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Regional specificity of the gut-incretin response to small intestinal glucose infusion in healthy older subjects

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ABSTRACT

The importance of the region, as opposed to the length, of small intestine exposed to glucose in determining the secretion of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) remains unclear. We sought to compare the glycemic, insulinemic and incretin responses to glucose administered to the proximal (12–60 cm beyond the pylorus), or more distal (>70 cm beyond the pylorus) small intestine, or both. 10 healthy subjects (9M,1F; aged 70.3 ± 1.4 years) underwent infusion of glucose via a catheter into the proximal (glucose proximally; GP), or distal (glucose distally; GD) small intestine, or both (GPD), on three separate days in a randomised fashion. Blood glucose, serum insulin and plasma GLP-1, GIP and CCK responses were assessed. The iAUC for blood glucose was greater in response to GPD than GP ($P < 0.05$), with no difference between GD and GP. GP was associated with minimal GLP-1 response ($P = 0.05$), but substantial increases in GIP, CCK and insulin ($P < 0.001$ for all). GPD and GD both stimulated GLP-1, GIP, CCK and insulin ($P < 0.001$ for all). Compared to GP, GPD induced greater GLP-1, GIP and CCK responses ($P < 0.05$ for all). Compared with GPD, GD was associated with greater GLP-1 ($P < 0.05$), but reduced GIP and CCK ($P < 0.05$ for both), responses. We conclude that exposure of glucose to the distal small intestine appears necessary for substantial GLP-1 secretion, while exposure of both the proximal and distal small intestine result in substantial secretion of GIP.

1. Introduction

Administration of macronutrients into the gut triggers the release of glucose-dependent insulinotropic polypeptide (GIP) from enteroendocrine K-cells, situated most densely in the duodenum and jejunum, and glucagon-like peptide-1 (GLP-1) from L-cells, which are predominate in the distal small intestine and colon [1]. GIP and GLP-1 are "incretin" hormones, accounting for the substantially greater insulin response to an enteral glucose load than an isoglycemic intravenous glucose infusion (the "incretin effect") [2]. In type 2 diabetes, the insulinotropic effect of GIP is markedly diminished, whereas that of GLP-1 is relatively preserved [3]. GLP-1 also slows gastric emptying and suppresses glucagon secretion and energy intake [1]. Diversion of nutrients from the proximal into the distal small intestine probably underlies the enhanced GLP-1 secretion which is likely to contribute to the improved glycemic control induced by Roux-en-Y gastric bypass (RYGB) [4,5].

The mechanisms by which GLP-1 is released from the intestine are incompletely understood. In humans, unlike rodents, exogenous GIP does not stimulate GLP-1 [6]. Unlike GIP, the release of GLP-1 in response to intraduodenal glucose infusion displays a threshold, so that only infusions at rates sufficient to exceed the absorptive capacity of the proximal small intestine stimulate substantial GLP-1 release [7]. In healthy humans we reported that GLP-1 was stimulated when intraduodenally infused glucose (3.5 kcal/min) was allowed to access the entire small intestine, but not when restricted to the proximal 60 cm [7]. In contrast, GIP and cholecystokinin (CCK) responses were comparable in the two conditions, supporting the concept that the proximal gut is the origin of the latter two hormones [7]. While there was a greater GLP-1 response to an additional load of glucose beyond the proximal 60 cm of the small intestine with access to the entire small intestine,

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the increases in blood glucose and plasma insulin were also greater [7]. Importantly, the design of this study did not allow the potential effects of the region of small intestine exposed, from those of the length of intestine exposed, to be discriminated, simply because the lengths of exposure of the small intestine to glucose differed substantially. That this is important is supported by the outcomes of an unpaired study which evaluated the effects of glucose infused into either the duodenum or proximal jejunum (50 cm from the pylorus) [8]. Plasma GLP-1 and insulin responses were shown to be greater following intrajejunal versus intraduodenal glucose, supporting the concept that diversion of nutrients to the more distal small intestine enhances GLP-1 and insulin secretion [8], however, the unpaired design represents a significant limitation, particularly given the small number of subjects [8]. Moreover, the rate of glucose infusion (2 kcal/min) is known to result in substantial GIP, but minimal GLP-1, release [8,9]. After RYGB, very high GLP-1 and insulin responses are associated with extremely rapid emptying of glucose from the gastric pouch (~100 kcal/min). However, when the rate of intestinal glucose delivery is slowed to the upper end of the normal range for gastric emptying, both incretin hormone and blood glucose responses are comparable to those in healthy controls following an identical rate of intraduodenal glucose infusion [10]. These, and other, observations challenge the concept that direct exposure of distal L-cells to luminal content is the primary mechanism for GLP-1 stimulation. CCK-signaling arising from the proximal gut is known to potentiate GLP-1 release in response to fat [11].

The current study was designed to evaluate (i) the relative importance of the proximal (12–60 cm beyond the pylorus) versus distal (>70 cm beyond the pylorus) exposure of the small intestine in incretin, CCK and insulin responses to luminal glucose, and (ii) the hypothesis that diversion of glucose from the proximal to the distal small intestine would increase GLP-1 secretion. The rate of small intestinal glucose infusion used (3 kcal/min) was known to result in substantial stimulation of both GIP and GLP-1 [9,12].

2. Materials and methods

2.1. Subjects

Thirteen healthy older subjects (12 male and 1 female, aged 71.3 ± 1.3 years) were recruited through an existing database. All were non-smokers and none had a history of cardiovascular, hepatic, renal or gastrointestinal disease or alcohol abuse. None had diabetes, or took medication known to influence gastrointestinal function. Older, rather than young, subjects were studied because of our interest in incretin responses in this group [12].

2.2. Protocol

Subjects were studied on 3 occasions, separated by at least 7 days. On each study day, the subject attended our laboratory at the Royal Adelaide Hospital at 0800 h after an overnight fast (14 h for solids and 12 h for liquids) [7]. Upon arrival, a silicone-rubber, multilumen catheter (external diameter 4.2 mm) (Dentsleeve International, Mui Scientific, Mississauga, Canada) (Fig. 1) was inserted into the stomach via an anesthetised nostril, and allowed to pass into the duodenum by peristalsis. The catheter included two infusion channels, positioned in the duodenum (~12 cm distal to the pylorus; channel 1) and the jejunum (~70 cm distal to the pylorus; channel 2), a ~10 cm long balloon (~60 cm distal to the pylorus to be inflated for isolating proximal and distal segments of the small intestine), and an aspiration channel (~3 cm proximal to the balloon to aspirate duodenal contents). The catheter also incorporated two 'air-return' channels, to equalise duodenal pressure during infusion and aspiration. Two other channels, located in the antrum (~2.5 cm proximal to the pylorus) and duodenum (~2.5 cm distal to the pylorus), were perfused continuously with saline (0.9%) and the correct positioning of the catheter was maintained by continuous measurement of the transmucosal potential difference (TMPD) in the antral (−40 mV) and duodenal (0 mV) channels [13,14]. For the purpose of measurement of TMPD, a 0.9% saline-filled cannula was inserted subcutaneously into the subject's forearm [13]. After the catheter had been positioned correctly, the subject was placed in a recumbent position and an IV cannula inserted into the left antecubital vein for blood sampling.

Commencing at t = −30 min, the balloon was inflated with air using a hand-held syringe until the subject reported a sensation of pressure, without discomfort (approximately 35 ml) [7,15]. The balloon was then deflated and the subject allowed to 'rest' for ~15 min. At t = 0 min, the balloon was re-inflated to the pre-determined volume, and intra-balloon pressure monitored throughout the study to ensure sustained inflation [7,15]. We have shown, using the non-absorbable marker PEG 4000, that this technique achieves total occlusion of the small intestine [15].

Between t = 0–60 min, each subject received the following three infusions via infusion channel 1 and infusion channel 2:

a) infusion channel 1: 25% glucose at 3 kcal/min + infusion channel 2: 0.9% saline (ie. proximal small intestinal segment only exposed to glucose; "GP"),
b) infusion channel 1: 25% glucose at 3 kcal/min + infusion channel 2: 25% glucose infused at an adjustable rate to allow the glucose recovered from the proximal segment to be infused concurrently (ie. both the proximal and distal segments exposed to glucose; "GPD"), or
c) infusion channel 1: 0.9% saline + infusion channel 2: 25% glucose at 3 kcal/min (ie. distal segment only exposed to glucose; "GD").

All infusions were administered at 3.15 ml/min, with the exception of the variable rate glucose infusion during b). During the GPD infusion, for the first 10 min, glucose was infused into the proximal segment only, before the glucose concentration in the luminal aspirate was measured to determine the appropriate infusion rate for the distal small intestine, which was then re-calculated and adjusted every 10 min. At the end of each 10 min epoch, 1 ml of the aspirate was diluted with 49 ml of saline (0.9%) and the glucose concentration measured with a 2300 Stat Plus glucose analyser (Yellow Springs Instruments (YSI), Ohio, USA). The glucose concentration in the aspirate was derived by multiplying this figure by 50, which was then multiplied by the volume of aspirate to yield the total amount of glucose in the aspirate. This glucose load was replaced by infusion into the distal small intestine over the subsequent 10 min. At t = 60 min the infusion catheter and IV cannulae were removed and the subject offered a meal prior to leaving the laboratory.

The three study days were undertaken in single-blind, randomised fashion; the investigator delivering the infusions could not be blinded, but was independent of any other measurements. All infusions were performed using a volumetric infusion pump (Gemini PC-1, IMED, San Diego, CA, USA).

The protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital, and each subject provided written, informed consent prior to their inclusion. All experiments were carried out in accordance with the Declaration of Helsinki.

2.3. Blood glucose, serum insulin, plasma GLP-1, plasma GIP and plasma CCK

Venous blood samples (18 ml) were collected into ice-chilled EDTA-treated tubes prior to balloon inflation (t = −15 min) and at 15 min intervals between t = 0–60 min. Samples were separated