Short-Term Impact of Sucralose Consumption on the Metabolic Response and Gut Microbiome of Healthy Adults

Pamela Thomson¹*, Rodrigo Santibañez¹*, Carolina Aguirre², Jose E. Galgani²,³, Daniel Garrido¹

¹Department of Chemical and Bioprocess Engineering, School of Engineering, Pontificia Universidad Católica de Chile; ²Departamento Ciencias de la Salud, Carrera de Nutrición y Dietética, Facultad de Medicina, Pontificia Universidad Católica de Chile; ³Departamento de Nutrición, Diabetes y Metabolismo, Facultad de Medicina, Pontificia Universidad Católica de Chile.

* Both authors contributed similarly in this study

Corresponding author: Daniel Garrido

Department of Chemical and Bioprocess Engineering
School of Engineering
Pontificia Universidad Católica de Chile
Email: dgarridoc@ing.puc.cl

Running Title: Impact of sucralose on the metabolic response and gut microbiome

Keywords: Sucralose, non-caloric artificial sweeteners, glucose control, insulin, gut microbiome

This peer-reviewed article has been accepted for publication but not yet copyedited or typeset, and so may be subject to change during the production process. The article is considered published and may be cited using its DOI

10.1017/S0007114519001570
Abstract
Sucralose is an artificial non-nutritive sweetener used in foods aimed to reduce sugar and energy intake. While thought to be inert, the impact of sucralose on metabolic control has shown to be the opposite. The gut microbiome has emerged as a factor shaping metabolic responses after sweetener consumption. We examined the short-term effect of sucralose consumption on glucose homeostasis and gut microbiome of healthy male volunteers. We performed a randomized, double-blind study in 34 subjects divided into two groups, one that was administered sucralose capsules (780 mg/day for seven days; n=17), and a control group receiving placebo (n=17). Before and after the intervention, glycemic and insulinemic responses were assessed with a standard oral glucose load (75 g). Insulin resistance was determined using HOMA-IR and Matsuda indexes. The gut microbiome was evaluated before and after the intervention by 16S rRNA sequencing. During the study, body weight remained constant in both groups. Glycemic control and insulin resistance were not affected during the 7-day period. At the phylum level, gut microbiome was not modified in any group. We classified subjects according to their change in insulinemia after the intervention, to compare the microbiome of responders and non-responders. Independent of consuming sucralose or placebo, individuals with a higher insulinemic response after the intervention had lower Bacteroidetes and higher Firmicutes abundances. In conclusion, consumption of high doses of sucralose for 7 days does not alter glycemic control, insulin resistance, or gut microbiome in healthy individuals. However, it highlights the need to address individual responses to sucralose.

Introduction
Sucralose (1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside) is a non-caloric artificial sweetener (NAS) synthesized by the selective halogenation of sucrose. Approved by the FDA for use in humans, it is 600 times sweeter than sucrose. Due to its low production cost, high thermostability and solubility, sucralose has emerged as an important sugar
substitute in foods and drinks. The acceptable daily intake (ADI) of sucralose has been established at 15 mg/kg body weight\(^2\).

The concept that replacing sucrose with NAS in foods and drinks improves metabolic control has been challenged\(^1\). In mice, sucralose added to drinking water for 11 weeks impaired oral glucose tolerance when compared with water alone or water with sucrose or glucose\(^4\). Such deleterious effect was prevented when mice were treated with broad-spectrum antibiotics against Gram-Negative or Gram-Positive bacteria. The fact that sucralose displays bacteriostatic action on several gut microbes\(^5,6\), and that most of the sucralose is not absorbed in the intestine\(^1,7,8\), gives support to observations showing that sucralose can alter gut microbiome composition\(^6,9\). Taken together, the notion that sucralose influences glucose control through alterations in intestinal microbiota has emerged.

In humans, consumption of high doses of sucralose for three months has been assessed in non-diabetic\(^10\) and type-2 diabetic\(^11\) individuals. Those studies showed no influence of sucralose on glycemic control when compared with placebo. However, between-subject variability in given markers of glycemic control appears higher after sucralose vs. placebo, particularly in non-diabetic individuals. Eventually, the glycemic response to sucralose in humans, as in mice, is also mediated through changes in gut microbiota\(^12\).

Considering the relevance of chronic diseases and the wide availability of sucralose in foods and drinks, it is critical to determine the effect of sucralose on metabolic responses and the gut microbiome. The goal of this study was to evaluate the short-term effect of sucralose on glycemic control and its interaction with the microbiota in healthy subjects.

**Experimental Methods**

**Subjects.** This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Medicine, Pontificia Universidad Católica de Chile. All participants provided a written informed consent. Thirty-four healthy men between 18 and 50 years with stable weight (variation <2 kg in the last three months) and body mass index (BMI) between
20 - 30 kg/m² were recruited. None of them carried out intense physical activity regularly or received any drug treatment during the last three months. The fulfillment of all the inclusion criteria was evaluated in an initial screening, and individuals not meeting the requirements were excluded. Only males were included in order to avoid potential menstrual cycle-related changes in insulin sensitivity.

**Study design.** A parallel, double-blind, placebo-controlled study was performed. Selected participants were requested to fast overnight and instructed to avoid intense physical activity the day before the evaluation, in addition to smoking, alcohol, and energy drinks consumption 12 h previous to the evaluation. On the evaluation day, subjects were requested to bring or collect in the clinical facility a fecal sample in a 15 ml anaerobic container. The fecal sample was immediately stored at -80 °C for gut microbiome analysis. Body weight was measured followed by the insertion of an intravascular cannula in a peripheral vein of the arm. After resting for 30 min, two 10-min apart blood samples were taken followed by the administration of an oral glucose load (75 g in 290 ml solution). Blood samples were obtained after 30, 60, 90 and 120 min of glucose ingestion. Once finished this procedure, volunteers were randomly assigned to an intervention group with sucralose (n = 17) or placebo (n = 17). For this we used an online resource (https://www.randomizer.org/). Randomization was conducted by one of the members of our group. This person was not involved in recruiting, selecting, or performing any of the measurements of this study, and broke the labels after data analysis. Four subjects did not finish the study, resulting in 16 subjects in the sucralose group and 14 in the placebo group. Individuals were instructed to ingest one capsule containing sucralose or placebo, three times a day for 7 days. Sucralose was purchased from VitaSweet (Beijing, China). Each sucralose capsule contained 260 mg sucralose and 70 mg calcium carbonate; thus the three capsules consumed daily were equivalent to the 75% of the Acceptable Daily Intake (ADI)² (15 mg/kg/d) of a subject weighing 70 kg. Each placebo capsule contained 250 mg of calcium carbonate. Each subject was instructed to register and report any adverse events. After 7
days, subjects were requested to attend the clinical facility to repeat the same procedures. Volunteers were instructed to record any adverse events or change in their customary diet.

**Glucose and insulin tests.** Plasma concentrations of glucose were determined by the glucose oxidase method, in a dry chemistry equipment (Vitros 4600, Ortho Clinical Diagnostics, Raritan, NJ). Serum concentrations of insulin were quantified by a capture chemiluminescent immunoassay (Centaur XPT, Siemens, Henkestr, Germany). Insulin resistance was calculated as the HOMA-IR index (Homeostasis Model Assessment of Insulin Resistance), using the formula by Matthews et al.\(^{13}\): \(\text{fasting insulin} [\mu\text{U/ml}] \times \text{fasting glucose} [\text{mmol/l}] / 22.5\). Alternatively, the ISI-Composite index was also determined, using the formula proposed by Matsuda and De Fronzo\(^{14}\): \(10,000 / \text{(fasting glycemia} [\text{mg/dl}] \times \text{fasting insulin} [\mu\text{U/ml}] \times \text{average glycemia in the oral test (30-120 min)} [\text{mg/dl}] \times \text{average insulin in the oral test (30-120 min)} [\mu\text{U/ml}])^{0.5}\). Total Area Under the Curve (AUC) for insulinenic and glycemic responses were also calculated, using the trapezoidal rule\(^{15}\).

**Statistical analysis.** Clinical data were expressed as the average ± SD. Statistical analyses were run on SASv9.2 (SAS Institute, Cary, NC). Subject’s characteristics at screening were compared between groups using the Student’s t-test. Differences in the AUC for the glycemic and insulinenic responses were assessed through the Wilcoxon’s rank sums test and the Mann-Whitney U-test. Differences in clinical variables before and after the intervention were determined using a repeated-measure ANOVA including group, time (repeated), and the group×time interaction. The significance level for all analyses was set at p-value < 0.05.

**Sample size.** The sample size needed to detect a difference in glycemia (i.e., mean of glycemia between 30-120 min after OGTT) after one-week sucralose ingestion was estimated from two 4-week apart OGTTs performed in healthy, young, non-obese individuals\(^ {16}\). Considering a within-subject SD of ±11 mg/dL as the expected variability without intervention, and a between-subject SD of 21 mg/dL, 15 subjects will allow detecting a difference of 13 mg/dL in glycemia after one-week
sucralose ingestion, with a power of 82% for paired samples two-sided and significance level of 5%.

**Gut microbiome analysis.** Fecal samples stored at -80 °C were first thawed and 150 mg were used for total DNA extraction (Quick-DNA Fecal/Soil Microbe Miniprep Kit; Zymo Research, Irvine, CA) by using a Disruptor Genie device (Scientific Industries, USA)\(^\text{17}\). DNA samples were quantified (NanoDrop 2000c; Thermo Fisher Scientific USA) and diluted to 20 ng/µl in nuclease-free water (IDT; Coralville, IA). DNA samples were submitted for Illumina MiSeq sequencing at Integrated Microbiome Resource (Halifax, NS). The 16S rRNA gene V3-V4 variable region was amplified using V3-V4 primers\(^\text{18}\), which also included a barcode in the forward primer. The DADA2 v1.10 R package was used to analyse the 16S rRNA gene sequences\(^\text{19}\), following a modified procedure\(^\text{19}\). Briefly, the sequences were filtered by quality and trimmed to remove the barcode and low-quality nucleotides prior to estimating the sequencing error. Then, sequences were denoised to identify Amplicon Sequence Variants\(^\text{20}\), then merged and used to assign microbial taxonomy following a Naïve Bayesian classifier\(^\text{21}\), employing the SILVA database version 132\(^\text{22,23}\). Raw data paired-end reads obtained from the MiSeq platform were stored in the European Nucleotide Archive database (ENA), under study accession number PRJEB27704.

**Bioinformatic analysis.** Microbiome composition similarity at phylum level was determined employing Principal Coordinates Analysis with distances calculated with the weighted UniFrac method\(^\text{24,25}\). The weighted UniFrac quantitatively estimates the beta-diversity taking into account phylogenetic distances between microbial taxa and their relative abundance. The similarity between microbiome compositions was calculated with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Algorithm. Fold changes in the relative microbiome composition at the phylum level (values after/before intervention), for each group of subjects and subgroups were determined using the Mann-Whitney U-test. The significance level for all analyses was set at \(p < 0.05\).

**Results**
Subjects characteristics at the screening

Age, weight and height were similar between groups (Table 1), while BMI was higher in the placebo compared with the sucralose group (p=0.04). Such difference in BMI was accompanied by higher blood cholesterol concentration (p<0.01), but similar blood glucose concentration (Table 1).

Metabolic responses to sucralose

Body weight remained stable throughout the study, with an average change of 0.16 ± 0.74 kg (p=0.44) and 0.21 ± 1.17 kg (p=0.49) in the placebo and sucralose groups, respectively. Volunteers in the placebo and sucralose groups did not report adverse events or changes in their usual dietary pattern. As observed at screening, fasting plasma glucose concentration was similar between groups, and not affected by the consumption of placebo or sucralose (Table 2). In turn, a borderline higher fasting serum insulin concentration was observed in the placebo vs. sucralose group (p=0.07; Table 2). Similar to fasting plasma glucose, consumption of sucralose or placebo did not affect fasting serum insulin concentration (Table 2).

Upon glucose ingestion, the average glycemic and insulinemic responses were similar between groups and not affected by the consumption of sucralose or placebo (Table 2). Regarding insulin resistance (sensitivity) markers, no differences by group and intervention were detected (Table 2).

One further assessment included the analysis of the changes in the aforementioned variables after both interventions. Consistent with ANOVA, none of the changes were different from zero (Table 2). We calculated the area under the curve for glycemic and insulinemic responses for each subject (Figure 1). This analysis did not show alterations in glycemia or insulinemia in any group.

Changes in gut microbiome composition

The gut microbiome of all subjects in each group, both before and after the 7-day intervention period, was analysed. As expected, the Firmicutes and Bacteroidetes phyla were dominant in the microbiome of these subjects (Figure S1). Smaller representations of Actinobacteria, Verrucomicrobia and Proteobacteria were also observed. In average, a higher relative abundance of phylum Firmicutes in the placebo vs. sucralose group was observed before initiating the treatments
(Figure 2). However, microbiome composition remained stable throughout both interventions (Figure 2).

We performed a principal component analysis (PCA) to identify variations in the gut microbiome composition for each individual before and after interventions (Figure 3A). This PCA represents the normalized absolute abundance for all 14 phyla identified in every microbiome sample evaluated. Consistent with the aforementioned analysis, most subjects displayed modest variations in their microbiome composition (Figure 3A).

We also calculated the weighted UniFrac distances for each gut microbiome (Figure 3B), in order to determine biological diversity among the microbiomes. This analysis showed that microbiomes in the sucralose group before and after the intervention (S1/S2) tend to cluster together, as well as microbiomes from subjects in the placebo group before and after the intervention (P1/P2). Again, this analysis indicates that both treatments did not substantially modify the microbiome of these subjects, while differences detected before interventions remained.

**Correlations between gut microbiome and metabolic markers**

Next, we classified individuals according to their metabolic responses, by calculating the after-to-before ratio for serum insulin and glucose AUCs, BMI and insulin resistance (sensitivity) markers. This was aimed to determine if within the same treatment certain subjects responded differently, identifying “responders” (subjects with a ratio higher than 1), and “non-responders” (a ratio smaller or equal to 1)\(^4\).

Independent of the treatment received, *responders vs. non-responders* according to insulin AUC had higher Firmicutes and lower Bacteroidetes abundances in their microbiomes (Figure 4A, Figure 4B). Such differences were also noted when classifying subjects according to their change in glucose AUC, albeit with borderline significance (Figure S2). Bacteroidetes relative abundance was also lower in *responders* according to changes in HOMA (Figure S3). Finally, classifying individuals by their BMI (overweight or normal weight) did not show significant changes in their gut microbiomes regardless of the treatment received. Only for individuals in the placebo group, we
observed that overweight individuals had higher Firmicutes and Actinobacteria compared to those with normal weight (Figure S4).

Discussion

**Evidence of metabolic impairments for NAS**

Sucralose is one of the most consumed NAS in the world\(^{26}\), with several foods and beverages being supplemented with this sweetener. While recognized as safe by several studies, recent evidence has shown that sucralose among other NAS may promote weight gain and metabolic disturbances such as glucose intolerance\(^{27}\). Therefore, it is critical to understand the actual impact of NAS in our metabolism.

Previous findings in mice showed no adverse effects of sucralose on inflammatory markers or fasting glucose levels, even at large doses for up to 2 years\(^{28,29}\). A study in diabetic subjects administrating sucralose at a dose of 667 mg (~7.5 mg/kg/day) for 13 weeks did not observe changes in glycated hemoglobin, fasting glycemia or fasting C-peptide against placebo\(^{11}\), which are similar observations compared to this work. Another study conducted in non-diabetic individuals who consumed 1000 mg/day of sucralose (~13.2 mg/kg/day) for 12 weeks did not detect differences relative to placebo in fasting glycemia, insulinemia or glycated hemoglobin\(^{10}\). Sucralose consumption at the population level may reach up to 15% ADI, which is evidently lower compared with the amount provided in these previous reports\(^{30}\). None of these studies evaluated the gut microbiome composition of these subjects. Here we performed a short-term study with small sample size, finding that sucralose consumption at high doses does not alter the glycemic response of healthy individuals.

Recently the gut microbiome has emerged as a factor that could contribute to the biological effects of NAS, in particular sucralose\(^ {31}\). Early environmental studies revealed a strong bacteriostatic effect of several NAS, including sucralose and saccharine\(^ {5,6,31,32}\). Wang et al. also showed increases in Firmicutes in mice exposed to sucralose\(^ {6}\). Thus, alterations in gut microbiome have been shown to be associated with NAS exposure, and these alterations could be causative of metabolic
impairments such as glucose intolerance and insulin resistance. In C57/BL6 mice sucralose altered microbiome composition, increased pro-inflammatory fecal metabolites, and induced hepatic pro-inflammatory markers. Suez et al. reported that in addition to saccharine, sucralose induced glucose intolerance in mice, and importantly this metabolic effect is transmittable to germ-free mice through their gut microbiome, indicating causality.

The Suez’s study was pioneer in showing that after short-term saccharine exposure, individuals display contrasting responses in terms of glucose tolerance and microbiome composition. Some of the observations raised have however been questioned by other studies. Interestingly, that contrasting glucose tolerance was emulated in germ-free mice transplanted with the microbiome of the human donors. That study also provided insights into what microorganisms are being altered in the complex gut microbiome. Saccharine consumption in the short-term resulted in enrichment in Bacteroides phyla, especially B. vulgatus and B. fragilis. Interestingly this exposure in mice also resulted in decreased abundance of Akkermansia muciniphila, a key gut microbe that has been associated with anti-inflammatory properties. While short-term saccharin consumption resulted in an increase in Bacteroidales and decrease in Clostridiales in responders, here we observed that sucralose administration at 75% of the maximum ADI for a similar period of time resulted in no major changes in the gut microbiome composition in healthy subjects. Evidently, metabolic and microbiome responses to saccharin could be very different compared to those of sucralose, considering their chemical properties and their differences in absorption and metabolism. The absence of effect of sucralose consumption on gut microbiome composition could be explained for powering the study on glycemia and not on gut microbiome data.

We observed that at the beginning of the study, subjects in the placebo group had a different gut microbiome (higher Firmicutes and lower Bacteroidetes) compared to the sucralose group. Interestingly this correlated with these subjects having higher initial BMI and cholesterol compared to the sucralose group. These differences were not intentional and could be expected from the randomization of a population of healthy subjects. Moreover, subjects in the placebo group with
higher insulinemia, HOMA or BMI presented significant changes in their gut microbiomes (Figure 4, Figure S3 and Figure S4). These changes were a higher abundance of the Firmicutes phylum and lower Bacteroidetes. These observations indicate that the placebo group had substantial differences regarding glycemic response and gut microbiome compared to the sucralose group, and even inside the placebo group there were interesting differences between these individuals. In spite of these baseline differences, neither group presented changes in glycemic response or gut microbiome associated with the treatment received.

In addition, we did not observe differences in the gut microbiome between respondents and non-responders associated with sucralose or placebo consumption, using criteria changes in serum insulin AUC. However, subjects who had a higher insulin AUC after the intervention, and regardless of the treatment received, had a higher Firmicutes/Bacteroidetes ratio (Figure 4). This indicates that this metabolic difference could be more relevant than the intervention itself. This also correlates with the idea of this cohort being so healthy that only minimal or very sensitive changes in the gut microbiome were obtained. Changes in these phyla are probably relevant and implicate a considerable rearrangement of the community, considering they represent more than 90% of the total gut microbiome. This same phenotype (higher Firmicutes and lower Bacteroidetes, or a higher Firmicutes/Bacteroidetes ratio) has been observed in several studies reporting alterations in the gut microbiome in obese subjects and especially in type 2 diabetes. Mechanistically, these microbiomes have been associated with low-grade inflammation, higher gut permeability and higher circulating lipopolysaccharide.

**Conclusion**

This study shows that the consumption of high doses of sucralose for 7 days in healthy subjects does not alter glycemic control. There were no changes in the gut microbiomes of these subjects with respect to the consumption of sucralose or placebo. Independent of the intervention, subjects displaying an increase vs. decrease in insulinemia after either intervention had different gut
microbiome compositions. Thus, initial metabolic differences could have been more important than
the intervention itself in terms of altering the gut microbiome. Further studies should study the
impact of other important non-caloric sweeteners, but including potential responder-non responder
differences among subjects.

Acknowledgments
We appreciate the contribution to this work of Angélica Ubilla, Daniel Medina, Verónica Ortúzar,
Camila Solar and Carolina Ulloa.

Financial Support
This project was funded by VRI-UC, Concurso de Investigación Interdisciplinaria 2015, VRI
13/2015 and Fondecyt Postdoctorado 3170609.

References
1. Magnuson BA, Carakostas MC, Moore NH, Poulos SP, Renwick AG. Biological fate of
3. Shearer J, Swithers SE. Artificial sweeteners and metabolic dysregulation: Lessons learned
6. Wang Q-P, Browman D, Herzog H, Neely GG. Non-nutritive sweeteners possess a
7. Sylvetsky AC, Bauman V, Blau JE, Garraffo HM, Walter PJ, Rother KL. Plasma
8. Roberts A, Renwick AG, Sims J, Snodin DJ. Sucralose metabolism and pharmacokinetics in


Table 1. Clinical parameters at screening (mean ± SD [range])

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=14)</th>
<th>Sucralose (n=16)</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.5 ± 2.9</td>
<td>22.8 ± 3.0</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>[18.2 – 29.3]</td>
<td>[18.7 – 30.2]</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.0 ± 8.3</td>
<td>73.2 ± 6.9</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>[57.9 – 88.0]</td>
<td>[60.9 – 83.7]</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73 ± 0.04</td>
<td>1.75 ± 0.07</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>[1.67 – 1.80]</td>
<td>[1.63 – 1.90]</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.7 ± 2.9</td>
<td>23.8 ± 1.7</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>[20.8 – 28.9]</td>
<td>[21.1 – 26.6]</td>
<td></td>
</tr>
<tr>
<td>Glycemia (mg/dl)</td>
<td>84 ± 8</td>
<td>85 ± 6</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>[67 – 95]</td>
<td>[72 – 94]</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>173 ± 23</td>
<td>147 ± 22</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>[134 – 219]</td>
<td>[106 – 184]</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Metabolic response to intervention (mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=14)</th>
<th>Sucralose (n=16)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Change</td>
</tr>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycemia (mg/dl)</td>
<td>82 ± 5</td>
<td>79 ± 4</td>
<td>-2.2 ± 5.0</td>
</tr>
<tr>
<td>Insulinemia (µU/ml)</td>
<td>12 ± 5</td>
<td>11 ± 4</td>
<td>-1.0 ± 3.3</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.4 ± 1.1</td>
<td>2.1 ± 0.8</td>
<td>-0.3 ± 0.7</td>
</tr>
<tr>
<td><strong>After oral glucose</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycemia (mg/dl)**</td>
<td>115 ± 17</td>
<td>112 ± 21</td>
<td>-3.0 ± 17.4</td>
</tr>
<tr>
<td>Insulinemia (µU/ml)**</td>
<td>81 ± 38</td>
<td>87 ± 51</td>
<td>5.6 ± 38.1</td>
</tr>
<tr>
<td>ISI-Composite</td>
<td>4.1 ± 2.1</td>
<td>4.7 ± 3.0</td>
<td>0.5 ± 2.4</td>
</tr>
</tbody>
</table>

*Calculated as values after minus before intervention.

**Mean of the respective response over the 30 to 120 min period after glucose ingestion.
Figure legends

**Figure 1:** Changes in metabolic responses upon oral glucose consumption before and after the intervention. A: Total Glycemic Area Under the Curve (AUC) for each group; B: insulinemic total AUC. Subjects with insufficient data to calculate the AUC were not included. The kernel density estimation shows the probability of the values.

**Figure 2:** Gut microbiome compositions for each group before and after each treatment. Bars show the average relative abundance of the four dominant phyla of the human gut microbiome.
**Figure 3:** Comparisons of gut microbiome composition between subjects. A: Principal Coordinates Analysis of all phyla identified showing close similarity for groups including both Placebo and Sucralose at both intervention times. Arrows show the trajectories of changes in microbiome composition for each individual in the study. B: Heatmap of distances calculated as the weighted UniFrac and clustering of closest distances using the UPMGA Algorithm.
Figure 4: Pairwise correlations between fold changes in microbiome phyla and insulinemia responder status. The figure shows the fold change in all major four phyla before and after the intervention, correlated with subgroups in the x-axis. Insulinemia AUC ratios were calculated with AUC values after/before the intervention. A: Fold changes in Firmicutes; B: Bacteroidetes; C: Actinobacteria; D: Proteobacteria. Boxplots indicate the median and the interquartile range, with whiskers determined as the 1.5 range of the box. Asterisk denotes significant differences (p-value < 0.05) determined with the Mann-Whitney U-test.

Supplementary Figures
Figure S1: Relative abundance of identified phyla in each sample. A: The 28 taxonomic assignment and determined abundance (before and after intervention) for the subjects in the sucralose treatment. B: Taxonomic assignment and abundance for the subjects in the placebo treatment.
Figure S2: Pairwise correlations between fold changes in microbiome phyla and glycemia responder status. The figure shows the fold change in all major four phyla before and after the intervention, correlated with subgroups in the x-axis. Glycemia AUC ratios were calculated with AUC values after/before the intervention. A: Fold changes in Firmicutes; B: Bacteroidetes; C: Actinobacteria; D: Proteobacteria. Boxplots indicate the median and the interquartile range, with whiskers determined as the 1.5 range of the box. The asterisk denotes significant differences (p-value < 0.05) determined with the Mann-Whitney U-test.
Figure S3: Pairwise correlations between fold changes in microbiome phyla and HOMA responder status. HOMA ratios were calculated with AUC values after/before the intervention. A: Fold changes in Firmicutes; B: Bacteroidetes; C: Actinobacteria; D: Proteobacteria. Boxplots indicate the median and the interquartile range, with whiskers determined as the 1.5 range of the box. Asterisk denotes significant differences (p-value < 0.05) determined with the Mann-Whitney U-test.
Figure S4: Pairwise correlations between fold changes in microbiome phyla and BMI. BMI values considered were only at day 1 of the study, and subjects were classified as overweight (BMI > 25) or normal weight (BMI < 25). Then the abundances of the four gut microbiomes were correlated with these groups. A: Fold changes in Firmicutes; B: Bacteroidetes; C: Actinobacteria; D: Proteobacteria. Boxplots indicate the median and the interquartile range, with whiskers determined as the 1.5 range of the box. Asterisk denotes significant differences (p-value < 0.05) determined with the Mann-Whitney U-test.