Initially more rapid small intestinal glucose delivery increases plasma insulin, GIP, and GLP-1 but does not improve overall glycemia in healthy subjects

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Chaikomin, Reawika, Selena Doran, Karen L. Jones, Christine Feinle-Bisset, Deirdre O’Donovan, Christopher K. Rayner, and Michael Horowitz. Initially more rapid small intestinal glucose delivery increases plasma insulin, GIP, and GLP-1 but does not improve overall glycemia in healthy subjects. Am J Physiol Endocrinol Metab 289:E504–E507, 2005. First published May 10, 2005; doi:10.1152/ajpendo.00099.2005.—The rate of gastric emptying of glucose-containing liquids is a major determinant of postprandial glycemia. The latter is also dependent on stimulation of insulin secretion by glucose-dependent insulinoctropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Although overall emptying of glucose approximates 1–3 kcal/min, the “early phase” of gastric emptying is usually more rapid. We have evaluated the hypothesis that increased stimulation of incretin hormones and insulin by a more rapid initial rate of small intestinal glucose delivery would reduce the overall glycemic response to a standardized enteral glucose load. Twelve healthy subjects were studied on two separate days in which they received an intraduodenal (id) glucose infusion for 120 min. On one day, the infusion rate was variable, being more rapid (6 kcal/min) between \( t = 0 \) and 10 min and slower (0.55 kcal/min) between \( t = 10 \) and 120 min, whereas on the other day the rate was constant (1 kcal/min) from \( t = 0 \)–120 min, i.e., on both days 120 kcal were given. Between \( t = 0 \) and 75 min, plasma insulin, GIP, and GLP-1 were higher with the variable infusion. Despite the increase in insulin and incretin hormones, blood glucose levels were also higher. Between \( t = 75 \) and 180 min, blood glucose and plasma insulin were lower with the variable infusion. There was no difference in the area under the curve 0–180 min for blood glucose. We conclude that stimulation of incretin hormone and insulin release by a more rapid initial rate of id glucose delivery does not lead to an overall reduction in glycemia in healthy subjects.

intraduodenal glucose; incretin hormones

Although the relative importance of the factors that determine postprandial blood glucose concentrations is controversial (10), we and others have established that, in both healthy subjects (3, 12) and patients with diabetes mellitus (15, 24, 26), the rate of carbohydrate entry into the small intestine is important. The concept that postprandial glycemia is a major determinant of average glycemic control, as assessed by glycated hemoglobin (1, 9), and is likely to represent an independent risk factor for cardiovascular disease (5, 10), has focused attention on dietary and pharmacological strategies aimed at reducing postprandial glycemic excursions, including by modulating the rate of gastric emptying (24).

It is well established that enterally administered glucose stimulates insulin secretion by more than a comparable amount of glucose given intravenously (5, 7). This so-called “incretin” effect is due to the secretion of gut hormones, including glucose-dependent insulinoctropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (7). Type 2 diabetes is characterized by a reduced “early” (and frequently increased “late”) postprandial insulin response. In animals, a small, early increase in portal vein/peripheral blood insulin levels is more effective than a larger, later increase in reducing blood glucose levels (8). Furthermore, the release of GIP and GLP-1 is related to the rate of carbohydrate entry into the small intestine (25). Therefore, it is possible that an initially more rapid rate of small intestinal delivery of a standardized glucose load would be associated with a reduction in the overall glycemic response.

Gastric emptying of glucose is closely regulated (14, 17, 18) so that, in humans, duodenal glucose delivery remains relatively constant at a rate of 1–3 kcal/min over a wide range of concentrations. This regulation is due to small intestinal feedback, the extent of which is dependent on the length of small intestine contacted by glucose (17). The initial rate of gastric emptying of liquids, sometimes referred to as “gastric emptying during gastric fill” (16), is, however, usually more rapid than the subsequent linear delivery, presumably, at least in part, because of the time taken to initiate effective small intestinal inhibitory feedback. This early phase of emptying is usually 5–15 min in duration and is influenced by intragastric drink volume, and associated with duodenal glucose delivery of the order of 6 kcal/min (4, 13, 14, 16).

We (23) have recently sought to determine the impact of changes in the rate of glucose entry into the small intestine on the secretion of GIP, GLP-1 and insulin, and blood glucose. Healthy subjects and patients with type 2 diabetes received an identical intraduodenal glucose load over 120 min on 2 days; on one day the infusion rate was variable, being more rapid initially (3 kcal/min for 15 min) and subsequently slower, whereas on the other day, the infusion was constant (1 kcal/min). This modest variation in the initial rate of small intestinal glucose entry, within the physiological range, resulted in marked early differences in glycemic, insulin, and incretin responses. However, any subsequent reduction in glycemia on the variable infusion day was minimal, and the overall area under the blood glucose curves did not differ (23). From the previously mentioned discussion, it is apparent that this negative outcome may have reflected the fact that the initial rate of glucose delivery with the variable glucose infusion (3 kcal/min) was less than that which may occur physiologically. In the present study we have, accordingly, evaluated the effects of...
a more rapid rate of intraduodenal glucose infusion (6 kcal/min for 10 min) that is still within the physiological range for the early phase of gastric emptying.

METHODS

Subjects. Twelve healthy male subjects (age, 33 ± 3 yr; body mass index, 24 ± 1.0 kg/m²) were recruited by advertisement. No subject had a history of gastrointestinal disease or surgery, significant respiratory or cardiac disease, alcohol abuse, or epilepsy, smoked more than 10 cigarettes a day, or was taking medication known to affect gastrointestinal function.

The protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital, and each subject provided written, informed consent.

Protocol. Each subject underwent paired studies, separated by an interval of 4–7 days, in randomized order. Following an overnight fast (14 h for solids and 12 h for liquids), the subject attended the laboratory at 0900. A manometric assembly (diameter 4 mm) was inserted into the stomach via an anesthetized nostril and allowed to pass into the duodenum by peristalsis (23). The assembly included an infusion channel with a port located 10 cm distal to the pylorus and two other channels positioned in the antrum and duodenum, respectively, and perfused with saline at 0.15 ml/min (22). The position of the assembly was monitored by measurement of the antroduodenal transmucosal potential difference, using a reference electrode (a 20-gauge cannula filled with sterile saline) placed subcutaneously in the forearm (6). An intravenous cannula was inserted in a forearm vein for blood sampling; the forearm was heated (using a heat pad) to obtain “arterialized” samples. The subject was then allowed to rest comfortably in the recumbent position for −20 min.

At time t = 0 min, an intraduodenal infusion of 50% glucose, or a mixture of 50% glucose and water, was infused at a rate of 3 ml/min between 0 and 120 min. On one day, the rate of energy delivery was 6 kcal/min between 0 and 10 min and 0.55 kcal/min from 10 to 120 min; on the other day, the energy delivery was maintained at 1 kcal/min from 0 to 120 min; i.e., on both days a total of 360 ml and 120 kcal of glucose was infused intraduodenally. Furthermore, the volumes infused from t = 0–10 and t = 10–120 min were the same on both days. At t = 240 min, the manometric assembly was removed. Blood samples (~20 ml volume) were obtained immediately before the commencement of the intraduodenal infusions (t = 0) and subsequently at t = 4, 6, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 min for measurement of glucose, insulin, GLP-1, and GIP, using established methods (22).

Blood glucose and plasma insulin, GLP-1, and GIP concentrations. Blood samples for determination of plasma insulin, GLP-1, and GIP were collected in ice-chilled tubes containing EDTA and 400 kIU of aprotonin (Trasylol; Bayer Australia, Pymple, Australia) per liter of blood. Plasma was separated by centrifugation and stored at −70°C for subsequent analysis.

Blood glucose concentrations were determined immediately by using a portable glucose meter (Medisense Precision QID; Abbott Laboratories, Bedford, MA). The accuracy of this method has been confirmed in our laboratory using the hexokinase technique (12).

Plasma insulin concentrations were measured by ELISA immunoassay (Diagnostics Systems Laboratories, Webster, TX); the intra-assay coefficient of variation was 2.6% and the interassay coefficient of variation 6.2% (11).

Plasma GLP-1 concentrations were measured by RIA; the intra-assay coefficient of variation was 17% and the interassay coefficient of variation 18% (23, 31). Plasma GIP was measured by RIA; intra- and interassay coefficients of variation were both 15% (23, 30).

Blood glucose and hormone concentrations were measured by methods identical to those used in our previous study (23).

Statistical analysis. Data were evaluated for two time periods (0–75 min and 75–180 min) using repeated-measures ANOVA, with treatment and time as factors. Areas under the curves (AUCs, 0–180 min) for blood glucose and plasma GLP-1, GIP, and insulin were calculated using the trapezoidal rule and compared using the Student’s t-test. Statistical significance was accepted at P < 0.05, and data are presented as means ± SE.

RESULTS

All subjects tolerated the study well. Fasting concentrations of glucose, insulin, GIP, and GLP-1 did not differ between the 2 days.

Blood glucose. Blood glucose (Fig. 1A) increased from baseline during each infusion (P < 0.05). Between 0 and 75 min, blood glucose concentrations were greater during the variable compared with the constant infusion (P < 0.05). In contrast, between 75 and 180 min, blood glucose concentrations were less (P < 0.05) during the variable infusion. There was no difference in the AUC (0–180) of blood glucose between the 2 study days (1,164 ± 44 vs. 1,124 ± 46).

Plasma insulin. Plasma insulin concentrations (Fig. 1B) increased from baseline during each infusion (P < 0.05). Between 0 and 75 min, plasma insulin concentrations were higher during the variable compared with the constant infusion (P < 0.05). Between 75 and 180 min, plasma insulin was less (P < 0.05) during the variable infusion. The AUC 0–180 min for plasma insulin was greater (5,153 ± 609 vs. 3,462 ± 493, P < 0.05) during the variable infusion.

Plasma GIP. Plasma GIP (Fig. 1C) concentrations increased from baseline during each infusion (P < 0.05). Between 0 and 75 min, plasma GIP concentrations were higher (P < 0.05) during the variable compared with the constant infusion. Between 75 and 180 min, there was no difference in plasma GIP between the two infusions. The AUC 0–180 min for plasma GIP was greater (5,838 ± 623 vs. 4,580 ± 640, P < 0.05) with the variable infusion.

Plasma GLP-1. Plasma GLP-1 (Fig. 1D) concentrations increased from baseline during each infusion (P < 0.05). Between 0 and 75 min, plasma GLP-1 concentrations were greater during the variable compared with the constant infusion (P < 0.05). Between 75 and 180 min, there was no difference in plasma GLP-1 between the two infusions. The AUC 0–180 min for plasma GLP-1 was greater (2,796 ± 238 vs. 1,807 ± 148, P < 0.05) with the variable infusion.

DISCUSSION

Our observations indicate that the stimulation of incretin hormones and “early” insulin release by a more rapid initial rate of duodenal glucose delivery, designed to approximate the early phase of liquid gastric emptying, does not lead to an overall reduction in the glycemic response to a standardized enteral glucose load given over a fixed time in healthy subjects.

The present study represents a logical development from our recent report (23), which evaluated the effects of more minor variations in the pattern of small intestinal delivery of glucose on glycemic, insulin, and incretin hormone responses in both healthy subjects and patients with type 2 diabetes. In that study, the rates of intraduodenal glucose infusion (0.71–3 kcal/min) were selected to be within the reported range observed for the linear emptying phase of glucose-containing liquids in healthy subjects (3, 4, 14). Hence, the impact of the normal, initially more rapid emptying phase was not evaluated. When the two
studies were compared, there were substantial differences in the patterns of blood glucose, insulin, and incretin hormone responses. In our initial study, the variable glucose infusion was 3 kcal/min between 0 and 15 min, followed by 0.71 kcal/min between 15 and 120 min; the present study employed 6 kcal/min between 0 and 10 min, followed by 0.55 kcal/min from 10 to 120 min. Compared with the previous study, 1) the magnitude of the initial rises in blood glucose, plasma insulin, and plasma GLP-1, but not GIP, in response to the variable infusion, were substantially greater; 2) during the variable infusion, blood glucose concentrations between 75 and 180 min were significantly less compared with those that resulted from the constant infusion; and 3) the overall glycemic response was the same as the variable when compared with the constant infusion, rather than greater.

The substantial initial increase in blood glucose in response to the variable infusion is not surprising; even in healthy subjects, relatively minor differences in duodenal glucose delivery may have a major effect on the initial glycemic response to carbohydrate meals (2, 12, 20, 21, 26). The increased stimulation of insulin can be accounted for by the greater rise in blood glucose and GLP-1. We confirmed that there is modest stimulation of GLP-1 in response to a 1-kcal/min duodenal glucose infusion (23), which apparently conflicts with the report by Schirra et al. (25), suggesting that a threshold of duodenal glucose delivery in excess of ~1.8 kcal/min is required for GLP-1 release. It has been assumed that the stimulation of GLP-1 by enteral glucose is a load- rather than concentration-dependent phenomenon, as has been documented to be the case for the regulation of gastric emptying of glucose (14, 17), but this has not been formally evaluated and may account for the discrepancy. Nevertheless, the stimulation of GLP-1 secretion by the 6-kcal/min infusion was substantially greater than the response to 3 kcal/min, which is not surprising (25). Further studies are needed to define the small intestinal glucose load, which results in maximum stimulation of GLP-1 release.

In contrast, there was no difference in the initial GIP response between the two variable infusions in our current and previous studies, suggesting that stimulation by 3 kcal/min may have already been maximal. In the study by Schirra et al. (25), the GIP response to intraduodenal glucose infusion at 2.2 kcal/min was greater than that at 1.1 kcal/min. A difference in the effect of small intestinal glucose on GIP and GLP-1 is not unexpected. GIP is released from duodenal K-cells, whereas GLP-1 is released from L-cells whose concentration is greatest in the distal jejunum.

Although the lowering of blood glucose between 75 and 180 min was likely to be attributable to the greater stimulation of plasma insulin that preceded it, it should be recognized that the rate of duodenal glucose delivery during this time was less with the variable, compared with the constant, infusion. We did not use glucose tracer techniques in this study that might have clarified which of these phenomena made the greater contribution.

Our study was designed to provide insights potentially relevant to the management of postprandial glycemia in patients with type 2 diabetes. Although we did not study type 2 patients, it is well documented that first phase insulin secretion is impaired in this group, as is the incretin effect (19). The latter appears to reflect impaired secretion of GLP-1 (27, 28) and a reduced insulinitropic effect of GIP (20, 29). Hence, our observations in healthy subjects add to the rationale for the use of dietary and pharmacological strategies designed to reduce postprandial glycemic excursions by slowing gastric emptying in this group, rather than initially accelerating it (24).
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