Effects of different sweet preloads on incretin hormone secretion, gastric emptying, and postprandial glycemia in healthy humans

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ABSTRACT
Background: Macronutrient “preloads” can stimulate glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), slow gastric emptying, and reduce postprandial glycemic excursions. After sweet preloads, these effects may be signaled by sodium-glucose cotransporter-1 (SGLT1), sweet taste receptors, or both.

Objective: We determined the effects of 4 sweet preloads on GIP and GLP-1 release, gastric emptying, and postprandial glycemia.

Design: Ten healthy subjects were studied on 4 separate occasions each. A preload drink containing 40 g glucose, 40 g tagatose/isomalt mixture (TIM), 40 g 3-O-methylglucose (3OMG; a nonmetabolized substrate of SGLT1), or 60 mg sucralose was consumed 15 min before a 13C-octanoic acid–labeled mashed potato meal. Blood glucose, plasma total GLP-1 and GIP, serum insulin, and gastric emptying were determined.

Results: Both glucose and 3OMG stimulated GLP-1 and GIP release in advance of the meal (each \(P < 0.05\)), whereas TIM and sucralose did not. The overall postprandial GLP-1 response was greater after glucose, 3OMG, and TIM than after sucralose (\(P < 0.05\), albeit later after TIM than the other preloads. The blood glucose and insulin responses in the first 30 min after the meal were greatest after glucose (each \(P < 0.05\)). Gastric emptying was slower after both 3OMG and TIM than after sucralose (each \(P < 0.05\)).

Conclusions: In healthy humans, SGLT1 substrates stimulate GLP-1 and GIP and slow gastric emptying, regardless of whether they are metabolized, whereas the artificial sweetener sucralose does not. Poorly absorbed sweet tastants (TIM), which probably expose a greater length of gut to nutrients, result in delayed GLP-1 secretion but not in delayed GIP release. These observations have the potential to optimize the use of preloads for glycemic control. This trial was registered at www.actr.org.au as ACTRN12611000775910. Am J Clin Nutr 2012;95:78–83.

INTRODUCTION
Major determinants of postprandial blood glucose include the rate of gastric emptying (1, 2) and the postprandial insulin response, of which \(\geq 50\%\) is stimulated by the secretion of GLP-1 (3) and GIP, the so-called incretin hormones, from the gut in healthy humans (3). In patients with type 2 diabetes, the incretin effect is impaired (4). Incretin-based therapies for diabetes have focused on GLP-1 rather than on GIP, because the insulinotropic action of the latter is diminished in these patients (5). Other actions of GLP-1 include slowing of gastric emptying (6) and reduction of appetite and energy intake (7).

One promising strategy to stimulate endogenous GLP-1 is the “preload” concept, in which a small load of macronutrient is given a fixed interval before a meal, to induce the release of gut peptides such as GLP-1 and GIP to slow gastric emptying and stimulate insulin secretion in advance of the main nutrient load. We have shown that both fat and protein preloads markedly reduce postprandial glycemic excursions in patients with type 2 diabetes by these mechanisms (8, 9). However, a potential disadvantage is that the preload could increase overall energy intake; preloads that entail minimal additional energy would be advantageous.

Incretin stimulation by carbohydrates may be signaled by sweet taste receptors and/or SGLT1. Data from in vitro, animal, and human studies in this area have been inconsistent. For example, stimulation of sweet taste receptors by sucralose (a noncaloric artificial sweetener) was reported to release GLP-1 from enterocanalytic L cells in vitro (10, 11) but has no effect on GLP-1 secretion and gastric emptying in humans (12). It has also been postulated that SGLT1 may be involved in incretin hormone secretion (13). Monosaccharides that are substrates for SGLT1, including glucose, galactose, and 3OMG, stimulate GLP-1 release in vitro and in perfused ileum in animal studies (14–16), an effect inhibited by phlorizin, which blocks SGLT1 (13). Similarly, in ob/ob mice, GIP is secreted in response to glucose, galactose, and 3OMG (17), but not to fructose and mannose, which are not SGLT1 substrates (18, 19). 3OMG is a glucose analog that is absorbed from the small intestine via SGLT1, but it is not metabolized and makes no contribution to energy intake. Whether 3OMG stimulates GIP and GLP-1 secretion in humans is unknown.

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4 Abbreviations used: GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; iAUC, incremental AUC; SGLT1, sodium-glucose cotransporter-1; T50, half-emptying time; TIM, tagatose/isomalt mixture; 3OMG, 3-O-methyglucose.

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The length of small intestine exposed to carbohydrate is another determinant of GLP-1 release (20). Secretion of GLP-1 in response to sucrose increases markedly when malabsorption is induced by the α-glucosidase inhibitor acarbose (21, 22). Poorly absorbed sugars such as tagatose and isomalt could potentially have a similar effect (23, 24).

The current study was designed to determine the comparative effects of preloads of glucose, 3OMG, a TIM, and sucralose on the release of GLP-1 and GIP, as well as gastric emptying of, and the glycemic response to, a subsequent meal in healthy humans.

SUBJECTS AND METHODS

Subjects

Ten healthy subjects [7 men and 3 women; mean ± SE age: 28.8 ± 4.0 y; BMI (in kg/m²): 25.5 ± 1.5] took part in the protocol, which was approved by the Research Ethics Committee of the Royal Adelaide Hospital, after subjects provided written informed consent.

Study protocol

Each subject was studied on 4 occasions, separated by ≥3 d, in a single-blind, randomized fashion. Female subjects were studied exclusively during the follicular phase of the menstrual cycle to limit potential variations in gut hormone concentrations secondary to changes in gastric emptying (25). On the evening before each study day (~1900), subjects consumed a standardized evening meal (McCain’s frozen beef lasagna, 2472 kJ; McCain Foods Proprietary Ltd) with bread, a nonalcoholic beverage, and one piece of fruit. Subjects were then asked to refrain from consuming solids and were asked to refrain from consuming liquids after 2200.

Subjects attended the laboratory at ~0830 and were seated comfortably for the duration of the study. An intravenous cannula was inserted into a forearm vein for repeated blood sampling. On each day, between t = −22 to −20 min, subjects consumed a preload drink containing either (1) 40 g glucose, (2) 40 g 3OMG (Sigma-Aldrich), (3) 40 g TIM [Tagatesset; ingredients: tagatose (39.92%), isomalt (39.92%), dietary fibers (20.14%: inulin and oligosaccharides), and sucralose (0.02%); glycemic index: 7.5; caloric value: 2.76 kcal/g; Damhert Nutrition], or (4) 60 mg sucralose (equivalent sweetness to glucose), each dissolved in 400 mL water. Fifteen minutes later (t = −5 to 0 min), they ate a solid meal consisting of 65 g powdered potatoes (Deb; Unilever Australia Ltd) and 20 g glucose, reconstituted with 200 mL water and one egg yolk containing 100 μL 13C octanoic acid. Breath samples were collected immediately before and every 5 min after meal ingestion in the first hour, and every 15 min for a further 3 h. Venous blood was sampled immediately before preload administration (t = −25 min) and then at t = −10, 0, 15, 30, 60, 90, 120, and 240 min. Blood samples were placed in ice-chilled EDTA tubes containing 400 kallikrein inhibitor units aprotinin (Trasylo; Bayer Australia Ltd) per milliliter of blood and were centrifuged at 3200 rpm for 15 min. Plasma was separated and stored at −70°C for subsequent analysis. At the same intervals as used for blood sampling, gastrointestinal sensations, including hunger, fullness, desire to eat, and projected consumption, were assessed by 100-mm visual analog scales (26). Volunteers were also asked to distinguish the sweetness of preloads.

Data analysis

13CO2 concentrations in breath samples were measured by an isotope ratio mass spectrometer (ABCA 2020; Europa Scientific) with an online gas chromatographic purification system. The T50 was calculated by using the formula described by Ghoos et al (27). This method has been validated against scintigraphy for the measurement of gastric emptying (28).

Blood glucose concentrations were measured immediately by using a glucometer (Medisense Precision QID; Abbott Laboratories). The accuracy of the method has been validated against the hexokinase technique (1).

Serum insulin was measured by ELISA immunoassay (catalog no. 10-1113; Mercodia). The sensitivity of the assay was 1.0 mU/L, and the CV was 2.1% within assays and 6.6% between assays. Plasma total GLP-1 was measured by radioimmunoassay (GLPIT-36HK; Linco Research). The sensitivity was 3 pmol/L, and the interassay CV was 9.2%. Plasma GIP was measured by radioimmunoassay (29), with a sensitivity of 2 pmol/L, an interassay CV of 11.2%, and an intraassay CV of 10.2%.

Statistical analysis

The iAUC was calculated by using the trapezoidal rule (30) for glucose, insulin, GLP-1, and GIP concentrations and analyzed by 1-factor ANOVA. These variables were also assessed by using repeated-measures ANOVA, with treatment and time as factors. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed if ANOVAs showed significant effects. Gastric emptying (T50) and the overall iAUC for insulin, GLP-1, and GIP after each preload were also compared with sucralose (negative control) by 2-tailed paired Student’s t tests adjusted by Bonferroni’s correction. All analyses were performed with SPSS software (version 17.0; SPSS Inc). The sample size of 10 subjects was calculated to have a power of 80% at an α = 0.05 to detect ~150% difference in GLP-1 secretion (31) and a ~20% difference in gastric T50 (32), when compared with the negative control (sucralose). Data are presented as means ± SEs. P < 0.05 was considered significant.

RESULTS

All subjects tolerated the study well. Most subjects perceived TIM as being sweeter than the other preloads but could not discriminate between the glucose, 3OMG, and sucralose drinks.

Blood glucose concentrations

Fasting blood glucose did not differ between the 4 study days. There was an increase in blood glucose immediately after the glucose preload but not after sucralose, 3OMG, or TIM. The increases in blood glucose after the glucose preload at t = −10 and 0 min were 1.5 ± 0.4 and 2.3 ± 0.4 mmol/L, respectively. Blood glucose concentrations increased after the meal on each day. Although the overall iAUC for blood glucose did not differ significantly, there was a treatment effect on iAUC for blood glucose in the first 30 min after the meal (P < 0.001) (Table 1, Figure 1A), such that blood glucose was greater after the glucose than after the sucralose (P = 0.002), 3OMG (P = 0.002), or TIM (P = 0.009) preloads but was less after 3OMG than after the sucralose (P = 0.038); there was no significant difference between TIM and sucralose.
There was a prompt increase in plasma GLP-1 after the glucose and sucralose preloads but not after the 3OMG or TIM preloads (P < 0.05 for each). Serum insulin concentrations increased after the meal on each day, with a significant treatment effect on the overall iAUC (P = 0.007) (Table 1, Figure 1B), such that serum insulin was greater after the glucose than after the sucralose (P = 0.022), 3OMG (P = 0.015), or TIM (P = 0.008), without any differences between sucralose, 3OMG, and TIM.

Plasma GLP-1 concentrations

Fasting plasma GLP-1 did not differ between the 4 study days. There was a prompt increase in plasma GLP-1 after the glucose and sucralose preloads (P < 0.05 for each), whereas GLP-1 concentrations after sucralose and TIM were unchanged before the meal. After the meal, plasma GLP-1 concentrations increased on each day. When compared with sucralose, the increase in plasma GLP-1 was greater between t = −10 and 15 min after the glucose and 3OMG preloads, and between t = 0 and 120 min after the TIM preload (P < 0.05 for each comparison). There was a significant treatment effect on the overall iAUC for plasma GLP-1 (P = 0.005) (Table 1, Figure 1C), such that plasma GLP-1 was greater after 3OMG (P = 0.018), TIM (P = 0.015), and glucose (P = 0.042), when compared with sucralose, and there was a trend for higher GLP-1 concentrations after 3OMG than after glucose (P = 0.052). Although TIM did not stimulate GLP-1 before the meal, the overall iAUC for plasma GLP-1 after TIM was comparable to preloads of both glucose and 3OMG.

**TABLE 1**

iAUCs for blood glucose concentrations in the first 30 min and overall after the meal and overall insulin, GLP-1, and GIP concentrations in response to a preload of glucose, sucralose (control), 3OMG, or TIM.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood glucose iAUC_{240} (mmol/L × min)</th>
<th>Serum insulin iAUC_{240} (mU/L × min)</th>
<th>Plasma GLP-1 iAUC_{240} (pmol/L × min)</th>
<th>Plasma GIP iAUC_{240} (pmol/L × min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucralose</td>
<td>48.1 ± 7.1</td>
<td>196.2 ± 33.6</td>
<td>818.4 ± 220.3</td>
<td>3931.2 ± 609.1</td>
</tr>
<tr>
<td>Glucose</td>
<td>114.4 ± 15.3</td>
<td>279.0 ± 41.4</td>
<td>1627.2 ± 356.8</td>
<td>5938.1 ± 851.0</td>
</tr>
<tr>
<td>3OMG</td>
<td>29.6 ± 6.8</td>
<td>226.5 ± 70.4</td>
<td>2341.1 ± 397.4</td>
<td>5199.1 ± 693.1</td>
</tr>
<tr>
<td>TIM</td>
<td>44.6 ± 7.3</td>
<td>196.7 ± 38.6</td>
<td>2309.5 ± 447.2</td>
<td>4142.3 ± 510.0</td>
</tr>
</tbody>
</table>

All values are means ± SE; n = 10. AUs were calculated by using the trapezoidal rule. GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; iAUC, incremental AUC; TIM, tagatose/isomalt mixture; 3OMG, 3-O-methylglucose.

Results

Fasting serum insulin did not differ between the 4 study days. There was an increase in serum insulin immediately after the glucose preload but not after the sucralose, 3OMG, or TIM (treatment effect at t = −10 min (P = 0.001) and 0 min (P < 0.001)). Serum insulin concentrations increased after the meal on each day, with a significant treatment effect on the overall iAUC (P = 0.007) (Table 1, Figure 1B), such that serum insulin was greater after the glucose than after the sucralose (P = 0.022), 3OMG (P = 0.015), or TIM (P = 0.008), without any differences between sucralose, 3OMG, and TIM.

**FIGURE 1.** Mean (±SEM) effects of a preload of glucose, sucralose (control), 3OMG, or TIM on blood glucose (A), serum insulin (B), plasma GLP-1 (C), and GIP (D) in response to a carbohydrate meal (n = 10). Repeated-measures ANOVA was significant (P < 0.01) for A through D; post hoc comparisons were adjusted by Bonferroni’s correction. A: *P < 0.05, glucose compared with sucrase; TIM, and 3OMG. B: *P < 0.05, glucose compared with sucralose; TIM, and 3OMG. C: *P < 0.05, glucose compared with sucralose; 3OMG compared with sucralose; TIM, and 3OMG. D: *P < 0.05, glucose compared with sucralose; 3OMG compared with sucrase; TIM; 3OMG compared with sucralose; TIM, and 3OMG compared with sucralose; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; TIM, tagatose/isomalt mixture; 3OMG, 3-O-methylglucose.
Plasma GIP concentrations

Fasting plasma GIP did not differ between the 4 study days. There was a prompt increase in plasma GIP after glucose and 3OMG preloads (P < 0.05 for each), whereas GIP concentrations were unchanged after sucralose and TIM before the meal. After the meal, plasma GIP concentrations increased on each day. There was a significant effect of treatment on iAUC for plasma GIP (P = 0.001) (Table 1, Figure 1D), such that plasma GIP, although comparable after glucose and 3OMG, was greater than after sucralose and TIM, with significant differences at t = −10, 0, and 15 min (P < 0.05 for each).

Gastric emptying (T50)

Gastric emptying was slower after 3OMG (T50: 207 ± 17 min) and TIM (T50: 275 ± 39 min) than after sucralose (T50: 165 ± 12 min) (P = 0.012 and 0.033, respectively). The difference between glucose (T50: 245 ± 59 min) and sucralose was not significant (P = 0.45). There was no significant difference between glucose, 3OMG, and TIM (Figure 2).

Appetite sensations

There were no differences in hunger, desire to eat, or prospective consumption between any of the preloads (data not shown). However, after the meal, fullness tended to be greater after glucose, 3OMG, and TIM than after sucralose (P = 0.06 for each) (Figure 3).

DISCUSSION

This study shows that, in healthy humans, a nonmetabolized SGLT1 substrate, 3OMG, stimulates GLP-1 and GIP release, whereas TIM probably stimulates a long length of gut, and therefore results in later GLP-1 secretion but does not stimulate GIP. Preloads of 3OMG and TIM slowed gastric emptying and induced fullness, when compared with sucralose, and 3OMG attenuated the early postprandial glycemic response to a carbohydrate meal. The interaction of ingested nutrients with the small intestine to stimulate the release of gut peptides, including GLP-1 and GIP, represents a major mechanism in the regulation of gastrointestinal feedback. Osmolarity and viscosity could not readily be matched without changing the taste, but this is unlikely to have had a major impact; high osmolarity induced by saline has no effect on GLP-1 secretion (34), whereas postprandial GLP-1 and cholecystokinin secretion are comparable between liquid and semiliquid meals of matched macronutrient composition, despite major differences in viscosity (35). We previously showed that stimulation of sweet taste receptors alone by sucralose in humans is not sufficient to stimulate incretin hormone or insulin release or slow gastric emptying (12). Despite a report of synergy between acesulfame-K and sucralose on glucose-induced GLP-1 release (36), we were unable to show any effect of sucralose alone on the GLP-1 response to a subsequent glucose load (34). Therefore, sucralose was chosen as a negative control in the present study. Conversely, glucose is known to be a potent stimulus of small intestinal feedback. As expected, plasma GLP-1, GIP, and insulin concentrations increased rapidly after the glucose preload, but they only began to increase after the subsequent meal, and to a lesser extent than for glucose, when the sucralose preload was given.

Both glucose and 3OMG are absorbed predominantly via SGLT1 (37, 38), with a minor role for other transporters (eg, glucose transporter 2) (38, 39). Studies in cell lines and rodents have confirmed the expression of SGLT1 in K and L cells (33) and showed that incretin hormone secretion induced by glucose or 3OMG is blocked by phloridzin (13). Consistent with this, we found that preloads of glucose and 3OMG stimulated both GLP-1 and GIP secretion in advance of the mashed potato meal. However, the overall GLP-1 response tended to be greater after 3OMG than after glucose, possibly because glucose is absorbed faster than 3OMG (40), resulting in greater exposure of 3OMG to more distal regions of the small intestine where L cell density is greater. It is also possible that gastric emptying was initially slower by stimulating GLP-1 secretion and slowing of gastric emptying, entail consumption of additional calories, which might limit their long-term benefits in diabetes management. The findings in the present study indicate that nonnutrient substrates of SGLT1 (eg, 3OMG) or poorly absorbed sweeteners (eg, TIM) could be used as alternative preloads to minimize additional energy intake.
after glucose due to relatively higher blood glucose concentrations; acute hyperglycemia, even within physiologic range (ie, 8 mmol/L), slows gastric emptying (41), and the stimulation of GLP-1 and GIP is known to be dependent on the glucose load entering the small intestine (42). However, the method we used to measure gastric emptying may not have been sufficiently sensitive to detect this. In contrast, 3OMG and glucose stimulated GIP secretion to a comparable degree both before and after the meal. This is not surprising, because the majority of K cells are located in the proximal, rather than distal, small intestine (33), and GIP secretion in response to intraduodenal glucose increases by elevated glycemia per se in healthy humans (43), which might have counterbalanced any tendency for diminished GIP stimulation due to slower gastric emptying.

We also observed that a mixture of poorly absorbed sugars failed to induce a plasma incretin response in advance of the potato meal, but the overall GLP-1 response was greater than with sucralose, and relatively later than after the glucose and 3OMG preloads, which is consistent with previous findings that indigestible carbohydrates, such as resistant starch, can induce sustained GLP-1 release and improve glucose tolerance (44). Given that the stimulation of GLP-1 was relatively late, it is not surprising that postprandial blood glucose was not lowered when compared with sucralose, but it would be of interest to evaluate in future studies the effects of a longer interval between ingestion of TIM and administration of the subsequent meal. In contrast, TIM did not result in greater GIP secretion than did sucralose, suggesting that it has no effect on K cell stimulation. This may increase the appeal for the use of TIM, given that some actions of GIP, particularly those on adipocytes resulting in fat deposition and impaired insulin sensitivity, may be deleterious (45).

The mechanism by which TIM stimulates GLP-1 secretion is uncertain. Oral tagatose is reported to stimulate GLP-1 to a comparable degree to fructose, whereas neither stimulates GIP (23). Because neither is a SGLT1 substrate, it follows that signaling pathways other than SGLT1 are likely to be involved in GLP-1 secretion. The exposure of poorly absorbed sugars to the distal gut, with production of short-chain fatty acids by bacterial fermentation, may represent an important mechanism of GLP-1 stimulation (33). Xylose, a poorly absorbed pentose, is a potent stimulus for GLP-1 (31), and tagatose may act in a similar manner, given its relatively low absorption rate (~25%) (46). Nevertheless, GLP-1 was stimulated to a greater level by the TIM than by the sucralose preload immediately after the meal, and tagatose was also shown to slow gastric emptying rapidly (32), so that early gastrointestinal responses to tagatose might be mediated by other pathways, such as GLUT5 (the fructose transporter). Isomalt is a mixture of disaccharide alcohols with a slow rate of hydrolysis, which results in limited absorption in the small intestine and subsequent fermentation in the colon (47). Although incorporation of isomalt into a diabetic diet has been reported to improve glycemic control (24), the capacity of isomalt to stimulate GLP-1 secretion has not been evaluated.

Despite the stimulation of incretin hormones by 3OMG and TIM, the insulin response after these preloads was less than after the glucose preload and was comparable among 3OMG, TIM, and sucralose. This is likely to reflect the fact that the insulinotropic effect of the incretins is glucose dependent (48) and highlights the concept that the slowing of gastric emptying can potentially outweigh the insulinotropic effect of GLP-1 in reducing postprandial glycemic excursions (49). It is also well established that GLP-1, even in physiologic concentrations, suppresses glucagon secretion in a glucose-dependent manner (33). However, we did not measure plasma glucagon in the present study.

We showed a trend for postprandial fullness to increase more after preloads of glucose, 3OMG, and TIM than after sucralose, indicating a potential inhibitory effect of these preloads on energy intake. This is likely to be related to increased GLP-1 concentrations. However, the differences failed to reach statistical significance, probably due to the small sample size.

The reduction in early postprandial glycemia after the 3OMG preload was modest in our study’s healthy subjects. It would be of interest to extend our investigation to patients with type 2 diabetes who are likely to have greater postprandial blood glucose excursions and consequently a larger margin for glycemic improvement. A better outcome in postprandial glycemia might also be achieved by enhancing endogenous active GLP-1 concentrations by concurrent administration of a dipeptidyl peptidase IV inhibitor. Although the cost of 3OMG renders its use in a diabetic diet unfeasible, our findings support the concept that endogenous GLP-1 can be stimulated with minimal cost in additional energy intake. This has significant implications for efforts to refine preload strategies to minimize postprandial glycemic excursions in diabetes.

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The authors’ responsibilities were as follows—TW: was involved in study design and coordination, subject recruitment, performing the study, data interpretation, statistical analyses, and drafting of the manuscript; BRZ, MB and HLC: were involved in performing the study, data interpretation, and drafting of the manuscript; MB: performed breath sample assays and gastric half-emptying time calculations; TJL and RLY: were involved in study design, data interpretation, and drafting of the manuscript; KLJ and MH: were involved in conception of the study, data interpretation, and drafting of the manuscript; and CKR: was involved in conception and design of the study, data analysis and interpretation, and drafting of the manuscript and had overall responsibility for the study. None of the authors had any personal or financial conflicts of interest to declare.

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