Not so sweet: Stevia fails to rescue glucose intolerance or microbiome dysbiosis on high fat diet

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Abstract

Non-caloric artificial sweeteners have been correlated with reduced glucose tolerance and obesity in both mice and humans, however the effect of the non-caloric plant-derived sweetener stevia on the gut microbiota has yet to be characterized, though other non-caloric sweeteners are known to induce changes in glucose tolerance through gut-mediated mechanisms, especially saccharin (Suez et al., 2014). We tested the hypothesis that stevia would reduce high fat diet-induced glucose intolerance which would be correlated with a significant change in the microbiome. We evaluated physiologic markers including caloric intake, liquid consumption, and bodyweight as well as glucose tolerance tests at the initiation and end of treatment. Fecal samples were collected at start and end of treatment. Male and female C57BL/6J mice (n=40) were randomly assigned to one of four treatment groups: low fat diet and water (LF), high fat diet and water (HF), HF diet and Saccharin (saccharin), or HF diet and stevia (Stevia). Over 10 weeks of sweetener treatment, LF mice ate fewer calories, drank more water, and gained less weight than the other groups. No significant differences in any of these measures were found among the groups on HF diet. Sweetener treatment did not affect glucose tolerance, with no difference between HF, Saccharin, and Stevia in AUC. We found distinct microbiome profiles for each sweetener beyond changes due simply to a HF diet. Stevia had a significant effect on alpha-diversity as measured by the Shannon index which was significantly different than both Saccharin and HF groups. Two measures of beta-diversity did not show a significant effect of treatment (weighted UniFrac and Bray-Curtis). These data suggest cautionary use of stevia as a sugar alternative, as it significantly alters alpha diversity and appears to have similar effects as saccharin on glucose control, weight gain, caloric intake, and beta-diversity.

Introduction

Artificial sweeteners have been shown to decrease glucose tolerance and induce dysbiosis in the gut microbiota of both mice and humans (Suez et. al, 2014).

Saccharin thought to have the most pronounced effect on glucose tolerance (Suez et al., 2014).

Stevia thought to have an anti-hyperglycemic effect, but its effect on the gut microbiome has yet to be characterized (Gregersen et al., 2004).

Hypothesis

Stevia will correct HF diet-induced glucose tolerance which will be correlated with changes in the microbiome

Materials and Methods

Methods: Male (n=20) and female (n=20) *C57BL/6J*. Housed individually at 23°C.
10 animals per treatment group (5 males, 5 females).

LF = Consume low fat food (15% calories from fat) with regular drinking water (n=10)
HF = Consume high fat food (60% calories from fat) with regular drinking water (n=10)
Saccharin = Consume HF food with drinking water containing saccharin (n=10)
Stevia = Consume HF food with drinking water containing stevia (n=10)

Food intake, bodyweight, and liquid consumption measured tri-weekly.
Saccharin and Stevia given at 5mg $kg^{-1}day^{-1}$ in drinking water
Dosage calculated weekly
Fecal samples collected before HF diet and sweetener treatment and after treatment.
Baseline data (water, BW, and food intake) monitored for eight days prior to day 0, when the HF diet was initiated. After 6 days on the HF diet, the sweetener protocol began.

GTT: Glucose tolerance test before and after treatment
Fasted for 6hrs during light phase, testing done during light phase.
dose 1mg glucose per kg BW injected into the peritoneal cavity.

Microbiome: DNA isolation from fecal samples
Sequencing of V4 region of 16s rRNA by Univ. Wisconsin
Mothur pipeline and R-based analysis

Results

Sweetener does not change calorie consumption

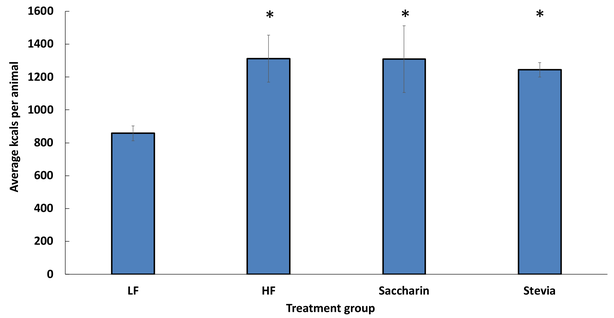

Figure 1. *Average caloric intake over 10 week treatment. * indicates p<0.05 vs LF. Sweetener addition did not significantly effect calorie consumption vs. HF.*

Sweetener does not change body weight

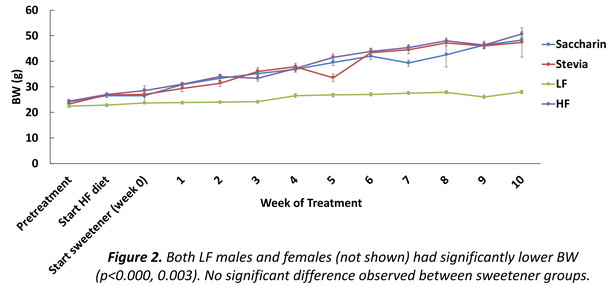

Figure 2. *Both LF males and females (not shown) had significantly lower BW (p<0.000, 0.003). No significant difference observed between sweetener groups.*

Average glucose tolerance test

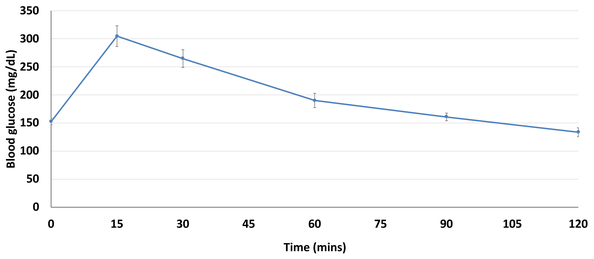

Figure 3. *Glucose tolerance test of initial animals, all on LF diet. Representative of a standard glucose tolerance test. Used to calculate area under the curve (AUC).*

Stevia does not cause a difference in HF diet-induced glucose intolerance

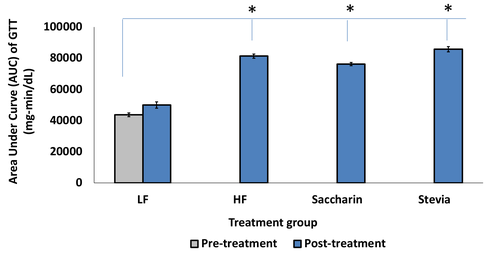

Figure 4. *Area under the curve (AUC) analysis of GTTs. Stevia did not significantly affect AUC vs HF, though LF was significantly lower than all other groups.*

Distinct differences in gut microbiome

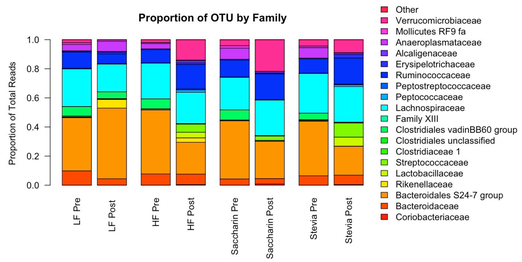

Figure 5. *Relative abundances based on OTU counts of experimental groups before and after treatment at the family level. OTU = operational taxonomic units, and is a measure of abundance of bacteria at various taxonomic classifications.*

Significant difference in alpha diversity by treatment group

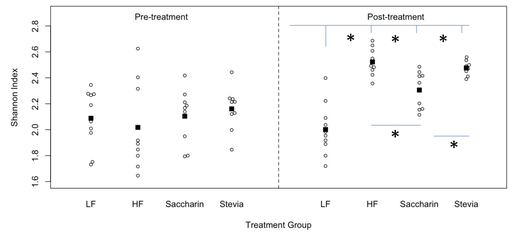

Figure 6. *Shannon index, a measure of richness (number of species present in a sample) and evenness (distribution of these samples based on relative abundance) in a sample which is used to evaluate alpha-diversity. A higher Shannon value indicates a greater degree of diversity. Each group has two time points: pre and post treatment.*

Results cont.

Diet appears to be strongest driver of beta-diversity

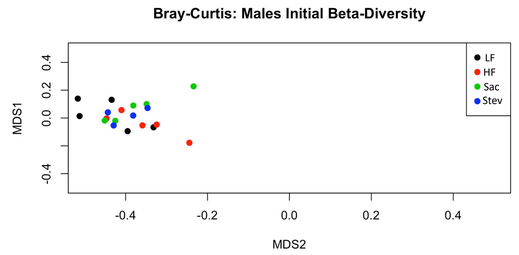

Figure 7. *Bray-Curtis plot of males at the beginning of treatment. Bray-Curtis is a principal coordinate analysis which compares pairwise dissimilarity between two points using a rank-based approach. It is a measure of beta-diversity of a population and each circle on the plot represents the microbiome of an entire animal. Animals are initially clustered together, showing there is little diversity initially between groups.*

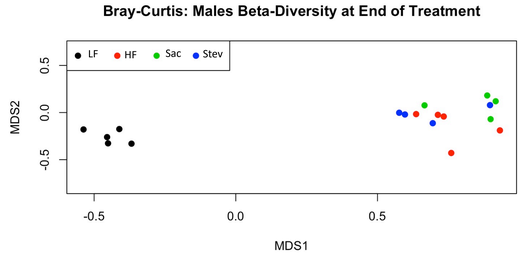

Figure 8. *Bray-Curtis of males at end of sweetener treatment. Groups have clustered based on treatment, with most of the variation coming from the interaction of group and time (p=0.003996). The HF and Saccharin groups display a trend toward a difference in beta diversity (p=0.015984).*

Conclusions

- Stevia does not reduce caloric consumption or weight gain on a HF diet.
- Stevia does not rescue HF diet-induced glucose intolerance.
- Stevia supplementation results in significant differences in alpha-diversity of the gut microbiome, but not in beta-diversity.

References:

Suez, J., et al., *Artificial sweeteners induce glucose intolerance by altering the gut microbiota.* Nature, 2014. **514**(7521): p. 181-6. Gregersen, S., et al., *Antihyperglycemic effects of stevioside in type 2 diabetic subjects.* Metabolism, 2004. **53**(1): p. 73-6.

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