Importance of changes in gastric emptying for postprandial plasma glucose fluxes in healthy humans

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jective: Regulation of postprandial (pp) plasma glucose excursions is complex. Insulin and glucagon are thought to play the predominant role. Nevertheless, only 50% of the variation in pp glucose excursions is explained by variations in the latter. Theoretically, gastric emptying (GE) should be another important factor. However, its impact on pp glucose homeostasis is unknown. Research Design and Methods: We examined the consequences of pramlintide-induced delay in GE on pp glycemia and glucose fluxes, determined isotopi-

cally. GE was recorded by scintigraphy. Fourteen healthy subjects (8 men, 6 women; age 40 ± 3 yr, body mass index 27.8 ± 1.1 kg/m2) ate a mixed meal, and 30 µg of pramlintide (PRAM) or placebo (PBO) were injected subcutaneously. Results: At 60 min, greater proportions of the initial gastric contents remained in the stomach (PBO vs. PRAM). Thereafter, GE slopes paralleled until 240 min. Fifty percent retention times were lower when PBO was given (P < 0.001). GE was greater from 240 min to the end of the PRAM experiments, so that only slightly greater proportions of the meal remained in the stomach at 330 min. Reductions of GE lowered pp glucose (P < 0.001), even though plasma insulin was lower with PRAM (164 ± 13 vs. 138 ± 13 pmol/ml, both P < 0.10). Reduction in total glucose appearance (P < 0.001) was due to reduced meal-derived glucose appearance (10.2 ± 0.5 vs. 7.0 ± 0.4 µmol·kg⁻¹·min⁻¹, P < 0.001). Endogenous glucose appearance was greater with PRAM (P < 0.001). Splanchnic glucose uptake was greater with PRAM (26.5 ± 1.6 vs. 32.5 ± 2.1%, P = 0.014). Conclusions: These data support the concept that GE is an important physiological regulator of pp glucose homeostasis in humans.

Glucose fluxes have generally been attributed to an interaction between pancreatic islet cell function (insulin and glucagon secretion) and hepatic and peripheral sensitivity to insulin (1, 2). Although this approach can explain to a large extent fasting glucose homeostasis, it can explain only ~50% of the variation in postprandial glucose excursions (20, 22). The importance of postprandial hyperglycemia has recently been emphasized, because it precedes fasting hyperglycemia as a precursor of diabetes and has been identified as an independent risk factor for cardiovascular disease (9).

Prominent among the other factors that may determine postprandial glucose excursions is glucose influx from the gut (12). This appears to be determined to a certain extent by the rate of gastric emptying, since previous studies suggest that as much as 40–50% of variation in postprandial glucose excursions may be explained by differences in the rate of gastric emptying (11).

The present studies were therefore undertaken to test the hypothesis that slowing of gastric emptying would reduce postprandial entry of glucose into the systemic circulation and thus lower postprandial plasma glucose excursions. For this purpose, we administered a physiological meal, delayed gastric emptying with the amylin analog pramlintide, and assessed the consequences on postprandial plasma glucose concentrations, endogenous glucose production, exogenous (meal derived) glucose appearance, splanchnic sequestration, and peripheral plasma glucose disposal.

Methods

Subjects. Informed written consent was obtained from 14 healthy volunteers after approval of the protocol by the Ludwig Maximilian University of Munich Institutional Review Board. The subjects (8 men, 6 women; age 40 ± 3 yr, body weight 78 ± 4 kg, body mass index 27.8 ± 1.1 kg/m2) had normal physical examinations, no gastrointestinal symptoms, and normal routine laboratory tests as well as normal glucose tolerance [assessed by oral glucose tolerance testing according to World Health Organization criteria (24)]. None of the subjects had a family history of diabetes. For 3 days before the study, all subjects had been on a weight-maintaining diet containing at least 200 g of carbohydrate and had abstained from alcohol and exercise.

Protocol. Subjects restrained from food intake at least 10 h before admission to the Clinical Research Center, between 7:00 and 7:30 AM on the study day. A dorsal hand vein was cannulated, and temperature was maintained at 40°C with a thermoregulated lamp for arterialized venous blood sampling (20). At 8 AM, a primed (25 µmol) continuous (~0.25 µmol·kg⁻¹·min⁻¹) infusion of [1-13C]glucose was started via a forearm vein of the contralateral arm for measurements of plasma glucose turnover. At least 3 h were allowed for achievement of isotope equilibration. Before meal ingestion, three baseline blood samples were collected for fasting glucose, insulin, glucagon, and [1-13C]glucose enrichments, and subjects ingested a standardized meal containing ~500 kcal within 5 min thereafter. The meal consisted of three scrambled eggs and 100 ml of Jell-O containing 50 g of glucose; 3 of the 50 g of glucose were 6,6-dideuteroglucose for measurements of meal-derived glucose. Consumption of 200 ml of sparkling water was allowed throughout the 330-min postprandial period. With the meal, 30 µg of pramlintide was injected subcutaneously. Results: At 60 min, greater proportions of the meal remained in the stomach (PBO vs. PRAM). Thereafter, GE slopes paralleled until 240 min. Fifty percent retention times were lower when PBO was given (P < 0.001). GE was greater from 240 min to the end of the PRAM experiments, so that only slightly greater proportions of the meal remained in the stomach at 330 min. Reductions of GE lowered pp glucose (P < 0.001), even though plasma insulin was lower with PRAM (164 ± 13 vs. 138 ± 13 pmol/ml, both P < 0.10). Reduction in total glucose appearance (P < 0.001) was due to reduced meal-derived glucose appearance (10.2 ± 0.5 vs. 7.0 ± 0.4 µmol·kg⁻¹·min⁻¹, P < 0.001). Endogenous glucose appearance was greater with PRAM (P < 0.001). Splanchnic glucose uptake was greater with PRAM (26.5 ± 1.6 vs. 32.5 ± 2.1%, P = 0.014). Conclusions: These data support the concept that GE is an important physiological regulator of pp glucose homeostasis in humans.

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or placebo (Amylin Pharmaceuticals, San Diego, CA) were injected into the subcutaneous tissue of the lower abdominal wall. All subjects were studied on two occasions separated by at least 1 day in randomized, single blinded order (pramlintide or placebo). Results of the placebo experiments will be partially reported elsewhere under different aspects. Subjects remained in a semisupine position throughout the study period. Over the initial 90 min of the postprandial period, blood samples were taken at 15-min intervals. Thereafter, blood samples were taken at 30-min intervals until 330 min. The scrambled eggs and Jell-O were labeled with 99m Tc-Sn-colloid at ~70 MBq for measurements of gastric emptying by high-resolution scintigraphy (20 images/min; Orbiter, Siemens, Erlangen, Germany) starting immediately after completion of the meal and continuing for the remainder of the experiment (14, 15).

Analytic procedures. Gastric outlines on the maximum intensity image were defined as the region of interest. Completion of gastric emptying was assumed at a reduction of the initial activity to <5%. Loss of activity was corrected for the radioactive half-life of 99mTc (14). According to the procedure guidelines of The Society of Nuclear Medicine (6), a continuous framing rate of 30–60 s is recommended to accurately determine lag phase and half-time of gastric emptying. Therefore, on the basis of our “high-resolution scintigraphy” (20 images/min), we calculated the time course of gastric emptying in 1-min intervals. As recommended in the Procedure Guideline for Gastric Emptying and Motility (6), we acquired the scintigraphic data in the left anterior oblique view with a single-head camera to avoid a (mathematical) tissue attenuation correction. Since all subjects were examined in a convenient semisupine position, movement artefacts were largely bypassed. The cine display was used to confirm the stomach outline and to determine the extent of patient motion so that the region of interest was adjusted appropriately. In the very rare cases of a significant subject motion, a nuclear medicine postprocessing software package (Hermes, Nuclear Diagnostics) provides a software tool for motion correction.

Blood samples were collected for glucose concentrations and [1-13C]glucose and [6,6-2H2]glucose enrichments in oxalate/flouride tubes and for insulin and glucagon concentrations in EDTA tubes.
containing a protease inhibitor. Samples were immediately placed in a 4°C ice bath, and plasma was separated within 30 min by centrifugation at 4°C. Plasma glucose concentrations, [1-13C]glucose enrichments, and [6,6-2H2]glucose enrichments were measured by previously described methods (4, 18). Plasma insulin and glucagon concentrations were determined by standard radioimmunoassays, as previously described (19). Coefficients of variation for insulin and glucagon assays were 2.2 and 4.0% within assay, respectively.

Calculations. Systemic release and uptake of glucose were calculated with steady-state equations before meal ingestion (23) and subsequently with the non-steady-state equations of DeBodo et al. (5) using a pool fraction of 0.65 and a volume of distribution of 200 ml/kg. The rate of appearance of the oral glucose load in the systemic circulation was calculated from [6,6-2H2]glucose data with the equation of Chiasson et al. (3, 13). Endogenous glucose release was calculated as the difference between the overall rate of plasma glucose appearance and the rate of appearance of exogenous glucose (13, 16). Overall splanchnic glucose disposal of the ingested glucose load was calculated as the difference between the amount of glucose ingested and the total appearance of the ingested glucose in the systemic circulation during the 330-min postprandial period, based on the proportion of the meal being emptied, as determined by measurements of gastric emptying at the end of the experiment.

Statistical analysis. Data are given as means ± SE, unless otherwise specified, using Statistica statistical software (1998 edition; Statsoft, Tulsa, OK). Normality of the distribution was assessed using the Kolmogorov-Smirnov test. For comparison of the two groups and to compare baseline data with mean data after meal ingestion, ANOVA for repeated measurements followed by post hoc comparison for paired groups was performed. A P value <0.05 was considered statistically significant. Determination of correlation between variables was performed using Spearman regression analysis. Values for the description of dynamic changes represent absolute values, unless otherwise specified.

RESULTS

Percent gastric retention, gastric retention slopes, lag periods, 50% retention time, and percent retention at 60 min. Data are presented in Fig. 1. Gastric content decreased rapidly within 60 min to a mean value of 77 ± 2% when placebo was administered. At the end of the experiment, only 6 ± 2% of the initial 99mTc counts could be detected in the region of interest. In contrast, subcutaneous pramlintide injection with the meal resulted in a significantly greater percentage of gastric content remaining in the stomach at 60 min (92 ± 1%, P = 0.0001 compared with placebo). Pramlintide prolonged the lag period (25 ± 8 vs. 74 ± 11 min, P = 0.0008), defined as the delay between ingestion of food and a 10% reduction of isotope counts in the region of interest. A significant overall effect of pramlintide on gastric emptying slopes could be detected. Assessment of retention slopes at 0–60, 60–240, and 240–330 min yielded significantly lower a-values for 0–60 min (a = −0.38 ± 0.03 vs. −0.13 ± 0.02, P < 0.001), comparable slopes for 60–240 min (a = −0.31 ± 0.02 vs. −0.27 ± 0.02, P = 0.08), and significantly greater a-values for 240–330 min (a = −0.17 ± 0.03 vs. −0.33 ± 0.04, P = 0.004) in the pramlintide experiment. Thus the significantly greater proportion of the meal remaining at the end of the experiment (14 ± 4%, P = 0.009) was largely due to the delay in gastric emptying within the first 60 min after meal ingestion, which resulted in a significant delay of the 50% retention time (T50) from 177 ± 9 to 227 ± 14 min (P = 0.001). Within the first 60 min, a total of 23 ± 2 vs. 8 ± 1% (P = 0.0001) of the meal was emptied by the stomach.

Plasma glucose. Data are presented in Fig. 2. Plasma glucose concentrations before meal ingestion were comparable on both study days (4.5 ± 0.1 vs. 4.6 ± 0.1 mmol/l, P = 0.47, placebo vs. pramlintide, respectively). In the placebo experiments, plasma glucose concentrations rose to a maximum of 7.5 ± 0.3 mmol/l at 60 min and returned to baseline levels at 180 min. In the pramlintide experiments, peak postprandial plasma glucose concentrations were significantly lower (6.0 ± 0.2 mmol/l at 120 min compared with 7.5 ± 0.3 mmol/l at 60 min, P = 0.0002). Mean postprandial plasma glucose concentrations were significantly reduced after pramlintide administration (5.6 ± 0.1 vs. 5.2 ± 0.1 mmol/l, P = 0.004). Areas under the plasma glucose curves over the entire postprandial period were lower with pramlintide (354.2 ± 31.1 vs. 207.0 ± 25.9 mmol/l·min, P = 0.003).

Fig. 2. Plasma glucose, insulin, and glucagon concentrations and insulin-to-glucagon molar ratio.
Plasma insulin and glucagon. Data are presented in Fig. 3. Plasma insulin concentrations paralleled those of plasma glucose concentrations (see above). Peak plasma insulin concentrations were significantly greater and earlier when placebo was administered (346 ± 110/1100637 pmol/ml at 45 min vs. 211 ± 42 pmol/ml at 90 min, both P < 0.001) compared with pramlintide. Average plasma insulin concentrations were significantly greater during the placebo experiment compared with pramlintide (188 ± 68 vs. 134 ± 13 pmol/ml, P < 0.001).

Basal plasma glucagon concentrations were comparable on both study days (59.7 ± 13.4 vs. 58.1 ± 5.8 pg/ml, P = 0.68), decreased to a comparable extent (nadir 48.1 ± 5.8 vs. 50.0 ± 3.7 pg/ml at 90 min, P = 0.70, placebo vs. pramlintide, respectively), and overall were not significantly different (65.6 ± 4.7 vs. 59.3 ± 5.0 pg/ml, P = 0.074, placebo vs. pramlintide, respectively).

As a result of the above differences, attributable primarily to differences in plasma insulin concentrations, the plasma insulin-to-glucagon molar ratio during the initial 90 min after meal ingestion was significantly greater after placebo administration compared with pramlintide (18.7 ± 15 vs. 9.0 ± 1.9, P = 0.001) and significantly lower between 150 and 330 min, averaging 5.0 ± 0.6 vs. 8.7 ± 1.1 (P = 0.001).

Rates of total plasma glucose appearance, exogenous (meal) plasma glucose appearance, and endogenous plasma glucose appearance. Data are presented in Fig. 4. Rates of basal glucose turnover were comparable on both study days (9.0 ± 0.3 vs. 9.4 ± 0.5 μmol·kg⁻¹·min⁻¹, P = 0.11, placebo vs. pramlintide, respectively). Pramlintide reduced peak (18.6 ± 1.6 μmol·kg⁻¹·min⁻¹ at 30 min vs. 12.7 ± 1.0 μmol·kg⁻¹·min⁻¹ at 120, P < 0.01) and overall rates of total glucose appearance in plasma (12.0 ± 0.5 vs. 10.7 ± 0.5 μmol·kg⁻¹·min⁻¹, P = 0.005).

Endogenous glucose production decreased more promptly in the placebo experiment, reaching a nadir of 1.4 ± 1.0 μmol·kg⁻¹·min⁻¹ at 45 min compared with a nadir of 3.1 ± 0.7 μmol·kg⁻¹·min⁻¹ at 90 min in the pramlintide experiment. On average, 3.5 ± 0.2 vs. 4.4 ± 0.2 μmol·kg⁻¹·min⁻¹ (P < 0.001) glucose were endogenously released in the placebo and pramlintide experiments, respectively. This corresponded to a 60.6 ± 1.5 vs. 55.0 ± 1.0% (P = 0.008) suppression of endogenous glucose production in the postprandial state.

Rates of exogenous glucose appearance (meal-derived glucose) increased to a maximum of 15.4 ± 1.1 μmol·kg⁻¹·min⁻¹ within 45 min after meal ingestion and declined steadily thereafter when placebo was administered with the meal.
Pramlintide decreased peak (15.4 ± 1.1 vs. 8.9 ± 0.7 μmol·kg⁻¹·min⁻¹, P < 0.001) and overall rates of exogenous glucose appearance (8.5 ± 0.4 compared with 6.3 ± 0.4 μmol·kg⁻¹·min⁻¹, P < 0.0001). Exogenous glucose appearance accounted for 70.7 ± 10.0% of total glucose appearance in the placebo experiment compared with 58.6 ± 1.6% in the pramlintide experiment (P < 0.001). The differences in rates of total glucose appearance (1.3 ± 0.4 μmol·kg⁻¹·min⁻¹, P = 0.005, see above) could thus be solely explained by differences in exogenous glucose appearance (2.2 ± 0.3 μmol·kg⁻¹·min⁻¹), since endogenous glucose appearance rates were greater in the pramlintide experiment (0.9 ± 0.2 μmol·kg⁻¹·min⁻¹, P = 0.001).

Splanchnic glucose uptake. Splanchnic glucose uptake estimated from the calculated proportion of the emptied gastric content, which did not appear in the systemic circulation, was significantly greater when pramlintide was administered (26.5 ± 1.6 vs. 32.5 ± 2.1%, P = 0.014) at the end of the experiment.

Rates of glucose disappearance. Data are presented in Fig. 5. In placebo experiments, rates of glucose disappearance were not significantly different from baseline values during the initial 45 min after meal ingestion, averaging 9.2 ± 0.8 μmol·kg⁻¹·min⁻¹ (P = 0.8), increasing to maximum values of 17.1 ± 1.5 μmol·kg⁻¹·min⁻¹ at 90 min, and decreasing steadily thereafter. In the pramlintide experiment, rates of disappearance were not significantly different from baseline during the first 90 min, averaging 8.8 ± 0.5 μmol·kg⁻¹·min⁻¹ (P = 0.17), with peak values of 14.5 ± 0.9 μmol·kg⁻¹·min⁻¹ at 210 min that were significantly lower compared with the placebo experiment (P = 0.001).

In the placebo experiment, rates of plasma glucose appearance exceeded rates of its removal by 5.6 ± 0.6 μmol·kg⁻¹·min⁻¹, corresponding to a total excess of 332 ± 35 μmol·kg⁻¹. In the pramlintide experiment, only 1.7 ± 0.2 μmol·kg⁻¹·min⁻¹ had not been removed from the circulation, corresponding to 201 ± 20 μmol/kg excessive glucose, both being significantly lower in the pramlintide experiments (P = 0.001 and P = 0.0001, respectively), explaining differences in peak plasma glucose concentrations. Since rates of removal of excessive plasma glucose exceeded rates of appearance by only -2.84 ± 0.1 vs. -1.4 ± 0.2 μmol·kg⁻¹·min⁻¹ (placebo vs. pramlintide), baseline plasma glucose values were not achieved until 120 min after peak plasma glucose concentrations in both experiments, explaining differences in overall plasma glucose excursions above baseline.

Regression analyses using rates of gastric emptying as the independent variable and peak plasma glucose concentrations and rates of exogenous glucose appearance as dependent variables. A significant inverse correlation between rates of gastric retention at 45 min and incremental peak plasma glucose concentrations (at 60 min in the placebo and 120 min in the pramlintide experiment) was found (r = 0.702, P = 0.001) (Fig. 3, top). Furthermore, the differences in gastric retention between the placebo and the pramlintide experiment correlated significantly with differences in incremental peak plasma glucose concentrations at 60 and 120 min, respectively (r = 0.474, P = 0.001).

There was a significant correlation between overall rates of gastric emptying and rates of exogenous glucose appearance (r = 0.564, P = 0.001). Furthermore, the differences in rates of meal-derived glucose appearance between placebo and pramlintide (mean rates from 0 min to time point of peak rates: 0–60 and 0–120 min in placebo and pramlintide experiments, respectively) correlated significantly with the differences in
rates of gastric emptying over the prevailing time intervals \( (r = 0.580, P = 0.001) \) (Fig. 3, bottom).

**DISCUSSION**

The present studies were undertaken to test the hypothesis that slowing of gastric emptying would reduce postprandial entry of glucose into the systemic circulation and thus lower postprandial plasma glucose excursions. The hormone amylin is cosecreted with insulin from the pancreatic β-cell (7). Amylin and its analog pramlintide have been shown to markedly delay gastric emptying (17). Accordingly, we used pramlintide to delay gastric emptying and found that the delay of gastric emptying was associated with a marked reduction of meal-derived glucose in plasma, especially within the first 60 min after meal ingestion, and a significant reduction in postprandial plasma glucose excursions. The reductions in postprandial plasma glucose excursions could be explained by both a reduction in glucose appearance in plasma, solely due to meal-derived glucose, and a relatively greater proportional uptake of glucose by peripheral tissues.

Pramlintide administration was associated with a significant overall effect on gastric emptying. The delay in gastric emptying occurred primarily because of an \( \sim 20\% \) reduction in gastric emptying within the first 60 min, whereas gastric emptying was found to be comparable to placebo between 75 and 240 min and somewhat greater thereafter. This delay in gastric emptying was accompanied by a significant reduction in peak and overall postprandial plasma glucose concentrations. Rates of meal-derived glucose appearance in the systemic circulation are determined by rates of gastric emptying and by the amount of glucose being sequestrated in the splanchnic bed (22). Previous studies in healthy humans found \( \sim 25–30\% \) of the glucose load being sequestrated in the splanchnic bed (13). In our placebo experiment, we found 26.7 ± 1.6% of glucose being sequestrated. Estimation of splanchnic sequestration in the pramlintide experiment yielded a significant increase (32.5 ± 2.1%, \( P < 0.05 \)) in the proportion of the oral glucose load being sequestrated. However, these estimates have to be interpreted with caution, since calculations of sequestration depend on the amounts of glucose being absorbed rather than emptied by the stomach. Differences in glucose absorption and greater rates of nonoxidative glycolysis cannot be excluded and may abolish differences in calculated rates of glucose sequestration. On the other hand, even if splanchnic glucose sequestration had been comparable, this would have occurred in the face of significantly lower postprandial insulin-to-glucagon ratios, which should have been accompanied by lower splanchnic sequestration rather than comparable or even increased rates. Thus it appears that modulation of gastric emptying, especially a reduction of the initial emptying, may have indeed improved splanchnic sequestration.

Pramlintide has been reported to suppress postprandial glucagon concentrations (8). However, we found no significant differences in plasma glucagon concentrations and, even more importantly, greater rates of endogenous glucose production in the pramlintide experiments, presumably because of lower postprandial plasma insulin concentrations.

Plasma glucose concentration increases until rates of plasma glucose removal equal its appearance (21). Postprandially, rates of plasma glucose appearance are largely determined by meal-derived plasma glucose appearance, responsible for the disequilibrium between rates of glucose appearance and disappearance (22). Delay in gastric emptying was associated with a reduction in the amount of meal-derived glucose entering the circulation. This reduced the disequilibrium between the rates of entry and exit of glucose from the circulation and thus reduced postprandial peak plasma glucose excursions by \( \sim 40\% \). The more glucose is rapidly added to the plasma pool, the longer it will take to remove the additional amounts of glucose (10). Accordingly, overall postprandial plasma glucose excursions are largely determined by the initial efflux of glucose into the circulation. It is important to note that this occurred in the face of significantly greater plasma insulin and comparable glucagon concentrations. Thus modification of glucose efflux via the gut, especially deceleration of initial gastric emptying, led to a relatively more sufficient disposal of glucose in the face of lower plasma insulin concentrations.

Taken together, these studies highlight the importance of gastric emptying to determine postprandial glucose fluctuations. The pramlintide-induced initial delay in gastric emptying was associated with a reduction in meal-derived glucose appearance in plasma. Since endogenous glucose production was greater in the pramlintide experiment, reduction in total glucose appearance was solely attributable to reductions in meal-derived glucose appearance. A greater proportion of the meal-derived glucose was sequestrated in the splanchnic bed. Since the initial rapid efflux of glucose into the circulation largely determines postprandial plasma glucose excursions, delay in gastric emptying significantly reduced postprandial plasma glucose concentrations.

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