Circulating levels of glucagon-like peptide-2 in human subjects with inflammatory bowel disease

QIANG XIAO,1,* ROBIN P. BOUSHEY,2* MARIA CINO,3 DANIEL J. DRUCKER,3,4 AND PATRICIA L. BRUBAKER1,3

Departments of 1Physiology, 3Medicine, and 2Surgery, Mount Sinai Hospital and the Toronto General Hospital, Toronto M5G 2C4; and the 4Banting and Best Diabetes Centre, University of Toronto, Toronto, Ontario, Canada M5S 1A8

CROHN’S DISEASE (CD) and ulcerative colitis (UC) represent intestinal diseases characterized by repeated episodes of mucosal inflammation that may result in marked alterations in intestinal epithelial structure and function. Although the etiology of both conditions remains unknown, current therapeutic strategies aim to modulate the inflammatory response, minimizing further intestinal injury and allowing endogenous repair mechanisms to restore intestinal integrity (15). Failure of these reparative mechanisms, through either an inadequate reparative response or through repeated episodes of repair and remodeling, may result in fibrosis and stricture, often requiring surgical resection, leading to further compromise of the intestinal epithelium, especially in patients with CD.

Numerous cell populations within the intestine participate in the reparative response through the production and secretion of various cytokines, endocrine peptides, and growth factors with pleiotropic biological activities. The intestinal mucosa contains cells that secrete molecules important for cell proliferation and migration, extracellular matrix formation, immune regulation, and tissue remodeling. Several families of growth factors play an important role in the response to intestinal injury, including the epidermal growth factor family, the transforming growth factor (TGF) superfamily, insulin-like growth factors (IGF), fibroblast growth factors (FGF), hepatocyte growth factor, trefoil factors, platelet-derived growth factor, keratinocyte growth factor (KGF), and vascular endothelial growth factor (9, 23, 24), as well as several members of the cytokine family (15).

The intestinotropic and protective properties of various cytokines and growth factors have prompted analyses of their activity in animal models of experimental intestinal injury. The presence or absence of TGF-α correlates well with the susceptibility to chemically induced intestinal injury in mice (12, 13), whereas administration of either IGF-I or KGF attenuates mucosal injury in rodents with experimental colitis (17, 32). Similarly, deficiency or overexpression of intestinal trefoil factors correlates with increased or reduced susceptibility, respectively, to experimental intestinal injury in murine models in vivo (20, 22). These findings have led to the suggestion that one or more growth factors may be therapeutically useful for enhancing the reparative response to intestinal injury in patients with intestinal disease.

Regulatory peptides with intestinotrophic activity have also been implicated in the response to intestinal inflammation and injury. Co-infusion of peptide YY with parenteral nutrition in rats significantly augmented intestinal mass and protein content, compared with findings in rats infused with parenteral nutrition alone.
(4). Similarly, administration of neurotensin or bombesin results in stimulation of mucosal epithelial proliferation in rodents in vivo (5, 6). The findings that intestinal injury in rodents and humans is commonly associated with increased levels of the gut proglucagon-derived peptides (PGDPs; 1), taken together with observations of gut growth in patients with glucagon-producing tumors (14, 26), ultimately led to the identification of one of the PGDPs, GLP-2, as yet another member of the intestinal regulatory peptide family with trophic properties in vivo (10).

GLP-2 is an endocrine peptide derived from the posttranslational processing of proglucagon in the intestine. GLP-2 and the structurally related PGDP GLP-1 are derived from the same proglucagon precursor, and both peptides are produced and secreted in a nutrient-dependent fashion by the enteroendocrine L cells of the small and large intestine (8, 25, 31). Whereas GLP-1 regulates pancreatic endocrine function and gastric motility (8), GLP-2 is trophic to the intestinal mucosal epithelium via stimulation of crypt cell proliferation and reduction of enterocyte apoptosis (29). Despite the emerging interest in a potential role for GLP-2 in the pathophysiology and/or treatment of intestinal disease, little information is available about the circulating levels and/or the molecular forms of circulating GLP-2 in patients with intestinal injury (1). In rodents, GLP-2-(1—33), in rodents or human subjects. Furthermore, there is no information available regarding the levels of circulating GLP-2-(1—33) in patients with intestinal disease. As the levels of PGDPs have been reported to be altered in the adapting or injured intestine (1), we have now determined whether patients with intestinal injury exhibit abnormalities in the levels and/or the molecular forms of circulating GLP-2 in vivo.

MATERIALS AND METHODS

Study group. Blood for analysis of GLP-2 was collected from the following groups of patients after written informed consent: 1) normal healthy controls (n = 14, 6 males and 8 females, mean age 28.9 ± 4.8 yr); 2) immune controls (patients with rheumatological diseases, n = 18, 2 males and 16 females, mean age 57.3 ± 16 yr, mean duration of disease 12.4 ± 9.9 yr and patients with liver transplants, n = 20, 11 males and 9 females, mean age 53.2 ± 8 yr, mean number of years after transplant 7.6 ± 5.9); 3) patients with CD without bowel resection (n = 30, 17 males and 13 females, mean age 31.9 ± 11.8 yr, 12 with small bowel disease, 10 with large bowel disease, and 8 with combined small and large bowel involvement, mean duration of clinical disease 4.5 ± 5.1 yr); 4) patients with UC (n = 21, 17 males and 4 females, mean age 29.0 ± 11.0 yr, 20 with pancolitis, 1 with left-sided colitis, mean duration of disease 4.3 ± 5.9 yr); and 5) CD and intestinal resection (n = 9, 3 males and 6 females, mean age 39.3 ± 13.2 yr, 4 patients with distal small bowel resection, 1 patient with colonic resection, and 4 with combined small and large bowel resection, mean duration of disease 12.9 ± 12.7 yr). Blood for analysis of dipeptidyl peptidase IV (DPP IV) was collected from six healthy controls (3 males and 3 females, mean age 27.5 ± 4.9 yr), one patient with CD without bowel resection (female, age 27 yr, duration 8 yr), four patients with CD and bowel resection (3 males and 1 female, mean age 32.5 ± 3.0 yr, mean duration 14.3 ± 3.8 yr), and one patient with UC (male, age 23 yr, duration 1 yr).

Given the wide spectrum of clinical presentation in patients with inflammatory bowel disease (IBD), we have limited our investigation to patients with clinically active IBD requiring hospitalization for either complications of their underlying disease or due to refractoriness to conventional forms of medical management. All patients included in this study had a diagnosis of CD or UC established on the basis of 1) clinical history, 2) distribution of disease, and 3) histological diagnosis on previous intestinal biopsy or resection when available. All patients underwent diagnostic testing to localize areas of active intestinal disease, including endoscopy, intestinal contrast studies, or computerized-automated tomography after venipuncture during their hospitalization. All of the blood samples were obtained before any abdominal surgery. All patients and controls fasted from midnight on, and a blood sample was obtained via venipuncture the following morning between 8:00 and 10:00 AM. The characteristics of the 60 patients analyzed for levels of GLP-2 and 6 patients studied for DP IV activity in this study are shown in Table 1.

Sample collection. For RIA of immunoreactive (IR)-GLP-2, blood samples were collected on ice in 10% vol/vol of Trasylol-EDTA-Diprotin A (5,000 kallikrein inhibitory units of Trasylol/ml; Miles Canada, Etobicoke, Canada):1.2 mg/ml EDTA:0.1 mM Diprotin A (ILE-PRO-ILE; Sigma Chemical, St. Louis, MO), an inhibitor of DP IV activity, to prevent enzymatic degradation of intact GLP-2 as previously described (2, 11). For assay of DP IV activity, blood was collected in 10% vol/vol Trasylol-EDTA. After centrifugation, plasma was collected and stored at −70°C until extraction. All blood samples were obtained after patients gave signed informed consent under protocols approved by the Human Ethics Committee at the Mount Sinai and Toronto General Hospital (Toronto, ON, Canada).

Peptide extraction. Plasma samples were acidified by addition of two volumes of 1% trifluoroacetic acid (TFA; pH adjusted to 2.5 with diethylamine), and peptides were extracted by passage twice through a Sep-Pak C18 cartridge (Waters Associates, Milford, MA). After being washed with 0.1% TFA, the peptides adsorbed onto the cartridge were eluted with 80% isopropanol containing 0.1% TFA. Recovery of GLP-2 with this extraction technique is 84 ± 17%, as reported previously (2, 11).

RIA. RIA for GLP-2 was performed using antiserum UTH7, as described previously (2, 11, 31). This antiserum recognizes the midsequence of GLP-2 (amino acids 25–30), and thus cross-reacts equally with intact GLP-2-(1–33), biologically inactive GLP-2-(3–33), and the inactive pancreatic precursor, major proglucagon fragment (MPGF), but has no cross-reactivity with GLP-1, glucagon, or other structurally related peptides (Fig. 1A). Fifty percent binding of the tracer was observed at 125 pg/tube, and the sensitivity of the assay was 10 pg/tube.

Reversed-phase HPLC. HPLC was performed using a Waters system with a uBondapak C18 column (Waters Associates). The solvent systems used were 0.1% (vol/vol) TFA in water (solvent A) and 0.1% (vol/vol) TFA in acetonitrile (solvent B). All plasma samples were extracted by Sep-Pak before loading onto the HPLC column. GLP-2-(1–33) was separated from GLP-2-(3–33) with the use of a gradient of 30–60% solvent B over 45 min, followed by a purge with 99% solvent B for 10 min. The solvent flow rate was 1.5 ml/min, and 18-s fractions were collected (2, 11, 31).

DP IV assay. Ninety-six well plates were loaded with 50 µl of 0.1 mM Tris (pH 7.4), 60 µl of plasma, and 90 µl of 1.11 mM
Gly-Pro-p-nitroanilide (substrate; Sigma Chemical). Absorbance at 450 nm was recorded immediately on addition of the substrate and then at 5-min intervals for 30 min to monitor the appearance of the product p-nitroaniline, using a Packard SpectraCount Microplate Photometer (Canberra Packard Canada, Mississauga, ON, Canada). A standard curve was prepared using concentrations of p-nitroaniline (Sigma Chemical) ranging from 0 to 1 mM in 0.1 M Tris buffer. Enzyme activity was determined as the micromoles of p-nitroaniline produced per minute per milliliter of plasma (U/ml), as previously described (3).

Data analysis. All data are expressed as means ± SE. Statistical differences between groups were determined by unpaired Student’s t-test or by ANOVA using n = 1 post hoc custom hypotheses tests, as appropriate, on a SAS system (Statistical Analysis Systems, Cary, NC).

Table 1. Profiles of hospitalized patients with IBD analyzed in this study for levels of circulating GLP-2 (patients 1–60) or DP-IV activity (patients 61–66)

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>Location</th>
<th>Resection</th>
<th>Duration</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>M</td>
<td>CD I/C</td>
<td>N</td>
<td>15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>F</td>
<td>UC C</td>
<td>N</td>
<td>1</td>
<td>5-ASA, S</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>F</td>
<td>UC C</td>
<td>N</td>
<td>2</td>
<td>5-ASA, S</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>F</td>
<td>CD C</td>
<td>N</td>
<td>1</td>
<td>F, C</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>3</td>
<td>5-ASA</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>M</td>
<td>CD I/C</td>
<td>N</td>
<td>2</td>
<td>5-ASA</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>M</td>
<td>CD I/C</td>
<td>N</td>
<td>2</td>
<td>5-ASA, I, F, C</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>F</td>
<td>CD I/J jejunost</td>
<td>Y</td>
<td>5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
<td>F</td>
<td>CD C</td>
<td>Y</td>
<td>5</td>
<td>S, F</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>48</td>
<td>F</td>
<td>CD C</td>
<td>N</td>
<td>1</td>
<td>C, O</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>67</td>
<td>M</td>
<td>UC C/D/C</td>
<td>N</td>
<td>4</td>
<td>O</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>39</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>4</td>
<td>S, I</td>
<td>—</td>
</tr>
<tr>
<td>13</td>
<td>33</td>
<td>F</td>
<td>CD I/C</td>
<td>Y</td>
<td>8</td>
<td>S</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>26</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>1</td>
<td>P</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>26</td>
<td>F</td>
<td>UC C</td>
<td>N</td>
<td>1</td>
<td>P</td>
<td>—</td>
</tr>
<tr>
<td>16</td>
<td>28</td>
<td>M</td>
<td>CD I</td>
<td>N</td>
<td>1</td>
<td>S</td>
<td>—</td>
</tr>
<tr>
<td>17</td>
<td>19</td>
<td>F</td>
<td>CD I</td>
<td>N</td>
<td>3</td>
<td>S, F</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>45</td>
<td>M</td>
<td>CD C</td>
<td>N</td>
<td>2</td>
<td>3TC HBV</td>
<td>—</td>
</tr>
<tr>
<td>19</td>
<td>47</td>
<td>F</td>
<td>CD I</td>
<td>Y</td>
<td>4</td>
<td>S</td>
<td>—</td>
</tr>
<tr>
<td>20</td>
<td>18</td>
<td>F</td>
<td>CD I/C</td>
<td>N</td>
<td>6</td>
<td>F, C</td>
<td>—</td>
</tr>
<tr>
<td>21</td>
<td>23</td>
<td>F</td>
<td>CD I/C</td>
<td>N</td>
<td>1</td>
<td>S-ASA, F, C</td>
<td>—</td>
</tr>
<tr>
<td>22</td>
<td>25</td>
<td>F</td>
<td>CD I</td>
<td>N</td>
<td>1wk</td>
<td>F, C</td>
<td>—</td>
</tr>
<tr>
<td>23</td>
<td>38</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>1</td>
<td>S, V</td>
<td>—</td>
</tr>
<tr>
<td>24</td>
<td>24</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>2</td>
<td>S-ASA</td>
<td>—</td>
</tr>
<tr>
<td>25</td>
<td>16</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>2</td>
<td>S-ASA</td>
<td>—</td>
</tr>
<tr>
<td>26</td>
<td>54</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>3wks</td>
<td>S</td>
<td>—</td>
</tr>
<tr>
<td>27</td>
<td>39</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>1</td>
<td>S</td>
<td>—</td>
</tr>
<tr>
<td>28</td>
<td>31</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>3</td>
<td>S, F</td>
<td>—</td>
</tr>
<tr>
<td>29</td>
<td>22</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>2</td>
<td>S-ASA</td>
<td>—</td>
</tr>
<tr>
<td>30</td>
<td>15</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>4</td>
<td>S, F, I</td>
<td>—</td>
</tr>
<tr>
<td>31</td>
<td>31</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>14</td>
<td>S, F, I</td>
<td>—</td>
</tr>
<tr>
<td>32</td>
<td>21</td>
<td>M</td>
<td>CD I</td>
<td>N</td>
<td>3mo</td>
<td>S-ASA</td>
<td>—</td>
</tr>
<tr>
<td>33</td>
<td>24</td>
<td>M</td>
<td>CD I</td>
<td>N</td>
<td>8</td>
<td>S</td>
<td>—</td>
</tr>
<tr>
<td>34</td>
<td>31</td>
<td>M</td>
<td>CD I</td>
<td>N</td>
<td>3wks</td>
<td>F, C</td>
<td>—</td>
</tr>
<tr>
<td>35</td>
<td>24</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>6</td>
<td>S-ASA</td>
<td>—</td>
</tr>
<tr>
<td>36</td>
<td>43</td>
<td>F</td>
<td>UC C</td>
<td>N</td>
<td>2</td>
<td>S-ASA</td>
<td>—</td>
</tr>
<tr>
<td>37</td>
<td>37</td>
<td>F</td>
<td>CD C</td>
<td>N</td>
<td>3</td>
<td>S, C, F</td>
<td>—</td>
</tr>
<tr>
<td>38</td>
<td>35</td>
<td>M</td>
<td>CD I</td>
<td>N</td>
<td>1</td>
<td>S, C, F</td>
<td>—</td>
</tr>
<tr>
<td>39</td>
<td>33</td>
<td>F</td>
<td>CD I</td>
<td>N</td>
<td>3</td>
<td>S, C, F</td>
<td>—</td>
</tr>
<tr>
<td>40</td>
<td>49</td>
<td>M</td>
<td>CD I/C</td>
<td>Y</td>
<td>37</td>
<td>S, F, C, I</td>
<td>—</td>
</tr>
<tr>
<td>41</td>
<td>36</td>
<td>F</td>
<td>CD I/C</td>
<td>N</td>
<td>5mo</td>
<td>F, C</td>
<td>—</td>
</tr>
<tr>
<td>42</td>
<td>23</td>
<td>F</td>
<td>CD I</td>
<td>N</td>
<td>4</td>
<td>F, C</td>
<td>—</td>
</tr>
<tr>
<td>43</td>
<td>25</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>2</td>
<td>S-ASA</td>
<td>—</td>
</tr>
<tr>
<td>44</td>
<td>27</td>
<td>M</td>
<td>UC C/C</td>
<td>N</td>
<td>7</td>
<td>S, F, C</td>
<td>—</td>
</tr>
<tr>
<td>45</td>
<td>23</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>2</td>
<td>S, I, S-ASA</td>
<td>—</td>
</tr>
<tr>
<td>46</td>
<td>33</td>
<td>F</td>
<td>CD I/C</td>
<td>Y</td>
<td>15</td>
<td>S, F, C</td>
<td>—</td>
</tr>
<tr>
<td>47</td>
<td>26</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>4</td>
<td>S, F</td>
<td>—</td>
</tr>
<tr>
<td>48</td>
<td>47</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>2</td>
<td>S, F, V</td>
<td>—</td>
</tr>
<tr>
<td>49</td>
<td>61</td>
<td>M</td>
<td>CD I</td>
<td>Y</td>
<td>20</td>
<td>F, C</td>
<td>—</td>
</tr>
<tr>
<td>50</td>
<td>28</td>
<td>F</td>
<td>UC C</td>
<td>N</td>
<td>14</td>
<td>S, F, C</td>
<td>—</td>
</tr>
<tr>
<td>51</td>
<td>43</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>1.5</td>
<td>S, F</td>
<td>—</td>
</tr>
<tr>
<td>52</td>
<td>26</td>
<td>M</td>
<td>CD I</td>
<td>N</td>
<td>4</td>
<td>BD, F, C</td>
<td>—</td>
</tr>
<tr>
<td>53</td>
<td>33</td>
<td>M</td>
<td>CD I</td>
<td>N</td>
<td>13</td>
<td>BD, F, C</td>
<td>—</td>
</tr>
<tr>
<td>54</td>
<td>49</td>
<td>M</td>
<td>CD I/C</td>
<td>Y</td>
<td>29</td>
<td>S, F, C</td>
<td>—</td>
</tr>
<tr>
<td>55</td>
<td>53</td>
<td>F</td>
<td>CD I/C</td>
<td>N</td>
<td>20</td>
<td>S, F, C</td>
<td>—</td>
</tr>
<tr>
<td>56</td>
<td>27</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>7</td>
<td>S, F</td>
<td>—</td>
</tr>
<tr>
<td>57</td>
<td>22</td>
<td>M</td>
<td>CD I/C</td>
<td>N</td>
<td>8</td>
<td>S, C, BD</td>
<td>—</td>
</tr>
<tr>
<td>58</td>
<td>42</td>
<td>M</td>
<td>CD C</td>
<td>N</td>
<td>6</td>
<td>F, C, S-ASA</td>
<td>—</td>
</tr>
<tr>
<td>59</td>
<td>22</td>
<td>M</td>
<td>CD C</td>
<td>N</td>
<td>2</td>
<td>S, F, C</td>
<td>—</td>
</tr>
<tr>
<td>60</td>
<td>15</td>
<td>F</td>
<td>UC C</td>
<td>N</td>
<td>2wk</td>
<td>S, F</td>
<td>—</td>
</tr>
<tr>
<td>61</td>
<td>23</td>
<td>M</td>
<td>UC C</td>
<td>N</td>
<td>1</td>
<td>S</td>
<td>—</td>
</tr>
<tr>
<td>62</td>
<td>27</td>
<td>F</td>
<td>CD I/C</td>
<td>N</td>
<td>8</td>
<td>S, C, F</td>
<td>—</td>
</tr>
<tr>
<td>63</td>
<td>37</td>
<td>M</td>
<td>CD I/C</td>
<td>Y</td>
<td>17</td>
<td>S-ASA, BD</td>
<td>—</td>
</tr>
<tr>
<td>64</td>
<td>29</td>
<td>M</td>
<td>CD I/C</td>
<td>Y</td>
<td>22</td>
<td>BD, F, C, I</td>
<td>—</td>
</tr>
<tr>
<td>65</td>
<td>26</td>
<td>M</td>
<td>CD I/C</td>
<td>Y</td>
<td>14</td>
<td>S, C, F</td>
<td>—</td>
</tr>
<tr>
<td>66</td>
<td>29</td>
<td>F</td>
<td>CD I/C</td>
<td>Y</td>
<td>4</td>
<td>BD, F, C</td>
<td>—</td>
</tr>
</tbody>
</table>

CD, Crohn's Disease; UC, ulcerative colitis; I, ileum; C, colon; I/C, both ileum and colon; J/ejunost, jejunum plus jejunostomy; GD, gastroduodenal; S, glucocorticoids such as Prednisone, Solumedrol, or Solucortef; S-ASA, Pentasa or Asacol; F, Flagyl; BD, Budesonide; I, Imuran; C, Ciprofloxacin; V, vancomycin; CsA, cyclosporin; GLP, glucagon-like peptide; IBD, inflammatory bowel disease; DP IV, dipeptidyl peptidase IV; M, male; F, female; Y, yes; N, no. Duration of disease is indicated in years unless indicated as weeks or months.
RESULTS

It is well established that a number of growth factors demonstrate trophic effects on the intestinal epithelium. With the exception of nutrient ingestion (31), the factors that regulate production and secretion of human GLP-2-(1–33) have yet to be elucidated. As the proglucagon gene is expressed in the endocrine pancreas and gastrointestinal tract, circulating levels of total IR-GLP-2 are therefore composed of at least three different molecular forms (Fig. 1A) of GLP-2 (2, 11, 31), including bioactive GLP-2-(1–33) liberated from intestinal endocrine cells, inactive GLP-2-(3–33) (produced via DP IV-mediated cleavage at Ala2), and inactive MPGF (16, 21) containing the unprocessed carboxy terminal sequences of proglucagon, including both GLP-1 and GLP-2. Antiserum against the carboxy terminal region of the GLP-2 molecule will potentially recognize at least three different PGDPs, including MPGF, GLP-2-(1–33), and GLP-2-(3–33) (31).

As shown in Fig. 1B, plasma levels of total IR-GLP-2 were not different between normal healthy and immunocompromised control subjects (706 ± 44 pg/ml, n = 14 vs. 781 ± 75 pg/ml, n = 38, respectively). Total IR-GLP-2 levels were also not different from normal controls in patients with UC (660 ± 69 pg/ml, n = 21). However, circulating levels of total IR-GLP-2 in patients with CD were significantly decreased (532 ± 46 pg/ml, n = 30; P < 0.05 vs. normal controls). There were no significant differences in total IR-GLP-2 levels between the different patients with CD when these individuals were subgrouped according to the site of disease activity (e.g., small intestine: 526 ± 105 pg/ml, n = 12; large intestine: 547 ± 49 pg/ml, n = 10; and both small and large intestine: 521 ± 61 pg/ml, n = 8). However, a significant decrease in total IR-GLP-2 levels was observed in those patients with CD who had a history of intestinal resection (373 ± 44 pg/ml, n = 9; P < 0.01).

As total GLP-2-IR comprises multiple molecular forms of GLP-2 (Fig. 1A), including MPGF, GLP-2-(1–33), and its circulating degradation product GLP-2-(3–33), the plasma levels of total IR-GLP-2 are clearly much higher than the levels of intact bioactive GLP-2-(1–33) (31). Accordingly, reversed-phase HPLC was used to determine the circulating levels of GLP-2-(1–33) and GLP-2-(3–33) (Fig. 2). Plasma samples from all subjects contained two peaks of IR-GLP-2 that eluted with the same retention times as synthetic human GLP-2-(1–33) and GLP-2-(3–33) (as shown in Fig. 2A for normal controls or patients with UC or CD). Consistent with results of previous studies (11, 31), the concentration of circulating intestinal GLP-2-(1–33) plus GLP-2-(3–33) (as determined from area under the curve analyses of the HPLC profiles) in normal human subjects was 67.5 ± 3.2 pg/ml, and this was not significantly altered in patients with IBD (Fig. 2B). However, when the levels of GLP-2-(1–33) alone were determined, the levels of this bioactive peptide were increased to 229 ± 65% of normal in patients with UC and to 317 ± 89% (P < 0.05) in those with CD. Furthermore, the proportion of GLP-2-(1–33) compared with GLP-2-(3–33) was increased in patients with IBD; GLP-2-(1–33) accounted for 43 ± 3% of these peptides in normal subjects (ratio 1:1.4 ± 0.2), whereas the proportion of GLP-2-(1–33) was increased to 61 ± 6% (P < 0.01) of normal in patients with UC (ratio 1:0.7 ± 0.1) and to 59 ± 2% (P < 0.01) of normal in patients with CD (ratio 1:0.7 ± 0.1; Fig. 3). The relative proportions of GLP-2-(1–33) and GLP-2-(3–33) were not altered in immunocompromised control patients (data not shown).

The finding of an increase in the ratio of GLP-2-(1–33) to GLP-2-(3–33) in patients with IBD suggested that the rate of DP IV-mediated degradation to GLP-2-(3–33) might be reduced in some patients with this condition. To address this possibility, we measured DP IV enzyme activity in plasma (collected in the absence of DP IV inhibitors) from normal subjects and patients with IBD. As shown in Fig. 4, plasma DP IV activity was significantly decreased in patients with IBD compared with normal subjects (1.4 ± 0.3 vs. 5.0 ± 1.1 mU/ml, respectively; P < 0.05).

DISCUSSION

The results of the present study indicate that the circulating levels of total IR-GLP-2 are reduced in patients with CD, but not in those with UC, when compared with healthy or immunocompromised controls. Analysis of plasma total IR-GLP-2 in immunocompromised control patients did not demonstrate altered levels of circulating total IR-GLP-2, when compared with normal controls, suggesting that the decreased circulating levels of total IR-GLP-2 observed in patients with CD was not simply due to the inflammatory process or to the unique profile of medications administered to these patients. In addition, we have not observed changes in the intestinal levels of GLP-2 in mice administered combinations of agents used to treat human subjects with IBD (unpublished observations). However, as pancreatic MPGF accounts for much of the circulating total IR-GLP-2 in human plasma (31), the changes observed in patients with CD suggest that pancreatic MPGF secretion or clearance may be altered in these individuals. Furthermore, the most striking (~50%) reduction in circulating levels of total IR-GLP-2 was observed in patients with CD and previous ileal resection, in keeping with the localization and relative abundance of GLP-2-producing enteroendocrine L cells in the distal ileum.

The finding that patients with intestinal resection exhibit reduced levels of circulating GLP-2 is consistent with a recent report describing impaired meal-stimulated increases in circulating GLP-2 in patients with intestinal failure and ileal resection (18). In contrast, extensive damage to or surgical resection of the terminal ileum (18) produces a state of relative GLP-2 deficiency, due to impaired function or resection of enteroendocrine cells that produce GLP-2. The latter findings led Jepessen and colleagues (18) to postulate that restoration of adequate levels of GLP-2 in patients with intestinal failure may represent a physiologically
relevant form of intestinal hormone replacement in vivo.

In contrast to GLP-2 deficiency in patients with ileal resection, HPLC analysis demonstrated a striking two-to-threefold elevation in the level of bioactive GLP-2-(1—33): Two-to-threefold elevation in the level of bioactive GLP-2-(1—33) in nonresected IBD patients with either CD or UC. Interestingly, previous studies demonstrated elevated plasma levels of some of the intestinal PGDPs in rodents with intestinal injury and in several human...
activities was reduced in IBD patients (by GLP-2-(1—33) and GLP-2-(3—33) is also regulated by 
In keeping with the findings from studies of GLP-1, the 
tive degradation product GLP-1-(9—36) amide (7, 19). 
Indeed, much of the available literature assessing 
peptides highly susceptible to cleavage by DP IV. 
amino terminal alanine at position 2, rendering these 
amount of this peptide increases further after nutrient 
normal human plasma from fasted individuals, and the 
the biologically inactive GLP-2-(3—33) is present in 
forms of GLP-2 for interpretation of physiological 
importance of using antisera and/or separation tech-
iques that discriminate among the different molecular 
forms of GLP-2 for interpretation of physiological 
changes in the levels of I R-GLP-2 peptides that circu-
late in vivo.

We have recently shown that a significant amount of 
the biologically inactive GLP-2-(3—33) is present in 
normal human plasma from fasted individuals, and the 
amount of this peptide increases further after nutrient 
ingestion (31). Both GLP-1 and GLP-2 contain an 
amino terminal alanine at position 2, rendering these 
peptides highly susceptible to cleavage by DP IV. 
Indeed, much of the available literature assessing 
circulating levels of GLP-1 is difficult to interpret 
because of the use of antisera that did not discriminate 
between bioactive GLP-1-(7—36) amide and the 
inactive degradation product GLP-1-(9—36) amide (7, 19). 
In keeping with the findings from studies of GLP-1, the 
available evidence suggests that the proportion of 
GLP-2-(1—33) and GLP-2-(3—33) is also regulated by 
the activity of the enzyme DP IV (2, 11).

Our observation that circulating DP IV enzymatic 
activity was reduced in IBD patients (by ~3.5-fold) is 
consistent with the potential importance of circulating 
DP IV as a component of the adaptive response to 
testinal injury in vivo. These findings suggest that 
regulation of DP IV activity may reflect the physiologi-
ical importance of maintaining levels of bioactive GLP-2-
(1—33) in settings of intestinal injury such as human 
IBD. Whether the reduced levels of circulating DP IV 
activity in IBD patients reflect a decrease in synthesis, 
increased clearance, and/or attenuated enzymatic activity 
merits further exploration.

Perspectives

In summary, GLP-2 represents an intestinal-derived 
peptide with significant reparative activity for the 
mucosal epithelium of the small and large intestine. 
The current study demonstrates an increase in circulating 
levels of bioactive GLP-2-(1—33) in patients hospi-
talized for the treatment of IBD in association with 
reduced plasma activity of DP IV. As DP IV is the key 
enzyme responsible for regulating the biological activity 
of GLP-2-(1—33) in vivo, these findings suggest that 
regulation of DP IV activity may be a previously 
unrecognized adaptive mechanism accounting for 
increased circulating levels of biologically active GLP-2-
(1—33) in the setting of intestinal damage and/or 
inflammation. Our findings are consistent with the 
hythesis that maintaining an appropriate level of 
circulating GLP-2-(1—33) via increased synthesis or 
secretion and/or reduced degradation of the biologically 
active peptide may contribute to the capacity for endog-
ogenous repair of epithelial injury in the human intesti-
ne.

Q. Xiao was supported in part by an operating grant from NPS Allelix (Mississauga, ON, Canada). This work was supported by grants from NPS Allelix (to P. L. Brubaker), the Medical Research 
Council of Canada (to P. L. Brubaker and D. J. Drucker), and the 
Ontario Research and Development Challenge Fund (to D. J. Drucker).

D. J. Drucker is a Scientist of the Medical Research Council of 
Canada and is a consultant to NPS Allelix. GLP-2 is the subject of a 
licensing agreement between the Toronto General Hospital, the 
University of Toronto, and D. J. Drucker.

Address for reprint requests and other correspondence: P. L. Brubaker, 
Rm. 3366, Medical Science Bldg., Univ. of Toronto, 1 Kings College Circle, 
Toronto, Ontario, M5S 1A8 Canada (E-mail: p.brubaker@utoronto.ca).

Received 16 July 1999; accepted in final form 4 November 1999.

REFERENCES

adaptation [a major role for enteroglucagon]. Scand J Gastroen-
2. Brubaker PL, Crivici A, Izzo A, Ehrlich P, Tsai C-H, and 
Drucker DJ. Circulating and tissue forms of the intestinal growth 
4843, 1997.
3. Brubaker PL, Izzo A, Hill M, and Drucker DJ. Intestinal 
function in mice with small bowel growth induced by glucagon-
4. Chance WF, Zhang X, Balasubramaniam A, and Fischer 
J E. Preservation of intestine protein by peptide YY during total 
5. Chu KU, Higashide S, Evers BM, Ishizuka J, Townsend CM 
Jr, and Thompson JC. Bombesin stimulates mucosal growth in 
jejunal and ileal Thiry-Vella fistulas. Ann Surg 221: 602—611, 
1995.
6. Chung DH, Evers BM, Shimoda I, Townsend CMJr, Rajara-
man S, and Thompson JC C. Effect of neotensin on gut 
mucosal growth in rats with jejunal and ileal Thiry-Vella fistulas. 
7. Deacon CF, Johnson AH, and Holst JJ. Degradation of 
glucagon-like-peptide-1 by human plasma in vitro yields an 
N-terminally truncated peptide that is a major endogenous 
8. Drucker DJ. The glucagon-like peptides. Diabetes 47: 159—169, 
1998.
10. Drucker DJ, Ehrlich P, Asa SL, and Brubaker PL. Induction 
of intestinal epithelial proliferation by glucagon-like peptide 2. 
Proc Natl Acad Sci USA 93: 7911—7916, 1996.
11. Drucker DJ, Shi Q, Crivici A, Sumner-Smith M, Tavares W, 
Hill M, Deforest L, Cooper S, and Brubaker PL. Regulation 

of the biological activity of glucagon-like peptide 2 by dipeptidyl
12. Egger B, Carey HV, Procaccino F, Chai N-N, Sandgren EP,
Lakshmanan J, Buslon VS, French QW, Buchler MW, and
Eysselein VE. Reduced susceptibility of mice overexpressing
transforming growth factor α to dextran sodium sulphate colitis.
13. Egger B, Procaccino F, Lakshmanan J, Reinshagen M,
Hoffman P, Patel A, Reuben W, Gnanakkan S, Liu L,
Barajas L, and Eysselein VE. Mice lacking transforming
growth factor α have an increased susceptibility to dextran
RH. Endocrine tumour in kidney affecting small bowel structure,
841–848, 1996.
16. Holst JJ, Bersani M, J ohnsen AH, Kofod H, Hartmann B,
17. Howarth GS, Xian CJ, and Read LC. Insulin-like growth
factor-1 partially attenuates colonic damage in rats with experi-
mental colitis induced by oral dextran sulphate sodium. Scand J
18. J eppesen PB, Hartmann B, Hansen BS, Thulesen J, Holst
JJ, and Mortensen PB. Impaired meal-stimulated glucagon-
like peptide-2 response in ileal resected short bowel patients
19. Kieffer TJ, Mclntosh ChS, and Pederson RA. Degradation of
glucose-dependent insulinotropic polypeptide and truncated
glucagon-like peptide 1 in vitro and in vivo by dipeptidyl pepti-
Impaired defense of intestinal mucosa in mice lacking intestinal
21. Patzell C and Schliit E. Conversion of proglucagon in pancre-
aric alpha cells: the major endproducts are glucagon and a single
peptide, the major proglucagon fragment, that contains two
(glucagon-like sequences. Proc Natl Acad Sci USA 81: 5007–5011,
1984.
22. Playford Rj, Marchbank T, Goodlad RA, Chinery RA,
Poulson R, Hanby AM, and Wright NA. Transgenic mice that
overexpress the human trefoil peptide pS2 have an increased
resistance to intestinal damage. Proc Natl Acad Sci USA 93:
2137–2142, 1996.
23. Podolsky DK. Healing the epithelium: solving the problem from
24. Podolsky DK. Regulation of intestinal epithelial proliferation: a
few answers, many questions. Am J Physiol Gastrointest Liver
25. Roberge JN and Brubaker PL. Secretion of proglucagon-
derived peptides in response to intestinal luminal nutrients.
26. Stevens FM, Flanagan RW, O’Gorman D, and Buchanan
KD. Glucagonoma syndrome demonstrating giant duodenal villi.
27. Taylor RG, Beveridge DJ, and Fuller PJ. Expression of ileal
glucagon and peptid tyrosine-tyrosine genes. Response to inhibition
of polyamine synthesis in the presence of massive small
28. Taylor RG and Fuller PJ. Humoral regulation of intestinal
29. Tsai CH, Hill M, Asa SL, Brubaker PL, and Drucker DJ.
Intestinal growth-promoting properties of glucagon-like peptide-
30. Ulshen MH, Hoyt EC, Fuller CR, Ghatel MA, Bloom SR,
and Lund PK. Increased ileal proglucagon expression after jejuno-
ectomy is not suppressed by inhibition of bowel growth. Dis
Carcinoma 41: 677–683, 1996.
31. Xiao Q, Boushey RP, Drucker DJ, and Brubaker PL.
Secretion of the intestinotropic hormone glucagon-like peptide-2 is
differentially regulated by nutrients in humans. Gastroenterology
32. Zeeh JM, Procaccino F, Hoffmann P, Aukerman SL, McRob-
erts J A, Soltani S, Pierce GF, Lakshmanan J, Lacey D, and
Eysselein VE. Keratinocyte growth factor ameliorates mucosal
injury in an experimental model of colitis in rats. Gastroenterology