Exogenous GLP-2 and IGF-I induce a differential intestinal response in IGF binding protein-3 and -5 double knockout mice

Sangita G. Murali, Adam S. Brinkman, Patrick Solverson, Wing Pun, John E. Pintar, and Denise M. Ney

Departments of 1Nutritional Sciences and 2Surgery, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin; 3Department of Neuroscience and Cell Biology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey

Submitted 12 September 2011; accepted in final form 19 January 2012

Murali SG, Brinkman AS, Solverson P, Pun W, Pintar JE, Ney DM. Exogenous GLP-2 and IGF-I induce a differential intestinal response in IGF binding protein-3 and -5 double knockout mice. Am J Physiol Gastrointest Liver Physiol 302: G794–G804, 2012. First published January 26, 2012; doi:10.1152/ajpgi.00372.2011.—Glucagon-like peptide-2 (GLP-2) action is dependent on intestinal expression of IGF-I, and IGF-I action is modulated by IGF binding proteins (IGFBPs). Our objective was to evaluate whether the intestinal response to GLP-2 or IGF-I is dependent on expression of IGFBP-3 and -5. Male, adult mice in six treatment groups, three wild-type (WT) and three double IGFBP-3/-5 knockout (KO), received twice daily intraperitoneal injections of GLP-2 (0.5 μg/g body wt), IGF-I (4 μg/g body wt), or PBS (vehicle) for 7 days. IGFBP-3/-5 KO mice showed a phenotype of lower plasma IGF-I concentration, but greater body weight and relative mass of visceral organs, compared with WT mice (P < 0.001). WT mice showed jejunal growth with either IGF-I or GLP-2 treatment. In KO mice, IGF-I did not stimulate jejunal growth, crypt mitosis, sucrase activity, and IGF-I receptor (IGF-IR) expression, suggesting that the intestinotrophic actions of IGF-I are dependent on expression of IGFBP-3 and -5. In KO mice, GLP-2 induced significant increases in jejunal mucosal cellularity, crypt mitosis, villus height, and crypt depth that was associated with increased expression of the ErbB ligand epiregulin and decreased expression of IGF-I and IGF-IR. This suggests that in KO mice, GLP-2 action in jejunal mucosa is independent of the IGF-I system and linked with ErbB ligands. In summary, the intestinotrophic actions of IGF-I, but not GLP-2, in mucosa are dependent on IGFBP-3 and -5. These findings support the role of multiple downstream mediators for the mucosal growth induced by GLP-2.

IGF-I is a major regulator of systemic and tissue-specific growth, including the gastrointestinal tract. Circulating IGF-I is produced primarily by the liver under growth hormone control, and almost all tissues express the IGF-I receptor (IGF-IR) (41). IGF-I stimulates intestinal growth by the endocrine delivery of IGF-I as well as local synthesis of IGF-I in the mesenchyme providing paracrine delivery of IGF-I (reviewed in Ref. 28). IGF-I bioactivity is modulated by a family of at least six IGF binding proteins (IGFBPs) that can both inhibit and potentiate IGF-I action either by sequestering IGF-I from its receptor or concentrating IGF-I activity within discrete cellular regions and then releasing IGF-I by proteolytic cleavage to combine with its receptor (reviewed in Ref. 14).

IGFBP-3 and -5 are unique because they are the only IGFBPs known to contribute to formation of the ternary IGF-IGFBP-acid-labile subunit complex that normally stabilizes the IGFs (3). Under specific conditions both IGFBP-3 and -5 can potentiate the action of IGF-I in vitro and in vivo (34). For example, IGFBP-3 can enhance the IGF-I-mediated DNA synthesis in breast carcinoma cells (4) and osteoblasts (12) in vitro, and coadministration of IGF-I with IGFBP-3 is more anabolic than IGF-I alone in hypophysectomized rats (5). Moreover, local expression of IGFBP-5 potentiates the actions of IGF-I in the intestine as noted in rats and mice with attenuation of the mucosal atrophy induced by total parenteral nutrition (30, 35), transgenic mice that overexpress IGF-I in mesenchyme (40), and rats with resection-induced growth (16) and inflammatory bowel disease (43). The stimulatory effect of IGFBP-5 has been linked with the ability of IGFBP-5 to bind extracellular matrix and increase the availability of IGF-I to bind the IGF-IR (2, 37). Interestingly, we have shown that the intestinal tropic effects of IGF-I are not diminished in IGFBP-5 KO mice, although expression of IGFBP-3 is increased and may compensate for the lack of IGFBP-5 (29).

Glucagon-like peptide-2 (GLP-2) is a nutrient-dependent intestinotrophic peptide hormone derived from tissue-specific posttranslational processing of proglucagon in the enteroendocrine L cells of the ileum and colon (36). GLP-2 maintains gastrointestinal homeostasis by inhibiting gastric acid secretion and motility, upregulating intestinal blood flow, stimulating nutrient absorption, and reducing intestinal permeability (10, 17, 39). Moreover, GLP-2 is considered a key mediator of intestinal adaptive growth through stimulation of epithelial cell proliferation and inhibition of apoptosis (7, 11, 17, 31). Clinical studies evaluating native GLP-2 and (Gly2)GLP-2, a degradation-resistant analog of GLP-2, demonstrate improved intestinal morphology and absorption in humans with intestinal failure (13, 19), and studies have been conducted or are underway to evaluate the efficacy of GLP-2 in Crohn’s disease (22) and other conditions of intestinal dysfunction (www.npsp.com).

In contrast with IGF-I, GLP-2 induces intestine-specific growth because expression of the GLP-2 receptor (GLP-2R) is limited to the intestine and brain. Within the gastrointestinal tract, the GLP-2R has been localized to several cell types, including enteroendocrine cells, subepithelial myofibroblasts, vagal afferents, and enteric neurons (10, 32, 36). However, the GLP-2R is not localized on proliferating crypt cells, a primary target of GLP-2 action, and this has led to the view that GLP-2 action is dependent on downstream mediators. In particular, small intestine growth due to GLP-2 has been associated with...
ErbB ligands (1, 36, 42) and the IGF system via intestinal subepithelial myofibroblasts that express the GLP-2R (24). Evidence that IGF-I global knockout (KO) mice are resistant to the intestinal tropic effects of GLP-2 (11) and that GLP-2 increases IGF-I transcript levels in mouse and rat intestine (24, 25, 31, 39) supports the concept that IGF-I is a downstream mediator of GLP-2 action in the small intestine. Possible links with GLP-2 action and the modulation of IGF-I activity by IGFBPs have not been explored. Thus we hypothesized that stimulation of intestinal response with IGF-I or GLP-2 treatment is dependent on expression of IGFBP-3 and -5.

A better understanding of the role of the IGF-I system in modulating GLP-2 action is needed given the potential for therapeutic use of GLP-2 to treat a variety of diseases affecting intestinal dysfunction. Given the key role of IGFBPs in modulating IGF-I activity in the intestine (14, 29) and the evidence that GLP-2 action is dependent on IGF-I expression (11, 24), we have chosen to investigate how expression of both IGFBP-3 and -5 impact the tropic actions of IGF-I and GLP-2 in the intestine. Our objective was to determine whether stimulation of intestinal growth due to administration of IGF-I or GLP-2 is dependent on expression of IGFBP-3 and -5 in double KO mice. Results show a differential response in that GLP-2, but not IGF-I, stimulates intestinal growth in the absence of IGFBP-3 and -5. Moreover, our findings are consistent with GLP-2 actions in intestine reflecting multiple downstream mediators (36).

**MATERIALS AND METHODS**

**Animals and experimental design.** The animal facilities and protocols reported were approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee. The derivation of the double IGFBP-3/-5 knockout mice has been reported previously (34). IGFBP-3 and -5 null mice on a mixed C57BL6 and 129S6 background were bred and the double heterozygous mutants intercrossed to generate IGFBP-3/-5 null mice. Genotyping was performed by PCR analysis using genomic DNA isolated from tail snips of newborn mice. Mice were adapted to the animal facility for 2 wk in a room maintained at 22°C on a 12-h:12-h light/dark cycle with group housing and free access to water and a stock diet. For the experimental protocol, mice were individually housed in wire-bottom cages with free access to water and diet.

The experimental design consisted of male, adult mice 14–18 wk of age in six treatment groups that were fed a standard 20% casein diet that provided 18.8% kcal from protein, 64% kcal from carbohydrate, and 17.2% kcal from fat (TD.09669; Teklad, Madison, WI). Three groups of WT mice (C57BL6; Jackson Laboratories, Bar Harbor, ME) and three groups of double IGFBP-3/-5 KO mice (provided by J. Pintar) were given twice daily intraperitoneal injections at 7:30 AM and 7:30 PM of human GLP-2 (0.5 μg/g body wt; California Peptide Research, Napa, CA), rhIGF-I (4 μg/g body wt; Genentech, South San Francisco, CA), or PBS vehicle for 7 days. The doses of GLP-2 and IGF-I were within the physiological range as determined by preliminary studies. The GLP-2 dose of 0.5 μg/g body wt increased plasma GLP-2 concentration approximately fourfold, similar to that observed with meal-induced increases in GLP-2, with a trend for higher plasma GLP-2 concentrations in KO compared with WT mice. On day 8 mice were anesthetized with isoflurane and euthanized by cardiac exsanguinations 1 to 2 h after receiving the 7:30 AM growth factor injection. Blood was obtained by cardiac puncture into syringes preloaded with EDTA resulting in a final concentration of 2.7 mmol/L EDTA. Plasma was isolated by centrifugation at 1,700 g for 15 min at 4°C. Total plasma IGF-I concentration was determined by radioimmunoassay after IGFBP were removed by HPLC under acid conditions (33). Liver, kidneys, and spleen were removed, weighed, and analyzed relative to body mass (in gram per 100 gram body weight).

**Intestinal composition and histology.** The entire small intestine and colon were removed, flushed with ice-cold saline, and placed on a chilled glass plate. The weight and length of the small intestine (pylorus to ileocecal valve) and colon (postcecum to rectum) were recorded, as determined by hanging each section with a constant weight for a fixed period of time. The bowel was sectioned into duodenum (4 cm distal to pylorus), jejunum (from 4 cm distal to pylorus to 10 cm proximal to ileocecal valve), ileum (the distal 10 cm of small intestine up to ileocecal valve), and colon. The mass of intact duodenum was determined. The first 2 cm of jejunum were used to measure wet and dry intact mass; the next 4 cm were used for determining wet and dry mass of mucosa and muscularis. The jejunum was opened lengthwise, and the mucosa was scraped with a glass slide by a single individual; the remaining bowel was considered muscularis. The 7th cm of jejunum was fixed in 10% buffered formalin, transferred to 70% ethanol, paraffin embedded, cut into 5-μm sections, and stained with hematoxylin and eosin for histomorphometry as described previously (8). The 8–11th cm of jejunum were used to determine mucosal concentrations of protein (bicinchoninic acid protein assay; Pierce Chemicals, Rockford, IL), DNA (23), and sucrase activity (unit; units = μmol/min) expressed as segmental (units/cm) or specific activity (units/mg protein) (6). The concentrations of protein and DNA were determined in jejunal muscularis. The remaining (distal) jejunum was snap frozen for RNA extraction and PCR analysis. The first 2 cm of intact ileum were used for measuring mass and the next 2 cm for determining concentrations of protein and DNA. The first 2 cm of intact colon were used to determine mass and the next 2 cm for protein and DNA analysis.

To assess enterocyte proliferation, paraffin embedded jejunal sections were stained for Ki67 antigen using rabbit anti-mouse Ki67 antibody (Millipore, Billerica, MA; No. AB9260) (38). To unmask the antigens and epitopes, sections were treated with citric buffer at 95°C for 20 min before incubation in Ki67 antibody followed by immunohistochemistry (15). Ten crypts (1 side of the crypt in a longitudinal cross section) were assessed per animal, and the number of stained cells as well as the total number of crypt cells was counted. Data are presented as a mitotic index calculated as the percentage of the total number of crypt cells that showed positive Ki67 staining.

**Biochemical analyses.** Total RNA was extracted from liver and intact jejunum using the TRizol reagent (Gibco BRL Life Technologies, Grand Island, NY). All RNA extracts were quantitated by absorbance at 260 nm, and quantity and integrity were confirmed by electrophoresis through 1.25% agarose/2.2 M formaldehyde gels and staining with ethidium bromide to visualize ribosomal RNA bands. Expression of IGF-I, IGF-IR, glucagon/proglucagon, GLP-2R, and the ErbB ligands epiregulin, amphiregulin, and heparin binding (HB)-EGF mRNAs in the distal jejunum was assessed.

Real time quantitative PCR (RT-qPCR) was performed by reverse transcription of total RNA, followed by TaqMan Gene Expression assay (Applied Biosystems, Foster City, CA) using the following murine primer kits: IGF-I (Mm00439560_m1), IGF-IR (Mm00802831_m1), proglucagon/glucagon (Mm01269055_m1), GLP-2R (Mm00588355_m1), and ErbB ligands epiregulin (Mm00514794_m1), amphiregulin (Mm00437583_m1), and HB-EGF (Mm00439307_m1). Data were analyzed using 7000 system software (Applied Biosystems), and relative quantification was done using the ΔΔCt method with β-actin as the reference gene (Mm00607939_m1), which was stable across the treatment groups (27), and WT mice treated with PBS as the control group.

**Statistical analyses.** Data were analyzed by two-way ANOVA using PROC GLM with a model that included a term for genotype (WT or IGFBP-3/-5 KO), treatment (PBS, IGF-I, or GLP-2), and their interactions. Groups were compared using the protected least significant-differences technique (SAS version 8.2; SAS Institute, Cary, NC).
NC) to determine individual group differences. Differences of $P \leq 0.05$ were considered statistically significant; data are presented as means ± SE. The final sample size for each of the three treatments included 4 to 5 IGFBP-3/-5 KO mice (total $n = 14$) and 6–8 WT mice (total $n = 22$). For each parameter measured, data from genotype or treatment were pooled if there were no significant interaction. Statistics were performed on log-transformed data for results showing unequal variances among groups.

**RESULTS**

IGFBP-3/-5 KO mice show phenotype of lower plasma IGF-I with greater body weight and relative organ mass. The IGFBP-3/-5 KO mice showed significantly lower plasma total IGF-I concentration compared with WT mice as reflected in a significant main effect for genotype ($P < 0.001$; Fig. 1A). Despite lower circulating IGF-I levels, KO mice were significantly 18% heavier, ate more (4.2 ± 0.3 vs. 3.4 ± 0.5 g diet/day; $P < 0.01$), and showed significantly 10–20% greater relative renal and spleen mass compared with WT mice (Fig. 2). There were no significant differences in relative liver mass across the groups. IGF-I treatment significantly increased hepatic IGF-I expression by 2.5-fold in KO mice, but not WT

![Image of graphs showing plasma concentration of total IGF-I and hepatic IGF-I expression](http://ajpgi.physiology.org/)
mice (Fig. 1B). In contrast, GLP-2 treatment significantly decreased hepatic IGF-I expression by 75% in KO mice, but not WT mice. Both WT and KO mice showed ~40% higher plasma IGF-I concentrations in response to twice daily intraperitoneal injections of IGF-I (P < 0.001) when compared with respective controls treated with PBS (Fig. 1A). WT mice responded to higher circulating IGF-I levels with a small increase in body weight gain (1.5 ± 0.3 vs. 0.2 ± 0.3; g/7 days; P < 0.05) and relative mass of the IGF-I target tissues kidneys and spleen compared with WT mice treated with PBS. KO mice did not show increases in body weight and renal or spleen mass with IGF-I compared with PBS treatment. GLP-2 treatment did not increase body weight, renal, or spleen mass in either WT or KO mice.

IGFBP-3/-5 KO mice showed a significantly larger small intestine and colon as reflected in a significant main effect for genotype (P < 0.001; Fig. 3). The mass of the entire small intestine and colon relative to body weight (in g/100 g body wt) was significantly 28% greater in KO mice compared with WT mice. Moreover, the small intestine was significantly longer in KO compared with WT mice (45 vs. 36 cm; P < 0.05), and this is interesting given that IGFBP-5 is primarily expressed in the muscularis layer of intestine (35, 40). Individual sections of small intestine also showed a significant genotype effect with greater intact mass of duodenum, jejunum, and ileum in KO versus WT mice with the largest effect in jejunum. Jejunal morphology showed a significant increase in villus height (KO = 509 ± 28, n =

Fig. 3. Relative length (A) and mass (B) of small intestine and mass of intact colon (C) in WT and KO mice treated with PBS, IGF-I, or GLP-2 for 7 days. Values are means ± SE; P < 0.0001 vs. WT reflecting a significant main effect for genotype (KO, n = 14; WT, n = 22), but not treatment or interaction.

Fig. 4. Mass of intact jejunum (A) and jejunal crypt mitotic index (B) in WT and KO mice treated with PBS, IGF-I, or GLP-2 for 7 days. Values are means ± SE; means with different superscripts (a-e) are significantly different (P < 0.05). In WT mice both IGF-I and GLP-2 stimulated an increase in jejunal mass, whereas in KO mice only GLP-2 stimulated an increase in jejunal mass.
14; WT = 435 ± 14, n = 16; in μm), but not crypt depth, in KO compared with WT mice. The greater relative colon mass in KO mice was reflected in higher concentrations of protein and DNA compared with WT mice. Colon mass, protein, and DNA concentration did not show a response to either IGF-I or GLP-2 treatment, and, therefore, colon was not analyzed further. Overall, the IGFBP-3/-5 KO mice demonstrated a novel phenotype of lower plasma total IGF-I concentration but greater body weight and relative mass of visceral organs compared with WT mice, as well as differ-

Fig. 5. Jejunal mucosa dry mass (A), concentrations of protein (B) and DNA (C), and sucrase specific activity (D) in WT and KO mice treated with PBS, IGF-I, or GLP-2 for 7 days. Values are means ± SE; means with different superscripts (a-c) are significantly different (P < 0.05). There was a significant main effect for GLP-2 to decrease sucrase specific activity compared with PBS and IGF-I without interaction due to genotype (PBS = 0.15 ± 0.02a; IGF-I = 0.14 ± 0.02a; GLP-2 = 0.09 ± 0.01b; in μmol/min/mg protein; GLP-2 treatment main effect, P = 0.01). Representative jejunal histology from WT and KO mice treated with PBS, IGF-I, or GLP-2 is shown in E; magnification is 100X.
ential effects of IGF-I and GLP-2 treatment on hepatic IGF-I expression.

Jejunum responds to GLP-2, but not IGF-I, in IGFBP-3/-5 KO mice. In WT mice both IGF-I and GLP-2 treatments stimulated an increase in jejunal mass (in mg/cm of intact jejunum) with a larger magnitude of response observed with GLP-2 treatment (Fig. 4A). In contrast, only GLP-2 and not IGF-I treatment stimulated an increase in jejunal mass in IGFBP-3/-5 KO mice. Differential increases in mass due to treatment with IGF-I and GLP-2 were associated with parallel changes in jejunal crypt proliferation based on expression of Ki67. A significant increase in the mitotic index occurred in WT mice treated with IGF-I or GLP-2, whereas only KO mice treated with GLP-2, and not IGF-I, showed an increase in enterocyte proliferation (Fig. 4B). Although a similar response to GLP-2 and IGF-I treatment was observed in duodenum and ileum, we chose to focus further analysis on jejunal composition where the treatment effects were greatest.

The cellularity of jejunal mucosa showed similar differential growth in response to IGF-I and GLP-2 treatment in BP-3/-5 KO mice as noted for jejunal mass. Specifically GLP-2, but not IGF-I, stimulated significant increases in jejunal mucosa dry mass and concentrations of protein and DNA in KO mice compared with PBS KO mice (Fig. 5). Villus height (VH) and crypt depth (CD) were also significantly elevated in KO mice treated with GLP-2, but not IGF-I; PBS = 451 μm VH and 73 μm CD, IGF-I = 483 μm VH and 79 μm CD, and GLP-2 = 615 μm VH and 86 μm CD (P < 0.0001 GLP-2 vs. IGF-I and PBS). In contrast, both GLP-2 and IGF-I stimulated mucosal growth in WT mice compared with PBS treatment (Fig. 5). Representative jejunal histology from WT and KO mice is shown in Fig. 5E. There were no significant differences in the ratio of protein to DNA concentration in jejunal mucosa across the treatment groups consistent with increases in mucosal cellularity.

Mucosal sucrase activity reflects the digestive capacity of the small intestine. Sucrase activity in jejunal mucosa paralleled the differential growth induced by IGF-1 and GLP-2. In both WT and KO mice, sucrase specific activity was significantly reduced with GLP-2 compared with IGF-I and PBS (GLP-2 treatment main effect, P = 0.01; Fig. 5D) (25). In contrast, sucrase segmental activity, which reflects greater mucosal mass, was increased with either IGF-I or GLP-2 in WT mice (PBS = 0.13 ± 0.02μa; IGF-I = 0.19 ± 0.03μa; GLP-2 = 0.19 ± 0.02μa, units/cm mucosa; P = 0.01). In KO mice, only GLP-2 increased sucrase segmental activity (PBS = 0.15 ± 0.03μa; IGF-I = 0.15 ± 0.04μa; GLP-2 = 0.24 ± 0.02μa; units/cm mucosa; P = 0.03) consistent with the lack of IGF-I-induced mucosal growth.

Fig. 6. Jejunal muscularis dry mass (A), concentrations of protein (B) and DNA (D), and protein-to-DNA ratio (C) in WT and KO mice treated with PBS, IGF-I, or GLP-2 for 7 days. Values are means ± SE; means with different superscripts (a–c) are significantly different (P < 0.05).
The greater length of small intestine observed in IGFBP-3/-5 KO mice is consistent with growth of the intestinal muscularis layer. To further investigate this response, we measured jejunal muscularis mass and concentrations of protein and DNA after scraping off the mucosa (Fig. 6). As expected, IGF-I stimulated muscularis growth based on significant increases in dry mass, protein, and DNA in WT mice, suggesting increases in both cell number and size. In contrast, GLP-2 did not stimulate muscularis except for an increase in protein concentration in WT mice compared with PBS treatment, suggesting an increase in cell size. Interestingly, KO control mice given PBS showed a larger jejunal muscularis based on mass, protein, and DNA compared with WT control mice and administration of either GLP-2 or IGF-I attenuates this response. Thus neither GLP-2 nor IGF-I increased growth of the jejunal muscularis layer in BP-3/-5 KO mice.

Expression of IGF-I, GLP-2, and ErbB ligands in distal jejunum. To understand the role of the IGF-I and GLP-2 systems in the differential jejunal growth induced by GLP-2 and IGF in IGFBP-3/-5 KO mice, we assessed expression of IGF-I, IGF-IR, GLP-2R, proglucagon, and ErbB ligands. KO mice treated with PBS showed 2.5- and fourfold greater expression of IGF-I and proglucagon, respectively, in the distal jejunum compared with WT control mice treated with PBS (P < 0.0001; Fig. 7, A and C). Expression of IGF-I and the IGF-IR were examined to account for the ability of IGF-I to stimulate jejunal growth in WT, but not KO mice. IGF-I treatment of either WT or KO mice did not alter the expression of IGF-I, proglucagon, or the GLP-2R compared with PBS control groups. However, expression of the IGF-IR was significantly increased in IGF-I-treated WT mice who demonstrated jejunal growth, whereas KO mice treated with IGF-I demonstrated a lack of jejunal growth and lower expression of the IGF-IR (P = 0.06; Fig. 7B). This suggests that failure of IGF-I to induce jejunal growth in IGFBP-3/-5 KO mice may be associated with the absence of upregulation of the IGF-IR and/or postreceptor signaling.

In association with robust jejunal growth, GLP-2 treatment in KO mice significantly reduced expression of IGF-I, IGF-IR, proglucagon, and the GLP-2R compared with KO PBS mice. This suggests that mediators other than the IGF-I/IGF-IR are associated with the ability of GLP-2 to induce jejunal growth in KO mice. Thus we assessed the expression of ErbB ligands as putative mediators of GLP-2 action in jejunum. The ability of GLP-2 to induce mucosal growth in KO mice was associated with 2.2-fold greater expression of the ErbB ligand epiregulin (P < 0.0001) but no significant increased expression of amphiregulin or HB-EGF as compared with PBS (Fig. 8).

![Figure 7](http://ajpgi.physiology.org/images/ajpgi_tv3.png)

Fig. 7. Jejunum IGF-I (A), IGF-I receptor (IGF-IR; B), proglucagon (C), and GLP-2R (D) mRNA expression in WT and KO mice treated with PBS, IGF-I, or GLP-2 for 7 days and euthanized 1 to 2 h following growth factor injection. Values are means ± SE; means with different superscripts (a-c) are significantly different (P < 0.05).
IGF-I and GLP-2 are potent intestinal growth factors, and evidence indicates that GLP-2 action is dependent on intestinal expression of IGF-I (11, 24) and also linked with ErbB ligands (1, 42). Given the key role of IGFBPs in modulating IGF-I activity (9, 14, 35) and potential interactions of GLP-2 action with IGFBP expression in intestine, we have investigated how expression of both IGFBP-3 and -5 impact the trophic actions of GLP-2 and IGF-I in IGFBP-3/-5 double KO mice. Results indicate a differential intestinal response in KO mice such that the proliferative effects of IGF-I are dependent on expression of IGFBP-3 and -5, whereas GLP-2 action is independent of IGFBP-3/-5.

Interestingly, the IGFBP-3/-5 KO mice showed a phenotype of lower plasma concentration of total IGF-I, but greater body size and relative mass of organs including small intestine, colon, spleen, and kidneys compared with adult WT mice. The reduction in plasma concentration of total IGF-I is consistent with the absence of IGFBP-3/-5, which forms the ternary IGF-IGFBP-acid-labile subunit complex (3) that prolongs the half-life of IGF-I in plasma and restricts access to tissue receptors. Lower plasma IGF-I concentration in KO mice would be expected to stimulate hepatic IGF-I synthesis, which was only observed in KO mice treated with IGF-I who paradoxically showed an increase in both plasma IGF-I concentration and hepatic IGF-I expression. This interesting observation suggests that increased circulating IGF-I in the absence of the IGFBP-3/-5 ternary IGF-I complex alters feedback regulation of hepatic IGF-I synthesis. The data suggest that KO mice show greater expression of the IGFR mRNA and may have higher concentrations of free IGF-I that is available to tissue receptors. This is consistent with the observation of larger body size and organ mass in KO compared with WT mice. This interpretation is supported by studies in liver-specific IGF-I-deficient mice who show a 75% decrease in plasma concentrations of total IGF-I and IGFBPs without significant impairment of somatic growth or increased expression of tissue IGF-I (41). In contrast, triple KO mice with mutations in IGFBP-3, -4, and -5 show diminished IGF-I bioactivity and growth, 78% smaller compared with adult WT mice (34), which suggests combinatorial effects of IGFBP-3, -4, and -5 in mice.

The failure to further augment intestinal growth in KO mice treated with IGF-I compared with PBS suggests that the intestinotrophic action of IGF-I is dependent on expression of both IGFBP-3 and -5. Because IGF-I action was not impaired in IGFBP-5 KO mice (29), this result demonstrates that IGFBP-3 and -5 can substitute for each other in modulating IGF-I bioactivity. The mechanism of impaired intestinal growth in KO mice treated with IGF-I may involve the IGFR as well as postreceptor events (Fig. 9). Decreased interaction with the IGFR in KO mice is supported by the observation that the IGFR is not increased in KO mice treated with IGF-I compared with PBS, whereas the ability of IGF-I to increase jejunal cellularity in WT mice was associated with a significant increase in IGF-IR expression. Thus failure to upregulate expression of the IGFR may account for the absence of jejunal growth in KO mice treated with IGF-I. An alternative explanation for the absence of intestinal growth in KO mice treated with IGF-I is that loss of IGFBP-3 and -5 resulted in enhanced jejunal IGF-I activity and IGF-IR expression that could not be further augmented by systemic administration of IGF-I. Additional research with a range of time points following growth factor administration is needed to characterize how IGFBP-3/-5 modulate receptor and postreceptor signaling events in the intestine.

GLP-2 induced dramatic intestinal growth based on mucosal cellularity and histology in both WT and KO mice, demon-
suggesting that GLP-2 action is not dependent on the presence of IGFBP-3 and -5. Consistent with the strong proliferative effects of GLP-2, sucrase specific activity was reduced, whereas segmental activity was increased with GLP-2 treatment (26). This decrease in sucrase activity when normalized to protein concentration instead of unit length reflects a greater proportion of immature, less differentiated enterocytes, which are known to have lower expression of disaccharidases than do mature cells (18). The dramatic intestinal growth induced by GLP-2 in KO mice was not associated with increased expression of IGF-I or its receptor as noted in fasted mice or rats who show refeeding induced mucosal growth and parallels increases in plasma GLP-2 and intestinal IGF-I mRNA expression (31, 39). In fact, expression of IGF-I and the IGF-IR were both significantly decreased in KO mice who showed dramatic intestinal growth in response to GLP-2 (Fig. 9). GLP-2-induced intestinal growth in KO mice was associated with increased expression of the ErbB ligand epiregulin, but expression of amphiregulin and HB-EGF was not significantly increased. This response was seen only in the absence of IGFBP-3/5 since there was no increase of epiregulin in WT mice given GLP-2. Prior studies have demonstrated increased jejunal epiregulin expression in WT mice treated with GLP-2 with maximal expression of epiregulin 4 h following injection and maximal amphiregulin and HB-EGF expression 1 h following exogenous GLP-2 administration (42). In keeping with past studies, our results demonstrate increased epiregulin expression 1 to 2 h following GLP-2 administration but did not show increased amphiregulin and HB-EGF expression, which may be related to the timing of jejunum collection 1 to 2 h following exogenous GLP-2 administration. Taken together, the data suggest that there are multiple downstream mediators of GLP-2 action (36) and selective expression of these mediators appears to reflect the different physiological states of the various rodent model systems used to study intestinal growth.

Consistent with greater bioavailability or expression of IGF-I in mesenchymal cells (20, 40), IGFBP-3/-5 KO mice showed a significantly longer small intestine than WT mice. However, unlike mucosa, further growth of muscularis in KO mice was not observed with either GLP-2 or IGF-I treatment. This may reflect a need for IGFBP-5 to stimulate further growth of muscularis in response to GLP-2 since overexpression of IGF-I in mesenchyme induces muscularis growth throughout the bowel in association with increased expression of IGFBP-5 in muscularis (40). In our rat model of short bowel syndrome we have noted that GLP-2 increases small intestine length (21), an important goal of rehabilitation therapy in patients with intestinal failure (20). Thus, given the therapeutic potential of GLP-2 to improve intestinal adaptation (13, 19), further studies are needed to investigate the role of IGFBP-3/5 in the growth of muscularis and subsequent lengthing of the bowel in response to GLP-2.

In summary, this study provides novel information about the phenotype associated with IGFBP-3/5 KO mice and how expression of IGFBP-3 and -5 exert differential effects on the ability of IGF-I and GLP-2 to stimulate intestinal growth. The data suggest that in our IGFBP-3/5 KO mouse model the proliferative action of IGF-I in small intestine is dependent on expression of IGFBP-3 and -5 and is associated with increased expression of the IGF-IR. In contrast, GLP-2 action in small intestine mucosa is not dependent on IGFBP-3 and -5 alone and is associated with increased expression of the ErbB ligand epiregulin in the absence of IGFBP-3/5. These data suggest that different genetic models and physiological states may rely on the IGF-I system, ErbB ligands, and possibly other systems to mediate the intestinotrophic action of GLP-2, consistent with multiple downstream mediators.
ACKNOWLEDGMENTS

We thank Michael J. Grahn for expert technical assistance and Jens J. Holst for assistance with the plasma GLP-2 assay.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants R01-DK-42835 and T32-DK-07665.

DISCLOSURES

No conflicts of interest, financial or otherwise, are disclosed by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


