The influence of the colon on postprandial glucagon-like peptide 1 (7–36) amide concentration in man

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Abstract

Glucagon-like peptide (7–36) amide (GLP-1) is an incretin hormone of the enteroinsular axis released rapidly after meals despite the fact that GLP-1 secreting cells (L-cells) occur predominantly in the distal gut. The importance of these colonic L-cells for postprandial GLP-1 was determined in healthy control subjects and in ileostomy patients with minimal small bowel resection (<5 cm). Subjects were fed a high complex carbohydrate test meal (15·3 g starch) followed by two carbohydrate-free, high fat test meals (25 g and 48·7 g fat respectively). Circulating levels of glucose, insulin, glucagon, glucose insulinoetric peptide (GIP) and GLP-1 were measured over a 9-h postprandial period. For both subject groups the complex carbohydrate test meal failed to elicit a rise in either GIP or GLP-1. However, both hormones were elevated after the fat load although the GLP-1 concentration was significantly reduced in the ileostomist group when compared with controls (P=0·02). Associated with this reduction in circulating GLP-1 was an elevation in glucagon concentration (P=0·012) and a secondary rise in the plasma glucose concentration (P=0·006). These results suggest that the loss of colonic endocrine tissue is an important determinant in the postprandial GLP-1 concentration. Ileostomists should not be assumed to have normal enteroinsular function as the colon appears to have an important role in postprandial metabolism.


Introduction

The gene encoding proglucagon (PG), the biosynthetic precursor of glucagon, encodes a polypeptide of 160 amino acids (Bell et al. 1983). Expression occurs in both the pancreatic islets and the endocrine cells of the gastrointestinal mucosa (Novak et al. 1987). In the pancreas, glucagon is the major biologically active hormone cleaved from proglucagon but in the small intestine cleavage sites differ and the glucagon sequence is contained within a larger molecule called glicentin which corresponds to PG 1–69 (Thim & Moody 1981). Cleavage of PG occurs at the single basic amino acid residue in position 77 of PG and at pairs of basic residues at positions 109 and 110, 124 and 125, and 159 and 160. The peptides formed correspond to PG 78–108 (glucagon-like peptide 1, GLP-1), PG 111–123 (intervening peptide 1, IP-1) and PG 126–158 (glucagon-like peptide 2, GLP-2). Post translational modification results in the secretion of amidated and glycine extended GLP-1 in the ratio of 4:1 respectively in humans (Holst et al. 1987).

Cells exhibiting GLP-1 immunoreactivity have been detected from the proximal jejunum to the rectum using a monocular antibody specific to the C-terminus of GLP-1 (Eisselle et al. 1992). Staining was associated with the so-called open-type endocrine L-cells of the mucosa predominantly in the basal portion. There was a continuous increase in the density (cells/mm² mucosal area) of immunoreactive cells from the proximal to the distal colon with the highest numbers found in the rectum itself. Proglucagon cleaved in these large intestinal L-cells results in the formation of fully processed, biologically active GLP-1 (Deacon et al. 1995) so that the large bowel is potentially a major site of GLP-1 expression.

GLP-1 is the most potent insulinoetric hormone known (Holst et al. 1987) and, together with the gastrointestinal hormone glucose-dependent insulinoetric polypeptide (GIP), acts as an incretin (Zunz & LaBarre 1929) influencing insulin secretion via the enteroinsular axis. GLP-1 inhibits glucagon secretion and thereby inhibits hepatic glucose production.

GLP-1 is released within a few minutes after ingestion of mixed meals containing carbohydrates and lipids.

Table 1 Meal composition and time of feeding to subjects

<table>
<thead>
<tr>
<th>Meal</th>
<th>Time of meal (min)</th>
<th>Energy* (kJ)</th>
<th>Protein* (g)</th>
<th>Fat* (g)</th>
<th>Starch* (g)</th>
<th>NSP (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (pea soup)</td>
<td>1000 h (0)</td>
<td>600</td>
<td>13:0</td>
<td>0:9</td>
<td>15:3**</td>
<td>9:6**</td>
</tr>
<tr>
<td>2 (scrambled egg)</td>
<td>1200 h (120)</td>
<td>1178</td>
<td>15:0</td>
<td>25:0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 (omelette)</td>
<td>1600 h (360)</td>
<td>2493</td>
<td>38:8</td>
<td>48:7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*By calculation from Paul & Southgate (1978); **determined by the method of Englyst & Cummings (1985).

NSP, non-starch polysaccharide.

(Orskov et al. 1994). The exact stimulus for release remains to be clarified although it has been proposed that early postprandial release in rodents is due to neuroendocrine stimulation brought about by GIP (Roberge & Brubaker 1993). Current evidence, however, suggests that the neuropeptide gastrin-releasing peptide (GRP) is the major determinant of GLP-1 release in man (Brubaker 1997). Luminal contact with nutrients escaping small intestinal digestion may also provide an important stimulus for the colonic L-cells (Qualmann et al. 1995).

The objective of this study was to determine the potential contribution of the colon as a source of circulating GLP-1. Our approach was to compare postprandial changes in GLP-1 concentration in healthy volunteers and in ileostomists given the same test meals. Ileostomist volunteers in whom the small bowel has been sectioned very close to the ileo-caecal junction because of colonic disease (ulcerative colitis) whilst retaining normal small bowel function (Andersson 1992) should provide a reasonable estimate of GLP-1 responses in the absence of the colon.

Materials and Methods

Subjects

Six male subjects (mean age 52·8 years, body mass index (BMI) 25·4 kg/m²) with a surgical ileostomy performed for ulcerative colitis each with less than 5 cm of small bowel resection, and six control male subjects (mean age 39·8 years, BMI 23·2 kg/m²) participated in this study. All subjects were healthy and none was currently taking any medication known to affect carbohydrate metabolism or gastrointestinal motility.

All subjects gave written informed consent and the study was approved by the joint ethical committee of the University of Newcastle and Newcastle and North Tyneside Health Authorities.

Study protocol

After an overnight fast (12–14 h), subjects were admitted to the Wellcome Research Laboratory, Royal Victoria Infirmary, Newcastle-upon-Tyne. An intravenous cannula was inserted under local anaesthetic (1% lignocaine) into a radial vein in a retrograde manner and the hand was placed in a perspex box at 55 °C to provide arterialised venous blood. Baseline (fasting) blood samples were taken at regular intervals for 120 min. A high complex carbohydrate/low fat test meal consisting of cooked peas (dry weight 50 g) prepared as a soup was fed to all subjects at time 0 followed by two further high fat, carbohydrate-free meals (scrambled eggs and a cheese omelette) after 120 and 360 min respectively. Drinking water was provided ad libitum. Blood samples were taken at frequent intervals for 9 h postprandially.

Test meals

The compositions of the experimental test meals are shown in Table 1.

Blood analysis

Whole blood for plasma glucose analysis was collected into sodium fluoroxolate and the concentration measured using the glucose oxidase method with an automated glucose analyser (Beckman Instruments). For hormone determination, blood was collected into potassium EDTA and all samples from an individual subject were assayed at the same time to minimise errors due to inter-batch variation. Insulin concentration was determined using unextracted plasma as described by Hampton (1984) using the double antibody plus polyethylene glycol radioimmunoassay (RIA) method. The sensitivity of the assay was 16 pmol/l. Blood for GLP-1 determination was collected into potassium EDTA containing 200 KIU aprotinin/ml blood (Bayer plc, Newbury, Berks, UK). GLP-1 was assayed on unextracted plasma according to Elliott et al. (1993) by a double antibody disequilibrium RIA method. The antisera used in this assay exhibited no detectable cross-reactivity with human GIP, GLP-2, glucagon, vasoactive intestinal peptide (VIP), secretin or motilin. It was specific for the C-terminal amidated form of GLP-1, cross-reacting 100% with GLP-1 (7–36 amide). GIP was assayed as previously described by Morgan et al. (1978).
Cross-reactivity of the antiserum with porcine secretin, VIP, glucagon, bovine pancreatic polypeptide, synthetic human C-peptide and bovine insulin was found to be negligible. The assay for pancreatic glucagon was performed on unextracted plasma using a commercially available glucagon RIA kit (Euro Diagnostica, Metachem Diagnostics, Northampton, Northants, UK) which utilised a rabbit antiserum raised against a glucagon–albumin conjugate. The antisera used in this assay exhibited less than 0·1% cross-reactivity with enteroglucagon. All the hormones assayed exhibited both inter- and intra-assay coefficients of variation of less than 10%.

Statistical analysis

The time-course of the glucose and hormone responses to meals are illustrated in the figures as means and their corresponding standard errors (S.E.M.) at each sampling time. Statistical analysis (ANOVA; MINITAB 10, Minitab Inc. State College, PA, USA) was performed on summary data: peak heights, times-to-peak and area under the curve (AUC) which was obtained by integration using the trapezoid approach. The statistical significances of differences between ileostomists and controls were tested using paired-independent Student’s t-tests.

Results

Glucose

The mean fasting plasma glucose concentrations were slightly (but not significantly) higher for the ileostomists than for the control group (Table 2). The time-course of the glucose response to the test meals differed between the experimental groups as illustrated in Fig. 1. The start of the glucose peak was delayed by 15 min in the ileostomist group (P=0·001) and the time-to-peak was also significantly (P=0·05) delayed (59 vs 41 min for the intact group). In both groups, however, the high starch test meal providing just 15·3 g starch resulted in a modest glycaemic response. Meals 2 and 3 appeared to have little effect on plasma glucose concentrations in control subjects but after test meal 3 there was a secondary peak in plasma glucose concentration in the ileostomist group. The AUC for the whole period of study did not differ between the subject groups but the AUC for the latter half of the study (post 180 min) was higher in the ileostomist subjects (P=0·042).

Insulin

Basal insulin concentrations were similar for the two groups (Table 3). The change in plasma insulin followed a similar pattern to the plasma glucose response with plasma insulin concentrations increasing rapidly to a maximal mean peak (3 to 4 times the fasting levels) at

Table 2 Parameters of the plasma glucose response following a starch-rich test meal and two subsequent high fat carbohydrate-free meals

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>Ileostomist (n=6)</th>
<th>Pooled S.E.M.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal glucose (mmol/l)</td>
<td>5-1</td>
<td>5-3</td>
<td>0-09</td>
<td>0-286</td>
</tr>
<tr>
<td>( \text{AUC}_{180} ) (min.mmol/l)</td>
<td>984</td>
<td>1040</td>
<td>31-2</td>
<td>0-388</td>
</tr>
<tr>
<td>( \text{AUC}_{480-180} ) (min.mmol/l)</td>
<td>1819</td>
<td>1963</td>
<td>31-0</td>
<td>0-042*</td>
</tr>
<tr>
<td>( \text{AUC}_{480} ) (min.mmol/l)</td>
<td>2779</td>
<td>3005</td>
<td>56-2</td>
<td>0-071</td>
</tr>
<tr>
<td>First peak height (mmol/l)</td>
<td>6-3</td>
<td>6-6</td>
<td>0-16</td>
<td>0-571</td>
</tr>
<tr>
<td>Time to first peak (min)</td>
<td>41</td>
<td>59</td>
<td>3-3</td>
<td>0-050*</td>
</tr>
<tr>
<td>Post nadir glucose peak (mmol/l)</td>
<td>5-1</td>
<td>5-8</td>
<td>0-10</td>
<td>0-006**</td>
</tr>
</tbody>
</table>

Basal glucose is the mean of three samples taken in the fasting state prior to test meal 1. The AUC for glucose is expressed as mmol/l180 min−1, mmol/l480 min−1 or the difference between the two values.

For control versus ileostomist subjects, a statistical significance is represented by *P≤0·05 and **P≤0·01.

Figure 1 Plasma glucose concentrations after a starch-rich test meal and two subsequent high fat carbohydrate-free meals in control (○) and ileostomist (●) subjects. Values are means ± S.E.M., n=6 for both groups.
approximately 150 min for both groups. No differences in the peak concentration or the AUC were noted between the two groups.

**GIP**

Both of the experimental groups exhibited very low fasting GIP concentrations (Table 3) which were at or below the lower limit of detection of this assay (24 pM) (Elliott et al. 1993). There was no appreciable change in the GIP concentration until after meal 2 (Fig. 2). Although concentrations tended to be higher for ileostomists in the period 180–360 min because of the substantial inter-individual variation, no differences in the AUC between experimental groups were detected.

**Glucagon**

Basal glucagon concentrations were similar in the two groups (Table 3). Between 3 and 8 h the plasma glucagon concentration rose steadily in all subjects with the ileostomist group exhibiting significantly higher concentrations ($P=0.012$) at the end of the sampling period (Fig. 3).

**GLP-1**

Fasting concentrations of GLP-1 were similar in the two groups (Table 4). A significant elevation in the circulating GLP-1 concentration was not apparent until 180 min after the initial test meal with concentrations in the control group rising more rapidly. In both groups GLP-1 concentrations peaked after 8 h with the circulating level of GLP-1 in the control group (58.8 pM) being almost double that measured in the ileostomist group (33.7 pM) (Fig. 4). The AUC for the first 180 min of the study was similar for the two groups but the AUC for the whole experimental period was significantly reduced in the ileostomist group ($P=0.039$).

**Discussion**

In this study the high carbohydrate meal (test meal 1) failed to elicit the early rise in plasma GLP-1 normally associated with the consumption of carbohydrate and mixed test meals. The rapid release of GLP-1 normally observed is thought to be due to a neuroendocrine loop (Roberge & Brubaker 1993) originating from nutrient stimulation of endocrine cells in the duodenum. In our study the lack of a GLP-1 response also coincided with a very limited rise in the plasma GIP concentration within the first 180 min after the oral carbohydrate (Figs 2 and 4). These findings agree with those of Elliott et al. (1993) who found no GLP-1 response when cereal test meals were consumed. GIP secretion is stimulated by the absorption of luminal fat and is ultimately dependent on the rate of

| Table 3 Plasma insulin, glucagon and GIP parameters in control and ileostomist subjects after a starch-rich test meal and two subsequent high fat carbohydrate-free meals |
|-------------------------------------------------|-------------------|-------------------|-------------------|
| **Control subjects (n=6)**                       | **Insulin (pmol/l)** | **Glucagon (pmol/l)** | **GIP (pmol/l)** |
| Basal concentration                              | 52.8 ± 5.3         | 43.4 ± 0.9         | <24.0            |
| Peak concentration                               | 168.6 ± 22.8       | 59.2 ± 2.6         | 192.4 ± 22.6     |
| AUC$_{180}$                                      | 14.408 ± 2173      | 8301 ± 462         | 4428 ± 55        |
| AUC$_{480–180}$                                   | 25.157 ± 3922      | 21665 ± 1210       | 54392 ± 5125     |
| AUC$_{480}$                                      | 41.637 ± 4796      | 29966 ± 1646       | 58821 ± 5096     |
| **Ileostomist subjects (n=6)**                   | **Insulin (pmol/l)** | **Glucagon (pmol/l)** | **GIP (pmol/l)** |
| Basal concentration                              | 66.5 ± 10.5        | 43.4 ± 1.0         | <24.0            |
| Peak concentration                               | 250 ± 29.9         | 73.7 ± 3.8*        | 329.5 ± 95.8     |
| AUC$_{180}$                                      | 17.863 ± 2199      | 9188 ± 385         | <4320            |
| AUC$_{480–180}$                                   | 31.943 ± 3543      | 25034 ± 1386       | 79524 ± 26952    |
| AUC$_{480}$                                      | 49.806 ± 4462      | 34222 ± 1682       | 83844 ± 26952    |

The AUC is expressed as pmol/l.180 min$^{-1}$ or 480 min$^{-1}$.
*P≤0.05 statistically significant difference between ileostomist and control subjects.

![Figure 2 GIP concentrations in plasma after a starch-rich test meal and two subsequent high fat carbohydrate-free meals in control (○) and ileostomist (●) volunteers. Values are means ± S.E.M., n=6 for both groups.](image)
glucose or fat absorption from the duodenum (Ellis et al. 1995). The first meal provided only 15·3 g starch so that the resulting modest glucose supply appears to have been responsible for the limited early glycaemia and hence the lack of a postprandial rise in plasma GIP.

The absence of a GLP-1 response over the first 150 min and the differences in GLP-1 concentration between the two subject groups are interesting aspects of this study. There are three possible explanations for the apparently late appearance of the GLP-1 peak; (i) the nutrient load in the first test meal was insufficient to trigger GLP-1 release, (ii) ileal and colonic luminal stimulation by the complex carbohydrate provided by test meal 1 and (iii) duodenal stimulation of GLP-1 release due to the relatively high fat content of test meals 2 and 3.

The orocaecal transit time of a starchy meal has been reported to be in the region of 5–6 h (Englyst & Cummings 1985) which might at first indicate that direct luminal stimulation in the ileum and colon due to the presence of complex carbohydrate is responsible for the observed difference in GLP-1 concentration. In a study by Qualmann et al. (1995), acarbose, an α-glucosidase inhibitor, was given in conjunction with a sucrose test meal in order to delay the duodenal hydrolysis of sucrose and move carbohydrate into the lower regions of the gastrointestinal tract. This led to a delayed and prolonged GLP-1 response coinciding with a rise in breath hydrogen (Qualmann et al. 1995) indicative of colonic fermentation (Bond & Levitt 1974). Feeding larger quantities of complex carbohydrate failed to elicit a GLP-1 response in an earlier study (Elliott et al. 1993) but the blood sampling protocol for this experiment was much shorter (only 3 h post-prandially) which would not have been long enough to demonstrate GLP-1 release due to direct luminal stimulation in the ileum and colon. Interpretation of these data is complicated because the release of GLP-1 is also stimulated by the presence of lipid in the upper gastrointestinal tract. GLP-1 is usually released within 150 min of fat ingestion (Elliott et al. 1993) with the time delay due to the slower gastric emptying of fat. The peak plasma GLP-1 concentration in this study coincided with fat absorption from the duodenum as indicated by the timing of the postprandial

| Table 4 Glucagon-like peptide 1 response in control and ileostomist subjects after a starch-rich test meal and two subsequent high fat carbohydrate-free meals. Values are means ± S.E.M. |
|---------------------------------------------|------------------|------------------|------------------|------------------|
| Control (n=6) | Ileostomist (n=6) | Pooled S.E.M | P value |
| Basal concentration (pmol/l) | 8.4 | 9.6 | 0.95 | 0.864 |
| Peak concentration (pmol/l) | 58.8 | 33.7 | 4.53 | 0.020* |
| Time-to-peak (min) | 395 | 320 | 12.5 | 0.409 |
| AUC_{180} (min.pmol/l) | 1642 | 1964 | 173 | 0.286 |
| AUC_{480–180} (min.pmol/l) | 14 618 | 9320 | 1389 | 0.024* |
| AUC_{480} (min.pmol/l) | 16 260 | 11 285 | 1474 | 0.039* |

*P≤0.05, statistically significant difference between ileostomist and control subjects.
rise in GIP. No statistically significant differences were noted in the GIP response between groups indicating the normal absorption of fat from the small intestine of the ileostomists.

Although we were not able to determine the exact stimulus for this later postprandial rise in GLP-1 concentration, it is evident that the loss of colonic tissue had significant effects on the circulating levels of GLP-1. There was a twofold difference in the peak GLP-1 concentration between the two groups. The integrated response also differed significantly between the subject groups. It is also possible that the difference in the GLP-1 response between the normal subjects and ileostomy patients may be due to reduced capacity to absorb glucose in the lower intestine rather than an overall decreased capacity for GLP-1 secretion although there is no evidence for this in the literature.

In this study the decreased GLP-1 concentration in the ileostomist group coincided with an elevation in the plasma glucagon concentration (Fig. 3). GLP-1 has been shown to inhibit glucagon secretion strongly (Orskov et al. 1988) although the situation is now believed to be more complex with recent evidence suggesting that pancreatic glucagon may actually inhibit GLP-1 secretion (Ranganath et al. 1998). The main effect of glucagon is to increase glucose output from the liver by the inhibition of glycogen synthesis and the stimulation of both glyco- genolysis and gluconeogenesis (Stalmans 1983). This increase in the glucagon/insulin ratio corresponds well with the observed secondary rise in the plasma glucose concentration in the ileostomy group (Fig. 1). The rise in GLP-1 had no effect on the plasma insulin concentration in euglycaemic conditions which is as expected.

Decreased GLP-1 secretion has been associated with impaired insulin secretion and reduced tissue sensitivity to insulin (insulin resistance) (Vaag et al. 1996). In this study, alterations in the postprandial concentrations of glucose, glucagon and GLP-1 in the ileostomy group could not be attributed to differences in tissue insulin resistance or β-cell function measured in the fasting state (Table 5). Nauk et al. (1996) and Hansen et al. (1997) claimed to have found a link between surgical ileostomy and the presence of tissue insulin resistance. Although there were differences in the designs of the two studies, the common finding was an elevation in both the basal insulin concentration and the integrated insulin response in colectomized subjects. We found no difference in the insulin response between control and ileostomy subjects. The reduced GLP-1 concentrations and moderate glucagonaemia which were observed in the ileostomy group in this study are known to stimulate the rate of appearance of both glycerol and free fatty acids (FFA) (Carlson et al. 1993). Higher plasma FFA are indeed linked with a reduced tissue insulin sensitivity (Reaven 1988) and, although FFA were not measured during this study, we found no evidence to suggest greater insulin resistance in our ileostomy subjects.

The present study was not designed to identify the gastrointestinal trigger for GLP-1 release. Instead, healthy male ileostomists and healthy male normal subjects were fed identical diets over a 9-h period and responses in GLP-1 measured. The relatively late rises in plasma GLP-1 concentrations suggest that the nutrient supply in the first test meal was insufficient to elicit a GLP-1 response. Given that the oral dose combined with the rate and extent of starch digestion will determine both the glycaemic response and passage of carbohydrates to the distal intestine, future studies should explore GLP-1 responses to higher doses of both slowly and rapidly digested starches.

In conclusion, the colon appears to be an important source of circulating GLP-1 constituting about half of this hormone secreted in response to the consumption of meals rich in complex carbohydrate, protein and lipid. However, the physiological significance, if any, of a reduction in GLP-1 remains to be determined.

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References


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