

Impaired Hyperglycemia-Induced Delay in Gastric Emptying in Patients With Type 1 Diabetes Deficient for Islet Amyloid Polypeptide

HANS J. WOERLE, MD¹
 MAX ALBRECHT¹
 RAINER LINKE, MD²
 SILVIA ZSCHAU, MD³
 CHRISTOPH NEUMANN, MD³

MATHIAS NICOLAUS, MD¹
 JOHN E. GERICH, MD⁴
 BURKHARD GÖKE, MD¹
 JOERG SCHIRRA, MD¹

OBJECTIVE — Slowing of gastric emptying by hyperglycemia, a physiological response to minimize postprandial hyperglycemia, may be impaired in patients with type 1 diabetes. The causes and consequences on glucose homeostasis are unknown.

RESEARCH DESIGN AND METHODS — Consequences of euglycemia- and hyperglycemia-induced changes in gastric emptying on postprandial glucose fluxes and excursions were studied in 10 healthy subjects and 15 type 1 diabetic subjects after ingestion of a mixed meal using the double isotope approach ($[6,6\text{-}^2\text{H}_2]$ and $[1\text{-}^{13}\text{C}]\text{glucose}$) and scintigraphic measurements of gastric emptying.

RESULTS — Gastric emptying was greater in type 1 diabetic subjects (90–120 min, $P < 0.03$), and 50% retention times were comparable in healthy subjects and type 1 diabetic subjects (167 ± 8 vs. 152 ± 10 min, $P = 0.32$). Hyperglycemia markedly delayed gastric emptying in healthy subjects but did not alter it in type 1 diabetic subjects (50% retention time 222 ± 18 vs. 167 ± 8 min, $P = 0.003$ and 148 ± 9 vs. 152 ± 10 min, $P = 0.51$). Plasma islet amyloid polypeptide (IAPP) increased approximately fourfold in healthy subjects ($P < 0.001$), whereas it was undetectable in type 1 diabetic subjects. IAPP replacement, using the analog pramlintide, in type 1 diabetic subjects slowed gastric emptying to a comparable extent, as did hyperglycemia in healthy subjects ($P < 0.14$), and greatly reduced postprandial hyperglycemia ($P < 0.001$). Meal-derived glucose appearance in plasma (10.7 ± 0.5 vs. $6.8 \pm 0.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.001$) was reduced, and splanchnic glucose sequestration increased (14.0 ± 3.0 vs. $25.0 \pm 6.0\%$, $P = 0.04$).

CONCLUSIONS — In patients with type 1 diabetes the ability to delay gastric emptying in response to hyperglycemia is impaired. This impairment contributes to exaggerated rates of meal-derived glucose appearance and, ultimately, postprandial glucose excursions.

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The importance of insulin and glucagon in maintaining postprandial glycemic excursions within a narrow range is well established (1). However, alterations in gastric emptying, another potentially important factor (2),

are not generally considered to be of clinical significance for postprandial hyperglycemia in diabetes unless diabetes late complications, such as gastroparesis, have emerged (3,4).

Gastroparesis is a relatively rare dia-

betes late complication resulting from irreversible intestinal nerve damage (5) and has to be distinguished from the physiological inhibitory effects of acute hyperglycemia on gastric motility (6,7). The latter has been proposed as a defense mechanism to minimize postprandial hyperglycemia by reducing the rate of efflux of glucose into the circulation from the gut (8). This process may be of special importance for patients with type 1 diabetes because they have been reported to have a reduced ability to delay gastric emptying in response to hyperglycemia (9).

The pancreatic β -cell hormone islet amyloid polypeptide (IAPP) is cosecreted with insulin in a fixed molar ratio (10) and reduces gastric emptying. Thus, patients with type 1 diabetes even without concomitant enteric neuropathy should have increased rather than delayed rates of gastric emptying, because they are IAPP deficient (11). Accordingly, the present studies were undertaken to test the hypothesis that impairment in hyperglycemia-induced delay in gastric emptying should result in greater meal-derived glucose appearance in the systemic circulation and thus should contribute to postprandial hyperglycemia in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Written informed consent was obtained from 10 healthy subjects and 15 subjects with type 1 diabetes after the Ludwig Maximilians University of Munich Institutional Review Board had approved the protocol. Healthy subjects (seven men and three women, 39 ± 4 years of age, body weight 80 ± 4 kg) had normal routine laboratory blood test results as well as no family history of diabetes and had normal glucose tolerance (assessed by oral glucose tolerance tests according to World Health Organization criteria [12]). Healthy subjects and type 1 diabetic subjects (eight men and seven women, 37 ± 2 years of age, body weight 76 ± 3 kg) had normal physical examina-

From the ¹Department of Internal Medicine II, Grosshadern, Clinical Research Unit, Ludwig Maximilians University, Munich, Germany; the ²Department of Nuclear Medicine, Grosshadern, Ludwig Maximilians University, Munich, Germany; the ³Outpatient Diabetes Center, Munich, Germany; and the ⁴Department of Medicine, University of Rochester School of Medicine, Rochester, New York.

Corresponding author: Hans J. Woerle, juergen.woerle@med.uni-muenchen.de.

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tions and no gastrointestinal symptoms. No type 1 diabetic subject had symptoms of cardiac autonomic or peripheral sensory neuropathy assessed by electrocardiogram breath-dependent variability and clinical examination of peripheral sensory function. None had symptoms or a history of gastrointestinal neuropathy (i.e., bloating, vomiting, constipation, diarrhea, or redundant postprandial hypoglycemia) or evidence for nephropathy, assessed by microalbumin excretion. Retinal fundus photography showed only mild background retinopathy in three type 1 diabetic subjects. All type 1 diabetic subjects had been receiving continuous subcutaneous insulin infusion therapy for at least 3 months before the study with good glycemic control as assessed by A1C ($7.3 \pm 0.2\%$) and seven-point self-obtained blood glucose profiles without severe hypoglycemic episodes. Three days before the study, all subjects had been consuming a weight-maintaining diet containing at least 200 g of carbohydrates and had abstained from alcohol, smoking, and exercise.

Protocol

Healthy subjects were studied on two occasions (euglycemia and hyperglycemia), and type 1 diabetic subjects were studied on three occasions (euglycemia and hyperglycemia with and without 30 μg pramlintide [Amylin Pharmaceuticals, San Diego, CA], injected subcutaneously in the lower abdominal wall with the meal, which was designed to replace absent IAPP secretion as indicated by its effects on gastric emptying compared with hyperglycemia in healthy subjects estimated from previous studies [13,14]). Healthy subjects received only placebo injections. Experiments were separated by at least 1 day and undertaken in a randomized, single-blinded order. Subjects refrained from food intake at least 10 h before admission to the clinical research unit between 7:00 and 7:30 A.M. 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 (8). On the nights before the study days, type 1 diabetic subjects were instructed to measure plasma glucose concentrations at 10 P.M. and 2 A.M. to adjust basal insulin infusion with the aim of average plasma glucose concentrations of ~ 5 or 10 mmol/l, respectively (15). After admission to the clinical research unit, the continuous subcutaneous insulin infusion was discontin-

ued, and an intravenous insulin infusion was adjusted accordingly to maintain preprandial plasma glucose concentrations at ~ 5 and ~ 11 mmol/l, respectively.

Postprandial intravenous insulin infusion rates were based on the individual bolus requirements from the subcutaneous pump therapy. Postprandial insulin infusion rates of euglycemic and hyperglycemic experiments were 6.4 ± 0.9 , 6.8 ± 0.8 , 3.5 ± 0.8 , 1.4 ± 0.3 , and 0.9 ± 0.1 islet equivalents/h at 0–30, 35–60, 65–120, 125–240, and 240–330 min, respectively.

At 8 A.M., a primed (25 μmol) continuous ($\sim 0.25 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) infusion of [$1\text{-}^{13}\text{C}$] glucose was started via a forearm vein for measurements of plasma glucose turnover in all except the hyperglycemic experiments of healthy subjects. 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 IAPP concentrations and [$1\text{-}^{13}\text{C}$]glucose enrichments. Thereafter, subjects ingested a standardized meal within 5 min. The meal (450 kcal, $\sim 45\%$ carbohydrates, $\sim 30\%$ fat, and $\sim 25\%$ protein) consisted of three scrambled eggs and 100 ml jelly containing 50 g of glucose enriched with 3 g 6,6-dideutero-glucose. Consumption of 200 ml sparkling water was allowed with the meal. 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 and thereafter at 30-min intervals until completion of the experiment at 330 min except for the hyperglycemic experiments in healthy subjects, which were terminated at 240 min. The scrambled eggs and jelly were additionally labeled with $^{99\text{m}}\text{Tc-Sn-colloid}$ (~ 70 MBq) for measurements of gastric emptying by high-resolution scintigraphy (20 images/min; Orbiter, Siemens, Erlangen, Germany), starting immediately after food ingestion for the remainder of the experiment.

After the hyperglycemic experiments in type 1 diabetic subjects had been completed, we performed hyperglycemic experiments in healthy subjects isoglycemic to those of the hyperglycemic placebo experiments of type 1 diabetic subjects using a glucose infusion algorithm as described previously (16,17). Fasting plasma glucose was clamped at 11.03 ± 0.1 mmol/l, not significantly different from preprandial plasma glucose concen-

trations in type 1 diabetic subjects. Glucose infusion rates in hyperglycemic isoglycemic experiments in healthy subjects were started 2.5 h before meal ingestion. No insulin was infused in healthy subjects. Mean glucose infusion rates before meal ingestion were $49.1 \pm 8.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ after an intravenous bolus of 16.2 ± 2.2 g glucose. Postprandial rates of glucose infusion in healthy subjects were adjusted to produce postprandial plasma glucose excursions not significantly different from those in hyperglycemic experiments in type 1 diabetic subjects. Mean postprandial rates of glucose infusion were $96.5 \pm 9.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. The hyperglycemic experiments in healthy subjects were terminated 4 h after meal ingestion. In type 1 diabetic subjects, no glucose was infused in the postprandial state. Healthy subjects served in part as a control group in studies in which effects of pramlintide administration on postprandial glucose fluxes in 14 healthy subjects are reported (8).

Analytical 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 $^{99\text{m}}\text{Tc}$ (8).

Blood samples were collected for plasma glucose concentrations and [$1\text{-}^{13}\text{C}$]- and [$6,6\text{-}^2\text{H}_2$]glucose enrichments in oxalate-fluoride tubes and for plasma insulin, glucagon, and IAPP concentrations in EDTA tubes containing a protease inhibitor. Samples were immediately placed on ice, and plasma was separated within 30 min by centrifugation at 4°C. Plasma glucose concentrations and [$1\text{-}^{13}\text{C}$]- and [$6,6\text{-}^2\text{H}_2$]glucose enrichments were measured as described previously (8). Plasma insulin and glucagon concentrations were determined by standard radioimmunoassay (8), and IAPP concentrations were determined using an enzyme-linked immunosorbent assay (Linco Research).

Calculations

Systemic release and uptake of glucose were calculated with steady-state equations before meal ingestion and subsequently with the non-steady-state equations of DeBodo et al. (8) using a pool fraction of 0.65 and a volume of distribution of 200 ml/kg. Rates of appearance of the oral glucose load in the systemic cir-

ulation were calculated from [6,6-²H₂]glucose enrichments using the equation of Chiasson et al. (8). The endogenous glucose release was calculated as the difference between the overall rate of plasma glucose appearance and the rate of appearance of exogenous glucose (8). Splanchnic glucose disposal of the ingested glucose load was calculated as the difference between the amount of glucose emptied by the stomach and the amount of glucose that appeared in the systemic circulation at the end of the experiments.

Statistical analysis

Data are means ± SEM unless otherwise specified using Statistica statistical software (1998 edition; Statsoft, Tulsa, OK). Normality of the distribution was assessed using the Kolmogorov-Smirnov test. Comparisons between the groups and baseline with postprandial values were performed using ANOVA for exclusion of carryover effects followed by post hoc comparison with paired and unpaired *t* tests within and between patient groups, respectively. *P* < 0.05 was considered statistically significant. Correlation between variables was performed using Spearman's regression analysis.

RESULTS

Gastric retention, lag period, 50% retention time, 60-min retention

Initial rates of gastric emptying during euglycemia were greater in type 1 diabetic subjects, with significantly greater rates between 90 and 120 min (all *P* < 0.03). However, lag periods, 60-min retention, and 50% retention times (T50) were not statistically different in healthy subjects and type 1 diabetic subjects (all *P* > 0.3) (Fig. 1). Hyperglycemia markedly slowed gastric emptying in healthy subjects with greater lag periods, 60-min retention, and T50 (all *P* < 0.001), with the most pronounced effects occurring within the first 60 min after meal ingestion. In contrast, hyperglycemia had no effects on these parameters in type 1 diabetic subjects.

Pramlintide administration markedly delayed gastric emptying in type 1 diabetic subjects (lag period, 60-min retention, and T50, all *P* < 0.001) compared with placebo, with the most pronounced effects occurring within the initial 60 min after meal ingestion. As a consequence, lag period, 60-min retention time, and T50 were restored to values comparable to those observed in hyperglycemic ex-

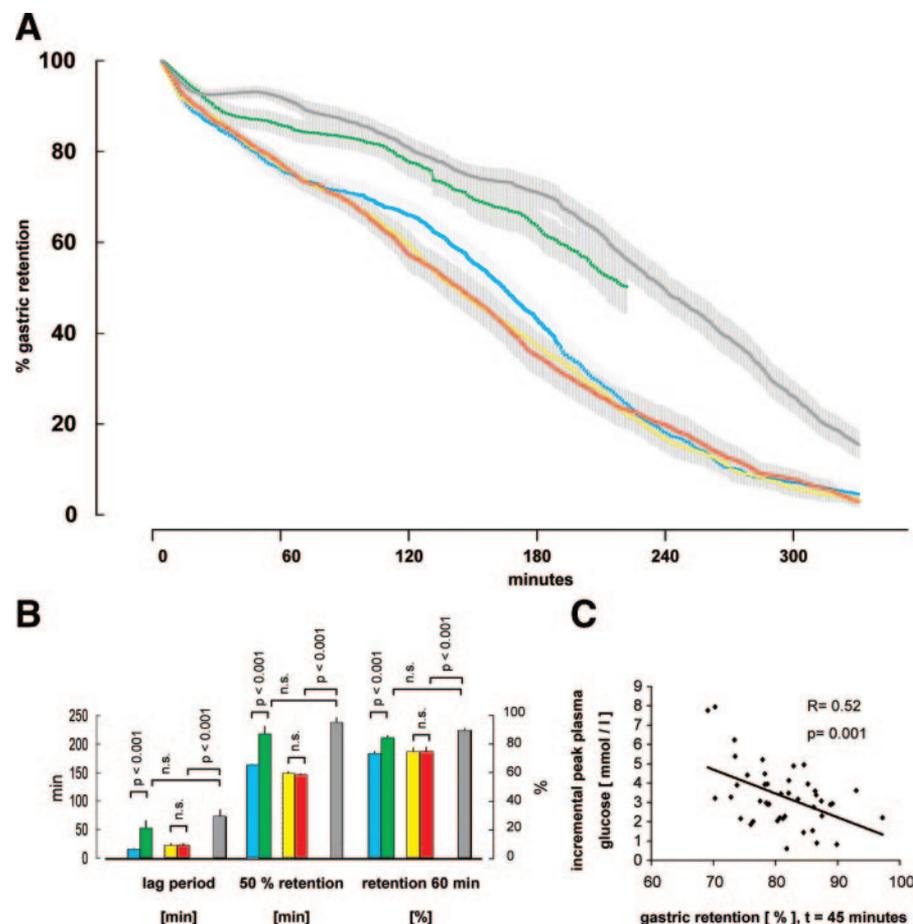


Figure 1—Percent gastric retention versus minutes (A), percent gastric retention, lag periods, 50% retention time, percent retention at 60 min (B), and inverse correlation of gastric retention at 45 min and incremental plasma glucose concentrations at 60 min (C) in all placebo experiments. blue, healthy subjects, euglycemia, placebo, n = 10; green, healthy subjects, hyperglycemia, placebo (isoglycemic to hyperglycemia placebo in type 1 diabetic patients), n = 10; yellow, type 1 diabetic patients, euglycemia, placebo, n = 15; red, type 1 diabetic patients, hyperglycemia, placebo, n = 15; gray, type 1 diabetic patients, hyperglycemia, pramlintide (30 μg), n = 15. Note that initial gastric emptying was greater in type 1 diabetic patients (*P* < 0.03). Hyperglycemia delayed gastric emptying in healthy subjects (*P* < 0.001) without effects in type 1 diabetic patients (n.s.). In type 1 diabetic patients, pramlintide reduced gastric emptying (*P* < 0.001) compared with placebo but not significantly different from gastric emptying under hyperglycemic conditions in healthy subjects. Comparison was made using paired and unpaired *t* tests within and between patient groups.

periments in healthy subjects (*P* = 0.29, 0.06, and 0.29, respectively).

Plasma glucose and correlation between peak plasma glucose at 60 min and gastric content at 45 min

Fasting plasma glucose concentrations in the euglycemic experiments were slightly but significantly greater in type 1 diabetic subjects (area under the curve [AUC] -60 to 0 min: 265 ± 7 vs. 334 ± 13 mmol · l⁻¹ · min⁻¹, *P* < 0.001); however, after meal ingestion plasma glucose increased comparably in type 1 diabetic and healthy subjects (increase in AUC 0 to 60 min: 131 ± 15 vs. 162 ± 20 mmol · l⁻¹ · min⁻¹, *P* = 0.45) (Fig. 2). In the hyper-

glycemic experiments, fasting and postprandial glucose concentrations did not differ between healthy subjects and type 1 diabetic subjects in the placebo experiments (AUC -60 to 0 min and 0–330 min: 665 ± 4 vs. 686 ± 9 and 2,959 ± 45 vs. 2,915 ± 80 mmol · l⁻¹ · min⁻¹, *P* = 0.08 and 0.69, respectively).

Pramlintide administration completely prevented any increase in postprandial plasma glucose above baseline during the entire postprandial period so that mean plasma glucose concentrations were reduced by 3 mmol/l (*P* < 0.001). The AUCs for 0–330 min were 3,739 ± 123 vs. 2,995 ± 86 mmol · l⁻¹ · min⁻¹

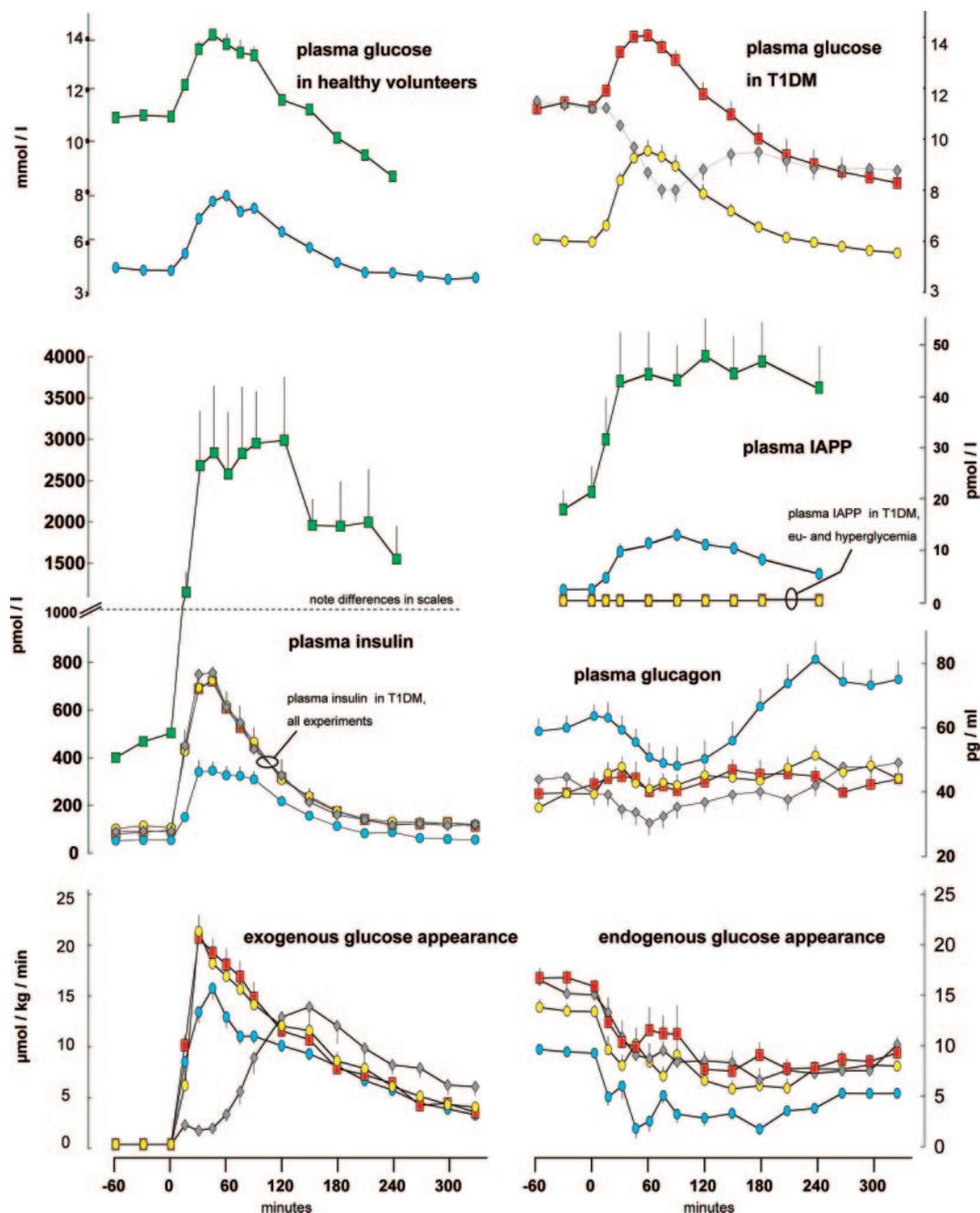


Figure 2—Plasma glucose concentrations, plasma insulin, glucagon, and IAPP concentrations, and rates of exogenous (meal) and endogenous plasma glucose appearance. yellow circles, type 1 diabetic patients (T1DM), euglycemia, placebo, n = 15; red squares, type 1 diabetic patients, hyperglycemia, placebo, n = 15; gray diamonds, type 1 diabetic patients, hyperglycemia, pramlintide (30 μg), n = 15; blue circles, healthy subjects, euglycemia, placebo, n = 10; green squares, healthy subjects, hyperglycemia, placebo (isoglycemic to hyperglycemia placebo in type 1 diabetic patients), n = 10. Note that subcutaneous pramlintide reduced postprandial glucose in type 1 diabetic patients (P < 0.001). Plasma insulin in type 1 diabetic patients was not different (n.s.). Matching isoglycemic hyperglycemia in healthy subjects resulted in greater insulin concentrations (P < 0.001). Plasma IAPP increased as a response to hyperglycemia in healthy subjects (P < 0.001) with no detectable IAPP concentrations under euglycemia and hyperglycemia in type 1 diabetic patients (n.s.). Pramlintide reduced glucagon from 0 to 90 min in type 1 diabetic patients (P < 0.01). Endogenous glucose production was greater in type 1 diabetic patients (P < 0.001); pramlintide showed no effect (n.s.). Exogenous glucose was greater in type 1 diabetic patients (P < 0.005) compared with healthy subjects and was reduced when pramlintide was given (P < 0.001). Comparison was made using paired and unpaired t tests within and between patient groups.

(P < 0.001). Changes in postprandial plasma glucose concentrations at 60 min correlated with gastric content at 45 min (r = 0.52, P = 0.001) (Fig. 1).

Plasma insulin, glucagon, and IAPP
Plasma insulin concentrations paralleled those of plasma glucose concentrations in healthy subjects and were on average

~11-fold greater in the hyperglycemic than in the euglycemic experiments. AUCs over the entire experimental period were 52,838 ± 3,975 versus 606,929 ±

135,590 pmol · l⁻¹ · min⁻¹ ($P < 0.001$). In type 1 diabetic subjects, plasma insulin concentrations were comparable in all experiments (AUCs over the entire experimental period: 114,146 ± 9,344, 113,551 ± 10,157, and 114,778 ± 7,514 pmol · l⁻¹ · min⁻¹, all $P > 0.70$) but on average approximately twofold greater than those for healthy subjects ($P < 0.001$) (Fig. 2).

Plasma glucagon concentrations decreased significantly after meal ingestion in healthy subjects (AUC -60 to 0 min vs. 0-60 min: 3,574 ± 279 vs. 3,283 ± 294 pg · ml⁻¹ · min⁻¹, $P = 0.01$) but not in type 1 diabetic subjects (AUC -60 to 0 min vs. 0-60 min: 2,433 ± 196 vs. 2,597 ± 203 pg · ml⁻¹ · min⁻¹, $P = 0.16$) (Fig. 2). When pramlintide was given in type 1 diabetic subjects, plasma glucagon concentrations decreased to a nadir at 60 min and were significantly lower within the first 90 min (AUC 0-90 min: 3,829 ± 280 vs. 3,085 ± 289 pg · ml⁻¹ · min⁻¹, placebo versus pramlintide, $P = 0.01$).

In euglycemic experiments in healthy subjects, plasma IAPP concentrations increased from 2.1 ± 0.6 pmol/l to peak values of 12.4 ± 1.3 pmol/l at 90 min, averaging 8.8 ± 0.9 pmol/l postprandially. In the hyperglycemic experiments, plasma IAPP concentrations were significantly greater before meal ingestion (on average 18.9 ± 4.2 pmol/l, AUC -30 to 0 min: 63 ± 17 vs. 566 ± 126 pmol · l⁻¹ · min⁻¹, $P < 0.001$) and increased to an average of 42.8 ± 7.3 pmol/l (AUC 0-240 min: 2,104 ± 206 vs. 10,261 ± 1,746 pmol · l⁻¹ · min⁻¹, $P < 0.0001$) (Fig. 2). In type 1 diabetic subjects, plasma IAPP was undetectable under euglycemic and hyperglycemic conditions both pre- and postprandially.

Rates of exogenous (meal) and endogenous plasma glucose appearance

In type 1 diabetic subjects, preprandial endogenous glucose production was significantly greater in both the euglycemic and hyperglycemic experiments than that in healthy subjects (AUC -60 to 0 min: 545 ± 21 μmol/kg in healthy subjects vs. 788 ± 41, 963 ± 38, and 911 ± 65 μmol/kg, respectively, in the experiments with type 1 diabetic subjects, all $P < 0.001$).

In healthy subjects, postprandial endogenous glucose production was suppressed by 60.6 ± 1.5%. In type 1 diabetic subjects, the degree of suppression was comparable in the euglycemic

and hyperglycemic experiments (45.4 ± 2.5 and 45.1 ± 1.6%, respectively, $P > 0.50$) and lower than that in healthy subjects (both $P < 0.001$). Postprandial endogenous glucose production did not differ after placebo and pramlintide administration in type 1 diabetic subjects (AUC 0-330 min: 2,991 ± 154 vs. 2,773 ± 199 μmol/kg, $P > 0.21$) (Fig. 2).

Rates of exogenous glucose appearance (meal-derived glucose) increased from 30 to 90 min in type 1 diabetic subjects compared with those of healthy subjects in the euglycemic placebo experiments (AUC 30-90 min: 743 ± 47 vs. 1,012 ± 67 μmol/kg, $P < 0.005$) and were unaffected by hyperglycemia (AUC 30-90 min in the hyperglycemic placebo experiment: 1,054 ± 50 μmol/kg, $P > 0.80$ compared with placebo euglycemia in type 1 diabetes). Administration of pramlintide significantly reduced rates of exogenous glucose appearance in type 1 diabetic subjects from 15 to 90 min (AUC 15-90 min: 1,220 ± 58 vs. 269 ± 47 μmol/kg, $P < 0.001$) with slightly but significantly greater rates for the remainder of the experiment. Overall, they were not significantly different from those of healthy subjects during euglycemia (AUC 0-330 min in healthy subjects and type 1 diabetic subjects after pramlintide administration, respectively: 2,750 ± 144 vs. 2,251 ± 241 μmol/kg, $P = 0.145$) (Fig. 2).

CONCLUSIONS— Major findings of these studies are that the physiological defense mechanism to delay gastric emptying in response to postprandial hyperglycemia is impaired in patients with type 1 diabetes. Moreover, our studies demonstrate that this impairment leads to exaggerated rates of meal-derived glucose appearance in plasma and thus contributes to postprandial hyperglycemia. IAPP increased markedly in healthy subjects and was associated with a profound delay in gastric emptying. A delay in gastric emptying in type 1 diabetic patients comparable to that found in healthy subjects markedly improved postprandial glucose excursions in type 1 diabetic patients. Delayed gastric emptying decreased profoundly initial appearance of meal-derived glucose in the systemic circulation in conjunction with increased splanchnic glucose sequestration.

These findings appear at first glance to contradict those of previously published studies reporting that physiological hyperglycemia delays gastric emptying in

patients with type 1 diabetes (9). As in our studies, gastric emptying was not found to be delayed in patients with type 1 diabetes under euglycemic conditions, but a blunted responsiveness to hyperglycemia was reported with a >50% reduction in the delay of gastric emptying in patients with type 1 diabetes. These findings and the results of the present studies support the concept that the physiological defense mechanism to delay gastric emptying in response to postprandial hyperglycemia is impaired in patients with type 1 diabetes. The complete unresponsiveness to hyperglycemia in our studies may be explained by different study designs. In our studies typical postprandial hyperglycemic glucose fluctuations were allowed, whereas Schvarcz et al. (9) used the continuous hyperglycemic clamp technique.

Under euglycemic conditions, plasma IAPP concentrations paralleled those of insulin concentrations in healthy subjects. When exposed to hyperglycemia, IAPP concentrations increased from ~9 to 43 pmol/l postprandially. No apparent IAPP secretion could be detected either under euglycemic or hyperglycemic conditions in patients with type 1 diabetes. Pramlintide administration in type 1 diabetic patients delayed gastric emptying to an extent comparable to that with hyperglycemia in healthy subjects. This result, however, does not necessarily imply concentrations comparable to those of endogenous IAPP found in healthy subjects but rather a comparable pharmacodynamic effect on gastric emptying. Taking this into consideration, IAPP deficiency may be seen as one cause for the unresponsiveness in delaying gastric emptying. To prove that IAPP deficiency was the sole reason for the lack of delay in gastric emptying, direct inhibition of the IAPP effects using a specific antagonist would be required. Such an antagonist, however, is not available for human use, but the view that IAPP deficiency plays an important role in the lack of delay in gastric emptying in response to hyperglycemia is further supported by the highly potent inhibitory effects of IAPP on gastric emptying (18).

In studies suggesting that insulin may be another important regulator of gastric emptying in healthy volunteers (19), the effects of hyperinsulinemia on gastric emptying were found to be marginal (19). Interestingly, in studies undertaken in patients with type 1 diabetes, no effect of insulin on gastric emptying was detected (20). Gastric emptying was found to be

increased in patients with type 1 diabetes under euglycemic conditions in our studies even though plasma insulin concentrations were significantly higher compared with those of healthy volunteers. This result also argues against an important effect of insulin on gastric emptying in patients with type 1 diabetes.

Gastric emptying is modulated by feedback mechanisms arising from the interaction of nutrients with the small intestine (21). Both intestinal vagus nerve activity and intestinal peptides regulate gastric emptying. Glucagon-like peptide 1 (GLP-1) inhibits gastric emptying (22). Its secretion, however, is stimulated by the intestinal nutrient content and flow rather than by the plasma glucose concentration itself (23). Furthermore, the fact that gastric emptying and thus nutrient flow to the intestine, which should reduce direct intestinal L-cell-stimulated and also cholinergic GLP-1 secretion, was delayed in the hyperglycemic experiments in healthy subjects speaks against a major role of GLP-1 as a mediator of hyperglycemia-induced delay in gastric emptying.

Direct inhibition of vagal nerve activity induced by hyperglycemia could be another important factor to delay gastric emptying. To our knowledge there is no convincing evidence that hyperglycemia per se as applied in our studies affects vagal activity. Interestingly, in healthy humans hyperglycemia has been reported to cause profound inhibition of vagal activity accompanied by substantial IAPP secretion (24). In contrast, however, in IAPP-deficient patients with type 1 diabetes, hyperglycemia did not affect vagal activity (9). Because the inhibitory effect of IAPP on gastric emptying seems to be mediated via inhibition in vagal nerve activity (24), our experiments are consistent with the concept that the hyperglycemia-induced delay in gastric emptying may be at least partially regulated via an IAPP-mediated inhibitory effect on vagal nerve activity.

Recent studies showed that IAPP and pramlintide suppress postprandial glucagon secretion (25,26). Indeed, we found greater suppression of postprandial glucagon in type 1 diabetic patients when pramlintide was given. This suppression may have occurred either directly by an inhibitory effect of pramlintide on the pancreatic α -cell or indirectly via reduced efflux of nutrients from the gut, because amino acids such as arginine are known to stimulate glucagon secretion (27). Thus, it remains unclear whether the greater

suppression of glucagon secretion is attributable to a direct inhibition of the pancreatic α -cells or to reduced influx of nutrients from the gut. However, because endogenous glucose production was comparable in the placebo and pramlintide experiments in type 1 diabetic patients, we believe that the pramlintide-induced reduction of postprandial glucose concentrations was primarily due to the delay in gastric emptying.

Rates of meal-derived glucose appearance were significantly greater in the early postprandial period in type 1 diabetic subjects when placebo was given and would correspond to a ~30% reduction in splanchnic glucose sequestration. If we assume that all of this glucose had been used for glycogen formation, our estimates are in close agreement with nuclear magnetic resonance spectroscopic studies revealing a 30% reduction in glycogen content in moderately hyperglycemic patients with type 1 diabetes (28). Interestingly, in the pramlintide experiments a nearly identical proportion of hepatic glucose sequestration was found in patients with type 1 diabetes compared with our healthy subjects. This result could have been related to the greater suppression of postprandial glucagon secretion or initially reduced influx of glucose from the gut and more efficient uptake by the liver or increased rates of glycolysis in the gut.

Teleologically, the slowing of gastric emptying during hyperglycemia can be seen as an important defense mechanism to prevent hyperglycemia. IAPP secretion is linked to insulin release (10). As a response to hyperglycemia, the pancreatic β -cell with its glucose sensor increases insulin and IAPP secretion. Insulin suppresses hepatic glucose output and increases peripheral glucose uptake (17). IAPP reduces the release of nutrition from the gut and thus reduces the efflux of glucose into the system and thereby prevents aggravation of postprandial hyperglycemia.

Taken together, these studies highlight the importance of a delay in gastric emptying as a response to hyperglycemia to minimize postprandial glucose excursions, a defense mechanism not operative in patients with type 1 diabetes, which may be explained at least partially by IAPP deficiency.

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