Am J Physiol Regulatory Integrative Comp Physiol
278: R1057–R1063, 2000.

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

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

Xiao, Qiang, Robin P. Boushey, Maria Cino, Daniel J. Drucker, and Patricia L. Brubaker. Circulating levels of glucagon-like peptide-2 in human subjects with inflammatory bowel disease. *Am J Physiol Regulatory Integrative Comp Physiol* 278: R1057–R1063, 2000.— Glucagon-like peptide-2 (GLP-2) is a recently characterized intestine-derived peptide that exerts trophic activity in the small and large intestine. Whether circulating levels of GLP-2 are perturbed in the setting of human inflammatory bowel disease (IBD) remains unknown. The circulating levels of bioactive GLP-2-(1—33) compared with its degradation product GLP-2-(3—33) were assessed using a combination of RIA and HPLC in normal and immunocompromised control human subjects and patients hospitalized for IBD. The activity of the enzyme dipeptidyl peptidase IV (DP IV), a key determinant of GLP-2-(1—33) degradation was also assessed in the plasma of normal controls and subjects with IBD. The circulating levels of bioactive GLP-2-(1—33) were increased in patients with either ulcerative colitis (UC) or Crohn's Disease (CD; to 229 ± 65 and 317 ± 89%, $P < 0.05$, of normal, respectively). Furthermore, the proportion of total immunoreactivity represented by intact GLP-2-(1—33), compared with GLP-2-(3—33), was increased from 43 ± 3% in normal healthy controls to 61 ± 6% ($P < 0.01$) and 59 ± 2% ($P < 0.01$) in patients with UC and CD, respectively. The relative activity of plasma DP IV was significantly reduced in subjects with IBD compared with normal subjects (1.4 ± 0.3 vs. 5.0 ± 1.1 mU/ml, respectively; $P < 0.05$). These results suggest that patients with active IBD may undergo an adaptive response to intestinal injury by increasing the circulating levels of bioactive GLP-2-(1—33), facilitating enhanced repair of the intestinal mucosal epithelium in vivo.

Crohn's disease; ulcerative colitis; short bowel syndrome; intestine

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. Coinfusion 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

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(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 of the biologically active form of the molecule, 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 (DP 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 C_{18} 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 UTTH7, 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. 1*A*). 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 C_{18} 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

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)*

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

CD, Crohn's Disease; UC, ulcerative colitis; I, ileum; C, colon; I/C, both ileum and colon; J/Jejunost, jejunum plus jejunostomy; GD, gastroduodenal; S, glucocorticoids such as Prednisone, Solumedrol, or Solucortef; 5-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, depeptidyl peptidase IV; M, male; F, female; Y, yes; N, no. Duration of disease is indicated in years unless indicated as weeks or months.

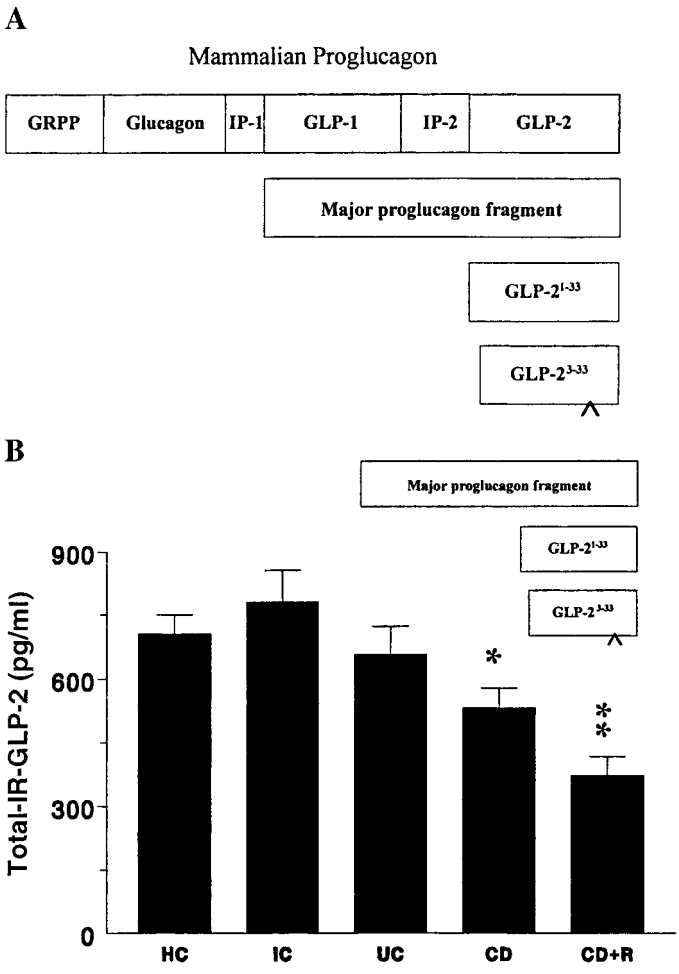

Fig. 1. *A*: structure of mammalian preproglucagon encoding glucagon-like peptide (GLP)-2. In the pancreas, incompletely processed major proglucagon fragment is liberated, whereas in the intestine, GLP-2-(1—33) that is generated undergoes further cleavage in the circulation to produce GLP-2-(3—33). Antiserum used for these studies, UTTH7 (arrowhead), cross-reacts with all of these molecular forms. GRPP, glicentin related pancreatic polypeptide; IP-1, intervening peptide-1; IP-2, intervening peptide-2. *B*: circulating levels of total immunoreactive (IR)-GLP-2 in normal healthy control subjects (HC; $n = 14$), immunocompromised controls (IC; $n = 38$), patients with ulcerative colitis (UC; $n = 21$), patients with unresected Crohn's Disease (CD; $n = 30$), and patients with CD with previous intestinal resection (CD+R; $n = 9$). $^*P < 0.05$ and $^{**}P < 0.01$ vs. normal controls. Arrowhead, proglucagon-derived peptides (PGDPs) recognized by GLP-2 assay for total IR-GLP-2.

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).

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. 1*A*) 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 Ala^2), and inactive MPGF (16, 21) containing the unprocessed carboxy terminal sequences of proglucagon, including both GLP-1 and GLP-2. Antisera 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. 1*B*, 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. 1*A*), 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. 2*A* 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. 2*B*). 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 Jeppesen and colleagues (18) to postulate that restoration of adequate levels of GLP-2 in patients with intestinal failure may represent a physiologically

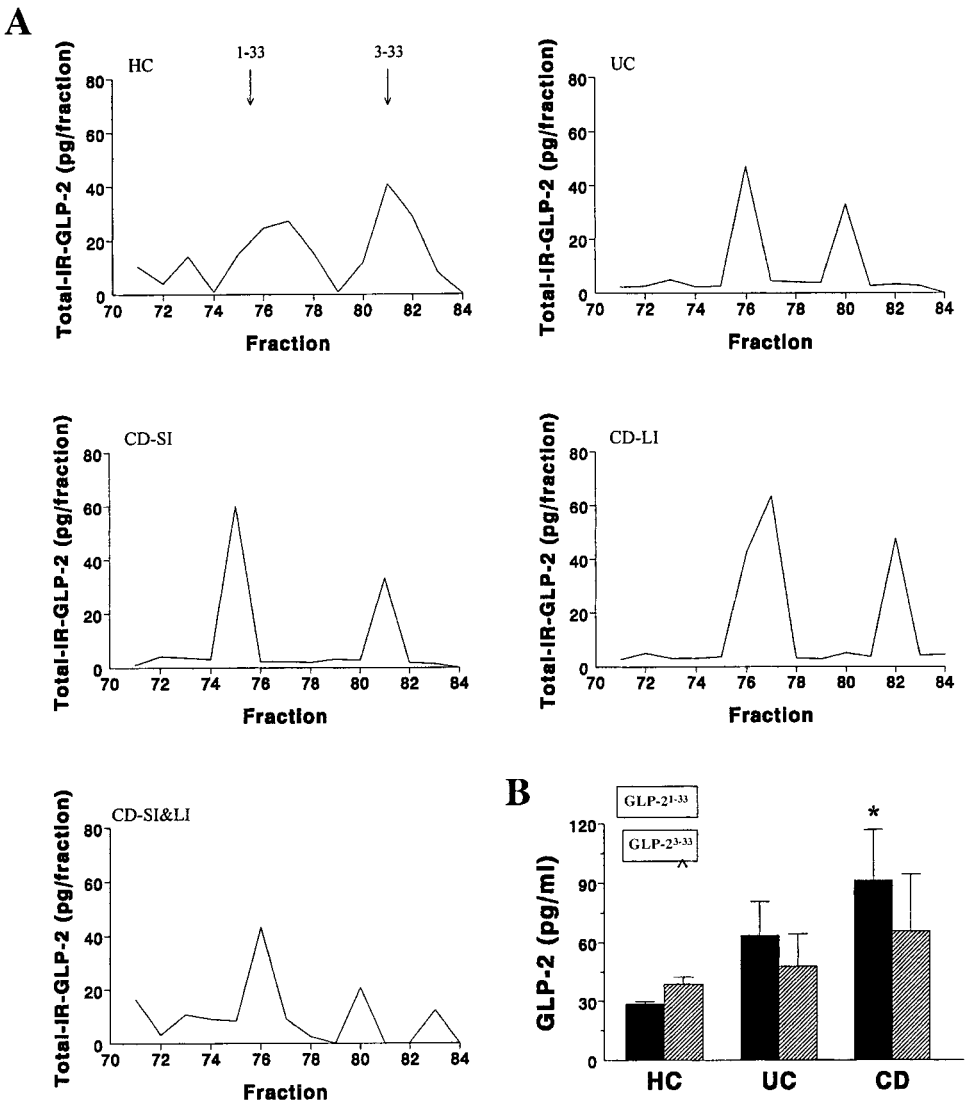

Fig. 2. *A*: representative HPLC profiles of IR-GLP-2 in 1 ml plasma from normal HCs or patients with UC or CD with disease focus in small intestine (CD-SI), large intestine (CD-LI), and both small and large intestine (CD-SI&LI). Arrows, elution positions of synthetic GLP-2-(1—33) and GLP-2-(3—33). *B*: absolute values for areas under the curve for GLP-2-(1—33) (filled bars) and GLP-2-(3—33) (hatched bars) in normal HC (n = 4) compared with patients with either UC (n = 4) or CD without intestinal resection (n = 7: n = 2 for disease focus in small intestine, n = 3 for disease focus in large intestine, and n = 2 for disease focus in both small and large intestine). $^*P < 0.05$ for GLP-2-(1—33) levels in CD vs. normal HC.

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) 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

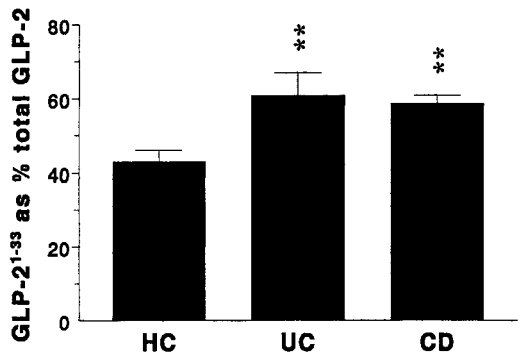

Fig. 3. Proportion of IR-GLP-2 found as intact GLP-2-(1—33) in HPLC profiles (as determined from areas under the curve of data presented in Fig. 2) of plasma from normal HC (n = 4), patients with UC (n = 4), and patients with CD (n = 7; n = 2 for SI, n = 3 for LI, and n = 2 for SI&LI). $^{**}P < 0.01$ vs. normal HCs.

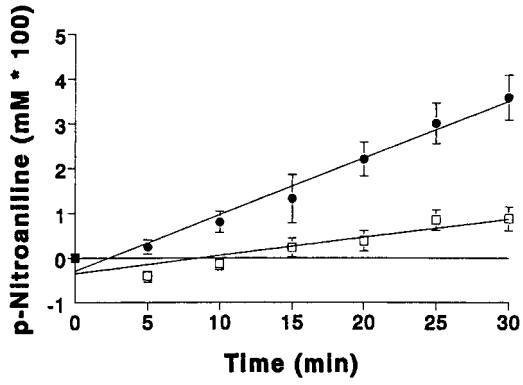

Fig. 4. Dipeptidyl peptidase IV (DP IV) activity as measured by temporal production of *p*-nitroaniline from Gly-Pro-*p*-nitroanilide in plasma from normal HCs (●; n = 6) and patients with inflammatory bowel disease (IBD; □; n = 5 with CD and n = 1 with UC). $P < 0.05$ for IBD vs. normal HCs.

diseases (including acute infective diarrhea, intestinal resection, jejunoileal bypass, celiac disease, and tropical sprue) as part of the normal intestinal adaptive response to injury or inflammation (1, 27, 28). Nevertheless, the relative levels or the molecular forms of circulating GLP-2 were not specifically examined in these previous studies. Our studies establish, for the first time, that levels of the intestinotrophic bioactive form of GLP-2 are clearly elevated in human patients with intestinal injury in the setting of IBD.

One hypothesis to explain the increased levels of GLP-2-(1—33) in patients with active IBD is that of enteroendocrine L cell adaptation as a component of the response to mucosal injury, leading to enhanced GLP-2 synthesis and/or secretion. For example, ileal proglucagon gene expression is increased in the intestinal remnant, and increased circulating levels of enteroglucagon and GLP-1 were observed in the rat after experimental intestinal resection (30). Unexpectedly, however, the proportions of GLP-2-(1—33) and its degradation product GLP-2-(3—33) were also altered in subjects with IBD, such that patients with IBD exhibited increased relative amounts of the bioactive GLP-2-(1—33) peptide. These findings highlight the importance of using antisera and/or separation techniques that discriminate among the different molecular forms of GLP-2 for interpretation of physiological changes in the levels of IR-GLP-2 peptides that circulate 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 intestinal injury in vivo. These findings suggest that regulation of DP IV activity may reflect the physiological 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 hospitalized 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 hypothesis 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 endogenous repair of epithelial injury in the human intestine.

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.

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

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