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Sucralose decreases insulin sensitivity in healthy subjects: a randomized controlled trial

Alonso Romo-Romo,[1] Carlos A Aguilar-Salinas,[1,2,4] Griselda X Brito-Córdova,[1] Rita A Gómez-Díaz,[3] and Paloma Almeda-Valdes[1,2]

[1]Departamento de Endocrinología y Metabolismo, and [2]Unidad de Investigación en Enfermedades Metabólicas, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico; [3]Unidad de Investigación Médica en Epidemiología Clínica, UMAE Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico City, Mexico; and [4]Tecnológico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Monterrey, Mexico

ABSTRACT

Background: Recently, the absence of metabolic effects from nonnutritive sweeteners has been questioned.

Objective: The aim of this study was to evaluate the effects of sucralose consumption on glucose metabolism variables.

Design: We performed a randomized controlled trial involving healthy subjects without comorbidities and with a low habitual consumption of nonnutritive sweeteners ($n = 33$/group).

Methods: The intervention consisted of sucralose consumption as 15% of Acceptable Daily Intake every day for 14 d using commercial sachets. The control group followed the same procedures without any intervention. The glucose metabolism variables (insulin sensitivity, acute insulin response to glucose, disposition index, and glucose effectiveness) were evaluated by using a 3-h modified intravenous-glucose-tolerance test before and after the intervention period.

Results: Individuals assigned to sucralose consumption showed a significant decrease in insulin sensitivity with a median (IQR) percentage change of −17.7% (−29.3% to −1.0%) in comparison to −2.8% (−30.7% to 40.6%) in the control group (P= 0.04). An increased acute insulin response to glucose from 577 mU · L^{-1} · min (350–1040 mU · L^{-1} · min) to 671 mU · L^{-1} · min (376–1010 mU · L^{-1} · min) ($P = 0.04$) was observed in the sucralose group for participants with adequate adherence.

Conclusions: Sucralose may have effects on glucose metabolism, and our study complements findings previously reported in other trials. Further studies are needed to confirm the decrease in insulin sensitivity and to explore the mechanisms for these metabolic alterations. This trial was registered at www.clinicaltrials.gov as NCT02589002. *Am J Clin Nutr* 2018;108:485–491.

Keywords: nonnutritive sweeteners, insulin resistance, sweetening agents, sucralose, glucose intolerance, intravenous glucose tolerance test

INTRODUCTION

The nonnutritive sweeteners (NNSs) are a class of food additives commonly used in products to provide a sweet taste and to reduce sugar and caloric content (1). The absence of metabolic effects by these substances has been questioned. Sucralose consumption before an oral-glucose-tolerance test (OGTT) produced higher glucose and insulin concentrations in comparison to water and a significant decrease in insulin sensitivity and insulin clearance (2). Saccharin ingestion increased glucose concentrations in 4 of 7 subjects after 7 d. Alterations in gut microbiota were associated with glucose intolerance. This finding was confirmed by performing fecal transplantation from humans to mice and observing the same effect on glucose tolerance 7 d after transplant (3). However, the findings among studies are not consistent; therefore, it is not possible to establish a certain conclusion (4). The NNS-induced effects may be related to the interaction with the heterodimeric sweet taste receptors type 1 members 2 and 3 (T1R2 and T1R3) in the gut and pancreas (5, 6). In vitro studies have shown that activation of sweet taste receptors in murine pancreatic β cells produced by sucralose, acesulfame potassium, and saccharin stimulates insulin secretion (7). Sucralose stimulates the release of glucagon-like peptide (GLP) types 1 and 2 in

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Address correspondence to PA-V (e-mail: paloma.almedav@incmnsz.mx).

Abbreviations used: ADI, Acceptable Daily Intake; FSIVGTT, frequently sampled intravenous-glucose-tolerance test; GLP, glucagon-like peptide; NNS, nonnutritive sweetener; OGTT, oral-glucose-tolerance test; Si, insulin sensitivity; T1R, taste receptor type 1.

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mice enteroendocrine L cells (8). Nevertheless, higher GLP-1 concentrations after sucralose ingestion has been reported in only 2 human studies (9, 10), and others have not replicated this effect (2, 11–15). In trials reporting higher GLP-1 concentrations, sucralose was administered before a glucose load, suggesting that the combination of sucralose with carbohydrates potentiates the GLP-1 response. In addition, it has been shown that NNSs increase the expression of the sodium-glucose cotransporter 1 (SGLT-1) and the glucose transporter 2 (GLUT-2) in the small intestine, enhancing glucose absorption (16, 17). The objective of this study was to assess the effects of sucralose consumption in glucose metabolism, controlling for some of the factors that other short-term studies did not take in consideration (i.e., control group, parallel design, and inclusion of healthy nonconsumers of NNSs).

METHODS

Description of participants

We performed an open-label, parallel, randomized clinical trial. Inclusion criteria were women or men aged 18–55 y, with a normal BMI (kg/m^2; 18.5–24.9) and low habitual consumption of NNSs. The exclusion criteria included the presence of diabetes or prediabetes, taking medications that could interfere with insulin sensitivity, severe gastrointestinal diseases, pregnancy or lactation, and history of bariatric surgery. All of the procedures were performed at the Unidad de Investigación de Enfermedades Metabólicas at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico City. The study was registered at clinicaltrials.gov (identifier code: NCT02589002).

Ethics

The Clinical Research and Bioethics Committees of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán approved the study and it was performed in accordance with the Helsinki Declaration. To obtain their acceptance, all participants received a full explanation of the purpose and procedures used in the study and all signed the informed consent.

Measurements at baseline

The study consisted of 3 visits. In the initial visit, the fulfillment of the selection criteria was confirmed. The absence of diabetes or prediabetes was established with a glucose concentration <140 mg/dL at 2 h after a 75-g OGTT according to the American Diabetes Association guidelines (18). In addition, in this visit we collected other data, such as physical activity, food intake, anthropometric measures (weight, height, waist circumference, and hip circumference), body composition (muscle and fat mass), biochemical variables (fasting blood glucose, insulin, and lipid profile), and a physical examination (blood pressure and heart rate). We used the physical activity questionnaire developed at Laval University, which has been validated for the Mexican population. This instrument measures total daily energy expenditure (19). The basal food intake was evaluated with 24-h dietary recalls collected at visits 1 and 2. Information on food intake was analyzed using the Food Processor Analysis Software version 11.4.412 by ESHA Research 2016. To confirm the low consumption of NNSs, a previously validated food-frequency questionnaire adapted to products containing NNSs was used (20). Low consumption was defined as the intake of ≤5 portions/wk regardless of the product (sachets, beverages, yogurts, gums, candies, jelly, etc.). Anthropometric measurements were performed by a certified level 1 anthropometrist and performed according to the technique described by the International Society for the Advancement of Kinanthropometry using a SECA mechanical weight scale with height rod model 700 and a Lufkin Executive Thinline diameter pocket tape measure (6 mm × 2 m; model W606PM). Body composition was evaluated by bioelectrical impedance analysis using Jawon Medical tetrapolar equipment (model ioi 353). Blood samples were collected at 0 and 120 min. Glucose and lipid profile were measured with a Beckman Coulter automatized Unicel DxC 600 Synchron Clinical System and insulin with Beckman Coulter Access 2 equipment. Blood pressure and heart rate were measured using an Omron automatic digital blood pressure monitor (model HEM-781INT).

Glucose metabolism evaluation

In the second visit, a modified, frequently sampled intravenous-glucose-tolerance test (FSIVGTT) administering an infusion of regular insulin at minute 20 over 5 min was performed (21, 22). Blood samples were collected at −15, −10, −5, 0, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 min. Glucose and insulin concentrations were measured in all of the blood samples and the results were entered into the statistical program MinMod Millenium to calculate the parameters related to glucose metabolism: insulin sensitivity (Si), acute insulin response to glucose, disposition index, and glucose effectiveness. After the FSIVGTT, the participants were randomly assigned to 1 of 2 groups (intervention or control) using the website Randomization.com (http://www.randomization.com) with a balanced block design of 11 blocks with 6 subjects each. The random allocation sequence was done by an external collaborator using sequentially numerated sealed envelopes, and the enrollment process was performed by the investigators. The intervention consisted of the daily ingestion of 15% of the Acceptable Daily Intake (ADI) for sucralose (15 mg · kg^{-1} · d^{-1}), as proposed by the Joint FAO/WHO Expert Committee on Food Additives using commercial sachets (Splenda) over 14 d. Each commercial sucralose sachet (1 g) contains 958 mg dextrose, 30 mg maltodextrin, and 12 mg sucralose. Participants assigned to sucralose were advised to consume the sachets 3 times/d adding them to beverages at meals. The control group followed the same procedures except for sucralose consumption.

Both groups were instructed to maintain their habitual food intake and physical activity during the intervention period. These variables were evaluated with a 3-d food record for 2 weekdays and 1 weekend day over the 14-d intervention and by repeating the physical activity questionnaire in visit 3. In addition, both groups were advised to avoid the consumption of any other products containing NNSs during the study. Adherence was evaluated with a specific format in which participants registered the number of sucralose sachets consumed each day and the method of consumption. At visit 3, study subjects returned the

empty and unused sucralose sachets. We established adequate adherence when the following criteria were achieved: compliance (consumption ≥80% of the prescribed sucralose sachets) and persistence (consumption of sucralose sachets on ≥12 d of the 14-d intervention period). The changes in glucose metabolism variables were evaluated in the third visit with a second modified FSIVGTT. The changes in weight, food intake, and physical activity habits during the intervention were also assessed on this day.

Statistical analysis

The sample size was calculated to have a power of ≥80% to detect a 20% change in Si with a probability of a type I error (α) of 0.05. The estimated study sample was 33 subjects/group including an extra 20% for probable losses of follow-up. Variable distributions were evaluated with a Shapiro-Wilk test for each group and are presented as means ± SDs or as medians (IQR). The differences in baseline characteristics between groups were evaluated by using Student's *t* test or Mann-Whitney *U* test, as appropriate. Qualitative variables are described as frequencies and percentages and were compared between groups using the chi-square test.

Two analyses were performed to evaluate the effects of sucralose on glucose metabolism variables according to intervention adherence: an intention-to-treat analysis (considering all of the participants) and per-protocol analysis (considering participants who fulfilled both adherence criteria). Differences between baseline and final glucose metabolism variables in each group were evaluated with Wilcoxon matched-pairs test, and the differences in percentage change between groups with Mann-Whitney *U* test, according to the nonparametric distribution of the data. To evaluate the influence of other variables in the results, a multiple linear regression model and a multiple logistic regression analysis were performed to adjust the effect of sucralose in glucose metabolism outcomes. Data were collected and analyzed with IBM SPSS Statistics version 21.0, and a *P* value <0.05 was considered significant.

RESULTS

From June 2015 to March 2017 we screened 104 subjects for inclusion in the study; 38 were excluded for different reasons and 66 were randomly assigned to 1 of the 2 groups. At the end of the study, there were 3 losses in the intervention group and 2 in the control group, not exceeding the extra 20% added to the sample size estimated. **Figure 1** shows a flow diagram of the participants according to the CONSORT (Consolidated Standards of Reporting Trials) guidelines.

Table 1 shows the baseline characteristics of the population. Participants were otherwise healthy adults, mostly female (74.2%), with a median age of 23 y, a normal BMI (21.6), and biochemical variables in the normal range, and free of metabolic disorders. There were no significant differences between groups. The energy intake did not match the total energy expenditure because these variables were estimated with indirect methods. However, the aim for quantifying these variables was to evaluate differences at baseline between groups and the changes after the intervention period in each group.

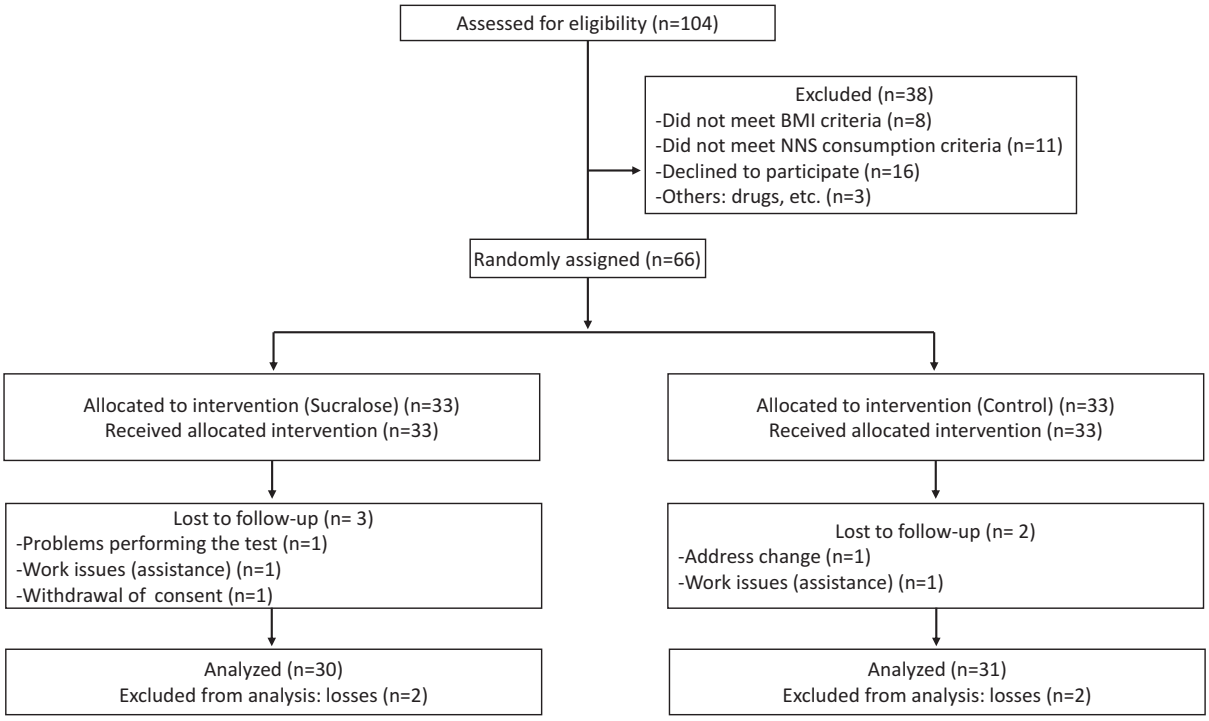

FIGURE 1 Flow diagram of the participants showing the process of recruitment, randomization, follow-up, and data analysis in the study. NNS, nonnutritive sweetener.

TABLE 1
Baseline characteristics of the participants by group[1]

	Intervention group ($n = 33$)	Control group ($n = 33$)	P[2]
Female sex, *n* (%)	24 (72.7)	25 (75.8)	0.77
Age, y	23 (22.5–26.5)	23 (22.0–25.0)	0.63
Weight, kg	58.0 ± 9.0	58.0 ± 8.1	0.99
Height, m	1.63 ± 0.09	1.62 ± 0.08	0.88
BMI, kg/m^2	21.6 ± 1.7	21.7 ± 1.6	0.80
Waist circumference, cm			
Women	71.0 ± 5.6	70.4 ± 4.3	0.63
Men	78.5 ± 3.9	79.6 ± 2.9	0.53
Hip circumference, cm	92.8 ± 4.3	93.6 ± 3.9	0.47
Body fat, %			
Women	28.1 ± 3.2	28.1 ± 3.4	0.96
Men	20.7 ± 2.3	21.3 ± 4.8	0.73
Muscle mass, kg			
Women	35.7 (32.2–40.1)	36.7 (34.2–38.3)	0.86
Men	51.5 (46.9–53.0)	49.6 (46.7–53.9)	0.81
Fasting glucose, mg/dL	84.9 ± 6.1	85.2 ± 6.5	0.84
Two-hour postload glucose, mg/dL	83 (66–87)	85 (73–99)	0.24
Fasting insulin, mU/L	5.6 (3.7–7.5)	4.9 (3.5–6.6)	0.24
HOMA-IR	1.16 ± 0.5	1.03 ± 0.4	0.28
Triglycerides, mg/dL	78 (63–110)	82 (57–96)	0.34
Total cholesterol, mg/dL	160 (150–187)	165 (155–182)	0.72
HDL cholesterol, mg/dL			
Women	53.9 ± 11.7	53.6 ± 9.9	0.90
Men	37.7 ± 5.5	42.8 ± 14.7	0.38
LDL cholesterol, mg/dL	99.8 ± 24.2	96.2 ± 19.0	0.50
SBP, mm Hg	102.5 ± 9.3	100.9 ± 11.6	0.53
DBP, mm Hg	69.2 ± 5.4	66.4 ± 7.5	0.08
Heart rate, bpm	69.1 ± 10.1	68.3 ± 7.1	0.71
Intake			
Energy, kcal/d	1748 (1435–2074)	1719 (1397–2066)	0.33
Carbohydrate, g/d	210 ± 65	211 ± 70	0.97
Protein, g/d	89 (77–111)	89 (71–108)	0.74
Lipids, g/d	66 ± 24	57 ± 22	0.16
Fiber, g/d	18 (13–23)	18 (13–26)	0.82
Sugars, g/d	63 (45–87)	58 (39–76)	0.25
Energy expenditure, kcal/d	2364 ± 457	2383 ± 444	0.86

[1] Values are means ± SDs or medians (IQRs) unless otherwise indicated. bpm, beats per minute; DBP, diastolic blood pressure; SBP, systolic blood pressure.
[2] Calculated with Student's *t* test, Mann-Whitney *U* test, or chi-square test according to the type and distribution of the variables.

The global adherence to the intervention was high (88.1%); mean ± SD sucralose consumption was 157.7 ± 12.9 mg/d for men and 123 ± 12.9 mg/d for women.

The changes in glucose metabolism variables in both groups are presented in **Table 2**. In the intention-to-treat analysis, sucralose consumption resulted in a significant decrement of Si from 5.8 (4.4–7.6) to 4.9 (3.4–6.7) × 10^{-4} min^{-1} · $(mU/L)^{-1}$ ($P < 0.01$) and in the per-protocol analysis from 6.0 (3.9–7.6) to 4.9 (3.4–6.7) · 10^{-4} min^{-1} · $(mU/L)^{-1}$ ($P < 0.01$). In the intention-to-treat analysis, the Si percentage change was −17.7% (−29.3% to −1.0%) in the sucralose group compared with −2.8% (−30.7% to 40.6%) in the control group ($P = 0.04$ between groups), with similar results in the per-protocol analysis. To corroborate the effects of sucralose and to evaluate if other variables could be intervening in the results, we adjusted the change of Si in a multiple linear regression model by the changes in weight, physical activity, calories, and sugars consumed throughout the intervention in both groups. We also considered the adjustment of other variables that showed a tendency ($P \leq 0.20$) in bivariate correlations with the percentage change for Si. These variables were age, BMI, waist circumference, and percentage of body fat; however, only age and BMI were included due to multicollinearity between the other variables. The 988 mg dextrose and maltodextrins provided in each sucralose sachet consumed by the participants were added to sugar consumption in the intervention group to eliminate any influence that this factor could produce. The only variable that significantly modified the Si was sucralose consumption ($P = 0.02$), with a β-coefficient of −0.278 (95% CI: −0.419, −0.023) and R^2 of 0.254 for this model; the other factors were not significant.

To calculate the RR for decreasing Si, we created a dichotomous variable according to the change in Si (reduction or no change/increment). The RR for decreasing Si in the intervention group was 1.48 (95% CI: 1.00, 2.20; $P = 0.04$). In the multiple logistic regression analysis, we adjusted this RR by the same variables included in the linear regression and we obtained similar results. The only factor associated with

TABLE 2
Changes in glucose metabolism variables[1]

	Intervention group ($n = 30$)			Control group ($n = 31$)						
	Baseline	Final	% Change	Baseline	Final	% Change	P^2	P^3	P^4	P^5
Intention-to-treat analysis										
Si, x 10^{-4} min^{-1}· (mU/L)$^{-1}$	5.8 (4.4–7.6)	4.9 (3.4–6.7)	−17.7 (−29.3 to −1.0)	5.8 (4.4–8.3)	5.3 (4.3–9.0)	−2.8 (−30.7 to 40.6)	0.88	<0.01	0.65	0.04
AIRg, mU· L^{-1}· min	594 (378–1096)	703 (389–1020)	9.5 (−7.1 to 53.2)	586 (372–798)	524 (377–737)	6.0 (−28.6 to 20.9)	0.60	0.19	0.73	0.37
DI, x 10^{-4} [Si x AIRg]	3832 (2557–5756)	3813 (1962–5858)	−13.1 (−34.1 to 25.2)	3520 (2242–4804)	3264 (2320–4744)	−9.7 (−25.8 to 44.8)	0.47	0.16	0.78	0.44
Sg, min^{-1}	0.022 (0.019–0.025)	0.021 (0.016–0.025)	−5.9 (−40.3 to 23.0)	0.020 (0.017–0.024)	0.020 (0.016–0.024)	−1.0 (−23.7 to 33.7)	0.25	0.36	0.65	0.70
Per-protocol analysis[6]										
Si, x 10^{-4} min^{-1} · (mU/L)$^{-1}$	6.0 (3.9–7.6)	4.9 (3.4–6.7)	−17.9 (−29.5 to 1.4)	5.8 (4.4–8.3)	5.3 (4.3–9.0)	−2.80 (−30.7 to 40.6)	0.96	<0.01	0.65	0.04
AIRg, mU · L^{-1} · min	577 (350–1040)	671 (376–1010)	16.2 (−3.6 to 55.9)	586 (372–798)	524 (377–737)	6.0 (−28.6 to 20.9)	0.94	0.04	0.73	0.16
DI, x 10^{-4} [Si x AIR g]	3640 (2545–5170)	3590 (1734–4980)	−3.7 (−31.8 to 26.8)	3520 (2242–4804)	3264 (2320–4744)	−9.7 (−25.8 to 44.8)	0.82	0.50	0.78	0.72
Sg, min^{-1}	0.021 (0.019–0.026)	0.021 (0.016–0.025)	−3.8 (−39.9 to 25.3)	0.020 (0.017–0.024)	0.020 (0.016–0.024)	−1.0 (−23.7 to 33.7)	0.40	0.54	0.65	0.88

[1] Values are medians (IQRs) according to the variable's distribution. AIRg, acute insulin response; DI, disposition index; Sg, glucose effectiveness; Si, insulin sensitivity.
[2] Differences between baseline values of the 2 groups using the Mann-Whitney *U* test.
[3] Differences between baseline and final values in the intervention group using a Wilcoxon matched-pairs test.
[4] Differences between baseline and final values in the control group using a Wilcoxon matched-pairs test.
[5] Differences between percentage change values of the 2 groups using the Mann-Whitney *U* test.
[6] $n = 27$ for the intervention group.

the decrease in Si was sucralose consumption, with an OR of 5.34 (95% CI: 1.39, 20.51; P= 0.01). The other factors (age, BMI, changes in weight, physical activity, calories, and sugars consumed throughout the intervention in both groups) were not significant.

DISCUSSION

A moderate consumption of sucralose (15% of the ADI) over 14 d was associated with a decreased Si in healthy adults. The randomized controlled design of this report allows us to avoid potential confounding factors that have resulted in controversial results in observational studies. Our study provides confirmatory evidence that sucralose has a negative impact on insulin action, even in healthy individuals.

There are several potential mechanisms to explain the decreased insulin sensitivity associated with sucralose consumption, such as the interaction with sweet taste receptors T1R2 and T1R3 in pancreatic β cells and enteroendocrine cells promoting insulin and GLP-1 release, respectively (5–8). In addition, the increased expression of SGLT-1 and GLUT-2 transporters stimulates active and passive glucose transport (16, 17). All of these factors combined may produce a constant state of higher glucose and insulin concentrations in habitual NNS consumers. Changes in gut microbiota produced by NNS consumption may generate a dysbiosis affecting glucose metabolism. Suez et al. (3) performed experiments in rats and reported marked glucose intolerance caused by different NNSs, with saccharin having the worst effect. They carried out a small study in 7 subjects who consumed 100% of the saccharin ADI over 7 d and observed the same increment in glucose concentrations in 4 participants. Significant changes in the gut microbiota of the participants designated as NNS-responders were observed. Later, a fecal transplant was performed in mice, which reproduced the effect of increased glucose concentrations 7 d after the transplant, with a significant increase in *Bacteroides fragilis* and *Weissella cibaria* combined with a decrease in *Candidatus arthromitus*; suggesting that saccharin modifies gut microbiota composition, altering glucose tolerance. Other studies in animal models have shown that sucralose and aspartame negatively modify gut microbiota; specifically, sucralose reduced helpful bacteria and fecal pH (23, 24).

The findings of Pepino et al. (2) are in agreement with the results of our study. They performed a crossover study using an OGTT preceded by consumption of 48 mg sucralose in a single dose (acute exposure) in comparison to only water consumption in 17 morbidly obese participants (15 women and 2 men, 13 African Americans and 4 whites) and measured insulin sensitivity with the minimal model approach. They found a significant decrease in insulin sensitivity and insulin clearance in addition to higher glucose and insulin concentrations. The differences in design, population, and intervention between these studies along with their similar conclusions complement the evidence for the effects of sucralose on glucose metabolism.

In our study, for participants with adequate adherence to the intervention, sucralose consumption was also associated with an increase in pancreatic response. This may represent a compensatory effect for the decrease in Si. However, the percentage change in pancreatic response between groups showed only a tendency toward statistical significance. This may be due to a lack of statistical power or a short exposure duration. The mechanisms for the increased insulin secretion might include interaction with sweet taste receptors in pancreatic β cells and a possible increment in GLP-1 induced by sucralose.

There is an emerging need to explore the proposed pathways by which NNSs may generate alterations in glucose metabolism in humans. Evaluation of the impact on insulin sensitivity by each

NNS should be considered in further randomized controlled long-term studies.

Cohort studies have shown an association between the consumption of artificially sweetened beverages and an increased risk to develop type 2 diabetes. However, the evidence is questionable due to reverse causality (i.e., overweight or obese people tend to consume this kind of product and they are at risk of developing metabolic diseases) (4, 25). Randomized controlled long-term studies are needed to assess the impact of sucralose in people at risk of type 2 diabetes. The lack of effect observed in other trials and a recent meta-analysis (26) could be related to the difficulty detecting significant changes in glucose metabolism with the selected outcome variables (e.g., glycated hemoglobin, fasting glucose, and/or insulin), coupled with short-term exposure to the NNS. We used the modified FSIVGTT, which is an accurate test to evaluate the pancreatic response and has good correlation with the euglycemic-hyperinsulinemic clamp to determine insulin sensitivity (22).

The limitations of this study include that an identical placebo was not available for the control group. The commercial sachets used in the intervention group contain a small quantity of caloric sweeteners (dextrose and maltodextrins); however, this represents a minimal amount of the participants' sugar consumption and was adjusted for in the multivariable analysis. In addition, we selected the sucralose dose on the basis of regular consumption, which might not be enough to detect other significant changes. In addition, the exposure period (14 d) might not be long enough to modify significantly other glucose metabolism variables, and we cannot affirm that these effects will remain, or improve or worsen, with sucralose consumption for a longer duration. Finally, we did not explore mechanisms for the observed decrease in insulin sensitivity, but this was not the aim of this study and will be the subject of future investigations. The strengths of our report are the study design and a homogenous population with healthy characteristics and good adherence to the intervention.

This research highlights the need for further studies to confirm the observed metabolic effects of sucralose and their mechanisms. The nutritional treatment of people with obesity and diabetes should focus on making lifestyle changes, such as consuming a healthful diet and increasing physical activity on a regular basis, without promoting sweet-tasting foods regardless of whether or not they contain caloric sweeteners or NNSs.

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