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RESEARCH ARTICLE | *Translational Human Pathophysiology*

Synergy of glucagon-like peptide-2 and epidermal growth factor coadministration on intestinal adaptation in neonatal piglets with short bowel syndrome

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Lim DW, Levesque CL, Vine DF, Muto M, Koepke JR, Nation PN, Wizzard PR, Li J, Bigam DL, Brubaker PL, Turner JM, Wales PW. Synergy of glucagon-like peptide-2 and epidermal growth factor coadministration on intestinal adaptation in neonatal piglets with short bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 312: G390–G404, 2017. First published January 19, 2017; doi: 10.1152/ajpgi.00281.2016.—Glucagon-like peptide-2 (GLP-2) and epidermal growth factor (EGF) treatment enhance intestinal adaptation. To determine whether these growth factors exert synergistic effects on intestinal growth and function, GLP-2 and EGF-containing media (EGF-cm) were administered, alone and in combination, in neonatal piglet models of short bowel syndrome (SBS). Neonatal Landrace-Large White piglets were block randomized to 75% midintestinal [jejunoileal (JI) group] or distal intestinal [jejunocolic (JC) group] resection or sham control, with 7-day infusion of saline (control), intravenous human GLP-2 (11 $nmol \cdot kg^{-1} \cdot day^{-1}$) alone, enteral EGF-cm (80 $\mu g \cdot kg^{-1} \cdot day^{-1}$) alone, or GLP-2 and EGF-cm in combination. Adaptation was assessed by intestinal length, histopathology, Üssing chamber analysis, and real-time quantitative PCR of intestinal growth factors. Combined EGF-cm and GLP-2 treatment increased intestinal length in all three surgical models ($P < 0.01$). EGF-cm alone selectively increased bowel weight per length and jejunal villus height in the JI group only. The JC group demonstrated increased intestinal weight and villus height ($P < 0.01$) when given either GLP-2 alone or in combination with EGF-cm, with no effect of EGF-cm alone. Jejunal permeability of mannitol and polyethylene glycol decreased with combination therapy in both SBS groups ($P < 0.05$). No difference was observed in fat absorption or body weight gain. IGF-1 mRNA was differentially expressed in JI vs. JC piglets with treatment. Combined treatment with GLP-2 and EGF-cm induced intestinal lengthening and decreased permeability, in addition to the trophic effects of GLP-2 alone. Our findings demonstrate the benefits of novel combination GLP-2 and EGF treatment for neonatal SBS, especially in the JC model representing most human infants with SBS.

NEW & NOTEWORTHY Glucagon-like peptide-2 (GLP-2) and epidermal growth factor (EGF) are intestinotrophic, with demonstrated benefit in both animal models and human studies of short bowel syndrome (SBS). The current research shows that over and above known trophic effects, the combination of GLP-2 and EGF synergistically lengthens the bowel in neonatal piglet models of SBS. Most notable benefit occurred with resection of the terminal ileum, the common clinical anatomy seen in neonatal SBS and associated with least de novo lengthening postsurgery.

intestinal failure; growth factors; trophic peptides; preclinical; intestinal growth

THE TREATMENT AND MANAGEMENT of infants and children with short bowel syndrome (SBS) remains an ongoing challenge to healthcare practitioners. The diseases that lead to major intestinal resection and SBS in children include congenital anomalies (e.g., intestinal atresia and gastroschisis), intestinal volvulus, and, most commonly, necrotizing enterocolitis (NEC), which premature infants are especially at risk of developing (19). Infants with SBS are dependent on parenteral nutrition (PN) therapy to sustain health and normal growth and development. However, many infants with SBS succumb to PN-associated complications, such as liver disease, infection, and sepsis, accounting for 1.4% of all deaths in children of <4 yr of age (48).

Intestinal adaptation to massive resection refers to the gradual anatomical and physiological changes that occur in the remnant intestine to restore nutrient absorptive function. Failure of the intestine to adapt results in irreversible intestinal failure, with infants requiring long-term PN therapy and, potentially, liver and/or intestinal transplantation (19). Therapies that augment intestinal adaptation are therefore desired to promote enteral function and weaning from PN and improve

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long-term health outcomes. At the present time, no such therapies are approved for treatment of children with SBS.

Glucagon-like peptide-2 (GLP-2) is a distal intestine-derived hormone that mediates the endogenous intestinal adaptive response to feeding (37). In normal rodents and rodent models of SBS, exogenous GLP-2 administration stimulates intestinal mucosal hyperplasia, upregulates the expression of nutrient transporters, and decreases intestinal permeability to fluorescein isothiocyanate-dextran (5, 8, 13, 28). In adult humans with SBS, treatment with teduglutide, a long-acting GLP-2 analog, for 24 wk reduces PN volume requirements and enhances morphological adaptation, including intestinal villus height (22, 24). Although teduglutide has been approved by the Food and Drug Administration for adult SBS, preclinical data on the efficacy of growth factors such as GLP-2 in neonates, where SBS is most frequently encountered, are limited.

The mature rodent models that have been used in preclinical SBS studies have limited clinical relevance to the human neonate because of differences in ontogeny and physiology (33). Neonates and infants have an innate gut growth potential that may be augmented with growth factor therapies, in comparison with adults, whose adaptive capacity is more limited (18). Hence we developed models of SBS using the neonatal piglet, a validated model for the human neonatal intestine with similarities in ontogeny and physiology (25, 33). Diseases that lead to SBS in human neonates frequently affect and require removal of the ileum, a site of physiological significance given that GLP-2-producing L cells are largely found in the distal intestine. Most preclinical models utilize a midintestinal resection with retained ileum, because of the feasibility of maintaining this model, but the midintestinal resection model has less translational relevance for human neonates (25, 41). Given that remnant anatomy is a significant predictor of pathophysiology and outcome in SBS, we therefore developed two neonatal piglet SBS models, one with midintestinal resection and another with distal intestinal resection (25). GLP-2 or teduglutide administration for 7 or 14 days in neonatal piglet SBS models stimulates structural adaptation, including increases in remnant villus height and crypt depth, but has limited or transient functional effects on digestive enzyme activity or nutrient transport (30, 40, 42, 44).

Because the GLP-2 receptor (GLP-2R) is not expressed by intestinal epithelial cells, the intestinotropic effects of GLP-2 are believed to be indirect (29, 51). Several downstream mediators have been implicated in the growth effects of GLP-2 including the ErbB ligand, epidermal growth factor (EGF; 15, 50). In mice, GLP-2 administration upregulates expression of ErbB ligands, an effect that is lost in GLP-2R knockout mice and in mice administered a pan-ErbB inhibitor and significantly diminished in *waved-2* mice that harbor a mutated EGF receptor (ErbB1; 50). The ErbB pathway also plays a role in the GLP-2-mediated intestinal adaptive response to refeeding, which is lost in GLP-2R knockout mice but rescued with EGF administration (1). Similar to GLP-2, exogenous EGF administration induces structural and functional adaptation in rodent models of SBS, including increased villus and crypt lengths and decreased permeability to macromolecules (35, 36). In unresected piglets, EGF administration stimulates body weight gain and reverses the changes in intestinal structure and inflammatory indexes associated with weaning (4). Furthermore, in a pilot study, enteral EGF (100 $\mu g \cdot kg^{-1} \cdot day^{-1}$) administration for 6 wk in five children with SBS improved 3-*O*-methylglucose absorption and enteral tolerance, although weight gain and intestinal permeability were not affected (39).

Given the utility of teduglutide in adult SBS and the demonstrated relationship between GLP-2 and EGF in the regulation of intestinal growth, the aim of our study was to determine the preclinical efficacy and physiological outcomes of GLP-2 and EGF administration, alone and in combination, on intestinal structure and function in two translational piglet models of neonatal SBS (47).

METHODS

Animals and surgery. Animal studies were conducted in compliance with the Canadian Council on Animal Care guidelines and approved by the Animal Policy and Welfare Committee at the University of Alberta. Neonatal Landrace-Large White cross F1 male piglets [4 ± 2 (SE) days old, 2.3 ± 0.54 kg] obtained from the University of Alberta Swine Research and Technology Center underwent general anesthesia for jugular venous catheterization and laparotomy with measurement of intestinal length, followed by assigned surgical procedure and insertion of a Stamm gastrostomy, as previously described (43). Piglets were block randomized to 75% midintestinal resection (leaving equal lengths of remnant jejunum and ileum) with jejunoileal anastomosis (JI group), 75% distal intestinal resection (including all of the ileum and proximal 5 cm of colon) with jejunocolic anastomosis (JC group), or sham control (exteriorization of the intestine, measurement of intestinal length and return to the abdominal domain without transection; Fig. 1).

Animal care. Postoperatively, piglets were secured to a swivel-tether system (Lomir Biomedical, Notre-Dame-de-l'Île-Perrot, QC, Canada) and maintained in metabolic cages lined with Plexiglas at 25°C with a 12-h light/dark cycle. For the first 3 study days, buprenorphine hydrochloride (Buprinex; Rekitt and Colman Pharmaceutical, Richmond, VA) and oral meloxicam (Metacam; Boehringer Ingelheim, Burlington, ON, Canada) were given for analgesic support, and ampicillin sodium (Sandoz, Boucherville, QC, Canada) and trimethoprim-sulfadoxine (Borgal; Merck Animal Health, Kirkland, QC, Canada) were given for prevention of venous catheter sepsis, as described (40).

Piglet activity, body weight, urine output, and fluid balance were assessed daily. Piglets with clinical evidence of dehydration, including significant diarrhea and inadequate (<400 ml/day) fluid balance, were given intravenous (IV) normal saline (0.9% sodium chlorine; Baxter, Mississauga, ON, Canada) boluses. If piglets developed fever, vomiting, or lethargy suggestive of sepsis, blood cultures were taken, and antibiotics were resumed. IV enrofloxacin (Baytril; 5 mg/kg; Bayer Animal Health, Toronto, ON, Canada) and clindamycin (3 mg/kg; Sandoz) were added if piglets did not improve after 24 and 48 h, respectively. Piglets were included in the study analysis if they improved on antibiotics and were blood culture negative.

Nutrition. Immediately after surgery, all piglets received PN via the venous catheter to meet 100% of daily caloric intake. On *postoperative day 2*, piglets commenced enteral nutrition (EN) at 20% of daily caloric intake via the gastrostomy tube. Given the diarrhea and malabsorption expected with introducing EN to piglets with SBS, the PN delivery rate was not correspondingly decreased to 80% of the total nutritional fluid rate. Both PN and EN were delivered by pressure-sensitive Alaris infusion pumps (CareFusion, San Diego, CA). As previously described, the PN and EN solutions were prepared in our laboratory on the basis of a commercially available formula (Vaminolact; Fresenius Kabi, Bad Hömburg, Germany; 40, 43). Target nutrient intakes were derived from proof-of-concept studies, as follows: 1,100 $kJ \cdot kg^{-1} \cdot day^{-1}$, 27% of energy from amino acids, 37% from carbohydrate, and 36% from fat (49).

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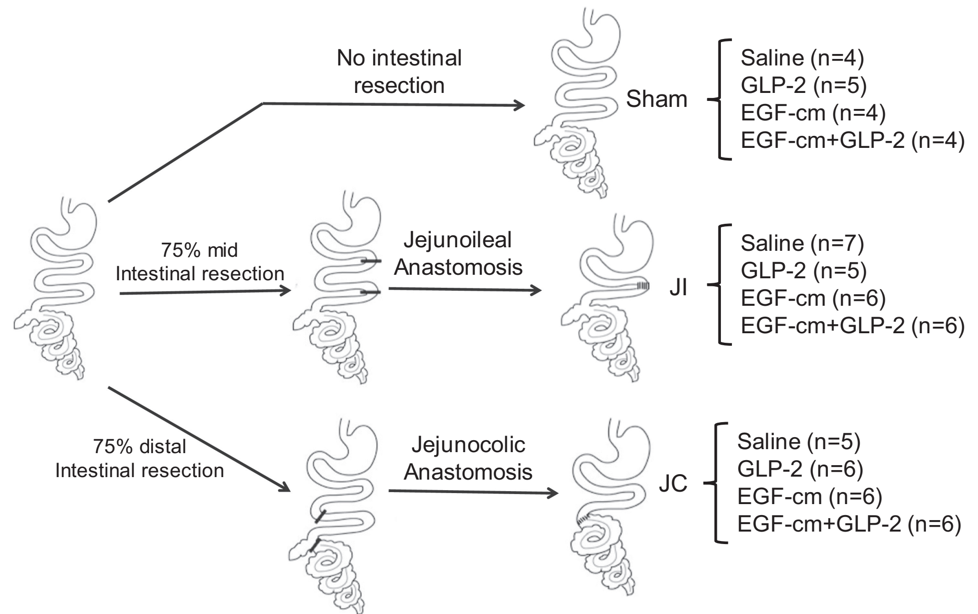

Fig. 1. Study flowchart. The number of piglets per group is illustrated. JI, jejunoileal; JC, jejunocolic; GLP-2, glucagon-like peptide-2; EGF-cm, epidermal growth factor-containing media.

Peptides. Piglets were block randomized to receive either IV saline (control), IV human GLP-2 alone, enteral EGF-containing media (EGF-cm), or GLP-2 and EGF-cm in combination (Fig. 1). Normal saline and human GLP-2 (1-33) (11 nmol·kg^{-1}·day^{-1} or 42 μg·kg^{-1}·day^{-1}; catalog no. CS9065; lot I074 with 96.83% purity; CS Bio, Menlo Park, CA) (38) were delivered continuously through the venous catheter by a syringe pump (NE-300 Just Infusion Syringe Pump; New Era Pump Systems, Farmingdale, NY) at 0.42 ml·kg^{-1}·h^{-1} beginning immediately postoperatively. EGF (80 μg·kg^{-1}·day^{-1}; 5.5 ml/kg) was administered via the gastrostomy tube beginning on *postoperative day 2*, delivered in the form of EGF-secreting *Lactococcus lactis* (*L. lactis*) culture supernatant (EGF-cm), the generation of which has been previously described and administered in studies of EGF supplementation in weanling piglets (4, 10). Briefly, the mature EGF sequence was amplified from porcine RNA and ligated into an expression vector that was transformed into *L. lactis*. Porcine EGF-expressing *L. lactis* was then fermented in M17 media (Oxoid, Basingstoke, United Kingdom) supplemented with 1% glucose and 1 μg/ml erythromycin (M17GE broth) at continuous agitation for 22 h in a Winpact fermentation system (Montreal Biotech, Montreal, QC, Canada) filled with M17GE broth at 32°C. The supernatant was isolated by centrifuging the whole fermentation product at 10,000 *g* for 15 min, followed by removal of the bacterial pellet. The purity of EGF in the supernatant was verified by using a specific antibody against EGF (anti-EGF, 1:500 dilution; Cell Sciences, Canton, MA) using Western blot, as previously described (4, 10). The concentration of EGF in the fermentation product was also quantified by Western blot analysis by comparing the intensities of bands corresponding to purified recombinant human EGF protein standards with those of the bands derived from the supernatant samples.

Enteral fat absorption. Fecal effluent was collected for 48 h, beginning on *study day 5*, into drainable ostomy appliances (Two-Piece Pouch System; Hollister, Aurora, ON, Canada). Samples were freeze dried, and fat was extracted by petroleum ether distillation for 6 h (20). Enteral fat absorption was calculated by subtracting the average fecal fat content per pig from the total amount of lipid infused and adjusted for the total duration of fecal collection (expressed as g·kg^{-1}·day^{-1}).

Tissue collection and morphology. On *study day 7*, piglets were anesthetized and underwent terminal laparotomy, where final intestinal lengths were measured as previously described (43), followed by euthanasia. The entire small intestine from ligament of Treitz to ileocecal valve or jejunocolic anastomosis was removed and emptied of fecal matter, and weight was measured. Mucosal scrapings from 20-cm segments of jejunum and ileum (in sham and JI piglets) also were weighed. A 20-cm-proximal jejunal segment was used to assess intestinal permeability and electrical activity using Üssing chamber analysis under physiological conditions (45). Cross-sectional jejunal and ileal (in JI and sham piglets) segments were preserved in 10% buffered formaldehyde for histology and immunohistochemistry, while adjacent segments were preserved in RNAlater Stabilization Solution (ThermoFisher Scientific, Waltham, MA; https://www.thermofisher.com/order/catalog/product/AM7020) or flash frozen in liquid nitrogen and stored at −80°C for gene expression analyses.

Villus height and crypt depth were measured on H&E-stained jejunal and ileal cross sections (Nikon Eclipse 80i; Nikon, Tokyo, Japan) by a certified veterinary pathologist blinded to treatment. Ten well-oriented villi and crypts were measured on 2–3 different cross sections per piglet. Mucosal crypt cell proliferation was determined using Ki-67 immunohistochemistry staining, as previously described (40), on formalin-fixed, paraffin-embedded, and sectioned (~5 μm) distal intestinal segments taken 5 cm proximal to either the ileocecal valve (in sham and JI piglets) or jejunocolic anastomosis (in JC piglets). The proportion of proliferating crypt cells in 3–5 well-oriented crypts was quantified by a blinded observer.

Intestinal alkaline phosphatase activity. Frozen distal intestinal segments (weighing 1.3 g) taken either 5 cm proximal to the ileocecal valve (in sham and JI piglets) or jejunocolic anastomosis (in JC piglets) were thawed in ice-cold homogenization buffer (50 mmol/l D-mannitol, 0.20 mmol/l phenylmethane sulfonyl fluoride, and protease inhibitors at pH 7.4) at a ratio of 20 ml homogenization buffer/g frozen tissue and homogenized using a polytron homogenizer. Tissue homogenate protein content was determined according to the Lowry procedure. Intestinal alkaline phosphatase (IAP) activity assays were performed at 37°C for 10 min, and activity was expressed as nanomoles *p*-nitrophenol liberated per milligrams protein per minute.

Intestinal permeability and electrical activity. Intestinal paracellular transport of radiolabeled mannitol and polyethylene glycol (PEG) (M_r of 180 and 380–420, respectively) was determined in jejunal segments (taken 20 cm distal to the ligament of Treitz) using a modified Üssing chamber (Harvard Apparatus, Holliston, MA) procedure, as previously described (45), by a team blinded to surgical

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Table 1. *Intestinal growth and function*

	Gene	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
		Target	
Trefoil factor 3 (tff3)	*TFF3*	GGGAGTATGTGGGCCTGTC	AGGTGCATTCTGTTTCCTGC
Antigen Ki-67	*MKI67*	TGGAGGGAAAGGCTTTTTAAGT	GCAGCCCTGCATCTGTGTAA
Homeobox protein cdx2	*CDX2*	CTAAAACAGACACGAGCCTTTCG	GCAACCAGTCGATGCATCCT
Caspase-3 (c3)	*CASP3*	TGCATATTCTACAGCACCTGGTTACT	CTGCACAAAGTGACTGGATGAAC
Intestinal alkaline phosphatase (IAP)	*ALPI*	AGCCATATACCTCCATCCTTTATG	GTACATGCGGTCGCTAATCT
Claudin-7	*CLDN7*	GGGAGACGACAAAGTGAAGAA	CATACCAGGAGCAAGCTATCAA
Claudin-15	*CLDN15*	GCGCTGCACGAACATTG	GTTGAAGGCATACCAGGAGATAG
		Housekeeping	
Beta-2-microglobulin	*B2M*	CGGAAAGCCAAATTACCTGAAC	TCTCCCCGTTTTTCAGCAAAT
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	*GAPDH*	CAGCAATGCCTCCTGTACCA	ACGATGCCGAAGTTGTCATG

List of target and housekeeping primers used to evaluate genes related to intestinal growth and function by RT SYBR Green qPCR. All primers were purchased from Integrated DNA Technologies (Coralville, IA).

group and treatment. The apparent permeability coefficients (P_{app}, cm/s) were calculated at steady state as follows: $P_{app} = dQ/dt \times [1/(A \times C_0)]$, where dQ/dt is the appearance rate of radiolabeled marker in the receiver chamber, A is the exposed surface area of intestine, and C_0 is the initial concentration in the donor chamber. The spontaneous transepithelial potential difference (PD) and the short-circuit current (I_{sc}) required to reduce the PD across the tissue were used to calculate transepithelial electrical resistance (TEER), as described (45).

Real-time PCR. Expression of genes related to tissue growth and function was assessed using total RNA isolated (UltraClean Tissue & Cells RNA Isolation kit; MoBio Laboratories, Carlsbad, CA) from distal intestinal segments, taken 5 cm proximal to the ileocecal valve in sham and JI piglets or jejunocolic anastomosis in JC piglets, and subjected to reverse transcription (High Capacity cDNA Reverse Transcription kit; Applied Biosystems, Foster City, CA). Real-time semiquantitative PCR was performed in triplicate on an Agilent Technologies Stratagene thermocycler with RT SYBR Green ROX qPCR Mastermix (QIAGEN, Germantown, MD) using primers (Integrated DNA Technologies, Coralville, IA) listed in Table 1. Relative mRNA expression was quantified using the $2^{-\Delta CT}$ method with β-2-microglobulin (B2M) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as controls, as previously validated for selection of stable porcine intestinal tract reference genes (32).

Expression of intestinal growth factor and receptor genes was assessed using total RNA isolated from whole jejunum and ileum (RNeasy Plus mini kit with Qiashredder; QIAGEN; https://www.qiagen.com/ca/shop/sample-technologies/rna/total-rna/rneasy-plus-micro-and-mini-kits/) and subjected to reverse transcription (5X All-in-One RT MasterMix; Applied Biological Materials, Richmond, BC, Canada; https://www.abmgood.com/5X-All-In-One-RT-MasterMix-G486.html). Real-time semiquantitative PCR was performed in duplicate on an Applied Biosystems thermocycler with TaqMan Gene Expression Assays (Life Technologies, Carlsbad, CA; https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/taqman-gene-expression.html) as listed in Table 2. Relative mRNA expression was quantified using the $2^{-\Delta CT}$ method using 18S rRNA as the internal control, as validated (15).

Statistical analyses. Sample size was determined on the basis of the outcomes of intestinal and crypt lengthening, with 6–8 piglets per group generating 80 and 87% power (2-sample t-test; $\alpha = 0.05$), respectively. Results are expressed as means ± SE per experimental group. Data were analyzed by two-way ANOVA followed by Bonferroni post hoc analysis. Some data were transformed to normalize variance. Significance was set at $P < 0.05$. Gross morphological data were further analyzed by multiple regression, with the adjusted R^2 value representing the variance in the dependent variable attributable to both surgical model and treatment. For histology and permeability data, with multiple repeated observations per piglet, a linear mixed-model analysis of the relationship between outcome measures and surgical anatomy and treatment was performed. As fixed effects, surgery type and treatment were entered (with the interaction term) into the model whereas intercepts for subjects were entered as random effects. Jejunal electrical activity was analyzed by Kruskal-Wallis ANOVA at the 0-min time point for each surgical model because data transformation could not satisfy parametric test assumptions. SPSS software, version 23 (IBM, Armonk, NY), was used for statistical analyses. Ki-67 immunohistochemical staining, IAP activity assays, and gene expression studies on intestinal growth and function were analyzed separately using the CONTRAST statement in a one-way ANOVA with SAS software, version 9.2 (SAS Institute, Cary, NC), comparing treatment groups with the saline control within each of the JI and JC models, as these specific outcomes were not studied in the sham group.

RESULTS

Sixty-four piglets completed the study, with the number of piglets per group presented in Fig. 1. Twelve piglets were excluded for encountered complications: three sham piglets (2 for severe dehydration and ataxia and 1 for intractable bowel obstruction), five JI piglets (2 for severe dehydration, 1 for

Table 2. *Intestinal growth factors and their receptors*

Peptide	Gene	NCBI Gene Names	NCBI mRNA Accession No.	Life Technologies TaqMan Assay ID
GLP-2R	*Glp2r*	Glucagon-like peptide-2 receptor	XM_003133457.3	AJFASAZ*
IGF-1	*Igf1*	Insulin-like growth factor 1	AF403247.1, DQ530510.1	Ss03373437m1
IGF-1R	*Igf1r*	Insulin-like growth factor 1 receptor	XM_003131575.4	AJVI4JV*
Proglucagon	*Gcg*	Glucagon	NM_001005352.2	Ss03378689u1
ErbB1 (main EGFR)	*Egfr*	Epidermal growth factor receptor	NM_214426.1	Ss03384833u1
18S	*RN18S*	18S ribosomal RNA	NM_001243304.1	Ss03377319u1

TaqMan gene expression assays using porcine-specific primers purchased from LifeTechnologies for RT-qPCR analysis. NCBI, National Center for Biotechnology Information. *Custom made using the Custom TaqMan Assay Design Tool from Life Technologies (Carlsbad, CA) available at https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/taqman-gene-expression.html.

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aspiration pneumonitis, 1 for bowel obstruction and sepsis, and 1 sudden death of unknown etiology), and four JC piglets (2 for severe dehydration, 1 for iatrogenic intravenous EGF administration, and 1 for intraperitoneal gastrostomy tube displacement). Baseline characteristics are presented in Table 3. Piglets gained 1.1–1.5 kg in body weight during the study period, with no difference between groups ($P = 0.38$; Fig. 2*A*). There was no difference between groups regarding the amount of PN (over 80% of expected, $P = 0.6$) and EN (over 84% of expected, $P = 0.2$) delivered.

Gross morphology. There was no significant interaction between surgery and treatment on the change in remnant intestinal length between groups [$F(6,50) = 1.54$, $P = 0.2$]; therefore both main effects were analyzed separately. Remnant anatomy influenced the change in intestinal length [$F(2,50) = 11.4$, $P < 0.001$], with the JI model demonstrating a 17.4% [95% confidence interval (CI): 8.2–26.7] and 11.6% (95% CI: 1.5–21.8) greater change in length than the JC and sham groups, respectively (Fig. 2*B*). Although no effects of GLP-2 alone or EGF-cm were detected, combination therapy resulted in a 15.4% (95% CI: 2.4–28.4) and 13.2% (95% CI: 0.8–25.7) greater change in length over EGF-cm alone and saline control, respectively, regardless of anatomy [$F(3,50) = 4.52$, $P < 0.01$; Fig. 2*B*]. Surgical anatomy and treatment independently predicted 33.6% of the variance in the change in intestinal length ($P < 0.001$).

There was a significant interaction between remnant anatomy and treatment on bowel weight per length [$F(6,50) = 3.6$, $P < 0.01$]. Although no treatment differences were observed in the sham piglets, EGF-cm increased bowel weight per length by 29.5% compared with saline ($P < 0.01$) in JI piglets and GLP-2 increased bowel weight per length by 26.8% compared with EGF-cm alone ($P < 0.05$) in JC piglets (Fig. 2*C*). In addition, in piglets given EGF-cm alone, bowel weight per length was 58.5 and 105.2% greater in the JI group compared with the JC ($P < 0.01$) and sham groups ($P < 0.01$), respectively. In piglets given combination therapy, bowel weight per length was 30.0% greater in the JC group ($P = 0.01$) and 44.3% greater in the JI group ($P < 0.01$) compared with sham. Surgical anatomy and treatment independently predicted 59.4% of the variance in intestinal weight per length ($P < 0.001$).

There was a significant interaction between remnant anatomy and treatment on normalized intestinal weight [$F(6,50) = 2.78$, $P = 0.02$]. In the sham group, GLP-2 alone and combination therapy increased normalized intestinal weight over saline control ($P < 0.01$) while in the JC group, GLP-2 alone and in combination with EGF-cm increased normalized intestinal weight vs. EGF-cm alone and saline control ($P < 0.01$); no treatment differences were observed in the JI model (Fig. 2*D*). Furthermore, for each treatment, all pairwise comparisons between the three surgical anatomies differed significantly ($P < 0.01$). Surgical anatomy and treatment independently predicted 93.6% of the variance in normalized intestinal weight ($P < 0.001$).

There was no significant interaction between surgery and treatment on jejunal mucosal weight ($P = 0.9$); therefore main effects were analyzed separately. Regarding surgical anatomy, both JI and JC anatomy demonstrated increased jejunal mucosal weight compared with the sham group [$F(2,50) = 31.7$, $P < 0.01$; Fig. 2*E*]. Regarding treatment, GLP-2, alone or in combination with EGF-cm, increased jejunal mucosal weight over both EGF-cm alone and saline control [$F(3,50) = 14.9$, $P < 0.01$], with no effect of EGF-cm alone and no difference between GLP-2 alone and combination therapy.

Histopathology. There was a significant interaction between surgical anatomy and treatment on remnant jejunal villus height [$F(6,50) = 2.3$, $P < 0.05$; Fig. 3*A*]. In the sham group, combination therapy demonstrated a 59.9% greater increase in jejunal villus height vs. saline control ($P < 0.01$), while EGF-cm alone and GLP-2 alone had no effect. In the JI group, GLP-2 and combination therapy increased jejunal villus height by 31.0% ($P < 0.05$) and 34.1% ($P < 0.05$), respectively, over saline control. In the JC group, GLP-2 alone increased jejunal villus height by 56.5, 31.3, and 60.7% over saline, combination therapy, and EGF-cm alone, respectively ($P < 0.05$). Regarding differences based on anatomy, JC GLP-2 pigs demonstrated 30.8% ($P < 0.05$) greater jejunal villus height compared with the sham GLP-2 group, and JI EGF-cm pigs had 35.2% ($P < 0.05$) greater jejunal villus height than the JC EGF-cm group.

Table 3. *Baseline data*

Groups	Initial Age, Days Old	Initial Weight, kg	Presurgery Intestinal Length, cm	Postsurgery Intestinal Length, cm
Sham				
Saline	3.8 ± 0.3	2.4 ± 0.06	626.6 ± 34.3	626.6 ± 34.3
GLP-2	3.8 ± 0.5	2.3 ± 0.1	598.6 ± 39.6	598.6 ± 39.6
EGF-cm	3.7 ± 0.9	2.0 ± 0.2	663.9 ± 27.4	663.9 ± 27.4
EGF+GLP-2	3.5 ± 0.5	2.3 ± 0.2	672.4 ± 60.7	672.4 ± 60.7
JI				
Saline	4.6 ± 0.4	2.3 ± 0.07	604.3 ± 17.3	151.3 ± 4.3
GLP-2	3.8 ± 0.2	2.3 ± 0.09	606.3 ± 18.0	151.2 ± 4.8
EGF-cm	4.0 ± 0.4	2.3 ± 0.07	593.6 ± 25.4	148.2 ± 6.3
EGF+GLP-2	3.5 ± 0.3	2.3 ± 0.04	592.6 ± 21.2	148.3 ± 5.4
JC				
Saline	3.8 ± 0.6	2.3 ± 0.09	582.1 ± 28.5	145.4 ± 7.2
GLP-2	3.3 ± 0.3	2.2 ± 0.05	625.1 ± 22.4	156.3 ± 5.7
EGF-cm	4.8 ± 0.6	2.5 ± 0.08	603.9 ± 20.0	151.0 ± 5.0
EGF+GLP-2	4.3 ± 0.3	2.4 ± 0.06	568.4 ± 8.9	142.0 ± 2.3
P value	0.4	0.8	0.4	0.5 (between JI and JC)

Baseline characteristics of piglets treated with GLP-2, EGF-cm, and combined treatment in sham, JI, and JC models of SBS. Data are means ± SE, analyzed by two-way ANOVA.

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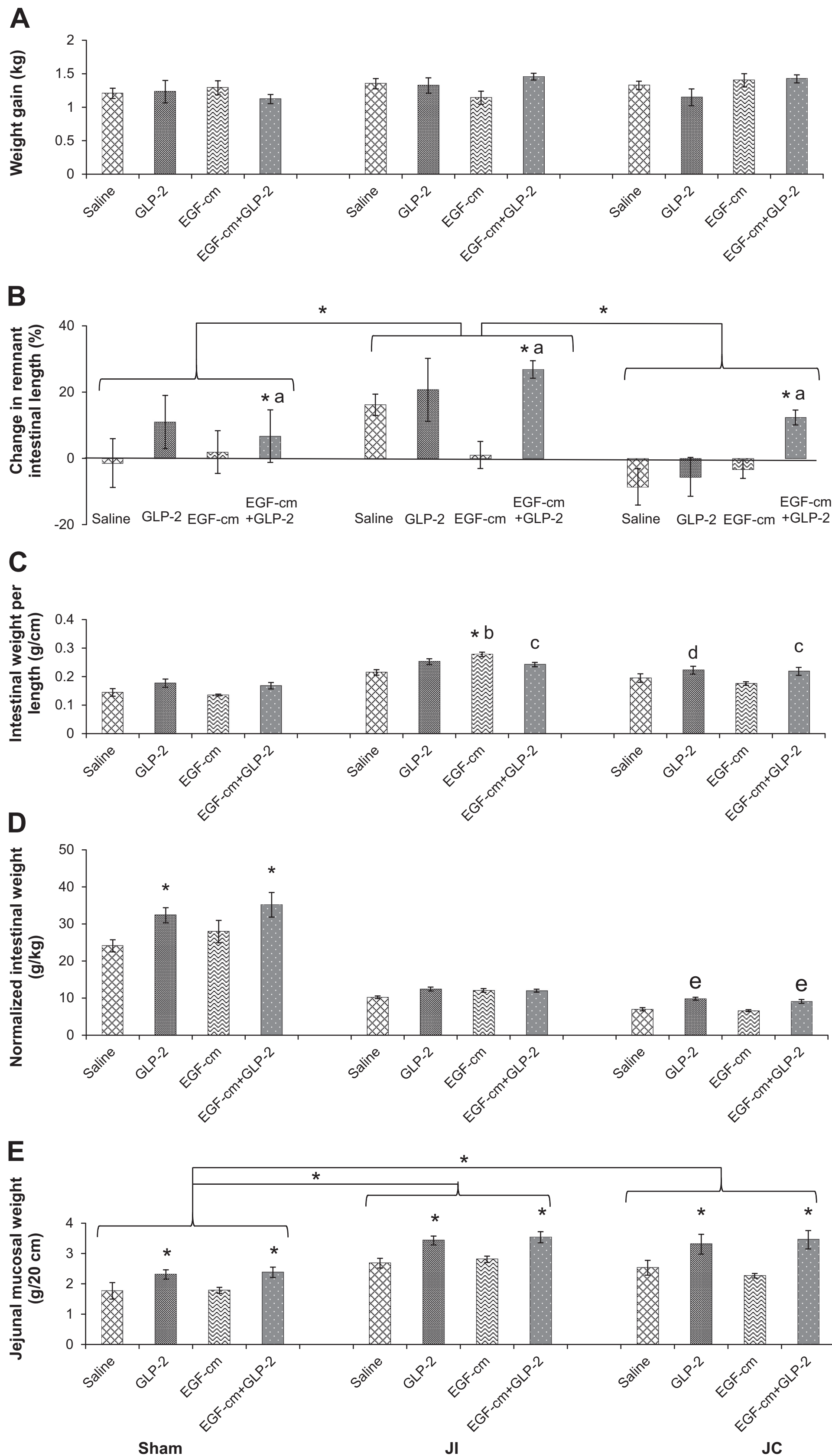

Fig. 2. Weight gain and gross morphology. Body weight gain (*A*), change in remnant intestinal length (*B*), bowel weight per length (*C*), normalized intestinal weight (*D*), and jejunal mucosal weight (*E*) following GLP-2, EGF-cm, and combined treatment in sham, JI, and JC piglet SBS models. Means ± SE; two-way ANOVA. Note that for normalized intestinal weight, all pairwise comparisons between the three surgical anatomies differed significantly (sham > JI > JC) for each treatment (not depicted). *$P < 0.05$ (as denoted or vs. saline), $^{a}P < 0.05$ vs. EGF-cm, $^{b}P < 0.01$ vs. sham EGF-cm and JC EGF-cm, $^{c}P < 0.01$ vs. sham EGF+GLP-2, $^{d}P < 0.05$ vs. JC EGF-cm, $^{e}P < 0.05$ vs. JC saline and JC EGF-cm.

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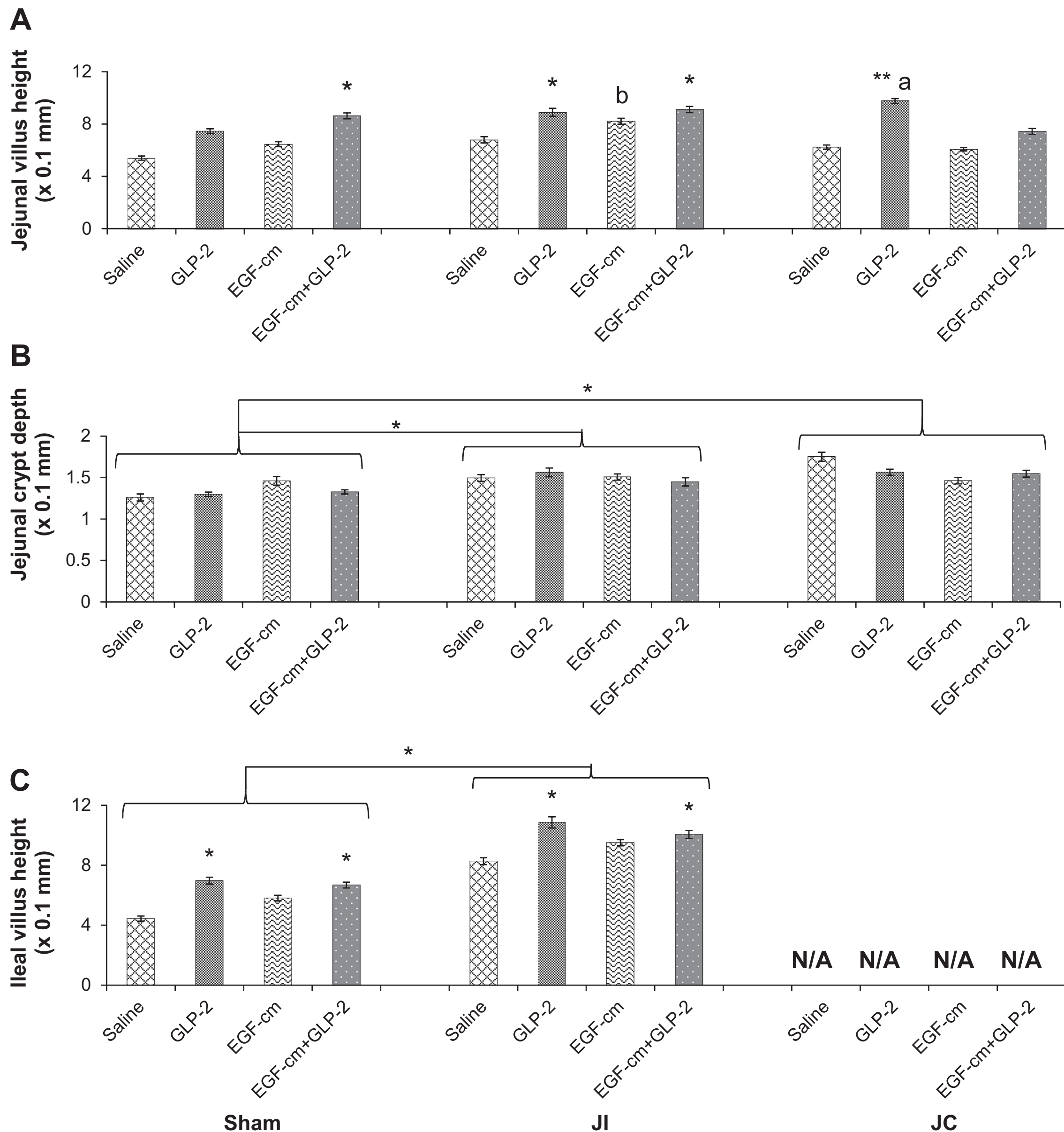

Fig. 3. Histopathology. Jejunal villus height (*A*) and crypt depth (*B*) and ileal villus height (*C*) following GLP-2, EGF-cm, and combined treatment in sham, JI, and JC piglet SBS models. Means ± SE; linear mixed-model analysis. N/A, not applicable. $^{*}P < 0.05$ vs. saline or sham, $^{**}P < 0.05$ vs. JC saline, JC GLP-2, and JC EGF+GLP-2, $^{a}P < 0.05$ vs. sham GLP-2, $^{b}P < 0.05$ vs. JC EGF-cm.

Jejunal crypt depth differed as a function of surgical anatomy but not by treatment ($P < 0.01$). Thus the JI and JC groups demonstrated 19% ($P < 0.01$) and 13.1% ($P < 0.05$) greater jejunal crypt depth, respectively, than the sham group (Fig. 3*B*).

In sham and JI piglets, there was no interaction between surgical anatomy and treatment on remnant ileal villus height ($P = 0.97$); therefore main effects were analyzed separately. The JI anatomy demonstrated 58.1% ($P < 0.001$) greater ileal villus height than the sham group. GLP-2 alone and combination therapy increased ileal villus height in these animals by 29.6% ($P < 0.05$) and 26.5% ($P < 0.05$) over saline control, respectively (Fig. 3*C*). Ileal crypt depth did not differ between groups as a function of either remnant anatomy or treatment (not shown).

Intestinal permeability and electrical activity. In the sham group, EGF-cm alone increased jejunal mucosal-to-serosal (M-to-S) permeability of mannitol compared with saline control by 5.52-fold ($P < 0.05$), while GLP-2 had no effect. In contrast, in both JI and JC models, combination therapy decreased M-to-S permeability to mannitol by >70% ($P < 0.05$ and $P = 0.01$, respectively), compared with saline, while monotherapy with either GLP-2 alone or EGF-cm alone had no effect on permeability (Fig. 4*A*). A similar pattern was observed with the jejunal serosal-to-mucosal (S-to-M) permeability of mannitol (not shown).

Results for the jejunal permeability of PEG were consistent with those found for mannitol. Hence EGF-cm alone increased M-to-S permeability ~6-fold ($P < 0.05$) compared with saline in sham piglets, whereas combination therapy in the JI and JC

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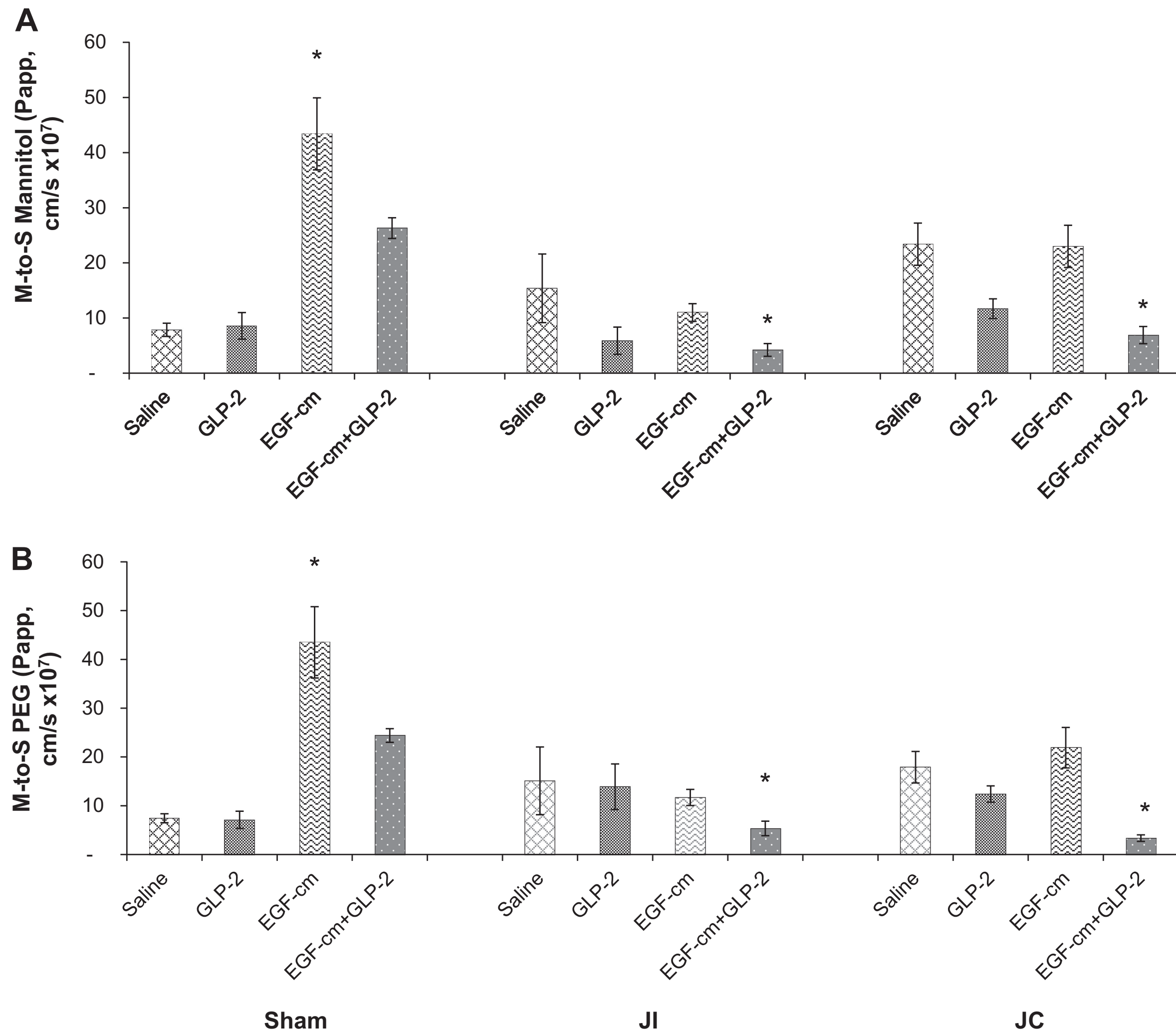

Fig. 4. Jejunal permeability. Jejunal mucosal-to-serosal (M-to-S) permeability of mannitol (*A*) and PEG (*B*) in sham, JI, and JC models of SBS. Papp, apparent permeability. Means ± SE; linear mixed-model analysis. $^*P < 0.05$ vs. saline.

groups decreased M-to-S PEG permeability by ~60% ($P < 0.05$) and 80% ($P = 0.01$), respectively, vs. saline (Fig. 4*B*); GLP-2 alone and EGF-cm alone had no effect. In piglets receiving combination therapy, the JI and JC groups also demonstrated decreased M-to-S permeability of PEG compared with sham animals by >75% ($P < 0.01$) and 85% ($P < 0.05$), respectively. A similar pattern was observed for the jejunal S-to-M permeability to PEG (not shown).

All intestinal segments established and maintained a transepithelial potential difference (PD) >2 mV, indicating that intestinal integrity was maintained throughout the Üssing experiment (Fig. 5*A*). There was no treatment-related difference in PD at $t = 0$ between the sham and JC groups. In the JI group, combination therapy increased the PD across the intestine 2.6-fold compared with EGF-cm alone at $t = 0$ ($P < 0.01$) and increased over time (Fig. 5*A*). I_{sc} is a summation of all ionic currents across the epithelium and a measure of active transport processes (11). In the sham group, I_{sc} at $t = 0$ was increased 3.1- and 17.1-fold in response to EGF-cm alone compared with GLP-2 alone ($P < 0.001$) and saline ($P < 0.001$), respectively, and maintained over time (Fig. 5*B*). There were no treatment-related differences in the JI model. In the JC model, combination therapy decreased I_{sc} 2.8-fold at $t = 0$ compared with GLP-2 alone ($P < 0.01$).

TEER is a measure of tissue integrity and barrier function (11). In the sham model, EGF-cm alone and combination therapy decreased TEER 9-fold ($P < 0.001$) and 7.6-fold ($P = 0.001$), respectively, compared with saline, while GLP-2 alone had no effect. In the JI model, there was no effect of treatment at $t = 0$, but over time there was a gradual increase in TEER with combination therapy, with no effect of either GLP-2 alone or EGF-cm alone. In the JC model, combination therapy increased TEER at $t = 0$ by 9.8-fold ($P < 0.01$) compared with GLP-2 and over time, which likely reflects the increased PD (Fig. 5*C*).

Fat absorption. Total fat absorption was affected by the SBS resection model, but not by treatment. Thus the JC anatomy demonstrated 19.7 and 26.0% lower fat absorption compared with both the JI and sham groups, respectively ($P < 0.01$, Fig. 6).

Intestinal gene expression, IAP activity, and proliferation. Administration of GLP-2 and EGF-cm, alone or in combination, increased trefoil factor 3 (tff3) expression by 150% over saline ($P < 0.01$) in the JI group (Fig. 7*A*), with a similar trend

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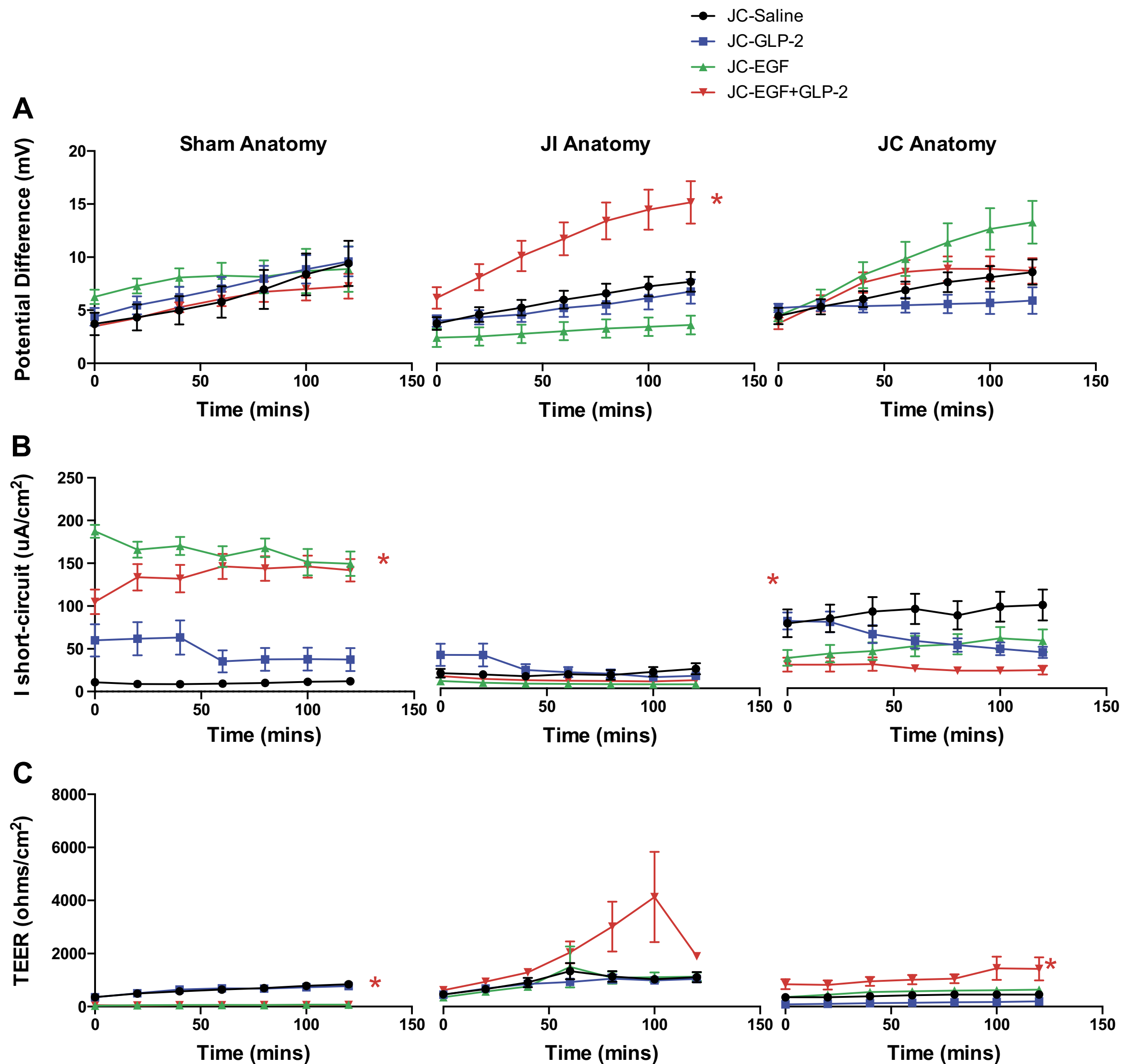

Fig. 5. Electrical parameters of jejunum. Potential difference (PD; *A*), short-circuit current (I_{sc}; *B*), and transepithelial electrical resistance (TEER; *C*) in GLP-2, EGF-cm, and combination treatment groups in sham, JI, and JC models of SBS. Means ± SE; Kruskal-Wallis ANOVA at $t = 0$. **P* < 0.05.

in the JC group (Fig. 7*B*). Although treatment did not affect Ki-67 expression in either JI or JC piglets, Ki-67 staining of distal intestine in JI piglets demonstrated a trend toward increased Ki-67-positive staining cells with combination therapy vs. saline control ($P = 0.08$; Fig. 7*C*). Treatment did not affect relative cdx2, caspase-3 (c3), or IAP expression in JI or JC piglets (Fig. 7, *A* and *B*). However, there was a 50% decrease in IAP activity in the JI group for all treatments compared with saline control ($P < 0.01$; Fig. 7*D*). In the JI piglets, GLP-2 alone and EGF-cm alone increased claudin-7 expression by 40% ($P < 0.05$) while GLP-2 alone and combination therapy increased claudin-15 expression by 150% ($P < 0.05$) vs. saline (Fig. 7*A*). A similar trend in claudin-15 expression was seen with combination therapy vs. saline in JC piglets (Fig. 7*B*).

Growth factor and receptor gene expression. Jejunal *Glp2r* expression differed as a function of surgery ($P < 0.01$) but not treatment, such that the JI and JC anatomies demonstrated a 48% ($P < 0.01$) and 43% ($P < 0.05$) decrease in jejunal *Glp2r* expression vs. sham, respectively (Fig. 8*A*). Ileal *Glp2r* expression also differed as a function of surgery ($P < 0.01$) but not treatment, with 22% greater ileal *Glp2r* expression in the JI vs. sham animals (Fig. 8*B*).

There was a significant interaction between surgery and treatment on jejunal *Igf1* expression ($P < 0.05$). There was no difference in *Igf1* expression compared with saline in all surgical models. However, in the JC anatomy, *Igf1* expression was 76% greater with EGF-cm compared with GLP-2 treatment (Fig. 8*C*). Furthermore, differential jejunal *Igf1* expression between anatomies was evident in the JI GLP-2 group demonstrating 76% greater expression compared with the JC GLP-2 group ($P < 0.01$). There was no difference in ileal *Igf1* or jejunal *Igf1r* expression (not shown). However, ileal *Igf1r* expression differed as a function of surgical anatomy ($P < 0.02$) but not treatment, with the JI group demonstrating 50% increased expression over sham animals (Fig. 8*D*). There was no difference in jejunal or ileal *Gcg* expression or jejunal

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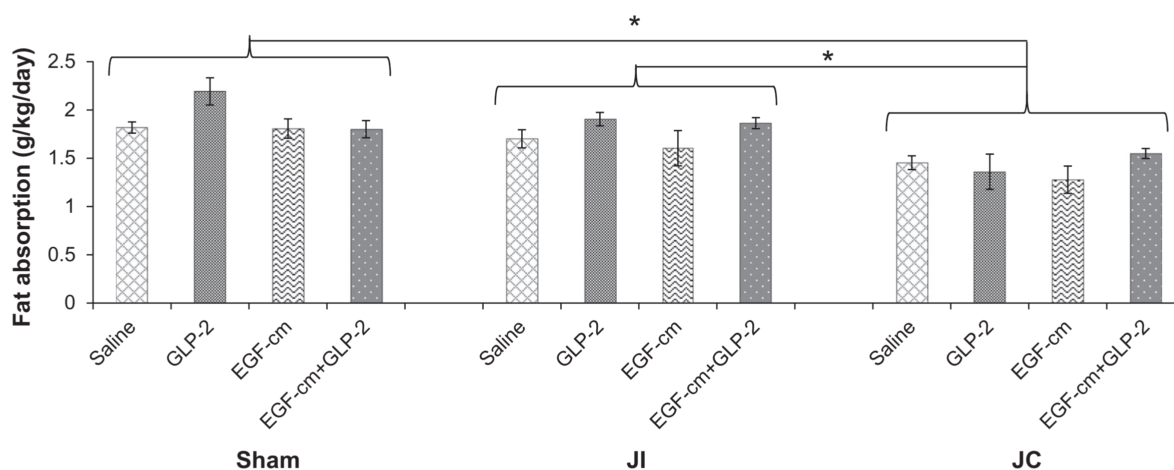

Fig. 6. Fat absorption. Total fat absorption in sham, JI, and JC models of SBS. Means ± SE; two-way ANOVA. *$P < 0.05$.

expression of ErbB1, the main EGF receptor (*Egfr*, not shown). Treatment with EGF-cm alone decreased ileal *Egfr* expression compared with saline control in JI piglets ($P < 0.01$; Fig. 8*E*).

DISCUSSION

In the present study, we investigated the preclinical physiological efficacy of novel administration of combined GLP-2 and EGF-cm therapy compared with each treatment alone and saline in the setting of neonatal SBS. We used two relevant translational animal models, the JI and JC anatomies, with the latter lacking ileum and representing most human infants with SBS and physiological biometrics as the major outcome of these preclinical studies. Preclinical efficacy studies using the piglet as an established model of the neonatal intestine are timely, as clinical trials with GLP-2 analogs in pediatric SBS are currently in progress. Furthermore, emerging mechanistic work has identified common downstream pathways between GLP-2R and ErbB1 signaling, suggesting synergy between GLP-2 and EGF, a natural ErbB1 ligand, in stimulating intestinal growth (1, 50).

Combination treatment with GLP-2 and EGF-cm was associated with tropic intestinal effects and structural adaptation in both the JI and JC models. In parameters such as normalized intestinal weight, mucosal weight, and villus height, there was no difference between combination therapy and GLP-2 alone, but both treatments were superior to saline control and/or EGF-cm alone. This suggests that GLP-2 is the main factor stimulating increases in these parameters, consistent with prior studies demonstrating that GLP-2 administration in rodents and piglets expands the intestinal mucosal epithelium (13, 28, 30, 37, 40, 42, 44). However, EGF-cm alone appeared to be selectively beneficial in increasing bowel weight per length and jejunal villus height in the JI group only. This finding may relate to the fact that the JI anatomy retains GLP-2-producing L cells that have been shown to increase their GLP-2 production postresection to mediate intestinal adaptation (21, 23). We observed evidence of this intrinsic adaptation in our JI model, with intestinal lengthening and increased intestinal weight and ileal villus height. Thus, although additional exogenous GLP-2 administration did not augment structural adaptation in the JI model, administration of EGF-cm alone may have acted synergistically with endogenous circulating GLP-2. In contrast, the JC model, with markedly reduced numbers of L cells and thus of endogenous GLP-2, did not exhibit intrinsic adaptation and demonstrated tropic effects only when given GLP-2, either alone or in combination with EGF-cm.

Aside from mucosal expansion, combined administration of GLP-2 and EGF-cm increased intestinal length in all three surgical models. The length of remnant intestine is a predictor of clinical outcomes in SBS (19), but preclinical studies to date have been inconsistent in demonstrating a GLP-2 effect on intestinal lengthening. Martin et al. previously suggested that the EGFR plays a role in intestinal smooth muscle adaptation following resection, as the EGFR mutant *waved-2* mice fail to demonstrate normal smooth muscle proliferation and intestinal lengthening as seen in control mice (27). Interestingly, our findings show that only coadministration of EGF-cm and GLP-2 increased intestinal length, suggesting a requirement for both factors.

In this study, we measured the jejunal permeability of mannitol (a small sugar alcohol molecule) and PEG (a larger-molecular weight molecule representative of the size of bacterial toxin or peptide), which use paracellular pathways of transport. Studies in healthy rodents have previously demonstrated that GLP-2 decreases permeability through an IGF-1R-dependent mechanism (12). However, our data showed no difference in intestinal permeability following GLP-2 treatment alone. In contrast, we observed that combination therapy with GLP-2 and EGF-cm decreased jejunal permeability of both mannitol and PEG in JI and JC models compared with the sham and saline treatments, suggesting a benefit in both resection models. These findings may be associated with the tropic effects observed in jejunal mucosal weight and villus height, reflecting an increase in overall epithelial biomass and thickness of the intestinal wall, which may have resulted in a decrease in permeability. This hypothesis is further suggested by the observed differences in TEER, with combination therapy leading to the greatest intestinal transepithelial resistance in the JI and JC models but not the sham. Modulation of tight junctional complex expression may underlie the improvements in intestinal permeability. Claudin-7 and claudin-15 are expressed all along the mammalian intestine (26) but have contrasting roles, with claudin-7 weakening the intestinal barrier (7) and claudin-15 maintaining barrier function (46). We ob-

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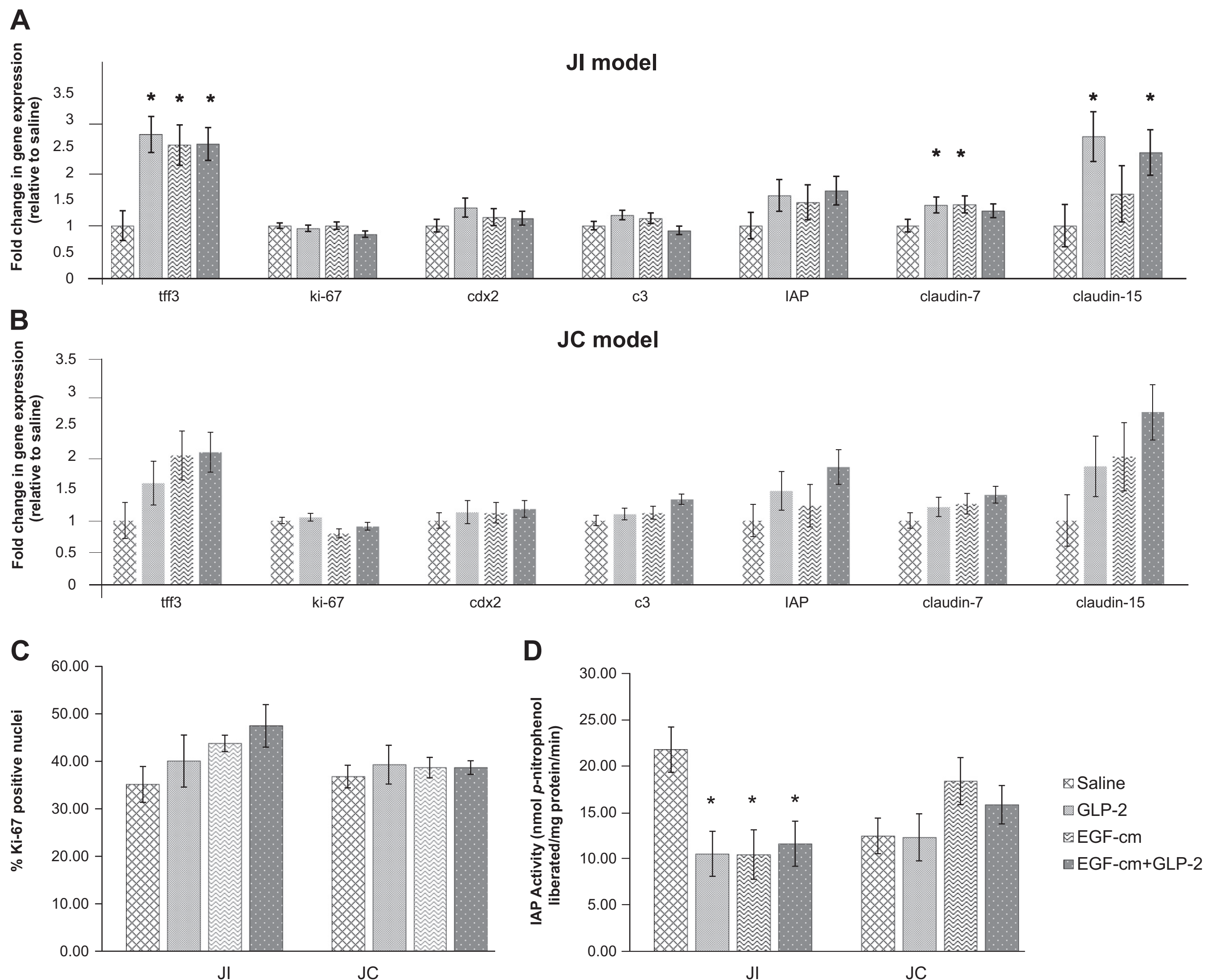

Fig. 7. Intestinal growth and function. Distal intestinal expression of genes involved in intestinal repair [trefoil factor 3 (tff3)] and cell proliferation (Ki-67), differentiation (cdx2), apoptosis [caspase-3 (c3)], function [intestinal alkaline phosphatase (IAP)], and permeability (claudin-7 and claudin-15) in JI (*A*) and JC (*B*) piglet SBS models, and Ki-67 immunohistochemistry (*C*) and IAP activity (*D*) in neonatal piglet SBS models following GLP-2, EGF-cm, and combination treatment. Means ± SE. *$P < 0.05$ vs. saline (1-way ANOVA and post hoc CONTRAST statement).

served that both combination therapy and GLP-2 monotherapy increased claudin-15 expression, while GLP-2 alone or EGF-cm alone increased claudin-7 mRNA expression in the JI model, while in the JC model, combination therapy increased claudin-15 expression, although not significantly, thus suggesting that these effects may vary according to the SBS resection model. In addition, we observed an increase in mannitol and PEG permeability and decrease in TEER in response to EGF-cm alone in the sham group but not the JI or JC resection groups. The sham group also had increased permeability with combination treatment, which was likely to be due to the EGF effect rather than the combination with GLP-2. The mechanisms underlying these effects remain speculative, but EGFR signaling has been implicated in the setting of oxidant-induced intestinal hyperpermeability relevant to inflammatory bowel disease (17), which may be translatable to human neonates with SBS that demonstrate impaired intestinal barrier function.

Although we observed expansion of the mucosal epithelium and intestinal lengthening with combination therapy, we did not observe parallel improvement in nutrient absorptive functional adaptation, except for an upregulation in tff3 expression with GLP-2 and EGF-cm in the JI and JC models. Collectively, the lack of functional findings suggests that while combination therapy may augment the intestinal absorptive surface area, functional adaptation related to nutrient absorption and active transport processes may remain unaffected or immature because of proliferating epithelial mucosal cells that are not yet fully differentiated. The nutrient absorptive effects of GLP-2 administration in healthy animals and SBS animal models are inconsistent and appear to depend on gestational age (31), with some authors reporting increased nutrient transporter expression (9, 34), digestive enzyme activity (6, 44), and relative macronutrient absorption (44) while others suggest that such changes are acute and transient (30). In our model, it is conceivable that improved intestinal function resulting from growth factor treatment occurs mainly because of increased absorptive surface area. If we had not pair fed these piglets and allowed their enteral intake to increase as per tolerance, we

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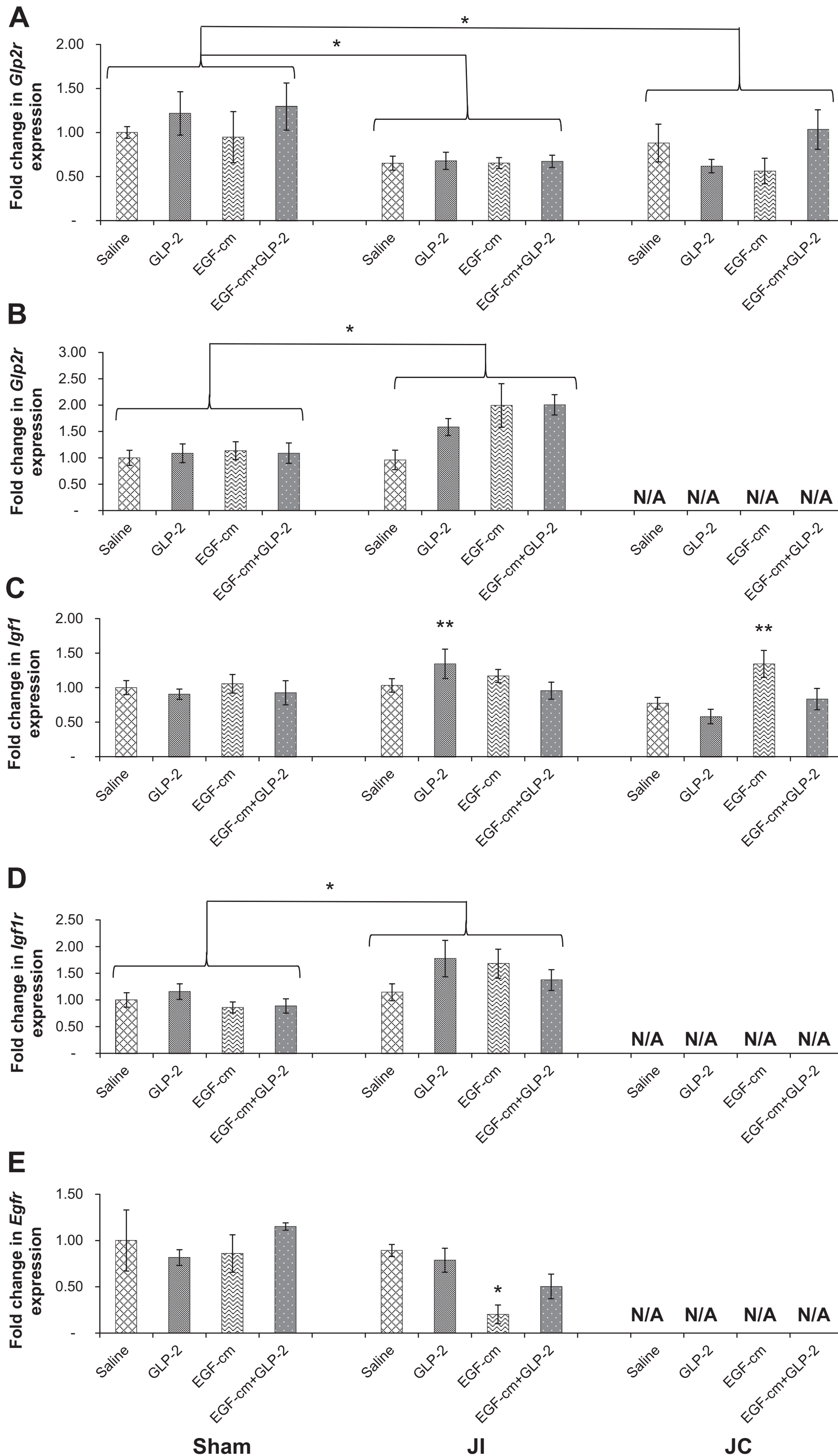

Fig. 8. Intestinal gene expression of growth factors and their receptors. Gene expression of jejunal (*A*) and ileal (*B*) *Glp2r*, jejunal *Igf1* (*C*), ileal *Igf1r* (*D*), and ileal *Egfr* (*E*) following GLP-2, EGF-cm, and combined treatment in piglet SBS resection models. Means ± SE; two-way ANOVA. N/A, not applicable. $*P < 0.05$ vs. sham or saline, $**P < 0.05$ vs. JC GLP-2.

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may have then appreciated treatment-related differences in intestinal function, as we have previously shown that JC piglets, following 14 days of GLP-2 treatment, tolerate more EN and wean off PN sooner compared with control (40).

Although the focus of our study was on preclinical physiological efficacy, we did attempt to address mechanistic pathways related to GLP-2 and EGF receptors and growth factors such as IGF. However, we recognize that these findings may not necessarily translate to the physiological outcomes reported. For example, we observed decreased jejunal *Glp2r* expression with either JI or JC resection compared with sham but no effect of treatment. In JI piglets, we did observe increased ileal *Glp2r* and *Igf1r* expression compared with sham, potentially an adaptive response to resection. Expression of jejunal *Igf1* differed according to surgical model: expression increased in JC piglets given EGF-cm alone compared with GLP-2 while JI piglets given GLP-2 alone had significantly greater *Igf1* expression compared with JC piglets given GLP-2 alone. This finding suggests that GLP-2 may differentially upregulate *Igf1* expression to a greater extent in the JI anatomy compared with the JC anatomy. Interestingly, the rodent studies linking GLP-2 signaling and IGF-1 were performed in unresected rodents that still had ileum (16). Finally, our finding of decreased ileal *Egfr* expression in JI piglets given EGF-cm alone compared with saline may represent a ligand-activated negative feedback loop, a hypothesis that requires further study. These results support the complexity of gut adaptation and growth pathways that require further investigation. We acknowledge that for many intestinotrophic effects of growth factors, especially GLP-2, the mechanisms remain incompletely understood (14).

It is possible that greater treatment-related differences in the functional outcomes of gene expression, fat absorption, and/or weight gain might be realized with a longer study period. However, a major limitation of study extension is significantly increased mortality in SBS piglets beyond 2 wk because of line infection and sepsis, as also often found in infants with SBS. Although no differences were observed in fat absorption, there may be differences in amino acid or glucose transport that were not measured in this study. More importantly, we acknowledge that in administering EGF in the form of *L. lactis*-expressed EGF, we provided supernatant that may contain nutrients that may affect enteral intake and intestinal adaptation or act synergistically with GLP-2. The medium used for *L. lactis* culture is M17 (Oxoid) and contains tryptone (5 g/l), soya peptone (5 g/l), beef extract (5 g/l), and yeast extract (2.5 g/l) (10). Although most of these nutrients were consumed by *L. lactis* during the fermentation process, there may be some trace amounts of them still present in the supernatant administered to piglets in the study. Furthermore, by-products of *L.* lactis metabolism such as short-chain fatty acids, which are known to augment intestinal adaptation, may also have been present in the EGF supernatant (3). However, while previous data do demonstrate some proliferation in mice given the supernatant containing the empty *L. lactis* vector, this level of proliferation is clearly nowhere near the level of proliferation seen in mice given supernatant containing the *L. lactis* vector with EGF. For most parameters measured, there was indeed no difference between supernatant containing the empty *L. lactis* vector and phosphate-buffered saline control. On the basis of this finding, we collectively felt that nutrients from bacterial metabolism are playing a minor role, if any, in the observed adaptive responses. We also felt that the supernatant from the empty *L. lactis* vector did not contain enough nutrients to stimulate adaptation, as Burrin et al. (6) previously demonstrated that the minimal enteral amounts necessary to stimulate jejunal and ileal adaptation in unresected neonatal piglets were 40 and 60% of caloric intake, respectively. For this reason, and because of funding limitations, we did not include a control group of piglets administered supernatant containing the empty *L. lactis* vector. The lack of an intestinal structural benefit with EGF-cm alone may relate to the fact that the EGFR is restricted to the basolateral epithelial membrane and is thus normally exposed only in the setting of epithelial injury (2). Effects of EGF may therefore be better appreciated in using a preclinical model that combines both epithelial injury, such as NEC, and resection, but such animal models carry significant morbidity and mortality. Finally, to better understand our findings in intestinal permeability and TEER, further analysis of cell structural proteins and lipids and measurement of receptor/transporter proteins such as EGFR signaling pathways, tight junction proteins, and nutrient (amino acid and glucose) transporters could be valuable but were beyond the scope of the present study.

In summary, our study demonstrates beneficial effects of combined administration of GLP-2 and EGF-containing media on intestinal morphology, histology, and barrier function in a preclinical model of neonatal SBS. Given these findings, this novel treatment combination has the potential to improve clinical outcomes by improving barrier function (thus decreasing risk for bacterial translocation and infection) and improving nutrient absorption and weaning of PN. Importantly, these growth and permeability effects were observed in the piglet SBS model lacking ileum, representing the remnant anatomy most seen in human neonates, thus illustrating the potential clinical utility of combined GLP-2 and EGF treatment for human infants with SBS.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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AUTHOR CONTRIBUTIONS

D.W.L., C.L.L., D.F.V., J.L., D.L.B., P.L.B., J.M.T., and P.W.W. conceived and designed research; D.W.L., C.L.L., D.F.V., M.M., J.R.K., P.N.N., P.R.W., and P.W.W. performed experiments; D.W.L., C.L.L., D.F.V., M.M., J.R.K., P.N.N., P.R.W., P.L.B., J.M.T., and P.W.W. analyzed data; D.W.L., C.L.L., D.F.V., J.R.K., J.L., D.L.B., P.L.B., J.M.T., and P.W.W. interpreted results of experiments; D.W.L., C.L.L., and J.R.K. prepared figures; D.W.L., C.L.L., and D.F.V. drafted manuscript; D.W.L., C.L.L., D.F.V., M.M., J.R.K., P.N.N., P.R.W., J.L., D.L.B., P.L.B., J.M.T., and P.W.W. edited and revised manuscript; D.W.L., C.L.L., D.F.V., M.M., J.R.K., P.N.N., P.R.W., J.L., D.L.B., P.L.B., J.M.T., and P.W.W. approved final version of manuscript.

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