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ORIGINAL ARTICLE

Sucralose enhances GLP-1 release and lowers blood glucose in the presence of carbohydrate in healthy subjects but not in patients with type 2 diabetes

S Temizkan[1], O Deyneli[1], M Yasar[1], M Arpa[2], M Gunes[1], D Yazici[1], O Sirikci[2], G Haklar[2], N Imeryuz[3] and DG Yavuz[1]

BACKGROUND/OBJECTIVE: Artificial sweeteners were thought to be metabolically inactive, but after demonstrating that the gustatory mechanism was also localized in the small intestine, suspicions about the metabolic effects of artificial sweeteners have emerged. The objective of this study was to determine the effect of artificial sweeteners (aspartame and sucralose) on blood glucose, insulin, c-peptide and glucagon-like peptide-1 (GLP-1) levels.

SUBJECTS/METHODS: Eight newly diagnosed drug-naive type 2 diabetic patients (mean age 51.5 ± 9.2 years; F/M: 4/4) and eight healthy subjects (mean age 45.0 ± 4.1 years; F/M: 4/4) underwent 75 g oral glucose tolerance test (OGTT). During OGTT, glucose, insulin, c-peptide and GLP-1 were measured at 15- min intervals for 120 min. The OGTTs were performed at three settings on different days, where subjects were given 72 mg of aspartame and 24 mg of sucralose in 200 ml of water or 200 ml of water alone 15 min before OGTT in a single-blinded randomized order.

RESULTS: In healthy subjects, the total area under the curve (AUC) of glucose was statistically significantly lower in the sucralose setting than in the water setting ($P = 0.002$). There was no difference between the aspartame setting and the water setting ($P = 0.53$). Total AUC of insulin and c-peptide was similar in aspartame, sucralose and water settings. Total AUC of GLP-1 was significantly higher in the sucralose setting than in the water setting ($P = 0.04$). Total AUC values of glucose, insulin, c-peptide and GLP-1 were not statistically different in three settings in type 2 diabetic patients.

CONCLUSIONS: Sucralose enhances GLP-1 release and lowers blood glucose in the presence of carbohydrate in healthy subjects but not in newly diagnosed type 2 diabetic patients.

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INTRODUCTION

Artificial sweeteners are frequently used to sweeten beverages and to decrease caloric intake by diabetic or nondiabetic adults in daily life. They were thought to be metabolically inactive, but after demonstrating that the gustatory mechanism was localized not only at the tongue but also in the small intestine, suspicions about the metabolic effects of artificial sweeteners have emerged.[1–5]

About 6 years ago, in *in vitro* studies, it was shown that G-protein gustducin and three subunits (α, β and γ) and other elements of the taste signals (T1r1, T1r2, T1r3, TRPM5, PLCβ2) were localized on the enteroendocrine L cells, and they are responsible for the incretin effect.[3,5] Artificial sweeteners are capable of stimulating T1r2 and T1r3 receptors in the taste cells. Therefore, it is concluded that the gut directly senses glucose and sweet compounds such as artificial sweeteners, which leads to glucagon-like peptide-1 (GLP-1) release from enteroendocrine cells. However, there are only few studies in humans, especially in diabetic patients.

In this study, we aimed to examine the acute effect of artificial sweeteners (aspartame, sucralose) on gut hormones (GLP-1), insulin, c-peptide and blood glucose.

MATERIALS AND METHODS

Subjects

The study included eight healthy volunteers (four male and four female, mean age 45.0 ± 4.1 years and mean body mass index (BMI) 30.3 ± 4.5 kg/m^2) and eight newly diagnosed, drug-naive type 2 diabetic patients (four male and four female, mean age 51.5 ± 9.2 years and mean BMI 33.7 ± 5.4 kg/m^2). The criteria for exclusion were smoking, substance abuse, chronic medical or psychiatric illness, history of gastrointestinal disease or surgery and pregnancy. The study subjects were not using any medications (antihipertansives, statins, antidiabetics and so on). Newly diagnosed patients with type 2 diabetes had a fasting plasma glucose ⩾ 126 mg/dl on two occasions and/or hemoglobin A1c ⩾ 6.5 % (48 mmol/mol).

The study was conducted prospectively at Marmara University Medical School Endocrinology Outpatients Clinic between 1 March 2012 and 15 November 2013. The study protocol was approved by the Local Ethics Committee of Marmara University and was performed in accordance with the Declaration of Helsinki II. All study subjects gave oral and written informed consent before the examination.

Study design

On three settings, a 75 g oral glucose tolerance test (OGTT) was performed for each study subject on different days. Fifteen minutes before OGTT, subjects drank 72 mg of aspartame in 200 ml of water or 24 mg of

[1]Department of Endocrinology and Metabolic Diseases, Marmara University Faculty of Medicine, Istanbul, Turkey; [2]Department of Biochemistry, Marmara University Faculty of Medicine, Istanbul, Turkey and [3]Department of Gastroenterology, Marmara University Faculty of Medicine, Istanbul, Turkey. Correspondence: Dr S Temizkan, Department of Endocrinology and Metabolic Diseases, Marmara University Faculty of Medicine, Pendik, Yukari Kaynarca, Istanbul 34890, Turkey.
E-mail: suletemizkan@yahoo.com
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sucralose in 200 ml of water or 200 ml of water alone in a single-blinded randomized manner. Sweeteners (aspartame—Canderel, sucralose—Splenda) were purchased from a pharmacy. As the aspartame is 200 times and the sucralose is 600 times sweeter than sucrose, we gave sucralose in 1/3 dosage of aspartame to have the same sweet-taste stimulation.[6] On each test day, after 10 h of fast, the tests began in the morning at 0900 hours. Blood was drawn at regular time intervals (−15, 0, 15, 30, 45, 60, 75, 90, 105 and 120 min). Minute −15 was the time at which the artificial sweetener in water or nonsweetened water was given and minute 0 was the time point of the beginning of OGTT. Blood samples were collected into plain tubes and BD P800 tubes (Becton Dickinson Company, Franklin Lakes, NJ, USA), which contained the DPP-4 inhibitor and EDTA. After centrifugation at 4 °C and at 3000 *g*, plasma was separated and stored at −80 °C.

Laboratory analysis

Active GLP-1 was measured using a commercially available ELISA Kit (IBL, Hamburg, Germany). This kit is for nonradioactive quantification of biologically active forms of GLP-1 (7–36 amide). Measurement range was 1.25–80 pmol/l. Insulin and C-peptide were measured with electrochemiluminescence immune assay (ECLIA) in a modular analytics E170 autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany). Glucose was measured in a spectrophotometric Cobas 8000 autoanalyzer with the hexokinase method (Roche Diagnostics GmbH).

Statistical analysis

Descriptive statistics was used for demographic variables, such as age, weight, height and BMI. Individual hormone concentrations versus time data were used to obtain area under the curve (AUC). Total AUC for each hormone (GLP-1, insulin and C-peptide) and glucose was calculated by using the trapezoidal method. Differences between water and either sweeteners were assessed using paired *t*-test or Wilcoxon's signed-rank test according to the distribution of the data. Differences between three settings were assesed with one-way analysis of variance for repeated measurements. Differences were considered to be significant with $P < 0.05$. Data are presented as means with their s.d.

RESULTS

Demographic characteristics of eight patients with newly diagnosed type 2 diabetes and eight healthy subjects are in Table 1. The curves of the glucose, insulin, c-peptide and GLP-1 concentrations during 75 g OGTT in patients with type 2 diabetes and in healthy subjects are given in Figures 1 and 2, respectively.

Only 60th-min glucose concentration was statistically lower in the sucralose setting compared with the water setting in patients with type 2 diabetes ($P = 0.002$); however, other time points were similar. There were no statistically significant individual time-point differences in insulin, c-peptide and GLP-1 concentrations in the three settings in patients with type 2 diabetes. Total AUC values of glucose, insulin, c-peptide and GLP-1 were similar in the three settings (aspartame, sucralose and water) in patients with type 2 diabetes (Table 2).

Time of glucose peak was earlier (30–45 *vs* 60 min) in aspartame and sucralose settings compared with the water setting in healthy subjects. Thirty-minute blood glucose levels were lower in both sweeteners than in the water setting (aspartame *vs* water, $P = 0.01$, sucralose *vs* water, $P = 0.01$) in healthy subjects. However, 60th- and 120th-min glucose concentrations were similar in three settings. Total AUC of glucose was significantly lower in the sucralose setting than in the water setting ($P = 0.002$) in healthy subjects. Total AUC values of glucose in aspartame and water settings were similar in healthy subjects ($P = 0.53$).

There were no statistically significant individual time-point differences in insulin, c-peptide and GLP-1 concentrations in three settings in healthy subjects. Total AUC values of insulin and c-peptide were similar in all settings in healthy subjects (Table 3).

Total AUC of GLP-1 was significantly higher in the sucralose setting than in the water setting ($P = 0.04$), but in the aspartame setting it was not different from that in the water setting ($P = 0.48$) in healthy subjects (Table 3).

GLP-1 peak was earlier in healthy subjects than in patients with type 2 diabetes in three settings (15th min *vs* 45th min).

Table 1. Demographic features of the participitants

	Healthy subjects	*Type 2 diabetic patients*
Number (F/M)	8 (4/4)	8 (4/4)
Age (years)	45.0 ± 4.1	51.5 ± 9.2
BMI (kg/m^2)	30.3 ± 4.5	33.7 ± 5.4
Glucose (mmol/l)	4.5 ± 0.3	6.2 ± 0.8
HbA1c (mmol/mol)	38 ± 3	54 ± 5
(%)	5.6 ± 0.3	7.1 ± 0.5

Values are means ± s.d. Abbreviations: BMI, body mass index; hbA1c, hemoglobin A1c.

DISCUSSION

Although most of the *in vitro* studies support the idea that artificial sweeteners might increase GLP-1 secretion from enteroendocrine cells, *in vivo* studies in humans are conflicting.[3,5,7–14] Our study is one of the few human studies that examines the effects of artificial sweeteners (aspartame and sucralose) on blood glucose, insulin, c-peptide and gut hormone GLP-1 in newly diagnosed type 2 diabetic patients and in healthy subjects. It is really important to determine whether artificial sweeteners are metabolically inert or active, as they are frequently used to sweeten beverages or foods by diabetic patients and healthy subjects in daily life, and in our study we found that sucralose enhances GLP-1 release and lowers blood glucose in healthy subjects.

Until now, there are studies that only show the acute effects of artificial sweeteners on intestinal taste receptors and gut hormones, but long-term effects are not investigated. Mostly, the studies are about the effects of the chronic consumption of these sweeteners on blood glucose regulation.[15–18] Our study is also an acute study. In this study, we used sweeteners separately on different settings and we used sweeteners in lower dosages compared with prior studies. We used aspartame or sucralose with similar doses in our daily life. Aspartame and sucralose were used in dosages of 72 mg (18- mg tablet × 4) and 24 mg (6- mg tablet × 4) in 200 ml of water, respectively. As sucralose is 600 and aspartame is 200 times sweeter than sucrose, sucralose was given in a 1/3 dosage of aspartame to sweeten 200 ml of water to obtain similar tastes.

In a prior study from Brown *et al.*,[7] diet soda was used to evaluate the effects of sweeteners in type 1 and type 2 diabetic patients and healthy subjects. In this study, diet soda contained a mixture of sweeteners (26 mg of asesulfam K and 46 mg of sucralose) and was given 10 min before glucose load. In this study, it was found that GLP-1 AUC was significantly higher in sweetened soda setting in healthy subjects and type 1 diabetic patients but not in type 2 diabetic patients, although glucose and insulin AUC was not different from the nonsweetened soda setting. In our study, as in a prior study, we found GLP-1 total AUC to be higher in the sucralose setting; however, we also found glucose total AUC to be lower in the sucralose setting than in the water setting in healthy subjects. In our study, glucose total AUC was lower and GLP-1 AUC was higher only in the sucralose setting but not in the aspartame setting. This result might be explained by different structures of the sweeteners, which may have different effects on glucose metabolism. It is known that aspartame is metabolized by proteolytic enzymes in the gastrointestinal system to aspartic acid, phenylalanine and methanol, but sucralose is a nonmetabolizable, stable sweetener.[6]

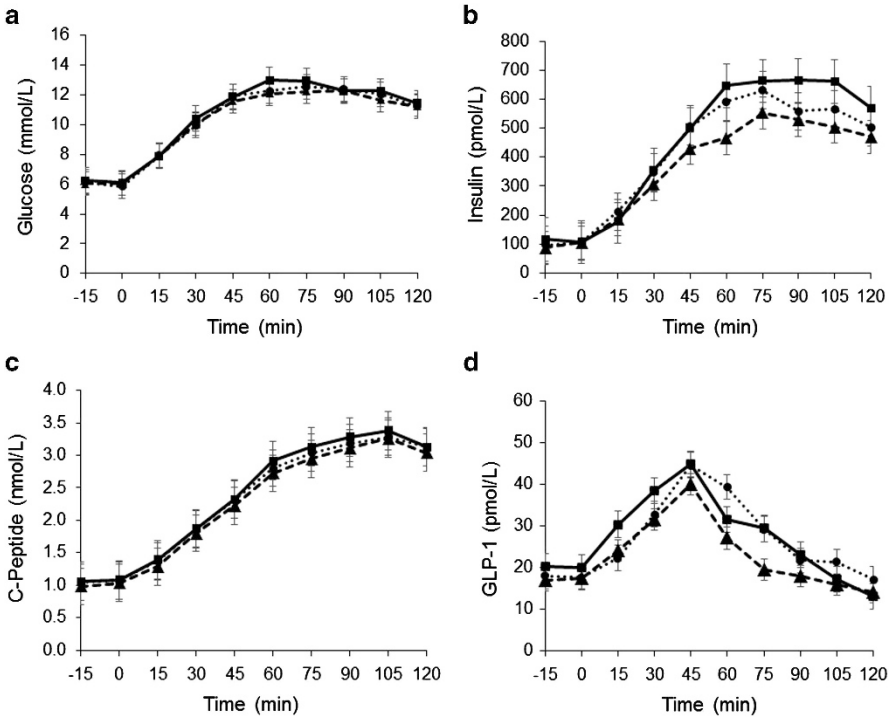

Figure 1. Glucose (**a**), insulin (**b**), C-peptide (**c**) and GLP-1 (**d**) levels during 75 g OGTT in newly diagnosed type 2 diabetes mellitus patients. Non-sweetened water (■) or aspartame-sweetened water (●) or sucrolose-sweetened water (▲) was given at – 15 min and glucose was given at 0 min. Values are means with their standard errors.

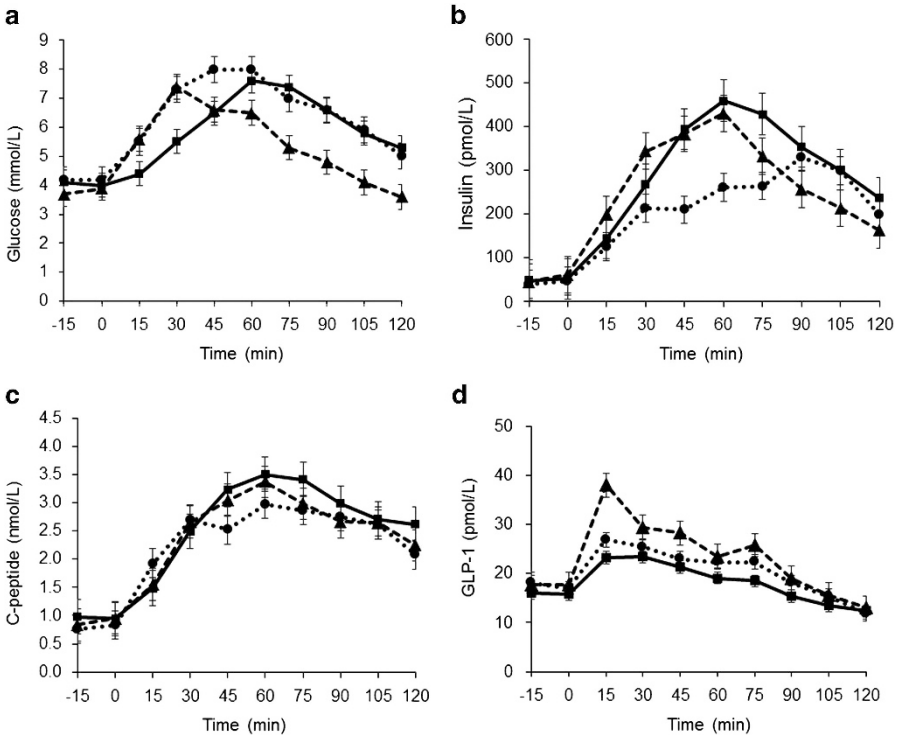

Figure 2. Glucose (**a**), insulin (**b**), C-peptide (**c**) and GLP-1 (**d**) levels during 75 g OGTT in healthy subjects. Non-sweetened water (■) or aspartame-sweetened water (●) or sucrolose-sweetened water (▲) was given at – 15 min and glucose was given at 0 min. Values are means with their standard errors.

In a study from Pepino *et al.*,[19] which was conducted among healthy subjects, sucralose (48 mg in 60 ml of water) or an equivalent volume of water alone was given before a 75 g OGTT.[19] The peak increases in plasma glucose, c-peptide, insulin concentrations and total insulin AUC were greater when subjects consumed sucralose compared with water alone. In this study, sucralose ingestion did not affect the GLP-1 response to a glucose load.

Table 2. AUC of glucose, insulin, c-peptide and GLP-1 in newly diagnosed type 2 diabetic patients

	Sucralose	*Aspartame*	*Water*	P-*value*[a]	P-*value*[b]	P-*value*[c]
Glucose (mmol/l 120 min)	1388 ± 307	1405 ± 306	1434 ± 284	0.54	0.09	0.45
Insulin (pmol/l 120 min)	50 ± 9	57 ± 15	62 ± 18	0.26	0.08	0.07
C-peptide (nmol/l 120 min)	303 ± 48	309 ± 66	322 ± 37	0.44	0.24	0.46
GLP-1 (pmol/l 120 min)	3139 ± 1298	3699 ± 1504	3777 ± 1706	0.86	0.54	0.17

Paired *t*-test or Wilcoxon's signed-rank test was used according to the distribution of the data for comparing two settings. One-way ANOVA for repeated measurements was used for comparing three settings. Values are means ± s.d. Abbreviations: ANOVA, analysis of variance; AUC, area under the curve during 75 g OGTT; GLP-1, glucagon-like peptide-1; OGTT, oral glucose tolerance test. [a]Difference between aspartame-sweetened water and nonsweetened water. [b]Difference between sucralose-sweetened water and nonsweetened water. [c]Difference between three settings ($P < 0.05$).

Table 3. AUC of glucose, insulin, c-peptide and GLP-1 in healthy subjects

	Sucralose	*Aspartame*	*Water*	P-*value*[a]	P-*value*[b]	P-*value*[c]
Glucose (mmol/l 120 min)	385 ± 68	855 ± 165	787 ± 272	0.53	0.002	< 0.001
Insulin (pmol/l 120 min)	35 ± 13	28 ± 17	38 ± 25	0.14	0.69	0.29
C-peptide (nmol/l 120 min)	321 ± 82	309 ± 116	339 ± 102	0.66	0.74	0.87
GLP-1 (pmol/l 120 min)	3192 ± 1108	2778 ± 1491	2463 ± 772	0.48	0.04	0.32

Paired *t*-test or Wilcoxon's signed-rank test was used according to the distribution of the data for comparing two settings. One-way ANOVA for repeated measurements was used for comparing three settings. Values are means ± s.d. Abbreviations: ANOVA; analysis of variance; AUC, area under the curve during 75 g OGTT; GLP- 1, glucagon-like peptide-1; OGTT, oral glucose tolerance test. [a]Difference between aspartame-sweetened water and nonsweetened water. [b]Difference between sucralose-sweetened water and nonsweetened water. [c]Difference between three settings ($P < 0.05$).

Another recent report different from our study was made again in healthy subjects by Wu *et al.*[20] In this study, 52 mg of sucralose or 200 mg of acesulfam K or 46 mg of sucralose plus 26 mg of acesulfam K in 240 ml of water or 240 ml of water alone was given before a 75 g OGTT.[20] Blood glucose, plasma insulin and total GLP-1 concentrations were not different after neither sweetened water nor only water settings. In other studies, sucralose was given directly through a nasogastric tube, and glucose was not given simultaneously. In these studies, GLP-1 release was not stimulated.[9–11]

There is evidence in animal studies that activation of sweet-taste receptors by artificial sweeteners increases SGLT-1 expression and enhances intestinal glucose absorption via the upregulation of GLUT2,[5,21,22] but in our study glucose AUC was lower in the sucralose setting in healthy subjects. GLP-1's insulinotropic effect might be the reason, but insulin and C-peptide UAC were not different from the water setting. It is known that increased GLP-1 inhibits glucagon secretion.[23] In our study, apart from GLP-1's insulinotropic effect, the inhibition of glucagon might be the reason for lower glucose AUC in healthy subjects; unfortunately, we did not study glucagon.

Although we observed a differential effect of sucralose between patients with diabetes type 2 and healthy subjects, it is known that GLP-1 release is not substantially affected and the transcription of the taste molecules in the gut is not decreased in type 2 diabetes.[24,25] Brown *et al.*[7] found that diet soda (asesulfam K and 46 mg of sucralose) does not enhance GLP-1 release in type 2 diabetics but enhances GLP-1 release in healthy subjects like in our study. In a study from Gregersen *et al.*[12] in which 1 g of stevioside was given with a test meal in type 2 diabetic patients, postprandial glucose levels were lower, insulin levels were higher in the stevioside setting than in the placebo setting, although GLP-1 levels were similar. In another study, sucralose was given at a dosage of 1000 mg before breakfast in type 1 and type 2 diabetic patients.[13] In this study, glucose and c-peptide AUC was not different from placebo.

In summary, sucralose enhances GLP-1 release and lowers blood glucose in the presence of glucose in healthy subjects but not in newly diagnosed type 2 diabetic patients. Additional studies are needed to clarify conflicting results in the literature, and also additional studies are needed to examine the chronic consumption effects of artificial sweeteners on gastrointestinal taste molecules and gut hormones.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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