Am J Physiol Regul Integr Comp Physiol 292: R2144–R2150, 2007.
First published March 1, 2007; doi:10.1152/ajpregu.00903.2006.

Intestinotrophic effects of exogenous IGF-I are not diminished in IGF binding protein-5 knockout mice

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Submitted 27 December 2006; accepted in final form 27 February 2007

Murali SG, Liu X, Nelson DW, Hull AK, Grahn M, Clayton MK, Pintar JE, Ney DM. Intestinotrophic effects of exogenous IGF-I are not diminished in IGF binding protein-5 knockout mice. *Am J Physiol Regul Integr Comp Physiol* 292: R2144–R2150, 2007. First published March 1, 2007; doi:10.1152/ajpregu.00903.2006.—IGF binding protein-5 (IGFBP-5) modulates the availability of IGF-I to its receptor and potentiates the intestinotrophic action of IGF-I. Our aim was to test the hypothesis that stimulation of intestinal growth due to coinfusion of IGF-I with total parenteral nutrition (TPN) solution is dependent on increased expression of IGFBP-5 through conducting our studies in IGFBP-5 knockout (KO) mice. IGFBP-5 KO, heterozygote (HT) and wild type (WT) male and female mice were maintained with TPN or TPN plus coinfusion of IGF-I [recombinant human (rh)IGF-I; 2.5 $mg \cdot kg^{-1} \cdot day^{-1}$] for 5 days. The concentration of IGF-I in serum was 73% greater ($P < 0.0001$) in mice given TPN + IGF-I infusion compared with TPN alone. IGF-I attenuated the 2–3 g loss of body weight associated with TPN in WT mice, whereas KO and HT mice did not show improvement in body weight with IGF-I treatment. KO and HT mice had significantly greater levels of circulating IGF-I binding proteins (IGFBPs) compared with WT mice. Intestinal growth due to IGF-I was observed in all groups treated with IGF-I based on greater concentrations of protein and DNA in small intestine and colon and significantly greater crypt depth and muscularis thickness in jejunum. Jejunal expression of IGFBP-5 mRNA was greater in WT mice, whereas IGFBP-3 mRNA was greater in KO mice treated with IGF-I. In summary, the absence of the IGFBP-5 gene did not block the ability of IGF-I to stimulate intestinal growth, possibly because greater jejunal expression of IGFBP-3 compensates for the absence of IGFBP-5.

total parenteral nutrition; jejunum; IGF-binding proteins-3 and 5

IGF-I PLAYS A CRUCIAL ROLE in regulating cellular growth, differentiation, and apoptosis through endocrine, as well as paracrine and autocrine actions. IGF-I action is controlled by a complex system consisting of the IGF-I peptide hormone, cell surface IGF-I receptors, and six, well-characterized IGF-binding proteins (IGFBPs 1-6) that modulate the availability of IGF-I to bind to its receptor (1, 6). IGFBPs are secreted by many cell types, and the profile of expressed IGFBPs is regulated in a tissue-specific manner (7). Over 95% of the IGF-I in serum and other biological fluids is bound by high-affinity IGFBPs (K_d ~0.1 nM), which restrict access to cell surface receptors and prolong the circulating half-life of IGF-I (1). In tissues, IGFBPs can both inhibit and potentiate IGF-I action either by sequestering IGF-I from its receptor or by releasing IGF-I to bind to its receptor (6, 7). In addition, IGFBPs show IGF-independent effects in some tissues (18). Knowledge of the physiological function of the IGFBPs is limited because most of the data addressing IGFBP action are derived from in vitro culture systems.

IGFBP-5, considered to be a stimulatory IGFBP, is the most conserved IGFBP across species (26). It plays an important role in biological processes in bone (11), mammary tissue (8), kidney (12, 25), and intestine (17). The gastrointestinal tract is a major target organ for IGF-I, and local expression of IGFBP-5 is known to potentiate the endocrine and paracrine effects of IGF-I in the intestine. We have noted in mice and rats that infusion of IGF-I attenuates the mucosal atrophy induced by total parenteral nutrition (TPN) and that increased expression of IGFBP-5 is the strongest correlate of the mucosal growth response (19, 24). This stimulatory effect of IGFBP-5 has been linked with the ability of IGFBP-5 to bind extracellular matrix, which reduces the affinity of the IGF-I-IGFBP interaction and increases the availability of IGF-I to bind to its receptor (1, 26). Other model systems demonstrating the ability of IGFBP-5 to potentiate the intestinotrophic effects of IGF-I include transgenic mice that overexpress IGF-I in mesenchyme (28), rats with resection-induced intestinal growth (10), a rat model of inflammatory bowel disease (29), and cultured smooth muscle cells derived from the intestine (3). The mouse TPN model is a suitable system to study how IGF-I and IGFBPs interact to promote intestinal growth and repair because of the significant 25% intestinal atrophy induced by TPN that is attenuated by IGF-I infusion (19), the ability to provide intravenous coinfusion of IGF-I with TPN solution, and the clinical relevance of using growth factors to reduce the complications associated with TPN.

Knowledge about the function of IGFBP-5 in vivo is limited because there are few published studies of genetic mouse models with overexpression or deletion of IGFBP-5 (21, 26, 27). The goal of this study was to further understanding of the role of IGFBP-5 in modulating the ability of IGF-I to stimulate whole body and tissue-specific growth. Our objective was to determine whether deletion of the IGFBP-5 gene blocks the intestinotrophic effects of exogenous IGF-I by conducting studies in IGFBP-5 knockout, heterozygote, and wild-type mice given coinfusion of recombinant human IGF-I (rhIGF-I) with TPN solution.

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MATERIALS AND METHODS

Animals. The animal facilities and protocols reported were approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee. Targeting of the IGFBP-5 gene was used to generate IGFBP-5-deficient mice as previously reported (21). Briefly, part of exon 1, including the IGFBP-5 translation start site was replaced with a neomycin resistance cassette. Progeny heterozygous for the mutated gene, identified by Southern blot analysis of tail DNAs, were mated, and offspring from these heterozygous matings were genotyped as IGFBP-5 knockout (KO, −/−), heterozygote (HT, +/−) and wild type (WT, +/+). The absence of the IGFBP-5 gene in KO mice was reconfirmed by ribonuclease protection assay (RPA) to measure IGFBP-5 mRNA in jejunum. The mice were bred on two backgrounds, C57BL6 (Jackson Laboratories, Bar Harbor, ME) and 129S6 (Taconic, Germantown, New York). Male and female mice were individually housed in stainless-steel, wire-bottom cages in a room maintained at 22°C on a 12:12-h light-dark cycle. The mice were adapted to the facility for 8–9 days and had free access to water and a stock rodent diet (Productf 8604; Harlan Teklad, Madison, WI) that contained (in g/kg) 240.0 crude protein, 40.0 crude fat, 45.0 crude fiber, and 78.4 ash.

Experimental design. The experimental design was a randomized complete block with three experimental factors: infusion of IGF-I (treatment), genotype, and sex. The 12 experimental groups included −IGF-I, KO, female; −IGF-I, KO, male; +IGF-I, KO, female; +IGF-I, KO, male; −IGF-I, HT, female; −IGF-I, HT, male; +IGF-I, HT, female; +IGF-I, HT, male; −IGF-I, WT, female; −IGF-I, WT, male; +IGF-I, WT, female; and +IGF-I, WT, male. The female heterozygote group was eliminated from the final sample due to the absence of an increase in serum concentration of IGF-I in response to IGF-I infusion. Mean initial body weight was significantly greater in male (29 ± 1, $n = 26$) compared with female (25 ± 1, $n = 20$) mice but otherwise not significantly different among groups.

All mice were maintained with TPN for 5 days, as previously described (19). On postoperative *days 2–5* when 9 ml/day TPN solution was provided, mice received 59 kJ/day or 881 kJ/(kg body $wt^{0.75}$·day) and 0.41 g protein or 65 mg nitrogen/day. This level of nutrition is sufficient to meet the energy requirements of mice, 674–1,100 kJ/(kg body $wt^{0.75}$·day) for maintenance and growth (12a). Body weight was measured on the day of surgery (*day 0*) and the day animals were killed (*day 6*).

Mice were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg body wt) and xylazine (10 mg/kg body wt). A silicone rubber catheter (0.30 mm, inner diameter; 0.64 mm, outer diameter) was inserted into the vena cava through the right jugular vein and tunneled subcutaneously to the back of the animal to exit the tail. Mice were immediately connected to an infusion pump (Harvard Apparatus, Holliston, MA) and saline (9 g sodium chloride/l) was infused at a rate of 4 ml/day. Mice were allowed free access to rodent diet on the day of surgery. All mice had free access to water throughout the experiment. Postoperative *day 1*, rodent diet was removed, and the saline infusion was replaced with nutritionally adequate TPN solution (19). The +IGF-I groups received 2.5 $mg \cdot kg^{-1} \cdot day^{-1}$ rhIGF-I (Genentech, South San Francisco, CA), coinfused with TPN solution. The infusion rate was gradually increased from 4 ml/day to 9 ml/day by *day 2*. After 5 days of TPN infusion, mice were weighed, anesthetized using isoflurane via an anesthesia machine (IsoFlo, Abbot Laboratories, North Chicago, IL), and killed by cardiac exsanguination. Blood was obtained by cardiac puncture and centrifuged at 1,700 *g* for 15 min to isolate the serum. Total serum IGF-I concentration was determined in duplicate samples by radioimmunoassay in a single assay; the intra-assay coefficient of variation was 6% (20). Liver, kidney, and spleen were removed, weighed, and snap-frozen.

Collection and analysis of intestinal tissue. The entire small intestine from pylorus to ileocecal valve, as well as colon were rapidly removed and flushed with ice-cold saline. The weight and length of the small bowel and colon were recorded. Different segments of small bowel were collected as follows: duodenum (from pylorus, proximal 4-cm piece of small bowel), jejunum (after 4 cm, until 10 cm proximal to ileocecal valve) and ileum (distal 10 cm of the small bowel up to ileocecal valve). Length of jejunum was measured, and each segment was weighed individually after being cut into sections for various analyses. The first 6 cm of jejunum were used for measurement of mass. The next 0.5 cm of jejunum was fixed in a 10% buffered formalin solution (Fisher Scientific) for morphometric analysis. The following 4 cm of jejunum were homogenized for the measurement of protein (bicinchoninic acid protein assay; Pierce Chemical, Rockford, IL) and DNA (16) content. For the measurement of sucrase activity, tissue homogenate was incubated with substrate solution containing 0.06 M sucrose in maleate buffer for 60 min and then with Tris-glucose oxidase reagent (4). The concentration of sucrase in the homogenate was calculated by measuring the amount of glucose liberated and expressed as micromoles glucose per minute per milligram protein. Intestinal assays were conducted in triplicate samples in a single assay with intra-assay coefficient of variation of 5–10%. The remainder of the jejunum (5–7 cm) was snap frozen in liquid nitrogen for RNA extraction. Duodenum, ileum, and colon were snap frozen for protein and DNA content and/or RNA extraction.

The fixed tissue from proximal jejunum was embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin. Villus height, crypt depth, and muscularis thickness were measured using a light microscope and SigmaScan software (SPSS, Chicago, IL) (19).

Western ligand blot analysis. Western ligand blotting was done according to the procedure previously described (9). Briefly, 2 μl of serum were fractionated by 12% SDS-PAGE, and proteins were transferred to a polyvinylidene difluoride membrane. The membrane was incubated overnight in biotinylated IGF-I (40 ng/ml) and then with streptavidin-horseradish peroxidase (1:500 dilution). Using enhanced chemiluminescence, the signal was captured on autoradiography film exposed for controlled time intervals to prevent band saturation and obtain a response linear to the concentration of the bound ligand. Bands were detected and quantified in the two molecular weight ranges: lighter band in the range of 38–43 kDa and darker band in 30–34 kDa ranges. The band intensities were quantified by OptiQuant software (Perkin Elmer Life Sciences, Boston, MA) and expressed as mean density light units.

IGFBP-3 and IGFBP-5 mRNA protection assay. RPA was used to measure IGFBP-3 and IGFBP-5 mRNA in intact sections of jejunum. Probes were derived from cDNAs subcloned into pGEM4z. The vectors were kindly provided by Dr. M. L. Adamo (San Antonio, TX). Both plasmids were linearized with EcoR1, and RNA polymerase T7 was used to generate radiolabeled antisense RNA probes (MaxiScript, Ambion, Austin, TX). The 135 bp IGFBP-3 antisense probe protected a band at 105 bp. The 316 bp IGFBP-5 probe protected a band at about 286 bp. 18S ribosomal RNA was measured along with mRNA as an internal, as well as experimental, control. An antisense pTRI RNA 18S control template (Ambion) was used to generate a labeled RNA probe, which protects an 80-bp fragment of 18S ribosomal RNA. After extraction using TRIzol reagent (GIBCO-BRL, Gaithersburg, MD), RNA integrity was confirmed by ethidium bromide staining of 28S and 18S ribosomal RNAs on an agarose formaldehyde gel. Fifty micrograms of jejunum total RNA were hybridized with radiolabeled antisense mRNA (IGFBP-3 or IGFBP-5) and 18S probes, and protected bands were quantified as previously described (9). Band intensities of IGFBP-3 were expressed as fold difference relative to band intensity of -IGF-I, WT group. Relative band intensities of IGFBP-5 were calculated by dividing the mRNA band intensity with the 18S band intensity in each sample and then expressed as a fold difference relative to band intensity of -IGF-I, WT group.

Statistics. Most data were analyzed using PROC GLM; gel data were analyzed using PROC MIXED. Models included a term for

block, the three experimental factors (genotype, IGF-I, and sex), and all interactions of these factors. Because the sample sizes for some factor combinations were small, backward elimination was used to remove insignificant terms from the model. Analysis of the data using serum concentration of IGF-I or body weight as a covariate did not significantly improve the model. Groups were compared using the protected least significant difference technique to determine individual group differences (SAS Institute, Cary, NC). Differences of $P \leq 0.05$ were considered statistically significant. Values in the text are means ± SE. The final sample size included 46 mice distributed across the three experimental factors as follows: −IGF-I = 26 and +IGF-I = 20; WT = 16, KO = 19 and HT = 11; and males = 26 and females = 20 . For each parameter measured, data from one or more experimental factors were pooled if there were no significant interaction, and in figures, for summary purposes, nonsignificant effects are not displayed.

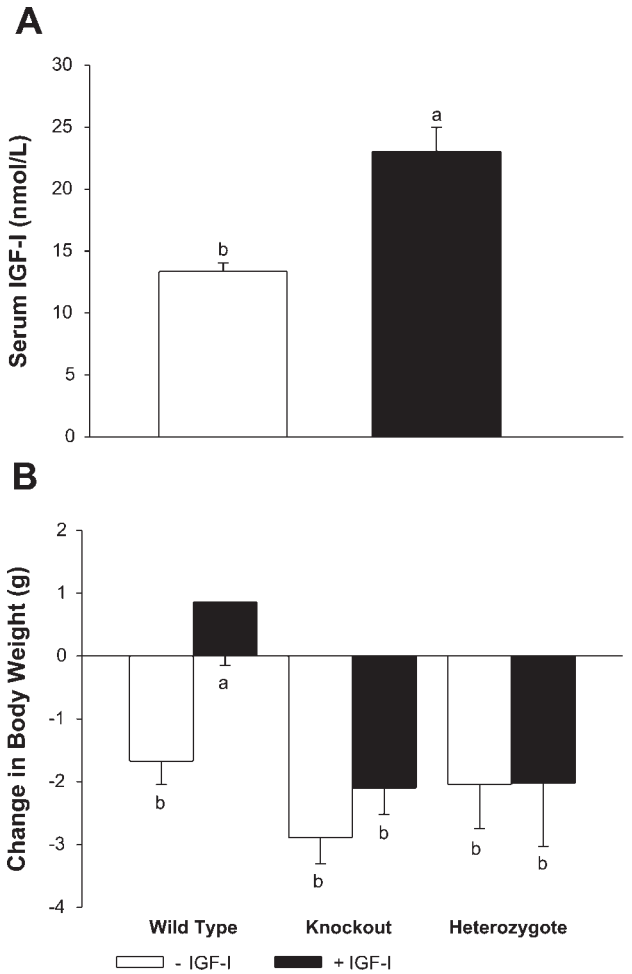

Fig. 1. Serum concentration of IGF-I (*A*) and net change in body weight over 6 days (*B*) in mice maintained with total parenteral nutrition (TPN) alone (−IGF-I) or TPN with IGF-I infusion (+IGF-I). Values are means ± SE. [a,b]Means with different superscripts are statistically different. IGF-I treatment significantly increased ($P < 0.0001$) the concentration of IGF-I in serum by 73% (*A*; −IGF-I, $n = 24$; +IGF-I, $n = 19$). There was a significant difference in body weight change due to IGF-I treatment in mice with different genotypes (Treatment × Genotype, $P = 0.04$). Because this difference was independent of sex (Treatment × Genotype × Sex, $P > 0.05$), male and female mice were pooled (*B*; −IGF-I, $n = 26$; WT, $n = 10$; KO, $n = 9$; HT, $n = 7$ and +IGF-I, $n = 20$; WT = 6, KO = 10 and HT = 4). IGF-I attenuated the loss of body weight associated with TPN in WT but not in KO and HT mice.

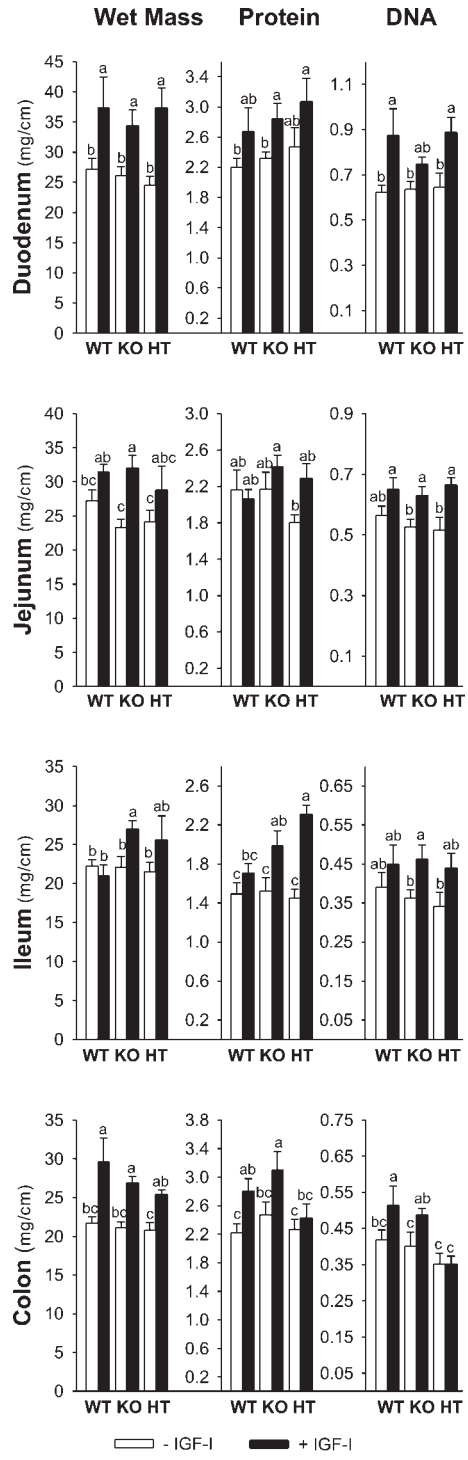

Fig. 2. Composition of duodenum, jejunum, ileum, and colon in mice maintained with TPN alone (−IGF-I, $n = 26$; WT = 10, KO = 9, and HT = 7) or TPN with IGF-I infusion (+IGF-I, $n = 20$; WT = 6, KO = 10, and HT = 4). Values are expressed as means ± SE. [a,b,c]Means with different superscripts are statistically different (protected least significant difference, $P < 0.05$) . There was a significant main effect of IGF-I treatment to induce growth in the small intestine and colon based on greater mass and concentrations of protein and DNA ($P = 0.0001$ to $P = 0.05$). There was no significant effect due to genotype or sex in duodenum, jejunum, or colon. In the ileum, there was a significant main effect of IGF-I treatment to induce greater concentration of DNA. However, the mass and concentration of protein in ileum showed a significant interaction (Treatment × Genotype, $P < 0.05$), such that knockout (KO) and heterozygote (HT), but not wild-type (WT) mice, showed greater ileal mass and protein concentration with IGF-I treatment.

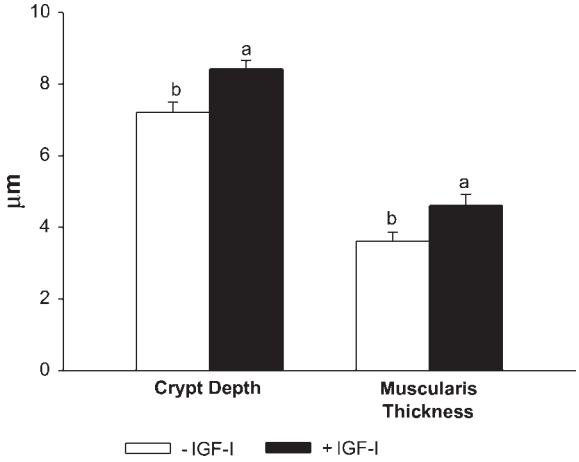

Fig. 3. Jejunum crypt depth and muscularis thickness of mice maintained with TPN alone (−IGF-I, $n = 23$) or TPN with IGF-I infusion (+IGF-I, $n = 19$). Values are expressed as means ± SE. [a,b]Means with different superscripts are statistically different. For each mouse, the mean of 20–30 measurements was calculated. Muscularis thickness includes the inner circular and outer longitudinal muscle layer. IGF-I treatment significantly increased crypt depth ($P = 0.003$) and muscularis thickness ($P = 0.03$).

RESULTS

Serum IGF-I and body weight. The concentration of IGF-I in serum was significantly greater by 73% due to coinfusion of IGF-I with TPN solution compared with TPN alone (Fig. 1*A*). This increase in serum concentration of IGF-I is within the physiological range of IGF-I concentration seen with fasting and feeding. There were no significant differences in the concentration of IGF-I in serum due to the main effect of genotype or sex. Female mice displayed significantly lower initial and final body weights (~4 g) compared with male mice. Mice in the current study with mixed background of C57BL6 and 129S6 lost 2–3 g of body weight over 6 days during TPN infusion. There was a significant difference in body weight change due to IGF-I treatment in mice with different genotypes (Treatment × Genotype, $P = 0.04$). Because this difference was independent of sex (Treatment × Genotype × Sex, $P > 0.05$), male and female mice were pooled. IGF-I attenuated the loss of body weight associated with TPN in WT mice (Fig. 1*B*). In contrast, KO and HT mice showed a similar loss of 2–3 g of body weight over 6 days with both TPN alone and TPN plus IGF-I treatment. Thus, the absence of the IGFBP-5 gene blocked the ability of IGF-I treatment to attenuate the weight loss associated with TPN in spite of a 73% increase in serum concentration of IGF-I.

Organ mass. To account for differences in body weight, liver, spleen, and kidney mass are expressed as g organ/100 g body wt. There were no significant differences in the mass of spleen or liver due to genotype. Spleen mass increased significantly with IGF-I treatment in male mice (−IGF-I = 0.44 ± 0.03, $n = 16$; +IGF-I = 0.77 ± 0.09, $n = 10$; $P = 0.001$). Female mice did not show a significant increase in spleen mass with IGF-I treatment (−IGF-I = 0.72 ± 0.06, $n = 10$; +IGF-I = 0.75 ± 0.05, $n = 9$; $P > 0.05$). Treatment with IGF-I resulted in a significantly lower mass of liver (−IGF-I = 4.38 ± 0.12, $n = 26$; +IGF-I = 3.93 ± 0.10, $n = 19$; $P = 0.01$).

Kidney mass was significantly greater in WT mice in response to IGF-I treatment consistent with successful administration of IGF-I (−IGF-I, WT = 1.39 ± 0.04, $n = 10$; +IGF-I, WT = 1.66 ± 0.09, $n = 5$; $P = 0.04$). In contrast, this response to IGF-I treatment was not observed in KO mice (−IGF-I, KO =1.66 ± 0.04, $n = 9$; +IGF-I, KO = 1.65 ± 0.05, $n = 10$). KO mice maintained with TPN alone for 5 days had a significantly greater kidney mass compared with WT mice (WT = 1.39 ± 0.04, $n = 10$; KO = 1.66 ± 0.04, $n = 9$; $P = 0.001$).

Mass and cellularity of intact intestine. IGF-I treatment induced significant intestinal growth in the small intestine and colon based on increases in mass and concentrations of protein and DNA across treatment groups (Fig. 2). The proliferative effect of IGF-I coinfused with TPN solution compared with TPN alone was relatively greater in duodenum (~37% greater mass) compared with jejunum (~25% greater mass) and ileum (~13% greater mass). There was no significant main effect of genotype and sex in duodenum and jejunum. There was a significant main effect of IGF-I treatment to induce greater concentration of DNA in ileum. However, ileum showed a significantly different response to IGF-I treatment with different genotype (Treatment × Genotype, $P < 0.05$, Fig. 2), such that KO and HT, but not WT, mice showed greater ileal mass and protein concentration. Increases in jejunal histology support the greater intestinal cellularity induced by IGF-I treatment based on increases in mass and concentrations of protein and DNA in jejunum. IGF-I treatment induced significantly greater jejunal crypt depth and muscularis thickness compared with TPN alone in all of the treatment groups (Fig. 3). Overall, the intestinal growth response is consistent with IGF-I treatment, inducing intestinal cellular hyperplasia. Thus, the intestinotrophic effects of IGF-I were not diminished in IGFBP-5 KO mice treated with TPN plus IGF-I.

Sucrase activity. There was a significant effect of genotype but no significant effect of IGF-I treatment or sex on sucrase-specific activity. In jejunum, WT mice had significantly 64%

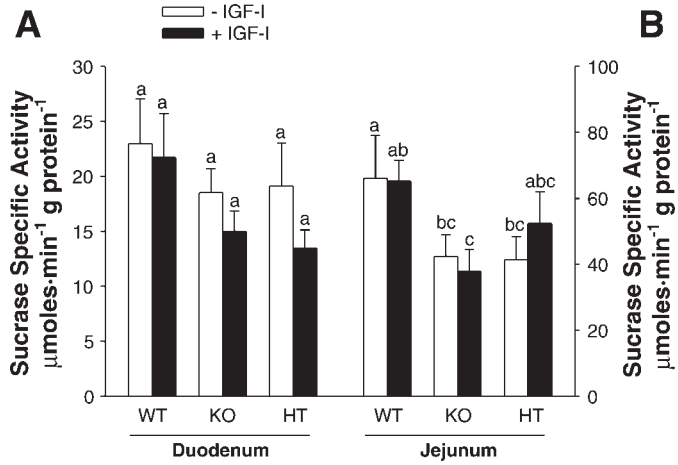

Fig. 4. Sucrase-specific activity in duodenum (*A*) and jejunum (*B*) of WT ($n = 16$; −IGF-I = 10, + IGF-I = 6), KO ($n = 18$; −IGF-I = 9, + IGF-I = 9) and HT ($n = 11$; −IGF-I = 7, + IGF-I = 4) mice maintained with TPN alone (−IGF-I) or TPN with IGF-I infusion (+IGF-I). Values are expressed as means ± SE. [a,b,c]Means with different superscripts are statistically different. There was a significant main effect of genotype but no significant effect of IGF-I treatment or sex on sucrase-specific activity. In jejunum, WT mice had 45–64% greater sucrase-specific activity ($P = 0.03$) compared with KO and HT mice. A similar trend though not statistically significant was observed in the duodenum.

greater sucrase-specific activity (μmol·min^{-1}·mg protein $^{-1}$) compared with KO mice and significantly 45% greater sucrase specific activity compared with HT mice (Fig. 4*B*). A similar trend though not statistically significant was observed in duodenum (Fig. 4*A*) and ileum (data not shown). Sucrase segmental activity (μmol·min^{-1}·cm jejunum $^{-1}$, data not shown) in duodenum, jejunum, and ileum was not significantly different due to IGF-I treatment, genotype, or sex.

Serum IGFBPs. There was a significant effect of genotype to alter the serum profile of IGFBPs. KO mice had significantly greater levels of IGFBPs at 38–43 kDa (IGFBP-3) compared with WT and HT mice (Fig. 5*A*). Both KO and HT mice had significantly greater levels of IGFBPs at 30–34 kDa (IGFBP-1, 2, and 5) (14), as well as significantly greater levels of total serum IGFBPs compared with WT mice. The serum profile of IGFBPs was not altered by IGF-I treatment (Fig. 5*B*).

IGFBP-5 and IGFBP-3 mRNA expression in jejunum. As expected, IGFBP-5 mRNA was not detectable in KO mice either with TPN alone or with IGF-I treatment. When treated with IGF-I, WT mice showed greater expression of IGFBP-5 mRNA (Fig. 6*A*) but no significant difference in IGFBP-3 mRNA expression (Fig. 6*B*). In contrast, KO mice showed a significant 360% greater expression of IGFBP-3 mRNA with IGF-I treatment compared with TPN alone (Fig. 6*B*). Thus, IGF-I treatment in WT mice stimulated IGFBP-5 mRNA expression but had no effect on IGFBP-3 mRNA, whereas IGF-I treatment in KO mice stimulated IGFBP-3 mRNA expression.

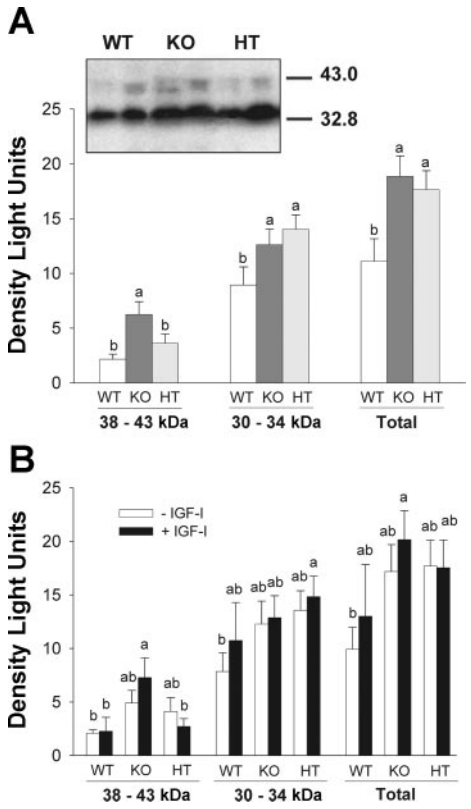

Fig. 5. Western ligand blot of serum insulin-like growth factor binding protein-5 (IGFBPs). Data are presented to show the significant main effect of genotype (*A*: WT, $n = 8$; KO, $n = 16$; HT, $n = 11$) and the effect of IGF-I treatment in the three genotypes (*B*: −IGF-I, $n = 19$; + IGF-I, $n = 16$). The bands were quantified and expressed as density light units. Values are expressed as means ± SE. [a,b]Means with different superscripts are statistically different. KO mice had significantly greater levels of total IGFBPs ($P = 0.007$), and IGFBPs in the 38–43 kDa ($P = 0.04$) and 30–34 kDa ($P = 0.01$) size ranges compared with WT mice (*A*).

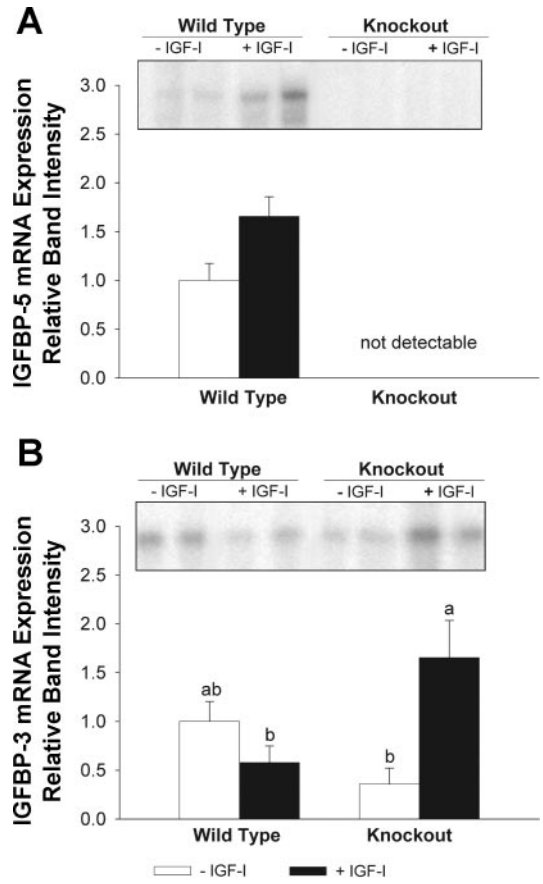

Fig. 6. Expression of IGFBP- 5 (*A*; WT, $n = 6$; KO, $n = 7$) and IGFBP-3 (*B*; WT, $n = 8$; KO, $n = 8$) mRNA in jejunum with the band intensity quantified by phosphorimage analysis and expressed as fold difference relative to the WT, −IGF-I group. Values are expressed as means ± SE. [a,b]Means with different superscripts are statistically different. IGF-I treatment increased expression of IGFBP-5 in WT mice by ~40% ($P = 0.07$) and IGFBP-3 in KO mice ($P = 0.01$).

DISCUSSION

The IGFBPs are known to both inhibit and potentiate IGF-I action, although the precise roles of individual IGFBPs in vivo are largely unknown (6, 7, 21). Previous studies suggest that IGFBP-5 potentiates the intestinotrophic actions of IGF-I (3, 10, 19, 24, 28, 29). Thus, we have tested the hypothesis that the intestinotrophic actions of IGF-I are dependent on IGFBP-5 by coinfusing IGF-I with TPN solution in WT, IGFBP-5 KO, and HT mice. To our knowledge, no previous studies have reported the anabolic effects of IGF-I infusion in an IGFBP KO model.

Our findings indicate that IGFBP-5 is not required for exogenous IGF-I to stimulate intestinal growth. Intestinal growth consistent with hyperplasia due to IGF-I was observed in WT, IGFBP-5 KO, and HT mice given IGF-I infusion compared with TPN alone, on the basis of significantly greater concentrations of protein and DNA in duodenum, jejunum, and ileum and significantly greater crypt depth and muscularis thickness in jejunum. These findings are consistent with our

earlier report that IGF-I infusion prevents the ~25% atrophy of the small intestine due to TPN in mice (19). We noted that IGF-I infusion induced greater mass and concentration of protein in ileum of KO and HT mice compared with WT mice. However, we believe this finding has little biological significance regarding the role of IGFBP-5 in intestinal growth because the concentration of DNA in ileum was not different due to genotype, and ileum showed only a modest 13% increase in mass after IGF-I treatment. In the current study, IGF-I infusion also stimulated growth of colon in TPN+IGF-I groups as shown by significantly greater mass and concentrations of protein and DNA in colon; a similar response, which did not achieve statistical significance, was noted in our previous report (19).

One explanation for the intestinal growth induced by IGF-I despite the absence of IGFBP-5, is that greater expression of IGFBP-3 compensated for the absence of IGFBP-5 in IGFBP-5 KO mice. IGFBP-5 KO mice given TPN+IGF-I compared with TPN alone showed 360% greater expression of IGFBP-3 in jejunum, whereas WT mice treated with IGF-I compared with TPN alone showed greater expression of IGFBP-5 mRNA in jejunum, as previously noted (19), but no change in jejunal IGFBP-3 mRNA. IGFBP-5 is thought to potentiate IGF-I action in intestine in a paracrine manner by binding extracellular matrix, which reduces the affinity of the IGF-I - IGFBP interaction and increases the availability of IGF-I to bind its receptor (1, 26). Perhaps greater local expression of IGFBP-3 acts in a similar manner to increase the bioavailability of IGF-I to its receptor in the intestine, resulting in intestinal hyperplasia. In support of this speculation that IGFBP-3 compensates for the absence of IGFBP-5, a recent study indicates that both IGFBP-3 and -5 single KO mice exhibit normal growth and serum concentration of IGF-I, suggesting that they have overlapping roles (21). Alternatively, previous associations between intestinal growth, IGF-I, and elevated IGFBP-5 mRNA may be indirect markers of another mechanism for the intestinotrophic effects of IGF-I.

Sucrase activity is a measure of differentiated epithelial cell function (17, 28). Interestingly, sucrase-specific activity was lower in KO and HT mice compared with WT mice in duodenum, jejunum, and ileum, irrespective of IGF-I infusion. This suggests that IGFBP-5 gene expression may be needed for differentiation and maturation of enterocytes. In vivo and in vitro studies suggest that IGFBP-3 may play a role in stimulating enterocyte differentiation (13, 23, 28). However, our data do not support this notion as sucrase activity was not greater in KO mice treated with IGF-I that showed greater expression of IGFBP-3.

The absence of IGFBP-5 blocked the ability of IGF-I treatment to attenuate the weight loss associated with TPN as reflected by a lack of improvement in body weight in KO and HT mice. Our experience conducting TPN studies in rodents suggests that TPN is more stressful to mice than rats, as rats show gain in body weight with TPN and, in particular, with TPN+IGF-I (5) whereas, mice show maintenance of body weight with TPN (19) . Surprisingly, mice in the current study with a mixed genetic background of C57BL6 and 129S6 showed a 2–3 g loss of body weight during TPN when receiving a level of nutrition sufficient to maintain body weight in our earlier study with C57BL6 mice (19). This difference in response may be due to the difference in genetic background of the mice (15) as well as the inclusion of females in the current study. One explanation for the failure of elevated serum IGF-I to improve the anabolic response to TPN in IGFBP-5 KO and HT mice may be the observed increase in total circulating IGFBPs in IGFBP-5 KO and HT mice compared with WT mice that may impair the release of IGF-I from its ternary complex to bind the IGF-I receptor (2).

The observed differential effects of TPN alone and TPN+IGF-I infusion on kidney mass in KO and WT mice suggests that deletion of the IGFBP-5 gene impacts kidney growth and development. For example, IGF-I infusion was associated with significantly greater kidney mass in WT, but not KO mice, and TPN alone was associated with significantly greater kidney mass in KO, but not WT mice. In our previous study, C57BL6 mice showed a significantly lower kidney mass with TPN compared with oral feeding suggesting that TPN alone does not increase kidney mass (19). Data for kidney mass in KO mice maintained on a standard oral mouse diet is not available to further interpretation of the effects of TPN on kidney mass in KO mice. Given the interest in the role that IGFBP-5 expression may play in the renal complications associated with diabetes (12, 25), further studies with IGFBP-5 KO mice are needed to understand the role of IGFBP-5 and kidney function.

In conclusion, we provide novel findings regarding how the absence of a single IGFBP, IGFBP-5, affects the ability of IGF-I infusion to stimulate intestinal growth. The absence of IGFBP-5 does not block the ability of IGF-I to stimulate intestinal growth, possibly because greater intestinal expression of IGFBP-3 compensates for the absence of IGFBP-5 by acting in a paracrine fashion. In contrast, from an endocrine perspective, the absence of IGFBP-5 blocks the ability of IGF-I infusion to attenuate the loss of body weight associated with TPN infusion. These observations suggest that IGFBP-5 and IGFBP-3 have both overlapping and distinct physiological roles in vivo. Additional studies are needed to understand the binding characteristics of IGF-I and IGFBPs in models with different IGFBP genotypes to further understanding of the distinct and overlapping roles of IGFBPs.

GRANTS

This work was supported by the National Institute of Health Grants R01-DK-42835 and T32-DK-07665 (D. M. Ney), RO1-NS-21970 (J. E. Pintar) and by funds from the College of Agricultural and Life Sciences, University of Wisconsin-Madison.

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