Effects of delayed gastric emptying on postprandial glucose kinetics, insulin sensitivity, and β-cell function

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Am J Physiol Endocrinol Metab 307: E494–E502, 2014. First published July 29, 2014; doi:10.1152/ajpendo.00199.2014.—Controlling meal-related glucose excursions continues to be a therapeutic challenge in diabetes mellitus. Mechanistic reasons for this need to be understood better to develop appropriate therapies. To investigate delayed gastric emptying effects on postprandial glucose turnover, insulin sensitivity, and β-cell responsivity and function, as a feasibility study prior to studying patients with type 1 diabetes, we used the triple tracer technique C-peptide and oral minimal model approach in healthy subjects. A single dose of 30 μg of pramlintide administered at the start of a mixed meal was used to delay gastric emptying rates. With delayed gastric emptying rates, peak rate of meal glucose appearance was delayed, and rate of endogenous glucose production (EGP) was lower. C-peptide and oral minimal models enabled the assessment of β-cell function, insulin sensitivity, and β-cell responsivity simultaneously. Delayed gastric emptying induced by pramlintide improved total insulin sensitivity and decreased total β-cell responsivity. However, β-cell function as measured by total disposition index did not change. The improved whole body insulin sensitivity coupled with lower rate of appearance of EGP with delayed gastric emptying provides experimental proof of the importance of evaluating pramlintide in artificial endocrine pancreas approaches to reduce postprandial blood glucose variability in patients with type 1 diabetes.

postprandial glucose excursions (22, 41, 42) due to alterations in glucose turnover estimated with the dual-isotope technique (9, 15, 23, 24, 29, 41, 42).

However, the dual-isotope technique utilized for these studies (41, 42) underestimates rate of meal glucose appearance (MRa) and rate of glucose disappearance (Rd) while providing inaccurate estimates of rate of endogenous glucose production (EGP) due to non-steady-state errors (34). The triple-tracer approach minimizes these errors, enabling accurate estimation of the components of glucose turnover after a meal (3, 34). It does so by permitting simultaneous assessment of MRa and EGP by infusing [6-3H]glucose to mimic systemic appearance of [1-13C]glucose contained in the meal and infusing [6-3H]glucose in a pattern mimicking anticipated changes in EGP after a mixed meal (3). This approach was subsequently shown to be better compared with the dual-tracer approach (34). Furthermore, to our knowledge, no prior study has examined the effect of delayed gastric emptying on insulin sensitivity (Si), β-cell responsivity, and disposition index (DI) by applying state-of-the-art C-peptide and oral minimal models.

To better understand the role of delayed GE on postprandial glucose metabolism, insulin action, and β-cell responsivity and function after a mixed meal, we applied the triple-tracer technique with the above models in healthy individuals, using pramlintide as a probe.

RESEARCH DESIGN AND METHODS

After approval by the Mayo Institutional Review Board, 16 healthy subjects were screened for the study after providing informed consent. Inclusion criteria included age 18–60 yr, BMI <40 kg/m², HbA1c ≤6.0% (42.1 mmol/mol), serum creatinine ≤1.5 mg/dl, normal fasting and 2-h post-75-g oral glucose tolerance test (OGTT), and normal GE for solids and liquids measured with scintigraphy (4). Exclusion criteria included family history of diabetes in first-degree relatives, significant gastrointestinal symptoms by Bowel Symptom Questionnaire (33), disorders of the gastrointestinal tract, medications known to affect gastric emptying (e.g., erythromycin), or glucose metabolism (e.g., corticosteroids), pregnancy, breast feeding, and any active systemic disease.

Screen Visit 1

After an overnight fast, subjects reported to Clinical Research Unit (CRU) of the Mayo Clinic Center for Clinical and Translational Science in the morning. To confirm eligibility, a medical history, physical examination, and blood tests, including a pregnancy test in female subjects with child-bearing capacity, were evaluated; dietician consultation ensured subjects were on a weight-maintaining diet that met American Diabetes Association standards of macronutrient and...
calorie composition. A 75-g OGTT was performed to ensure normal glucose tolerance, and body composition was measured by dual-energy X-ray absorptiometry (1).

Screen Visit 2

GE of solids and liquids was measured by established and validated scintigraphic techniques (4) in subjects that were eligible to participate after the first screening visit. Data were summarized as the half-time (T1/2) separately for liquids and solids (4). Subjects with normal GE were scheduled for inpatient studies.

Inpatient Study Visits

Glucose metabolism was evaluated by the triple-tracer technique on two occasions, during which subjects were treated with pramlintide or no pramlintide in randomized order.

Day 1. Subjects reported to the CRU at 4 pm. A DexCom 7 plus continuous glucose monitor (CGM) and a modular signal recorder (MSR) accelerometer (MSR Electronics, Seuzach, Switzerland) were placed and maintained for the rest of the inpatient study period as part of separate analyses. A mixed meal (10 kcal/kg, 55% carbohydrates, 30% fat) was consumed at 6 PM. No other food was provided until the next morning. An intravenous cannula was placed and maintained for the rest of the inpatient study period as part of a separate study. Dinner was provided at 7 PM. The following morning, the CGM and accelerometers were removed; subjects were provided breakfast and subsequently dismissed.

GE Test With Pramlintide

Within 1 wk after completion of the second inpatient study visit, subjects underwent a repeat GE test as described in screen visit 2 above. However, during this visit, 30 μg of pramlintide was administered subcutaneously at the start of the test to determine the effects of pramlintide on liquid and solid GE rates.

Analytical Methods

Insulin was measured with a two-site electrochemiluminescence immunoenzymatic assay by DXI automated system (Beckman Instruments, Chaska, MN). C-peptide was measured by the Cobas e411 (Roche Diagnostics, Indianapolis, IN) using a two-site electrochemiluminescence immunoassay. Glucagon was measured with a direct double-antibody radioimmunoassay (Linco Research, St. Charles, MO).

Plasma samples were placed on ice, centrifuged, and then separated and stored at −80°C until assay. Plasma glucose concentration was measured using a glucose oxidase method (YSI, Yellow Springs, OH). Plasma [6-3H]glucose-specific activity (SA) was measured by liquid scintillation counting as described (1). Plasma enrichment of [1-13C]glucose and [6, 6-2H2]glucose were measured using gas chromatography-mass spectrometry (Thermoquest, San Jose, CA) as described (3).

Calculations

Glucose kinetics. Fasting and postprandial rates of glucose turnover were calculated as described (3). Briefly, the systemically infused [6-3H]glucose was used to trace the systemic rate of appearance of [1-13C]glucose contained in the meal, and [6, 6-2H2]glucose was used to trace the rate of appearance of endogenously derived glucose.

The ratio of plasma concentration of [6-3H]glucose to [1-13C]glucose [SA(t)] was used to calculate the rate of appearance of ingested [1-13C]glucose:

\[
R_{13C}(t) = \frac{INF_{3H}(t)}{SA(t)} = -\frac{p \cdot V \cdot G_{13C}(t)}{SA(t)} \cdot \frac{dSA(t)}{dt} \tag{1}
\]

where INF3H is the infusion rate of [6-3H]glucose, G13C is the plasma concentration of [1-13C]glucose V is the volume of distribution and p is the pool fraction fixed to 200 and 0.65 ml/kg, respectively.

The total rate of appearance can be thus calculated as

For the rest of the study day, the subject ambulated periodically on a treadmill at a pace (1.9 km/h) that mimicked activities of daily living as part of a separate study. Dinner was provided at ~7 PM. The following morning, the CGM and accelerometers were removed; subjects were provided breakfast and subsequently dismissed.

Table 1. Baseline characteristics of the subjects completing the 2-meal study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Means ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>36.0 ± 11.9</td>
<td>21.0–56.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72.4 ± 15.8</td>
<td>51.9–104.7</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>9/3</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.3 ± 4.0</td>
<td>20.0–32.7</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>48.5 ± 10.7</td>
<td>37.7–72.3</td>
</tr>
<tr>
<td>Percent body fat, %</td>
<td>32.4 ± 8.9</td>
<td>16.7–45.1</td>
</tr>
</tbody>
</table>

Laboratory results

Fasting blood glucose, mM 4.6 ± 0.4 4.3–5.5
2-h Postprandial glucose, mM 5.3 ± 1.2 3.3–7.1
HbA1c % 5.1 ± 0.4 4.6–6.0
mmol/mol 32.0 ± 4.0 26.8–42.1
Hemoglobin, g/dl 13.4 ± 1.1 11.6–15.7
Creatinine, mg/dl 0.8 ± 0.2 0.6–1.2
BUN 14.1 ± 4.4 8.0–22.0
TSH, IU/l 2.0 ± 1.1 0.6–4.6

Data are means ± SD; n = 12 (9 males and 3 females). BUN, blood urea nitrogen.

Table 2. Meal content

<table>
<thead>
<tr>
<th>Variables</th>
<th>No Pramlintide</th>
<th>Pramlintide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>582.7 ± 110.5</td>
<td>574.9 ± 94.4</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>76.2 ± 0.5</td>
<td>76.2 ± 0.4</td>
</tr>
<tr>
<td>%</td>
<td>53.5 ± 8.4</td>
<td>53.9 ± 7.7</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>25.4 ± 9.6</td>
<td>24.7 ± 8.2</td>
</tr>
<tr>
<td>%</td>
<td>16.9 ± 3.0</td>
<td>16.8 ± 2.7</td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>19.8 ± 7.5</td>
<td>19.3 ± 6.6</td>
</tr>
<tr>
<td>%</td>
<td>29.6 ± 5.9</td>
<td>29.3 ± 5.5</td>
</tr>
<tr>
<td>Fibers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>%</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = 12.
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Glucose rate of disappearance can then be calculated as

$$R_d(t) = R_{\text{meas}}(t) - EGP(t) = p \cdot \frac{dG(t)}{dt}$$  (4)

where $$R_{\text{meas}}$$ is the ratio of [13C]glucose and unlabeled glucose in the meal.

Similarly, the ratio of plasma concentration of [6,6-2H2]glucose to endogenously produced glucose [tracer-to-tracee ratio (TTR)] was used to calculate EGP:

$$EGP(t) = \frac{INF_{2H2}}{TTR(t)} - \frac{p \cdot V \cdot G_{\text{end}}(t) \cdot dTTR(t)}{dt}$$  (5)

where $$INF_{2H2}$$ is the infusion rate of [6,6-2H2]glucose, $$G_{\text{end}}$$ is the plasma concentration of endogenous glucose [calculated by subtracting the concentration of exogenously derived (ingested) glucose (i.e., plasma [13C]glucose enrichment) from total plasma glucose concentration, and V is the volume of distribution and p is the pool fraction, fixed to 200 and 0.65 ml/kg, respectively.

Glucose rate of disappearance can then be calculated as

$$R_d(t) = R_{\text{meas}}(t) + EGP(t) - p \cdot \frac{dG(t)}{dt}$$  (4)

Table 3. Outcome measurements of 2 study visits without and with pramlintide results

<table>
<thead>
<tr>
<th>Rate of gastric emptying</th>
<th>No Pramlintide</th>
<th>Pramlintide</th>
<th>Difference</th>
<th>95% CI for mean</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid, minutes to 50% clearance</td>
<td>30 ± 9</td>
<td>110 ± 29</td>
<td>80 ± 33</td>
<td>59–101</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Solid, minutes to 50% clearance</td>
<td>173 ± 26</td>
<td>63 ± 26</td>
<td>46–79</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Glucose, mM iAUc 120 min</td>
<td>324.0 ± 94.3</td>
<td>280.9 ± 101.7</td>
<td>-43.2 ± 89.6</td>
<td>-100.1 to 13.8</td>
<td>0.12</td>
</tr>
<tr>
<td>Glucose, mM iAUc 360 min</td>
<td>365.8 ± 144.4</td>
<td>375.0 ± 122.1</td>
<td>9.2 ± 120.6</td>
<td>-67.4 ± 85.8</td>
<td>0.80</td>
</tr>
<tr>
<td>Glucose, mM iAUc 120 min</td>
<td>9.7 ± 1.3</td>
<td>9.3 ± 1.1</td>
<td>-0.4 ± 1.4</td>
<td>-1.3 ± 0.5</td>
<td>0.38</td>
</tr>
<tr>
<td>Time to peak, min</td>
<td>44 ± 12</td>
<td>86 ± 22</td>
<td>43 ± 26</td>
<td>26–59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin, pM iAUc 120 min</td>
<td>23,509.2 ± 7,769.4</td>
<td>14,641.8 ± 10,101</td>
<td>-8,868.0 ± 7,565.4</td>
<td>-1,367,464 ± 4,060.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Insulin, pM iAUc 360 min</td>
<td>31,136.4 ± 10,012.8</td>
<td>2,731,56 ± 13,848</td>
<td>-3,819 ± 8,878.2</td>
<td>(-9,460.2 ± 1,821.6)</td>
<td>0.16</td>
</tr>
<tr>
<td>Glucagon, pg/mL iAUc 120 min</td>
<td>388.8 ± 163.8</td>
<td>281.4 ± 118.8</td>
<td>-108 ± 135.6</td>
<td>-193.8 ± 21.6</td>
<td>0.019</td>
</tr>
<tr>
<td>Glucagon, pg/mL iAUc 360 min</td>
<td>51 ± 20</td>
<td>104 ± 23</td>
<td>53 ± 34</td>
<td>(31, 74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-peptide, nM iAUc 120 min</td>
<td>223.2 ± 78.4</td>
<td>127.9 ± 68.3</td>
<td>95.4 ± 68.9</td>
<td>-139.1 ± 51.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-peptide, nM iAUc 360 min</td>
<td>380.3 ± 146.4</td>
<td>326.0 ± 126.3</td>
<td>-54.3 ± 79.7</td>
<td>-104.9 ± 3.6</td>
<td>0.038</td>
</tr>
</tbody>
</table>
| Glucose rate of disappearance can then be calculated as

$$R_d(t) = R_{\text{meas}}(t) + EGP(t) - p \cdot \frac{dG(t)}{dt}$$  (4)

Meal Indices

The oral glucose minimal model (8, 11, 12) was used to interpret plasma glucose and insulin concentrations measured during the meal test. The meal was a mixed meal (10 kcal/kg) with 75 g of dextrose flavored with Jell-O, as described previously (3). Meal macronutrient contents are provided in Table 2. The model assumes that insulin action on glucose production and disposal emanates from a compartment remote from plasma, which is usually identified with the interstitium. The most important parameter of the model estimated from data is net insulin sensitivity (SI), which measures the overall effect of insulin to stimulate whole body (liver and periphery) glucose disposal and inhibit glucose production; details and calculations have been published previously (30). The oral C-peptide minimal model (35) was used together with the oral insulin minimal model (6) to interpret the interaction of plasma glucose with C-peptide and insulin, respectively. The oral C-peptide minimal model provides index of total β-cell responsivity (Φ) (10). In addition, we calculated total DI, a composite measurement of insulin secretion appropriate to the prevailing degree of insulin sensitivity (8), thus representing a marker of β-cell function.

Index of glucose action

$$S_t = \frac{1}{\text{Plasma glucose concentration (mg/dL)}}$$

$$\phi = \frac{1}{\text{Plasma glucose concentration (mg/dL)}}$$

$$DI = \frac{1}{\text{Plasma glucose concentration (mg/dL)}}$$

Data are mean ± SD; n = 12. CI, confidence interval; AUC, incremental area under the curve; EGP, endogenous glucose production; FFM, fat-free mass; SI, insulin sensitivity; DI, disposition index.
Statistical Analyses

The sample size for this study was estimated on the basis of the determination that a sample size of 12 was the minimum sample size required to achieve stable variance estimates for estimation purposes (19, 36). Results were expressed as means ± SD with 95% confidence interval (CI) for the mean, unless otherwise stated. Given the fact that the study was designed as a crossover study, the considerations for carryover (treatment contamination from the first study visit carried forward to the second study visit) and period (or order) effects required attention. The study made an assumption that a carryover effect was not biologically plausible given the short half-life of pramlintide (~3 h). A decision to not make a statistical adjustment for the period effect was determined based on the small sample size and the potentially advantageous larger residual error estimation (i.e., the variation in change in response attributable to the order effect would remain in the error term). As with the carryover effect, there was no a priori evidence to anticipate a period effect, as the study involved eating a labeled meal followed by standard experimental procedures. Thus, comparisons between visits with and without pramlintide were analyzed using a paired t-test with the assumption of no period or carryover effect. This analytical strategy was used for all outcome measurements presented in Table 3. For incremental area under the curve (iAUC), time 0 was used for baseline for all measurements. Differences were considered as statistically significant with \( P < 0.05 \) (2-sided). Analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC).

RESULTS

Subject Characteristics

Of the 16 subjects screened for the study, one subject failed the first screen visit due to abnormal OGTT, one refused to consume eggs during the mixed meal, and one had IV access difficulty. Thirteen subjects entered the crossover phase of the study, but data from one subject were excluded because the subject could not ingest the entire meal. Demographic of subjects is shown in Table 1. Briefly, the mean (SD) age and body mass index were 36 (12) yr and 25.3 (4.0) kg/m², respectively. Fasting plasma glucose concentrations, Hb A1C, and OGTT were normal, and subjects met inclusion criteria for normal GE prior to entry into the crossover phase of the study.

Mixed-Meal Content

The mixed meal composition is provided in Table 2.

GE Percentages for Solids and Liquids

With pramlintide, the \( T_{1/2} \) (750) for solid and liquid emptying was prolonged (Table 3). For liquids, an additional 80 min (95% CI: 59–101 min, \( P < 0.001 \)) was required to reach 50% clearance during the GE study. Similarly, solids required an additional 63 min (95% CI: 46–79 min, \( P < 0.001 \)). However, between-group difference in the prolongation of GE attributable to pramlintide was not statistically different for liquids and solids (mean: 18 min; 95% CI: −42 to 7 min; \( P = 0.14 \)).

Plasma Glucose, Insulin, C-peptide, and Glucagon Concentrations

The peak postprandial glucose was delayed on average 43 min (95% CI: 26–59 min, \( P < 0.001 \)) with the introduction of pramlintide (Fig. 1 and Table 3). This delay did not affect the

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Fig. 1. Glucose and islet hormone concentrations during triple-tracer meal studies without and with pramlintide in healthy subjects A: plasma glucose concentrations (time to peak delayed by pramlintide, \( P < 0.001 \)). B: plasma insulin concentrations [incremental area under the curve (iAUC) 0–120 min, \( P = 0.002 \)]. C: plasma C-peptide concentrations (iAUC 0–360 min, \( P = 0.038 \)). D: plasma glucagon concentrations (iAUC 0–120 min, \( P = 0.037 \)).

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The iAUC of MR_a was lower with pramlintide for 0–120 min after the meal [difference in iAUC: −1,440.1 μM·kg⁻¹·min over 2 h; 95% CI: −2,075.3 to −804.9 μM·kg⁻¹·min over 2 h; P < 0.001; Fig. 3 and Table 3]. However, the iAUC of MR_a during the entire 0–360 min did not differ with or without pramlintide (difference in iAUC: 693.7 μM·kg⁻¹·min over 6 h; 95% CI: −46.0 to 1,433.4 μM·kg⁻¹·min over 6 h; P = 0.063). The time to peak of rate of MR_a was delayed (difference: 70 min; 95% CI: 40–100 min; P < 0.001) with pramlintide.

In contrast, the iAUC of EGP did not differ with pramlintide for 0–120 min after the meal (difference in iAUC: 47.3 μM·kg⁻¹·min over 2 h; 95% CI: −121.6 to 216.1 μM·kg⁻¹·min over 2 h; P = 0.55). However, the iAUC of EGP during the entire 0–360 min was lower (difference in iAUC: −514.6 μM·kg⁻¹·min over 6 h; 95% CI: −943.1 to −86.0 μM·kg⁻¹·min over 6 h; P = 0.023) with pramlintide, whereas there were no differences in time to nadir of EGP (difference: −60 min; 95% CI: −181 to 62 min; P = 0.30) with or without pramlintide.

The iAUC of R_d was lower (difference in iAUC: −1,665.6 μM·kg⁻¹·min over 2 h; 95% CI: −2,367.9 to −963.3 μM·kg⁻¹·min over 2 h; P < 0.001) with pramlintide for 0–120 min after the meal. However, the iAUC of R_d during the entire 0–360 min did not differ with or without pramlintide (difference in iAUC: 124.7 μM·kg⁻¹·min over 6 h; 95% CI: −63.4 to 885.9 μM·kg⁻¹·min over 6 h; P = 0.73). The time to peak R_d was delayed (difference: 59 min; 95% CI: 39–78 min; P < 0.001) with pramlintide.

Insulin Action (S_b), β-Cell Responsivity (Φ), and DI

Whereas whole body insulin sensitivity was higher (difference: 5.0 × 10⁻⁴ dl·kg⁻¹·min⁻¹ per μU/ml; 95% CI: 0.8 to 9.2 × 10⁻⁴ dl·kg⁻¹·min⁻¹ per μU/ml; P = 0.024), Φ was lower (difference: −1.4 × 10⁻⁹/min; 95% CI: −2.5 to −0.2 × 10⁻⁹/min; P = 0.023) with pramlintide (Table 3 and Fig. 4). This resulted in an unchanged DI (difference: 70.0 × 10⁻¹⁴ dl·kg⁻¹·min² per pmol/l; 95% CI: −17.1 to 157.1 × 10⁻¹⁴ dl·kg⁻¹·min² per pmol/l; P = 0.10).

DISCUSSION

Applying state-of-the-art methods to estimate postprandial glucose turnover and β-cell function, this study demonstrates that delay in gastric emptying obtained with the administration of 30 μg of pramlintide administered at the start of a mixed meal in healthy subjects delays peak rate of meal glucose appearance. Furthermore, delayed gastric emptying lowered rate of EGP and improved insulin sensitivity while reducing β-cell responsivity, resulting in a net unchanged disposition index.

Pramlintide was used as a probe to delay gastric emptying, confirming prior studies in animals and in subjects with and without diabetes (17, 31, 41–43). This effect is probably mediated by vagal inhibition (24).

Delayed gastric emptying of liquids probably explains the marked delay in the time to peak meal glucose appearance rate and hence, time to peak postprandial glucose concentration. It is noteworthy that the carbohydrate (dextrose) component of

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**Fig. 2.** A: [6-3H]glucose/[1-13C]glucose concentrations during triple-tracer studies without and with pramlintide in healthy subjects. B: [6,6-2H₂]glucose/endogenous glucose concentrations during triple-tracer studies without and with pramlintide in healthy subjects.

**Total postprandial glucose excursion (difference: 9.2 mM over 6 h; 95% CI: −67.4 to 85.8 mM over 6 h; P = 0.80) or insulin concentration (difference: −3,819.0 pM; 95% CI: −9,460.2 to 1,821.6 pM over 6 h, P = 0.16) after the mixed meal, but the delay did have an impact on the measurements in the 2 h immediately following the meal (Table 3). Postprandial excursions of insulin (difference in iAUC: −8,868.0 pM over 2 h; 95% CI: −13,674.6 to −4,060.8 pM over 2 h; P = 0.002), C-peptide (difference in iAUC: −95.4 nM over 2 h; 95% CI: −139.1 to −51.6 nM over 2 h; P < 0.001), and glucagon (difference in iAUC: −1,140.6 pg/ml over 2 h; 95% CI: −2,198.5 to −82.8 pg/ml over 2 h; P = 0.037) were lower with pramlintide. Only the difference in C-peptide remained statistically significant over the entire 6-h period following the meal (P = 0.038; Table 3).

**Ratio of [6-3H]glucose to [1-13C]glucose and Ratio of [6,6-2H₂]glucose to Endogenous Glucose**

As shown in Fig. 2A, the tracer/tracée ratio applied to calculate EGP was fairly constant apart from a change during the pramlintide study day between 150 and 240 min. As shown in Fig. 2B, the tracer/tracée ratio applied to calculate the MR_a was also fairly constant for almost the entire duration (10–360 min) of the study apart from the initial perturbations (0–10 min) that are unavoidable when both the intravenously infused tracer and orally ingested tracée are entering the systemic circulation. Therefore, the triple-tracer approach applied here was successful in minimizing fluctuations in tracer/tracée ratios, enabling accurate measurements of postprandial glucose turnover.
the mixed meal was ingested in the semisolid form induced by mixing with Jell-O, which liquefies at body temperature in the stomach. The consequent slower rise in postprandial plasma glucose concentrations caused a delay in rise in insulin concentration, likely resulting in a delayed peak in the rate of Rd. However, despite the lower insulin concentrations during the first 2 h after the meal, the iAUC of EGP during the entire 0–360 min was lower with pramlintide. Furthermore, iAUC of C-peptide was lower with pramlintide, implying that portal insulin concentrations were also lower with than without pramlintide, further strengthening the conclusion that hepatic insulin action improved with pramlintide use.

It is noteworthy that although delayed gastric emptying delayed postprandial rise in glucose and insulin concentrations and time to peak in rates of MRa and Rd, the integrated postprandial glucose and insulin concentrations and those of MRa and Rd over the entire period of 6 h were not affected. The higher rate of appearance of meal glucose resulting in higher glucose and insulin concentrations during the later postprandial period and, at the end of the study, plasma glucose, plasma hormone concentrations, and glucose turnovers all returning to baseline levels suggest that effects of a single dose of pramlintide last for a short period of time, as expected, and that it does not alter net systemic appearance of meal glucose and, therefore, splanchnic glucose uptake in healthy subjects. It is noteworthy that integrated response of EGP for the 6 h after the meal was lower with delayed gastric emptying, which was likely influenced by lower postprandial glucagon concentrations.

It is intriguing that delayed gastric emptying improved postprandial insulin sensitivity significantly, as measured by the oral minimal model. Whereas the insulin sensitivity change is impressive, the β-cell response is likely compensatory. The glucagon response over 6 h was not different, but the iAUC EGP was different. The improved insulin sensitivity coupled with lower rate of EGP with delayed gastric emptying suggests that delayed gastric emptying improved whole body insulin sensitivity by likely improving hepatic insulin action. In addition, iAUC of C-peptide was lower with pramlintide, implying that portal insulin concentrations were also lower with than without pramlintide. The observation that iAUC of EGP was lower with pramlintide despite a lower projected portal insulin concentrations further strengthens our conclusion that hepatic insulin action was improved with pramlintide use.

Furthermore, although delayed gastric emptying reduced direct β-cell responsivity to glucose, the net effect on DI, a measure of β-cell function, i.e., β-cell response appropriate to the degree of insulin resistance, remained unaltered. It is apparent from the unchanged DI that the true underlying change is the improvement in insulin sensitivity, and the observed changes in β-cell response are compensatory. The reasons for the change in insulin sensitivity need to be investigated in more detail. Since glucagon concentrations were not different, the changes of glucagon made by pramlintide only cannot explain this effect. It has been well known that some peptides and nutrients, such as free fatty acids, modify hepatic insulin sensitivity (5, 28, 38, 39), and the improved whole body insulin sensitivity could be mediated by changes in

![Fig. 3. A: rate of meal glucose appearance (MRa) during triple-tracer studies without and with pramlintide in healthy subjects (iAUC 0–120 min, P < 0.001). B: rates of endogenous glucose production (EGP) during the same studies without and with pramlintide (iAUC 0–360, P = 0.023). C: rates of whole body glucose disappearance (Rd) without and with pramlintide in healthy subjects (iAUC 0–120 min, P < 0.001).]
other peptides or changes in other nutrients, such as free fatty acids.

This study is underpinned by state-of-the-art assessments of gastric emptying of solids and liquids and glucose metabolism with the triple-tracer approach in healthy subjects with normal glucose tolerance and gastric emptying. However, our study has its limitations. First, these assessments were limited to a single dose of pramlintide, resulting in an acute episode of delayed gastric emptying. Future studies should examine the effects of long-term alterations in gastric emptying caused by long-term pramlintide use but increased numerically, indicating modest effect size ($P = 0.10$). Additional studies using selective agents that separately manipulate gastric emptying and glucagon secretion are necessary in this context.

To conclude, we have demonstrated that delayed gastric emptying using pramlintide acutely improves insulin sensitivity in healthy subjects in the postprandial state with state-of-the-art techniques. Recent studies have shown promising effects of pramlintide on postprandial glucose excursions during closed-loop control in individuals with type 1 diabetes (25, 40). Our study sets the stage for investigating the effects of delayed gastric emptying induced by pramlintide on metabolic parameters in response to different types of meals.
postprandial glucose turnover and insulin action in closed-loop settings.

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DISCLOSURES

Dr. Yogish C. Kudva is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of the data and the accuracy of data analysis. There are no conflicts of interest, financial or otherwise, to declare for any of the authors.

AUTHOR CONTRIBUTIONS


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