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## Supplemental Information

# Short-Term Consumption of Sucralose with, but Not without, Carbohydrate Impairs Neural and Metabolic Sensitivity to Sugar in Humans

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# Short-term consumption of sucralose with, but not without, carbohydrate impairs neural and metabolic sensitivity to sugar

## SUPPLEMENTARY INFORMATION

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**Data S1.** (Related to Star Methods Section: General Procedure)

**Sweet taste preference**

*Stimuli*

We used the sucrose concentrations 3% - 0.09M, 6% - 0.18M, 12% - 0.35M, 24% - 0.70M, and 36% - 1.05M.

*Procedure*

The M-STP (*6*) task was performed pre and post beverage exposure to investigate psychophysiological changes in sweet taste preference. Subjects sampled (sip-and-spit) two cups containing 10 ml sucrose dilutions and chose the one they preferred in a forced choice setting. If the subject selected the higher concentration, the next two cups presented was the selected beverage plus the next highest concentration. If the subject selected the weaker concentration, the next two presented cups were the weak solution plus the next lowest concentration. Forced choices were presented until the subject selected the same concentration two times in a row. The procedure was completed twice; starting at 3% and 36% sucrose, respectively.

*Analysis and results*

Statistical analysis was performed in R (version 3.1.3, 2015-03-09). First, we made the step size in the concentration range equal by converting the preferred concentrations to a log scale (correlation between log concentrations and linear scale, r = 0.996). The preferred level of sucrose was estimated by averaging the two chosen concentrations (on log scale) for the pre and post measurement, separately. Subsequently, difference scores were calculated (post minus pre beverage exposure) and submitted to a type III Analysis of Variance (ANOVA) model to investigate changes in sweet taste preferences across groups and to investigate whether changes in sweet taste preference were associated with changes in insulin sensitivity. ΔLog sucrose preference was entered as dependent variable whereas group * ΔInsulin $iAUC_{0\text{-}30m}$were entered as independent variables. Results are reported in the Supporting information.

We found no differences across groups ($F(2,32)=0.16$, $p=0.85$). We found no association between changes in sucrose preference and changes in insulin sensitivity ($F(1,32)=0.05$, $p=0.82$), nor did we find a group * ΔInsulin $iAUC_{0\text{-}30m}$ interaction ($F(2,32)=2.08$, $p=0.14$). These results indicate that we found no evidence that our experimental manipulation affected sucrose preference. Though, we are unable to rule out that the M-STP was not sensitive enough.

**Psychophysiological measurements**

*Stimuli*

Taste stimuli included a sweet sucrose solution (0.32 M), a sour citric acid solution (0.0056 M), a salty sodium chloride solution (0.14 M), an umami monopotassium glutamate solution (68 mM), a sweet sucralose solution (0.588 mM) and a sweet and sour sucralose + citric acid solution (0.588 mM + 0.009 M).

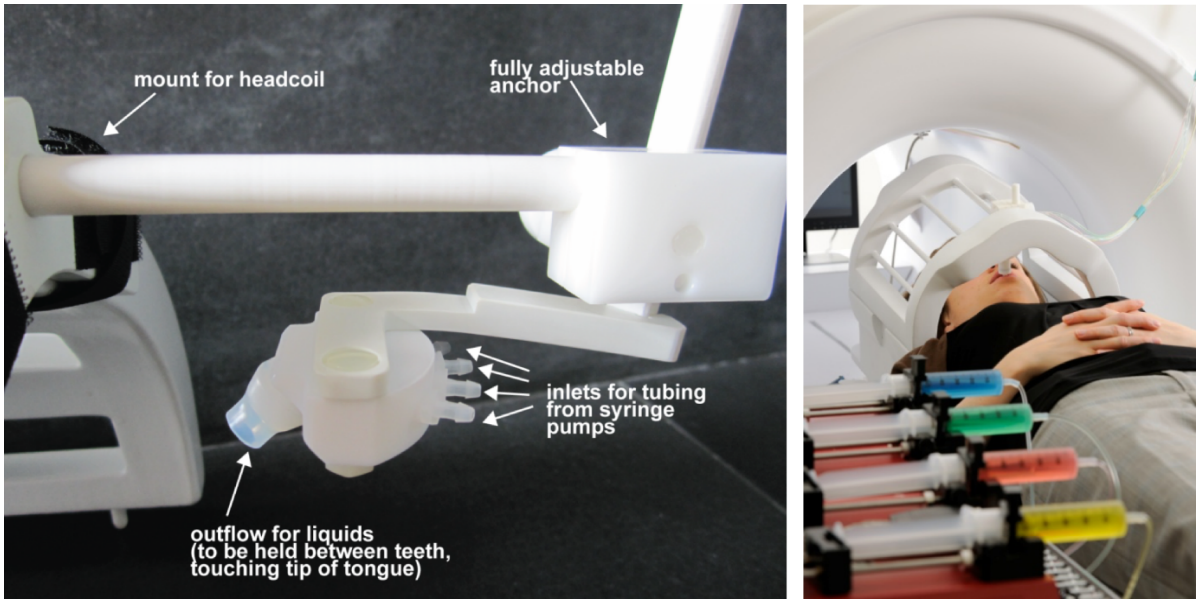
*Procedure*

Prior to each beverage exposure, we measured taste intensity perception to test for possible changes as a function of group during each exposure session. Participants were presented with a tray of 18 medicine cups, containing 10 ml of 3x the six taste and one mixture solution (Sucrose, Sucralose, Citric Acid, NaCl, MPG, Sucralose+Citric Acid, see section 1.3). All tastes were presented three times in a randomized order. Participants were asked to sip the solution, swirl it around in their mouth and spit it in the sink, after which they made ratings of the sweetness, saltiness, sourness, umami and general intensity of the solution. A 30 second wait-period between trials was used to rinse at least three times with deionized water. After completing the ratings for all 18 samples, participants were provided with their respective exposure beverage and were asked to finish the drink within five minutes.

*Analysis and results*

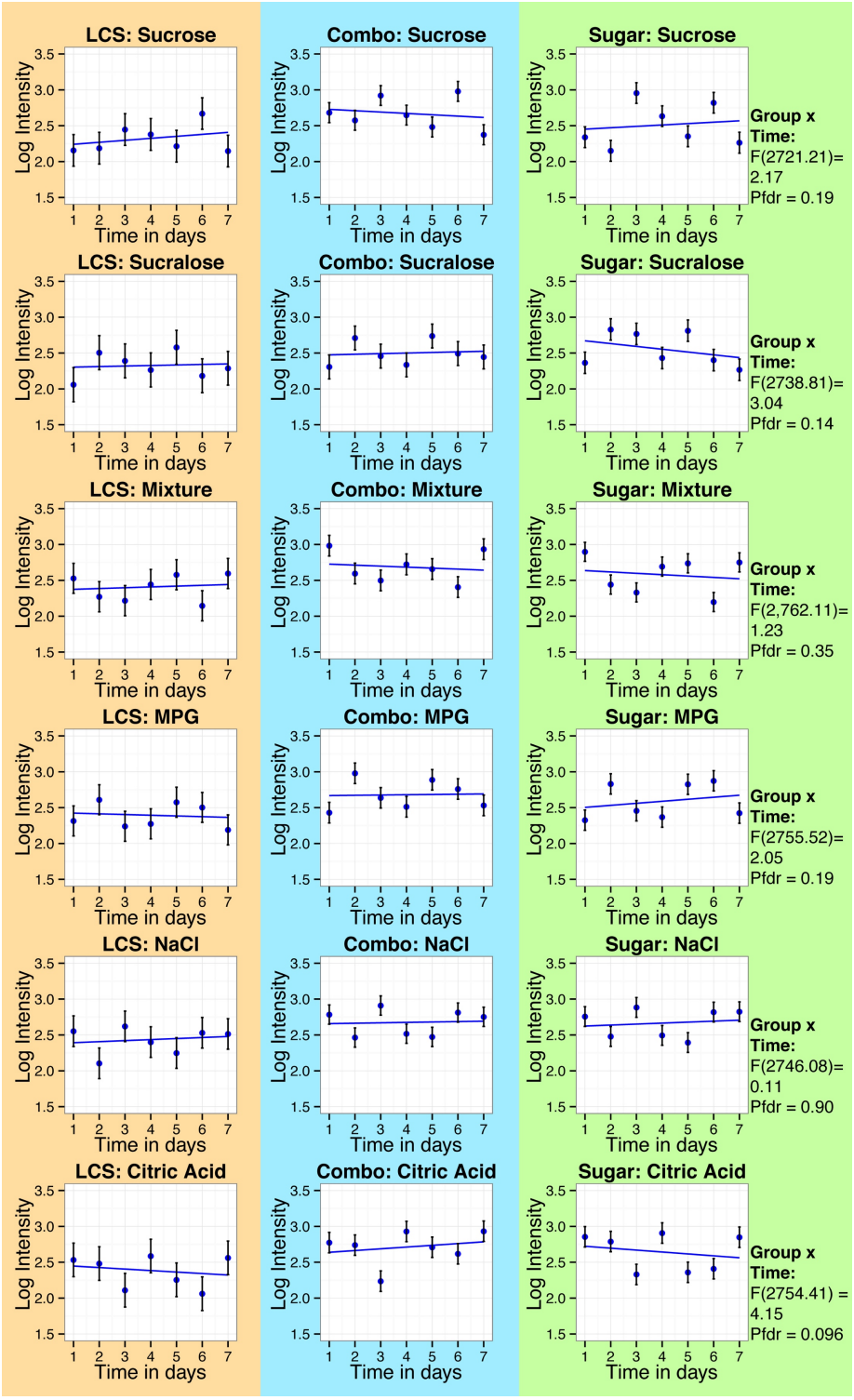
To investigate whether beverage exposure affected taste intensity ratings, we performed LMMs in R using packages LME4 and lmerTest. Models were performed for each taste solution separately. Within each model, log transformed intensity ratings were entered as dependent variable, whereas time in days, experimental group, and their interaction were entered as independent variables. Finally, participant ID was entered as random variable. To test for any interaction with $\Delta$Insulin $iAUC_{0\text{-}30m}$ we also performed similar models that included an interaction between change in insulin and time in days. We found no time x group interactions nor a time x group x $\Delta$Insulin $iAUC_{0\text{-}30m}$ interaction that survived a multiple comparison correction. The results of the intensity ratings together with the group x time interaction F-statistic derived from a mixed-effects repeated-measures ANOVA (type III) are shown in Figure S2.

**Figure S1. Related to Figures 1 and 3. Gustatory Manifold & System**




A) The gustatory manifold is photographed from a side view, showing the silicone tube that sits in between the subject's teeth, touching the tip of the tongue. The mouthpiece has between 4-9 inlets arrayed on the semicircular bottom end parallel to the subject's body. This configuration was designed to occupy as little space as possible inside the head coil. B) The subject is depicted in the mock scanner with gustatory manifold in position and the gustometer system in the foreground.

**Figure S2. Related to Figures 2 and 3. Intensity perception changes across taste solutions and groups.**




The plots indicate the average log transformed gLMS intensity ratings (±SEM) per exposure day and the linear trend across time per taste solution (rows) and group (columns). We found no group x time interaction surviving family wise error (FWE) correction. For each taste solution, F and P values are reported for the group x time interaction derived from a

mixed-effects repeated-measures ANOVA. LCS: Low Calorie Sweetener; NaCl: sodium chloride (salty); MPG: monosodium glutamate (umami); Combo: sucralose + maltodextrin.

**Table S1. Detailed Participant demographics and beverage consumption, Related to Figures 2 and 3.**

| **Adults** | | | | |
|---|---|---|---|---|
| Group | LCS | Combo | Sugar | |
| n | 13 | 13 | 13 | - |
| Age in years (mean, sd) | 26.54 (3.78) | 29.15 (3.76) | 27.69 (4.19) | F(2,36)=1.46, p=0.24 |
| BMI in kg/m$^2$ (mean, sd) | 22.77 (3.12) | 24.78 (2.88) | 23.62 (3.27) | F(2,36)=1.39, p=0.26 |
| Bodyfat in % (mean, sd) | 23.6 (10.45) | 25.11 (6.40) | 29.03 (8.56) | F(2,36)=1.38, p=0.26 |
| Female:Male | 7:6 | 6:7 | 8:5 | $\chi^2(2)=0.62$, p=0.73 |
| Education in years (mean, sd) | 16.92 (2.10) | 17.38 (2.50) | 19.15 (3.91) | F(2,36)=2.08, p=0.14 |
| Ethnicity | | | | |
| Arabic | 0 | 0 | 1 | - |
| Asian | 2 | 6 | 4 | |
| Black | 1 | 1 | 0 | |
| Hispanic | 2 | 2 | 0 | |
| White | 8 | 4 | 8 | |
| Pre TLFB LCS consumption in ml over 2 weeks (median, maximum)[1] | 0 (1656.12) | 0 (3193.94) | 0 (2839.06) | Kruskal-Wallis $\chi^2(2)$= 2.55, p=0.28 |
| Post TLFB LCS consumption in ml over 2 weeks (median, maximum)[1] | 0 (4140.29) | 0 (12420.88) | 0 (3075.65) | Kruskal-Wallis $\chi^2(2)$= 1.48, p=0.48 |
| Pre TLFB LCS consumption in drinking days over 2 weeks (median, maximum)[1+] | 0 (4) | 0 (6) | 0 (8) | Kruskal-Wallis $\chi^2(2)$= 2.62, p=0.27 |
| Post TLFB LCS consumption in drinking days over 2 weeks (median, maximum)[1+] | 0 (4) | 0 (14) | 0 (5) | Kruskal-Wallis $\chi^2(2)$= 1.62, p=0.45 |
| Pre TLFB SSB consumption in ml over 2 weeks (mean, sd) | 4497.45 (4168.04) | 3917.36 (3022.12) | 1574.22 (2267.77) | Kruskal-Wallis $\chi^2(2)$= 8.62, p= 0.01 |
| Post TLFB SSB consumption in ml over 2 weeks (mean, sd) | 4186.93 (3946.01) | 3671.67 (3240.76) | 1625.41 (1371.59) | Kruskal-Wallis $\chi^2(2)$= 3.82, p= 0.15 |
| Pre TLFB SSB consumption in drinking days over 2 weeks (mean, sd) [+] | 8.62 (5.08) | 8.08 (4.77) | 3.23 (3.77) | Kruskal-Wallis $\chi^2(2)$= 9.96, p= 0.007 |
| Post TLFB SSB consumption in drinking days over 2 weeks (mean, sd) [+] | 7.38 (4.96) | 7.85 (4.72) | 4.23 (3.54) | Kruskal-Wallis $\chi^2(2)$= 4.59, p= 0.10 |

| **Adolescents** | | | | |
|---|---|---|---|---|
| Group | Sugar | Combo | Sugar | |
| n | 4 | 3 | 4 | - |
| Age in years | [13 14 15 17] | [16 17 17] | [14 15 15 17] | - |
| BMI in kg/m$^2$ | [18.4 19.5 26.6 26.9] | [19.6 20.4 28.8] | [19 20.2 21.4 22.6] | - |
| Bodyfat in % | [14.6 36.1 36.6 28.9] | [35 24.9 18.3] | [12.5 14.0 27.9 22.3] | - |
| Female:Male | 2:2 | 3:0 | 3:1 | - |
| Education in years | [11 10 8 9] | [10 12 11] | [9 10 10 11] | - |
| Ethnicity | | | | |
| Asian | 0 | 1 | 0 | - |
| Black | 0 | 0 | 1 | |
| Hispanic | 1 | 2 | 2 | |
| White | 3 | 0 | 1 | |
| Pre TLFB LCS consumption in ml over 2 weeks | [0 0 0 0] | [0 0 0] | [0 1182.94 0]* | - |
| Post TLFB LCS consumption in ml over 2 weeks | [0 0 0 0] | [0 0 0] | [295.74 709.76 0]* | |
| Pre TLFB LCS consumption in drinking days over 2 weeks | [0 0 0 0] | [0 0 0] | [0 4 0]* | |

| | | | | |
|---|---|---|---|---|
| Post TLFB LCS consumption in # of drinking days over 2 weeks | [0 0 0 0] | [0 0 0] | [1 2 0]* | |
| Pre TLFB SSB consumption in ml over 2 weeks | [7275.09 709.76 2395.46 10587.32] | [4790.91 5175.37 4820.49] | [7156.79 10823.91 4820.49]* | |
| Post TLFB SSB consumption in ml over 2 weeks | [8546.75 0 3607.971 10528.18] | [6594.90 5175.37 8221.44] | [4199.44 11770.26 1596.97]* | |
| Pre TLFB SSB consumption in drinking days over 2 weeks | [13 2 9 14] | [13 12 12] | [14 14 13]* | |
| Post TLFB SSB consumption in drinking days over 2 weeks | [12 0 10 14] | [14 12 14] | [12 14 9]* | |
| **Maltodextrin Control Group** | | | | |
| n | 15 | - | - | - |
| Age in years (mean, sd) | 29.40 (4.81) | - | - | - |
| BMI in kg/m$^2$ (mean, sd) | 24.88 (3.54) | - | - | - |
| Bodyfat in % (mean, sd) | 27.06 (9.82) | - | - | - |
| Female:Male | 8:7 | - | - | - |
| Education in years (mean, sd) | 18.07 (3.41) | - | - | - |
| Ethnicity | | | | |
| Arabic | 0 | | | |
| Asian | 8 | - | - | - |
| Black | 2 | | | |
| Hispanic | 0 | | | |
| White | 5 | | | |
| Pre TLFB LCS consumption in ml over 2 weeks (median, maximum)[1] | 0 (710) | - | - | - |
| Post TLFB LCS consumption in ml over 2 weeks (median, maximum)[1] | 0 (946) | - | - | - |
| Pre TLFB LCS consumption in drinking days over 2 weeks (median, maximum)[1+] | 0 (2) | - | - | - |
| Post TLFB LCS consumption in drinking days over 2 weeks (median, maximum)[1+] | 0 (2) | - | - | - |
| Pre TLFB SSB consumption in ml over 2 weeks (mean, sd) | 1765.33 (1562.53) | - | - | - |
| Post TLFB SSB consumption in ml over 2 weeks (mean, sd) | 1362.60 (835.87) | - | - | - |
| Pre TLFB SSB consumption in drinking days over 2 weeks (mean, sd) [+] | 4.60 (4.24) | - | - | - |
| Post TLFB SSB consumption in drinking days over 2 weeks (mean, sd) [+] | 3.67 (2.19) | - | - | - |

[1]Data contained mostly zeros with a few outliers.
*Data was not recorded for 1 participant.
[+]Total number of days on which LCS or SSB were consumed over the past 2 weeks.
Abbreviations: **TLFB**: Time line follow back measurement (for the preceding 2 weeks, see section 1.7); **LCS**: low calorie sweetener; **SSB**: sugar sweetened beverage.

## Table S2. Group comparisons for the OGTT indices, Related to Figures 2 and 3.

| Measure | Sugar<br>Mean (sd) | LCS<br>Mean (sd) | Combo<br>Mean (sd) | statistic |
|---|---|---|---|---|
| **Matsuda** | | | | |
| Absolute change | 0.61 (1.45) | 1.12 (2.19) | -0.35 (2.62) | F(2,36)=1.59, p=0.22 |
| Percent change | 18.41% (38.70%) | 30.73% (45.52%) | -3.02% (27.46%) | F(2,36)=2.63, p=0.09 |
| **Hepatic insulin sensitivity** | | | | |
| Absolute change | -6.81 (16.85) | -6.15 (13.04) | 5.61 (7.04)[a,b] | F(2,36)=3.79, p=0.03 |
| Percent change | -8.05% (81.72%) | 3.88% (110%) | 32.68% (67.84%) | F(2,36)=0.73, p=0.49 |
| **Insulin incremental $AUC_{0-30}$** | | | | |
| Absolute change | -4.27 (9.71) | -4.88 (8.86) | 4.67 (5.68)[a,b] | F(2,36)=5.43, p=0.009 |
| Percent change | -9.85% (38.93%) | -12.45% (41.59%) | 27.15% (41.00%)[a,b] | F(2,36)=3.88, p=0.03 |
| **Glucose incremental $AUC_{0-30}$** | | | | |
| Absolute change | -0.10 (0.27) | -0.10 (0.59) | -0.02 (0.29) | F(2,36)=0.19, p=0.83 |
| Percent change | -10.12 (36.38%) | 8.72% (73.88%) | 4.14% (43.75%) | F(2,36)=0.43, p=0.65 |
| **Insulin $AUC_{0-30}$** | | | | |
| Absolute change | -5.06 (9.82) | -5.77 (9.91) | 4.45 (5.15) [a,b] | F(2,36)=5.75, p=0.007 |
| Percent change | -11.38% (30.77%) | -14.28% (33.86%) | 20.36% (29.86%) [a,b] | F(2,36)=4.82, p=0.014 |
| **Glucose $AUC_{0-30}$** | | | | |
| Absolute change | -0.09 (0.41) | -0.11 (0.66) | 0.03 (0.32) | F(2,36)=0.33, p=0.72 |
| Percent change | -2.35% (11.79%) | -1.21% (18.07%) | 1.11% (10.48%) | F(2,36)=0.21, p=0.81 |
| **Insulin incremental $AUC_{0-120}$** | | | | |
| Absolute change | -0.51 (22.33) | -24.51 (46.01) | 23.86 (32.46) [b] | F(2,36)=6.22, p=0.005 |
| Percent change | 5.43% (40.56%) | -14.34% (38.88%) | 24.13% (35.58%) [b] | F(2,36)=3.27, p=0.05 |
| **Glucose incremental $AUC_{0-120}$** | | | | |
| Absolute change | -0.51 (1.24) | -0.07 (2.41) | 0.01 (1.34) | F(2,36)=0.33, p=0.72 |
| Percent change* | -18.95% (47.05%) | -45.25% (98.89%) | 7.16% (136%) | F(2,35)=0.84, p=0.44 |
| **Insulin $AUC_{0-120}$** | | | | |
| Absolute change | -3.68 (21.77) | -28.14 (49.43) | 22.99 (30.92)[b] | F(2,36)=6.58, p=0.004 |
| Percent change | 0.95% (28.18%) | -14.78% (33.82%) | 20.31% (29.04%)[b] | F(2,36)=4.33, p=0.02 |
| **Glucose $AUC_{0-120}$** | | | | |
| Absolute change | -0.47 (1.80) | -0.08 (2.32) | 0.22 (1.49) | F(2,36)=0.42, p=0.66 |
| Percent change | -3.43% (12.62%) | 0.27% (17.33%) | 2.34% (12.85%) | F(2,36)=0.53, p=0.59 |

[a] Different from LCS, $p_{fdr} < 0.05$
[b] Different from Carb, $p_{fdr} < 0.05$
* One extreme outlier omitted.