Novel antidiabetic nutrients identified by in vivo screening for gastric secretion and emptying regulation in rats

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Jordi J, Herzog B, Lutz TA, Verrey F. Novel antidiabetic nutrients identified by in vivo screening for gastric secretion and emptying regulation in rats. Am J Physiol Regul Integr Comp Physiol 307: R869–R878, 2014. First published August 6, 2014; doi:10.1152/ajpregu.00273.2014.—Diabetes mellitus is a disease characterized by elevated blood glucose levels and represents a worldwide health issue. Postprandial hyperglycemia is considered a major predictor of diabetic complications, and its reduction represents a specific treatment target in Type 1 and 2 diabetes. Since postprandial glucose excursions depend to a large extent on gastric secretion and emptying, amylin and glucagon-like peptide 1 analogs are prescribed to reduce them. Although gastric function is considered mainly sensitive to ingested calories, its chemospecificity is not well understood. To identify ingestible nutrients reducing postprandial hyperglycemia, we applied intragastrically more than 40 individual nutrients at an isomolar dose to rats and quantified their impact on gastric secretion and emptying using a novel in vivo computed tomography imaging method. We identified L-tryptophan, L-arginine, L-cysteine, and L-lysine as the most potent modulators with effective strength comparable to a supraphysiological dose of amylin. Importantly, all identified candidates reduced postprandial glucose excursion within an oral glucose tolerance test in healthy and diabetic rats. This clinical benefit effect originated predominantly from their impact on gastric function, as none of the candidates altered plasma glucose concentrations induced by intraperitoneal or intraduodenal glucose tolerance tests. Overall, these data demonstrate a remarkable chemospecificity of stomach function, unveil a strong role of the stomach for glycemic control and identifies nutrients with antidiabetic potential.

diabetes; postprandial hyperglycemia; computed tomography; exenatide; pramlintide; liraglutide

THE WORLD HEALTH ORGANIZATION estimates that 347 million people suffer from Type 1 or Type 2 diabetes worldwide (9). Although both diseases differ fundamentally in their etiology, they are both characterized by high blood glucose levels. Reducing hyperglycemia delays the onset of diabetes-related morbidities and, consequently, is the primary aim of current therapeutic interventions (7, 22, 42a). The daily blood glucose profile can be segmented into tailing peaks caused by food ingestion and plateaus during fasting. Preprandial and postprandial glycemia are elevated in diabetic patients, but their relative significance to high HbA1c values varies. In untreated Type 2 diabetic patients, fasting glycemia contribution is greater, whereas in well-controlled patients (HbA1c <7.3%) postprandial glycemia accounts for ~70% of the variability in HbA1c (32, 36). This is not surprising as the postprandial phase spans ~60–70% of a day, if three regular meals are ingested (28). Furthermore, postprandial hyperglycemia is also an independent risk factor for adverse cardiovascular outcomes in diabetic and nondiabetic subjects (7, 34, 38). Therefore, the search for therapeutic agents that lower cumulative and peak postprandial glycemia is important.

Upon meal ingestion, blood glucose levels are determined by numerous factors, e.g., gastrointestinal nutrient absorption, hormone secretion and sensitivity, metabolism, and many others. Their individual relevance for the control of postprandial glycemia is not well understood because they form an interdependent homeostatic feedback mechanism. This complexity theoretically enables multiple strategies to successfully reduce postprandial glycemia. Among them delaying gastric function has recently become a key approach. At least 35% of the variance in glycemic response to an oral glucose load or a normal meal are determined by gastric emptying in healthy and diabetic humans (16, 20). Consequently, pharmacologically delaying gastric emptying reduces postprandial hyperglycemia, a strategy that is clinically successfully exploited by the widely used analogs of amylin (pramlintide) and glucagon-like peptide 1 (GLP-1; exenatide and liraglutide) (12, 26). To date, these drugs require daily injections, cause side effects, and are expensive (30, 37). Hence, cheap and oral alternatives are highly desirable.

Altering gastric function is a druggable physiological function and offers a defined entry point for a screen aiming to identify compounds reducing postprandial hyperglycemia. However, assessing gastric function remains a technological challenge. Gastric emptying and secretion are fast, and together form the integrated functional output of the stomach. Most available techniques cannot assess both simultaneously, and they are also lethal or invasive to the experimental animal (13, 24, 39). These methodological constraints limit throughput and, thereby, disable systematic screens for compounds targeting gastric function. Here, we tested the individual impact of all major ingested nutrients on gastric function using computed tomography (CT) imaging, aiming to identify a natural compound to reduce postprandial hyperglycemia in rats.

MATERIALS AND METHODS

Animals. Male Wistar rats (Janvier, France) were group housed and adapted to housing conditions for at least 1 wk prior to experiments [room temperature 21 ± 1°C, artificial 12:12-h light-dark cycle, water ad libitum, rat chow ad libitum (3430 Kliba Nafag, Kaesersgut, Switzerland)]. Experiments were conducted during the light phase, and animals had a recovery time of at least 48 h between experiments. All procedures adhered to the Swiss Animal Welfare legislation and were approved by the Kantonales Veterinäramt Zürich.

In vivo screen for macronutrients with an impact on gastric function by in vivo CT. Four-hour food-deprived rats received an intragastric (1G, gavage tool diameter 2.2 mm) application of 2 ml of

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sodium diatrizoate hydrate ([SDH]; 200 mg/ml SDH solubilized in tap water and pH adjusted to 7.2 (Sigma-Aldrich, Buchs, Switzerland)] with individual nutrients added at a dose of 6.7 mmol/kg (freshly prepared, Sigma). Following the gavage, the amylin-treated group received an intraperitoneal (IP) injection of a supraphysiological dose of amyl (50 μg/kg; H9475, Bachem AG, Bubendorf, Switzerland). Amylin served as a reference to a current antidiabetic drug (positive control). After application, animals were returned to their home cage, where they did not have access to food or water. Thirty minutes postapplication, animals were anesthetized with isoflurane (5%), and a CT image was taken. To exclude simple osmotic effects, isomolar conditions were chosen, and statistical comparison was performed to l-αlanine (negative control). The dose of 6.7 mmol/kg was selected to enable comparison with previous publications and is suggested to reflect a physiological dose for most nutrients (23, 25). For instance, for l-glutamate 6.7 mmol/kg reflects 1 g/kg, which is ~27% of the daily l-glutamate intake in rats fed a standard protein diet (20%), or for D(+)-glucose 6.7 mmol/kg corresponds to 1.2 g/kg, which represents 60% of the dose commonly used in an oral glucose tolerance test (2 g/kg, GTT). Some nutrients did not dissolve completely at the given dose and, therefore, were applied as a dispersion (maximum particle size 2.2 mm). The specific individual nutrients were selected because they represent all major macronutrient classes (nonessential and essential amino acids, saturated and unsaturated fatty acids, monosaccharides and disaccharides, and artificial sweeteners).

Dose-dependent and time-dependent effect of individual amino acids on gastric function measured by in vivo CT. Four-hour food-deprived rats received an intraperitoneal (IP) Zoletil injection [Zoletil = Tiletamine base:Zolazepam base, 20 mg/kg ip (Virbac, Glattbrugg, Switzerland)]. This anesthesia protocol was previously demonstrated not to alter gastric function (24). After anesthesia induction, eyes were covered with vitamin A ointment, rats gavaged with 2 ml SDH (200 mg/ml) with individual doses of amino acids (0, 0.7, 2.7, 4.7, and 6.7 mmol/kg), and subsequently imaged immediately and at 10-min increments for 40 min.

Intravenous effects of individual amino acids on gastric function measured by in vivo CT. Four-hour food-deprived rats received an IP injection of 2 ml SDH or SDH with individual amino acids (0.7 mmol/kg or 1.4 mmol/kg) and an intravenous (ID) injection of 2 ml tap water or 2 ml tap water with individual amino acids (0.7 mmol/kg or 1.4 mmol/kg). After application, animals were returned to their home cages, where they did not have access to food or water. Thirty minutes postapplication, animals were anesthetized with isoflurane (5%), and a CT image was taken.

Intravenous effects of individual amino acids on gastric function measured by in vivo CT. Four-hour food-deprived rats received an IP Zoletil injection (20 mg/kg). After the induction of anesthesia, eyes were covered with vitamin A ointment, rats received an intravenous injection of Ringer lactate (RLA) or RLA with dissolved amino acids (0.7 mmol/kg, pH 7.2) into the lateral tail vein and were subsequently gavaged with 2 ml SDH (200 mg/ml) or SDH with l-lysine (6.7 mmol/kg). Here, gastric l-lysine served as a positive control. A CT image was taken 30 min after application. l-Tryptophan could not be tested intravenously because of its low solubility.

Oral GTT. Four-hour food-deprived rats received an IP injection of 2 ml tap water with l-α-amino acids (6.7 mmol/kg) and/or glucose (2 g/kg). The amylin-treated group received an intraperitoneal injection of amyl (50 μg/kg) following the gavage. An incision at the lateral tail vein enabled us to measure blood glucose levels and to collect blood before application (baseline, t = 0 min) and at 15-min increments afterward. Blood was collected into EDTA-coated tubes (Sarsted, Sevelen, Switzerland). Tubes were gently inverted and centrifuged (1,000 g, 10 min, 4°C), and plasma was collected and stored at −20°C for insulin measurement.

IP and ID GTT. Four-hour food-deprived rats received an IP application of 2 ml tap water with or without l-α-amino acids (6.7 mmol/kg). For the IP GTT, animals received an IP injection of a 0.5 g/ml glucose solution (2 g/kg, pH 7.2, 37°C) dissolved in saline following the gavage. For the ID GTT, animals received an ID bolus (within 30 s) injection of a 0.5 g/ml glucose solution dissolved in 0.9% NaCl (2 g/kg, pH 7.2, 37°C), a glucose solution with dissolved l-lysine (6.7 mmol/kg), or vehicle following the gavage. The amylin-treated group received an IP injection of amyl (50 μg/kg) following glucose administration. A puncture at the lateral tail vein was made to measure blood glucose levels before application (baseline, t = 0 min) and every 15 min following.

CT image acquisition, analysis, and presentation. Images were taken using Quantum FX 2.2 micro-CT (Perkin Elmer, Waltham, MA) and image analyses were performed using Caliper Analyse 11.0 (Analyse Direct, Stillwell, KS). The CT method was established and extensively validated; please see Ref. 24 for details. In brief, up to now, most techniques have been invasive, providing only and single measurements of gastric emptying or secretion. The CT method applied here overcomes these limitations and enables the simultaneous accurate and repetitive measurement of gastric emptying and secretion. The method was validated with the following parameters: 1) quantitative detection of contrast agent and volume in vitro and in vivo, 2) comparison to phenol red method, a standard method in the field, and 3) validation with well-established pharmacological (CCK, Histamin) and physiological interventions (gastric glucose load, semi-solid meal). Next, the method is briefly explained (for graphical abstract, please see Fig. 1A). The area of CT image acquisition (field of view: 7.3 cm) was centered in all three dimensions above the stomach of an anesthetized rat. X-ray images (26 mgY/scan; 148 μm3 voxel size) were captured from animals in a prone position within 34 s, and respiratory gating was applied to correct for motion artefacts during image capture. Acquired images were all treated equally (no filters and no change in contrast or intensity). Semiautomatic image segmentation was performed on the basis of a seed-point algorithm, which links all voxels connected to the seed point (in our case, the contrast agent in the stomach) if they are above a specific threshold (here, 3,200). The extracted object (in our case, the contrast agent in the stomach) was inspected and manually corrected, if the algorithm falsely assigned voxels within the esophagus or the small intestine by a treatment-blinded investigator. Subsequently, object mean intensity and volume were extracted, and three-dimensional volume renderings were generated, reflecting representative examples for a given condition (Fig. 1, A and E). The quantitative data were subsequently used to calculate the stomach SDH content and the nonadministered stomach volume, as seen in the following equations: stomach mean signal intensity = measured variable; total stomach volume t = x min = measured variable; SDH concentration t = x min = (stomach mean signal intensity − 3.093) / 26.20; stomach SDH content t = x min = [SDH concentration t = x min total stomach volume t = x min] + 110 / 0.96; SDH volume t = x min = 2 ml·stomach SDH content t = x min / 400 mg (we apply 400 mg SDH in 2 ml); nonadministered stomach volume t = x min = total stomach volume t = x min − SDH volume t = x min.

ID infusion catheterization surgery. Surgery was conducted similarly to what has been previously described (1). Briefly, a 3–4 cm midline laparotomy was performed in pretreated rats (anesthesia: isoflurane, 2.5%; analgesia: Carprofen, 5 mg/kg; antibiotic: Cefo-verin, 8 mg/kg; all Kantonsapotheke, Zürich, Switzerland). A small incision was made 1–2 cm distal to the pylorus and the distal end of the ID catheter (0.075/0.17 mm inner/outer diameter; Technical Products, Decatur, GA) was inserted 1–2 cm into the duodenum. The application site was located 2–4 cm distal from the pylorus (confirmed postmortem). The catheter was fixed to the serosal surface of the intestine using instant glue and a 5 x 5 mm square surgical mesh (Marley; Bard Implants, Billerica, MA). The proximal end of the catheter was channeled through a puncture in the abdominal muscle wall and then subcutaneously through the interscapular area. Here, the catheter was exteriorized using a custom-made stainless-steel cannula. The cannula was held in place with an angular surgical mesh to
promote adhesion. Animals reached presurgical weight within 3–5 days post-surgery, and the presence of the duodenal catheter did not appear to interfere with further body weight gain. Experiments with animals were started at least 10 days after surgery. Catheter placement was apparent when only intestinal segments showed staining rather than a “splash” (see Fig. 3A for examples). Catheter position was checked regularly after each experiment. After every ID application, the catheter was flushed with 0.5 ml 0.9% NaCl at the end of the experiment.

**Blood glucose and insulin measurement.** Blood glucose concentration was measured immediately using AccuCheck Aviva glucose meters and strips (Roche, Basel, Switzerland). A commercially available ELISA kit was used to measure plasma insulin concentration following the manufacturer’s instructions (ultra-sensitive rat insulin ELISA kit; CrystalChem, Downers Grove, IL).

**Streptozotocin-induced diabetes.** Diabetes was induced in 4-h fasted rats by IP injection of 50 mg/kg streptozotocin (STZ; Sigma) dissolved in citrate buffer (0.1 M sodium citrate, pH 4.5, 4°C, and dissolved for a maximum of 3 min prior to application). Fasting was continued for 3 h postinjection. The experiments were started 7 days postinjection.

**Statistics.** Rats were randomly allocated to treatment groups, and the order of application was randomized. Results are presented as means ± SE. The data were analyzed using GraphPad Prism 5.0. Statistical significance between the means was tested using paired two-tailed Student’s t-test, one- or two-way ANOVA and a Bonferroni or Dunnett post hoc test as indicated. Differences were considered significant at P < 0.05.

**RESULTS**

**In vivo screen for macronutrients with impact on gastric function.** To identify individual nutrients with a potent impact on gastric function, we administered isomolar doses of the nutritionally most relevant macronutrients by the physiological enteral route and measured their impact on stomach volume, gastric emptying (stomach SDH content), and gastric secretion (non-administered stomach volume) by applying CT imaging in rats (Fig. 1). Generally, gastric emptying was very similar between macronutrient classes with few exceptions, whereas gastric secretion was not induced by individual fatty acids and was differentially influenced by individual saccharides and amino acids (Fig. 1, C and D). For instance, the two related disaccharides with identical molar mass α-lactose and D-(+)-maltose had clearly different impacts on gastric secretion, whereas no difference was found in the case of the monosaccharides L-(+)-arabinose and D-(+)-xylose. The nutritional relevant D-(+)-fructose and D-(+)-glucose did not alter gastric emptying differentially, but gastric secretion was induced by D-(+)-glucose and not by D-(−)-fructose. Importantly, the amino acids L-tryptophan, L-arginine, L-cysteine, and L-lysine...
had the most potent impact on gastric emptying and secretion compared with more than 40 other individual nutrients (Fig. 1, B and E), and, therefore, were selected as interesting candidates for further study. Consistent with previous studies, amylin solely delayed gastric emptying but did not induce gastric secretion, thereby, confirming the sensitivity of the CT method (Fig. 1, C and D) (18, 43). Subsequent temporal and dose-dependent studies demonstrated that candidate nutrients did alter gastric function at doses above 0.7 mmol/kg and that l-alanine behaved similarly to an aqueous solution even at 6.7 mmol/kg (Fig. 2). In summary, we reveal a remarkable chemospecific effect of individual nutrients on gastric function in vivo with L-tryptophan, L-arginine, L-cysteine, and L-lysine as the most potent modulators.

L-tryptophan, L-arginine, L-cysteine, and L-lysine impact on gastric function via postgastric and precirculatory mechanisms. We applied candidate nutrients by distinct routes to delineate where they induce their gastric effect. To bypass the stomach, we surgically placed an ID infusion catheter (Fig. 3A). Surgical procedure did not alter the gastric response to intragastrically applied L-lysine (Fig. 3B), nor did we observe long-term surgical effects on body weight development (weight gain of 4 ± 1 g/day in sham and surgical animals 1 wk postsurgery). We infused candidate nutrients intraduodenally at a dose of 0.7 mmol/kg, which had no impact when given intragastrically (Fig. 4A). Given intraduodenally, this dose of l-arginine, l-cysteine, and l-lysine altered gastric function, but no apparent effect was observed for l-alanine and l-tryptophan. Consequently, we doubled the dose and observed that 1.4 mmol/kg l-tryptophan given intraduodenally altered gastric function, whereas l-alanine did not. Next, we tested whether candidate nutrients applied intravenously altered gastric function (Fig. 4B). None of the candidate nutrients had a significant impact on gastric function if given intravenously. These findings indicate that candidate nutrients induce their gastric effect by a postgastric and precirculatory mechanism.

L-tryptophan, L-arginine, L-cysteine, and L-lysine reduce postprandial hyperglycemia in healthy rats via their gastric effect. To test whether our identified candidate nutrients are natural compounds able to reduce postprandial hyperglycemia, we performed an oral GTT. A supraphysiological dose of amylin served as a positive control to enable analogy with a U.S. Food and Drug Administration-approved antidiabetic agent. In agreement with its pharmacological role, amylin reduced postprandial hyperglycemia and plasma insulin concentration (Fig. 5, A and G) (11, 27), whereas l-alanine had no significant effect (Fig. 5, B and H). Importantly, all candidate nutrients identified within our screen significantly reduced postprandial hyperglycemia with distinct temporal kinetics (Fig. 5, C–F). For instance, l-lysine reduced postprandial hyperglycemia already at 15 min postapplication. L-cysteine and l-lysine significantly reduced plasma insulin concentrations, whereas all other candidate nutrients had no significant effect (Fig. 5, H–L). Taken together, these data demonstrate that candidate nutrients reduce the acute rise in hyperglycemia and, therefore, exert a clinical beneficial effect in healthy rats.

Next, we tested whether the differences in glucose excursion observed within the oral GTT are primarily caused by the impact of candidate nutrients on gastric function. Therefore, we administered glucose intraperitoneally and candidate nutrients intragastrically, thereby maintaining candidate nutrients’ effect on gastric function but detracting glucose solely from the gastrointestinal effects. We did not observe a reduction of blood glucose concentrations within this modified IP GTT for candidate nutrients, whereas amylin had a significant effect as expected (Fig. 6, A–F) (11, 27). To further delineate whether only the impact of candidate nutrients on gastric function or alternatively on the small intestine is relevant for the observed reduction in postprandial hyperglycemia, we administered glucose by an ID infusion catheter (Fig. 3A). We established that catheter implantation did not impair the reduction of postprandial hyperglycemia observed after l-lysine and glucose coad-

![Fig. 2. Dose- and time-dependent impact of L-alanine (A), L-tryptophan (B), L-arginine (C), L-cysteine (D) and L-lysine (E) on gastric function. After 4 h of food deprivation, rats were gavaged with contrast agent and a different dose of individual nutrients (as indicated), and their subsequent impact on gastric function was measured with computed tomography (CT). Values are expressed as means ± SE; n = 4–6.](http://ajpregu.physiology.org/content/early/2017/07/04/ajpregu.00273.2014/additional/Figures/2.png)
administration in an oral GTT (Fig. 3C), and that the experimental paradigm of ID bolus injection of glucose triggered very similar temporal changes in blood glucose levels as after oral GTT (Fig. 3C). To exclude that the coadministration of glucose and candidate nutrients alters intestinal glucose absorption, we coadministered glucose and l-lysine intraduodenally. We did not observe altered blood glucose excursion (Fig. 3D). Using these surgically modified animals and the established experimental paradigm, we administered candidate nutrients by the intragastric route and injected glucose as a bolus directly into the duodenum. Crucially, none of the candidate nutrients altered blood glucose concentration in this ID GTT, whereas amylin significantly reduced blood glucose levels (Fig. 6, A–L). Hence, these data demonstrate that the main mechanism by which candidate nutrients reduce postprandial hyperglycemia is their effect on gastric emptying and secretion.

l-tryptophan, l-arginine, l-cysteine, and l-lysine reduce postprandial hyperglycemia in diabetic rats. To demonstrate the full potential of candidate nutrients to reduce postprandial hyperglycemia, we tested their effect in a diabetic rat model. Diabetes was induced by injecting STZ, which causes hyperglycemia by β-cell death (40). STZ-treated animals exhibited marked hyperglycemia (Fig. 7). Next, we performed an oral GTT in the diabetic rats, where glucose was coadministered with candidate nutrients, and subsequent blood glucose concentrations were measured. As expected, amylin reduced postprandial hyperglycemia, and l-alanine had no significant effect (Fig. 7, A and B). All candidate nutrients caused a remarkable reduction in postprandial hyperglycemia similar to or exceeding the effect of amylin (Fig. 7, C–F). These data demonstrate the potential of candidate nutrients to reduce postprandial hyperglycemia in diabetic animals.

DISCUSSION

Gastric emptying and secretion are the two major physiological functions of the stomach that are critical for nutrient digestion and release into the absorptive small intestine. Numerous methods enable the measurement of one or the other of these stomach functions. However, until recently, no method was able to measure both gastric emptying and secretion simultaneously in small animals; techniques were lethal or invasive, and additionally, they were limited by spatial, temporal, or quantitative resolution, thereby constraining throughput and observatory value (13, 39). We recently overcame these limitations and established a CT method enabling the simultaneous quantification of gastric emptying and secretion in vivo (24). Applying this innovative method, we assessed now the impact of more than 40 individual macronutrients on gastric function, a previously unachieved endeavor, and identified l-tryptophan, l-argi-
nine, L-cysteine, and L-lysine as strong modulators of gastric function (Fig. 1).

Gastric function adapts quickly to an ingested meal based on intertwined autonomous, endocrine, and neuronal control systems that are sensitive to meal-specific properties such as volume, osmolarity, and caloric content (6). A long-standing concept states that gastric function and particularly gastric emptying is primarily regulated by the caloric content of an ingested meal. In line with this view, different nutrient solutions were shown to calibrate the gastric emptying rate to 2–2.5 kcal/min in humans and 30–45 cal/min in rats, respectively (15, 21, 24, 29). Consequently, meal macronutrient composition was considered insignificant for the control of gastric function, but the specificity to individual nutrients was never tested systematically. This concept states that gastric function and particularly gastric secretion are mainly modulated by the caloric content of nutrients (Fig. 1). Within our screen, we maintained volume and osmolarity constant to exclude them as confounders, but significant caloric differences between the applied individual nutrients exist. The combustion energy ranges between 0.9 and 5.6 kJ/mol, 4.7 and 11.2 kJ/mol, and 2.3 and 5.7 kJ/mol for individual amino acids, fatty acids, and saccharides, respectively (19). Consequently, we did not find a correlation between applied calories and the effect observed on gastric emptying or secretion for individual nutrients (Fig. 1). Importantly, we identified L-tryptophan, L-arginine, L-cysteine, and L-lysine as the most potent modulators at an isomolar dose. Mechanistically our data suggest a role for the small intestine for the regulation of gastric function. Small ID doses of candidate nutrients were able to inhibit gastric emptying and induce gastric secretion but were inefficient if given intragastrically or into common circulation (Fig. 4). Consequently, candidate nutrients are likely sensed in the small intestine or alternatively in the hepatic portal vein, where nutrients reach a higher concentration than in the common circulation. Both areas are known for distinct physiological mechanisms. The portal vein is innervated by the vagus nerve, which fires in response to certain amino acids (33). In the intestinal mucosa, distinct endocrine cells reside and excrete a variety of hormones into the peritoneal cavity upon nutrient binding (33). Currently, the precise mechanism of L-tryptophan, L-arginine, L-cysteine, and L-lysine sensing at a molecular or
Fig. 5. Impact of amylin, L-alanine, L-tryptophan, L-arginine, L-cysteine, and L-lysine on an oral glucose tolerance test (GTT). Food-deprived rats received a gavage of glucose combined with individual nutrients and their impact on blood glucose (A–F) and plasma insulin (G–L) was measured. Amylin was injected intraperitoneally. Values are expressed as means ± SE; n = 8, using unpaired two-way ANOVA, Bonferroni post hoc test; *P < 0.05, **P < 0.01, ***P < 0.001. DOB, delta over baseline. Baseline blood glucose concentrations (in mg/dl) were the following: glucose, 119 ± 04; glucose + amylin, 120 ± 8; glucose + Ala, 118 ± 10; glucose + Trp, 112 ± 7; glucose + Arg, 116 ± 7; glucose + Cys, 112 ± 9; glucose + Lys, 112 ± 6. Baseline insulin concentrations (ng/ml) were the following: glucose, 1.7 ± 0.5; glucose + amylin, 1.7 ± 0.7; glucose + Ala, 2.2 ± 1.2; glucose + Trp, 1.3 ± 0.8; glucose + Arg, 1.4 ± 1.0; glucose + Cys, 1.4 ± 0.8; glucose + Lys, 1.7 ± 0.5.

Cellular level is unknown and offers a rich avenue for future research. Overall, we propose that gastric emptying and secretion are also regulated by calorie-independent mechanisms based on ID and/or portal sensing mechanisms that enable meal-specific digestive activity.

Reduction of cumulative and also peak postprandial hyperglycemia is important because these glucose excursions are considered a major predictor of diabetic complications and greatly contribute to high HbA1c in diabetic patients (7, 34). Slowing down nutrient efflux from the stomach is one desirable strategy to increase time for peripheral glucose absorption and to compensate for the delay in insulin release or resistance to its action in diabetes (28). Consequently, diets rich in fiber are recommended for diabetic patients as they may delay gastric emptying (8). Their impact remained limited due to issues with dietary compliance and limited effective strength (14). The effect of two other classes of antidiabetic drugs i.e., amylin and GLP-1 analogs, rely, at least in part, on their gastric action. Amylin’s or its synthetic analog pramlintide’s antidiabetic effects act on three fronts: they inhibit glucagon secretion, they inhibit food intake, and they delay gastric emptying (2, 11, 27, 37). Our data support this view, as amylin reduced postprandial hyperglycemia, independent of whether glucose was applied intraduodenally or intraperitoneally (Fig. 6); hence, suggesting that amylin reduces postprandial hyperglycemia mainly by other mechanisms than its gastric effect. In contrast to amylin, GLP-1 or its analog exenatide reduced postprandial hyperglycemia predominantly by delaying gastric emptying (26). Analogous to GLP-1, the newly identified antidiabetic nutrients described here reduced postprandial hyperglycemia mainly by delaying gastric emptying and inducing gastric secretion, as they did not alter blood glucose concentration following ID or IP glucose administration (Fig. 6). Thus, the result of this experiment indicates that potential effects of our candidate nutrients on gastrointestinal hormone release have little impact on blood glucose. Importantly, candidate nutrients reduced postprandial hyperglycemia to larger extents than a supraphysiological dose of amylin in healthy and, particularly, in diabetic rats (Figs. 5 and 7). Therefore, we suggest L-tryptophan, L-arginine, L-cysteine, and L-lysine for use as novel antidiabetic nutrients. They offer significant advantages over current treatment options, as they are cheap natural products, can be ingested orally, act immediately, and do not require dietary adaptation. Similar to amylin, L-arginine and L-lysine have a potent anorectic effect, whereas L-tryptophan and L-cysteine reduce food intake to a smaller extent (23). Future work should, therefore, test the effect of these candidate nutrients on gastric function and postprandial hyperglycemia in humans within double-blinded clinical control trials to clarify the translation potential and clinical importance of the present findings.

In today’s society, meals are normally ingested as solids, and the study performed here is based on a liquid experimental strategy. Solids and liquids were shown to empty at a similar rate from the stomach if they have comparable macronutrient composition (31). A characteristic lag phase precedes solid emptying, in which they are churned to smaller particle size by propulsive stomach contractions (41). This lag phase seems not to have a major impact on blood glucose concentrations. Wolever et al. (42) compared a classical oral GTT (liquid) to
the ingestion of a test meal and did not find significant differences. Consequently, the use of liquid meals represents an experimental simplification, but the results likely hold for the ingestion of solids (24).

**Perspectives and Significance**

The work presented here highlights a remarkable chemospecificity of stomach function, uncovers a strong role of the stomach for glycemic control, and identifies nutrients with antidiabetic potential. Future basic work should aim to identify the underlying molecular, cellular, and endocrinial mechanisms of these effects, and clinical studies assess the efficiency of candidate nutrients in diabetic patients. These amino acids have the advantage of being cheap to obtain and orally ingestible and may become natural alternatives to the administration of exenatide, pramlintide, and liraglutide for reducing post-
prandial hyperglycemia in the treatment of Type 1 and 2 diabetes.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: J.J., B.H., T.A.L., and F.V. conceived and designed of research; J.J. and B.H. performed experiments; J.J. analyzed data; J.J., B.H., T.A.L., and F.V. interpreted results of experiments; J.J. prepared figures; J.J. drafted manuscript; J.J., B.H., T.A.L., and F.V. edited and revised manuscript; J.J., B.H., T.A.L., and F.V. approved final version of manuscript.

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