal·kg⁻¹·day⁻¹ from days 7 to 16 for T, R, and R + I, respectively, P = 0.02, T vs. R + I and R vs. R + I). IGF-I-treated resected rats gained more weight for the same nutrient intake during TPN and IGF-I infusion and maintained a significantly greater body weight with oral feedings than vehicle-treated resected rats. However, resected rats, irrespective of IGF-I treatment, had copious watery diarrhea with oral feedings, which indicated malabsorption of ingested diet compared with transection and oral controls. Thus the transected animals gained more weight than the IGF-I-treated resected rats, which gained more weight than vehicle-treated resected rats with the same nutrient intake.

Results of the body composition analysis are shown in Table 1. The absolute carcass weights and amounts of water, fat, and protein per carcass are consistent with changes in body weight. That is, oral controls had a significantly greater carcass weight and significantly more carcass water, fat, and protein than the transection controls. Transection controls had greater carcass weight and more carcass water, fat, and protein than the resected groups. IGF-I-treated resected rats had significantly more carcass water and protein than vehicle-treated resected rats, indicating accretion of lean body mass. There was no difference in percent carcass water or fat between the two groups of resected rats, and there was no difference in percent carcass protein or residue between all four groups. Thus there was a 13% greater carcass mass but no difference in the proportional body composition between IGF-I- and vehicle-treated resected rats.

**Jejunal mucosal composition.** Resected rats acutely treated with IGF-I had significantly greater jejunal mucosal wet weights than oral and transection controls and significantly greater concentrations of protein and DNA than oral and transection controls as well as vehicle-treated resected rats (Fig. 2). A parallel increase in the concentrations of protein and DNA indicates that the greater mass in resected rats treated with IGF-I is due to cellular hyperplasia. Mucosal protein concentration was significantly greater in vehicle-treated resected rats than in oral and transection controls, but there was no significant difference in mucosal DNA concentration between vehicle-treated resected rats, oral controls, and transection controls, indicating that resection induced mucosal hypertrophy. Thus IGF-I-treated resected rats had an increased mucosal cellularity compared with all other treatment groups and an increased mucosal mass compared with oral and transection controls. There was no significant difference in sucrase-specific or segmental activity between resected rats acutely treated with IGF-I, vehicle-treated resected rats, and transection controls (Table 2).

Despite differences in jejunal mucosal composition, there was no difference in jejunal villus height or crypt depth between IGF-I- and vehicle-treated resected rats (Table 2). Villus height and crypt depth were greater in both resected groups than in transection controls. There was no difference in jejunal villus height and crypt depth between oral and transection controls.

**Colonic composition.** Intact colon wet weight was significantly greater in oral controls and resected
groups than in transection controls (Fig. 3). Colon protein and DNA contents were significantly greater in both groups of resected rats than in oral and transection controls. Colonic crypt depth was also greater in both groups of resected rats than in transection controls. Parallel increases in protein, DNA, and crypt depth in resected rats independent of IGF-I treatment indicate cellular hyperplasia of the colon due to resection. A similar pattern was observed in the colon of rats maintained exclusively with TPN (6) or fed enterally (19) after this same intestinal resection.

Serum and tissue immunoreactive IGF-I. Serum total IGF-I levels are shown in Fig. 4A. Serum IGF-I levels were significantly greater (26–59%) at the end of the experiment in transection and oral controls than in resected rats. On day 17, serum IGF-I was significantly greater (17%) in resected rats treated with IGF-I on days 1–6 than in non-IGF-I-treated resected rats. Serum IGF-I levels followed a pattern similar to that observed in final body weights. There was no significant difference in liver immunoreactive IGF-I between groups (range 49–55 ng/g tissue). Jejunal immunoreactive IGF-I levels were greater in oral and transection controls than in the resected groups (Fig. 4B). There was no difference in jejunal immunoreactive IGF-I between the two groups of resected rats. There was no significant difference in colonic immunoreactive IGF-I levels between all four treatment groups (Fig. 4C).

Serum IGFBPs. Serum IGFBP-3 (38–43 kDa) was lower in both groups of resected animals than in transection or oral controls (Fig. 5). Serum binding proteins at 30–34 kDa (IGFBP-1, -2, and -5) were significantly lower in IGF-I-treated resected rats than in oral controls but were not different from the other two treatment groups. Resection lowered total circulating IGFBPs by decreasing circulating IGFBP-3.

Tissue IGF-I mRNA. There were no significant differences in jejunal IGF-I mRNA between groups (Fig. 6A). The differences in jejunal immunoreactive IGF-I were not observed in jejunal IGF-I mRNA, suggesting a posttranscriptional regulation of IGF-I protein levels in jejunal tissue. Colonic IGF-I mRNA was significantly lower in vehicle- than in IGF-I-treated resected animals but was not different from the other groups.

Table 2. Sucrase activity of jejunal mucosal homogenates and jejunal and colon histology of animals transitioned from parenteral to enteral feeding after resection and/or IGF-I

<table>
<thead>
<tr>
<th>Group</th>
<th>Jejunum Specific, U/mg protein</th>
<th>Jejunum Segmental, U/cm</th>
<th>Colon Villus height, mm</th>
<th>Colon Crypt depth, mm</th>
<th>Colon Crypt depth, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>0.078 ± 0.004*†</td>
<td>0.243 ± 0.027†</td>
<td>0.37 ± 0.01*†</td>
<td>0.09 ± 0.00†</td>
<td>0.15 ± 0.01†</td>
</tr>
<tr>
<td>T</td>
<td>0.105 ± 0.002*†</td>
<td>0.340 ± 0.014*†</td>
<td>0.29 ± 0.03†</td>
<td>0.07 ± 0.00*†</td>
<td>0.13 ± 0.01*†</td>
</tr>
<tr>
<td>R</td>
<td>0.103 ± 0.007*</td>
<td>0.402 ± 0.033*</td>
<td>0.42 ± 0.01*†</td>
<td>0.11 ± 0.00*</td>
<td>0.18 ± 0.01*†</td>
</tr>
<tr>
<td>R + I</td>
<td>0.090 ± 0.008*†‡</td>
<td>0.419 ± 0.028*</td>
<td>0.38 ± 0.01*†</td>
<td>0.10 ± 0.01*†</td>
<td>0.18 ± 0.01*†</td>
</tr>
<tr>
<td>P</td>
<td>0.0165</td>
<td>0.0009</td>
<td>0.0131</td>
<td>0.0019</td>
<td>0.0041</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6–10/group). Values in the same column with different symbols (*, †, ‡) are significantly different (1-way ANOVA, least significant difference).

Fig. 3. Colon wet weight (A), protein content (B), and DNA content (C) of rats treated as described in Fig. 1 legend. Resected rats had a significantly greater colon wet weight and protein and DNA content than Oral and T independent of IGF-I treatment. Values are means ± SE (n = 8–10). Means with different superscripts are significantly different.
Despite significant differences in colonic IGF-I mRNA between resected groups, colonic immunoreactive IGF-I was not significantly different, suggesting that tissue protein levels are regulated post-transcriptionally.

DISCUSSION

The whole body and intestine-specific growth effects of IGF-I in animals have been repeatedly demonstrated in orally fed, parenterally fed, and/or resected animal models. Although the enterotrophic effects of IGF-I have not been studied in humans, Leinskold et al. (16) demonstrated nitrogen-sparing effects of short-term IGF-I therapy in patients after radical large bowel resection. IGF-I may be an ideal growth factor to induce small bowel adaptation in humans with SBS. Using a novel approach, we have demonstrated that the improved weight gain and increased serum IGF-I concentrations and jejunal mucosal cellularity after acute IGF-I treatment in resected rats were sustained after IGF-I treatment was stopped.

During acute IGF-I administration, weight gain was improved after resection surgery, similar to other reports (6, 18, 21, 32). The anabolic effects of IGF-I in resected rats were observed, despite nutrient intake equal to that of vehicle-treated resected rats that lost weight. This suggests that IGF-I alters nutrient availability and/or utilization to favor whole body anabolism in an otherwise catabolic condition. We previously demonstrated that IGF-I promotes accretion of lean body mass (19) by increasing fat oxidation and decreasing protein oxidation (20) in parenterally fed rats, but the effects of IGF-I appear to be modulated by the amount and composition of the TPN. Kee et al. (11) and Sevette et al. (26) found no effect of IGF-I on weight gain or body composition in parenterally fed rats given TPN solutions with equal amounts of carbohydrate and lipid, high carbohydrate-low lipid, or high lipid-low carbohydrate. There are several differences between the reports by Kee and Sevette and co-workers and our study: our parenteral solution provided ~30% more protein and 20% less energy, and our animals had significant surgical stress with a massive gut resection. The anabolic actions of IGF-I are dependent on ade-
quate protein and enhanced in conditions of stress (14). In our study, rats given IGF-I for 6 days after resection surgery maintained a significantly greater body weight with the same oral intake as vehicle-treated rats for 10 days after the cessation of TPN and IGF-I. Thus resected rats previously treated with IGF-I consumed less energy per kilogram body weight (kcal/kg) and maintained a greater body weight, suggesting improved nutrient absorption and/or utilization in these animals compared with vehicle-treated resected rats.

The increased body weight in IGF-I-treated resected rats was due to proportionate increases in lean body mass and fat, similar to our previous reports (19, 31). As mentioned above, several studies found no effect on body composition in parenterally fed rats given similar amounts of IGF-I (11, 26). These authors suggest that this finding may be related to a lack of stress in their studies and/or the use of young animals already growing maximally. Consistent with the hypothesis that IGF-I effects are enhanced in conditions of stress, Lemmy et al. (18) reported increased nitrogen retention in orally fed animals given IGF-I after 80% jejunooileal resection compared with resected rats given vehicle with no increase in food intake. Taken together, these data suggest that IGF-I may enhance protein accretion and/or reduce protein loss after massive bowel resection. Our study suggests that the increase in body protein can be maintained after cessation of IGF-I treatment, which has not been observed with other growth factors. In a previous study of growth hormone treatment in humans with SBS, an increase in body weight and lean body mass was observed with treatment, but these increases were not sustained after cessation of growth hormone administration (5).

Interestingly, serum IGF-I levels were significantly greater in resected rats given IGF-I for 6 days than in resected rats not given IGF-I 10 days after IGF-I infusion had stopped. Although our RIA does not distinguish between endogenous and rhIGF-I, it is unlikely that infused rhIGF-I contributes to serum IGF-I levels at day 17. The half-life of free IGF-I is ~10 min, and the half-life of IGF-I bound to IGFBPs in serum is 10–12 h (29). The liver produces and secretes the majority of circulating IGF-I, but liver immunoreactive IGF-I was not different between groups, despite significantly greater serum IGF-I levels in rats given IGF-I. This suggests that resected rats given IGF-I for 6 days

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**Fig. 6.** Left: RNase protection assays utilizing an IGF-I antisense RNA probe in jejunum (A) and colon (B) from rats treated as described in Fig. 1 legend. Jejunal RNA (30 μg, A) and colonic RNA (12 μg) were hybridized with a ^32P-labeled rat IGF-I (50 pg) probe and then subjected to RNase digestion and electrophoresis of protected bands. Lanes labeled – and + represent 50 μg of yeast RNA hybridized with the probe and treated without and with RNase, respectively. Lane labeled M is a size marker, and band sizes are identified at left. Right: density light units gathered by phosphorimaging analysis corrected for 18S and expressed as fold difference relative to T. There was no significant difference in jejunal IGF-I mRNA but significantly greater colonic IGF-I mRNA in IGF-I- than in vehicle-treated resected rats. Values are means ± SE (n = 4–6). Means with different superscripts are significantly different.
after surgery have a higher endogenous production of IGF-I with increased secretion into circulation or that they have a reduced clearance of circulating IGF-I 10 days after IGF-I infusion has stopped. Increased hepatic production of IGF-I is consistent with enhanced nutrient absorption and maintenance of a greater body mass in rats treated acutely with IGF-I after resection.

Serum IGFBP-3 was lower in both groups of resected animals than in transection and oral controls. We previously reported that serum IGFBP-3 and IGFBP-1, -2, and -5 were increased 7 days after resection surgery and/or IGF-I treatment (7). The difference in serum IGFBP levels between the two studies may be related to the time from surgery (7 vs. 17 days) and a change in the adaptive response at various time points from surgery. Resection surgery may initially result in up-regulation of the serum IGFBPs (and other components of the IGF-I axis) followed by a later downregulation in serum IGFBPs. Alternatively, IGFBPs may be lower because of poorer nutritional status in resected than in control animals, possibly reflected in decreased serum IGF-I concentrations. Synthesis and secretion of IGFBPs are highly sensitive to the nutritional status of the animal, and both resected groups exhibited malabsorption of nutrients after introduction of oral feeding (29). Despite similar serum IGFBP levels, serum IGF-I concentration was significantly greater in IGF-I- than vehicle-treated resected rats, suggesting greater bioavailability of IGF-I.

We previously reported that IGF-I treatment increased jejunal mucosal cellularity in resected rats maintained with TPN for 7 days (6, 7). In the present study, we report that this increase in mucosal cellularity was sustained for 10 days after the cessation of IGF-I treatment and transition to oral feedings, although greater mucosal cellularity was not associated with changes in jejunal sucrase activity or histology. We previously reported that IGF-I treatment reduced sucrase-specific activity in rats treated with IGF-I (6). This is most likely related to the mitogenic effects of IGF-I and the presence of enterocytes, which are less differentiated. Equal sucrase activity suggests that the enterocytes from the TPN treatment groups were in a similar state of differentiation on day 17. Whether the increased mucosal cellularity with no effect on jejunal sucrase activity or histology is related to a return to baseline after cessation of IGF-I treatment or to jejunal changes with the introduction of oral feedings is not clear.

Differences in jejunal weight, protein, and DNA content between groups did not correlate with jejunal immunoreactive IGF-I or IGF-I mRNA. That is, there were no differences in jejunal immunoreactive IGF-I or IGF-I mRNA between resected groups, but there were significant differences in jejunal mucosal cellularity. We previously reported that IGF-I treatment increases jejunal immunoreactive IGF-I in resected rats maintained exclusively with TPN for 7 days (7). In this study, IGF-I treatment may have transiently increased jejunal IGF-I levels during the 6 days of IGF-I and TPN infusion, thus establishing a new rate of enterocyte turnover, which can be maintained after cessation of growth factor treatment (4).

Colon protein and DNA contents and crypt depth were significantly greater in both groups of resected rats than in oral and transection controls. These changes in colonic structure occurred despite no significant differences in colonic immunoreactive IGF-I levels between groups. Studies in enterally and parenterally fed rats have measured a remarkable increase in colonic IGF-I mRNA after a 60% jeunoileal resection + cecectomy (6, 21). The absence of a resection-induced increase in colonic IGF-I in the present study may again be related to length of time from surgery (17 vs. 7 days). Thus resection may induce an early rise in IGF-I mRNA followed by a later return to baseline levels of message. Despite significant increases in colonic structure, resected rats transitioned to oral feedings developed substantial diarrhea. This is consistent with our previous report of no changes in electrogenic ion transport of colonic tissues from resected rats maintained with TPN for 7 days compared with transection controls (6).

In conclusion, we demonstrate for the first time that acute IGF-I treatment in rats with SBS produced increases in serum IGF-I concentration, body weight, and jejunal mucosal cellularity that were sustained after cessation of IGF-I treatment, intravenous nutrition support, and transition to oral feedings. Further study is needed to determine whether these positive short-term treatment effects of IGF-I are permanent or transient in nature.

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