The effect of the artificial sweeteners on glucose metabolism in healthy adults: a randomized double-blinded crossover clinical trial

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<td>Ahmad, Samar; University of Manitoba, Food and human nutritional sciences; Kuwait Ministry of Health, Food and nutrition department Friel, James; University of Manitoba, Human Nutritional Sciences MacKay, Dylan; University of Manitoba, 1Department of Food and Human Nutritional Sciences, Richardson Centre for Functional Foods and Nutraceuticals. Department of Community Health Sciences, University of Manitoba, Rady Faculty of Health Sciences</td>
</tr>
<tr>
<td>Novelty bullets: points that summarize the key findings in the work:</td>
<td>Daily consumption of pure aspartame or sucralose for 2 weeks had no effect on glucose metabolism., Daily consumption of pure aspartame or sucralose for 2 weeks had no effect on insulin sensitivity among healthy adults.</td>
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<td>Keyword:</td>
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The effect of the artificial sweeteners on glucose metabolism in healthy adults: a randomized double-blinded crossover clinical trial

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Abstract

**Background:** This study aims to determine the effect of pure forms of sucralose and aspartame, in doses reflective of common consumption, on glucose metabolism.

**Methods:** Healthy participants consumed pure forms of a non-nutritive sweetener (NNS) mixed with water that were standardized to doses of 14% (0.425 g) of the acceptable daily intake (ADI) for aspartame and 20% (0.136 g) of the ADI for sucralose every day for two weeks. Blood samples were collected and analysed for glucose, insulin, active glucagon-like peptide-1 (GLP-1), and leptin.

**Results:** Seventeen participants (10 females and 7 males; age 24 ± 6.8 years; BMI 22.9 ± 2.5 kg/m²) participated in the study. The total area under the curve (AUC) values of glucose, insulin, active GLP-1 and leptin were similar for the aspartame and sucralose treatment groups compared to the baseline values in healthy participants. There was no change in insulin sensitivity after NNS treatment compared to the baseline values.

**Conclusions:** These findings suggest that daily repeated consumption of pure sucralose or aspartame for 2 weeks had no effect on glucose metabolism among normoglycaemic adults. However, these results need to be tested in studies with longer durations.

**Novelty:**

- Daily consumption of pure aspartame or sucralose for 2 weeks had no effect on glucose metabolism.
- Daily consumption of pure aspartame or sucralose for 2 weeks had no effect on insulin sensitivity among healthy adults.

**Keywords:** non-nutritive sweetener, aspartame, sucralose, protocol, glucose metabolism, insulin, glucose, active GLP-1, leptin
Introduction

Non-nutritive sweeteners (NNSs) are novel chemosensory compounds in the food additive class that have been commonly used in different foods and beverages to provide an intense sweet taste and decrease caloric content (Gardner et al. 2012). NNSs are also used and recommended for managing weight and controlling blood glucose levels in individuals with obesity and diabetes (Gardner et al. 2012; Mattes and Popkin 2009). The US Food and Drug Administration (FDA) has approved some artificial sweeteners, including acesulfame potassium, neotame, saccharin, sucralose, aspartame and some natural sweeteners, including monk fruit extract and plant-derived stevia (U.S. Food & Drug Administration 2018). In Canada, acesulfame potassium, neotame, sucralose, aspartame, monk fruit extract, steviol glycosides and erythritol have been approved by Health Canada (Government of Canada 2019).

Evidence shows conflicting results regarding the effects of NNSs on health. For example, some studies have reported that NNSs can be associated with weight loss (Benton 2005; Mattes and Popkin 2009). Other observational and cohort studies have found that repeated consumption may be associated with an increased risk of overweight and obesity, diabetes mellitus, cardiovascular diseases and metabolic syndrome (Azad et al. 2017; Swithers 2013). In particular, repeated consumption of diet soda sweetened with NNSs may be associated with an increased risk of type 2 diabetes mellitus (T2DM) and metabolic syndrome, including abdominal obesity, impaired glucose tolerance, insulin resistance, high blood pressure (BP) and dyslipidaemia (Nettleton et al. 2009).

There are many proposed mechanisms for how NNSs may alter glucose metabolism and glycaemic control, such via cephalic phase insulin response, which might be weakened by repeated NNS use, causing eventual failure of the body to respond to actual sugar appropriately (Swithers et al. 2009, 2010). Another interesting possible mechanism is that
changes in glucose metabolism could be mediated by alterations in the gut microbiota (dysbiosis) caused by NNS consumption. Suez et al. (2014) demonstrated that daily intake of saccharine for 7 days caused glucose intolerance in four out of seven individuals. Furthermore, the change in glucose metabolism was shown to be mediated by gut microbiota dysbiosis, which was demonstrated when the faecal microbiota of humans were transplanted into germ-free mice. The recipient mice developed glucose intolerance as well (Suez et al. 2014). It is possible that NNSs might influence the growth of certain gut bacteria, leading to a microbial imbalance (Abou-Donia et al. 2008; Palmnas et al. 2014).

Recent studies that have investigated the effect of aspartame and sucralose on glucose metabolism and gut hormones are limited and have conflicting results (Ahmad et al. 2019). Randomized clinical trials have reported an effect of repeated daily doses of sucralose on insulin sensitivity and acute insulin response (Lertrit et al. 2018; Romo-Romo et al. 2018) and active glucagon-like peptide 1 (GLP-1) concentrations (Lertrit et al. 2018). Other randomized clinical trials have reported an acute effect of a single doses of sucralose on glucose (Pepino et al. 2013; Temizkan et al. 2015), insulin and insulin sensitivity (Pepino et al. 2013), and active GLP-1 concentrations (Brown et al. 2012; Temizkan et al. 2015). These results have not always been consistently replicated in other studies (Baird et al. 2000; Brown et al. 2011; Grotz et al. 2003, 2017; Sylvestsky et al. 2016; Wu et al. 2012, 2013).

Additionally, a few clinical trials have reported an acute effect of a single doses of aspartame on glucose (Melanson et al. 1999; Moller 1991), insulin (Horwitz et al. 1988), and active GLP-1 concentrations (Hall et al. 2003), while other studies investigating either repeated daily doses of aspartame (Bonnet et al. 2018; Higgins et al. 2018) or a single dose of aspartame (Anton et al. 2010; Bryant et al. 2014; Temizkan et al. 2015; Tey et al. 2017) could not confirm these results.
A recent systematic review analysed twenty-eight clinical trials evaluating the effects of NNSs on glucose metabolism. Most of the evaluated studies measured the acute effect of a single dose of an NNS (n=20), and the remaining studies (n=8) evaluated the effect of repeated doses of NNSs. In a systematic review, it was concluded that the effects of NNS consumption on glucose metabolism are still unclear and incomparable due to major protocol differences that exist between studies (Romo-Romo et al. 2016). Another systematic review and meta-analysis of randomized clinical trials (RCTs) (n=21) assessed the effect of aspartame consumption on fasting blood glucose and insulin concentrations. They reported that aspartame consumption had no association with changes in blood glucose or insulin concentration compared to the concentrations observed in the control group (Santos et al. 2018).

To date, few studies have assessed the effect of repeated daily consumption of aspartame and sucralose in beverages on glucose metabolism, insulin and GLP-1 hormone (Bonnet et al. 2018; Colagiuri et al. 1989; Lertrit et al. 2018; Romo-Romo et al. 2018). Aspartame and sucralose are the most commonly used sweeteners in diet beverages in Canada; therefore, these two artificial NNSs were investigated in this study (Garriguet 2008; Nikpartow et al. 2012). The acceptable daily intake (ADI) in Canada is 9 mg/kg body weight for sucralose, while that for aspartame is 40 mg/kg body weight (Pepsico Canada 2011).

Therefore, we decided to investigate the effect of pure aspartame and sucralose, without contamination from other ingredients present in diet sodas and at intakes reflecting normal daily consumption, on glucose metabolism in healthy adults.

Materials and methods

Recruitment and population
Participants were recruited in Winnipeg, Canada by using posters, flyers and advertisements around the University of Manitoba campus. Participants aged 18-45 years old were included if they had a body mass index (BMI) of 20-25 kg/m$^2$ and a fasting blood glucose (FBG) < 5.7 mmol/L (i.e., normal FBG) and were not regular users of NNSs. We defined regular users of NNSs as those consuming ≥ 1 can of diet beverages, one spoonful of NNSs or the equivalent per week in food products. We used the web-based version of the Canadian Diet History Questionnaire II (C-DHQ II) (Lo Siou et al. 2017) to screen for NNS intake over the last 12 months and to assess nutrient intake. The DHQ II includes questions that inquire about the type, quantity and frequency of consumption of artificial sweeteners used for tea, coffee, and other drinks and the intake of diet beverages (including fruit drinks, diet soda, iced tea and flavoured water).

Adherence to dietary recommendations was evaluated once a week during the treatment periods, and a 3-day food record was completed (Yang et al. 2010). Women who were taking oral contraceptive pills and/or who had irregular menstrual cycles were excluded from the study. Individuals were excluded from the trial if they were pregnant or lactating, had a history of alcohol or drug abuse, were on antibiotic medication or took probiotics within the 6-month period before the study, had any past or present medical conditions including metabolic or gastrointestinal disorders, or used medications known to impact glucose metabolism, gastric pH or gastric emptying. The protocol was reviewed and approved by the University of Manitoba Bannatyne Campus Biomedical Research Ethics Board (BREB) in Winnipeg, Manitoba, Canada. This trial was registered at clinicaltrials.gov under the number NCT02569762.

**Study design**
This study used a randomized, controlled, double-blinded, crossover design to investigate the effect of NNS consumption on glucose metabolism. This trial was conducted from 2016-2018 at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) at the University of Manitoba in Winnipeg. The trial consisted of two 2-week periods separated by a minimum of a 4-week washout period during which the participants were instructed to maintain their regular water intake and habitual diet. Participants were randomly assigned to 1 of 2 treatment orders by simple randomization after enrolment in the trial. Treatments were beverages containing either 14% of the ADI for aspartame or 20% of the ADI for sucralose to be consumed by participants daily. The doses of NNSs used in this study were similar to the amount of aspartame or sucralose present in ~3 cans of diet soda (355 ml can) (Franz 2010; Garriguet 2008).

For the first 2-week intervention period, participants consumed either an aspartame beverage, which contained 1000 ml water, 0.425 g (425 mg) of pure aspartame powder (HerbStoreUSA, Walnut, CA 91788, USA), 0.08 g of citric acid and 0.037 g of pure lemon extract (Club House brand, McCormick London On, Canada N6A 4Z2), or a sucralose beverage, which contained 1000 ml water, 0.136 g (136 mg) of sucralose pure powder (HerbStoreUSA, Walnut, CA 91788, USA), 0.08 g of citric acid and 0.037 g of pure lemon extract, depending on the group to which they were randomized. During the second 2-week intervention period, participants received the treatment they did not receive in the first 2-week intervention period. Beverages were given to the participants in a blinded fashion in identical bottles labelled “A” or “B”. Participants were instructed to drink their beverages throughout the day. Participants were instructed to consume their habitual diets and maintain their physical activity levels throughout the entire study duration. Additionally, they were advised to avoid consuming food or drink products that contained NNSs during the entire
duration of the study. Participants were asked to complete a 3-day food diary for 2 weekdays and 1 weekend day over the 14-day intervention period.

To increase compliance, participants were also asked to complete a daily checklist to verify beverage consumption and to return all empty beverage containers each week for counting purposes.

Palatability, motivation to eat, energy, fatigue, and physical comfort were measured by visual analogue scale (VAS) in participants when they received the aspartame and sucralose drinks. Participants were asked to rate themselves after having their first drinks during the treatment period.

**Blood sampling and analysis**

Participants were instructed to abstain from consuming caffeinated beverages for 12 hours and alcoholic beverages for 48 hours prior to blood draws.

On the first and last day of each period, 12-hour fasting blood samples were collected. A registered nurse collected blood samples immediately before and 15, 30, 45, 60, 90 and 120 minutes after the administration of a 75 g glucose challenge. For female participants, glucose challenges were scheduled after the cessation of menses. Plasma was separated from whole blood samples within 1 hour of collection. Blood was centrifuged at 3000 × g for 15 minutes at 4°C. Plasma aliquots were treated with 20 µl (10 µl/1 mL blood) of dipeptidyl peptidase-4 (DPP-IV) inhibitor before they were stored at -80°C. Untreated aliquots of plasma were stored immediately at -80°C for further analysis.

Glucose and fructosamine concentrations were measured in plasma by a Cobas 311 analyser (Roche Diagnostic, Germany) at baseline and after each treatment period. Insulin, active GLP-1 and leptin contents in plasma were measured at baseline and after each treatment phase by a Meso Scale Discovery (MSD) multiplex assay (Meso Quickplex SQ
120, Rockville, Maryland, USA) according to the manufacturer’s protocol. The incremental area under the curve (AUC) values were calculated for glucose, insulin, active GLP-1 and leptin by the trapezoidal method (Allison et al. 1995). The homeostasis model assessment-insulin resistance (HOMA-IR) was calculated using the formula ([insulin, μIU/L]*[glucose, mg/L])/405. Additionally, HOMA-%beta was calculated as 20 × fasting insulin (μIU/ml)/fasting glucose (mmol/ml) – 3.5 (Matthews et al. 1985).

**Statistical analysis**

A sample size of 12 was sufficient to detect a difference in treatment effect on glucose AUC of 139 mmol/l at 120 minutes with 85% power and a 5% level of significance. Considering a dropout rate of 35%, the sample size required was 19 (Suez et al. 2014).

Statistical analyses were performed using SPSS 22.0 for Macintosh. The normality of the data was assessed using the Shapiro Wilk test, and non-normal variables were normalized using log transformation. The results are expressed as estimated least-squares means ± standard errors of the means (SEMs) for all values unless otherwise stated, and statistical significance was set at p<0.05 for all analyses. Changes in the data from baseline within treatment groups were assessed using a linear mixed model with REML estimation. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. P-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question.

**Results**

**Participants**
A total of ten female and seven male participants completed the 2-stage trial and were included in all analyses. Two participants dropped out without providing a reason.

Fig. 1 is a flow diagram of the trial according to the CONSORT (Consolidated Standards of Reporting Trials) guidelines. The participants were young healthy adults with a mean age of 24±6.8 years, a BMI of 22.9±2.5 kg/m$^2$ and biochemical variables within the normal range. The participants’ BP and FBG levels were within the normal range. Table 1 shows the baseline characteristics of the participants who completed the trial.

Based on returned empty bottle counts, there were no significant violations of the protocol. The mean bottle count adherence for sucralose drinks was 100% compared to 99.47±1.49% for the aspartame treatment.

**Fasting glucose, insulin, active GLP-1 and leptin concentrations following OGTT**

There were no differences in mean fasting glucose, insulin, active GLP-1 or leptin concentrations between the baseline visit and the visit at the start of period 2 after the washout period (Table 2).

There were no significant differences in glucose, insulin, active GLP-1 or leptin concentrations between the aspartame or sucralose treatments and baseline. The curves of mean fasting glucose, insulin, active GLP-1 and leptin concentration during the 75 g OGTT in the 17 healthy participants are given in Fig. 2.

The total AUC values for glucose, insulin, GLP-1 and leptin were not different between baseline and the aspartame or sucralose treatments (Table 3). There were no differences in the total AUC for glucose (p=0.54), insulin (p=0.38), active GLP-1 (p=0.67) or leptin (p=0.80) between the end of the sucralose treatment and baseline. There were no differences in the total AUC for glucose (p=0.65), insulin (p=0.16), active GLP-1 (p=0.63) or leptin (p=0.32) between the end of the aspartame treatment and baseline (Table 3).
change in AUC from baseline to after sucralfose treatment was -4.2% for glucose, +19.8% for insulin, +8.2% for active GLP-1 and +7.8% for leptin. The % change in AUC from baseline to after the aspartame treatment was +3.1% for glucose, +31.9% for insulin, +9.2% for active GLP-1 and +31% for leptin. There were no differences in the percentage change between the treatment groups and baseline (Table 3).

**Insulin secretion and sensitivity derived from OGTTs**

The linear mixed model showed no difference in HOMA-IR, HOMA-%B, or HOMA-%S in healthy participants after sucralfose or aspartame consumption compared to the baseline values (Table 4). There were no differences in HOMA-IR (p=0.35), HOMA-%B (p=0.16) or HOMA-%S (p=0.59) after sucralfose treatment compared to the values at baseline. There was no difference in HOMA-IR (p=0.46), HOMA-%B (p=0.60) or HOMA-%S (p=0.61) after aspartame treatment compared to the values at baseline.

**Discussion**

To the best of our knowledge, this study is the first to evaluate repeated oral daily consumption of beverages sweetened with pure aspartame or sucralfose powder in healthy adults in a randomized, double-blind, crossover trial. The primary outcome of this study was the effect of repeated daily consumption of NNSs on glucose metabolism. Daily oral ingestion of flavoured beverages containing pure aspartame or sucralfose for 2 weeks did not affect plasma glucose, insulin, active GLP-1 or leptin concentrations in healthy participants. HOMA-IR, HOMA-%B and HOMA-%S were also unaffected by aspartame and sucralfose ingestion, suggesting that daily consumption of aspartame or sucralfose does not impact the outcomes measured here, at least at the doses of 425 mg/day of aspartame and 136 mg/day of...
sucralose, which corresponded to an intake of ~3 cans (355 ml) of NNS sweetened beverages/day (Garriguet 2008).

Our results confirm and add to the present understanding of the effects of aspartame and sucralose on glucose metabolism. Many recent clinical trials have shown that NNSs, especially repeated daily doses of aspartame or sucralose, do not alter glucose metabolism in healthy or unhealthy individuals (Ahmad et al. 2019; Bonnet et al. 2018; Grotz et al. 2017; Higgins et al. 2018; Lertrit et al. 2018; Romo-Romo et al. 2018).

The effect of repeated daily doses of aspartame has been examined in recent studies. A study in 8 patients with newly diagnosed type 2 diabetes and 8 healthy participants found that consuming a daily table top formulation containing aspartame did not alter glucose, insulin or GLP-1 concentrations (Temizkan et al. 2015). Another study of 93 healthy participants found that consuming different doses of aspartame lower (350 mg) and higher (1050 mg) than the aspartame dose (425 mg) we used daily for 12 weeks had no effect on glucose, insulin or GLP-1 concentrations in all groups (Higgins et al. 2018). Similarly, a study in 50 healthy men found that daily oral ingestion of 2 cans of carbonated beverages containing aspartame and acesulfame potassium for 12 weeks did not change glucose or insulin concentrations (Bonnet et al. 2018).

The effect of repeated daily doses of sucralose has been examined previously in a few studies, which have shown mixed results. Two studies examined the effect of daily sucralose consumption; the first study was conducted with 77 participants and the second with 8 healthy participants, and both found that daily consumption of a beverage containing different doses of sucralose for >17 days did not alter blood glucose or insulin levels (Baird et al. 2000). Furthermore, a study of 67 patients with obesity and T2DM showed that consuming a daily sucralose dose 3x the estimated maximum intake for 13 weeks had no effect on blood glucose or insulin concentrations (Grotz et al. 2003). A recent study in 47
healthy males demonstrated that consuming a high dose of sucralose in capsule form daily for 12 weeks did not affect glucose metabolism (Grotz et al. 2017).

Another recent trial of 66 healthy female participants reported that the daily consumption of commercial sucralose sachets of unknown concentration did not have an effect on glucose but did decrease insulin sensitivity and increase the acute insulin response (Romo-Romo et al. 2018). Another study in 15 healthy participants showed that oral ingestion of sucralose capsules for 4 weeks enhanced GLP-1 secretion and decreased insulin sensitivity without any effect on glucose levels (Lertrit et al. 2018).

These differing results could be due to the longer period of exposure to sucralose or the high dose of NNSs used in this trial, which is 1.47-times higher than the dose we used in our trial.

Previous studies have used a wide range of designs, and only two studies have assessed pure forms of sucralose in beverages in healthy individuals and individuals with obesity (Pepino et al. 2013; Sylvetsky et al. 2016). Most previous human trials were carried out with oral ingestion of NNS to measure the acute single dosing effects (Anton et al. 2010; Brown et al. 2009, 2012; Bryant et al. 2014; Pepino et al. 2013; Sylvetsky et al. 2016; Temizkan et al. 2015; Tey et al. 2017; Wu et al. 2012), but studies assessing the repeated daily consumption of aspartame or sucralose are far less common than studies of a single dosing (Bonnet et al. 2018; Grotz et al. 2017; Higgins et al. 2018; Lertrit et al. 2018; Romo-Romo et al. 2018).

The strengths of our current trial include its double-blind, crossover design and the selection of a healthy population with good adherence to the protocol and the inclusion of male and female participants, extending the generalizability of the results of this trial to the general population. Additionally, females were assessed after the cessation of their menses to avoid diminished insulin sensitivity during some phases of the menstrual cycle (Diamond et
Moreover, the NNSs used in this study were in a pure form to avoid any contamination by any other ingredients present, for example, in varying types of diet soda. However, this study has some potential limitations. We did not measure aspartame or sucralose consumption compliance through urinary biomarkers, and our study intervention period was only 2 weeks. This intervention period length may have been too short to observe a change, especially in healthy participants. Additionally, we did not have equal numbers of men and women in our RCT.

Future studies should recruit participants with higher BMI values, including individuals with obesity and people with prediabetes or type 2 diabetes. Additionally, more studies are needed with longer exposure durations to assess the effect of the chronic use of NNSs on metabolism.

In our future research, we plan to examine the impact of the consumption of the pure form of sucralose and aspartame on the gut microbiome using samples from this trial. We will explore the potential changes in the gut microbiota that might be induced by regular oral consumption of NNSs in humans. These changes in the gut microbiome could have occurred prior to the development of glucose metabolism dysregulation, so they may be captured in the timescale of this study even though no changes in glucose metabolism were observed.

In conclusion, our study showed that sucralose or aspartame consumption, reflecting high but realistic daily intakes, for 2 weeks had no effect on glucose, insulin, active GLP-1 or leptin concentrations in healthy participants. Further research is needed to confirm the findings of this trial.

Acknowledgements
The authors would like to thank all the participants for their effort and time to take part in this study. We would like to thank the clinical coordinators, laboratory staff, and nurses for their help in this trial.

Conflicts of interest
D.M. was an invited speaker at a seminar entitled ‘Conflicting Outcomes from Systematic Reviews: Is the Consumption of Low-Calorie Sweeteners a Benefit or a Risk for Weight Management?’ at Nutrition 2018 in Boston, Massachusetts, USA, which was sponsored by PepsiCo. PepsiCo paid for his accommodation, conference fee and honorarium. PepsiCo is a company that sells products that contain non-nutritive sweeteners. The other authors declare no conflicts of interest.

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References


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### Tables

#### Table 1. Characteristics of participants at baseline.

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<th>Value</th>
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<tbody>
<tr>
<td>Total participants (F/M)</td>
<td>17 (10 / 7)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>$24 \pm 6.8^*$</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>$68.9 \pm 10.5^*$</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>$22.9 \pm 2.5^*$</td>
</tr>
<tr>
<td>Race (n)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>4</td>
</tr>
<tr>
<td>Asian</td>
<td>10</td>
</tr>
<tr>
<td>Middle Eastern</td>
<td>2</td>
</tr>
<tr>
<td>Not reported</td>
<td>1</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>$119.9 \pm 2.5$</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>$78 \pm 1.8$</td>
</tr>
<tr>
<td>FBG</td>
<td>$4.8 \pm 0.1$</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>$5.3 \pm 0.1$</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/L)</td>
<td>$67.7 \pm 8.5$</td>
</tr>
<tr>
<td>Fasting plasma GLP-1 (pmol/L)</td>
<td>$3.2 \pm 0.5$</td>
</tr>
<tr>
<td>Fasting plasma leptin (ng/ml)</td>
<td>$7.6 \pm 1.2$</td>
</tr>
<tr>
<td>Fasting plasma fructosamine (µmol/L)</td>
<td>$248.6 \pm 4.9$</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>$1.3 \pm 0.2$</td>
</tr>
<tr>
<td>HOMA-%B</td>
<td>$98.5 \pm 6.6$</td>
</tr>
<tr>
<td>HOMA-%S</td>
<td>$90.6 \pm 6.8$</td>
</tr>
</tbody>
</table>

1Values are means ± SEMs unless otherwise indicated; * standard deviation; Concentrations were determined from plasma; M, males; F, Females; FBG, fasting blood glucose; GLP-1, glucagon-like peptide-1; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-%B, homeostasis model assessment of beta cell function; HOMA-%S, homeostasis model assessment of insulin sensitivity.
Table 2. Mean fasting glucose, insulin, active GLP-1 and leptin concentrations between the baseline visit and the baseline visit after the washout period measured in healthy participants, n=17.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Values at baseline</th>
<th>Values after washout period</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean fasting plasma glucose (mmol/L)</td>
<td>5.3 ± 0.1</td>
<td>5.9 ± 0.6</td>
<td>0.30</td>
</tr>
<tr>
<td>Mean fasting plasma insulin (pmol/l)</td>
<td>67.7 ± 8.5</td>
<td>81.9 ± 8.0</td>
<td>0.51</td>
</tr>
<tr>
<td>Mean fasting plasma GLP-1 (pmol/L)</td>
<td>3.2 ± 0.5</td>
<td>3.6 ± 0.4</td>
<td>0.99</td>
</tr>
<tr>
<td>Mean fasting plasma leptin (ng/ml)</td>
<td>7.6 ± 1.2</td>
<td>14.3 ± 4.4</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Values are means ± SEMs unless otherwise indicated; concentrations were determined from plasma; GLP-1, glucagon-like peptide-1. T-test was used to compare values between phases. *Significant value if p < 0.05
**Table 3.** Changes in the AUCs of glucose, insulin, GLP-1 and leptin in healthy participants.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Sucralose</th>
<th>Aspartame</th>
<th>% change a</th>
<th>% change b</th>
<th>P-value c</th>
<th>P-value d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l 120 min)</td>
<td>833 ± 143</td>
<td>798 ± 145</td>
<td>860 ± 205</td>
<td>- 4.2</td>
<td>+ 3.1</td>
<td>0.54</td>
<td>0.65</td>
</tr>
<tr>
<td>Insulin (nmol/l 120 min)</td>
<td>68 ± 39</td>
<td>81 ± 50</td>
<td>89 ± 42</td>
<td>+ 19.8</td>
<td>+ 31.9</td>
<td>0.38</td>
<td>0.16</td>
</tr>
<tr>
<td>GLP-1 (pmol/l 120 min)</td>
<td>695 ± 386</td>
<td>752 ± 373</td>
<td>759 ± 404</td>
<td>+ 8.2</td>
<td>+ 9.2</td>
<td>0.67</td>
<td>0.63</td>
</tr>
<tr>
<td>Leptin (ng/ml 120 min)</td>
<td>898 ± 512</td>
<td>968 ± 960</td>
<td>1177 ± 915</td>
<td>+ 7.8</td>
<td>+ 31.0</td>
<td>0.80</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Abbreviations: AUC, area under the curve during 75 g OGTT; GLP-1, glucagon-like peptide-1; OGTT, oral glucose tolerance test; a % change in AUC from baseline to after sucralose treatment; b % change in AUC from baseline to after aspartame treatment; c differences between the end of the sucralose-sweetened beverage treatment and baseline; d differences between the end of the aspartame-sweetened beverage treatment and baseline; Values are means ± SDs, * Linear mixed model with REML estimation.
Table 4. Summary of insulin sensitivity and insulin secretion derived from OGTT results in healthy participants (n=17) who had consumed aspartame or sucralose for 14 days.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Sucralose</th>
<th>Aspartame</th>
<th>% change (^a)</th>
<th>% change (^b)</th>
<th>P-value (^c)</th>
<th>P-value (^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR</td>
<td>1.2 ± 0.6</td>
<td>1.5 ± 0.7</td>
<td>1.4 ± 0.5</td>
<td>+17.3</td>
<td>+13.3</td>
<td>0.35</td>
<td>0.46</td>
</tr>
<tr>
<td>HOMA-%B</td>
<td>98.4 ± 27.9</td>
<td>115.4 ± 40.1</td>
<td>104.6 ± 34.4</td>
<td>+17.24</td>
<td>+6.30</td>
<td>0.16</td>
<td>0.60</td>
</tr>
<tr>
<td>HOMA-%S</td>
<td>90.5 ± 28.9</td>
<td>83.5 ± 40.9</td>
<td>83.8 ± 43.3</td>
<td>-7.7</td>
<td>-7.4</td>
<td>0.59</td>
<td>0.61</td>
</tr>
</tbody>
</table>

OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-%B, homeostasis model assessment of beta cell function; HOMA-%S, homeostasis model assessment of insulin sensitivity; \(^a\) % change from baseline to after sucralose treatment; \(^b\) % change from baseline to after aspartame treatment; \(^c\) differences between the end of the sucralose-sweetened beverage treatment and baseline; \(^d\) differences between the end of the aspartame-sweetened beverage treatment and baseline. (\(p<0.05\)); Values are means ± SDs; *Linear mixed model with REML estimation.
Figure captions

**Fig. 1.** CONSORT trial flow diagram. CONSORT, Consolidated Standards of Reporting Trials.

**Fig. 2.** Mean plasma glucose (A), insulin (B), active GLP-1 (C) and leptin (D) concentrations during oral glucose tolerance test of healthy participants (n=17) at baseline (closed circle) and after daily consumption of beverages containing aspartame (closed triangle) or sucralose (closed square) for 2 weeks, P< 0.05. Values are expressed as the means ± SEMs.
**Figures**

![CONSORT trial flow diagram](image)

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