Trefoil peptide expression and goblet cell number in rat intestine: effects of KGF and fasting-refeeding


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Submitted 17 July 2002; accepted in final form 3 October 2002

Fernández-Estivariz, Concepción, Li H. Gu, Liang Gu, Carolyn R. Jonas, Timothy M. Wallace, Robert R. Pascal, Kathryn L. Devaney, Catherine L. Farrell, Dean P. Jones, Daniel K. Podolsky, and Thomas R. Ziegler. Trefoil peptide expression and goblet cell number in rat intestine: effects of KGF and fasting-refeeding. Am J Physiol Regul Integr Comp Physiol 284: R564–R573, 2003. First published October 10, 2002; 10.1152/ajpregu.00428.2002.—The trefoil factor family peptides TFF1, TFF2, and TFF3 are important for gut mucosal protection and restitution. Keratinocyte growth factor (KGF) stimulates proliferation and differentiation of epithelial cells with potent effects on goblet cells. To investigate interactions between food intake and KGF, rats were fed ad libitum (control), fasted for 72 h, or fasted for 72 h and then refed for 72 h with or without KGF (3 mg·kg⁻¹·day⁻¹). With fasting, goblet cell number in duodenum increased, TFF3 mRNA in duodenum and jejunum decreased, and TFF3 protein did not change or increased. KGF during fasting stimulated colonic growth, normalized TFF3 mRNA in duodenum and jejunum, and broadly upregulated gut goblet cell number and TFF3 protein expression. With fasting-refeeding, KGF increased small bowel and colonic mucosal growth, goblet cell number, and TFF3 protein but had variable effects on TFF3 mRNA. KGF induced TFF2 mRNA and protein in duodenum and jejunum with both nutritional regimens. We conclude that nutrient availability modifies rat intestinal goblet cell number, TFF3 mRNA, and the gut-trophic effects of KGF in a region-specific manner. KGF enhances TFF2 expression in proximal small bowel and increases goblet cell number and TFF3 protein content throughout the intestine independent of food intake.

colon; intestinal mucosa; keratinocyte growth factor

TOGETHER WITH MUCINS, trefoil factor family (TFF) peptides are major constituents of the mucus layer that protect the gastrointestinal mucosa from injurious agents (2, 5, 13, 15, 26, 29, 34, 38, 41). TFF1 (formerly pS2) is synthesized primarily in gastric mucosal pit cells (TFF1). TFF2 (formerly spasmyloytic polypeptide or SP) is produced by gastric mucous neck cells and duodenal submucosal Brunner’s glands (8, 22, 41). TFF3 (formerly intestinal trefoil factor) is synthesized by goblet cells throughout small intestine and colon (36, 40). Trefoil peptides are resistant to protease digestion, and their cellular localization is ideal for gut epithelial protection (7, 28, 41). In vitro and in vivo studies strongly link trefoil peptides with gastrointestinal mucosal restitution (7, 19, 28, 41). TFF1, TFF2, and TFF3 are upregulated at sites of gastric and intestinal mucosal injury, where these proteins stimulate gut epithelial cell migration and mucosal repair (16, 20, 25, 33, 45, 46). However, the role of TFF peptides in physiological and adaptive gut epithelial cell proliferation, apoptosis, and turnover remains uncertain (17, 28, 31, 38, 45). Recent studies in TFF2-deficient mice clearly show that TFF2 stimulates gastric mucosal cell proliferation (12); in contrast, epithelial cell proliferation and apoptosis in jejunum were unaltered in mice with targeted transgenic expression of TFF3 in jejunal mucosa (27).

Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor family (FGF-7) synthesized by stromal cells (18). The presence of KGF and the KGF receptor in gastrointestinal mucosa suggests the involvement of the endogenous KGF action pathway in gut physiology (9, 18). KGF specifically stimulates the proliferation and differentiation of epithelial cells, including cells of the gastrointestinal mucosa (18, 24). KGF treatment augments small bowel and colonic mucosal growth, repair, and barrier function in models of malnutrition, parenteral nutrition, chemotherapy/irradiation, and intestinal inflammation (4, 10, 11, 23, 24, 32). KGF appears to have particularly trophic effects on intestinal goblet cell expression in vivo (10, 18). KGF also increased small intestinal TFF3 mRNA in a

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rat model of short bowel syndrome (23) and TFF3 protein in murine models of inflammatory bowel disease (4). In undifferentiated colonic HT-29 subclone H2 cells, KGF upregulated TFF3 mRNA and protein expression and stimulated differentiation into goblet cells through regulation of the goblet cell silencer inhibitor, a goblet cell-specific transcription factor (21).

Gut mucosal growth, function, and repair are highly dependent on nutrient availability (3, 14, 49). Nutritional status also regulates expression of various endogenous intestinal growth factors, including insulin-like growth factor-I and KGF and its receptor (9, 44, 47–49). However, there have been few studies on nutrient regulation of intestinal goblet cells (6, 30, 35, 37) or TFF peptide expression. Our hypotheses were as follows: 1) gut mucosal atrophy induced by fasting would be associated with a reduction in the number of goblet cells and decreased TFF expression; 2) KGF would increase gut mucosal growth, goblet cell number, and TFF expression; and 3) effects of KGF would be modified by food intake. Therefore, we used rat models of fasting and fasting-refeeding, with or without administration of KGF, to assess mucosal growth, goblet cell number, and expression of TFF peptide mRNA and protein in rat small bowel and colon.

MATERIALS AND METHODS

Animal Protocols

Male Sprague-Dawley rats weighing 170–210 g were housed in individual cages in the Emory University Animal Care Facility on a 12:12-h light-dark cycle. Animals were acclimatized to laboratory conditions for 3 days with ad libitum water and standard pelleted rat food (Laboratory Rodent Chow 5001, PMI Feeds, St. Louis, MO). Water was provided ad libitum to all rats, and animal food intake was measured daily. Initial body weight of each of the study groups was not different, and all control animals gained the expected amount of body weight over time (Table 1). The animal procedures were approved by the Institutional Animal Care and Use Committee of Emory University and followed the newest guiding principles of research (1).

Study 1: food deprivation. Weight-matched rats (n = 8/group) were fed ad libitum for 6 consecutive days (control) or fasted for 3 days and then refed ad libitum for 3 days. We previously showed in rats that gut mucosal atrophy induced by 3 days of fasting can be reversed by 3 days of oral refeeding (10, 47). During the entire 6-day protocol, fasted-refed rats were given daily intraperitoneal injections of KGF (3 mg/kg, refed-KGF) or saline (refed-Sal); control rats were given intraperitoneal saline. To ensure identical food intake between groups during refeeding, the refed-KGF rats were pair fed the average daily food intake consumed by the refed-Sal rats. Animals were killed on the morning of day 7.

Study 2: food deprivation and refeeding. Weight-matched rats (n = 8/group) were fed ad libitum for 6 consecutive days (control) or fasted for 3 days and then refed ad libitum for 3 days. Animals were not monitored for coprophagia. We previously showed in rats that a 3-day period of fasting induces gut mucosal atrophy (47). Rats were also given daily intraperitoneal injections of recombinant human KGF (Amgen, Thousand Oaks, CA; 3 mg/kg, fasted-KGF) or saline (fasted-Sal). The fed control group was given daily intraperitoneal saline injections. Animals were killed on the morning of day 4. As expected, at death, fasted animals had lost ~20% of their initial body weight, whereas control animals had continued to gain weight (Table 1).

Table 1. Changes in rat body weight with fasting and refeeding

<table>
<thead>
<tr>
<th>Initial</th>
<th>Fasted</th>
<th>Fasted-Refed</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>216 ± 2</td>
<td>238 ± 3</td>
</tr>
<tr>
<td>Fasted-Sal</td>
<td>218 ± 2</td>
<td>172 ± 3*</td>
</tr>
<tr>
<td>Fasted-KGF</td>
<td>219 ± 2</td>
<td>174 ± 2*</td>
</tr>
<tr>
<td>Control</td>
<td>222 ± 1</td>
<td>250 ± 4</td>
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<tr>
<td>Refed-Sal</td>
<td>222 ± 2</td>
<td>182 ± 1*</td>
</tr>
<tr>
<td>Refed-KGF</td>
<td>224 ± 3</td>
<td>172 ± 2†</td>
</tr>
</tbody>
</table>

Values are means ± SE in g; n = 8. Control, rats fed ad libitum for 3 days (fasting study) or 6 days (fasting-refeeding study); fasted-Sal, fasted for 3 days + saline; fasted-KGF, fasted for 3 days + keratinocyte growth factor (KGF, 3 mg·kg⁻¹·day⁻¹); refed-Sal, fasted for 3 days-refed for 3 days + saline; refed-KGF, fasted for 3 days-refed for 3 days + KGF (3 mg·kg⁻¹·day⁻¹). *P < 0.05 vs. control; †P < 0.05 vs. fasted-Sal.

Gut Mucosal Growth Indexes

Paraffin-embedded sections of duodenum, jejunum, ileum, and colon were stained with hematoxylin and eosin. Crypt depth and villus height were measured manually using a viewing Olympus BH-2 light microscope and calibrated ocular micrometer by two pathologist coinvestigators who were blinded to study group. A total of 15–25 well-oriented crypts and villi from each small bowel segment and 15–25 colonic crypts from each colon segment per animal were measured and averaged.

Gut Mucosal Goblet Cell Number

Goblet cells were identified by classical morphology in each small bowel and colonic section in all animals. Total goblet cell number was determined along the combined crypt-villus axis of 10 well-oriented crypt-villus units in each small bowel section and 10 well-oriented colonic crypt units, from the crypt base to the luminal surface. To confirm goblet cell...
numbers identified by morphology, goblet cells in duodenal and colonic sections from the same animals (n = 5–6/group) were localized using Alcian blue staining (19). Positive staining with Alcian blue indicates the presence of acid mucins within goblet cells and was quantitated as outlined for morphological assessment.

**RNA Preparation and Northern Blot Analysis**

Northern blotting was performed as previously described (49). Total cellular RNA was isolated using TRI-reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s instructions. RNA integrity was confirmed by ethidium bromide staining.

The rat TFF1 cDNA was a 135-bp KpnI/SacI fragment based on the rat TFF1 cDNA sequence made by PCR and subcloned into pGEMT (Promega, Madison, WI) (20). The TFF2 cDNA was a 267-bp EcoRI fragment of the rat TFF2 cDNA sequence (22) made by RT-PCR and subcloned into pGEMT. The TFF3 cDNA probe used for hybridization was a 500-bp KpnI/BglII fragment of the rat TFF3 cDNA sequence (36) subcloned into pGEMT. The cDNAs were labeled with [32P]dCTP by random priming (Prime-It II Random Primer Labeling Kit, Stratagene, La Jolla, CA) followed by spin column removal of unincorporated nucleotides. Total RNA (25 μg/lane) was loaded onto 1% agarose gels, electrophoresed, and transferred to nylon membranes. The membranes were hybridized with the respective TFF probes for 16–18 h at 42°C in hybridization solution, as described elsewhere (49), washed under high-stringency conditions, and exposed for various times at ~80°C using Kodak X-OMAT film with intensifying screens. To control for RNA loading, all membranes were stripped and rehybridized with a mouse 18S cDNA probe (American Type Culture Collection, Rockville, MD). Bands were quantitated by laser densitometry.

**Western Immunoblotting**

A rabbit monoclonal antibody against human TFF2 (provided by Dr. Lars Thim, Novo Nordisk, Malov, Denmark) and a rabbit anti-rat polyclonal TFF3 antibody raised against a rabbit anti-rat TFF3 antibody (1:500 dilution), respectively. Binding of primary antibody was visualized by incubation with goat anti-rabbit immunoglobulin G (1:500; Vector Laboratories, Burlingame, CA) and use of avidin, biotinylated horseradish peroxidase, and diaminobenzidine tetrahydrochloride reagents according to the manufacturer’s instructions (ABC-Peroxidase Elite, Vector Laboratories). The sections were counterstained with hematoxylin. Tissue sections processed without primary antibody or with overnight incubation of primary antibody with TFF2 or TFF3 protein (10 μM at 4°C) were used as negative controls.

**Statistical Analysis**

The abundance of TFF mRNA and protein was expressed in densitometry units, normalized against values obtained for control animals within each experiment. mRNA data from Northern blotting experiments were normalized to 18S mRNA expression. One-factor ANOVA was used to detect significant intergroup differences, in which case individual study groups were compared using Fisher’s protected least significant difference test. P < 0.05 was considered statistically significant in all analyses. Values are means ± SE.

**RESULTS**

**Gut Mucosal Growth Indexes**

Fasting for 3 days induced site-specific effects on gut mucosal growth indexes (Table 2). The significant changes were a modest decrease in jejunal and increase in ileal villus height (to 83 and 112% of control values, respectively) and a decrease in jejunal, ileal, and colonic crypt depth (to 85, 88, and 88% of control values, respectively). KGF administration completely prevented the decrease in colonic crypt depth during food deprivation but did not alter the small bowel mucosal growth indexes. Rats in the fasting-refeeding protocol exhibited site-specific effects on gut mucosal growth parameters, and KGF induced small bowel and colonic mucosal growth responses (Table 3).

**Goblet Cell Number**

Goblet cell number increased in a proximal-to-distal intestinal gradient in the control, nutrient-restricted, and refed rats (Table 4). Fasting alone increased duodenal goblet cell number by 25% compared with control fed values but did not modify goblet cell number in the

<table>
<thead>
<tr>
<th>Villus height, μm</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Colon</th>
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<tr>
<td>Control</td>
<td>510 ± 19</td>
<td>460 ± 16</td>
<td>176 ± 7</td>
<td></td>
</tr>
<tr>
<td>Fasted-Sal</td>
<td>459 ± 17</td>
<td>384 ± 13*</td>
<td>198 ± 7*</td>
<td></td>
</tr>
<tr>
<td>Fasted-KGF</td>
<td>469 ± 17</td>
<td>389 ± 22*</td>
<td>208 ± 5*</td>
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<table>
<thead>
<tr>
<th>Crypt depth, μm</th>
<th>Duodenum</th>
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<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>200 ± 3</td>
<td>128 ± 4</td>
<td>112 ± 3</td>
<td>196 ± 4</td>
</tr>
<tr>
<td>Fasted-Sal</td>
<td>198 ± 7</td>
<td>109 ± 4*</td>
<td>95 ± 6*</td>
<td>173 ± 4*</td>
</tr>
<tr>
<td>Fasted-KGF</td>
<td>182 ± 9</td>
<td>114 ± 5*</td>
<td>93 ± 2*</td>
<td>201 ± 9*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8. See Table 1 footnote for description of groups. *P < 0.05 vs. control; †P < 0.05 vs. fasted-Sal.
KGF INDUCTION OF GUT TREFOIL PEPTIDES

Table 3. Trophic effects of KGF in small bowel and colonic mucosa with nutrient repletion after fasting

<table>
<thead>
<tr>
<th></th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Colon</th>
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</thead>
<tbody>
<tr>
<td>Villus height, μm</td>
<td></td>
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<tr>
<td>Control</td>
<td>573 ± 11</td>
<td>571 ± 16</td>
<td>205 ± 8</td>
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<tr>
<td>Refed-Sal</td>
<td>596 ± 11</td>
<td>520 ± 14*</td>
<td>177 ± 7*</td>
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<tr>
<td>Refed-KGF</td>
<td>683 ± 28†</td>
<td>628 ± 13†</td>
<td>239 ± 7†</td>
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<tr>
<td>Crypt depth, μm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>183 ± 4</td>
<td>147 ± 6</td>
<td>133 ± 5</td>
<td>193 ± 6</td>
</tr>
<tr>
<td>Refed-Sal</td>
<td>196 ± 6</td>
<td>160 ± 3*</td>
<td>121 ± 6</td>
<td>197 ± 4</td>
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<tr>
<td>Refed-KGF</td>
<td>253 ± 16†</td>
<td>108 ± 3†</td>
<td>123 ± 5</td>
<td>234 ± 10†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8. See Table 1 footnote for description of groups. *P < 0.05 vs. control; †P < 0.05 vs. refed-Sal.

other gut segments. KGF markedly increased goblet cell proliferation in all gut segments during food deprivation (Table 4). In fasted-refed rats, goblet cell number in duodenum and colon increased (to 118 and 125% of control, respectively). In this model, KGF markedly increased goblet cell number in duodenum, jejunum, ileum, and colon (Table 4). The magnitude of the goblet cell response to KGF in refed rats was quantitatively similar to the response to KGF when given during food deprivation alone.

Alcian blue staining detects acid mucin-positive goblet cells and was performed in duodenal and colonic mucosal sections to confirm the morphological assessment. Results using Alcian blue were statistically similar to data derived from morphological determination of goblet cell number. The number of Alcian blue-positive cells per 10 crypt units in duodenal mucosa was 169 ± 18, 209 ± 3, and 343 ± 13 in control, fasted-Sal, and fasted-KGF groups, respectively (P < 0.05, fasted-Sal vs. control; P < 0.01, fasted-KGF vs. control and fasted-Sal). In colonic mucosa, the number of Alcian blue-positive cells was 294 ± 15, 295 ± 8, and 539 ± 9 in control, fasted-Sal, and fasted-KGF groups, respectively (P < 0.01, fasted-KGF vs. control and fasted-Sal). In the fasting-refeeding model, the number of Alcian blue-positive cells in duodenal mucosa was 156 ± 4, 184 ± 3, and 397 ± 13 in control, refed-Sal, and refed-KGF groups, respectively (P < 0.08, refed-Sal vs. control; P < 0.01, refed-KGF vs. control and refed-Sal). In colon, the number of Alcian blue-positive cells was 303 ± 7, 358 ± 16, and 578 ± 3 in control, refed-Sal, and refed-KGF groups, respectively (P < 0.05, refed-Sal vs. control; P < 0.01, refed-KGF vs. control and refed-Sal).

TFF1 and TFF2 Expression

The expected 0.5-kb TFF1 mRNA transcript was demonstrated in stomach (not shown), but TFF1 mRNA was not detected in duodenum, jejunum, ileum, or colon in any of the study groups, even after 8 days of autoradiographic exposure. The 0.6-kb rat TFF2 mRNA transcript was evident in stomach after 3 h of exposure (Fig. 1A, lane 1). TFF2 mRNA was not detected after 48 h of exposure in duodenum (Fig. 1, A and C) or jejunum (Fig. 1, C and D) in control, fasted, or fasted-refed animals given saline. However, KGF treatment induced expression of TFF2 mRNA in duodenum and jejunum in both nutritional models (Fig. 1). Upregulated TFF2 gene expression with KGF was particularly marked in the duodenum of food-deprived rats (Fig. 1A, lanes 4 and 7). TFF2 mRNA was not detected in ileum or colon after 8 days of exposure (not shown).

TFF2 immunoreactivity was consistently detected in duodenal submucosal Brunner’s glands in all study groups (Fig. 2, A and B). In jejunum, faint expression of TFF2 protein was present, but cellular localization was variable between animals; low-level staining was observed in goblet cells, along the apical border of villus epithelial cells, and in secreted mucus (not shown). TFF2 was not observed in the duodenal mucosa of saline-treated rats; however, KGF administration in both nutritional models ectopically induced TFF2 protein expression in goblet cells of duodenum and, to a lesser extent, jejunum (Fig. 2, C and D). TFF2 staining after KGF treatment appeared heaviest in the crypt and lower villus region, with decreased staining in the mid- to upper villus goblet cells.

Using Western blotting, we detected a ~16-kDa band in duodenal and jejunal tissue that was identical in size to purified TFF2 protein and the band observed in rat stomach (Fig. 3). Food deprivation alone did not alter TFF2 protein abundance in duodenum and jejunum; however, KGF given in this setting increased TFF2 abundance in duodenum and, to a lesser extent, jejunum compared with controls (Fig. 3). In the fasting-refeeding protocol, KGF did not significantly alter TFF2 protein abundance in duodenum and jejunum (100 ± 5, 121 ± 4, and 137 ± 24 in control, fasted-Sal, and fasted-KGF, respectively; not significant) or jejunum (100 ± 14, 153 ± 19, and 140 ± 29 in control, fasted-Sal, and fasted-KGF, respectively; not significant).

TFF3 Expression

Fasting decreased steady-state expression of TFF3 mRNA in duodenum and jejunum (to 55 and 30% of control values, respectively) but did not alter TFF3 mRNA values in ileum or colon (Fig. 4A). KGF treatment during food deprivation prevented the decrease in duodenum and jejunal TFF3 mRNA but did not alter TFF3 mRNA expression in ileum or colon. In contrast to changes in duodenal and jejunal TFF3 mRNA, fasting increased TFF3 protein levels in jejunum and did

Table 4. Nutrient and KGF regulation of goblet cell number in small bowel and colonic mucosa

<table>
<thead>
<tr>
<th></th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>159 ± 17</td>
<td>227 ± 12</td>
<td>240 ± 12</td>
<td>305 ± 8</td>
</tr>
<tr>
<td>Fasted-Sal</td>
<td>199 ± 4*</td>
<td>227 ± 3</td>
<td>246 ± 3</td>
<td>295 ± 6</td>
</tr>
<tr>
<td>Fasted-KGF</td>
<td>368 ± 7†</td>
<td>430 ± 16†</td>
<td>465 ± 14†</td>
<td>560 ± 12†</td>
</tr>
<tr>
<td>Control</td>
<td>164 ± 15</td>
<td>228 ± 6</td>
<td>252 ± 7</td>
<td>285 ± 8</td>
</tr>
<tr>
<td>Refed-Sal</td>
<td>194 ± 3*</td>
<td>237 ± 5</td>
<td>259 ± 3</td>
<td>357 ± 10†</td>
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<tr>
<td>Refed-KGF</td>
<td>410 ± 13†</td>
<td>450 ± 7†</td>
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<td>576 ± 0†</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as total goblet cells/10 crypt-villus units; n = 8. See Table 1 footnote for description of groups. *P < 0.01 vs. control; †P < 0.01 vs. fasted-Sal or refed-Sal.
KGF induction of gut trefoil peptides

Fig. 1. Keratinocyte growth factor (KGF) increases trefoil factor family (TFF2) mRNA expression in rat duodenum and jejunum. TFF2 mRNA was determined by Northern blotting in full-thickness portions of stomach antrum, duodenum, and jejunum from control, fasted, and refed rats. A: representative duodenal experiment (48-h exposure) from 2 sets of rats studied as follows: fed ad libitum for 3 days and treated daily with intraperitoneal saline [control (C), lanes 2 and 5], fasted for 3 days and treated daily with intraperitoneal saline (fasted-Sal, lanes 3 and 6), and fasted for 3 days and treated with KGF at 3 mg·kg⁻¹·day⁻¹ ip (fasted-KGF, lanes 4 and 7). B: representative jejunal experiment from 2 sets of rats studied as outlined for A. C: representative duodenal blot (48-h exposure) from 2 sets of rats studied as follows: fed ad libitum for 6 days with daily intraperitoneal saline (control, lanes 1 and 4), fasted for 3 days and then refeeding for 3 days with daily intraperitoneal saline treatment (refed-Sal, lanes 2 and 5), and fasted-refed with daily intraperitoneal KGF treatment (refed-KGF, lanes 3 and 6). D: representative jejunal blot from 2 sets of rats studied as outlined in C. TFF2 mRNA was not detected in ileum or colon in any of the study groups outlined above, even after 8 days of exposure (not shown). n = 5/group.

Fig. 2. KGF ectopically induces TFF2 protein expression in rat duodenal mucosa. Cellular localization of TFF2 protein in duodenal mucosa was determined using a rabbit monoclonal antibody against human TFF2 peptide in control, fasted-Sal, and fast-KGF rats. A: TFF2 staining localized to duodenal Brunner’s glands (black arrow) and no staining in crypt cells (white arrow) in a control rat. Cellular localization of TFF2 was similar in fasted rats given saline (B). KGF induced TFF2 expression in duodenal crypt and villus goblet cells in fasted rats (C and D). TFF2 staining in Brunner’s glands (black arrow) and heavy staining in crypt region (white arrow) are shown in C. KGF induction of TFF2 in villus cells is shown in D. Secreted TFF2 protein is also evident in the lumen.

not alter levels in the other intestinal segments (Fig. 4B). KGF treatment in this nutritional model also increased TFF3 protein abundance in duodenum, ileum, and especially colon (to 135, 263, and 1,578% of control values, respectively) and tended to increase values in jejunum (not significant).

Fasting-refeeding did not modify intestinal TFF3 mRNA levels compared with continuously fed control animals (Fig. 5A). KGF treatment in this nutritional setting increased TFF3 mRNA abundance in duodenum, ileum, and colon (to 238, 686, and 244% of control values) but did not alter values in jejunum. TFF3...
protein levels in fasted-refed rats were similar to those in fed controls (Fig. 5B). KGF administration in this model increased TFF3 protein expression by two- to fourfold in duodenum, jejunum, ileum, and colon compared with control values (Fig. 5B).

Immunoreactive TFF3 protein was readily detected within goblet cells in all bowel segments. In ileum, TFF3 protein was localized within goblet cells and along the overlying mucosal surface, reflecting secretion into the lumen (Fig. 6). Consistent with the Western blot data shown in Fig. 5, ileal mucosa TFF3 protein abundance and cellular expression were similar in fasted-refed and control rats (Fig. 6). Consistent with Western blot data, TFF3 protein was detected in goblet cells of all bowel segments. In ileum, TFF3 protein was localized within goblet cells and was unaltered by fasting-refeeding (Fig. 7, A and B). KGF treatment increased goblet cell number and TFF3 immunoreactivity in colon (Fig. 7C).

**DISCUSSION**

Nutritional status markedly influences gut mucosal growth and function, but surprisingly little is known about the effects of nutritional factors on intestinal goblet cells or their secreted proteins (6, 33, 42). Little is known regarding the influence of nutrient intake on gut mucosal responses to recombinant growth factors, including KGF (44, 48, 49). To our knowledge, this is the first study to evaluate effects of diet and KGF on concomitant gut goblet cell and TFF peptide expression and to determine whether KGF regulates TFF1 and TFF2 expression.

**Gut Mucosal Growth Indexes**

Enteral nutrient availability induced segment-specific effects on small intestinal and colonic mucosal growth, consistent with previous studies (10, 44, 47). In the absence of exogenous nutrients (fasted model), KGF did not increase small bowel crypt depth or villus height but prevented the decrease in colonic crypt depth. When given during fasting followed by refeeding, KGF increased duodenal, jejunal, and ileal villus height and duodenal and colonic crypt depth. We did not investigate the time course of response to KGF or administer KGF to control fed rats. However, KGF has been shown to increase small bowel mucosal growth as early as 1 day after treatment in fed rats (18). Also, we...
previously showed that KGF administration during refeeding induced greater duodenal and ileal mucosal growth when animals were refed at 25% of ad libitum intake (10). Thus the level of nutrient intake appears to influence KGF gut-trophic responses in small bowel, with the presence of some luminal food being important. In contrast, this study and our previous work in different nutritional models demonstrate that KGF potently stimulates colonic growth independent of the level of nutrition (10). Additional studies to determine responses to KGF in parenterally fed animals, in which luminal food is absent, would be of interest (32).

**Goblet Cell Number**

Intestinal goblet cell number and crypt depth/villus height during altered nutrient availability alone were generally dissociated (Tables 2–4). Goblet cell number increased in duodenum after fasting alone or fasting-refeeding. To our knowledge, this is the first demonstration of increased intestinal goblet cell number during altered nutrient availability. These data are consistent with another study showing increased mucus production in proximal small bowel after a 48-h fast in rats (37). Earlier investigations indicated that chronic protein depletion or protein-energy undernu-

**Fig. 5.** KGF upregulates TFF3 mRNA and protein in small bowel and colon during food deprivation-refeeding. TFF3 mRNA (0.4 kb) was determined by Northern blotting, normalized to mouse 18S mRNA expression, and TFF3 protein (6.5 kDa) was measured by Western immunoblotting in full-thickness intestinal portions. Solid bars, rats fed ad libitum for 6 days and treated daily with intraperitoneal saline (control); open bars, rats fasting for 3 days and then refeeding and then treated with KGF at 3 mg·kg⁻¹·day⁻¹ i.p (refeed-KGF). Values are means ± SE. Representative autoradiograms are shown above quantitative histograms. n = 8/group. *P < 0.05 vs. control; †P < 0.05 vs. refeed-Sal.

**Fig. 6.** KGF increases goblet cell number and TFF3 protein expression in ileum during fasting-refeeding. Ileal mucosal TFF3 protein expression within goblet cells was similar in ad libitum-fed control rats (A) and rats fasted for 3 days and then refeeding and then treated with KGF at 3 mg·kg⁻¹·day⁻¹ i.p (reefed-KGF). Values are means ± SE. Representative autoradiograms are shown above quantitative histograms. n = 8/group. *P < 0.05 vs. control; †P < 0.05 vs. refeed-Sal.
trition decreased goblet cell number or mucin synthesis in rodent and piglet proximal small intestine, respectively (30, 35). Differences between our results and these latter studies may relate to the type or duration of malnutrition or the animal species utilized. Colonic goblet cell number increased in the fasting-refeeding model but was unaltered with fasting alone. Different local or systemic nutrition-related factors thus govern expression of the goblet cell lineage in duodenum compared with colon. Increased goblet cell expression in these gut segments may represent a protective adaptation to diminished nutrient availability. KGF increased goblet cell number uniformly throughout the small and large intestine, and this effect was unrelated to nutrient availability. In vivo studies to determine whether malnutrition, enteral vs. parenteral feeding, or KGF alters goblet cell differentiation, proliferation, apoptosis, and production of specific mucins (6, 43) would be of interest.

**TFF1 and TFF2 Responses**

As expected, TFF1 and TFF2 mRNA were abundant in rat stomach tissue by Northern blotting but were not detected in intestine, with or without oral intake. KGF treatment did not induce TFF1 mRNA in any bowel segment or TFF2 mRNA in ileum and colon. Thus KGF stimulation of trefoil peptides is region and TFF specific. It is possible that a more sensitive assay, such as PCR or in situ hybridization, may have enabled detection of these mRNAs in the intestine.

We confirmed cellular localization of TFF2 protein in duodenal submucosal Brunner’s glands, as previously reported by others (22, 33, 34, 40, 45). However, we show for the first time that KGF upregulates TFF2 expression in rat proximal small bowel, with ectopic expression in goblet cells and secretion of the peptide into the lumen. Ectopic local upregulation of TFF2 was previously shown to occur in ulcer-associated cell lineages and in goblet cells at sites of gut mucosal injury (20, 25, 33, 46), suggesting a role for TFF2 in bowel mucosal restitution. Local KGF mRNA in gut mucosa is upregulated at sites of injury in inflammatory bowel disease and with mucosal ulceration (4, 48). Our results showing that exogenous KGF increased goblet cell TFF2 suggest that local upregulation of TFF2 in areas of mucosal inflammation may be due, in part, to increased local levels of KGF.

**TFF3 Responses**

The broad pattern of upregulated TFF3 protein after KGF injection occurred concomitantly with increased TFF mRNA in most gut regions. However, in ileum and colon after food deprivation (Fig. 4) and in jejenum after fasting-refeeding (Fig. 5), TFF3 mRNA levels were unchanged, despite an increase in TFF3 protein abundance. In addition, with fasting alone, TFF3 mRNA decreased in duodenum and ileum, whereas protein levels at these sites were maintained. Taken together, these data suggest that TFF3 expression is controlled at a posttranscriptional level in a region-
specific manner and differently depending on nutrient supply or KGF administration.

KGF-stimulated TFF3 production was localized to goblet cells, and abundant secreted TFF3 was present in the gut lumen. TFF3 mRNA and/or protein levels in some gut segments varied independently from changes in goblet cell number during altered nutrition (e.g., duodenum in both models and in colon with fasting-refeeding). Discordance between goblet cell number and TFF3 production was previously observed in mice with partial ablation of intestinal goblet cells, in which chemical mucosal injury increased goblet cell TFF3 expression (19). Increased goblet cell number with KGF treatment was invariably associated with increased TFF3 protein abundance in all gut segments. Previous studies show that KGF increases muc2 mRNA levels in HT-29 subclone H2 cells (21). Given the emerging roles for mucins such as muc2 in intestinal mucosal growth, protection, and barrier function (43, 45), in vivo studies to determine whether KGF or specific dietary factors differentially regulate goblet cell production of specific mucins would be of interest (43).

Changes in local TFF2 or TFF3 expression and mucosal crypt depth and villus height did not generally correlate in the present study. However, our study was not designed to test whether intestinal growth responses to diet or KGF are mediated by changes in goblet cell number or local TFF production. Studies of gut growth responses to nutritional alterations and KGF in TFF2 and TFF3 knock-out and transgenic mice are needed to address these questions.

In conclusion, in rat intestine, nutrient availability modulated goblet cell number, small intestinal TFF3 mRNA abundance, and trophic effects of KGF, but these effects were region specific. KGF markedly increased goblet cell number and TFF3 expression throughout the intestine, despite short-term lack of food intake. In addition, KGF induced ectopic TFF2 expression in proximal small bowel goblet cells. Further studies are necessary to determine the mechanisms involved in the nutritional and KGF modulation of goblet cells and their trefoil peptide products. In addition, more information on the specific roles of goblet cells and TFF peptides in mediating actions of nutrients and KGF in the gut is needed. Increased goblet cell number and TFF2 and TFF3 expression may mediate some of the beneficial effects of KGF observed previously (4, 10, 11, 24). Our results extend these studies and suggest that KGF may have potential therapeutic roles in disorders associated with malnutrition, including intestinal inflammation and short gut syndrome.

C. Fernández-Estivariz gratefully acknowledges the support of Dr. J. Moreiro (Son Dureta Hospital, Mallorca, Spain, and Nutricia).

This work was supported, in part, by National Institutes of Health Grants DK-53585 and RR-00639 (to T. R. Ziegler), ES-009047 (to D. P. Jones), and DK-43351, DK-66906, and DK-43351 (to D. K. Podolsky) and Spanish Instituto de Salud Carlos III del Ministerio de Sanidad y Consumo Grants BAE 97/5082 and 98/5057 (to C. Fernández-Estivariz).

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