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Experience with the high-intensity sweetener saccharin impairs glucose homeostasis and GLP-1 release in rats

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Abstract

Previous work from our lab has demonstrated that experience with high-intensity sweeteners in rats leads to increased food intake, body weight gain and adiposity, along with diminished caloric compensation and decreased thermic effect of food. These changes may occur as a result of interfering with learned relations between the sweet taste of food and the caloric or nutritive consequences of consuming those foods. The present experiments determined whether experience with the high-intensity sweetener saccharin versus the caloric sweetener glucose affected blood glucose homeostasis. The results demonstrated that during oral glucose tolerance tests, blood glucose levels were more elevated in animals that had previously consumed the saccharin-sweetened supplements. In contrast, during glucose tolerance tests when a glucose solution was delivered directly into the stomach, no differences in blood glucose levels between the groups were observed. Differences in oral glucose tolerance responses were not accompanied by differences in insulin release; insulin release was similar in animals previously exposed to saccharin and those previously exposed to glucose. However, release of GLP-1 in response to an oral glucose tolerance test, but not to glucose tolerance tests delivered by gavage, was significantly lower in saccharin-exposed animals compared to glucose-exposed animals. Differences in both blood glucose and GLP-1 release in saccharin animals were rapid and transient, and suggest that one mechanism by which exposure to high-intensity sweeteners that interfere with a predictive relation between sweet tastes and calories may impair energy balance is by suppressing GLP-1 release, which could alter glucose homeostasis and reduce satiety.

Keywords

Energy balance; glucose tolerance; artificial sweetener; learning

1. Introduction

Previous data from our lab, and others, indicates that sweet and fatty orosensory stimuli that do not reliably predict the post-ingestive energetic consequences of consumption can impair the ability of rats to regulate both short-term and longer-term food intake and body weight. For example, rats given dietary supplements with the high-intensity sweetener saccharin, which provides a strong sweet taste, but does not deliver calories, exhibit poorer caloric compensation for novel sweet-tasting pre-meals in short-term intake tests [1, 2]. Further, over the long-term, consumption of a saccharin-sweetened yogurt supplements resulted in

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increased energy intake, increased body weight gain and increased adiposity relative to consumption of the same supplements sweetened with glucose [3–5].

We have suggested that mechanisms that underlie the dysregulation of energy balance when consuming high-intensity sweeteners may involve reducing the validity of a predictive relationship between sweet taste and the delivery of energy or calories. Based on fundamental principles of Pavlovian conditioning, presentation of a cue without its anticipated consequence will weaken the ability of the cue to evoke the conditioned responses [6–8]. In the case of sweet tastes, high-intensity sweeteners provide a strong sweet cue, but without the delivery of an anticipated energetic or caloric outcome. This would be expected to weaken the validity of the sweet taste→calorie relationship. To the extent that the ability to anticipate the caloric consequences of intake contributes to energy, and ultimately body weight regulation, animals may both overeat and gain excess body weight when sweet tastes that do provide calories are encountered. Consistent with this hypothesis, we have recently demonstrated that consumption of non-caloric sweeteners selectively impairs the ability of rats to regulate intake and weight gain when they are maintained on a sweetened high-calorie diet [9]. We have recently extended the generality of this account by showing that energy balance can also be disrupted by exposure to fatty-tasting foods manufactured with fat substitutes that mimic the sensory properties of fat, but without calories [10]. Similar to effects seen with sweet tastes, positive energy balance is the result of the selective overconsumption fatty foods that do deliver energy.

While food intake, weight gain and adiposity are increased by exposure to such substitutes, the specific physiological mechanisms that contribute to overconsumption and impaired energy balance have not been specified. One potential mechanism is interference with physiological responses that anticipate the arrival of energy and nutrients upon consumption of foods. The role of experience with environmental cues in modulating such physiological responses have been the focus of study since the foundational work of Pavlov [11] and these “cephalic phase” responses have been documented in both pre-clinical rodent studies, as well as clinical studies in humans. Cephalic phase responses, such as the release of insulin, are thought to reflect fractional components of physiological responses recruited to efficiently metabolize ingested foods [11–15]. They may also serve to modulate meal size by generating satiety signals. The strength of such responses should be determined, at least in part, by the validity of the predictive relationship between taste cues and their consequences. Accordingly, the magnitude of cephalic phase responses should be weakened to the extent that exposure to taste cues that are not followed by energetic outcomes weakens the validity of the normal sweet taste→calorie predictive relationship.

Previous studies show that consuming noncaloric sweeteners can evoke cephalic phase responses in rats (e.g. [14, 16–19]). However, whether the magnitude of these responses is reduced following extended experience with these sweeteners is presently unknown. Thus, the goal of the present studies was to determine whether experience with sweet tastes that do not predict energetic outcomes alters cephalic phase responses as indexed by the ability of rats to modulate blood glucose levels in response to sweet-tasting, high calorie foods. The hypothesis was that compared to animals given diets in which sweet taste always predicted the delivery of increased calories and glucose, animals given diets in which sweet taste did not predict calories (using saccharin) would show a hyperglycemic response to a sweet-tasting caloric load. In contrast, glycemic responses to sweet-tasting, caloric diets which bypass the oral cavity, thereby precluding cephalic phases reflexes evoked by oral taste, were expected to be similar between groups. In a series of experiments, we examined the effects of exposure to yogurt diet supplements (Experiments 1–4, 6) and solutions (Experiment 5) on glycemic responses to a novel, sweet-tasting test meal (Experiment 1), or a glucose solution (Experiments 2–6) to determine whether experience consuming sweet

tastes that were not associated with calories or sugar resulted in alterations in blood glucose homeostasis. In addition, we examined whether levels of the peptide hormones insulin and GLP-1 were altered following experience with saccharin-sweetened diet supplements compared to glucose-sweetened diet supplements since release of both insulin and GLP-1 are critical in regulation of glucose homeostasis, and have been implicated in satiety and modulation of meal size (e.g. [20–32]).

2. Methods and Materials

2.1 General Procedures

2.1.1 Subjects—All animals were adult male Sprague-Dawley rats (Harlan, Indianapolis, IN). Animals were housed individually in the laboratory and given ad lib access to water and laboratory chow (Harlan 2018) for at least one week prior to being assigned to groups matched on body weight.

2.1.2 Diets and dietary supplements—In Experiments 1–4, animals were given access to 30 g yogurt (Dannon Lowfat yogurt; ~ 0.6 kcal/g) daily as a dietary supplement. Yogurt supplements were provided in plain, unsweetened form 3 days per week and in sweetened form on the remaining 3 days of the week. For one group, yogurt was sweetened with 20% (w/w) glucose. For the second group, yogurt was sweetened with 0.3% (w/w) saccharin. A single day of chow alone was provided per week. The order of yogurt presentation was semi-randomized so that animals did not receive plain or sweetened yogurt on more than 2 consecutive days per week. Yogurts were available for 3 hr per day in Experiment 1 and 24 hr per day in Experiments 2–4. Animals were maintained on standard laboratory chow (Harlan 2018) throughout Experiment 1–4.

In Experiments 5 and 6, the goal was to examine the consequences of diets that may more closely mimic current patterns of food intake in humans in the US. Thus, in place of a relatively low-calorie, low fat chow diet, animals were placed onto a high-fat, high-energy, sweetened chow diet which provided approximately 35% of calories from fat and 50% of calories from carbohydrate. In Experiment 5, sweeteners were provided as solutions, since beverages provide a major source of high-intensity sweeteners in the human population; rats received daily access to a bottle containing 30 g of a sweetened solution in addition to ad lib sweetened high-fat diet and water. For one group, the solution was 10% glucose and for the second group the solution was 0.3% saccharin. Solutions were available for 24 hr per day. In Experiment 6, plain and sweetened yogurt diets were provided to animals maintained on the high-fat, sweetened diet used in Experiment 5. Yogurt, chow and water were available for 24 hr per day.

2.1.3 Blood sampling and analysis—In Experiments 1 and 3–6, blood samples were taken from the tail vein. In Experiment 2, blood samples were collected from indwelling jugular cannulas. Glucose levels were determined immediately upon collection using a handheld glucometer (Bayer Contour). The remaining samples were then centrifuged, serum separated and frozen. Insulin (Experiments 2–4, 6) and total GLP-1 (Experiments 5 and 6) were assayed in duplicate using commercial ELISA kits (Crystal Chem, Inc. and Millipore, respectively).

2.1.4 Glucose tolerance tests—In all experiments, blood glucose testing occurred following an overnight fast. In Experiments 2–6, glucose tolerance tests were conducted using 20% (w/w) glucose solutions. In Experiment 2, the volume provided was 10 ml. Because solutions were delivered by gavage in Experiments 3–5, the volume was reduced to 5 ml, and the same volume of 5 ml was used in Experiment 6. In Experiment 1, glycemic

responses to oral consumption of 5 ml of a novel, sweet-tasting test meal were assessed. The test meal was Chocolate Ensure Plus thickened with 2% guar; we chose this meal because previous studies in the lab with this test meal had shown that animals given exposure to saccharin-sweetened yogurts evidence both reduced caloric compensation and a reduced thermic effect of food compared to animals given exposure to glucose-sweetened yogurts [2]. The test meal had a caloric density of approximately 1.4 kcal/g, with 57, 14.8 and 28.2 % of calories provided by carbohydrate, protein and fat, respectively; the sugar content was approximately 9% by weight.

2.1.5 Data analysis—Because the experiments were designed to assess the impact of experience consuming sweetened diets that were either associated with increased calories (glucose groups) or not associated with increased calories (saccharin groups) on glycemic responses, animals that failed to consume a minimum amount (at least 70%) of the dietary supplements during training were excluded from analysis. In addition, animals were excluded from analysis if they failed to consume at least 90% of the meal or glucose solution provided during testing; the duration of access to the meal and solution in Experiments 1 and 2 was 30 minutes. In Experiment 3, the solution was provided for 15 minutes, while in Experiments 4, 5 and 6, only animals that had consumed 90% of the glucose solution within 8 minutes were included. Animals were also excluded if difficulties with the gavage procedure were encountered. Finally, in Experiment 2, animals in which blood samples could not reliably be withdrawn by hand from the jugular catheters were not included. Body weight gain in each Experiment was analyzed with two-way (Day X Sweetener) repeated-measures ANOVAs, with day as a within-subjects factor and Sweetener group as a between subjects factor with post-hoc LSD tests conducted across groups on each day as indicated. Glycemic responses (glucose, insulin and GLP-1) were analyzed with separate three-way (Time X Method of delivery X Sweetener) repeated-measures ANOVAs with Time and Method of delivery as within-subjects factors and Sweetener as a between-subjects factor with post-hoc LSD tests conducted as indicated. Total caloric intake was analyzed with a two-way (Day X Sweetener) repeated measures ANOVA, with Day as a within-subjects factor and Sweetener as a between-subjects factor. Changes in body composition were analyzed separately for lean mass and fat mass using One-Way ANCOVAs (Sweetener) with starting lean mass or starting fat mass as the covariate, respectively. A $p < 0.05$ was taken as significant for all tests.

2.2. Experiment 1: Effects of prior exposure to saccharin versus glucose on glycemic responses to a novel sweet-tasting meal

2.2.1 Subjects—Subjects were 38 adult male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing approximately 350 – 375 g upon arrival who were given ad lib access to water and laboratory chow for one week prior to being assigned to one of two groups saccharin or glucose, matched on body weight and morning tail blood glucose levels.

2.2.2 Training and testing—Yogurt diets were available for 3 hr daily, for 14 days. Glycemic testing occurred after the end of 14-day yogurt exposure. All animals were tested twice, once while consuming 5 g of a novel, sweet-tasting test meal and once while no test meal was provided to control for potential circadian entrainment due to the fixed timing of presentation of the yogurt diets [33–35]. Animals were food deprived overnight and 3 baseline tail blood glucose samples were collected prior to the introduction of an enamel cup into the home cage. Tail blood glucose was measured every 15 min following presentation of the cup. To avoid possible effects of the odor of the test meal on glycemic responses during the no-test meal condition, animals were tested in two separate cohorts. For the first cohort, the cup presented during the first test was empty. For the second cohort, the cup presented during the first test contained the 5 g test meal test. Following the first test, ad lib

chow and water were provided for 5 days and animals were given two additional days exposure to their assigned sweetened yogurt before receiving a second test with the test meal conditions reversed. After excluding animals as described in section 2.1.5, final sample sizes were 16 animals in the glucose group and 15 animals in the saccharin group.

2.3 Experiment 2: Effects of prior exposure to saccharin versus glucose on glycemic responses to oral glucose intake

2.3.1 Subjects—Twenty adult male Sprague-Dawley rats weighing 275–300 g were received from Harlan (Indianapolis) and were given ad lib access to Harlan 2018 Chow and water for one week prior to being assigned to one of two groups matched on body weight.

2.3.2 Training—Rats in both groups were placed into plastic tub cages and given daily access to an enamel camping cup containing 30 g lowfat yogurt (Dannon, ~0.6 kcal/g) as a diet supplement for a total of 20 days.

2.3.3 Surgery—Following the 20 days of yogurt exposure, indwelling jugular catheters were implanted for collection of blood samples during oral glucose tolerance tests. Animals were anesthetized with isoflurane, and catheters (CBAS heparin-coated PU Catheters 3Fr; Instech) were inserted into the jugular vein and fitted to vascular access harnesses (Instech). Butorphanol tartrate was given subcutaneously for pain management for 3 days following surgery; Baytril was given prophylactically for two days following surgery.

2.3.4 Testing—To accommodate animals to the blood sampling procedure, after 5 days recovery, animals were moved in their home cages to the testing room where they were connected to automated blood sampling machines (ABS; Instech) and given 15 g of their assigned sweetened yogurt to consume while blood samples were collected from the jugular catheters. Two days later, animals were food deprived overnight and then attached to the ABS machines in their home cages in a testing room. After the collection of 3 baseline blood samples, 10 ml of a 20% glucose solution was provided in the same type of enamel cup previously used to deliver yogurt. Blood samples were collected at 4, 8, 12, 16, 20, 30, 48, 64, 96 and 120 minutes after the glucose solution was presented. After excluding animals as described in section 2.1.5, blood glucose samples were obtained from 5 animals in each sweetener group. Values for missing samples in this experiment (6 of the 140 samples) were estimated by averaging values from samples immediately prior to and immediately following the missing sample. Area under the curve for glucose and insulin were analyzed using separate one-way ANOVAs.

2.4 Experiment 3: Effects of prior exposure to saccharin versus glucose on glycemic responses to oral glucose intake versus glucose delivered by gavage

2.4.1 Subjects—Sixteen adult male Sprague-Dawley rats weighing 275–300 g were received from Harlan (Indianapolis) and were given ad lib access to Harlan 2018 Chow and water for one week prior to being assigned to one of two groups matched on body weight and being given access to 30 g yogurt as in Experiment 2.

2.4.2 Training and testing—Following 14 days of yogurt exposure, all animals were food deprived overnight, and then 2 baseline blood samples were taken from the tail vein and blood glucose was measured immediately with a handheld glucometer. Following the second baseline sample, half of the animals in each group were allowed to consume 5 ml of a 20% glucose solution provided in the same enamel cups used to provide yogurt. The remaining half of the animals in each group received 5 ml of 20% glucose solution delivered by intragastric gavage. To avoid animals receiving a taste of the glucose solution during gavage, gavage tubes were rinsed with deionized water. Blood glucose was measured every

15 min following introduction of the glucose solution for 90 minutes. Animals were then given food back and two days later were again food deprived and were given a second test with the gavage and oral glucose conditions reversed. After excluding animals as described in in section 2.1.5, blood glucose samples were obtained from 6 animals in the glucose group and 7 animals in the saccharin group.

2.5 Experiment 4: Effects of prior exposure to saccharin versus glucose on glycemic and insulin responses to oral glucose intake, glucose delivered by gavage and glucose delivered by gavage with oral stimulation

2.5.1 Subjects—Twenty-two adult male Sprague-Dawley rats weighing 275–300 g were received from Harlan (Indianapolis) and were given ad lib access to Harlan 2018 Chow and water for ten days prior to being assigned to one of two groups matched on body weight.

2.5.2 Training and Testing—Rats were given daily access to 30 g lowfat yogurt as a diet supplement 6 days per week for 24 days, with 1 day of chow and water alone every 6 days as in Experiments 1 and 2. Following training, all animals were food deprived overnight, and a baseline blood sample was taken from the tail vein. Following the baseline sample, one third of the animals in each group were given access to 5 ml of a 20% glucose solution provided in the same enamel cups used to provide yogurt (Oral condition). Another third of the animals in each group received 5 ml of 20% glucose solution delivered by intragastric gavage with care taken to avoid stimulation of the oral cavity by glucose (Gavage condition). For the final third of the animals in each group, the tongue was bathed with approximately 0.5 ml of the 20% glucose solution, and then the remaining 4.5 ml glucose solution was delivered into the stomach by gavage (Taste Gavage). Samples were collected 8, 16 and 32 minutes following presentation of the glucose solutions. Food was then returned to all animals for four days; on two of those days, animals also received 30 g of their assigned sweetened yogurts. Animals were food deprived again, and given one of the other two types of glucose tolerance tests. Food was then returned for four days, with two additional exposures to sweetened yogurt, and animals were then given a final glucose tolerance test using the third method of delivery. The order of testing was counterbalanced across animals. Blood glucose samples were obtained at all time points and all methods from 8 animals in the glucose group and 7 animals in the saccharin after excluding animals as described in section 2.1.5.

2.6 Experiment 5: Effects of exposure to glucose and saccharin solutions on glucose, insulin and GLP-1 levels in animals fed a high-fat, sweetened diet

2.6.1 Subjects—Thirty adult male Sprague-Dawley rats weighing 325 – 350 g were received from Harlan (Indianapolis) and were given ad lib access to Harlan 2018 Chow and water for twelve days in the lab prior to being assigned to one of two groups matched on body weight.

2.6.2 Training and Testing—Animals in each group then received daily access to a bottle containing 30 g of either a 10% glucose-sweetened solution or a 0.3% saccharin-sweetened solution in addition to a powdered high fat, high-sugar maintenance diet (Purina Test Diet #25312 modified with 20% glucose by weight) and water for 24 days. Chow intake and intake from the sweetened liquid bottle were measured daily by weighing, corrected for spillage. Chow spillage was excessive and precluded correction in 1 animal in each of the final groups, and these animals were excluded from intake analysis. Following training, animals were food-deprived overnight, and given a glucose tolerance test in which 5 g of a 20% glucose solution was delivered in a cup or by gavage as in Experiment 3. Half of the animals in each group received glucose by each method during the first test. Blood samples were taken from the tail vein at baseline, and 8, 16, and 32 min following delivery

of the glucose. Animals then received an additional 6 days exposure to their assigned solutions, followed by an overnight fast and a second glucose tolerance test with the method of delivery reversed. After excluding animals as described in the General Methods, blood glucose and GLP-1 data were obtained from 8 animals in the glucose group and 8 animals in the saccharin group were obtained at all time points following both oral consumption and gavage of the glucose solution.

2.7 Experiment 6: Effects of exposure to glucose- or saccharin-sweetened yogurt supplements on glucose, insulin and GLP-1 levels in animals fed a high-fat, sweetened diet

2.6.1 Subjects—Seventy-two adult male Sprague-Dawley rats weighing 350 – 375 g were received from Harlan (Indianapolis) and were given ad lib access to Harlan 2018 Chow and water for 5 days in the lab and then were given access to a powdered, high-fat unsweetened diet (Purina Test Diet #25312) for one week prior to being assigned to one of two groups matched on body weight. Body composition was assessed at this time using NMR (Echo-NMR).

2.6.2 Training and Testing—Animals in each group then received daily access to 30 g of plain or sweetened yogurt in addition to the same powdered high fat, high-sugar maintenance diet used in Experiment 5 and water ad lib for 24 days. Half of the animals received saccharin-sweetened yogurt, while the remaining half received glucose-sweetened yogurt. Yogurt diets were provided 6 days per week for 4 weeks, with a single day of chow and water alone per week. Chow and yogurt intake were measured daily by weighing, corrected for spillage. Following training, body composition was again assessed with NMR. Animals were then food-deprived overnight, and given an oral glucose tolerance test in which 5 g of a 20% glucose solution was delivered in a cup. Blood samples were taken from the tail vein at baseline, and 8, 16, and 32 min following delivery of the glucose. Animals that failed to consume at least 90% of the glucose solution within 8 minutes during the test the received an additional 2 days exposure to their assigned sweetened yogurt, were fasted overnight and then a given a second test in which 5 g of a 20% glucose solution was delivered in a cup and blood samples were taken from the tail vein at baseline, and 8, 16 and 32 min following delivery of the glucose. After excluding animals as described in the General Methods, blood glucose, insulin and GLP-1 data were obtained from 23 animals in both the glucose and saccharin groups.

3. Results

3.1 Experiment 1

3.1.1 Body Weight—Starting body weight did not differ between the two groups (means $\pm$ SEM = 390.0 ± 3.5 and 391.8 ± 3.6 for glucose and saccharin groups, respectively). Body weight gain was significantly affected by both the day of yogurt exposure and the Sweetener group (Main effect of Sweetener, $F_{1, 29} = 7.38$, $p = 0.011$; Main effect of Day, $F_{12, 348} = 343.8$, $p < 0.0000001$; Sweetener X Day interaction, $F_{12, 348} = 4.47$, $p = 0.000001$; Figure 1). Animals given the saccharin-sweetened yogurt gained significantly more weight than animals given the glucose-sweetened yogurt beginning on day 7.

3.1.2 Blood glucose—Analysis of blood glucose responses indicated that blood glucose was significantly higher on the day when the animals consumed a test meal compared to the day when no test meal was provided. Main effect of Test meal (Figure 2; $F_{1, 29} = 320.21$, $p < 0.0000001$; Main effect of Time, $F_{9, 261} = 39.481$, $p < 0.0000001$; Test meal X Time interaction, $F_{9, 261} = 67.42$, $p < 0.0000001$). Post-hoc comparisons revealed that animals previously given the saccharin-sweetened yogurt showed significantly higher blood glucose

levels 15 min following the introduction of the test meal compared to animals previously given the glucose-sweetened yogurt ($F_{1, 29} = 6.53$, $p = 0.016$). No difference in blood glucose was observed at the 15 min time point on the day that no test meal was provided.

3.2 Experiment 2

3.2.1 Body Weight—Body weight at the start of training did not differ between the groups (means $\pm$ SEM = 312.9 ± 0.95 and 313.4 ± 0.95 for glucose and saccharin groups, respectively). As in Experiment 1, animals given the saccharin-sweetened yogurt supplements gained significantly more weight than animals given the glucose-sweetened yogurt (Figure 3; Main effect of Day, $F_{20, 160} = 298.4$, $p < 0.0000001$; Day X Sweetener Interaction; $F_{20, 160} = 2.3$, $p = 0.0023$). Post-hoc comparisons indicated that body weight gain was significantly higher on days 16, 17, 20 and 21.

3.2.2 Blood glucose—Blood glucose levels during the oral glucose-tolerance test were also significantly affected by the type of sweetener consumed during training (Figure 4A; Main effect of Sweetener, $F_{1, 8} = 6.71$, $p = 0.032$; Main effect of Time, $F_{13, 104} = 26.9$, $p < 0.0000001$; Sweetener X Time interaction, $F_{13, 104} = 1.98$, $p = 0.029$), with glucose levels being significantly higher in Saccharin animals at 8, 12 and 48 minutes following the introduction of the glucose solution compared to Glucose animals. In addition, AUC of blood glucose levels indicated a significant increase in Saccharin animals compared to Glucose animals (Figure 4B; $F_{1, 8} = 5.84$, $p = 0.042$).

3.2.3 Insulin—In contrast, while insulin levels were significantly affected by the time after introduction of the glucose solution (Figure 4C; Main effect of Time, $F_{13, 104} = 36.00$, $p < 0.0000001$), neither the pattern of insulin levels across time (Main effect of Sweetener = 1.78, $p = 0.22$; Sweetener X Time interaction = 1.13, $p = 0.34$) nor the insulin AUC (Figure 4D; $F_{1, 8} = 1.9$, $p = 0.21$) differed between Saccharin and Glucose animals as indicated by ANOVA and planned comparisons at 0, 4, 8, 12 and 16 min.

3.3 Experiment 3

3.3.1 Body Weight—Starting body weights did not differ across the two groups of animals (means = 267.9 ± 2.3 for glucose animals and 271.7 ± 2.1 for saccharin animals). Although animals given saccharin-sweetened yogurt appeared to gain more weight than animals given glucose-sweetened yogurt, in this experiment, no significant differences in weight gain were found (Figure 5).

3.3.2 Blood Glucose—Despite the lack of significant differences in weight gain, glycemic responses to 5 ml of a 20% glucose solution were affected by the sweetener provided during training, the method used to deliver the glucose and the time of testing (Figure 6A and 6C; Method X Sweetener interaction, $F_{1, 11} = 7.222$, $p = 0.021$; Main effect of Time, $F_{7, 77} = 134.44$, $p < 0.0000001$; Time X Method X Sweetener interaction; $F_{7, 77} = 2.23$, $p = 0.040$). Post-hoc comparisons indicated that glucose levels were significantly higher 15 min following introduction of the oral glucose solution in animals previously exposed to the saccharin-sweetened yogurt compared to animals exposed to the glucose-sweetened yogurt. No differences were observed when the glucose solution was delivered by gavage. Analysis of AUC also indicated that both the sweetener experienced prior to testing and the method of delivery affected the glucose AUC (Figure 6B and 6D; Method X Sweetener interaction, $F_{1, 11} = 6.59$, $p = 0.026$) but post-hoc testing did not reveal any significant differences.

3.4 Experiment 4

3.4.1 Body weight—Starting body weights did not differ between groups (means = 312.2 ± 2.8 for glucose animals and 317.9 ± 3.0 for saccharin animals). Body weight gain was significantly greater during training in animals given the saccharin-sweetened yogurt compared to animals given the glucose-sweetened yogurt (Figure 7; Main effect of Day, $F_{22, 286} = 364.5$, $p < 0.0000001$; Day X Sweetener interaction, $F_{22, 286} = 2.12$, $p = 0.0029$), but post-hoc testing did not reveal significant differences on any individual day.

3.4.2 Blood glucose—Analysis of blood glucose levels indicated that glycemic responses were affected by both the sweetener to which animals had been exposed and the method of glucose delivery (Figure 8A–C; Main effect of Sweetener, $F_{1, 13} = 5.39$, $p = 0.037$; Method X Sweetener interaction, $F_{2, 26} = 3.71$, $p = 0.038$; Main effect of Time, $F_{3, 39} = 65.8$, $p < 0.0000001$; Method X Sweetener X Time, $F_{6, 78} = 3.03$, $p = 0.010$). When animals consumed the glucose orally from cups, blood glucose levels were significantly higher in animals that previously consumed saccharin-sweetened yogurt (Figure 8A; Main effect of Time, $F_{3, 39} = 54.3$, $p < 0.0000001$; Time X Sweetener interaction, $F_{3, 39} = 2.99$, $p = 0.043$), with post-hoc comparisons indicating higher blood glucose at 16 min. Similarly, when animals were allowed to taste the glucose prior to it being delivered by gavage, blood glucose levels were also affected by their previous exposure to sweetener (Figure 8B; Main effect of Sweetener, $F_{1, 13} = 9.93$, $p = 0.0080$; Main effect of Time, $F_{3, 39} = 33.8$, $p < 0.0000001$; Time X Sweet interaction, $F_{3, 39} = 4.08$, $p = 0.013$), with post-hoc tests indicating significant differences at 16 and 32 min. In contrast, when glucose was delivered by gavage directly into the stomach, bypassing the mouth, there were no effects of the previous sweetener consumed on blood glucose levels (Figure 8C; Main effect of Time, $F_{3, 39} = 29.8$, $p < 0.0000001$).

3.4.3 Insulin—Analysis of insulin levels revealed that insulin was not affected by the Method used to deliver glucose or by the sweetener to which animals had previously been exposed (Figure 8D–F).

3.5 Experiment 5

3.5.1 Body Weight—Starting body weight did not differ between groups (means = 367.8 ± 4.4 for glucose animals and 371.4 ± 4.3 for saccharin animals). Animals consuming the saccharin solution gained significantly more weight than animals consuming the glucose solution (Figure 9A; Main effect of Day, $F_{22, 308} = 142.34$, $p < 0.0000001$; Day X Sweetener interaction, $F_{22, 308} = 2.41$, $p = 0.00052$), with post-hoc tests indicating significant differences on Days 21, 22 and 23..

3.5.2 Food Intake—Analysis of food intake indicated that the total amount of energy consumed was affected by the day of testing as well as by the sweetened liquid provided (Figure 9B; Main effect of Day, $F_{22, 264} = 39.68$, Sweetener X Day interaction, $F_{22, 264} = 1.76$, $p = 0.021$), with significant differences on days 2, 5, 6, 10, 22 and 23.

3.5.3 Blood glucose—Analysis of blood glucose data indicated that blood glucose levels were affected by the method of delivery, as well as by the sweetener in the solution consumed during training (Figure 10A and 10D; Main effect of sweetener, $F_{1, 14} = 6.10$, $p = 0.027$; Main effect of method, $F_{1, 14} = 8.48$, $p = 0.011$; Main effect of time, $F_{3, 42} = 118.7$, $p < 0.0000001$; Time X Method interaction, $F_{3, 42} = 3.49$, $p = 0.024$). Post-hoc analyses indicated that animals previously given a saccharin solution had higher glycemic responses than animals previously given a glucose solution when the test solution was consumed orally (Figure 10A; Main effect of sweetener, $F_{1, 14} = 10.9$, $p = 0.0052$; Main effect of time, $F_{3, 42} = 79.54$, $p < 0.0000001$), with significant differences between glucose and saccharin

animals observed at 8 and 16 min. In contrast, there were no differences in blood glucose levels when the glucose was delivered by gavage (Figure 10D).

Analysis of glucose AUC indicated effects of the sweetener and the method of delivery (Main effect of Sweetener, $F_{1, 14} = 6.26$, $p = 0.025$; Main effect of Method, $F_{1, 14} = 9.08$, $p = 0.0093$). Post-hoc analyses indicated that blood glucose levels were significantly higher when the glucose was delivered by gavage than when animals consumed it orally, and that when animals consumed the glucose orally, saccharin-exposed animals had significantly higher blood glucose AUC compared to glucose-exposed animals (Figure 11A; $F_{1, 14} = 10.37$, $p = 0.0062$).

3.5.4 GLP-1—Levels of total GLP-1 were affected by the method of delivery and the sweetener previously consumed (Figure 10B and 10D; Main effect of method, $F_{1, 14} = 5.87$, $p = 0.029$; Main effect of time, $F_{3, 42} = 28.18$, $p < 0.0000001$; method X time interaction, $F_{3, 42} = 4.73$, $p = 0.00062$). Post-hoc analyses revealed that when glucose was consumed orally, GLP-1 levels were affected by the previous sweetener exposure (Figure 10B; Main effect of Sweetener, $F_{1, 14} = 11.75$, $p = 0.0040$; Main effect of Time, $F_{3, 42} = 22.24$, $p < 0.0000002$; Sweetener X Time interaction, $F_{3, 42} = 3.18$, $p = 0.033$), with GLP-1 levels being significantly lower in animals that had previously consumed the saccharin solution at 8 and 16 minutes compared to animals that had previously consumed the glucose solution. When glucose was delivered by gavage, there were no differences in GLP-1 levels across groups at any time (Figure 10D).

Analysis of GLP-1 AUC indicated that the AUC was significantly lower when glucose was consumed orally compared to when it was delivered by gavage (Figure 11B; Main effect of method of delivery, $F_{1, 14} = 6.72$, $p = 0.021$) and with post-hoc tests indicating that when animals consumed the glucose orally, GLP-1 AUC was significantly lower in saccharin-exposed animals compared to glucose-exposed animals ($F_{1, 14} = 12.56$, $p = 0.0032$) but not when the glucose was delivered by gavage.

3.6 Experiment 6

3.6.1 Body Weight—Starting body weight did not differ between groups (means = 404.6 ± 3.0 for glucose animals and 405.8 ± 3.0 for saccharin animals). Animals consuming the saccharin-sweetened yogurt gained significantly more weight than animals consuming the glucose-sweetened yogurt (Figure 12A; Main effect of Sweetener, $F_{1, 44} = 5.40$, $p = 0.025$; Main effect of Day, $F_{22, 968} = 721.48$, $p < 0.0000001$; Day X Sweetener interaction, $F_{22, 968} = 4.31$, $p < 0.0000001$), with post-hoc tests indicating significant differences beginning on Day 12 and continuing through the end of the exposure.

3.6.2 Food Intake—Analysis of food intake indicated that the total amount of energy consumed was affected by the day of testing as well as by the sweetened yogurt provided (Figure 12B; Main effect of Sweetener, $F_{1, 44} = 6.25$, $p = 0.016$; Main effect of Day, $F_{22, 968} = 9.29$, $p < 0.0000001$). Post-hoc analysis indicated that animals given saccharin-sweetened yogurt consumed significantly more total calories than animals consuming glucose-sweetened yogurt.

3.6.3 Body Composition—Analysis of body composition indicated that fat mass was not affected by the sweetener provided, but animals given access to saccharin-sweetened yogurt had greater lean mass at the end of testing than animals given access to glucose-sweetened yogurt (Figure 13; Main effect of starting lean mass, $F_{1, 43} = 112.3$, $p < 0.0000001$; Main effect of sweetener, $F_{1, 43} = 6.03$, $p = 0.018$).

3.6.4 Blood glucose—Analysis of blood glucose data during the oral glucose tolerance test indicated that blood glucose levels were affected by the sweetener provided in the yogurt during training and by the time of sampling (Figure 14A; Main effect of sweetener, $F_{1,44} = 6.16$, $p = 0.017$; Main effect of Time, $F_{3,44} = 287.85$, $p < 0.0000001$). Post-hoc analyses indicated that animals previously given saccharin-sweetened yogurt had significantly higher blood glucose levels 8 and 16 minutes after presentation of the glucose solution compared to animals previously given glucose-sweetened yogurt

AUC for blood glucose after both 16 and 32 min of the oral glucose tolerance test was affected by the sweetener previously consumed during training (Figure 15A; at 16 min, Main effect of Sweetener, $F_{1,44} = 6.30$, $p = 0.016$; at 32 min, $F_{1,44} = 6.11$, $p = 0.017$, data not shown). Animals previously provided with the saccharin-sweetened yogurt had significantly higher glucose AUC at 16 and 32 min.

3.6.5 GLP-1—When samples at 0, 8, 16 and 32 minutes were included, levels of GLP-1 were affected by the time of testing, and there were trends for levels of total GLP-1 to be affected by the sweetener consumed during training (Figure 14B; Main effect of Sweetener, $F_{1,44} = 3.04$, $p = 0.089$; Main effect of time, $F_{3,132} = 36.48$; Time X Sweetener interaction, $F_{3,132} = 2.23$, $p = 0.11$). When data from the 0, 8 and 16 min timepoints were analyzed, significant differences in levels of GLP-1 were observed (Main effect of Sweetener, $F_{1,44} = 4.63$, $p = 0.037$, Main effect of Time, $F_{3,132} = 36.48$, $p < 0.0000001$), with post-hoc analysis indicating that GLP-1 levels were significantly lower at 8 and 16 min in animals previously given access to saccharin-sweetened yogurt compared to those previously given glucose-sweetened yogurt. Analysis of GLP-1 AUC confirmed that the AUC after 16 min was significantly lower in saccharin-exposed animals (Figure 15B; Main effect of Sweetener, $F_{1,44} = 5.77$, $p = 0.021$), while the AUC after 32 min showed a trend to be smaller in saccharin-exposed animals compared to glucose-exposed animals (Main effect of Sweetener, $F_{1,44} = 3.50$, $p = 0.068$; data not shown).

3.6.6 Insulin—Insulin levels during the glucose tolerance test were affected by the time at which they were measured, but not the sweetener to which the animals had previously been exposed (Figure 14C; Main effect of Time, $F_{3,132} = 66.06$, $p < 0.0000001$). Insulin AUC at 16 min (Figure 15C) and 32 min (data not shown) were not affected by the sweetener to which the animals had previously been exposed.

4. Discussion

Previous work from our lab indicated that following exposure to dietary supplements of saccharin-sweetened yogurt, rats showed both impaired ability to compensate for calories and a diminished thermic effect of food following consumption of a novel, sweet-tasting test meal of thickened Chocolate Ensure Plus [2]. Therefore, the goal of Experiment 1 was to determine whether exposure to saccharin-sweetened yogurt altered glycemic responses to this novel test meal compared to exposure to glucose-sweetened yogurt. The results of this experiment indicated that animals with experience consuming saccharin-sweetened yogurts showed hyperglycemic responses when given a novel, sweet-tasting test meal compared to animals that had experience consuming glucose-sweetened yogurts, and that these differences appeared during the first blood sample, collected 15 min following delivery of the test meal. These data suggested that exposure to saccharin-sweetened diets may have resulted in altered cephalic phase responses, such as a diminished cephalic phase insulin release (CPIR), when a novel, sweet-tasting diet that delivered energy was consumed.

The goals of Experiment 2 were to examine how rapidly these changes in glycemic responses were observed and to measure whether in fact differences in the CPIR contributed

to differences in the glycemic response; data were collected during a standard glucose tolerance test rather than during consumption of a novel, complex test meal as was done in Experiment 1. To collect samples rapidly, indwelling jugular catheters were implanted following exposure to saccharin-sweetened or glucose-sweetened yogurt, and animals were then given access to an oral glucose solution while samples were collected every 4 min. The results demonstrated that prior exposure to saccharin-sweetened yogurt resulted in hyperglycemic responses to oral consumption of a glucose solution compared to prior exposure to glucose-sweetened yogurt, and that this hyperglycemic response appeared during the early stages of ingestion. Further, the increase in blood glucose did not appear to result from an impaired release of insulin, since insulin levels did not differ between the two groups during testing at any time point, even during the earliest sample collected at 4 min.

In Experiment 3, we compared glycemic responses to oral ingestion of a glucose solution to the same glucose solution delivered by gavage to bypass oral stimulation in animals exposed to saccharin-sweetened yogurt versus glucose-sweetened yogurt. The results indicated that differences in glucose tolerance following exposure to saccharin-sweetened yogurt compared to glucose-sweetened yogurt do not occur when glucose is delivered directly into the stomach, bypassing the oral cavity. This finding is consistent with the idea that saccharin consumption weakens the ability of a sweet taste in the mouth to evoke cephalic phase responses involved with controlling blood glucose levels.

Experiment 4 assessed the effects of oral taste stimulation and delivery rate on glycemic and insulin responses to glucose test solutions by rats that had previously consumed yogurt sweetened with saccharin or glucose. These responses were compared for rats that consumed the glucose test solution orally (Oral), had the solution administered by gavage (Gavage) or had the solution administered by gavage in conjunction with having the tongue rinsed with glucose solution. These results indicated that blood glucose responses were different in both conditions when animals tasted the solution (Oral and Taste Gavage), but not when the solution was delivered directly by gavage (Gavage). The use of the Taste Gavage meant that animals in both groups received the taste stimuli over a similar period of time, ruling out differences in the rate of consumption of the glucose solutions during the oral tests as a necessary condition for differences between groups. As in Experiments 2 and 3, changes in oral glucose tolerance following exposure to saccharin were not accompanied by changes in insulin levels in Experiment 4.

In the final two experiments, animals were maintained on a high-fat, glucose-sweetened diet that mimicked characteristics of the diet widely-consumed by humans in the U.S. Along with increased food intake, body weight gain, and impaired oral glucose tolerance, decreased release of GLP-1 during the oral glucose test was associated with prior consumption of saccharin (relative to glucose) in solution (Experiment 5) or mixed in yogurt (Experiment 6). Given findings that implicate GLP-1 in both satiety and glucose homeostasis, a reduction in GLP-1 response to glucose intake could contribute to increased intake and impaired glucoregulation by the rats that had consumed saccharin.

The results of this series of experiments also confirm [2–5, 9] that animals given access to dietary supplements containing the high-intensity sweetener saccharin show increased body weight gain relative to animals given access to the same supplements containing the caloric sweetener glucose. In 5 of the 6 experiments, such differences in weight gain were statistically significant, whether the sweetener was mixed in a yogurt supplement (3.1.1, 3.2.1, 3.4.1, 3.6.1) or provided in liquid form (3.5.1). In Experiment 3, animals did not show significant differences in weight gain after two weeks exposure to saccharin-sweetened yogurt compared to glucose-sweetened yogurt. This lack of differential weight gain may be related to the fact that animals were smaller at the start of this experiment, and were still in a

rapid growth phase. The high rate of growth in both groups in this experiment, which appear to be greater than in all of the other experiments, may have obscured differences in weight gain based on exposure to the differential relations between sweet tastes and calories. Nevertheless, when taken together with previous results [2–5, 9], the data are consistent with the hypothesis that exposure to a relation in which sweet taste reliably predicts the delivery of calories, such as that provided by glucose-sweetened diets, results in reduced weight gain relative to a diet in which sweet taste does not reliably predict calories, such as that provided by saccharin-sweetened diets. As previously demonstrated [2–5, 9] differences in body weight gain are related to differences in food intake; animals given saccharin-sweetened solutions consumed more total calories than animals given glucose-sweetened solutions in Experiment 5 (3.5.2) as did animals given saccharin-sweetened yogurt supplements in Experiment 6 (3.6.2).

In addition to these differences in body weight gain and food intake, oral glucose tolerance tests consistently revealed that animals given experience with saccharin-sweetened yogurt or liquid showed higher levels of blood glucose within the first 8 – 16 minutes of consuming either a glucose solution or a novel sweet-tasting caloric test meal. Thus, compared to experience with glucose-sweetened diets, experience with saccharin-sweetened diet appeared to impair glycemic responses. This impairment appeared to be dependent on stimulation of the oral cavity, since delivery of an identical glucose solution directly into the stomach by gavage did not produce differential glycemic responses (3.3.2, 3.4.2, 3.5.3). Differential glycemic responses were observed following gavage only after the tongue was first stimulated with the glucose solution (3.4.2). Thus, experience with a saccharin-sweetened diet led to exaggerated blood glucose levels when animals ate sweet-tasting foods that actually delivered energy and calories. This effect appeared early in testing (within the first 8 – 16 minutes), and was transient in nature, supporting the idea that alterations were related to an impaired ability to predict the arrival of energy in the saccharin-exposed animals, rather than a general impairment in glucose utilization.

The mechanism underlying changes in blood glucose regulation in saccharin-exposed animals does not appear to be related to decreased release of insulin since no significant differences in insulin release were observed in any experiments. However, given that the earliest time sample collected was 4 min (Experiment 2) or 8 min (Experiments 4–6) following presentation of the glucose solution it is possible that differences in CPIR were missed, and that samples collected more rapidly might reveal differences between the groups. Nevertheless, the current data do not provide evidence for altered insulin release in the observed effects on glycemic responses.

The results from Experiments 5 and 6 suggest that a primary deficit resulting from exposure to saccharin-sweetened diets may be a decreased secretion of GLP-1 in response to sweet tastes in the mouth. GLP-1 levels were similar at baseline, but secretion of GLP-1 was significantly lower, and blood glucose levels were significantly higher, in animals previously exposed to saccharin-sweetened liquids or saccharin-sweetened yogurts during the first two samples (8 and 16 min) after consumption of an oral glucose load (see 3.5.3, 3.5.4, 3.6.4 and 3.6.5).

Based on principles of associative learning, experience with consuming a sweet taste that is not followed by the anticipated energetic consequence could cause the sweet taste to become less effective at eliciting release of GLP-1 over time. Release of GLP-1 by sweet taste in the mouth would then become blunted even when caloric sweeteners are subsequently consumed. This diminished ability of sweet taste to release GLP-1 could underlie increased food intake, as both peripheral and central actions of GLP-1 during meals have been directly implicated in satiety (e.g. [20–22, 24–32, 36, 37]). A reduction in the release of GLP-1 could

also lead to increased blood glucose levels by a variety of mechanisms. For example, GLP-1 can contribute to glucose homeostasis independent of effects on insulin release, by enhancing glucose metabolism in skeletal muscle, liver and adipose tissue, regulating of glucose transporter expression, suppressing of glucagon release and slowing of gastric emptying (e.g. [23, 38–58]). Thus, for example, diminished release GLP-1 in response to a sweet-tasting food or glucose solution could promote more rapid gastric emptying which would then lead to more rapid delivery of glucose to the intestines, and more rapid elevations of blood glucose levels (e.g. [23, 38–58]). Increased gastric emptying would also lead to diminished gastric distension, reducing another potential source of satiety signals that could contribute to increased food intake. Further, decreased glucose utilization in muscle, liver or adipose tissue related to decreased levels of GLP-1 would lead to higher blood glucose levels (e.g. [23, 38, 39, 41, 50, 51, 53]). Lower GLP-1 release could also result in increased blood glucose levels due to diminished suppression of glucagon release (e.g. [40, 42, 45, 59]).

These data are consistent with the hypothesis that dysregulation of energy balance and glucose homeostasis can occur following exposure to high-intensity sweeteners. One might question this conclusion based on the facts that final sample sizes were relatively small, and in each experiment a number of animals were excluded, either due to technical difficulties with gavage or blood collection, or due the animals' failure to consume the diets or liquids during training or the glucose or test meal during testing. However, there did not appear to be systematic differences between groups in consumption of the assigned diets during training or in consumption of the glucose solution or Ensure test meal during glucose tolerance testing, thus differences between animals that fail to eat the assigned diets during exposure or testing and those that did eat do not appear to be directly related to sweetener exposure. There also could be differences in the intensity of the sweet taste arising resulting from the sweeteners used. For example, rats may perceive the sweetness of the 0.3% saccharin to be higher or lower in intensity than the 20% glucose added to the yogurts used in Experiments 1–4 and 6, or the 10% glucose solution used in Experiment 5. However, even if differences in perceived intensity existed, it would be difficult to see how they could account for the current findings.

Thus, the results from these experiments suggest that exposure to sweet tastes that do not reliably predict the delivery of energy or glucose produces alterations in glycemic responses to orally ingested glucose, but not to glucose delivered to the gut directly. These differences in glucose homeostasis were associated with decreased release of GLP-1 early in the meal, which may have contributed to the increases in food intake and body weight gain in saccharin-exposed animals. Such results are consistent with the hypothesis that rather than preventing or reversing overweight and obesity, consumption of foods or beverages manufactured with high-intensity sweeteners may contribute to dysregulation of body weight by altering cephalic phase responses. This could occur because high-intensity sweeteners interfere with conditioned cephalic phase responses, because glucose-sweetened foods enhance conditioned cephalic phase responses, or both. In any of those cases, consuming diets prepared with high-intensity sweeteners results in augmented food intake, body weight gain, altered glucose homeostasis and diminished release of GLP-1 compared to the same diets prepared with glucose.

To date, little attention has been paid to the possibility that such experiences affect glycemic or other responses in humans. Recent studies examining glycemic responses to delivery of high-intensity sweeteners alone, for example, demonstrate that human subjects consuming these sweeteners orally do not show increases in plasma GLP-1, PYY, insulin or glucose [60, 61] or that delivery of high-intensity sweeteners directly into the gut do not affect gastric emptying, blood glucose, insulin or release of GLP-1 (e.g. [62–64]). These results

have been used to argue that there are fundamental differences between humans and rodents, and that high-intensity sweeteners cannot or do not alter glucose homeostasis, satiety or energy balance in humans. However, this conclusion can be challenged on several grounds.

First, studies that only deliver sweeteners directly into the stomach or intestines do not test the hypothesis that glucose homeostasis and energy balance depend on the validity of the predictive relationship between sweet taste and caloric outcomes. Thus results showing that humans do not release hormones such as GLP-1 or insulin in response to delivery of sweeteners directly into the gut [62–64] do not speak to the hypothesis, and are consistent with some data in rats showing a similar lack of effect in rats when sweeteners are delivered directly into the gut in rats [65]. The critical role of taste is demonstrated in the present data. When animals consume a glucose solution oral during glucose tolerance testing, previous exposure to saccharin resulted in hyperglycemia relative to previous exposure to glucose. In contrast, no effects of previous exposure to saccharin on glucose homeostasis were observed in glucose tolerance tests where the glucose solution is delivered directly into the stomach.

Second, measuring acute metabolic or hormonal responses evoked by oral intake of non-caloric sweeteners consumed in isolation, does not address the more important hypothesis, from our perspective, that exposure to non-caloric sweeteners impairs the ability of consuming sweet, high-calorie, substances to evoke those responses. Third, most studies in humans fail to consider the possibility that the effects repeated or chronic exposure to high-intensity sweeteners and their consequences may be different compared to acute exposure. If consumption of high-intensity sweeteners interferes with conditioned responses, then previous exposure to the high-intensity sweeteners could affect responses during tests and a single exposure to a high-intensity sweetener might be insufficient to elicit any altered responses. Consistent with this hypothesis is recent data documenting differences in brain activity in response to an oral sucrose solution in human subjects with histories of high versus low consumption of high-intensity sweeteners [66]. Examining the effects of a single exposure to a sweetener, or failing to account for the amount of previous exposure to the sweeteners therefore complicates interpretation of human studies attempting to examine how high-intensity sweeteners might influence food intake, body weight and/or glucose homeostasis.

Within our theoretical framework, the critical questions are not about the acute effects of high intensity sweeteners when they are consumed alone or when they are delivered directly to the gut. The real issue is how does chronic, oral consumption of non-caloric sweeteners impact energy regulation when sweet caloric substances are orally consumed. This is the question we have tried to address in our animal studies. And the question is important because, in the current human food environment, high-intensity sweeteners are typically (a) consumed chronically, not acutely; (b) consumed not in isolation, but by people who also consume sweet high-calorie foods and beverages; (c) consumed and tasted orally, not in ways that bypass taste and the oral cavity.

There are clear differences between rats and humans that prescribe caution when applying the results of studies such as ours to regulation of human body weight. However, it is also important to avoid dismissing the potential implication of studies conducted in rats, such as those described above, on the basis of human studies that fail to adequately evaluate the hypothesis.

5. Conclusion

These studies support a role for interfering with learned relations between sweet tastes and calories in the regulation of not only body weight, but glucose homeostasis. Animals given

experience with sweet tastes that are not associated with calories show hyperglycemia and reduced secretion of GLP-1 during subsequent oral glucose tolerance tests, while secretion of insulin appears to be unchanged. The decrease in GLP-1 may play a direct role in the dysregulation of food intake and body weight. The results also highlight critical differences in physiological consequences when foods are consumed by mouth compared to when foods are placed directly into the stomach, with hyperglycemia evidenced by all animals in tests where glucose solutions were placed directly into the stomach compared to similar solutions placed into the mouth or consumed by the animals. Thus, while some caution is required in extending results of animal studies to mechanisms that operate in humans, the results are consistent with recent human epidemiological demonstrating a link increased availability and consumption of food products containing high-intensity sweeteners and increased prevalence of overweight and obesity (e.g. [67–69]), diet soda consumption and adverse cardiovascular events [67, 69–73], and adds to the known potential role of GLP-1 in modulating body weight and glucose homeostasis in humans (e.g. [40, 43, 59, 74–81]).

Highlights

Rats given dietary supplements sweetened with saccharin gained extra body weight
 Saccharin-exposed animals were hyperglycemic during oral glucose tolerance tests
 Hyperglycemia in saccharin-exposed animals was not due to changes in insulin release
 Reduced release of GLP-1 was observed in saccharin-exposed animals
 Decreased release of GLP-1 may explain both hyperglycemia and increased food intake

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Figure 1.

Body weight gain in animals given access to saccharin-sweetened yogurt for 3 hr per day was significantly higher than in animals given access to glucose-sweetened yogurt.

* $p < 0.05$ compared to Glucose

Figure 2.

Blood glucose levels were significantly higher 15 min after the introduction of a novel sweet-tasting meal in animals previously exposed to saccharin-sweetened yogurt compared to animals previously exposed to glucose-sweetened yogurt (left panel). No differences in blood glucose levels were observed when no meal was provided (right panel).

* $p < 0.05$ compared to glucose

Figure 3.

Body weight gain prior to surgery in male rats given access to saccharin-sweetened yogurt was significantly greater compared to those given access to glucose-sweetened yogurt.

* $p < 0.05$ compared to Glucose group

Figure 4.

A) In animals that consumed at least 90% of 10 g of a 20% glucose solution in the first 30 min, blood glucose levels were significantly higher at 8 min, 12 min and 48 min in male rats previously exposed to saccharin-sweetened yogurt compared to those previously exposed to glucose-sweetened yogurt. B) Glucose AUC was significantly higher in animals previously exposed to saccharin-sweetened yogurt. C) Neither patterns of insulin levels nor insulin AUC (D) were different in animals previously exposed to glucose versus saccharin-sweetened yogurt

* $p < 0.05$ compared to Glucose group

Figure 5.
Body weight gain was not significantly different in animals given glucose-sweetened versus saccharin-sweetened yogurt in Experiment 3.

Figure 6.

In animals that orally consumed at least 90% of 5 g of a 10% glucose solution within 15 minutes, blood glucose levels were significantly higher in male rats previously exposed to saccharin-sweetened yogurt compared to those previously exposed to glucose-sweetened yogurt (A and B). In contrast, no differences in glucose levels were observed when glucose was delivered directly into the stomach by gavage (C and D).

* $p < 0.05$ compared to Glucose group

Figure 7.
Body weight gain in Experiment 4 was significantly higher in animals given saccharin-sweetened yogurt compared to glucose-sweetened yogurt.

Figure 8.

Blood glucose levels were significantly higher in animals previously given saccharin-sweetened yogurt following 5 ml of a 20% glucose solution in animals that consumed at least 90% of the solution from a cup within 8 minutes (A) or when animals were allowed to taste the solution and then given a gavage directly into the stomach (B), but not when the glucose solution bypassed the oral cavity and was delivered directly into the stomach (C). There were no differences in insulin levels between groups regardless of the method of delivery.

* $p < 0.05$ compared to Glucose group

Figure 9.

Body weight gain (A) and energy intake (B) were significantly greater in animals given a saccharin-sweetened solution compared to animals given a glucose-sweetened solution when a high-fat, high sugar maintenance diet was provided.

* $p < 0.05$ compared to Glucose group

Figure 10.

Blood glucose levels were significantly higher in animals previously given access to a saccharin-sweetened solution in animals that consumed at least 90% of 5 ml of a 20% glucose solution orally (A) within 8 minutes, but not when the same solution was delivered directly into the stomach by gavage (C). Levels of total GLP-1 were significantly lower 8 and 16 min following presentation of the glucose solution orally (B) but not following delivery of the glucose solution directly into the stomach by gavage (D).

* $p < 0.05$ compared to Glucose group

Figure 11.

AUC for blood glucose (A) was significantly lower when animals consumed the glucose orally compared to when it was delivered by gavage. In addition, when animals consumed the glucose orally, blood glucose AUC was lower in animals that had previously been exposed to glucose compared to animals previously exposed to saccharin. AUC of GLP-1 (B) was significantly higher when glucose was delivered by gavage compared to being consumed orally, and significantly lower in saccharin-exposed animals compared to glucose-exposed animals when animals consumed the glucose solution orally during the test.

* $p < 0.05$ compared to Glucose group

Figure 12.

Body weight gain (A) was significantly higher in rats maintained on a high-fat, sweetened diet and given saccharin-sweetened yogurt supplements compared to glucose-sweetened yogurt supplements. Total energy intake across all days of training (B) was also significantly higher in rats given the saccharin-sweetened yogurt.

* $p < 0.05$ compared to Glucose group

Figure 13.

Analysis of body composition indicated that animals given access to saccharin-sweetened yogurt supplements and maintained on high-fat, sweetened diets had significantly greater lean mass at the end of exposure compared to animals given glucose-sweetened yogurt.

* $p < 0.05$ compared to Glucose group

■ Glucose ● Saccharin

Figure 14.

In rats maintained on a high-fat, sweetened diet and given saccharin-sweetened yogurt diets, blood glucose levels were significantly more elevated at 8 and 16 min following consumption of 5 ml of a 20% glucose solution compared to animals given glucose-sweetened yogurt diets (A), while release of GLP-1 was significantly lower at 8 and 16 min in animals given saccharin-sweetened yogurts (B). Insulin release did not differ at any time point between groups (C).

* $p < 0.05$ compared to Glucose group

Figure 15.

In rats maintained on a high-fat, sweetened diet and given saccharin-sweetened yogurt diets, AUC calculated over the first 16 min for blood glucose was significantly higher following consumption of 5 ml of a 20% glucose solution compared to animals given glucose-sweetened yogurt diets (A), AUC for GLP-1 was significantly lower in animals given saccharin-sweetened yogurts (B) and there were no differences in AUC for Insulin between groups (C).

* $p < 0.05$ compared to Glucose group