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Inhibition of sweet chemosensory receptors alters insulin responses during glucose ingestion in healthy adults: a randomized crossover interventional study[1,2]

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ABSTRACT

Background: Glucose is a natural ligand for sweet taste receptors (STRs) that are expressed on the tongue and in the gastrointestinal tract. Whether STRs directly contribute to the regulation of glucose homeostasis in response to glucose ingestion is unclear.

Objective: We sought to determine the metabolic effects of the pharmacologic inhibition of STRs in response to an oral glucose load in healthy lean participants.

Design: Ten healthy lean participants with a body mass index (in kg/m^2) of 22.4 $\pm$ 0.8 were subjected to an oral-glucose-tolerance test (OGTT) on 4 separate days with the use of a randomized crossover design. Ten minutes before the 75-g OGTT, participants consumed a preload solution of either 300 parts per million (ppm) saccharin or water with or without the addition of 500 ppm lactisole, a human-specific inhibitor of STRs. When present, lactisole was included in both the preload and OGTT solutions. We assessed plasma responses of glucose, insulin, C-peptide, glucagon, glucagon-like peptides 1 and 2, gastric inhibitory peptide, acetaminophen, and 3-*O*-methylglucose. With the use of mathematical modeling, we estimated gastric emptying, glucose absorption, β-cell function, insulin sensitivity and clearance, and the portal insulin:glucagon ratio.

Results: The addition of lactisole to the OGTT caused increases in the plasma responses of insulin ($P = 0.012$), C-peptide ($P = 0.004$), and the insulin secretory rate ($P = 0.020$) compared with the control OGTT. The addition of lactisole also caused a slight reduction in the insulin sensitivity index independent of prior saccharin consumption ($P < 0.025$). The ingestion of saccharin before the OGTT did not alter any of the measured variables but eliminated the effects of lactisole on the OGTT.

Conclusion: The pharmacologic inhibition of STRs in the gastrointestinal tract alters insulin responses during an oral glucose challenge in lean healthy participants. This trial was registered at clinicaltrials.gov as NCT02835859. *Am J Clin Nutr* doi: 10.3945/ajcn.116.146001.

Keywords: sweet taste receptors, lactisole, glucose intolerance, insulin, saccharin, artificial sweeteners, OGTT modeling analysis

INTRODUCTION

Epidemiologic data have suggested that the consumption of noncaloric artificial sweeteners (NCASs)[7] is associated with adverse metabolic effects, weight gain, and type 2 diabetes (1, 2). Although the interplay of complex factors likely accounts for these associations, some studies have shown that both NCASs and dietary sugars interact with and activate cell-surface sweet taste receptors (STRs) that are expressed in a variety of tissues, including the gastrointestinal tract (3) and pancreatic β cells (4). The physiologic significance of STR signaling in these non-gustatory tissues remains to be elucidated, but it is likely relevant to the metabolic effects of NCASs and dietary sugars. For instance, STRs in mouse and human islets regulate basal insulin secretion by sensing circulating glucose (5), but they also potentiate glucose-stimulated insulin secretion in response to fructose or saccharin (4). Similarly, intestinal STRs induce the secretion of glucagon-like peptide (GLP) 1 in mice and human cell lines and increase intestinal glucose transport in response to sugars or NCASs (6–8). These data suggest that dietary monosaccharides can activate chemosensory signaling pathways independent of their cellular uptake and metabolism and that NCASs are physiologically active despite not being metabolized by tissues.

Because dietary NCASs are slowly absorbed by the gastrointestinal tract, they are unlikely to reach substantial systemic concentrations after a mixed meal (9). As a result, STRs in the intestine, like those on the tongue, are exposed to high concentrations of both NCASs and sugars and thus are expected to

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[2] Supplemental Figures 1 and 2 and Supplemental Table 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[7] Abbreviations used: GIP, gastric inhibitory peptide; GLP, glucagon-like peptide; HGP, hepatic glucose production; NCAS, noncaloric artificial sweetener; OGTT, oral-glucose-tolerance test; ppm, parts per million; STR, sweet taste receptor.

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exert robust physiologic effects. Nevertheless, the acute consumption of NCASs does not alter plasma gastrointestinal hormones or glucose homeostasis in humans (10–13). In contrast, when NCASs are ingested immediately before sugar consumption the combination can potentiate insulin (14) or incretin (15, 16) responses. Thus, it is plausible that sugars provide the primary stimulus, whereas NCASs synergize to amplify hormone secretion and regulate glucose homeostasis. Although NCASs and sugars are bona fide ligands for STRs, their direct contribution has not been assessed in prior studies to our knowledge.

The main objective of this randomized crossover interventional study (NCT02835859) was to test whether the stimulation (i.e., saccharin) or inhibition (i.e., lactisole) of STRs in the gastrointestinal tract (tongue and small intestine) play a direct role in regulating glycemic and hormonal control in response to the oral ingestion of glucose. We recruited healthy lean participants because previous findings have suggested that disturbances in glucose homeostasis alter the expression and function of intestinal STRs (3, 17).

METHODS

Experimental design

This study was a 4-arm randomized crossover interventional study conducted at Florida Hospital from April 2014 to April 2015. Ten young lean healthy subjects without diabetes and who maintained a stable weight (±3.0 kg) during the 3 mo before enrollment and consumed less than a can of diet beverage or a spoonful of NCAS weekly (or each equivalent from foods) during the past month participated in the study. All potential subjects were recruited by local advertisements and interviewed before their participation. Subjects were excluded according to the following criteria: *1*) diagnosed with coronary artery disease, angina, heart failure, diabetes, bleeding disorders, infections, hepatitis and/or cirrhosis, severe asthma or chronic obstructive pulmonary disease, renal insufficiency, bariatric surgery, inflammatory bowel disease or malabsorption, cancer within the last 3 y (except nonmelanoma skin cancer or treated cervical carcinoma in situ), psychiatric or eating disorders, untreated or inadequately controlled thyroid or other endocrine disorders, or active rheumatoid arthritis or other inflammatory rheumatic disorder; *2*) pregnant or nursing women; *3*) the presence of substantial clinical abnormalities on the electrocardiogram; *4*) current smokers (smoking within the past 3 mo); *5*) known hypersensitivity to saccharin, lactisole, and acetaminophen or any of its excipients; *6*) history of difficult blood sample collections or unfavorable anatomy of venous access; *7*) the use of medications such as nitrates, β-blockers, digoxin, antidiabetic agents, topical steroids (inhaled steroids for mild asthma were acceptable), chronic use of aspirin or other nonsteroidal anti-inflammatory drugs (including cyclooxygenase 2 inhibitors), other drugs known to affect immune or metabolic function, and orlistat, phentermine, or other weight-loss or anorectic agents; and *8*) blood pressure ≥160/100 mm Hg or ≤100/50 mm Hg at screening. The menstrual phase of the women was not controlled because treatment order was randomized. The study was approved by the Florida Hospital Institutional Review Board.

Subjects participated in a randomized crossover design of 4 separate interventions that were performed 7 d apart. The order of the 4 interventions was randomized for each participant with the use of statistical software (www.randomize.net) by an unblinded trained pharmacist. Investigators and clinical personnel were blinded to the interventions. Diet-related instructions were provided to avoid the consumption of NCASs and Tylenol for the duration of the study. For each intervention, subjects reported to the Translational Research Institute at Florida Hospital at 0730 after a 10-h overnight fast. The following procedures were then performed: *1*) assessment of dietary compliance; *2*) assessment of vital signs (heart rate, blood pressure, respiration, temperature); *3*) measurements of weight; *4*) urine sample for pregnancy tests; and *5*) insertion of an intravenous catheter for blood draws. Blood samples were obtained to measure plasma glucose, insulin, C-peptide, glucagon, total GLP-1 and GLP-2, gastric inhibitory peptide (GIP), 3-*O*-methylglucose, and acetaminophen at 15, 10, and 2 min before and 30, 45, 60, 90, 120, 150, and 180 min after the ingestion of 75 g glucose in 600 mL (12.5% solution) with or without 500 ppm lactisole, a human specific STR inhibitor. The glucose solution also included 3.0 g 3-*O*-methylglucose and 1.4 g acetaminophen for the estimation of gastric emptying and glucose absorption, respectively. Ten minutes before the ingestion of the glucose solution, subjects consumed a 60-mL solution of distilled water or 300 parts per million (ppm) saccharin with or without 500 ppm lactisole. All solutions were consumed in <3 min. Participants were asked to report the general taste sensation of the ingested solutions (i.e., sweet, bitter, neutral, etc.). Saccharin, glucose, and lactisole doses were selected considering physiologic intake, pharmacokinetic relations, and stimulus potency (18). A saccharin concentration of 300 ppm elicits sweet responses equivalent to 12.5% glucose solution. At high concentrations (>10 mM), saccharin has been shown to engage with bitter taste receptors on the tongue (19). Therefore, the current dose (300 ppm = 1.46 mM) excluded the possibility of non-STR–mediated effects. As expected, all participants reported a strong sweet taste sensation upon ingesting the saccharin or glucose solution. Finally, the saccharin concentration also approximated the physiologic acute intake of a saccharin-sweetened solution [1 packet of Sweet'N Low (Cumberland Packing Corp.) in a cup of coffee; ∼0.24 mg/mL]. Lactisole is a competitive inhibitor of human STRs and at 500 ppm completely eliminates the stimulatory effects induced by either a 300-ppm saccharin solution or a 12.5% glucose solution (75.0 g/600 mL) (18). In the presence of lactisole, all participants reported a neutral taste sensation upon ingesting either the saccharin or glucose solution without any adverse aftertaste, consistent with previous findings (18). Lactisole was also administered to the upper safety limits established by the Food and Drug Administration (20).

Measurements

Glucose concentrations were measured with the use of the glucose oxidase method (YSI 2300 automated analyzer); insulin, C-peptide, total GLP-1, GLP-2, and GIP concentrations were measured with the use of an immunoassay (Milliplex Map Kit; Meso Scale Discovery); and glucagon was measured with the use of ELISA (Mercodia). Blood was also collected in K_2 EDTA-coated tubes with a cocktail of protease, esterase, and dipeptidyl peptidase IV inhibitors (BD P800 blood collection system; BD Bioscience). Glucose absorption and gastric emptying were

assessed by dissolving 3.0 g of the nonmetabolizable glucose analog 3-*O*-methylglucose and 1.4 g acetaminophen in the glucose solution, respectively. 3-*O*-Methylglucose is a glucose analogue that is transported by intestinal sodium and glucose cotransporter 1 and glucose transporter 2 but is not metabolized by enterocytes (21) and has the same affinity as glucose for STRs (22). The 3-*O*-methylglucose assay has been routinely used to assess glucose absorption (17, 21). Gastric emptying was estimated by mathematically modeling plasma acetaminophen concentrations. Plasma 3-*O*-methylglucose and acetaminophen concentrations were measured with the use of mass spectroscopy (Sanford Burnham Prebys Medical Discovery Institute).

Mathematical modeling and calculations

The baseline-adjusted AUC was calculated by the trapezoid rule with the use of all time points (0–180 min). β-Cell function was assessed from the oral-glucose-tolerance test (OGTT) with the use of a model that describes the relation between insulin secretion and glucose concentration, which has been described previously in detail (23, 24). The model expresses insulin secretion (pmol/min $\times$ m^2) as the sum of 2 components. The first component represents the dependence of insulin secretion on absolute glucose concentration at any time point during the OGTT through a dose-response function relating the 2 variables. Characteristic parameters of the dose response are the mean slope over the observed glucose range, denoted as β-cell glucose sensitivity, and insulin secretion at a fixed glucose concentration of 7.0 mmol/L (i.e., approximate mean peak of glucose). The dose response is modulated by a potentiation factor that accounts for the fact that during an acute stimulation insulin secretion is higher in the descending phase of hyperglycemia than at the same glucose concentration in the ascending phase. As such, the potentiation factor encompasses several potentiating mechanisms such as sustained hyperglycemia or gastrointestinal hormones. It is set to be a positive function of time and is constrained to mean unity during the experiment. In normal subjects, the potentiation factor typically increases from baseline to the end of a 2-h OGTT (25). To quantify this excursion, the ratio between the 2-h and baseline values was calculated. This ratio is denoted as the potentiation ratio. The second insulin secretion component represents the dependence of insulin secretion on the rate of change of glucose concentration. This component is termed the derivative component and is determined by a single parameter denoted as rate sensitivity. Rate sensitivity is related to early insulin release (25). The model parameters were estimated from glucose and C-peptide concentrations with the use of regularized least squares as previously described (23). Regularization involves the choice of smoothing factors that were selected to obtain glucose and C-peptide model residuals with SDs close to the expected measurement error ($\sim$1% for glucose and $\sim$4% for C-peptide). Insulin secretory rates were calculated from the model every 5 min. β-cell function was also estimated with the use of the insulinogenic index and calculated for the entire duration of the OGTT as $AUC_{\text{C-peptide (0–180 min)}}/AUC_{\text{glucose (0–180 min)}}$ (26). Insulin clearance was calculated as $AUC_{\text{insulin secretion}}/AUC_{\text{insulin}}$. The insulin sensitivity index was assessed as the metabolic clearance rate of glucose according to Stumvoll et al. (27). The estimate of the portal insulin:glucagon ratio is obtained with the use of insulin secretion and insulin and glucagon concentrations. It is based on estimates of glucagon clearance and portal flow (28) and uses the following formula:

$$\text{GGN/INS} = (\text{INS} + \text{ISR/FH})/[\text{GGN} \times (1 + \text{GGNCL/FH})] \quad (1)$$

INS is plasma insulin concentration (picomoles per liter), GGN is plasma glucagon concentration (picomoles per liter), ISR is insulin secretion calculated by C-peptide deconvolution (pmol/min $\times$ m^2), FH is hepatic plasma flow estimate (calculated as 3.2 L/min $\times$ m^2 cardiac output $\times$ 0.6 plasma fraction $\times$ 0.3 fraction of cardiac output represented by FH = 3.2 $\times$ 0.6 $\times$ 0.3 L/min $\times$ m^2), and GGNCL is the glucagon clearance estimate (0.537 L/min $\times$ m^2).

The acetaminophen rate of appearance (micromoles per minute) was calculated by deconvolution with the use of the 2-exponential model of acetaminophen kinetics (29). With the use of the rate of acetaminophen appearance, the following parameters were calculated: the amount (grams) of acetaminophen that appeared during the OGTT (0–180 min) and the mean time of acetaminophen appearance as the integral of the product of time and acetaminophen appearance. The models give estimates every minute, which is more often than the blood sampling.

Statistical analysis

Sample size calculations were performed to achieve $\geq$80% power based on the historical minimal detectable differences of glycemic responses during an OGTT performed in cohorts of normal compared with impaired glucose tolerance populations (Translational Research Institute, Florida Hospital). The general linear mixed models (PROC MIXED) with subject-level random intercepts were performed to detect treatment and time effects on the target variables. Treatment, time, and treatment-by-time interaction were treated as fixed effects for the variables with time-course measurements, with the mean basal as the covariate. Otherwise, treatment was the main effect with the period (visit order) as the covariate. Three multiple comparisons post hoc tests with Bonferroni correction were conducted: *1*) water followed by glucose without lactisole compared with water followed by glucose with lactisole, *2*) water followed by glucose without lactisole compared with saccharin followed by glucose without lactisole, and *3*) saccharin followed by glucose without lactisole compared with saccharin followed by glucose with lactisole. Data are presented as means $\pm$ SEMs unless otherwise stated. All analyses were performed in SAS version 9.4 (SAS Institute) and with α = 0.05.

RESULTS

Study participants

A total of 14 subjects were recruited. Ten participants completed the study and received all 4 treatments in a randomly assigned order. Four participants were excluded after screening because of dietary compliance or abnormal blood testing (**Supplemental Figure 1**). Subjects were recruited between August 2014 and December 2014. **Table 1** summarizes the baseline clinical characteristics of the participants that completed the study.

TABLE 1
Baseline characteristics of participants[1]

	Value
Total, *n*	
Men	3
Women	7
Age, y	33.5 ± 3.5
Height, cm	169 ± 2.6
Weight, kg	64.4 ± 3.4
BMI, kg/m^2	22.4 ± 0.8
Glucose, mg/dL	86.8 ± 1.7
Triglycerol, mg/dL	85.7 ± 12.0
Total cholesterol, mg/dL	184.4 ± 8.5
HDL, mg/dL	64.4 ± 3.8
LDL, mg/dL	103 ± 8.7
Cholesterol:HDL ratio	3.0 ± 0.3
LDL:HDL ratio	1.7 ± 0.2
VLDL, mg/dL	17.8 ± 2.6
Insulin, mU/L	7.1 ± 1.1
Glycated hemoglobin, %	5.1 ± 0.1

[1] All values are means ± SEMs unless otherwise indicated.

Inhibition of STRs alters plasma insulin responses during the OGTT

There were significant overall treatment effects for insulin ($P = 0.044$) and C-peptide ($P = 0.023$), but no significant treatment-by-time interactions were noted for the assessed variables (**Supplemental Table 1**). Specifically, the addition of lactisole, a human-specific inhibitor of STRs, did not induce statistically significant increases in glucose excursions during the OGTT (**Figure 1**A), but it caused significant increases in plasma insulin (post hoc $P = 0.012$) (Figure 1B) and C-peptide (post hoc $P = 0.004$) (Figure 1C) concentrations. Similar increases were noted in insulin (post hoc $P = 0.020$) and C-peptide (post hoc $P = 0.017$) AUCs (**Table 2**). It is worth noting that the ingestion of the STR agonist saccharin before the glucose load prevented the increases in plasma insulin and C-peptide induced by lactisole (Figure 1E–G). Lactisole with or without the prior ingestion of saccharin did not alter plasma glucagon responses (Figure 1D, H, Table 2). Finally, the ingestion of saccharin before the OGTT did not alter glycemic or insulin responses compared with OGTT alone (**Supplemental Figure 2**, Table 2).

Inhibition of STRs does not alter incretin responses or the estimated rate of gastric emptying and glucose absorption during the OGTT

Lactisole with or without the prior ingestion of saccharin did not alter total GLP-1 (**Figure 2**A, D, Table 2), GLP-2 (Figure 2B, E, Table 2), or GIP (Figure 2C, F, Table 2). The estimated rate of gastric emptying was assessed with the use of plasma concentrations of acetaminophen. The addition of lactisole to the glucose challenge did not alter plasma concentrations or the rate of acetaminophen appearance (**Figure 3**A, B, **Table 3**) regardless of the prior consumption of saccharin (Figure 3D, E, Table 3). The rate of glucose absorption was assessed by measuring plasma 3-*O*-methylglucose (21). None of the treatments altered plasma 3-*O*-methylglucose responses (Figure 3C, F, Table 3). The ingestion of saccharin before the OGTT did not alter incretin responses or the estimated rate of gastric emptying and glucose absorption compared with OGTT alone (Supplemental Figure 2, Table 3).

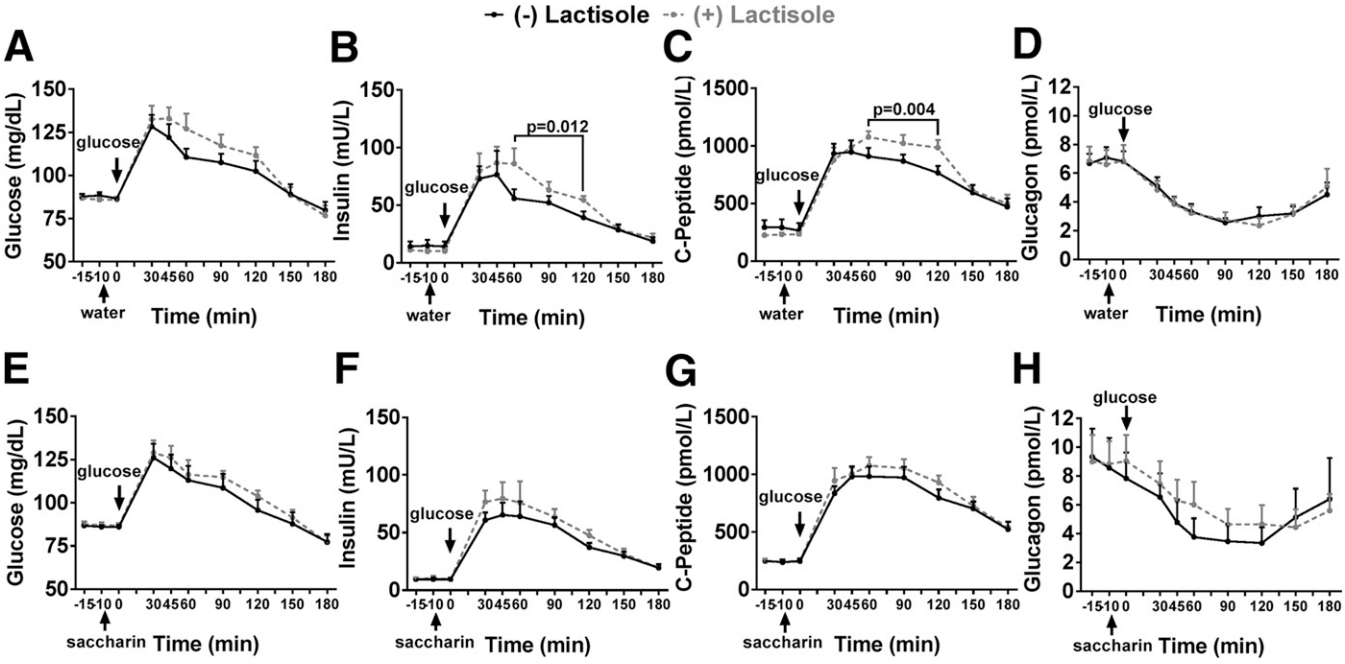

FIGURE 1 Mean + SEM plasma glucose (A and E), insulin (B and F), C-peptide (C and G), and glucagon (D and H) concentrations in 10 healthy lean participants after the oral consumption of either water (A–D) or 18.0 mg saccharin (E–H) 10 min before the ingestion of 75 g (12.5% solution) glucose given at baseline. Solid black lines represent responses in the absence of lactisole; dotted gray lines represent responses in the presence of 50 mg lactisole/dL added to both the water or saccharin and the glucose solution. Overall between-treatment comparisons were performed with the use of the general linear mixed model with subject-level random intercepts and post hoc Bonferroni correction for 3 multiple comparisons. Only significant *P* values are shown.

TABLE 2
Metabolic responses of an OGTT that was preceded by the ingestion of water or saccharin and in the presence or absence of lactisole[1]

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Fixed-effect *P* value
Peak glucose, mg/dL	135 ± 6	142 ± 7	141 ± 6	140 ± 6	0.609
Glucose AUC, mg · dL^{-1} · min^{-1}	18,564 ± 693	19,676 ± 788	19,086 ± 773	19,087 ± 604	0.354
Peak insulin, mU/L	97.7 ± 18.1	100 ± 12.9	98.5 ± 12.8	124 ± 21	0.590
Insulin AUC, mU · L^{-1} · min^{-1}	8131 ± 1015[#]	9914 ± 1008[#]	7922 ± 856	9338 ± 945	0.049
Peak C-peptide, pmol/L	1094 ± 72.3	1142 ± 63.9	1078 ± 74.6	1174 ± 83.4	0.909
C-peptide AUC, pmol · L^{-1} · min^{-1}	483,924 ± 32,055[#]	536,534 ± 28,585[#]	511,473 ± 34,483	534,002 ± 45,239	0.057
Nadir glucagon, pmol/L	2.50 ± 0.53	2.13 ± 0.55	3.34 ± 0.68	4.45 ± 0.59	0.336
Glucagon AUC, pmol · L^{-1} · min^{-1}	694 ± 67.2	690 ± 95.4	886 ± 278	1010 ± 209	0.347
Peak total GLP-1, pmol/L	57.6 ± 5.26	55 ± 4.77	54.9 ± 10.8	64.4 ± 7.21	0.787
Total GLP-1 AUC, pmol · L^{-1} · min^{-1}	22,736 ± 1527	21,734 ± 1226	21,907 ± 2858	24,858 ± 1871	0.632
Peak GLP-2, pmol/L	640 ± 60.5	663 ± 84	713 ± 123	754 ± 76.5	0.668
GLP-2 AUC, pmol · L^{-1} · min^{-1}	274 ± 32.7	276 ± 43.9	292 ± 55.7	314 ± 40.9	0.467
Peak total GIP, pmol/L	116 ± 22	108 ± 15	102 ± 18	109 ± 14	0.656
Total GIP AUC, pmol · L^{-1} · min^{-1}	14,686 ± 2766	13,017 ± 1701	12,757 ± 1983	13,672 ± 1758	0.542

[1] All values are means ± SEMs. $n = 10$. Treatment 1: water followed by glucose; treatment 2: water with lactisole followed by glucose with lactisole; treatment 3: saccharin followed by glucose; treatment 4: saccharin with lactisole followed by glucose with lactisole. Overall between-treatment fixed-effect *P* values were obtained with the use of a general linear mixed model with subject-level random intercepts. Post hoc analysis with Bonferroni's correction was performed to adjust for multiple testing. [#]Significant differences between corresponding treatments. GIP, gastric inhibitory peptide; GLP, glucagon-like peptide; OGTT, oral-glucose-tolerance test.

Inhibition of STRs does not alter β-cell function but reduces an index of insulin sensitivity during the OGTT

The pharmacologic inhibition of STRs with lactisole induced slight increases in the insulin secretory rate (post hoc $P = 0.020$) (**Figure 4**A) during the OGTT, whereas the consumption of saccharin before the OGTT abolished the effects of lactisole (Figure 4E). However, lactisole with or without the prior consumption of saccharin did not alter β-cell function assessed by the insulinogenic index (Table 3), β-cell glucose-stimulated insulin dose response (Figure 4B, F), and β-cell potentiation of insulin secretion (Figure 4C, G). Similarly, no significant differences in the estimated portal insulin:glucagon ratio responses were noted (Figure 4D, H). Interestingly, the addition of lactisole decreased the Stumvoll's insulin sensitivity index during the OGTT with (post hoc $P = 0.015$) or without (post hoc $P = 0.025$) the prior consumption of saccharin (Table 3). Finally, the ingestion of saccharin before the OGTT did not alter indexes of β-cell function and insulin sensitivity compared with OGTT alone (Supplemental Figure 2, Table 3).

DISCUSSION

The major findings of our study show that acute pharmacologic inhibition of STRs in the gastrointestinal tract alters insulin response to an OGTT in lean healthy adults who are not regular consumers of NCASs. These data demonstrate for the first time to our knowledge that chemosensory input derived from STRs on the tongue and in the intestine regulates acute metabolic responses to oral ingestion of glucose in humans.

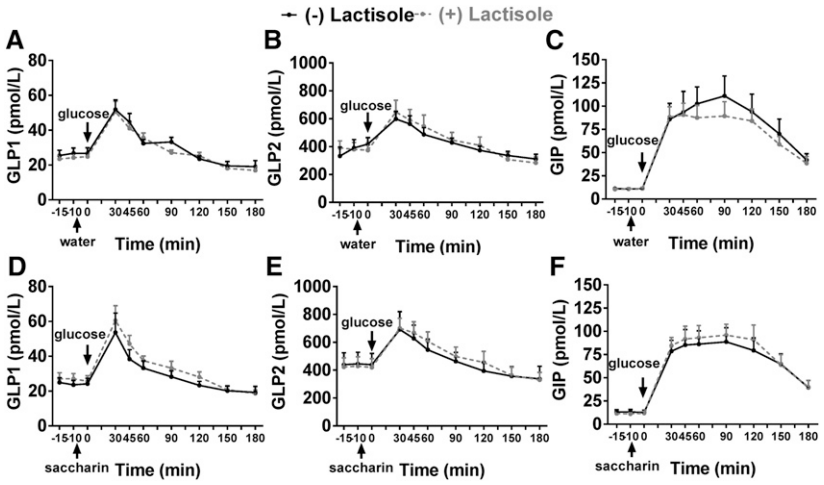

FIGURE 2 Mean + SEM plasma total GLP-1 (A and D), GLP-2 (B and E), and GIP (C and F) concentrations in 10 healthy lean participants after the oral consumption of either water (A–C) or 18.0 mg saccharin (D–F) 10 min before the ingestion of 75 g (12.5% solution) glucose given at baseline. Solid black lines represent responses in the absence of lactisole; dotted gray lines represent responses in the presence of 50 mg lactisole/dL added to both the water or saccharin and the glucose solution. Overall between-treatment comparisons were performed with the use of the general linear mixed model with subject-level random intercepts and post hoc Bonferroni correction for 3 multiple comparisons. No statistically significant differences were found. GIP, gastric inhibitory peptide; GLP, glucagon-like peptide.

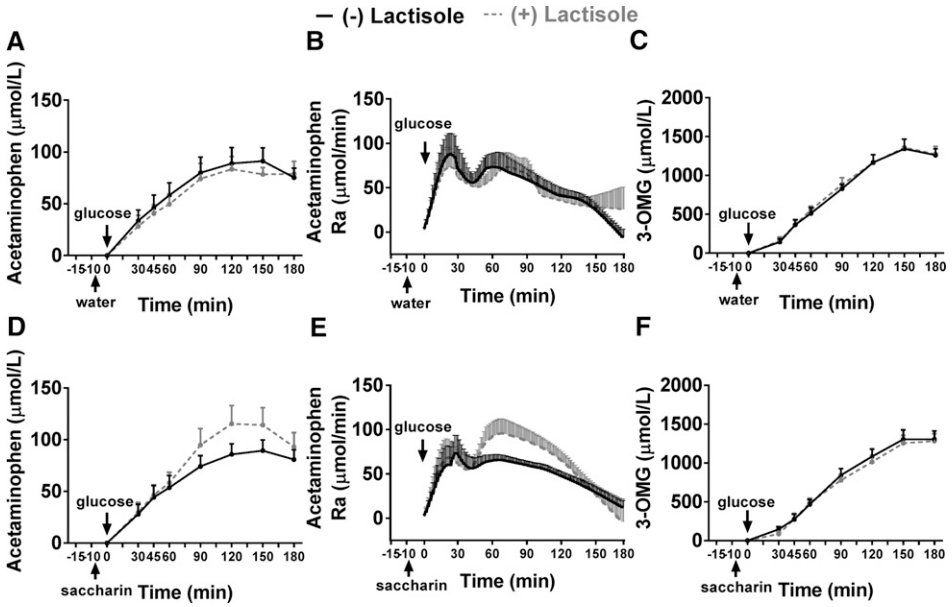

FIGURE 3 Mean + SEM plasma acetaminophen (A and D), acetaminophen Ra (B and E) based on modeling analysis of acetaminophen concentrations, and plasma 3-OMG (C and F) concentrations in 10 healthy lean participants after the oral consumption of either water (A–C) or 18.0 mg saccharin (D–F) 10 min before the ingestion of 75 g (12.5% solution) glucose given at baseline. Solid black lines represent responses in the absence of lactisole; dotted gray lines represent responses in the presence of 50 mg lactisole/dL added to both the water or saccharin and the glucose solution. Overall between-treatment comparisons were performed with the use of the general linear mixed model with subject-level random intercepts and post hoc Bonferroni correction for 3 multiple comparisons. No statistically significant differences were found. Ra, rate of appearance; 3-OMG, 3-*O*-methylglucose.

The inhibition of STRs with lactisole increased plasma insulin and C-peptide in response to the oral glucose load and marginally elevated glucose responses, although the responses were not statistically significant. These effects mirrored the increase in the insulin secretion assessed by the modeling analysis of the OGTT. However, the augmented insulin secretion occurred independent of plasma GLP-1 and GIP responses. In fact, the intragastric infusion of glucose plus lactisole has been shown to attenuate plasma concentrations of GLP-1 compared with glucose alone (30). GLP-1 can also reduce gastric emptying via gut-brain mechanisms (31, 32), but consistent with previous findings (30) we did not observe any differences in gastric emptying assessed by modeling plasma acetaminophen appearance. Orally administrated acetaminophen is rapidly absorbed by the small intestine but not the stomach. Thus, gastric emptying is the rate-limiting step for the rate of appearance of acetaminophen in the blood (33). Animal studies have also suggested that intestinal STRs may regulate glucose absorption (6, 8) via mechanisms that include local regulatory effects of GLP-2 (34), a gut peptide that is co-secreted with GLP-1 by L cells in an equimolar amount (35). However, STR inhibition did not alter plasma GLP-2 concentrations or the glucose absorption indirectly assessed by measuring plasma excursions of 3-*O*-methylglucose. To further test the involvement of STRs, we used NCASs that are bona fide agonists and have been shown to potentiate hormonal responses to a glucose load (14–16). Specifically, we investigated whether the prior ingestion of

TABLE 3
Model analysis of an OGTT that was preceded by the ingestion of water or saccharin and in the presence or absence of lactisole[1]

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Fixed-effect *P* value
Acetaminophen AUC, μmol · L^{-1} · min^{-1}	5554 ± 782	5309 ± 862	5746 ± 1089	6654 ± 1679	0.616
Appearance of acetaminophen from 0 to 180 min, g	1.30 ± 0.16	1.25 ± 0.14	1.30 ± 0.16	1.60 ± 0.22	0.354
Acetaminophen mean appearance time, min	78.4 ± 5.2	82.1 ± 7.5	82.5 ± 3.9	83.5 ± 4.2	0.864
3-OMG AUC, μmol · L^{-1} · min^{-1}	88,828 ± 9332	88,071 ± 10,094	81,479 ± 11346	73,953 ± 12,667	0.069
Insulinogenic index	26.1 ± 1.55	27.4 ± 1.18	27.1 ± 1.74	28.0 ± 2.24	0.421
Glucose sensitivity, pmol/m^2 × mmol/L	43.0 ± 3.47	60.8 ± 16.65	49.3 ± 6.45	49.8 ± 7.90	0.612
Insulin secretion rate at 7.0 mM, pmol/min × m^2	112 ± 18.7	173 ± 43.3	108 ± 14.6	111 ± 15.0	0.716
Potentiation factor ratio	1.18 ± 0.10	1.25 ± 0.15	1.22 ± 0.10	1.32 ± 0.12	0.827
Rate sensitivity, pmol/m^2 × mmol/L	587 ± 112	646 ± 173	275 ± 107	744 ± 138	0.108
Insulin sensitivity, μmol/min × kg	9.89 ± 0.35$^{\#}$	9.30 ± 0.34$^{\#}$	9.93 ± 0.30$^{\$}$	9.52 ± 0.33$^{\$}$	0.003
Insulin clearance, L/min × m^2	0.44 ± 0.05	0.38 ± 0.04	0.46 ± 0.04	0.40 ± 0.04	0.113

[1] All values are means ± SEMs. n = 10. Treatment 1: water followed by glucose; treatment 2: water with lactisole followed by glucose with lactisole; treatment 3: saccharin followed by glucose; treatment 4: saccharin with lactisole followed by glucose with lactisole. Overall between-treatment fixed-effect *P* values were obtained with the use of a general linear mixed model with subject-level random intercepts. Post hoc analysis with Bonferroni's correction was performed to adjust for multiple testing. $^{\#,\$}$Significant differences between corresponding treatments. OGTT, oral-glucose-tolerance test; 3-OMG, 3-*O*-methylglucose.

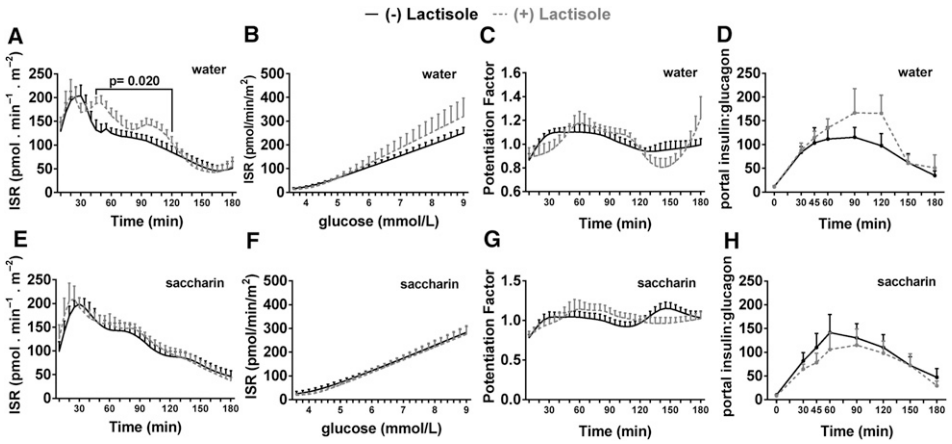

FIGURE 4 Mean + SEM insulin secretory rate based on time (A and E) and dose (B and F) responses, β-cell potentiation factor (C and G), and estimated portal insulin:glucagon ratio (D and H) in 10 healthy lean participants after the oral consumption of either water (A–D) or 18.0 mg saccharin (E–H) 10 min before the ingestion of 75 g (12.5% solution) glucose given at baseline. Solid black lines represent responses in the absence of lactisole; dotted gray lines represent responses in the presence of 50 mg lactisole/dL added to both the water or saccharin and the glucose solution. Overall between-treatment comparisons were performed with the use of the general linear mixed model with subject-level random intercepts and post hoc Bonferroni correction for 3 multiple comparisons. Only significant *P* values are shown. ISR, insulin secretory rate.

saccharin can alter glucose homeostasis in response to an OGTT and whether it can antagonize the effects of lactisole.

The acute effects of saccharin consumption on an OGTT have not been studied in humans to our knowledge (36). The ingestion of saccharin (18.0 mg/60 mL) before the oral glucose load did not affect any of the measured variables compared with oral glucose alone. This is consistent with some (10, 12, 13, 37) but not all studies (14–16) that have examined the effects of other NCASs, although results are variable even in studies showing a potentiating effect. For instance, diet soda (sucralose and acesulfame potassium) augmented plasma GLP-1 in healthy adults without affecting plasma insulin or glucose responses (15, 16). In contrast, the ingestion of sucralose (48.0 mg/60 mL) had no effect on GLP-1 responses in obese participants but increased plasma glucose, insulin, and C-peptide (14). Several factors could have contributed to these discrepancies, including the type and dose of the NCAS used or the characteristics of the studied population. Nevertheless, the ingestion of saccharin before the OGTT attenuated the effects of lactisole on glucose homeostasis. Saccharin is a potent STR ligand that, although it did not exhibit observable effects when consumed alone, may have antagonized the inhibitory effects of lactisole on STRs. These findings were not entirely unexpected because we deliberately considered pharmacokinetic relations between STR agonists (i.e., glucose and saccharin) and antagonists (i.e., lactisole) (18). We administered saccharin immediately before the glucose load to prestimulate STRs, whereas the addition of lactisole at the selected concentration completely eliminated the sweet sensation of both the saccharin and glucose solution.

Taken together, our findings corroborate the involvement of STRs in the regulation of postprandial glucose metabolism in humans. However, marginal changes in glycemic control induced by the acute inhibition of STRs in the gastrointestinal tract were not mediated by changes in incretin secretion, gastric emptying (estimated by acetaminophen appearance), or glucose absorption (estimated by 3-*O*-methylglucose appearance). It is therefore plausible that these effects are mediated by secondary mechanisms that require STR-derived chemosensory input. The intragastric administration of lactisole significantly reduced the glucose-stimulated secretion of GLP-1 and peptide YY, leading to increased plasma glucose and insulin concentrations (38). In our study, the oral delivery of lactisole induced similar insulinemic responses but in the absence of incretin effects, suggesting alternative mechanisms to explain our observations. Tasting of the sweet solutions could have triggered a cephalic phase of insulin secretion within 2–10 min of the taste stimulus (39), but we observed differences in insulin secretion between 60 and 120 min of the exposure. In agreement, β-cell rate sensitivity during the OGTT, which is a variable that describes first-phase insulin secretion, was not altered with the addition of lactisole. Another possibility is that, upon absorption, circulating lactisole could have directly targeted STRs on pancreatic β-cells, but this would have instead inhibited insulin secretion (4). We did not measure plasma concentrations of lactisole but, based on the selected oral dose and published pharmacokinetic data, lactisole could not have reached effective plasma concentrations to directly inhibit STRs in peripheral tissues (18, 40–42). To shed further light on the potential mechanisms, we implemented a modeling analysis of β-cell function. However, glucose sensitivity, which is the slope of the β-cell dose response, was not altered by lactisole. Similarly, no differences were observed in the insulinogenic index and insulin secretory rate (both of which assess insulin secretion in relation to glucose) at a fixed amount of glucose or in the potentiation factor, which is a variable that reflects the potentiation of insulin secretion caused by the incretins and other factors.

Finally, the augmented plasma insulin responses during STR inhibition may be an indirect manifestation of the gut-brain axis dysregulation. For instance, the jejunal infusion of glucose reduces hepatic glucose production (HGP) via vagal afferents (43). Similarly, GLP-1 infusion into the hepatic portal vein increases vagal nerve activity and enhances glucose disposal (44). GLP-2 also activates vagal afferent pathways (45), and the GLP-2 receptor in pro-opiomelanocortin C neurons is critical for suppressing HGP (46). Thus, STR-mediated chemosensory mechanisms in the gut may regulate glycemia in response to the

ingestion of glucose by indirect effects in the brain aiming to regulate peripheral glucose turnover. Therefore, suppressing this STR-mediated glucosensory mechanism with lactisole may increase HGP and/or reduce glucose disposal, leading to mild transient hyperglycemia and compensatory insulin hypersecretion. However, the addition of lactisole did not change plasma glucagon concentrations or the estimated portal insulin:glucagon ratio, likely excluding disturbances in HGP mediated by glucagon. In contrast, the presence of lactisole decreased Stumvoll's insulin sensitivity index independent of prior saccharin ingestion, suggesting a mild transient state of insulin resistance (hepatic or peripheral). This is also consistent with observations in patients with type 2 diabetes whose intestinal STR mRNA concentrations were inversely correlated with fasting glycemia (3). This intriguing hypothesis requires further investigation considering the relatively small sample size of our study, indirect assessment of insulin sensitivity, and potential unknown ectopic effects of lactisole.

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The authors' responsibilities were as follows—EKA and KRS: conducted the research; EKA, KRS, TFO, REP, and GAK: analyzed and interpreted the data; FY: performed the statistical analysis; RB: performed the acetaminophen modeling analysis; AM: performed the β-cell and insulin-sensitivity modeling analysis; REP and GAK: designed the research; GAK: conceived the study, wrote the manuscript, and had primary responsibility for final content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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