The Rate of Intestinal Glucose Absorption Is Correlated with Plasma Glucose-Dependent Insulinotropic Polypeptide Concentrations in Healthy Men

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ABSTRACT Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) both play a role in the control of glucose homeostasis, and GIP is implicated in the regulation of energy storage. The capacity of carbohydrates to induce secretion of these incretin hormones could be one of the factors determining the metabolic quality of different types of carbohydrates. We analyzed the correlation between the rate of intestinal absorption of (starch-derived) glucose and plasma concentrations of GLP-1 and GIP after ingestion of glucose and starchy foods with a different content of rapidly and slowly available glucose. In a crossover study, glucose, insulin, GLP-1, and GIP concentrations were monitored for 6 h after consumption of glucose, uncooked cornstarch (UCCS) or corn pasta in 7 healthy men. All test meals were naturally labeled with 13C. Using a primed, continuous 6,6-2H-glucose infusion, the rate of appearance of exogenous glucose (RaEx) was estimated, reflecting the rate of intestinal glucose absorption. GLP-1 concentrations increased significantly from 180 to 300 min after ingestion of UCCS, the starch product with a high content of slowly available glucose. A high GIP response in the early postprandial phase (15–90 min) occurred after consumption of glucose. There was a strong positive within-subject correlation between RaEx and GIP concentrations (r = 0.73, P < 0.01) across the test meals. Rapidly and slowly digestible carbohydrates differ considerably in their ability to stimulate secretion of incretin hormones; the metabolic consequences of such differences warrant exploration.


KEY WORDS: • starch • glucose absorption • glucagon like peptide-1 • glucose-dependent insulinotropic peptide • stable isotopes

Various types of dietary carbohydrates differ considerably in the effects they exert on metabolic parameters such as the postprandial glucose or insulin response. Reduction of postprandial hyperglycemia is of importance for patients with diabetes and persons with impaired glucose tolerance, but possibly also for the healthy population. The main determinants of the postprandial glucose response are the amount and type of the ingested carbohydrates, but other factors such as the composition of the meal and the rate of gastric emptying also play a role (1). Furthermore, it was shown that the composition of the preceding meal can affect the postprandial glucose response (2–4). In addition, incretin hormones, which are secreted by endocrine cells located in the gastrointestinal mucosa, influence postprandial glucose excursions by potentiating glucose-induced insulin secretion (5,6). The main incretin hormones are glucagon like peptide-1 (GLP-1)3 and glucose-dependent insulinotropic polypeptide (GIP, formerly known as gastric inhibitory polypeptide) (6), which also affects fat deposition (7–9). Both hormones are secreted in response to

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3 Abbreviations used: AUC, incremental area under the curves; Cmax, maximum paracetamol concentration; CP, corn pasta; cum dose %, percentage of the administered dose glucose equivalents; GIP, glucose-dependent insulinotropic polypeptide; GIP receptor knockout; GLP-1, glucagon-like peptide-1; IRS1−/−, insulin receptor substrate-1 knockout; RaEx, rate of appearance of exogenous glucose; RAG, rapidly available glucose; RaT, rate of appearance of total glucose; RS, resistant starch; SAG, slowly available glucose; T1/2, time to reach paracetamol peak concentration; UCCS, uncooked cornstarch.
Ingestion of a meal. In vitro and in vivo studies suggest that intraluminal glucose is one of the triggering factors for the secretion of these hormones (10,11). Because dietary carbohydrates vary in their rate of digestion and thus in glucose release and absorption, it is conceivable that the ability to stimulate incretin hormone secretion differs among the various types of carbohydrates. The capacity of carbohydrates to induce the secretion of incretin hormones could be one of the factors affecting the postprandial glucose response and therefore the "metabolic quality" of carbohydrates.

The aim of this study was to assess the effect of glucose and 2 starchy foods, varying in their content of rapidly and slowly available glucose, on plasma concentrations of GIP and GLP-1 in healthy volunteers and to establish whether the concentrations of those hormones are related to the rate of intestinal glucose absorption, which is reflected by the appearance of exogenous glucose in blood.

MATERIALS AND METHODS

Subjects

Healthy men [n = 7; age 23.4 ± 1.0 y, BMI = 21.6 ± 1.1 kg/m² (mean ± SEM)] were recruited by advertising. The criteria for exclusion were use of medications, blood donation in the previous 6 mo, use of antibiotics in the last 3 mo, gastrointestinal symptoms, diabetes mellitus, and gastrointestinal surgery. Each subject gave written informed consent for the study, and approval was obtained from the Medical Ethics Committee of the University Medical Center in Groningen.

Test meals. Three test meals were selected that were expected to differ in the rate at which glucose derived from these meals would appear in the systemic circulation. In addition, the carbohydrates in these test meals had to be derived from either C4 or CAM plants, because these carbohydrates are naturally labeled with $^{13}$C; this is necessary to be able to apply the dual isotope technique. For the test meals, 55 g glucose (90% carbohydrates; glucose-monohydrate, Natufood, Natuproduts BV) was dissolved in 250 mL of tap water; 53.5 g uncooked cornstarch (UCCS; 85% carbohydrates; Koopmans) was added to the same amount of tap water, and 50.4 g (dry weight) corn pasta (CP; 90% carbohydrates; Honig) was cooked for 10 min in 1 L of water. The drained pasta was served immediately after cooking. The test meals were planned to contain 50 g of glucose or glucose equivalents (UCCS, CP), and portion sizes were calculated on the basis of carbohydrate contents from the product information. The $^{13}$C abundance (atom %) of the corn-derived glucose was 1.09824%, for the UCCS, it was 1.09752%, and for the CP 1.09833%.

In vitro carbohydrate analysis of the starch-containing test meals was conducted according to the method of Englyst, as described previously (12). For the measurement of rapidly available glucose (RAG), slowly available glucose (SAG), and starch fractions, CP was milled to mimic chewing. Then, ∼500 mg carbohydrate of each starchy test meal was treated with pepsin and incubated with a mixture of hydrolytic enzymes under standardized conditions of mixing, pH, and temperature. Exactly 20 and 120 min after the start of the hydrolysis, samples were taken from the incubation mixture. RAG is defined as the glucose released in the first 20 min and SAG as the glucose released between 20 and 120 min, as measured by HPLC. The glucose released after dispersion and hydrolysis of the remaining starch into the incubation mixture is defined as resistant starch (RS).

Study protocol

The study was performed in a crossover manner, with each subject studied on 3 occasions at least 1 wk apart. The subjects were asked to refrain from consuming foods enriched in $^{13}$C, such as cane sugar, corn, corn products, and pineapple, for the 3 d preceding the experiments. The subject's food intake after 1700 the day before each experiment was individually standardized. Subjects refrained from alcohol and strenuous exercise for the 24 h before each study day.

They fasted and drank only water, coffee, or tea without sugar and milk from 2200 the night before the study. Subjects arrived at 0800 on each study day. Cannulas were inserted into veins in both forearms, one for the collection of blood, kept patent with heparin (50 kIE/L), and the other for infusion of $^{6,6-^{2}}$H$_{2}$glucose (98% $^{2}$H atom % excess; Isotec). Throughout the study, subjects were encouraged to relax by reading or watching videos.

A primed-continuous infusion of $^{6,6-^{2}}$H$_{2}$glucose (prime: 342 mg; continuous infusion: 3.5 mg/min (9.5 g/L)) was started at time minus 120 min, and blood samples were taken at frequent intervals for 8 h; 120 min after the beginning of the infusion (t = 0), the test meal was ingested. At the same time that the glucose and UCCS drink were ingested, 1.5 g paracetamol, as a marker of the gastric emptying rate, was administered in 100 mL tap water. The gastric emptying rate of CP was not measured because incorporation of the marker into the pasta was not possible.

Sample collection. Blood was collected throughout the study into tubes containing sodium fluoride potassium oxalate, into blank tubes, and into ice-chilled EDTA tubes containing aprotime (5 × 10³ Kallikrein Inhibitor Units/L of blood; Transyl, Bayer) (13). After centrifugation (1500 g, 7 min) at 4°C, the samples were stored at −20°C until assayed. Breath samples were collected into exetainers (Labco). Basal blood and breath samples were collected before the beginning of the infusion. Blood samples were taken every 30 min for 90 min, every 15 min for the next 150 min, and every 30 min thereafter. Breath samples were collected every 30 min for 90 min and every 15 min in the 390 min thereafter.

Analytical procedures. Glucose was measured with an ECA-180 glucose analyzer (Medingent). The interassay and intra-assay CV was 3 and 1%, respectively. Insulin concentrations were measured in duplicate using a commercially available RIA (Diagnostic Systems Laboratories). The interassay and intra-assay CV was 9.9 and 4.9%, respectively. The sample preparation procedure for the analysis of the isotopic enrichment of plasma glucose is described in detail elsewhere (14); only some minor modifications were made. Glucose was extracted with ethanol. The extract was dried under nitrogen gas; thereafter, glucose was derivatized to its penta-acetate-ester using acetic acid anhydride-pyridine. After evaporation of the reagent, the derivative was dissolved in 1.25 mL acetonitrile. The $^{13}$C-$^{14}$C isotope ratio measurement of the glucose penta-acetate derivative was determined by GC-Combustion-Isotope Ratio MS as described previously (14,15). The derivatization in the derivative was carried out by GC-MS (14). GIP and GLP-1 concentrations in plasma were measured after extraction of plasma with 70% ethanol (v:v, final concentration). For the GIP RIA (16), we used the C-terminally directed antiserum R 65, which cross-reacts fully with human GIP but not with the so-called C-terminus of GIP, 90% GIP and 125-I human GIP (70 MBq/nmol) were used for standards and tracer. The plasma concentrations of GLP-1 were measured (17) against standards of synthetic GLP-1 7-36 amide using antisemur code number 89390, which is specific for the amidated C-terminus of GLP-1 and therefore does not react with GLP-1 containing peptides from the pancreas. The results of the assay accurately reflect the rate of secretion of GLP-1 because the assay measures the sum of intact GLP-1 and the primary metabolite, GLP-1 9-36 amide, into which GLP-1 is rapidly converted (18). For both assays, sensitivity was <1 pmol/L. The intra-assay CV was <6% at 20 pmol/L, and recovery of standard, added to plasma before extraction, was within ±10% of expected values, when corrected for losses inherent in the plasma extraction procedure.

Serum paracetamol concentrations were determined by fluorescence polarization immunosassay on an Abbott Axsym full automatic analyzer. The CV was 3.8%. Breath hydrogen analysis was performed by GC (HP 6890 Agilent, Hewlett Packard), using a 5A column of 25 m × 0.53 mm (50-μm film thickness) (Chrompack International). All plasma samples of one subject were analyzed together to exclude the effects of interbatch variation.

Calculations. The molar percentage enrichment of [6,6-$^{2}$H] glucose and the $^{13}$C atom percentage were calculated as previously described (14), and smoothed using the Optimized Optimal Segments program developed by Bradley et al. (19). The rate of total (endogenous and exogenous) glucose appearance (RaT) in plasma was...
RESULTS

Test meals. Glucose per definition consists of 100% RAG. The RAG content in CP (89% of total amount of carbohydrates) was higher than in UCCS (27.8%) as expected, based on the starch characteristics. Of the total amount of carbohydrates in CP and UCCS, 6.8 and 45.3% respectively, was SAG.

The portion sizes of the test meals were calculated in such a way as to provide 50 g of glucose equivalents, based on the product information on the package. However, for the CP, after the experiment was completed, it was found that there was a loss of substrate to the cooking water during preparation. After this adjustment was made, the CP meal was calculated to contain 32.7 g of glucose equivalents.

Plasma glucose. Fasting plasma glucose concentrations did not differ on the glucose (5.3 ± 0.2), UCCS (5.2 ± 0.1), and CP (5.3 ± 0.1 mmol/L) study days, P = 0.776. The peak postprandial plasma glucose concentration was higher after glucose ingestion (8.8 ± 0.5 mmol/L) than after UCCS (6.5 ± 0.4 mmol/L, P = 0.009) and CP (6.7 ± 0.3 mmol/L, P = 0.004). The time to peak values did not differ (Fig. 1A). The AUC from 0 to 120 min was higher after the ingestion of glucose than that after either CP (P = 0.023) or UCCS ingestion (P = 0.025). The AUC from 120 to 240 min was lower after the glucose meal compared with that after the UCCS meal (P = 0.006) (Table 1).

TABLE 1
Postprandial plasma glucose, insulin, GIP, GLP-1 concentrations (2-h AUC), and rate of appearance of exogenous glucose (RaEx, 2-h cumulative dose %) in healthy men after ingestion of 55 g glucose, 53.5 g UCCS, and 50.4 g (dry weight) CP.

<table>
<thead>
<tr>
<th>Glucose</th>
<th>UCCS</th>
<th>CP</th>
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<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td></td>
<td></td>
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<tr>
<td>AUC 0–120 min</td>
<td>181.7 ± 40.1</td>
<td>74.7 ± 21.1</td>
</tr>
<tr>
<td>AUC 120–240 min</td>
<td>0.7 ± 0.5</td>
<td>20.2 ± 6.4</td>
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<tr>
<td>Insulin, pmol/L</td>
<td></td>
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<tr>
<td>AUC 0–120 min</td>
<td>13450 ± 2451</td>
<td>4964 ± 1077</td>
</tr>
<tr>
<td>AUC 120–240 min</td>
<td>529 ± 174</td>
<td>3726 ± 705</td>
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<tr>
<td>RaEx, cum dose %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC 0–120 min</td>
<td>62.4 ± 2.7</td>
<td>35.4 ± 3.9</td>
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<tr>
<td>AUC 120–240 min</td>
<td>13.4 ± 1.4</td>
<td>34.6 ± 2.7</td>
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<tr>
<td>GIP, pmol/L</td>
<td></td>
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<tr>
<td>AUC 0–120 min</td>
<td>3859 ± 531</td>
<td>1174 ± 172</td>
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<tr>
<td>AUC 120–240 min</td>
<td>365 ± 85</td>
<td>1183 ± 241</td>
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<tr>
<td>GLP-1, pmol/L</td>
<td></td>
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<tr>
<td>AUC 0–120 min</td>
<td>603 ± 80</td>
<td>324 ± 164</td>
</tr>
<tr>
<td>AUC 120–240 min</td>
<td>91 ± 44</td>
<td>928 ± 136</td>
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</table>

† Values are means ± SEM, n = 7. Means in a row with superscripts without a common letter differ, P < 0.05.
Plasma rate of appearance of total glucose. The basal RaT did not differ on the glucose (2.2 ± 0.1), UCCS (2.2 ± 0.1), and CP [2.4 ± 0.1 mg/(kg · min)] study days, *P* = 0.379. After ingestion of glucose, the RaT increased to a peak rate of 6.8 ± 0.4 mg/(kg · min) at 34 ± 3 min. The peak postprandial RaT [3.7 ± 0.3 mg/(kg · min)] at 56 ± 15 min on the UCCS study days was lower (*P* = 0.002) than that on the glucose study days. Also, after ingestion of CP, the peak RaT of 3.8 ± 0.3 mg/(kg · min) at 39 ± 3 min was lower than that after ingestion of glucose (*P* = 0.004).

Plasma rate of appearance of exogenous glucose. The RaEx after ingestion of glucose reached a peak of 5.2 ± 0.3 mg/(kg · min) (Fig. 2). After ingestion of both UCCS and CP, the peak RaEx was lower than that after glucose ingestion [2.6 ± 0.4 mg/(kg · min), *P* < 0.001 and 2.4 ± 0.4 mg/(kg · min), *P* = 0.002 respectively]. The time to peak values, for glucose (49 ± 3 min), UCCS (93 ± 20 min), and CP (47 ± 8 min) did not differ. The glucose meal resulted in a higher AUC from 0 to 120 min than the UCCS meal (*P* = 0.004) and a lower AUC from 120 to 240 min than either of the other test meals (UCCS: *P* < 0.001, CP: *P* = 0.002) (Table 1).

Gastric emptying. Based on the paracetamol results, gastric emptying was slower after ingestion of glucose than after ingestion of UCCS (T_{max}:C_{max} glucose: 6.1 ± 0.5, T_{max}:C_{max} UCCS: 2.1 ± 0.5, *P* = 0.018).

GIP and GLP-1. Plasma GIP concentrations at baseline for glucose (4.6 ± 1.8), UCCS (6.4 ± 1.2), and CP (5.0 ± 1.4 pmol/L), did not differ, *P* = 0.617. The peak GIP concentrations (54.9 ± 7.2 pmol/L) after ingestion of glucose were higher than that after CP (26.0 ± 3.5 pmol/L, *P* = 0.002) and UCCS ingestion (24.3 ± 3.1 pmol/L, *P* = 0.003). The time to peak values did not differ (Fig. 3A).

The AUC from 0 to 120 min after ingestion of glucose was higher for GIP than that after UCCS and CP ingestion (*P* = 0.004 and *P* = 0.002 respectively). The glucose meal significantly lowered the AUC from 120 to 240 min compared with the UCCS meal (*P* = 0.016) (Table 1).

Plasma GLP-1 concentrations at baseline for glucose (18.6 ± 1.6), UCCS (20.1 ± 2.2), and CP (19.9 ± 1.3 pmol/L) did not differ, *P* = 0.656. The peak concentrations after ingestion of glucose (35.6 ± 2.9 pmol/L) were significantly higher than those after ingestion of CP (23 ± 1.6 pmol/L, *P* = 0.005). The time to peak after ingestion of UCCS (141 ± 34 min) was delayed compared with that after ingestion of glucose (19 ± 3 min) (*P* = 0.030) (Fig. 3B).

DISCUSSION

This study was conducted to assess the effect of ingestion of glucose and 2 starchy foods with different contents of rapidly and slowly available glucose on the plasma concentrations of GIP and GLP-1 in healthy volunteers and to investigate whether the concentrations of these hormones are related to the rate of intestinal glucose absorption.

Little is known concerning whether stimulation of the secretion of incretin hormones is influenced by the type of carbohydrate ingested. The hormones studied play a role in the regulation of postprandial glucose homeostasis and energy storage (7–9). More knowledge about how different types of...
carbohydrates influence incretin hormone secretion could contribute to defining their "metabolic quality."

The in vitro characteristics of the starchy foods, CP and UCXS, were measured according to the method of Englyst. With this method, the glycemic glucose fraction is divided into RAG and SAG to reflect the likely rate of release and absorption of glucose. CP contained more RAG than UCXS (89 and 28% of the total amount of carbohydrates, respectively). These results thus confirmed our expectations regarding the digestive characteristics of CP and UCXS.

The main finding of this study is that the rate of exogenous glucose appearance is strongly correlated with the plasma concentrations of GIP. Furthermore, we showed that slowly available carbohydrates can induce late and prolonged GLP-1 and GIP responses.

GLP-1 is synthesized within L-cells that are found in high density in the ileum and colon (26,27) and recently were reported to be present in the duodenum in comparable amounts to GIP-releasing K-cells (28). Glucose and other nutrients stimulate GLP-1 secretion (29). In our study, glucose ingestion caused an early GLP-1 response that coincided with glucose absorption in the early postprandial phase, and a late GLP-1 response was observed after ingestion of slowly available carbohydrates (UCXS). However, the correlation between the rate of glucose absorption and plasma concentrations of GLP-1 was not strong, indicating that indirect stimulation via neural or hormonal pathways (30) may play a more important role. The late and prolonged rise in GLP-1 secretion is consistent with findings of studies in which the absorption of rapidly absorbable carbohydrates was delayed with an α-glucosidase inhibitor (31,32). A substantial part of the absorption of glucose derived from UCXS can be expected to take place more distally than that from glucose or CP because of the slowly digestible starch characteristics of this test meal.

The relevance of this late GLP-1 response is unclear. In view of the capacity of GLP-1 to decelerate the gastric emptying rate even at physiological concentrations (2,33), we speculate that an elevated GLP-1 concentration at the time point of the intake of a subsequent meal could affect nutrient delivery to the small intestine, resulting in decreased postprandial glucose concentrations. Indeed, improved glucose tolerance to meals after ingestion of foods rich in slowly available carbohydrates was reported (2–4). Further studies are warranted to provide evidence of such an association.

GIP is produced by the K-cells located in the proximal intestine and its secretion is stimulated by absorbable carbohydrates and lipids (6,7). Schirra et al. (10) showed that GIP release is proportional to the glucose delivery in the duodenum using duodenal glucose perfusion. On the other hand, a more recent study in rats (34) suggests an indirect role of GIP in the regulation of glucose absorption. GLP-2, which is released from intestinal L-cells in response to GIP, was shown to stimulate GLP-1 secretion (29). In our study, glucose ingestion caused an early GLP-1 response that coincided with glucose absorption in the early postprandial phase, and a late GLP-1 response was observed after ingestion of slowly available carbohydrates (UCXS). However, the correlation between the rate of glucose absorption and plasma concentrations of GLP-1 was not strong, indicating that indirect stimulation via neural or hormonal pathways (30) may play a more important role. The late and prolonged rise in GLP-1 secretion is consistent with findings of studies in which the absorption of rapidly absorbable carbohydrates was delayed with an α-glucosidase inhibitor (31,32). A substantial part of the absorption of glucose derived from UCXS can be expected to take place more distally than that from glucose or CP because of the slowly digestible starch characteristics of this test meal.

In conclusion, we found differences in incretin response after ingestion of rapidly and slowly available carbohydrates. These findings might be relevant for the investigation of beneficial "late" effects of slowly available carbohydrates and of possible associations between rapidly available carbohydrates and the development of obesity.

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LITERATURE CITED


