Stimulation of Intestinal Growth Is Associated with Increased Insulin-Like Growth Factor–Binding Protein 5 mRNA in the Jejunal Mucosa of Insulin-like Growth Factor–I–Treated Parenterally Fed Rats

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Abstract. **Surgically stressed rats maintained with total parenteral nutrition (TPN) exhibit jejunal atrophy, which can be attenuated by insulin-like growth factor–I (IGF-I) but not by growth hormone (GH) treatment. In order to understand the basis for the selective action of IGF-I, the levels of mRNAs encoding IGF-I, IGF-binding proteins (IGFBPs), IGF-I receptor, and GH receptor/binding protein (GHR/GHBP) were determined in rats given TPN and treated with GH, IGF-I, or GH + IGF-I. GH treatment significantly stimulated hepatic IGF-I mRNA. IGF-I treatment did not alter liver IGF-I mRNA, nor was there any evidence for interaction between GH and IGF-I. Jejunal mucosa IGF-I mRNA was extremely low and was not altered by TPN or by any of the hormonal treatments. The inability of GH to stimulate jejunal growth was not associated with a deficiency in GHR/GHBP mRNA. In jejunal mucosa, IGF-I and GH treatment independently and synergistically stimulated IGFBP-3 mRNA. IGF-I stimulated jejunal IGFBP-5 mRNA, but GH had no effect on IGFBP-5 mRNA. The levels of IGF-I receptor and IGFBP-1, 2, 4, and 6 mRNAs were extremely low and/or were not altered by any of the treatments. These results suggest that the ability of exogenous IGF-I, but not GH, to induce IGFBP-5 mRNA in jejunal mucosa may lead to the selective growth-promoting effect of IGF-I. Jejunal mucosa IGFBP-3 mRNA levels were not correlated with altered growth. We postulate that IGFBP-5 positively modulates the anabolic effects induced by exogenous IGF-I in the jejunum.** [P.S.E.B.M. 1997, Vol 216]

The growth-promoting effects of insulin-like growth factor–I (IGF–I) and growth hormone (GH) have led to an interest in the use of these agents therapeutically. IGF-I and GH treatment leads to a greater anabolic response than that seen with either hormone alone in calorically restricted human volunteers (1). GH may act to increase circulating levels of IGF-I of hepatic origin, increase local production of IGF-I, and/or directly stimulate tissue growth and anabolism (2). Moreover, GH and IGF-I regulate the production of circulating and locally produced IGF-binding proteins (IGFBPs), which modulate IGF-I action (3).

Gastrointestinal (GI) growth, anabolism, and function are regulated by IGF-I and GH (e.g., Ref. 4 and references therein). Surgically stressed rats maintained with hypoca-

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loric total parenteral nutrition (TPN) exhibited whole body weight loss as well as significant jejunal mucosa atrophy (5, 6). Combined GH and IGF-I treatment led to an independently additive increase in body weight. In contrast, jejunal mucosa atrophy was attenuated only by IGF-I treatment. GH alone was ineffective and the response to GH and IGF-I in combination was not different than the response to IGF-I alone (5, 6). Moreover, GH and IGF-I differentially increased protein synthesis in skeletal muscle and jejunal mucosa, respectively (7). In order to begin to understand the basis for the selective action of IGF-I on jejunal growth, we have determined the levels of mRNAs encoding GH receptor and GH-binding protein (GHR/GHBP) and components of the IGF-I system in the jejunal mucosa of surgically stressed rats maintained with TPN.

Materials and Methods

Animals and Experimental Design. The data presented in this paper are original and are derived from the same animals described in (5, 6). Male Sprague-Dawley rats, initially weighing 210–230 g, were used. Two surgeries were performed: placement of intravenous (iv) catheters in the superior vena cava by way of the external jugular vein (8), and a 6-cm midline laparotomy. After surgery, on Day 0, TPN solution was infused at 11 ml/day, followed by a gradual increase from 29 ml/day on Day 1, to 46 ml/day on Day 2, and to 60 ml/day on Days 3–5. This feeding regimen provided adequate parenteral energy and nutrients for the animals on Days 3–5 (9). The TPN solutions contained 45 g/l amino acids, 180 g/l dextrose, and 28 g/l lipid (142 ml of Intralipid), providing 1.1 kcal/ml, as previously reported (5).

Animals received recombinant human (rh) GH and/or rhIGF-I beginning at Day 1 after surgery. The doses of rhGH and rhIGF-I were 800 μg/rat/day. rhIGF-I was added to fresh TPN solution daily and infused concurrently with TPN solution. rhGH was dissolved in sterile saline solution and administered by subcutaneous (sc) injection twice a day. Animals not treated with GH had injection of carrier only. Although the doses of GH and IGF-I were not equal on a molar basis, these doses were used because they resulted in maximal response in previous studies of *ad libitum*–fed rats (10). In addition, the dose of IGF-I used was found to be optimal in promoting anabolism in glucocorticoid-treated rats (11).

The experimental design included four treatment groups arranged in a 2 × 2 factorial design, as follows: –IGF-I, – GH (– I – G); + IGF-I, – GH (+ I – G); – IGF-I, + GH (– I + G); and + IGF-I, + GH (+ I + G). Two age-matched control groups, namely reference and sham, were included. The reference group was fed *ad libitum* a semipurified diet containing similar amounts of carbohydrate, protein, and fat as in the TPN infusate. Sham animals received identical surgical procedures as did the TPN groups and were continuously infused with saline to control for the stress of parenteral infusion. These rats were orally fed graded amounts of the semipurified diet that was used to feed the reference group. Rats were sacrificed by exsanguination after anesthesia. The liver was dissected, immediately frozen in liquid nitrogen, and stored at −70°C. The jejunum (from the Ligament of Treitz to 25 cm proximal to the cecum) was removed and the lumen was flushed with cold saline. Jejunal mucosa was scraped from the upper 20 cm of the excised small intestine, and the mucosal scrapings and remaining submucosal tissue were frozen in liquid nitrogen and stored at −70°C.

RNA Extraction. Total RNA was isolated from frozen tissues using the Ultraspec Reagent (Biotexc, Houston, TX). Concentration of the aqueous RNA solutions was determined using the A_{260}. The integrity of the RNA was checked by visualizing the ethidium bromide stained 28S and 18S ribosomal RNAs on agarose/formaldehyde gels (12).

Antisense RNA Probes and Solution Hybridization/RNase Protection Assays.

IGF-I. A 464-bp DNA fragment containing contiguous rat IGF-I exon 2, exon 3, and exon 4 sequence was cloned into pGEM 4 (13). This plasmid was linearized using *Eco*RI, and T7 RNA polymerase was used with [α-^{32}P]-UTP (3000 Ci/mmol; Dupont/NEN, Boston, MA) to synthesize an antisense RNA probe (12–14). In RNase protection assays, IGF-I mRNAs transcribed from the exon 1 promoter result in a single protected band of 238 nucleotides (nt), while IGF-I mRNAs transcribed from the exon 2 promoter result in a band at 290 nt, representing the minor downstream initiation site, and a doublet at ~305 nt, representing the major upstream transcription initiation cluster.

IGF-I Receptor. A plasmid containing 265 bp of the rat IGF-I receptor cDNA (15) cloned into pGEM 3, was a gift from Drs. Werner, Roberts, and LeRoith (Diabetes Branch, NIDDK, National Institutes of Health, Bethesda, MD). The plasmid was linearized with *Eco*RI and antisense, ^{32}P-labeled RNA was prepared with SP6 RNA polymerase for use in RNase protection assays.

GH Receptor/GH-Binding Protein. A plasmid (pT7T318) containing rat GH receptor/GH-binding protein (GHR/GHBP) cDNA sequence (16) was kindly supplied by Drs. Gunnar Norstedt and Lawrence Mathews. The plasmid was linearized with *Bam*HI. Runoff transcription using T7 RNA polymerase produced an antisense probe of 449 b. Protected bands of 439 nt corresponding to GHR mRNA and 298 nt corresponding to GHBP mRNA are observed after RNase protection assay.

IGFBPs. All rat IGFBP (RBP) constructs were kindly provided by Drs. Shimisaki and Ling of the Whittier Institute (La Jolla, CA). All constructs were supplied in plasmid pBluescript SK+.

IGFBP-1. Plasmid pRBP-1-501 containing 407 bp of rat IGFBP-1 sequence was linearized with *Eco*RI, and antisense RNA was synthesized using T7 RNA polymerase.

IGFBP-2. Plasmid pRBP-2-501 containing 397 bp of rat IGFBP-2 sequence was linearized with *Eco*RI, and antisense RNA was synthesized using T7 RNA polymerase.

IGFBP-3. A 699-bp fragment of rat IGFBP-3 sequence was removed from pRBP-3-AR with *Apa*1 and *Bam*HI. The fragment was labeled by random priming using [α-^{32}P]-dCTP. Total RNA was electrophoresed through a 1.25% agarose/2.2 *M* formaldehyde gel. The gel was stained with ethidium bromide, and the RNA was transferred to a nitrocellulose filter using standard capillary blotting procedure. The filter was hybridized with the labeled probe and then washed under high-stringency conditions (12). Autoradiography revealed the 2.6-kb IGFBP-3 mRNA.

IGFBP-4. Plasmid-pRBP-4-SH containing 444 bp of the rat IGFBP-4 sequence was linearized with *Bam*HI. Antisense RNA probe was synthesized using T7 RNA polymerase.

IGFBP-5. A 300-bp rat IGFBP-5 insert was removed from pRBP-5-SH using *Hin*dIII and *Sac*I, and was cloned into the same sites in pGEM4Z. This plasmid was linearized with *Eco*RI, and an antisense RNA probe was synthesized using T7 RNA polymerase.

IGFBP-6. Plasmid pRBP-6-PP containing 246 bp of rat IGFBP-6 sequence was linearized with *Xba*I. An antisense RNA probe was synthesized using T7 RNA polymerase.

Solution hybridization/RNase protection assays were conducted as described (11–13). Reagents for runoff transcription were obtained from Promega (Madison, WI).

Quantitations. RNase protection assays for IGF-I, GHR/GHBP, and IGFBP-5 mRNAs, and the Northern blot for IGFBP-3 mRNA all used the same 13 jejunal mucosa total RNA samples. Autoradiograms were scanned using the Visage 110 system from BioImage (Millipore Corp., Ann Arbor, MI).

Statistical Analysis. The four TPN groups were compared in a two-way analysis of variance (ANOVA) that included IGF-I and GH main effects. All groups were also compared using one-way ANOVA (17). Group means were considered significantly different at $P < 0.05$, as determined by the protected least significant difference technique. Data are presented as mean +/– SEM.

Results

Systemic Growth and Jejunal Mucosa Cellularity. Body weight gain for the 6-day period was significantly greater in rats given either IGF-I (+10 g) or GH (+11 g) compared with maintenance with TPN alone, which resulted in the loss of 7 g of body weight for the 6-day period (5). However, treatment with IGF-I plus GH resulted in significantly greater weight gain (+25 g) than with either factor alone. Serum levels of total and free IGF-I, and GH are presented in Table I, which is adapted from data first shown in Reference 5. GH and IGF-I equally increased total serum IGF-I levels. Combined GH and IGF-I treatment additively increased total serum IGF-I levels. Free serum IGF-I levels were elevated by both GH and IGF-I compared with nontreated rats, and levels were slightly, but significantly, higher in IGF-I– than in GH–treated rats. However, combined GH and IGF-I treatment increased free serum IGF-I levels above those in either group of rats treated with each hormone alone. Serum rat GH levels were decreased in untreated rats maintained with TPN receiving either exogenous GH or exogenous IGF-I separately. Combined treatment lowered rat GH levels further. These effects probably reflect the effect of increased serum IGF-I to suppress endogenous GH secretion. Rats maintained with TPN showed jejunal mucosa atrophy when they were compared with orally fed rats. Treatment with IGF-I, but not GH, doubled the mass of the jejunal mucosa. The increased tissue mass was associated with parallel increases in DNA and protein content, suggesting hyperplasia (6). Treatment with IGF-I plus GH did not alter the responses compared with treatment with IGF-I alone.

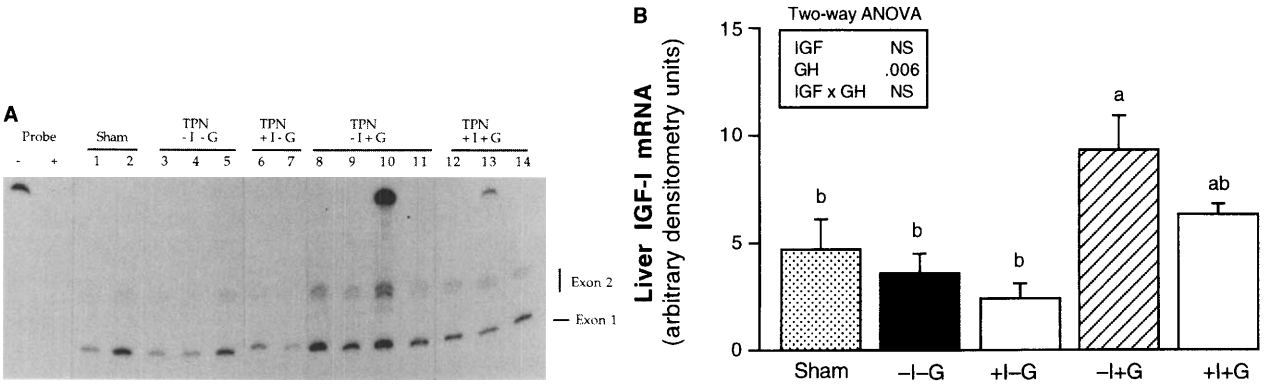
Table I. Serum Levels of Total and Free IGF-I and Rat Growth Hormone in Rats Maintained with TPN (μg/l serum)

Treatments	Total IGF-I	Free IGF-I	Growth hormone
Reference	313 ± 30^a	38 ± 3^b	7.9 ± 3.6^c
–I – G	279 ± 20^a	34 ± 2^b	5.7 ± 1.7^c
+I – G	511 ± 41^d	59 ± 2^d	2.1 ± 0.7^d
–I + G	454 ± 43^d	47 ± 3^a	2.0 ± 0.5^d
+I + G	684 ± 58^c	93 ± 2^c	0.8 ± 0.3^d

Note. Values are the mean ± SEM. Means in a column with different letter superscripts are significantly different, $P < 0.05$. (Data were taken from Ref. 5.)

IGF-I mRNA Levels in Liver and Jejunal Mucosa. Livers from rats maintained with TPN expressed IGF-I mRNAs resulting from the use of both the exon 1 and exon 2 promoters, consistent with the results in *ad libitum*-fed rats (Fig. 1, A and B) (12). There was no significant difference in hepatic IGF-I mRNA levels between the sham group and the TPN group receiving no hormonal treatments (Fig. 1, A and B). IGF-I treatment alone did not significantly alter liver IGF-I mRNA levels in rats maintained with TPN (Fig. 1, A and B). GH treatment alone resulted in a significant, 2.7-fold increase ($P < 0.05$) in hepatic IGF-I mRNA levels compared with rats maintained with TPN alone, and a significant, 3.8-fold increase ($P < 0.05$) over levels observed in rats treated with IGF-I alone (Fig. 1, A and B). Rats maintained with TPN and treated with IGF-I + GH had 1.8-fold and 2.6-fold higher hepatic IGF-I mRNA levels than did rats maintained with TPN alone or treated with IGF-I, respectively, although the effects were not statistically different. Two-way ANOVA showed that GH significantly ($P = 0.0058$) stimulated hepatic IGF-I mRNA levels, with no main IGF-I effect and no interaction between GH and IGF-I.

IGF-I mRNA levels were extremely low in the jejunal mucosa. Under the conditions of RNase protection assay that were employed, detection of protected bands corre-



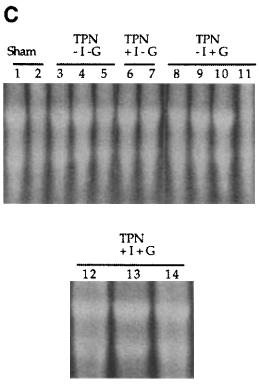


Figure 1. Hepatic IGF-I mRNA in rats maintained with TPN and receiving GH and/or IGF-I treatment. Rats were maintained as described in Materials and Methods with TPN for 6 days. The sham control group consisted of rats that underwent the same surgical procedures as the TPN rats. The sham rats were infused parenterally with saline and were fed orally with the same diet that was fed to the reference animals. Two sham control rats were studied. The TPN rats received no treatments (–I – G; $n = 3$); rhIGF-I infused at 800 μg/rat/day (+I – G; $n = 2$); rhGH injected at 400 μg/rat twice daily (–I + G; $n = 4$); or combined rhIGF-I and rhGH treatment (+I + G; $n = 3$). (A) The autoradiogram of a solution hybridization/RNase protection assay using the rat IGF-I exon 2–based antisense probe. The lanes labeled Probe, – and +, refer to probe alone that was carried through the assay and treated without (–) or with (+) RNases. The positions of the protected bands corresponding to exon 1 and exon 2 mRNAs are shown. Ten micrograms total RNA were used for each sample, and the autoradiogram was exposed for ~16 hr at –70°C. The band in lanes 10 and 13 which appears to co-migrate with probe probably represents probe that was not completely digested by the RNases during assay of those samples. (B) The results of scanning densitometry of the autoradiogram in Panel A. The height of the bars represents the mean and the smaller vertical bars are ± SEM. Bars with different superscripts in Panel B are significantly different ($P < 0.05$, one-way ANOVA). Results of two-way ANOVA are shown in the box in Panel B. (C) The ethidium bromide–stained 28S and 18S rRNA bands on an agarose/formaldehyde gel of 20-μg aliquots of total RNA corresponding to the samples shown in Panel A.

sponding to IGF-I mRNA required that gels be exposed for at least several days. As seen in Figure 2A, a band corresponding to protection of the common exon 3 and exon 4 sequence in the antisense IGF-I RNA probe was observed when jejunal mucosa RNA was used. Thus, jejunal mucosa expresses mostly, if not only, IGF-I mRNA transcripts resulting from the use of the exon 1 promoter. We did not use an exon 1–based antisense RNA probe to determine which exon 1 start sites were used in jejunal mucosa. There did not appear to be any differences in IGF-I mRNA levels among any of the groups, a conclusion that was confirmed in repetitive RNase protection assays.

GHR/GHBP mRNA Levels in Liver and Jejunal Mucosa. Among the liver RNA samples described in Figure 1, there were no differences in hepatic GHR or GHBP mRNA between any of the groups (data not shown). In jejunal mucosa, GHR mRNA levels were not different in the orally fed reference control animals and in the rats maintained with TPN alone (Fig. 3, A and B). IGF-I treatment alone or in combination with GH resulted in a significant, 2.8-fold decrease in GHR mRNA compared with rats treated with GH alone ($P < 0.05$). Two-way ANOVA confirmed that there was a main effect of IGF-I to decrease GHR mRNA levels ($P = 0.0045$), and that GH did not

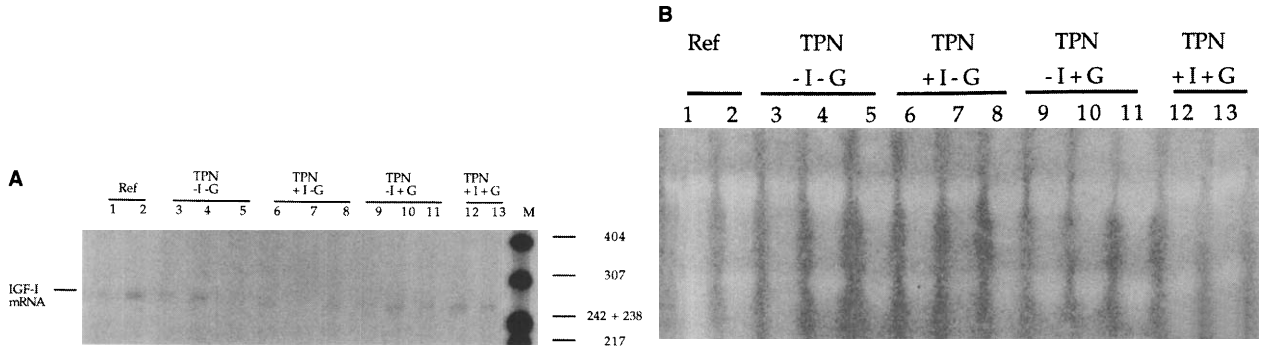


Figure 2. Jejunal mucosa IGF-I mRNA levels in rats maintained with TPN and receiving GH and/or IGF-I treatment. Rats were maintained as described in Materials and Methods with TPN for 6 days. The reference (Ref, $n = 2$) group was fed *ad libitum*. The TPN groups were as follows: –I – G, $n = 3$; +I – G, $n = 3$; –I + G, $n = 3$; +I + G, $n = 2$. IGF-I mRNA was determined on 20 μg total jejunal mucosa RNA using an exon 2–based antisense RNA probe as described in Materials and Methods. The autoradiogram (A) was exposed for 3 days at –70°C. M, a [^{32}P]-labeled *Msp* I digest of pBR322 DNA. The sizes of the labeled markers in nucleotides are shown to the right. The RNA samples shown in Panel A were treated with 1 μg/ml DNase I for 30 min at 37°C, extracted and precipitated, prior to RNase protection assay. Panel B shows the ethidium bromide stain of an agarose/formaldehyde gel on separate 4-μg aliquots of the samples (not pretreated with DNase I).

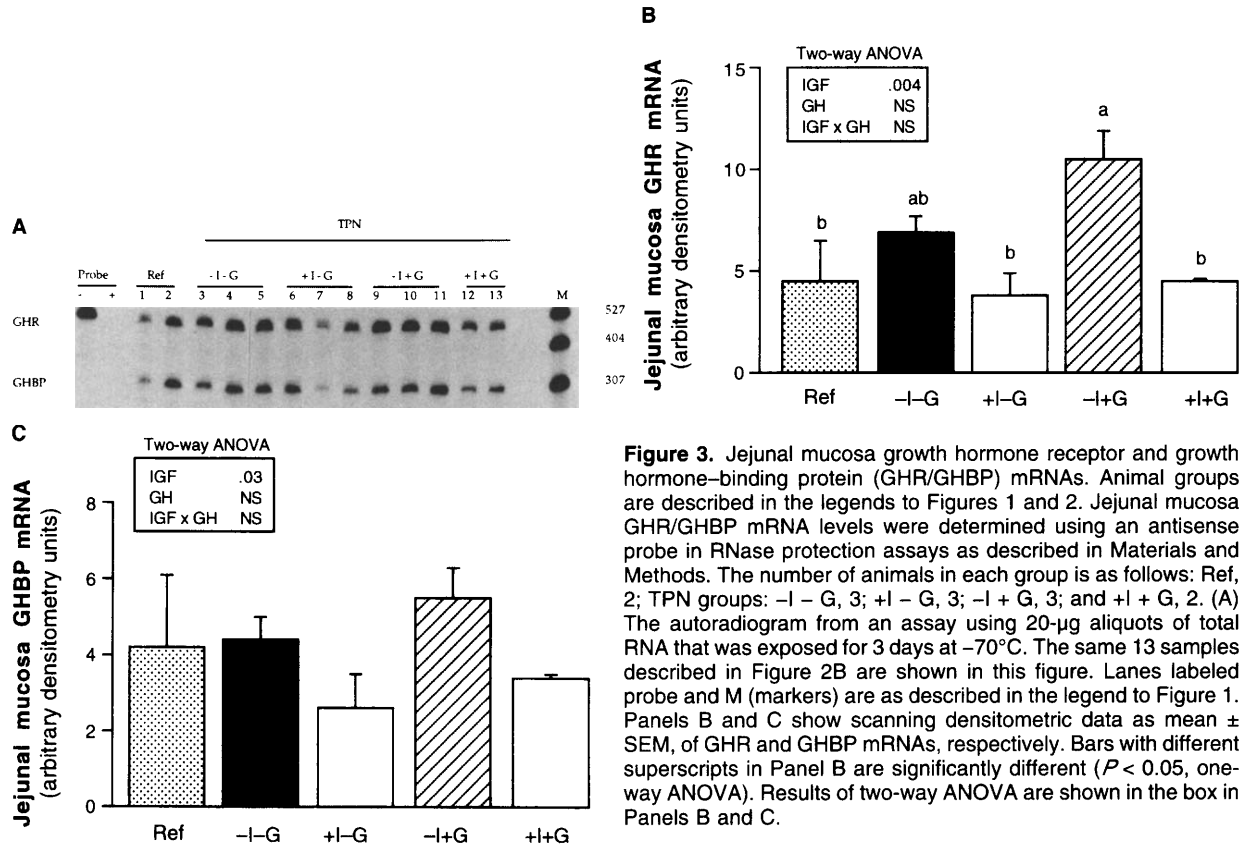


Figure 3. Jejunal mucosa growth hormone receptor and growth hormone–binding protein (GHR/GHBP) mRNAs. Animal groups are described in the legends to Figures 1 and 2. Jejunal mucosa GHR/GHBP mRNA levels were determined using an antisense probe in RNase protection assays as described in Materials and Methods. The number of animals in each group is as follows: Ref, 2; TPN groups: –I – G, 3; +I – G, 3; –I + G, 3; and +I + G, 2. (A) The autoradiogram from an assay using 20-μg aliquots of total RNA that was exposed for 3 days at –70°C. The same 13 samples described in Figure 2B are shown in this figure. Lanes labeled probe and M (markers) are as described in the legend to Figure 1. Panels B and C show scanning densitometric data as mean ± SEM, of GHR and GHBP mRNAs, respectively. Bars with different superscripts in Panel B are significantly different ($P < 0.05$, one-way ANOVA). Results of two-way ANOVA are shown in the box in Panels B and C.

significantly alter GHR mRNA levels. There were no significant differences in jejunal mucosa GHBP mRNA levels between any of the groups by one-way ANOVA (Fig. 3, A and C). Two-way ANOVA indicated a main effect of IGF-I to lower GHBP mRNA levels ($P = 0.0331$).

IGFBP-3 mRNA Levels in Jejunal Mucosa. In rats maintained with TPN, treatment with IGF-I alone resulted in a significant, 4.1-fold increase in IGFBP-3 mRNA levels in jejunal mucosa (Fig. 4, A and B; $P < 0.05$). GH treatment caused a significant, 6-fold increase in IGFBP-3 mRNA levels in jejunal mucosa, compared with nontreated rats maintained with TPN ($P < 0.05$). However, combined GH and IGF-I treatment led to a 15-fold increase in IGFBP-3 mRNA levels, which were significantly greater than the levels seen in rats treated with IGF-I or GH alone ($P < 0.05$). Two-way ANOVA indicated significant main effects for both GH ($P = 0.0005$) and IGF-I ($P = 0.0001$). In addition, the results showed a significant interaction between GH and IGF-I ($P = 0.0442$), indicating that GH and IGF-I synergized in stimulating IGFBP-3 mRNA in the jejunal mucosa.

IGFBP-5 mRNA Levels in Jejunal Mucosa. There were no significant differences in jejunal mucosa IGFBP-5 mRNA levels between the orally fed reference group and the group of rats maintained with TPN alone (Fig. 5). IGF-I treatment alone led to a 4.3-fold increase in jejunal mucosa IGFBP-5 mRNA levels compared with rats maintained with TPN alone (Fig. 5). This effect was significant at $P < 0.10$ with a P value of 0.07. The effect of IGF-I was not significant at the $P < 0.05$ level, probably because of the lower level of IGFBP-5 mRNA in Sample 7. This, in turn, may have been due to the lower level of total RNA in this sample (Fig. 2B). GH alone was without effect on IGFBP-5 mRNA levels. Combined IGF-I + GH treatment led to a significant, 8.6-fold increase in jejunal mucosa IGFBP-5 mRNA level compared with that observed in rats maintained with TPN that did not receive hormone treatment (Fig. 5; $P < 0.05$). Two-way ANOVA indicated that there was a significant main effect for IGF-I ($P = 0.0032$), but not GH ($P = 0.0739$), to stimulate IGFBP-5 mRNA in jejunal mucosa. The IGF-I effect was not apparent for IGF-I alone in the one-way ANOVA because of small sample size and variance resulting from inclusion of Sample 7.

The levels of mRNAs encoding IGF-I receptor and IGFBPs 1, 2, 4, and 6 in jejunal mucosa were extremely low and/or were not altered by any of the treatments (data not shown).

Discussion

In a previous study, rats maintained with TPN and treated with IGF-I + GH showed an additive increase in body weight (5). In contrast, jejunal mucosa mass and protein synthesis were stimulated by IGF-I, but not by GH (6, 7). The main goal of the present study was to determine the levels of mRNAs encoding components of the GH/IGF-I axis in order to understand the basis for increased jejunal growth in response to IGF-I in this model.

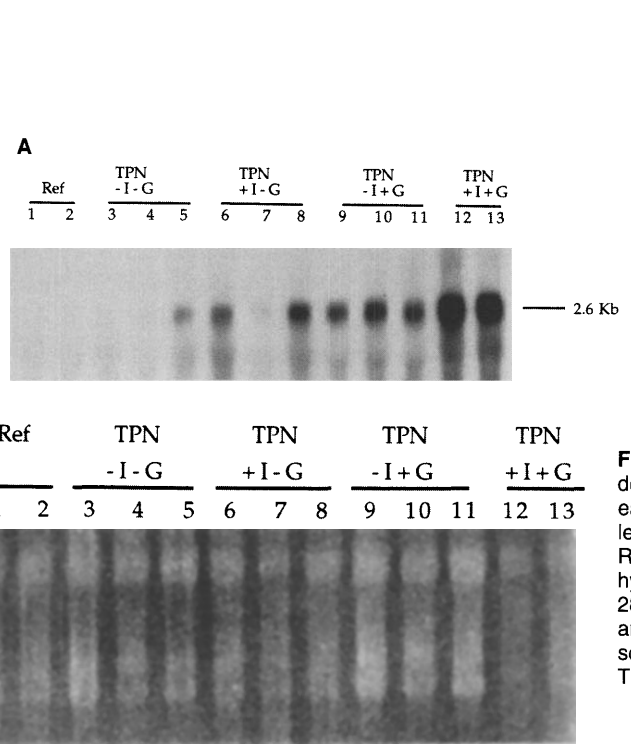


Figure 4. IGFBP-3 mRNA levels in jejunal mucosa. Animal groups were described in the legends to Figures 1 and 2. The number of animals in each group is listed in the legend to Figure 3. Jejunal IGFBP-3 mRNA levels were determined by Northern hybridization analysis on 20 μg total RNA as described in the text. (A) The autoradiogram resulting from the hybridization. (B) Densitometric data. (C) The ethidium bromide-stained 28S and 18S rRNA bands on the filter paper after transfer from the gel and before the hybridization was performed. Bars with different superscripts in Panel B are significantly different ($P < 0.05$, one-way ANOVA). The results of two-way ANOVA are shown in the box in Panel B.

IGF-I mRNA in liver showed the expected increase in response to GH infusion. Increased liver IGF-I mRNA by GH is consistent with increased plasma IGF-I levels in the GH-treated rats maintained with TPN (5). Moreover, in another study utilizing rats maintained with TPN, GH also increased hepatic IGF-I mRNA and plasma IGF-I levels (18). In that study, GH was also ineffective in preventing atrophy of the intestinal mucosa, whereas IGF-I treatment was effective in promoting mucosal growth. Thus, GH-stimulated increase in hepatic IGF-I production and circulating IGF-I is not able to prevent the atrophy that occurs in the jejunal mucosa of rats maintained with TPN.

We observed very low IGF-I mRNA levels in the jejunal mucosa, which did not appear to change in response to TPN or hormonal treatments. Using RNase protection assays, Winesett *et al.* (4) found no difference in jejunal IGF-I mRNA levels whether fasted rats were refed intravenously (iv) or orally, despite significant jejunal atrophy in the iv-refed rats. These results, combined with our current data, suggest that changes in locally produced IGF-I do not account for decreased jejunal growth in rats maintained with TPN.

In previous studies, fasting caused decreased GHR gene expression and decreased GH receptor binding in the liver (19, 20). These defects in GHR were suggested to contribute to decreased hepatic IGF-I production. However, fasting did not alter jejunal GHR/GHBP gene expression, despite decreased jejunal IGF-I gene expression (4). Our current results would further suggest that jejunal growth responses in rats maintained with TPN were not due to

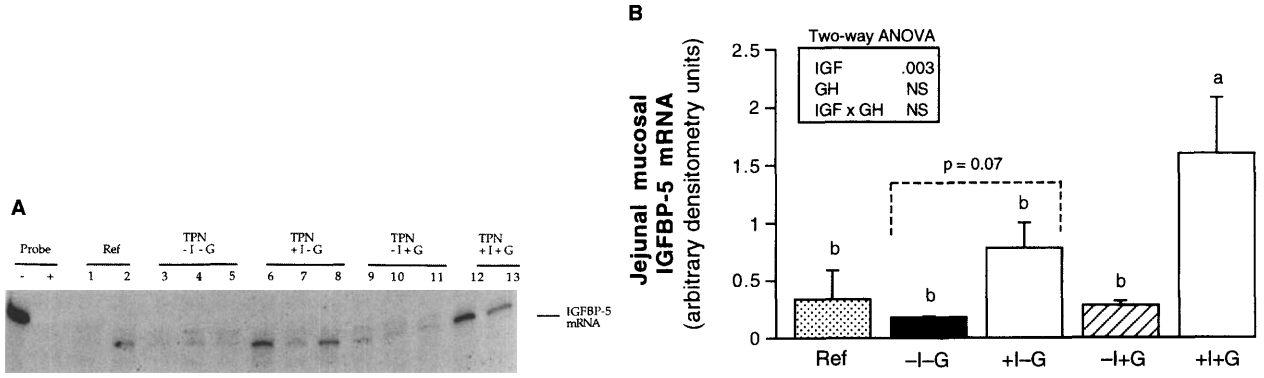


Figure 5. IGFBP-5 mRNA levels in jejunal mucosa. Animal groups and numbers per group are described in the legends to Figures 1–3. Jejunal mucosa IGFBP-5 mRNA levels were determined by RNase protection assay using the antisense RNA probe described in Materials and Methods. (A) The autoradiogram of the protection assay gel. (B) The scanning densitometry data. Bars with different superscripts in Panel B are significantly different ($P < 0.05$, one-way ANOVA). The results of two-way ANOVA are shown in the box in Panel B.

changes in GHR/GHBP gene expression, especially considering that IGF-I, which stimulated gut growth, inhibited GHR gene expression. There was a slight, but significant, increase in jejunal IGF-I receptor mRNA in the study of fasted rats which were refed iv nutrients (4). This increase occurred in the face of continued jejunal atrophy. Thus, changes in IGF-I receptor gene expression do not appear to underlie jejunal atrophy that occurs in rats maintained with TPN. We cannot rule out the possibility, however, that regulation of GHR/GHBP and/or IGF-I receptor occur at the level of protein expression or intrinsic binding activity in the jejunum of these rats. Moreover, there could be differences in GHR/GHBP and/or IGF-I receptor in different cell types in the jejunum that our analysis of total jejunal RNA levels cannot detect. These possibilities await further study.

GH and IGF-I both stimulated IGFBP-3 mRNA in the jejunal mucosa, and together GH and IGF-I synergistically induced IGFBP-3 mRNA. It has been suggested that IGF-I directly stimulates IGFBP-3 expression, and that the effect of GH is mediated through increased IGF-I (3). Moreover, exogenous IGF-I was reported to stimulate IGFBP-3 mRNA in the small intestine in another study (21). However, there is *in vivo* and *in vitro* evidence that GH alone can stimulate IGFBP-3 (22, 23). If the effect of GH is secondary to increased IGF-I, then plasma IGF-I is most likely responsible since it was doubled in the GH and IGF-I–treated rats compared with GH or IGF-I alone. The increased jejunal mucosa IGFBP-3 mRNA in response to GH alone or in response to GH + IGF-I is not, however, correlated with altered jejunal growth, since GH did not reverse jejunal mucosa atrophy in rats maintained with TPN, and since the effects of GH + IGF-I on jejunal growth were not different from those of IGF-I alone. Winesett *et al.* (4) localized jejunal IGFBP-3 mRNA to cells of the lamina propria. They found that fasting reduced jejunal IGFBP-3 mRNA levels, and that refeeding further decreased the levels of IGFBP-3 mRNA. These authors argued that the further decrease in IGFBP-3 mRNA in the refed rats may reflect an IGF-I–inhibitory role for IGFBP-3. It will be necessary to determine whether the changes in IGFBP-3 mRNA are paralleled by changes in IGFBP-3 protein levels, and whether changes in IGFBP-3 glycosylation or proteolysis (3) could be effected by GH and/or IGF-I treatment. Such effects could alter the biological activity of IGFBP-3 in ways not predicted by changes in gene expression.

The selective increase in jejunal IGFBP-5 mRNA caused by IGF-I, but not by GH, could modulate IGF-I action. Both stimulatory and inhibitory effects of IGFBP-5 on IGF-I action have been reported (24, 25) likely dependent on partitioning of IGFBP-5 between the cell surface, extracellular matrix, and soluble compartments (24). IGFBP-5 mRNA levels are directly stimulated by IGF-I *in vitro* (3). *In vivo,* IGFBP-5 mRNA levels in adipose tissue were decreased by hypophysectomy, and were increased by GH, but not by IGF-I treatment (26). Our current results suggest that in the jejunal mucosa, exogenous IGF-I alone regulated IGFBP-5 gene expression and that GH-induced changes in circulating IGF-I were without effect. Furthermore, the correlation of IGFBP-5 gene expression with hormonally induced attenuation of jejunal mucosa atrophy suggests that IGFBP-5 positively modulates IGF-I action. Recent preliminary *in situ* hybridization results from a different set of rats maintained with TPN suggest that IGF-I stimulates IGFBP-5 mRNA in the muscularis and lamina propria (27). Based on low expression and/or no alteration in their mRNA levels, IGFBPs other than IGFBP-3 and IGFBP-5 do not appear to play roles in the changes in jejunal mucosa growth that were observed in these rats.

The inability of GH to stimulate IGFBP-5 gene expression and jejunal growth, while stimulating IGFBP-3 gene expression, raises some interesting questions regarding GH action in the jejunal mucosa. It is apparent that exogenous, but not GH-stimulated endogenous, serum IGF-I can stimulate growth and IGFBP-5 gene expression. GH-induced increase in IGFBP-3 mRNA, on the other hand, may occur as the result of increased endogenous serum IGF-I. Conversely, if the effect of GH on IGFBP-3 in jejunal mucosa is direct, then this would mean that there are differential effects of GH on IGFBP gene expression and growth within the jejunal mucosa. It is also possible that the inability of GH to induce IGFBP-5 mRNA and intestinal growth is due to the fact that GH increases the level of the acid labile subunit (ALS) of the large molecular weight IGF binding ternary complex (3). In this case, it would be expected that ternary complex would reduce bioavailability of IGF-I in GH-treated rats, whereas bioavailable IGF-I would be high in IGF-I–treated rats. Indeed, serum free IGF-I levels were slightly, but significantly higher in IGF-I– than in GH–treated rats (Table I and Ref. 5). However, serum free IGF-I concentrations were higher in rats treated with GH than in nontreated rats, and were higher in GH– and IGF-I–treated rats than in rats treated with IGF-I alone, and yet intestinal growth was only stimulated as the result of exogenous IGF-I. Moreover, if ternary complex were inhibiting the action of GH-induced serum IGF-I levels, then the effect would have to be tissue specific, since there was a response to GH in terms of skeletal muscle and whole body growth (5).

In summary, the selective trophic effect of IGF-I, but not GH, in atrophied jejunal mucosa is correlated with increased IGFBP-5 expression, leading to the hypothesis that IGFBP-5 positively modulates IGF-I action. Understanding the role of GH and IGF-I on jejunal growth and function will require elucidation of the mechanisms by which GH and IGF-I signaling lead to specific effects on IGFBP gene expression and jejunal phenotype. Further studies will also be required to understand the consequences of changes in IGFBP-3 and IGFBP-5 on IGF-I action.

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