Short-chain fatty acid–supplemented total parenteral nutrition alters intestinal structure, glucose transporter 2 (GLUT2) mRNA and protein, and proglucagon mRNA abundance in normal rats

Kelly A Tappenden, Laurie A Drozdowski, Alan BR Thomson, and Michael I McBurney

ABSTRACT

Intestinal adaptation is a complex physiologic process that is not completely understood. Intravenous short-chain fatty acids (SCFAs) enhance intestinal adaptation after 80% enterectomy in rats. The purpose of this study was to examine rapid responses to SCFA-supplemented total parenteral nutrition (TPN) in the normal small intestine. After jugular catheterization, 31 Sprague-Dawley rats (weighing 258 ± 3 g) were randomly assigned to receive standard TPN or an isoenergetic, isonitrogenous TPN solution supplemented with SCFAs (TPN+SCFA). Intestinal samples were obtained after 24 or 72 h of nutrient infusion. TPN+SCFA for 24 h increased (P < 0.05) the ileal RNA concentration (µg RNA/mg ileum) whereas TPN+SCFA for 72 h increased (P < 0.05) the ileal DNA concentration (µg DNA/mg ileum) and decreased (P < 0.05) the ileal protein concentration (µg protein/mg ileum). Ileal proglucagon mRNA abundance was elevated (P < 0.05) after 24 h of TPN+SCFA infusion and returned to levels seen with control TPN by 72 h. Glucose transporter 2 (GLUT2) mRNA was significantly higher (P < 0.05) in the TPN+SCFA groups at both time points when compared with control TPN groups. Ileal GLUT2 protein abundance in the 72-h TPN+SCFA groups was significantly higher (P < 0.05) than that of all other groups. Sodium-glucose cotransporter (SGLT-1) mRNA and protein abundance and uptake of D-fructose and D-glucose did not differ between groups. Jejunal uptake of L-glucose and lauric acid was significantly higher (P < 0.05) after 72 h of TPN+SCFA than after 24 h, whereas the 24- and 72-h TPN groups did not differ. In summary, SCFAs led to rapid changes in ileal proglucagon and glucose transporter expression in rats receiving TPN and provide insights into therapeutic management of individuals with short bowel syndrome or intestinal malabsorption syndromes.

KEY WORDS

Short-chain fatty acids, total parenteral nutrition, intestinal adaptation, proglucagon, glucagon-like peptide 2, nutrient transport, SGLT-1, GLUT2, GLP-2, Sprague-Dawley rats, glucose transporter 2, sodium-glucose cotransporter

INTRODUCTION

Since its inception in the late 1960s, total parenteral nutrition (TPN) has become a powerful therapy used to prevent malnutrition in patients unable to absorb nutrients provided enterally. It is believed that TPN is the most important factor responsible for prolonging the lives of patients with short bowel syndrome (1–3). An increase in the survival of children with < 40 cm of small intestine from 42% before 1980 to 94% in the decade since has been documented (4). Despite these successes with TPN, intestinal atrophy and impaired functional activity occur when luminal nutrients are absent (5–11). Investigations aimed at identifying regulatory factors of intestinal adaptation will lead to revised TPN formulations capable of stimulating enteroplastic changes and the stable transition to total enteral nutrition.

Short-chain fatty acids (SCFAs) are the byproducts of dietary fiber fermentation in the colon. Acetate, propionate, and butyrate account for ~85% of SCFAs and are produced intraluminally in a nearly constant molar ratio of 60:25:15 (12). One week of SCFA supplementation has been shown to retard TPN-associated atrophy in rats with intact bowels (13) and after 80% intestinal resection (14). Recently, our laboratory reported that SCFA-supplemented TPN enhanced both structural (15) and functional (16) adaptation to 80% intestinal resection as early as 3 d after surgery. However, the acute effects (< 72 h) of SCFAs on intestinal structure and function are unknown.

SCFAs may influence small intestinal mucosal proliferation by stimulating secretion of proglucagon-derived peptides. Intestinal proglucagon mRNA abundance and plasma concentrations of proglucagon-derived peptides are strongly correlated with cellular proliferation during intestinal adaptation (17–20). Precise physiologic roles for each of the proglucagon-derived peptides continue to be elucidated; however, it was shown recently that glucagon-like peptide 2 (GLP-2) modulates basolateral membrane glucose transport in rats (21). We reported previously that SCFA-supplemented TPN increases proglucagon gene expression 3 and 7 d after intestinal resection (15). Addi-

1 From the Department of Agricultural, Food and Nutritional Science and the Department of Medicine, University of Alberta, Edmonton, Canada.
2 Supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada. KAT received an NSERC postgraduate industrial scholarship.
3 Address reprint requests to MI McBurney, WK Kellogg Institute, 2 Hamblin Avenue East, PO Box 3232, Battle Creek, MI 49016-3232. E-mail: michael.mcburney@kellogg.com.
Received January 16, 1997.
Accepted for publication November 25, 1997.

optimal sterile conditions and were filter sterilized before infusion (0.22-μm Millipore filter; Millipore Corporation, Bedford, MA). The SCFAs—acetate, propionate, and butyrate—were added as sodium salts (Sigma Chemical Co, St Louis) in the molar proportions found physiologically in the colon (12). The diets were infused daily to provide 217 kJ and 425 mg N. The nutrient solutions were administered by using a Harvard infusion pump (Harvard Apparatus, Wellesley, MA). All animals had free access to drinking water throughout the study period.

Tissue preparation

Twenty-four (TPN 24 h, n = 8; TPN+SCFA 24 h, n = 8) and 72 (TPN 72 h, n = 7; TPN+SCFA 72 h, n = 8) h after the operation, the animals were weighed and anesthetized with halothane. The intestine was rapidly excised from the ligament of Treitz to the ileocecal valve, freed of its mesenteric fat, rinsed in ice-cold saline, and weighed. The intestine was divided into three segments with the most proximal and distal segments representing the jejunum and ileum, respectively. Measuring from the ligament of Treitz or ileocecal valve, a 2-cm section of tissue was removed, snap frozen in liquid nitrogen, and stored at −70 °C for subsequent RNA isolation. A 1-cm segment was stored at −70 °C until assayed for DNA (24) and protein (25). A 15-cm segment of tissue was removed for intestinal glucose uptake studies and mucosal weight determination. Finally, a 10-cm segment was removed and mucosal scrapings were obtained for isolation of brush border and basolateral membranes.

RNA isolation and Northern blot analysis

Total cellular RNA was extracted by using the supplier’s instructions for the Trizol reagent (Gibco BRL, Burlington, Canada). Total cellular RNA (20 μg/lane) was size fractionated on a 1% (wt:vol) agarose gel with 0.66 mol formaldehyde/L and transferred to MSI Nitropure nitrocellulose membrane (MSI Laboratories, Westboro, MA) by capillary diffusion. The RNA was fixed to the membranes by baking under vacuum at 80 °C for 2 h. The proglucagon, GLUT2, and sodium-glucose cotransporter (SGLT-1) cDNA probes were labeled with [32P]dCTP [110 100 GBq/mmol (3000 Ci/mmol); Amersham Canada, Oakville, Canada] and hybridized with the membranes as described previously (16).

Relative mRNA concentrations were determined by using laser densitometry [model GS-670 imaging densitometer; BioRad Laboratories (Canada) Ltd, Mississauga, Canada]. The 28S ribosomal units were quantified from photographs of the ethidium bromide–stained membranes before hybridization (26). These values were used to normalize for any loading discrepancies, variations in RNA integrity, or nonspecific changes in RNA abundance. This method eliminates the issue of variability of expression of housekeeping genes within this model of intestinal proliferation (27).

The proglucagon cDNA probe, which detects a 1.2-kilobase (kb) proglucagon transcript, was a gift from PJ Fuller of Prince Henry’s Institute of Medical Research in Melbourne. The GLUT2 plasmid was donated by GI Bell of Howard Hughes Medical Institute, University of Chicago, and detects a 3.8-kb GLUT2 species. N Davidson (University of Chicago) supplied the SGLT-1 plasmid, which detects a 4.8-kb SGLT-1 transcript.

Brush border and basolateral membrane isolation

This method was derived from a combination of procedures published previously (28–30) for the simultaneous isolation of

### MATERIALS AND METHODS

**Animals**

Thirty-one adult, male Sprague-Dawley rats (weighing 258 ± 3 g), obtained from Health Sciences Laboratory Animal Services (University of Alberta, Edmonton, Canada), were acclimatized and housed in individual metabolic cages in a temperature- and humidity-controlled facility with 12-h light-dark exposure. Four days before surgery, the animals were given free access to a nutritionally complete elemental diet (22) and drinking water. The elemental diet was given to minimize the effect of residual dietary fiber fermentation and therefore decrease SCFA production in the gastrointestinal tract before the start of the experiment. All procedures received ethical approval from the University of Alberta Animal Policy and Care Committee and are consistent with the guidelines of the Canadian Council on Animal Care.

**Experimental model**

Before surgery, animals underwent 12 h of food restriction and were weighed and anesthetized with halothane. Animals underwent superior vena cava cannulations and swivel placement as outlined by Popp and Brennan (23). Sterile instruments and aseptic technique were used at all times. Postoperatively, animals were randomly assigned to receive either standard TPN or an isoenergetic, isonitrogenous TPN solution supplemented with SCFAs (TPN+SCFA). Within these two diet groups, animals were randomly assigned further to receive the parenteral solutions for either 24 or 72 h. The TPN solutions (Table 1) were prepared daily under a laminar flow hood to maintain

### TABLE 1

<table>
<thead>
<tr>
<th>Composition of nutrient solutions</th>
<th>TPN</th>
<th>TPN+SCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose (g/L)</td>
<td>204</td>
<td>199</td>
</tr>
<tr>
<td>Nitrogen (g/L)</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Lipid (g/L)</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>NaCl (mmol/L)</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Sodium acetate (mmol/L)</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>Sodium propionate (mmol/L)</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Sodium butyrate (mmol/L)</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>KCl (mmol/L)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>KPO4 (mmol/L)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Calcium gluconate (mmol/L)</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>MgSO4 (mmol/L)</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Multivitamins (mL/L)</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

1 TPN, a standard total parenteral nutrition formulation; TPN+SCFA, TPN plus short-chain fatty acids.
2 10% Travasol (Baxter, Toronto).
3 20% Intralipid (Kabi Pharmacia, Baie D’urfe, Canada).
4 Multi-1000 (Sabex Inc, Boucherville, Canada).

This method was derived from a combination of procedures published previously (28–30) for the simultaneous isolation of...
basolateral, microsomal, and brush border membranes. Mucosal scrapings were homogenized in sucrose-tris buffer (250 mmol sucrose/L, 2 mmol tris-HCl/L, 0.1 mmol phenylmethylsulfonylfluoride/L, pH 7.4) three times for 30 s by using a Polytron at setting 8 (Brinkman, Mississauga, Canada). The homogenate was centrifuged for 15 min at 2400 × g and 4°C with no brake. The pellet (unbroken cells and nuclear material) was discarded and the supernate was centrifuged for 20 min at 43 700 × g and 4°C with no brake. The resulting supernate was discarded. The upper white fluffy pellet (P1) was resuspended in sucrose-tris buffer and combined with P1. The lower dark pellet (P2) was resuspended in sucrose-tris buffer and centrifuged for 20 min at 43 700 × g and 4°C with no brake. The supernate was discarded and the upper white fluffy pellet resuspended in sucrose-tris buffer and combined with P1. The lower dark pellet was resuspended in sucrose-tris buffer and combined with P2.

The combined P1 suspension was homogenized for 15 s at Polytron setting 8, layered onto a 20% Percoll gradient, and centrifuged for 30 min at 46 000 × g and 4°C with maximum brake. The resulting white, fluffy basolateral membrane layer was then centrifuged for 30 min at 115 000 × g and 4°C with maximum brake. The membrane layer was removed, resuspended in sucrose-tris buffer, and homogenized for 15 s at Polytron setting 8. Calcium chloride (1 mol/L) was added to a final concentration of 10 mmol/L and the homogenate was stirred gently on ice for 10 min. The mixture was then centrifuged for 10 min at 7700 × g and 4°C. The supernate was discarded and the pellet resuspended in sucrose-tris buffer. The resuspended pellet was then homogenized for 15 s at setting 8 and centrifuged for 20 min at 46 000 × g and 4°C. The supernate was discarded and the final basolateral membrane pellet was resuspended in sucrose-tris buffer.

The combined P2 suspension was homogenized for 15 s at setting 8 and centrifuged for 15 min at 19 000 × g and 4°C. The resulting pellet was discarded and the supernate was centrifuged for 15 min at 14 600 × g and 4°C. The pellet was discarded. Calcium chloride (1 mol/L) was added to the supernate to a final concentration of 10 mmol/L and the homogenate was stirred gently on ice for 20 min. The mixture was then centrifuged for 10 min at 3000 × g and 4°C with maximum brake. The supernate was then centrifuged for 30 min at 46 000 × g and 4°C, and the resulting final brush border membrane pellet was resuspended in distilled deionized water. Membrane purity was confirmed through 10–20-fold enrichment of brush border and basolateral membrane markers, alkaline phosphatase, and Na+/K+-ATPase, respectively. Aliquots were stored at −80°C.

**Western blot analysis**

Brush border and basolateral membrane proteins were resolved by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis by using the Mini-Protean II electrophoresis system (BioRad Laboratories) and then transferred onto MSI Nitropure nitrocellulose membrane. The blotting conditions were optimized by multiple trials to ensure complete protein transfer from gel to membrane. After blotting, the membrane was stained with amido black 10B to ensure homogeneous protein transfer and the gel was stained with comassie blue to document complete protein transfer. Primary antibodies (OncoGene Science Inc, Uniondale, NY) included anti-GLUT2 purified rabbit polyclonal immunoglobulin (Ig) G antibody (GLUT2, molecular weight of 60 kDa) and anti-SGLT-1 purified rabbit polyclonal IgG antibody (SGLT-1, molecular weight of 73 kDa).

Immunoblotting was conducted according to the manufacturer's instructions. Briefly, nonspecific binding sites were blocked by immersing the membrane in 5% nonfat dry milk in tris-buffered saline with Tween 20 (TBST, 20 mmol tris/L, 137 mmol NaCl/L, 0.05% Tween 20, pH 7.6) for 1 h at room temperature on a metabolic shaker. The membrane was then washed 3 × 10 min in TBST at room temperature. Primary antibodies were diluted 1:500 in TBST and incubated with membranes for 4 h at room temperature. Blots were washed again 3 × 10 min as described above. The fluorescent reaction was developed by using the SuperSignal CL-HRP Substrate System (Pierce) and exposed to enhanced chemiluminescence detection with Hyperfilm-ECL (Amersham Canada). Relative protein concentrations were determined by using laser densitometry (BioRad model GS-670 imaging densitometer).

**Measurement of transport kinetics**

Determination of transport kinetics was done as described previously (31). Briefly, the 15-cm segment of jejunum and ileum removed from each animal was opened along its mesenteric border and the mucosal surface was carefully washed with cold saline to remove visible mucus and debris. Pieces of intestine (1 cm²) were cut out and the tissue was mounted as flat sheets in incubation chambers containing oxygenated Krebs bicarbonate buffer (pH 7.4) at 37°C. Tissue disks were preincubated in this buffer for 15 min to allow equilibration at this temperature. After preincubation, the chambers were transferred for 6 min to other beakers containing [3H]inulin and various [14C]probe molecules in oxygenated Krebs bicarbonate (pH 7.4 and 37°C).

The concentration of solutes was as follows: lauric acid, 0.1 mmol/L; d-fructose, 4, 8, 16, 32, or 64 mmol/L; d-glucose, 4, 8, 16, 32, or 64 mmol/L; and l-glucose, 1 or 16 mmol/L. The preincubation and incubation solutions were stirred at a rate of 600 rpm to achieve low effective resistance of the intestinal unstirred water (32). The experiment was terminated by removing the chambers and quickly rinsing the tissue in cold saline for <5 s. The exposed mucosal tissue was then cut out of the chambers and dried overnight in an oven at 155°C. The dry weight of the tissue was determined, the sample was saponified with 0.75 mol NaOH/L, scintillation fluid was added (Beckman Ready Solve HP, Beckman Instruments, Fullerton, CA), and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes. The mucosal weight was determined after scraping of adjacent intestinal samples not used for uptake studies. The weight of the mucosa in the samples used to measure uptake was determined by multiplying the dry weight of the intestinal sample by the percentage of the intestinal wall composed of mucosa. Because the proportion of mucosa in the total jejunum or ileum did not differ between groups, the uptake of nutrients was expressed as nmol·100 mg intestine⁻¹·min⁻¹.

**Glucagon-like peptide 2 radioimmunnoassay**

Blood was collected by cardiac puncture into a prechilled
TABLE 2
Effect of short-chain fatty acid (SCFA)–supplemented total parenteral nutrition (TPN) on jejunal and ileal protein and DNA and RNA concentrations after 24 or 72 h of nutrient infusion.1

<table>
<thead>
<tr>
<th></th>
<th>TPN</th>
<th>TPN+SCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h (n = 8)</td>
<td>72 h (n = 7)</td>
</tr>
<tr>
<td>Jejunal weight (mg/cm)</td>
<td>8.4 ± 0.7</td>
<td>8.7 ± 0.9</td>
</tr>
<tr>
<td>Ileal weight (mg/cm)</td>
<td>9.0 ± 0.5</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td>Jejunal mucosa (%)</td>
<td>57.2 ± 3.3</td>
<td>56.7 ± 3.8</td>
</tr>
<tr>
<td>Ileal mucosa (%)</td>
<td>55.7 ± 4.0</td>
<td>57.6 ± 7.3</td>
</tr>
<tr>
<td>Jejunal protein (µg protein/mg jejunum)²</td>
<td>110.9 ± 5.4</td>
<td>103.0 ± 6.3</td>
</tr>
<tr>
<td>Ileal protein (µg protein/mg ileum)</td>
<td>106.2 ± 3.9</td>
<td>112.9 ± 4.4</td>
</tr>
<tr>
<td>Jejunal DNA (µg DNA/mg jejunum)</td>
<td>4.0 ± 0.6</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>Ileal DNA (µg DNA/mg ileum)³</td>
<td>6.7 ± 0.8abc</td>
<td>5.6 ± 0.9 abc</td>
</tr>
<tr>
<td>Jejunal RNA (µg RNA/mg jejunum)</td>
<td>3.8 ± 0.2</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>Ileal RNA (µg RNA/mg ileum)⁶</td>
<td>4.0 ± 0.3abc</td>
<td>3.4 ± 0.4ac</td>
</tr>
</tbody>
</table>

¹ Standard error of the mean. Means within a row with different superscript letters are significantly different, P<0.05.
² Significant effect of time, P<0.05.
³ Significant interaction between diet and time, P<0.03.
⁴, ⁵ Significantly different from jejunal value: ⁴ P<0.01, ⁵ P<0.0001.
⁶ Significant effect of diet (P<0.05) and time, P<0.03.

RESULTS
Nutritional support

All animals received continuous nutrient infusion throughout the study period. Nutrient solutions were infused at a rate established previously to support growth comparable with nonsurgical, ad libitum pellet-fed controls (15).

Jejunal and ileal characteristics

SCFA-supplemented TPN had no influence on the intestinal weight (mg/cm) or percentage of the intestinal wall composed of the mucosa in the jejunum or ileum at either time point (Table 2). In the jejunum, protein, DNA, and RNA concentrations did not differ between groups (Table 2). However, the ileal protein concentration was lower (P<0.05) in both the 24- and 72-h TPN+SCFA groups than in the control TPN groups (Table 2). The ileal DNA concentration was significantly greater (P<0.05) in the 72-h TPN+SCFA than in the 24-h TPN+SCFA group; however, no change was noted within the TPN control groups (Table 2). The ileal RNA concentration increased significantly (P<0.05) in the TPN+SCFA groups; however, the ileal RNA concentration declined (P<0.03) with time irrespective of diet (Table 2).

GLUT2 and SGLT-1 mRNA and protein abundance

GLUT2 mRNA abundance was significantly higher (P = 0.03) in the TPN+SCFA groups at both time points when compared with the control TPN groups (Figure 1). The GLUT2 protein abundance within the jejunal basolateral membrane did not differ between groups (Figure 2). However, in the jejunal basolateral membrane, the abundance of the GLUT2 protein was significantly higher (P<0.04) after 72 h of SCFA-supplemented TPN than in all other groups (Figure 2). Ileal GLUT2 protein abundance in the 72-h TPN+SCFA group was significantly greater (P<0.05) than that of the jejunum.

The SGLT-1 mRNA abundance in jejunal total cellular RNA did not differ among groups (Table 3). Similarly, the SGLT-1 protein abundance in the jejunal and ileal brush border membrane was not altered by SCFA-supplemented TPN after 24 or 72 h of infusion (Table 3). After 72 h of TPN+SCFA, the abundance of SGLT-1 protein was significantly greater (P<0.05) in the ileum than in the jejunum (Table 3).
TABLE 3
Effect of short-chain fatty acid (SCFA)–supplemented total parenteral nutrition (TPN) on jejunal mRNA and protein abundance after 24 or 72 h of nutrient infusion

<table>
<thead>
<tr>
<th></th>
<th>TPN</th>
<th>TPN+SCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>72 h</td>
</tr>
<tr>
<td>Jejunal mRNA</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Jejunal protein</td>
<td>2.8 ± 1.3</td>
<td>0.3 ± 1.0</td>
</tr>
<tr>
<td>Ileal protein</td>
<td>1.7 ± 0.7</td>
<td>2.3 ± 0.7</td>
</tr>
</tbody>
</table>

1 SEM.
2 Significantly different from jejunal protein for time period, P < 0.05.

In vitro nutrient uptake

Jejunal and ileal uptake of 4–64 mmol D-glucose/L was not altered by administration of SCFA-supplemented TPN; there were no differences in the estimated values for the maximal transport rates (Vmax) and apparent Michaelis affinity constant (Km; Table 4). Jejunal uptake of l-glucose, used to estimate the apparent passive permeability coefficient, was significantly higher after 72 h of TPN+SCFA than after 24 h of TPN+SCFA (Table 5). Likewise, jejunal uptake of lauric acid, an indirect measure of unstirred water resistance, was significantly higher (P < 0.05) in the 72-h SCFA group than in the 24-h SCFA group, suggesting a lower unstirred water layer resistance in this group (Table 5). The lauric acid uptake did not differ between the 24- and 72-h control TPN groups (Table 5). SCFA-supplemented TPN had no influence on D-fructose uptake in the jejunum or ileum.

Proglucagon mRNA abundance and plasma GLP-2 concentration

As seen in Figure 3, the proglucagon mRNA abundance was significantly up-regulated (P < 0.05) after 24 h of SCFA-supplemented TPN when compared with the control TPN groups at both time points. The value for the 72-h TPN+SCFA group was not significantly lower than that of the 24-h TPN+SCFA group, nor did it differ from the control TPN groups. Plasma GLP-2 concentration was not significantly altered by SCFA-supplemented TPN after 24 or 72 h of infusion (Figure 4).

DISCUSSION

Intestinal adaptation has been explored primarily by resecting a portion of the small intestine (33) and monitoring the morphologic and functional reactions to this intestinal insult. SCFAs have been shown to promote intestinal adaptation after intestinal resection within 7 d of administration (13, 15, 16); however, few studies have examined the enteroplastic responses to SCFA-supplemented TPN in the normal, unresected gut (13). TPN therapy induces atrophy in patients with an intact small intestine, thus, information regarding the effect of gut-specific fuels in this physiologically discreet, yet prevalent clinical scenario is lacking. This experiment begins to elucidate the early effects of SCFAs on the intestine in normal rats. SCFA supplementation rapidly up-regulates jejunal GLUT2 mRNA, ileal GLUT2 protein, and ileal proglucagon mRNA.

During the first 72 h of SCFA supplementation, no changes in protein, DNA, or RNA concentration occurred in the jejunum.
TABLE 5
Effect of short-chain fatty acid (SCFA)–supplemented total parenteral nutrition (TPN) on L-glucose and lauric acid uptake

<table>
<thead>
<tr>
<th></th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h (n = 4)</td>
<td>72 h (n = 4)</td>
</tr>
<tr>
<td>1 mmol L-glucose/L</td>
<td>9.8 ± 0.8a</td>
<td>11.9 ± 1.2a</td>
</tr>
<tr>
<td>16 mmol L-glucose/L</td>
<td>157 ± 13a</td>
<td>191 ± 20a</td>
</tr>
<tr>
<td>0.1 mmol lauric acid/L</td>
<td>12.8 ± 1.8b</td>
<td>17.4 ± 2.0b</td>
</tr>
</tbody>
</table>

The short administration periods used in this study may not have allowed enough time to produce the well-established SCFA-induced increases in jejunal structural markers observed in previous studies (13–15). Early responses to systemic SCFAs in the ileum include decreased ileal protein contents. The lower ileal protein concentration within the SCFA group was unexpected because SCFAs are known to increase protein concentration after 1 wk of supplementation (14). The hyperplastic response in the small intestine occurs secondary to increased cell turnover in the proliferative zone of the crypts (18). Individual enterocytes thus engendered spend less time in the differentiation zone of the crypt and are believed to be functionally immature (34). At the early time points after SCFA administration examined in this study, the enterocytes may have begun to proliferate, yet have had inadequate time to differentiate, thus, being relatively immature with lower protein content. It appears that structural changes observed after 1 wk of SCFA supplementation cannot be measured with 72 h of SCFA administration in the normal, unresected small intestine. Further characterization and mechanistic understanding of the proliferative and functional response in the early periods after the administration of trophic nutrients is needed.

Systemic SCFAs do not appear to influence the brush border sodium-glucose cotransporter SGLT-1. The up-regulation of jejunal GLUT2 gene expression with SCFA supplementation is consistent with previous reports from our laboratory (16). It is unknown whether the trophic force of intestinal resection prompts a SGLT-1 response or if systemic SCFAs differentially affect brush border and basolateral glucose transporters. Increased ileal GLUT2 protein abundance after 72 h of SCFA-supplemented TPN suggests that this message is translated into protein and inserted into the basolateral membrane. This up-regulation of the basolateral GLUT2, rather than the brush border SGLT-1, may be limited to the parenteral model, wherein nutrients are available only at the basolateral membrane. Studies are currently underway to determine whether the route of SCFA administration has differential effects on nutrient transporter abundance around the enterocytes.

The lack of response of jejunal and ileal glucose uptake to systemic SCFAs was unexpected because ileal D-glucose uptake increased with SCFA supplementation for 3 and 7 d after an 80% intestinal resection (16). It appears that an intestinal insult may be required as trophic stimuli for measurable SCFA-induced changes in glucose uptake to occur within 3 d. In contrast with the short administration of parenteral nutrients presented here, many studies showing increased glucose uptake involve overt models of intestinal adaptation such as intestinal resection (35–37), diabetes (31, 38–44), pregnancy (45), and lactation (46).

The increased lauric acid uptake, which was used as an indirect measure for unstirred water layer resistance, indicates that after 72 h of SCFA supplementation there is less unstirred water layer resistance than after 24 h of SCFA supplementation. The increase in L-glucose uptake with time in the SCFA groups may directly reflect decreased unstirred water layer resistance or, alternatively, a mechanism whereby macromolecular nutrient transport is increased. Gut mucosal macromolecular transmission is believed to be an important phenomenon associated with TPN in response to shortage of substrate (47).

Rather than causing a generalized adaptation of the entire small intestine, the ileum appears to be particularly responsive to systemic administration of SCFAs. This ileal-specific response may be due to a local effect of L cells neighboring ileal enterocytes or enhanced sensitivity to plasma GLP-2. Physiologically, SCFAs are a logical mediator of trophic gastrointestinal hor-

FIGURE 3. Effect of 24 or 72 h of short-chain fatty acid (SCFA)–supplemented total parenteral nutrition (TPN) on ileal proglucagon mRNA abundance. Means with different letters are significantly different, P < 0.05. x ± SEM; n = 7–8.
mones (i.e., proglucagon-derived peptides) and ileal adaptation because they are produced distally within the gastrointestinal tract in response to malabsorbed substrate.

The manner in which SCFAs induce adaptive changes is not directly addressed in the current study. Many studies have established a strong relation between cellular proliferation and elevated amounts of proglucagon mRNA and proglucagon-derived peptides (17, 18, 48–50). SCFAs increased ileal proglucagon mRNA in normal, unresected rats after 24 h of supplementation; however, this increase was not maintained in the 72-h SCFA+TPN group. Taylor et al (20) reported that after intestinal resection proglucagon expression increased threefold, peaking 2 d after surgery and declining thereafter. We showed recently that SCFA-supplemented TPN increased proglucagon mRNA abundance both 3 and 7 d after intestinal resection (15). Within the intestinal resection model, SCFAs may exert their trophic effect by extending the duration of increased proglucagon expression. Although not addressed in the current study, SCFA-supplemented TPN is known to up-regulate the gene expression of ornithine decarboxylase, the rate-limiting enzyme in polyamine synthesis (15). It is possible that ornithine decarboxylase and subsequent polyamine synthesis represent a final pathway through which trophic substances, such as SCFAs and proglucagon-derived peptides stimulate intestinal adaptation.

In summary, SCFA-supplemented TPN rapidly up-regulates jejunal GLUT2 mRNA and ileal GLUT2 abundance and ileal proglucagon mRNA. This study provides insight into potential strategies to promote distal small bowel adaptation that could be useful in the therapeutic management of patients with malabsorption syndromes.

We are grateful for the technical assistance of M Keelan, K Doring, and E Wierzbicki.

**FIGURE 4.** Effect of 24 or 72 h of short-chain fatty acid (SCFA)-supplemented total parenteral nutrition (TPN) on the plasma glucagon-like peptide 2 (GLP-2) concentration. SCFA-supplemented TPN did not influence the plasma GLP-2 concentration after 24 or 72 h of infusion. $\bar{x} \pm$ SEM; $n = 7–8$.

**REFERENCES**

9. Castillo RO, Feng JJ, Stevenson DK, Kwong LK. Altered matura-

15. Tappenden KA, Thomson ABR, Wild GE, McBurney MJ. Short-


