Sweetness Perception is not Involved in the Regulation of Blood Glucose after Oral Application of Sucrose and Glucose Solutions in Healthy Male Subjects

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Scope: This study investigates the effect of the sweetness of a sucrose versus an isocaloric glucose solution in dietary concentrations on blood glucose regulation by adjusting the sweetness level using the sweet taste inhibitor lactisole.

Methods and Results: A total of 27 healthy males participated in this randomized, crossover study with four treatments: 10% glucose, 10% sucrose, 10% sucrose + 60 ppm lactisole, and 10% glucose + 60 ppm lactisole. Plasma glucose, insulin, glucagon-like peptide 1, and glucagon levels are measured at baseline and 15, 30, 60, 90, and 120 min after beverage consumption. Test subjects rated the sucrose solution to be sweeter than the isocaloric glucose solution, whereas no difference in sweetness is reported after addition of lactisole to the sucrose solution. Administration of the less sweet glucose solution versus sucrose led to higher blood glucose levels after 30 min, as reflected by a lower ΔAUC for sucrose (1072 ± 136) than for glucose (1567 ± 231). Application of lactisole leads to no differences in glucose, insulin, or glucagon responses induced by sucrose or glucose.

Conclusion: The results indicate that the structure of the carbohydrate has a stronger impact on the regulation of blood glucose levels than the perceived sweetness.

1. Introduction

Sweet taste is innately highly preferred by humans, but the global excessive consumption of sweet tasting carbohydrates largely contributes to the overall energy intake,[1] leading to an increased risk for obesity and comorbidities like type 2 diabetes.[2] One of the main sources for dietary sugars are sugar-sweetened beverages like fruit drinks, lemonades, and ice tea.[3] Beside the caloric load, the consumption of such sugar-sweetened beverages is associated with the exposure to a high level of sweetness. The perceived sweetness has been hypothesized to interact with signaling pathways of insulin secretion, also known as cephalic phase insulin release (CPIR), in the regulation of blood glucose levels.[4–7] The finding that chemosensory signaling pathways of sweet taste receptors are not only present in the oral cavity, but also in non-gustatory-tissues like the

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gastrointestinal tract and the pancreas, fueled the debate about the impact of an activation of hT1R2/hT1R3 on the regulation of blood glucose levels, which is also affecting the usage of non-caloric sweeteners.[8] However, results are conflicting, and the contribution of the perceived sweetness to its metabolic effect of a beverage remains unclear. For example, sweet taste receptors expressed in the gut directly affected glucose metabolism, independently of sweetness signaling in the mouth.[9] But also the activation of sweet taste receptors in the oral cavity influenced the blood glucose regulation by a higher secretion of insulin.[10] In contrast, the acute consumption of sweeteners without caloric load did not have an impact on the regulation of blood glucose,[11,12] arguing against the regulatory role of sweetness. A more recent study concluded that only the combination of the non-caloric sweetener sucralose and a carbohydrate, but neither sucralose nor the carbohydrate alone, impairs insulin sensitivity.[13] The regulatory process of blood glucose concentrations is characterized by a complex interaction of various hormones like insulin, glucagon-like peptide 1 (GLP-1), and glucagon and neuropeptides derived from brain, pancreas, liver, intestine, muscle, and adipocyte tissue.[14] A dysregulation of this complex interactions can lead to serious diseases like type 2 diabetes and associated comorbidities. Thus, a precise understanding of the blood glucose regulation affecting parameters is of high social and scientific relevance.

A sucrose solution is rated significantly sweeter than an iso-caloric glucose solution by trained panelists (own unpublished data, also described by Carocho et al.[15]) and a previous study by Crapo et al. showed that an iso-caloric administration of 100 g glucose or 100 g sucrose led to different peaks in blood glucose levels.[16] However, it is not yet known whether the different level of sweetness of glucose and sucrose contributes to the above-named differences in the blood glucose peaks, or whether these differences are only based on their different structures, namely a monosaccharide versus a disaccharide. The disaccharide sucrose consists of one molecule of glucose and fructose. Fructose has different metabolic effects than glucose, which can also contribute to differences in blood glucose metabolism. For example, fructose does not stimulate insulin secretion,[17] leading to reduced plasma glucose and insulin responses when fructose- opposed to glucose-sweetened beverages are consumed.[18] Another characteristic of fructose is that fructose metabolism occurs primarily in the liver.[19]

To determine the effect of sugars on blood glucose levels, most of the conducted studies used high amounts (up to 100 g of the tested sugars),[16,20–22] exceeding the quantities typically consumed at one time, leading to exalted effects regarding blood glucose levels, associated hormones, and neuropeptides. Thus, in the present study, test solutions were chosen to imitate a typical amount of sugar in sugar-sweetened soft drinks or juices. To test iso-caloric sucrose and glucose solutions with a similar sweetness level, the sweetness of the test solutions was modulated using lactisole. Lactisole, the sodium salt of 2-(4-methoxyphenol)propionic acid, is a selective competitive inhibitor of the T1R3 subunit of the human sweet taste receptor.[23] To the best of our knowledge, previous studies applied lactisole in high concentrations solely, completely eliminating the stimulatory effect of sugars and sweeteners.[20] However, no studies are currently available that applied lactisole for adjusting the sweet-

ness of different sugars in a human intervention trial to obtain equally sweet tasting solutions using the same concentrations of sugars.

In summary, in the context of the prevention of nutrition-dependent diseases, it is of special interest to understand whether the blood glucose response is modulated by the sweetness perception of the test solution, or mainly based on the structure of the carbohydrate. Thus, in the present study, we aimed to investigate the impact of a sweetness modulation of a sucrose solution in comparison to a glucose solution on blood glucose metabolism of male healthy subjects. We hypothesized here that the structure (monosaccharide vs disaccharide) as well as the sweetness may have an impact on the regulation of blood glucose levels in healthy subjects.

2. Experimental Section

2.0.0.1. Participants: Thirty-nine male subjects were recruited for a medical screening by advertisements in web forums and billboards at Universities in Vienna. The study inclusion criteria were metabolic healthy males aged between 18 and 45 years with a body mass index between 18.5 and 30 kg m^{-2} and no taste disorders. Fasting blood glucose <120 mg dl^{-1} was mandatory for registration. The exclusion criteria were major chronic diseases, metabolic diseases such as type 2 diabetes or lipometabolic disorders, tobacco consumption, medical treatment, alcohol or drug abuse, as well as intolerances or allergies to test products.

Female test persons were excluded from the study, because of fluctuations concerning blood glucose levels during menstrual cycle, which can distort the results of this study.[24]

The present study procedures were approved by the ethical committee of the University of Vienna (approval no. 00432). All study participants provided written informed consent prior to the interventions.

2.0.0.2. Design: This study was a single blinded, cross-over human intervention study with four different interventions and all measurements were conducted using coded samples. Participants received four different interventions on four study days. The four visits were carried out at least five days apart. Participants were blinded to the treatment allocation. All participants were randomly assigned to the treatments using the online tool "randomizer.org", and the sequence of the treatments was balanced.

2.0.0.3. Test Solutions: According to amounts commonly found in soft drinks or juices, a concentration of 10% (w/v) sucrose in 300 mL water was chosen. Hence, a solution using the same concentration of glucose was selected. A sucrose solution is rated to be sweeter than an iso-caloric glucose solution.[15] The sweetness of the iso-caloric glucose and sucrose solution (10% w/v) was adjusted to a similarly rated sweetness by the addition of 60 ppm lactisole to the sucrose solution in preliminary tests (*n* = 5, data not shown). The applied test solutions were thus as follows: 1) 10% (w/v) glucose in 300 mL water, 2) 10% (w/v) sucrose in 300 mL water, 3) 10% (w/v) sucrose in 300 mL water with 60 ppm lactisole, and 4) 10% (w/v) glucose in 300 mL water with 60 ppm lactisole as an additional control for the effect of lactisole.

2.0.0.4. Dosage Information: Participants ingested 30 g of glucose or 30 g of sucrose with or without 60 ppm lactisole

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respectively. Glucose, sucrose, as well as lactisole were dissolved in tap water. All four test solutions were ingested by every participant once, on different days, at least five days apart. Participants were instructed to drink the test solutions within five minutes after the first sip. The dose of glucose and sucrose corresponds to those typically found in soft drinks or juices. Lactisole has been used in a previous study with a dosage of 500 ppm.[20]

2.0.0.5. Procedure: All participants were asked to attend five sessions consisting of one medical screening session and four consecutive test sessions. Metabolic disorders were excluded during the medical screening session. Fasting hematological parameters, plasma lipids, as well as glucose concentrations in plasma and urine samples 60 and 120 min after an oral glucose tolerance test (oGTT) were analyzed by "Ihr Labor 1220" (Medical diagnostics laboratory, Dr. Gabriele Greiner, Vienna, Austria). The compliant elevation of blood glucose levels during the oGTT was additionally monitored after 15, 30, 60, 90, and 120 min with a blood glucose meter in the capillary blood of the fingertip (Accu-Chek Performa, Roche, Switzerland). Also, blood pressure measurements were conducted in triplicates. Basic anthropometric measurements were recorded, namely body height with a precision of 0.01 m by means of a stadiometer (Seca, Germany) and body weight to the nearest of 0.1 kg using a body scale (Soehnle, Germany). Participants were asked to fill out 1) a SCOFF questionnaire, to identify and exclude eating disorders,[25] and 2) a screening questionnaire including questions such as food allergies or intolerances, chronic diseases, and basic health information.

In addition, the sweet threshold level of the test persons was determined according to DIN EN ISO 3972:2013–12 in Höhl and Busch–Stockfisch (2015).[26] The sweet intensity was rated for each test solution on an unstructured scale [0–10] after pre-tasting five sweet solutions with 0 to 100 g L^{-1} sucrose as "not at all" to "very intensive" sweetness. The test compounds were dissolved in tap water. All sensory tests were conducted in a sensory laboratory. The test solutions were additionally rated on every test day as described above, but without pre-testing of different sucrose solutions, to ensure no different sweet perception between screening day and study day and between sensory laboratory and study room.

Power analysis by means of the software GPower 3.1 resulted in an estimated number of 26 test subjects based on a study of Pepino et al.,[27] with an effect size of 0.55 (power of 0.85, $\alpha = 0.05$). A total of 39 subjects was recruited, out of which 29 volunteers passed the medical screening. One volunteer did not finish the study due to personal reasons, and one participant was excluded due to obvious violation against the study protocol. Accordingly, 27 participants completed all four treatments and were included in the study. The mean characteristics of the participants are given in **Table 1**.

An overview of the study protocol is shown in **Figure 1**. On each study day, baseline blood collection (t0) after 12 h overnight fast was carried out. Further blood samples were collected 15, 30, 60, 90, and 120 min after administration of the test solution. The participants were asked to rate the sweetness of the respective test solution on a 10 cm unstructured scale (0 cm = not at all and 10 cm = very intensive). After the last blood collection, a standard continental breakfast was served as described in previous studies.[28,29]

Table 1. Study subjects´ characteristics.

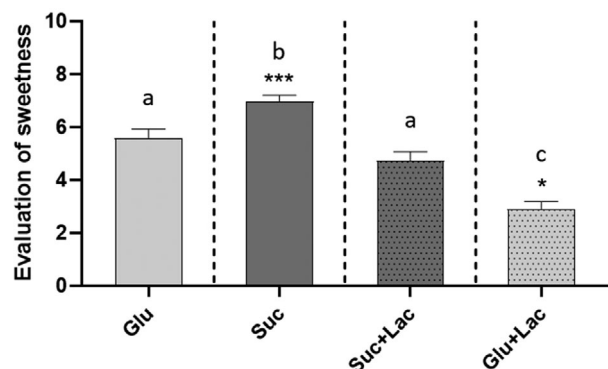| n	27
Gender	male
Age [years]	27.6 ± 0.88
Body Weight [kg]	77.8 ± 2.29
Height [m]	1.81 ± 0.01
BMI [kg m^{-2}]	23.7 ± 0.07

Data are depicted as mean ± SEM.
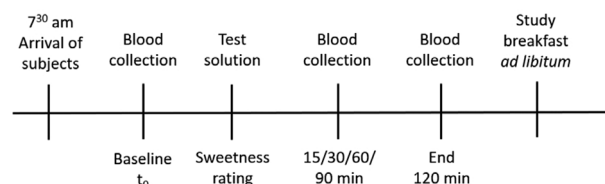
Figure 1. Flow chart showing the procedure of the study day. A total of 27 volunteers underwent the following four interventions as test solutions in a randomized order: 1) 10% sucrose in 300 mL water, 2) 10% sucrose in 300 mL water with 60 ppm lactisole, 3) 10% glucose in 300 mL water, and 4) 10% glucose in 300 mL water with 60 ppm lactisole.

2.0.0.6. Blood Sample Collection: Venous blood samples were collected in EDTA-coated monovettes (Sarstedt, Germany), centrifuged immediately at 1800 x g at 4 °C for 15 min and the plasma was stored at −80 °C until analysis for concentrations of GLP-1 and glucagon. In addition, blood was collected in fluoride-coated monovettes to determine plasma glucose and heparin-coated monovettes (both Sarstedt, Germany) were used for plasma insulin as described previously.[30]

2.0.0.7. Plasma Concentrations of total GLP-1, Glucagon, Glucose, and Insulin: Total GLP-1 (LOD: 2 pм, inter-assay CV 8 ± 4.8%, intra-assay CV 7.4 ± 1.1%) and glucagon (LOD: 2.5 pg mL^{-1}, inter-assay CV < 12%, intra-assay CV < 10%) plasma concentrations were determined by means of a sandwich ELISA (Merck Millipore, Darmstadt, Germany, and Thermo–Fisher Scientific, Waltham, USA, respectively). Plasma glucose concentrations were quantitated by a colorimetric assay with an LOD of 0.23 mg dl^{-1} (inter-assay CV 1.7%, intra-assay CV 4.6%) (Cayman Europe, Tallinn, Estonia). Insulin concentrations in the plasma were assessed using sandwich ELISA (LOD: 50 pg mL^{-1}, inter-assay CV 2.6%, intra-assay CV 5.99%) obtained from IASON (Graz, Austria).

2.0.0.8. Statistical Analyses: Statistical analyses were performed using GraphPad Prism 8. Normally distributed data sets, assessed by a Shapiro–Wilk test, are presented as means ± standard errors of the mean (SEM) unless stated otherwise. In case of no normal distribution, a non-parametric test was applied as indicated in the figure legends. Statistically significant differences were assumed at $P < 0.05$. Time dependent effects were determined by a mixed effect analysis with Tukey´s multiple comparison. To test for differences between two treatments, a two-tailed, paired t-test was conducted. Δ values were calculated by subtracting the baseline values (t0). Area under the curve (AUC) was calculated according to the trapezoidal rule. For glucose and insulin, the positive ΔAUC over time, and for GLP-1 and glucagon total ΔAUC over time was calculated. Correlation was assessed by

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Figure 2. Sensory evaluation of test solutions. Participants rated the sweetness of the four different test solutions on an unstructured scale [0–10]. Values are shown as mean ± SEM. Statistically significant differences were tested by one-way ANOVA on Ranks ($P < 0.05$). *** indicates statistically significant difference compared to glucose solution ($P < 0.001$) and distinct letters are used to mark statistically significant differences in the sweetness level ($P < 0.05$).

Pearson Correlation between glucose and threshold, sweet perception, BMI as well as for insulin and insulin/glucose ratio, respectively.

3. Results

3.1. Sensory Evaluation–Rating of Sweetness

As described above, unpublished data from sensory studies showed that sweetness of a 10% glucose and 10% sucrose can be adjusted by adding 60 ppm lactisole to the sucrose solution. This resulted in a similar sweetness, also called equi-sweetness, of 10% glucose and 10% sucrose with lactisole. Sensory evaluation of the test solutions by the sensorially untrained test subjects in the present study was carried out by rating the sweetness on the screening day in a sensory laboratory, and on each study day directly after application of the test solution. As displayed in **Figure 2**, the sensorially untrained test subjects rated the glucose solution to be less sweet than the sucrose solution ($P < 0.001$). After addition of 60 ppm lactisole to the 10% sucrose solution, there was no difference in the rating between glucose and the sucrose with lactisole solution in sweet sensation ($P = 0.85$). As expected, the glucose solution with 60 ppm lactisole was rated less sweet then the glucose solution ($P = 0.01$). The above presented sensory evaluation results originate from the screening day in the sensory laboratory. There was no significant different rating for sweetness level of the test solutions at the screening day or the study day.

3.2. Plasma Concentrations of Glucose and Insulin

The plasma glucose level was lower after the administration of 10% sucrose solution compared to 10% glucose solution after 30 min ($P = 0.01$, **Figure 3**A), which is mirrored by a reduced ΔAUC ($P = 0.023$, Figure 3B). The application of the glucose solution elicited a 31.56% ± 6.04% higher plasma glucose level
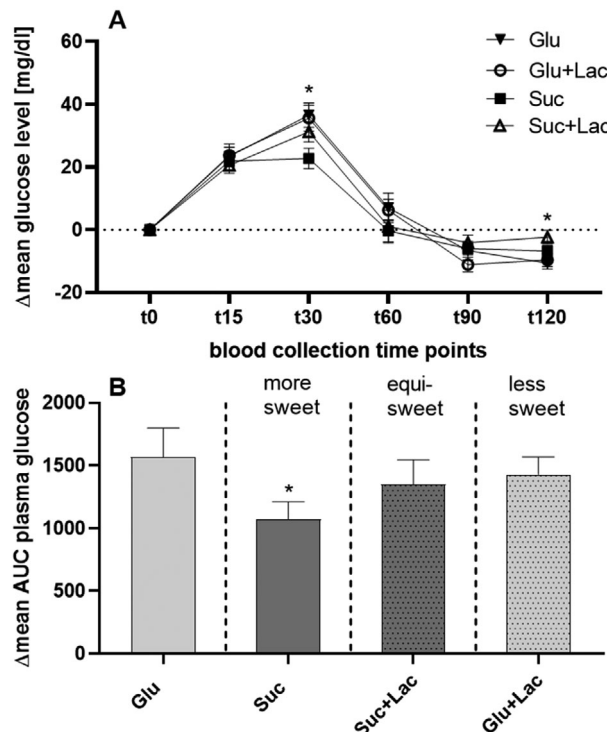
Figure 3. A) Mean change in plasma glucose level normalized to the level at fasting and 15, 30, 60, 90, and 120 min after administration of glucose (Glu), glucose with lactisole (Lac), sucrose (Suc), and sucrose with lactisole ($n = 27$ respectively). Values are shown as mean ± SEM. Statistical difference ($P < 0.05$) was determined by mixed effect analysis with multiple comparisons. * Indicates significant difference after 30 min (Glu vs Suc) and significant difference after 120 min (Glu vs Suc+Lac). B) Effect of glucose versus sucrose administration on plasma glucose; effect of glucose versus sucrose with lactisole administration on plasma glucose, and effect of glucose versus glucose with lactisole on plasma glucose (expressed as AUC [mg dl^{-1} × min] respectively) in 27 healthy volunteers. Values are shown as mean ± SEM. Statistically significant differences against glucose-treatment were tested by Student's t-test ($P < 0.05$). * indicates significant differences.

over time compared to sucrose (ΔAUC for plasma glucose 1567± 231 vs sucrose 1072 ± 136; $P = 0.02$). In contrast, the application of the equi-sweet test solutions (Figure 3B), 10% glucose versus 10% sucrose with 60 ppm lactisole, led to no significant difference in blood glucose levels over time (ΔAUC glucose 1567 ± 231 and ΔAUC sucrose with lactisole 1351 ± 193; $P = 0.29$). However, there was no effect of lactisole administration on 10% sucrose solution on blood glucose peaks ($P = 0.14$). In addition, there was no difference in blood glucose levels after application of the glucose solutions with or without the addition of lactisole (Figure 3B) (ΔAUC glucose 1567 ± 231 and ΔAUC glucose with lactisole 1427 ± 139; $P = 0.60$).

The regulation of insulin over time showed no differences in the time-dependent effect after administration of the test solutions (**Figure 4**A). However, the ΔAUC of insulin (Figure 4B) showed a trend ($P = 0.053$) towards a lower ΔAUC after administration of sucrose compared to the glucose solution (-21.4% ± 2.3%, ΔAUC glucose 2577 ± 278 and ΔAUC sucrose 2024 ± 219). Moreover, there was a significant difference ($P = 0.02$) in the

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Table 2. ΔPeak values of blood glucose, inlsun, GLP-1, and glucagon concentrations.

	Glu	Suc	Suc+Lac	Glu+Lac
ΔPeak glucose [mg dl^{-1}]	38.28 ± 3.68	30.98 ± 2.72#	37.61 ± 3.16	40.61 ± 3.49
ΔPeak insulin [μIU mL^{-1}]	61.98 ± 6.94	50.30 ± 4.70#	53.80 ± 6.61#	57.58 ± 6.02
ΔPeak GLP-1 [pM]	6.05 ± 0.93	5.49 ± 1.04	4.02 ± 0.80*	5.79 ± 0.96
ΔPeak glucagon [pg mL^{-1}]	0.56 ± 0.21	1.56 ± 0.77	1.69 ± 1.01	1.18 ± 0.65

Data are depicted as mean ± SEM. Statistical difference ($P < 0.05$) was determined by mixed effect analysis with multiple comparisons. *indicates statistically significant difference compared to glucose Treatment. #indicates a p for trend ($P < 0.1$).
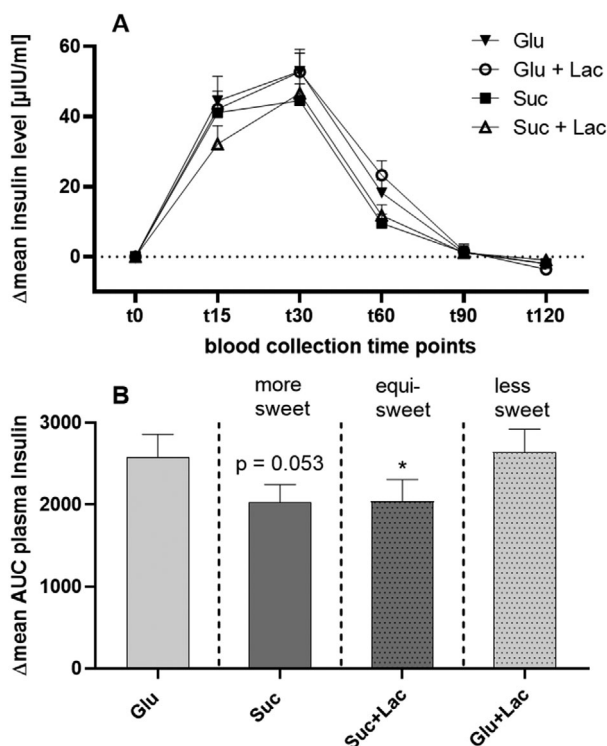
Figure 4. A) Mean change in plasma insulin level normalized to the level at fasting and 15, 30, 60, 90, and 120 min after administration of glucose (Glu), glucose with lactisole (Lac), sucrose (Suc) and sucrose with lactisole ($n = 27$ respectively). Values are shown as mean ± SEM. Statistical difference was determined by mixed effect analysis with multiple comparisons. B) Effect of glucose versus sucrose administration on plasma insulin, effect of glucose versus sucrose with lactisole administration on plasma insulin, and effect of glucose versus glucose with lactisole on plasma insulin (expressed as AUC [mg dl^{-1} × min] respectively) in 27 healthy volunteers. Values are shown as mean ± SEM. Statistically significant differences against glucose-treatment were tested by Student's-test ($P < 0.05$). * indicates significant differences.

ΔAUC for insulin after application of sucrose with lactisole compared to application of the glucose solution with a 21.08% ± 0.3% lower ΔAUC after the application of sucrose with lactisole (ΔAUC glucose 2577 ± 278 and ΔAUC sucrose with lactisole 2034 ± 271). Further, there was no difference in insulin levels neither between glucose without or with lactisole ($P = 0.76$), nor between sucrose and sucrose with lactisole ($P = 0.97$).

The Δpeak values for glucose and insulin reflect the results of the ΔAUC calculation, showing the lowest peak after administration of sucrose (**Table 2**). Also, the Δinsulin/Δglucose ratio (**Table 3**) was calculated for all treatments over time, there was no difference between the treatments and time points.

3.3. Plasma Concentrations of GLP-1 and Glucagon

For blood glucose regulation parameters, GLP-1 and glucagon concentrations were assessed at fasting and after administration over time for 120 min (t15, t30, t60, t90, t120). The administration of 10% glucose led to an increase in GLP-1 level compared to the equi-sweet solution 10% sucrose with 60 ppm lactisole at timepoint 30 min ($P = 0.01$, **Figure 5**A). This is also reflected in the ΔAUC values: the application of sucrose in combination with lactisole elicited a 102.66% decrease in plasma GLP-1 compared to the glucose solution ($P = 0.02$; ΔAUC glucose −219 ± 77 and ΔAUC sucrose with lactisole −446 ± 104). The application of the more sweet 10% sucrose solution compared to 10% glucose, as well as the less sweet solution 10% glucose with 60 ppm lactisole compared to glucose led to no difference ($P = 0.7$ and $P = 0.5$, respectively; Figure 5B). Lactisole had no influence on plasma GLP-1 concentrations after glucose administration ($P = 0.5$; glucose compared with glucose + lactisole). In contrast, GLP-1 levels were lower after the administration of sucrose in combination with lactisole compared to the administration of sucrose ($P = 0.04$).

The application of the different solutions led to no difference in the glucagon plasma levels neither at the time-response-curve, nor at the ΔAUC as depicted in **Figure 6**A,B ($P > 0.05$; Δ AUC glucose −51.42 ± 19.85, ΔAUC sucrose 69.71 ± 74.37, ΔAUC sucrose with lactisole 88.73 ± 103.48, ΔAUC glucose with lactisole 43.38 ± 71.73). Concomitant application of lactisole with either sucrose or glucose did not lead to differences in plasma glucagon concentrations. Notably, eight out of 27 participants were under the limit of detection, resulting in a number of 19 subjects for glucagon.

The Δpeak values for GLP-1 and glucagon are in accordance with the calculated ΔAUCs (Table 2). Further, the calculated Δglucagon/Δinsulin ratio showed no difference between treatments and time points (Table 3).

3.4. Correlation Analysis

To answer the question, if there is an association between the glucose regulation and their hormones with the sweet thresh-

Table 3. ΔRatios for insulin/glucose and glucagon/insulin over time.

	Treatment	t15	t30	t60	t90	t120	Fixed-effect P value
ΔInsulin/ΔGlucose	Glu	2.27 ± 0.29	2.48 ± 0.67	0.56 ± 0.69	−0.21 ± 0.40	0.50 ± 0.25	0.61
	Suc	4.69 ± 2.55	1.40 ± 0.60	−28.28 ± 29.36	2.53 ± 2.09	0.24 ± 0.32	
	Suc+Lac	2.24 ± 0.62	1.45 ± 0.57	1.93 ± 1.11	−0.03 ± 0.41	−0.81 ± 0.91	
	Glu+Lac	0.29 ± 1.86	1.37 ± 0.50	−5.94 ± 6.87	0.41 ± 0.56	0.37 ± 0.22	
ΔGlucagon/ΔInsulin	Glu	0.005 ± 0.008	−0.007 ± 0.003	0.280 ± 0.298	−0.412 ± 0.238	0.051 ± 0.233	0.56
	Suc	0.017 ± 0.011	0.008 ± 0.013	0.235 ± 0.278	−0.592 ± 0.587	−0.010 ± 0.149	
	Suc+Lac	−0.191 ± 0.214	0.053 ± 0.058	−0.230 ± 0.166	−0.275 ± 0.157	−0.118 ± 0.142	
	Glu+Lac	0.016 ± 0.016	0.006 ± 0.011	0.007 ± 0.020	0.150 ± 0.094	0.002 ± 0.174	

Data are depicted as mean ± SEM. Statistically significant differences ($P < 0.05$) were excluded by mixed effect analysis with multiple comparisons.
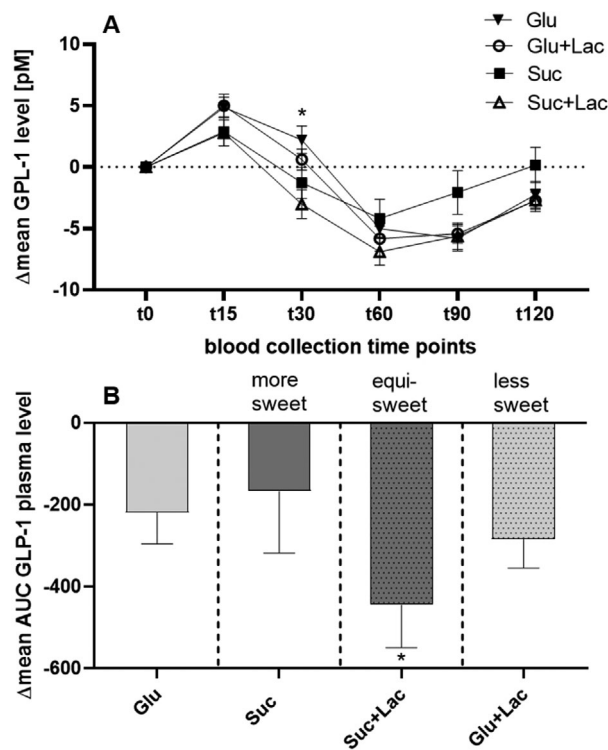
Figure 5. A) Mean change in plasma GLP-1 level normalized to the level at fasting and 15, 30, 60, 90, and 120 min after administration of glucose (Glu), glucose with lactisole (Lac), sucrose (Suc) and sucrose with lactisole ($n = 27$ respectively). Values are shown as mean ± SEM. Statistical difference ($P < 0.05$) was determined by mixed effect analysis with multiple comparisons. * Indicates significant difference after 30 min (Glu vs Suc+Lac). B) Effect of glucose versus sucrose administration on plasma GLP-1, effect of glucose versus sucrose with lactisole administration on plasma GLP-1 and effect of glucose versus glucose with lactisole on plasma GLP-1 (expressed as AUC [mg dl^{-1} × min] respectively) in 27 healthy volunteers. Values are shown as mean ± SEM. Statistically significant differences against glucose-treatment were tested by Student's t-test ($P < 0.05$). * indicates significant differences.
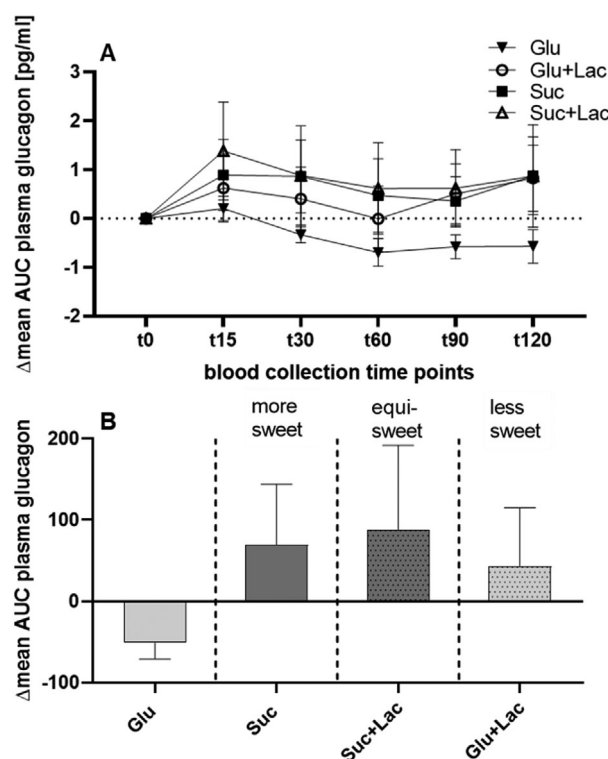
Figure 6. A) Mean change in plasma glucagon level normalized to the level at fasting and 15, 30, 60, 90, and 120 min after administration of glucose (Glu), glucose with lactisole (Lac), sucrose (Suc) and sucrose with lactisole ($n = 19$ respectively). Values are shown as mean ± SEM. Statistical difference ($P < 0.05$) was excluded by mixed effect analysis with multiple comparisons. B) Effect of glucose versus sucrose administration on plasma glucagon, effect of glucose versus sucrose with lactisole administration on plasma glucagon and effect of glucose versus glucose with lactisole on plasma glucagon (expressed as AUC [mg dl^{-1} × min] respectively) in 19 healthy volunteers. Values are shown as mean ± SEM. Statistically significant differences against glucose treatment were excluded by Student's t-test ($P > 0.05$).

old and sweet perception, a correlation analysis using Pearson's product moment correlation was carried out. However, neither a correlation between the regulation of glucose, GLP-1, and insulin with the individual sweetness rating, nor an association the threshold for sweet taste was found (data not shown). Furthermore, the results of correlation analysis of sweet perception and sweet threshold with BMI showed no association (data not shown).

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4. Discussion

Sweetness perception has been postulated to interact with hormones to regulate blood glucose levels via targeting sweet taste receptor signaling in the oral cavity and in non-gustatory tissues.[8] However, the impact of the sweetness level of sugar-sweetened beverages on blood glucose levels in typically consumed amounts is not well understood.

In the present study, we investigated the role of the different sweetness of isocaloric glucose and sucrose solutions via adjusting the sweetness level using the sweet taste inhibitor lactisole in amounts typically used in soft drinks. We hypothesized here that if the sweetness drives reinforcement on blood glucose levels, then equi-sweet solutions of the two different carbohydrates should induce a similar response on blood glucose levels.

Being the essential base for investigating this hypothesis, we confirmed that the sensorially untrained test persons were able to distinguish the different levels of sweetness of sucrose and glucose with or without the addition of lactisole. This was of importance, since other studies showed that untrained test persons not always rank the sweetness of sucrose as sweeter than an equicaloric glucose solution.[31] In addition, the participants of the present study rated the solutions containing glucose and sucrose in combination with lactisole with the same sweetness level. This result given, the participants underwent the four consecutive interventions in a cross-over design using 10% sucrose and 10% glucose without or with the addition of 60 ppm lactisole, analyzing plasma concentrations of glucose, insulin, glucagon, and GLP-1.

Results of the plasma glucose levels after application of the different test solutions showed a time-dependent difference after application of the glucose and the sucrose solution, due to the significant higher blood glucose plasma level at time point 30 min after glucose administration compared to the sucrose administration. This result confirmed the initial hypothesis of a differential blood glucose level after glucose and sucrose consumption and is in accordance with the results by Crapo et al., who showed a time-dependent difference in the increase of the plasma glucose levels when comparing a glucose or sucrose load.[16] This indicates a time-dependent component in the absorption of different sugars which may be attributed to the fact that glucose and sucrose vary in their structure, monosaccharide and disaccharide, leading to a delay in the absorption of glucose from the disaccharide sucrose. It has to be noticed that fructose, which is a part of the disaccharide sucrose, is differently metabolized as glucose.[17–19] After ingestion, glucose is taken up by enterocytes mainly via the sodium-dependent glucose transporter 1 (SGLT-1), whereas fructose is predominantly absorbed passively from the intestinal lumen via GLUT-5.[32] Moreover, in contrast to glucose, fructose is not an insulin secretagogue and is mainly metabolized by the liver.[33] Fructose sweetened beverages lead to a reduced plasma glucose and insulin response compared to glucose sweetened beverages,[18] which is likely to contribute to the lower glucose peak after 30 min. This result was also reflected by the incremental ΔAUC, at which glucose application resulted in higher plasma glucose levels over total time compared to sucrose application. In contrast, after application of the sucrose solution supplemented with lactisole as equi-sweet solution to the glucose solution this effect was abolished, there were no differences in

the glucose plasma levels over time. However, there was neither a direct effect of lactisole on sucrose-mediated plasma glucose levels, nor did the supplementation of the glucose solution with the same amount of lactisole, 60 ppm, change plasma glucose levels over time. This result suggests that the sweetness of the glucose and sucrose solutions had no impact on blood glucose regulation in the present study. Although it should be finally clarified, why the difference between glucose and sucrose was abolished when applied as equi-sweet solutions, the missing direct effect of lactisole on either sucrose- or glucose–induced blood glucose peaks strongly suggest that the structure plays a predominant role on blood glucose regulation. Notably, these are the results of healthy participants, in patients with type 2 diabetes, the regulation of the sweet taste receptors in response to glucose exposure is disordered,[34] which could modify short-term responses as well.

In the next step, we analyzed if and how hormones involved in the regulation of blood glucose levels are affected by the test solutions. First, the plasma insulin concentration was analyzed as one of the most important hormones regarding the regulation of the glucose homeostasis with blood glucose-lowering effects.[35] The time curve of plasma insulin showed no significant time-dependent effect following the different treatments. Comparison of the ΔAUCs after application of sucrose versus glucose indicated a trend toward a lower ΔAUC after oral ingestion of sucrose, which is in accordance with the data obtained for the blood glucose levels. Moreover, the application of the sucrose solution supplemented with lactisole resulted in a significant lower ΔAUC compared to the equi-sweet glucose solution, indicating that the structure of the carbohydrate has more impact than the sweetness perception. This is further supported by the comparison of the ΔAUC for insulin after ingestion of glucose with or without lactisole at which no difference was detected, although the glucose with lactisole was the least sweet solution tested in the present study. If the sweetness would have a regulatory impact on plasma insulin levels, no difference in the equi-sweet solutions, but a difference comparing glucose and glucose with lactisole would have been expected. Additionally, a direct effect of lactisole on sucrose-mediated insulin secretion was also not detected. To summarize the data obtained for insulin, the regulation of insulin levels was not associated with the sweetness of the test solution. However, Karimian Azari et al. reported that the application of 500 ppm lactisole prior to a 12.5% glucose solution increased plasma responses to insulin in ten healthy subjects.[20] The higher amount of lactisole used in the study by Karimain Azari et al., blocking the sweet sensation completely, in addition to the time-dependent effect caused by the prior application of lactisole may explain the difference to the results of our study. Moreover, it has to be noticed, that a study by Renwick et al. did not find an effect of the sweetness of a solution on insulin regulation.[8] In the 1990s, a number of studies hypothesized that the stimulation of the sweet taste receptor on the tongue can act as signaling for insulin release, known as CPIR.[36] There was no evidence of an increase in insulin after tasting various low-energy sweeteners, which led to the conclusion that the sweetness level has no effect on insulin release.[36] This issue has been raised again in a more recent study,[37] in which blood insulin levels were measured in volunteers who tasted different solutions for 45 s. The authors reported that both, sucrose and saccharin led to an increase in insulin, but the increases in insulin for starch and

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water were larger than those for the sweeteners. Further, a recent study showed that the taste of sugars which contain glucose, but not high-intensive sweeteners, elicited CPIR in mice.[38] The authors hypothesized that carbohydrates were hydrolyzed in the mouth, and that the released glucose triggered CPIR. Our results on insulin are in accordance with the described studies and provide evidence that the sweetness perception does not have a major effect on the insulin release, it is rather the carbohydrate itself. Also, a recently published study concluded that short-term consumption of sucralose decreased insulin sensitivity only when applied in the combination with a carbohydrate.[13] Also, the calculated peak values are in agreement with the ΔAUCs. The Δinsulin/Δglucose ratio shows no difference between the treatments, which does not support that the sweetness induces an exaggerated insulin response. Also, the Δglucagon/Δinsulin ratio shows no difference, indicating that the glucagon response fits to the insulin response and the sweetness does not influence the need for endogenous hepatic glucose production.

Blood glucose is not only regulated by insulin, but by a complex interaction of several hormones, amongst others GLP-1, which is known to enhance pancreatic insulin secretion, and to suppress pancreatic glucagon secretion.[39] In contrast to the results obtained for plasma glucose and insulin levels, lactisole administration reduced GLP-1 plasma levels applied in combination with sucrose, but not glucose. This result does not support a general impact of the sweetness, but points to the involvement of the different structure between sucrose and glucose and requires further investigation. We hypothesize that a different interaction with the sweet taste receptor may have an impact on GLP-1 secretion: lactisole targets the T1R3 subunit,[40] and while glucose and sucrose both target the venus flytrap domains of T1R2 and T1R3, a higher binding affinity of glucose for T1R2, and of sucrose for T1R3 is described.[41] This may explain that sucrose-mediated effects are more sensitive to lactisole-effects, but this requires further mechanistic studies. Due to the selected concentrations as typically found in soft drinks, 10% sucrose or glucose in 300 mL water corresponding to 30 g of sugar, lower GLP-1 peaks were expected compared to studies that applied a regular oGTT with 75 g of glucose.[42,43] The GLP-1 curve shows the peak value at time point 15 min which is in agreement with the insulin response with a peak value at time point 30 min, as insulin release is described to be controlled by GLP-1. In more detail, GLP-1 is secreted directly in response to glucose present in the chymus and subsequently promotes insulin secretion via GLP-1 receptors expressed in pancreatic beta cells.[44] Overall, the here presented results from plasma GLP-1 concentrations are in accordance with the insulin data and suggest as well that the sweetness plays a minor role on the regulation of GLP-1 release after sugar-sweetened beverages consumption. If the sweetness would have an impact on GLP-1 release, the equi-sweet solutions are expected to lead to no difference in plasma GLP-1 concentrations, whereas the different sweet tasting solutions, sucrose versus glucose, as well as glucose with lactisole versus glucose alone, would be expected to lead to differences in the time-dependent regulation of plasma GLP-1. The artificial sweetener sucralose has been reported to induce release of GLP-1 and GIP from the murine endocrine cell line GLUTag by activation of the sweet taste receptor,[45] which suggests that the sweetness plays a major role for GLP-1 release. However,

although mechanistically interesting, the tested concentration of 50 mM sucralose is very high and raises the question of the physiological relevance. Moreover, a study from Fujita et al. does not support the notion that sweeteners acutely induce the release of meaningful quantities of incretin hormones in rodents.[46] Further, Wu et al. conclude that the prior exposure to sweetness in form of the artificial sweeteners sucralose and acesulfame K did not influence GLP-1 concentration as well as blood glucose and plasma insulin after ingestion of 75 g glucose.[47] These results are in agreement with a clinical study that reported no effect of oral stevioside consumption on release of GIP and GLP-1 in subjects with type 2 diabetes,[48] supporting the assumption that the sweetness would have rather only little or even no impact on GLP-1 release.

The results of glucagon concentrations revealed no differences in the time-dependent regulation after application of the test solutions. The amount of carbohydrates administered in the present study was lower than the concentrations commonly used for an oGTT, which may have influenced the glucagon regulation. Since no differences in the regulation were found, no statement regarding a different impact of mono- or disaccharide or the sweetness can be made. However, since there was no effect of lactisole on sucrose- or glucose-mediated glucagon responses, a major impact of the sweetness can be excluded. We hypothesized that an influence of the individual sweetness perception on blood glucose parameters, especially insulin and GLP-1, would be mirrored in a correlation to the subject's sweet taste threshold and the sweet perception. More precisely, a higher sweet threshold and a low insulin release should then correlate with each other as well as a high sweet perception with a low insulin release. This should be also reflected in correlation analysis with GLP-1 release, at which also a high sweet threshold and a low GLP-1 release as well as a high sweet perception and a low GLP-1 release would be expected to correlate, if the sweetness is involved. However, the Pearson's product moment correlation analysis showed no effect between sensory parameters and blood glucose parameters insulin and GLP-1. This supports our results from insulin and GLP-1, that the sweetness has no or only a minor impact on blood glucose hormones.

This study has potential limitations. First, no female subjects were included in the study due to interaction of blood glucose regulation with female sex hormones. Second, the blood samples to analyze glucose and regulating hormones were drawn at six different time points, a higher resolution could have refined the data, especially at the earlier time points. Moreover, future studies should consider the incretin hormone GIP beside the here analyzed GLP-1 to analyze the lactisole-effect on sucrose and glucose. Also, long-term intervention studies are needed to investigate if a prolonged activation of sweet taste receptors would enhance glucose absorption via upregulation of intestinal glucose transporters. In addition, further studies are needed to clarify if lactisole mixed with glucose and sucrose maintained the same level of suppression on the sweet taste receptor signal throughout the observation window and to test the effects of higher concentrations of lactisole for complete inhibition of the sweet taste receptor.[23] Having that said, a major strength of the current study includes its robust and clear cross-over study design using the sweet taste inhibitor lactisole for adjusting the sweetness of a glucose and sucrose solution in typically consumed concentrations. Moreover,

the achieved power with the 27 test subjects is 82% (1-β) based on the reached effect size of 0.5 for the differences in the plasma glucose levels after glucose and sucrose consumption and an error probability of 0.05 (G-Power 3.1).

To summarize, the present study investigated for the first time the influence of the sweetness compared to the structure of glucose and sucrose on blood glucose regulation in dietary-relevant concentrations. The results obtained for insulin, GLP-1, and glucagon levels argue against a major role for the sweetness of the test solutions in the regulation of hormone levels. In addition, there was no association between the sweetness perception and the plasma glucose or the hormone levels. In conclusion, sweetness perception plays no major role in the differences in the time-dependent regulation of blood glucose following oral ingestion of a sucrose versus a glucose solution. Future studies with modulated sweetness are needed to analyze the differences in the impact of lactisole on glucose and sucrose in GLP-1 regulation. Moreover, the results provide a solid basis for future studies to unravel the role of sweetness perception in blood glucose levels after carbohydrate consumption in females, and to study the long-term impact of sweet carbohydrates.

Conflict of Interest

The authors G. Krammer and J. P. Ley are employees at Symrise AG, Holzminden, Germany.

Author Contributions

The authors´ responsibilities were as follows – B.L., V.S., J.P.L., and G.E.K. designed the research. V.G. and K.S. conducted this study. K.S. and C.G. did blood sample preparations. C.G. and J.T. analyzed the blood samples. C.M.K. and C.G. performed the sensory study. V.G. performed data and statistical analysis. J.K. provided access to a fully equipped sensory laboratory. V.G. and B.L. wrote the manuscript. B.L. had primary responsibility for the final content. All authors edited the manuscript, provided comments, and approved the final version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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