DIFFERENTIAL EFFECTS OF CHRONIC LOW CALORIE SWEETENER CONSUMPTION ON BODY WEIGHT, GLYCEMIA, AND INGESTIVE BEHAVIOR

by

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To my friends, family, and everyone who helped me along the way

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LIST OF ABBREVIATIONS

LCS: Low Calorie Sweeteners

FDA: Food and Drug Administration

GRAS: Generally Recognized as Safe

RCT: Randomized Control Trial

CPIR: Cephalic Phase Insulin Response

Reb A: Rebaudioside A

Ace-K: Acesulfame-potassium

NHANES: National Health and Nutrition Examination Survey

BMI: Body Mass Index

SSB: Sugar-sweetened beverages

T2D: Type 2 Diabetes

FFQ: Food Frequency Questionnaire

GLP-1: Glucagon-like Peptide-1

DTE: Desire to Eat

SSS: Sensory Specific Satiety

TFEQ: Three Factor Eating Questionnaire

PABA: Para-aminobenzoic acid

VAS: Visual Analog Scale

OGTT: Oral Glucose Tolerance Test

DEXA: Dual-energy x-ray absorptiometry

FMC: Fine Motor Control

TBW: Total Body Water

ASA24: Automated Self-Administered 24-hour Dietary Recall

TAG: serum triacylglycerol

HDL: High-density lipoprotein

LDL: Low-density lipoprotein

HbA1c: Hemoglobin A1c

TEE: Total Energy Expenditure

mBEV: Beverage Consumption Habits Questionnaire

AUC: incremental area under the curve

ABSTRACT

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Title: Differential Effects of Chronic Low Calorie Sweetener Consumption on Body Weight, Glycemia, and Ingestive Behavior
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Low calorie sweeteners (LCS) provide sweetness with little to no energy. Each sweetener has a unique chemical structure that possesses unique sensory and functional characteristics. While LCS are generally considered in aggregate, these unique chemical structures have potential implications for sensory, metabolic, and behavioral differences that may impact body weight and glycemia. Therefore, two, twelve-week experiments were conducted to determine the effect of chronic LCS consumption on body weight, glycemia, and ingestive behaviors.

The first experiment investigated the differential effects of four LCS (saccharin, aspartame, rebaudioside A, and sucralose) and sucrose consumed for twelve weeks on body weight, glycemia, and ingestive behaviors among healthy adults with overweight or obesity (body mass index (BMI) between 25 and 40 kg/m^2). In a parallel-arm design, 154 participants were randomly assigned to consume 1.25 to 1.75L of beverage sweetened with 1 of the 5 sweeteners daily for 12 weeks. Body weight was measured every two weeks; energy intake, energy expenditure, and appetite were assessed every 4 weeks; and glucose tolerance was measured at baseline and week 12. Every four weeks, participants completed 24-hour urine collections to determine study compliance via PABA excretion. Sucrose and saccharin consumption led to increased body weight across the 12-week intervention (Δ weight = +1.85 and +1.18kg, $p \leq 0.02$) and did not differ from each other. While there was no significant change in body weight with consumption of the other LCS treatments

compared to baseline, changes in weight in comparison to the sucrose treatment (sucrose – LCS) were significantly different for aspartame, rebA, and sucralose after 12 weeks (weight difference = 1.13, 1.25, 2.63kg, respectively; $p \leq 0.03$). In addition, change in body weight at week 12 was significantly lower between sucralose and all other LCS (weight difference ≥ - 1.37 kg, p=0.008).

The second experiment investigated the effect of daily aspartame ingestion on glycemia, body weight, and appetite. One hundred lean (BMI between 18 and 25 kg/m^2) adults were randomly assigned to consume 0, 350, or 1050 mg aspartame/day for twelve weeks in a parallel-arm design. This experiment followed a similar protocol but measured body weight and blood pressure weekly and contained a 240-min glucose-tolerance test (OGTT) with measurements of selected hormones at baseline and week 12. Participants also collected 24-h urine samples every four weeks. There were no group differences for glucose, insulin, resting leptin, glucagon-like peptide 1, or gastric inhibitory peptide at baseline or week 12. There also were no effects of aspartame ingestion on appetite, body weight, or body composition.

These trials demonstrate that all LCS contribute negligible energy but should not be aggregated because of their differing effects on body weight. Sucrose and saccharin consumption significantly increased body weight compared to aspartame, rebA, and sucralose. This differential change in body weight among LCS indicates individual LCS likely exert different physiological responses beyond the contribution of sweetness with negligible energy. Saccharin, rebA, sucralose, and aspartame (ingested at three doses) for twelve weeks had no effect on glycemia. These data do not support the view that LCS are problematic for the management of glycemia. If substantiated through additional testing, findings from this trial have implications for consumers, food industry, clinicians, and policy makers. Some LCS may not hold the anticipated beneficial effects on body weight (e.g., saccharin) and positive effects of one LCS (sucralose) may be attenuated if combined

with select other LCS. Going forward it will be important to consider each LCS as a distinct entity with respect to its potential health effects.

CHAPTER 1. INTRODUCTION

Dissertation Rationale

Low calorie sweeteners (LCS) are ubiquitous in the US food supply. Each sweetener possesses unique sensory and functional properties making them attractive for varied preferences and purposes. Food companies use LCS to moderate the sugar and energy content of their products while maintaining their appeal; consumers may select products containing LCS and/or add them to foods and beverages to achieve a similar sugar/energy reduction goal. While the Food and Drug Administration (FDA) has deemed the commercially available LCS as safe food additives or Generally Recognized as Safe (GRAS), controversies exist over the efficacy of LCS use for weight loss or weight maintenance. Results from meta-analyses of randomized controlled trials (RCT) (Azad et al., 2017; P. E. Miller et al., 2014; P. J. Rogers, Hogenkamp, de Graaf, et al., 2016) examining the effect of LCS consumption on body weight conclude there are beneficial or no effects of LCS consumption on body weight compared to sugar sweetened and water alternatives. Nevertheless, results from select epidemiological (Azad et al., 2017; Fowler et al., 2008), *in vitro* (Egan et al., 2008), and animal trials (J. Suez et al., 2014; S. E. Swithers et al., 2008) continue to raise consumer concerns over the safety and efficacy of LCS use because of their reported detrimental effects on glycemia and body weight. Absent concerns about safety, clinical and policy recommendations generally aggregate LCS. This reflects a view that their common mode of action is to provide sweetness while contributing little or no energy. However, each LCS has a unique chemical structure with potential implications for differences in sensory properties, digestion, metabolism, intestinal microbiota populations, brain reward activation, appetite, and impact on

body weight. If the latter is true, it may explain the variability in responses to varying LCS in the literature.

The current state of the literature is too limited to provide the public dietary recommendations to use or avoid select sweeteners. In fact, the United States Department of Agriculture 2015 Dietary Guidelines Advisory Committee made no recommendation regarding use of LCS despite the "moderate and consistent evidence" that substitution of LCS for nutritive sweeteners facilitates weight management (DGAC, 2015). If all LCS cause weight gain or impair glucose tolerance, then their consumption should be discouraged. If all LCS cause weight loss or improve glucose tolerance, then replacement of sugars with LCS can be a valuable tool for control of weight or glycemia. If there is no effect of LCS on body weight or glucose tolerance, then post-ingestive effects of LCS either do not occur or do not translate to clinical outcomes, and no one LCS should be recommended or avoided over another. If LCS have differential physiological effects, then substitution of select LCS for sugar-sweetened alternatives could be used to support and optimize weight loss or weight maintenance strategies or maintain euglycemia. Therefore, the purpose of this dissertation is to evaluate the effect of LCS consumption on body weight, glycemia, and ingestive behavior.

Objectives

The objectives of this dissertation are the following:

- Review of the literature on the effect of LCS consumption on body weight, ingestive behaviors, and glycemia.
- Evaluate the differential effects of twelve weeks of LCS consumption on body weight, energy intake, appetite, and glycemia in individuals with overweight or obesity.

- Examine the effect of twelve weeks of consumption of three dosages of aspartame on glycemia, appetite, and body weight among healthy, lean adults.

Organization of Dissertation

This dissertation contains a review of the literature and published or submitted peer-reviewed publications. Publication details are included at the beginning of each chapter.

Chapter 2 reviews the literature on the effect of LCS consumption on body weight, ingestive behavior, and glycemia, primarily from randomized controlled trials and prospective cohort trials in humans. An evaluation of three meta-analyses examining the effect of LCS on body weight summarizes the differential conclusions draw by these analyses. A focus on differentiation between LCS utilized in these trials and their associated outcomes is emphasized.

Chapter 3 reviews the proposed mechanisms by which LCS hypothetically affect body weight, ingestive behaviors, and glycemia. These mechanisms include energy dilution, sensory and pre-ingestive effects, uncoupling of sweetness and energy, gastric effects, gut peptide release, brain reward effects, informed compensation, and gut microbiota changes that accompany LCS consumption.

Chapter 4 presents the results from a RCT assessing the differential effects of four commonly consumed LCS and sucrose on body weight, energy intake, appetite, and glycemia with 12 weeks of consumption among healthy adults with overweight or obesity. While some LCS have no effect on body weight change (aspartame, rebaudioside A), consumption of saccharin for 12 weeks led to significant increases in body weight that were not significantly different than sucrose and sucralose tended to lower body weight.

Chapter 5 presents the results from a RCT examining the effect of two doses of aspartame equivalent to one can of soda and the 95 percentile of aspartame consumption on glycemia,

appetite, and body weight among healthy, normal weight adults. Aspartame consumption at either dose had no effect on glycemia, appetite, or body weight.

Chapter 6 summarized the findings of these trials and presents future directions for research on the effect of LCS consumption on body weight and glycemia.

Study Aims and Hypotheses

Specific Aim 1: Contrast the effect of consumption of four different LCS and sucrose on body weight, body composition, and ingestive behavior with 12 weeks of consumption among adults with overweight and obesity.

Hypothesis 1: Varying sensory, physiological, or behavioral responses to LCS will result in differential changes to body weight and body composition between LCS and compared to sucrose.

Hypothesis 1a: If energy intake alone promotes weight gain, then sucrose consumption will increase body weight and all LCS will have no effect or reduce body weight.

Hypothesis 1b: If sweetness alone promotes weight gain, then all sweeteners (LCS and sucrose) will lead to an increase in body weight.

Hypothesis 1c: If a cephalic phase insulin response (CPIR) is elicited without energy or a diminished CPIR with chronic LCS exposure promotes increased energy intake, then saccharin and sucralose consumption will increase body weight.

Hypothesis 1d: If release of glucagon-like peptide-1 (GLP-1) leads to satiety and decreased energy intake, then sucralose consumption will decrease body weight.

Hypothesis 1e: If LCS in circulation activate mechanisms that increase energy intake, saccharin and sucralose are predicted to lead to greater weight gain than the other LCS.

Hypothesis 1f: If LCS in the gut lead to changes to the microbiota that increase or decrease energy harvesting efficiency, then saccharin, sucralose and rebA consumption will either increase or decrease body weight.

Specific Aim 2: Contrast the effect of consumption of four different LCS and sucrose on glycemic response with 12 weeks of consumption among adults with overweight and obesity.

Hypothesis 2: LCS consumption will have no effect on glycemic response while sucrose consumption will lead to heighted glucose and insulin among adults with overweight and obesity.

Specific Aim 3: Assess the effect of daily aspartame ingestion for 12 weeks on glycemia among healthy, normal weight adults.

Hypothesis 3: Aspartame consumption will have no effect on glycemia with 12 weeks of consumption among healthy, normal weight adults.

Specific Aim 4: Assess the effect of daily aspartame ingestion for 12 weeks on appetite and body weight among healthy, normal weight adults.

Hypothesis 4: Aspartame consumption will have no effect on appetite and body weight with 12 weeks of consumption among healthy, normal weight adults.

CHAPTER 2. LITERATURE REVIEW OF THE EFFECT OF LOW CALORIE SWEETENER CONSUMPTION ON BODY WEIGHT AND GLYCEMIA

Low Calorie Sweetener Consumption Patterns

LCS are incorporated into foods and beverages to displace sugar and energy and are also used as tabletop sweeteners to increase sweetness. The commercially available LCS that are approved food additives by the Food and Drug Administration (FDA) are acesulfame-potassium (ace-K), advantame, aspartame, neotame, saccharin, and sucralose while steviol glycosides and Luo Han Guo fruit extracts (mongrosides) are generally recognized as safe (GRAS) steviol glycosides (FDA, 2018). LCS are selected by food companies and individuals based on sensory preference and their desired functionality. For example, aspartame's flavor profile is more similar to sucrose than other LCS, but is heat labile and unstable at neutral pH (O'Donnell et al., 2012); on the other hand, saccharin is stable during food processing but concentrations of saccharin approaching equivalent sweetness of 10% sucrose are accompanied by unwanted bitter and off-flavors.

Approximately 4.4% of products in the American food supply contain LCS (Dunford et al., 2018). LCS are most commonly consumed in beverages (consumed by 30.8% of American adults), but also as foods (10.3%) and LCS packets (14.1%) (A.C. Sylvetsky et al., 2017). Up to 60% of households purchase beverages with LCS and 19% purchase foods with LCS based on 2010 Nielsen Homescan data (C. Piernas et al., 2013). LCS consumption by Americans has increased from 32% in the 2007-2008 cycle of the National Health and Nutrition Examination Survey (NHANES) to 41.1% according to data from the 2009-2010 and 2011-2012 NHANES cycles (A. C. Sylvetsky et al., 2017; A. C. Sylvetsky et al., 2012).

Even though all LCS provide sweetness with limited to no energy, they vary in their chemical structure, receptor affinity, sensory properties, digestibility, enteroendocrine response, and metabolic fate. A comparison of LCS properties is found in **Table 1.** These differences have potential implications that may affect body weight. For example, LCS structure affects receptor affinity, either in the oral cavity or throughout the GI tract, which can affect endocrine signaling. Differences in these responses vary between sweeteners and between *in vitro* and *in vivo* trials. How these structural differences between LCS may differentially affect appetite and body weight is further explained in the **Chapter 3**.

LCS are commonly consumed as a method to lose or maintain body weight. According to the National Weight Control Registry (a database of individuals who successfully lost at least 13.6 kg and maintained the weight for a year), 53% of registry members reported consumption of at least one LCS beverage a day (Catenacci et al., 2014). Dietary recall data from 1999 to 2008 cycles of NHANES indicate that individuals who reported an attempt to lose weight over the past year were 64% more likely to consume LCS (Drewnowski et al., 2016). LCS consumption for weight loss is not always successful; epidemiologic evidence supports both weight loss and weight gain with LCS use. Individuals who lost or gained 50lbs over a ten-year time frame were 26% and 14% more likely to consume LCS compared to individuals who gained a pound a year over 10 years (Drewnowski et al., 2016). This positive and negative fluctuation in body weight among LCS consumers makes epidemiological associations between LCS consumption and body weight difficult to interpret. A positive association between LCS and body weight may be causal or an artifact of reverse causality (R. D. Mattes et al., 2009); individuals who have gained weight may seek out LCS sweetened products as a weight loss tool. This positive and negative association between LCS use and BMI is evident in conclusions from prospective cohort trials that LCS

increase risk of weight gain (Fowler et al., 2008) or pose no risk to weight gain (Pan et al., 2013). However, evidence from RCT indicates that LCS can be helpful weight loss and weight management tools (Paige E. Miller et al., 2014; P. J. Rogers, Hogenkamp, de Graaf, et al., 2016).

Low Calorie Sweetener Consumption and Body Weight

Low Calorie Sweetener Consumption and Body Weight in Prospective Cohort Trials

Prospective cohort and cross-sectional studies have attempted to explain the relationship between LCS use and body weight, but separate analyses have drawn conflicting conclusions. LCS use in prospective cohort trials is typically assessed by measuring LCS soda or beverage consumption. In the San Antonio Study, 3,682 participants had anthropometrics measured and reported LCS beverage consumption at baseline and seven to eight years later (Fowler et al., 2008). A positive, dose-dependent relationship between LCS beverage use and change in BMI was observed; participants who consumed at least 22 LCS beverages per week had 78-83% greater changes in BMI compared to non-users. This positive association was reduced (47%) but remained significant after controlling for demographics, education, socioeconomic status, BMI, exercise frequency, and smoking status. Conversely, analysis of beverage consumption via a food frequency questionnaire (FFQ) from three separate prospective cohort studies up to 20 years in duration (Nurses' Health Study (NHS), NHS II, and Health Professionals Follow-up Study) found LCS beverage consumption was negatively associated with weight gain: -0.10kg every 4 years on average with consumption of one serving per day (Pan et al., 2013). This effect was similar to the association of one serving of water and body weight (-0.13kg) and directionally different than the association with sugar sweetened beverage (+0.36kg).

In other epidemiological trials, the relationship between LCS use and BMI is not clear. In one trial, one-year change in BMI was positively associated with diet soda consumption for

adolescent males but not females (Berkey Catherine et al., 2012). In another, BMI and percent body fat was significantly higher among diet soda adolescent consumers compared to non-consumers cross-sectionally, but there was no longitudinal relationship between LCS beverage use and BMI or percent body fat (Laska et al., 2012). In a third trial, LCS beverage consumption was associated with increased risk of obesity (BMI>30kg/m2) over three years among adolescents (Macintyre et al., 2018) but was not associated with increased risk of overweight or obesity (BMI>25 kg/m2). This association was only significant among the middle tertial of LCS beverage consumption (1-6 times per week) after controlling for significant covariates of baseline BMI, maternal BMI, and processed meat consumption. These associations between LCS consumption and BMI may be specific to select populations, LCS doses, or a product of spurious correlations.

Saccharin is the only LCS that has been differentiated from total LCS consumption on body weight within epidemiological studies. In an assessment of dietary and lifestyle factors associated with weight change among data collected from the NHS, a prospective cohort trial of 30,000+ women, saccharin was positively associated with weight gain (Colditz et al., 1990). Similar results were found among FFQ records from 465 individuals in the Pawtucket Heart Health Program (Parker et al., 1997). In both assessments, no other LCS were evaluated exclusively, so whether this association is unique to saccharin or LCS in general cannot be determined.

Prospective cohort studies provide important information about the characteristics of LCS consumers in a free-living environment. However, results from these trials are associations, so a causal direction (if one exists) cannot be determined as to whether LCS consumption increases body weight or increases in body weight lead to increased LCS consumption. Individuals on a positive weight trajectory may use LCS as a diet tool and lose weight or continue on their weight trajectory if LCS use is not combined with energy restriction, explaining both the positive and

negative association between LCS use and body weight (Drewnowski et al., 2016). LCS consumption is also associated with a high Healthy Eating Index score and improved solid fats, added sugar and alcohol subscore (Drewnowski et al., 2014). This may suggest the inclusion of LCS in an overall healthy dietary pattern or sugar/fat reduction diet as a method to lose weight, but this but requires further investigation. Randomized control trials are necessary to answer these questions and determine a causal relationship between LCS consumption and body weight.

Low Calorie Sweetener Consumption and Body Weight in Randomized Controlled Trials

Change in body weight is determined by the difference in energy intake and energy expenditure over time. For weight loss to occur, an individual must increase energy expenditure or decrease energy intake. However, reducing the energy content of the diet can be difficult, partially due to the decrease in diet palatability when reduced energy versions of foods are substituted for foods with greater energy density. LCS are proposed as a method to reduce the sugar and energy content of the diet while maintaining its sweetness and palatability. Numerous long-term RCT have assessed the efficacy of LCS for weight loss or weight management. Many of these trials use beverages and/or foods sweetened with a combination of LCS or do not disclose the specific LCS used. Select RCT in humans use a single LCS, primarily aspartame. RCT investigating the long-term effects of saccharin, sucralose, and steviol glycosides consumption with body weight as a primary outcome have not been conducted. The results from RCT investigating the effect of select LCS on body weight are reviewed below, with an emphasis on differences between LCS. Animal trials will be reviewed when human trials have not been conducted. Conflicting results from three recent meta-analyses comparing the effect of LCS on body weight is also reviewed.

Interventions with Aspartame

Three RCT of at least six weeks in duration investigated the effect of aspartame consumed orally on body weight. These trials concluded aspartame consumption decreased body weight compared to a water control during weight maintenance but not during weight loss (Blackburn et al., 1997)); was not different than regular soda, milk or water (Maersk et al., 2012); and led to decreased body weight compared to water when consumed as part of a weight loss regimen among females but not males (Kanders et al., 1988). In the longest of these trials, women with obesity were asked to consume aspartame sweetened beverages and foods or no aspartame as part of a 16-week active weight loss intervention with one year of weight maintenance (Blackburn et al., 1997). There was no significant difference in weight loss during the active weight loss phase; yet, participants who consumed aspartame regained significantly less weight (2.6kg vs. 5.4kg in the no-aspartame group) after one year of weight maintenance and at two years follow-up (5.4kg vs. 9.4kg). The results from these trials indicate aspartame consumption does not promote weight gain, and may have benefits for weight maintenance compared to water.

Interventions with Sucralose

To date, no RCT has tested the effect of sucralose as the exclusive sweetener on body weight. A sucralose tolerance study in humans concluded that 12-week consumption of up to 500mg of sucralose per day did not lead to changes in body weight; however, select participants did report loss of appetite throughout the trial (Baird et al., 2000). The trial was designed to analyze sucralose tolerance (not body weight) and administered a dose that was approximately five times the estimated daily intake delivered as a 35g/L solution, limiting the ability to translate these findings to consumption of sucralose in naturalistic conditions. One exercise-focused family intervention included the replacement of sugar with sucralose and found a higher percentage of

children maintained or reduced body weight (Rodearmel et al., 2007); however, there was no difference in body mass index (BMI) z-scores in children and no difference in BMI among parents compared to a self-monitoring control group. It is not possible to determine whether these results are attributed to change in exercise or increased sucralose consumption, because there was no sucralose only or exercise only intervention. A RCT to explore the effect of sucralose consumption on body weight as the primary outcome is necessary to determine its efficacy for weight loss or weight maintenance.

Interventions with Saccharin

The long-term effects of saccharin consumption alone on body weight in a RCT has not been conducted. Prospective cohort trials conclude saccharin consumption is associated with weight gain (Colditz et al., 1990; Parker et al., 1997), though a causal association cannot be determined. Saccharin supplemented diets increase body weight in select rodent studies (Feijó et al., 2013; Ramirez, 1990; S. E. Swithers et al., 2010), while others find no difference in body weight compared to water (D'Anci, 1999; Yeomans et al., 1997). The enhancement of body weight with saccharin sweetened diets observed in these trials typically occurs when saccharin is intermittently provided along with nutritive sugars (sweet non-predictive) compared to diets sweetened with only nutritive sugars (sweet predictive). This paradigm is utilized repeatedly by one laboratory that consistently reports saccharin consumption increases body weight by uncoupling sweetness from energy (Davidson et al., 2004; S. E. Swithers et al., 2009; S. E. Swithers et al., 2008). However, trials by another group testing intermittent saccharin exposure found no difference in energy intake despite an increase in body weight (Feijó et al., 2013). This intermittent exposure to nutritive and nonnutritive sweeteners is likely the scenario for how

humans consume LCS in combination with other dietary sweeteners. The effect of chronic saccharin consumption on body weight in humans requires further investigation.

Interventions with Reb A

Few long-term trials have investigated consumption of rebA or other steviol glycosides on any health outcome, and the trials that do monitor body weight find no significant change (Hsieh et al., 2003; Maki, Curry, Reeves, et al., 2008). Adults with mild hypertension who consumed 1,500 mg of stevioside in a capsule daily for two years experienced no significant change in BMI compared to the placebo control (Hsieh et al., 2003). There were significant reductions in systolic and diastolic blood pressure (the primary outcome of the trial) with stevioside consumption. Another RCT aimed to measure the safety of consumption of 1,000 mg rebA via capsules daily among adults with type 2 diabetes (T2D) and found no change in body weight, glucose homeostasis, or blood pressure after 16 weeks of consumption (Maki, Curry, Reeves, et al., 2008). Consumption of steviol glycosides as a capsule eliminates the sweetness and associated sensory effects of sweetness exposure. Long-term oral exposure to steviol glycosides on body weight in healthy individuals with normal weight or obesity is yet to be determined.

LCS in Weight Loss Interventions and Interventions among Non-Dieting Populations

Multiple LCS interventions with a weight loss component have been conducted. The Choose Healthy Options Consciously Everyday (CHOICE) Trial investigated the effect of replacing sugar-sweetened beverages (SSB) with water or LCS beverages as part of a 6-month intervention (D. F. Tate et al., 2012). Participants were asked to replace at least two servings of SSB with LCS beverages or water or made no changes to their beverage consumption patterns. All treatment groups were provided dietary counseling for weight loss. All groups lost weight at the

end of the 6-month intervention, with no difference in magnitude of weight loss between treatment groups. However, participants who replaced SSB with LCS beverages had a greater likelihood of achieving a 5% weight loss (odds ratio (OR) = 2.29, 95% confidence interval (CI) (1.05, 5.01)) compared to individuals who continued consuming SSB, while the likelihood of 5% weight loss was not different between the water treatment and SSB control (OR=1.87, CI (0.08, 4.14)). In a 12-week weight loss intervention with one year weight maintenance follow-up period, 308 participants with overweight or obesity who were LCS beverage consumers consumed either 24oz of LCS beverages or water (J. C. Peters et al., 2016). While both groups lost weight, the LCS beverages treatment group lost significantly more weight at the end of the weight loss intervention (-5.95kg vs. 4.09kg) (J. C. Peters et al., 2014) and maintained greater weight loss during the maintenance period (-6.21kg vs. -2.45kg) (J. C. Peters et al., 2016).

Interventions investigating the role of LCS on body weight among non-dieting populations have also been conducted. Participants with overweight or obesity who consumed diets supplemented with LCS foods and beverages for 10 weeks had subtle, but significant, decreases in body weight (1.0kg) while the sugar sweetened control group gained 1.6kg (A. Raben et al., 2002). This body weight effect was attributed to differences in energy intake; the sugar sweetened treatment reported significantly greater energy intake compared to the LCS treatment group. A similar effect was documented among children age 4-11 enrolled in the 18-month Double-blind, Randomized Intervention Study in Kids (DRINK) trial (de Ruyter et al., 2013). Children who consumed a 250ml sucralose and ace-K sweetened beverage daily had significantly lower changes in BMI z-score and significantly less increase in fat mass, skinfold thickness measurements, and waist-to-height ratio compared to the control group who consumed beverages containing sugar.

Therefore, LCS do not pose a risk to increases in body weight when included into a diet without the intention of weight loss.

These trials provide information about the beneficial effect of energy dilution when LCS are included as part of a weight loss regimen and can help with weight maintenance without additional dietary energy restriction. LCS are most effective when LCS replace SSB or among individuals who already consume sweetened beverages but do not pose a risk of weight gain if LCS are added to the diet.

Conflicting Conclusions about Low Calorie Sweetener Consumption and Body Weight from Three Meta-Analyses

Three recent meta-analyses have sought to elucidate the relationship between LCS consumption and body weight in prospective cohort and randomized controlled trials (RCT) (Azad et al., 2017; P. E. Miller et al., 2014; P. J. Rogers, Hogenkamp, de Graaf, et al., 2016). Meta-analyses are designed to combine data from multiple trials to determine a more precise estimate of intervention effects on an outcome of interest. Theoretically, all three meta-analyses should arrive at similar conclusions, yet slight variation may exist due to data availability at the time of analysis. However, these meta-analyses make varying conclusions of the effect of LCS consumption on body weight ranging from LCS consumption is associated with weight gain to LCS consumption promotes weight loss when compared to a water or a non-energetic control.

Conclusions from Meta-Analyses of Prospective Cohort Trials

LCS consumption was positively associated with BMI in two meta-analyses and had no association in one meta-analysis. One meta-analysis of twelve prospective cohort trials found the overall effect of LCS consumption of BMI was a 0.002 kg/m^2 reduction in BMI per year (95% CI:

-0.009, 0.005) (P. J. Rogers, Hogenkamp, Graaf, et al., 2016). Another meta-analysis of nine cohorts concluded LCS intake was not associated with body weight or fat mass but had a "significant, albeit modest, positive association with BMI" compared to non-consuming controls (Paige E. Miller et al., 2014); the overall effect was 0.03 change in BMI (95% CI: 0.01, 0.06). The most recent analysis including two cohorts (none overlapping with papers included in the prior two meta-analyses) found LCS to be positively associated with increased BMI (Azad et al., 2017); the overall effect was 0.05 change in BMI (95% CI: 0.03, 0.06).

Conclusions from Meta-Analyses of Randomized Controlled Trials

The meta-analyses of RCT had strikingly different conclusions than the analyses of prospective cohort trials. LCS consumption had no effect or a beneficial effect on body weight compared to sugar sweetened or water controls in the RCT meta-analyses. LCS consumption was associated with no effect on body weight in one meta-analysis of five RCT (Azad et al., 2017); the mean difference between LCS groups and controls was -0.17kg (95% CI: -0.54, 0.21). LCS consumption was associated with decreased body weight among fifteen RCT (Paige E. Miller et al., 2014); the mean difference between LCS treatment groups compared to sugar sweetened or water control groups was -0.80kg (95% CI: -1.17, -0.43). Results from the third meta-analysis were LCS consumption was associated with decreased body weight compared to both sugar sweetened controls and compared to water controls in nine and three trials, respectively (P. J. Rogers, Hogenkamp, Graaf, et al., 2016). The overall effect was a mean difference of -1.35kg compared to sugar-sweetened controls (95% CI: -2.28, -0.42) and -1.24kg compared to water controls (95% CI: -2.22, -0.26).

Methodological Differences between the Low Calorie Sweetener and Body Weight Meta-Analyses

The conflicting results from these meta-analyses challenges the validity of the meta-analysis method, because different conclusions can be found with roughly the same data and approach. Discrepancies within these meta-analyses can be attributed to the studies included in each analysis, including different criteria of samples size, study duration, and study population (healthy versus cardiometabolic disease risk factors); availability of data based on time of analysis; primary outcomes of selected trials; and LCS delivery vehicle (i.e. beverage, food, or capsule). Interpretation of the meta-analysis findings recommend either LCS use or avoidance. While RCT are the gold standard of clinical research, the results from the meta-analyses of prospective cohort trials raise questions regarding the use of LCS among individuals looking to lose or maintain body weight. Therefore, even when the data from available prospective cohort trials is compiled, conclusions on how LCS consumption affects body weight outside of experimental trials cannot be definitively drawn.

Low Calorie Sweetener Consumption and Glycemia

LCS are utilized by diabetics as a mechanism to reduce sugar intake while maintaining palatability of the diet. The recommendations to replace dietary sugar with LCS is supported by the American Heart Association, American Diabetic Association (Gardner et al., 2012), Academy of Nutrition and Dietetics (Fitch et al., 2012), and Canadian Diabetes Association (R. Gougeon, 2004). Concerns related to an enteroendocrine response to LCS, disruption of the association between sweetness and glucose, changes to sweet preference, and alterations to the gut microbiota are proposed mechanisms by which LCS disrupt metabolic health, including disordered glycemia (A. C. Sylvetsky et al., 2018). Select LCS do bind to T1R2/T1R3 receptors on enteroendocrine

and pancreatic β-cells and increase intestinal glucose transport and increase release of incretins GLP-1 and GIP from enteroendocrine cells *in vitro* and in *in vivo* animal models (Margolskee et al., 2007). However, these results fail to replicate in *in vivo* animal or human trails, with minimal to no effect on glycemia with acute or chronic intake. The section below will review the results from *in vitro* and *in vivo* trails investigating the glycemic response to specific LCS (summarized in Table 1) with an explanation of the study design differences to explain discrepant findings between trials.

Saccharin

There is no documentation of enteroendocrine incretin responses to saccharin *in vitro* but *in vivo* trials in mice have been conducted. Wild-type mice fed a low carbohydrate diet supplemented with saccharin for two weeks expressed 1.8-fold higher levels of sodium-glucose transporter-1 (SGLT1) mRNA than non-LCS supplemented controls. Similar increased expression was documented with ace-K supplementation (1.9 fold increase) and sucralose supplementation (2.2 fold increase) but not aspartame (Margolskee et al., 2007). The increase in glucose transporters corresponds with an increase in glucose absorption measured in another experiment with glucose perfusions in the jejunum of rats. Glucose absorption increased with the addition of saccharin, ace-K, or sucralose; absorption with saccharin elicited only a fraction of the rate as ace-K or sucralose (Mace et al., 2007). Direct exposure of MIN6 pancreatic β-cells to saccharin or ace-K with 3 and 25mM glucose significantly increased insulin secretion (Nakagawa et al., 2009). However, oral administration of saccharin had no effect on blood glucose, GIP and GLP-1 levels during a two-hour intraperitoneal glucose tolerance test in rats (Fujita et al., 2009).

There is no documentation of an effect of saccharin on glycemia in humans. The effect of saccharin consumption on gut peptide release has not been measured in a clinical trial to date (C.

Bryant et al., 2016). Consumption of 135mg saccharin had no effect on glucose or insulin levels in the three hours post-consumption compared to an unsweetened control among healthy subjects and subjects with T2D (D. L. Horwitz, 1988). Saccharin consumption led to a lower insulin AUC than after aspartame consumption in healthy individuals only, but the response to saccharin was not significantly different than the unsweetened control. Similar null effects were found when saccharin, aspartame, or ace-K were consumed with a glucose load; despite an increase in glucose AUC with ace-K, none of the LCS had a significant effect on blood glucose throughout the 60 minute post-prandial period (C. Bryant et al., 2016).

Aspartame

Aspartame, which is hydrolyzed to aspartate, phenylalanine, and methanol in the lumen of the small intestine, does not bind to T1R2/T1R3 sweet taste receptors past the oral cavity and theoretically has little effect on glucose absorption or incretin release in enteroendocrine cells or pancreatic β-cells. There is no documentation of endocrine effects with aspartame exposure to enteroendocrine or pancreatic β-cells *in vitro* (C. Bryant et al., 2016). Aspartame consumption with a low-carbohydrate diet had no effect on SGLT mRNA expression in wild-type mice Margolskee et al., 2007) and had no effect on blood glucose and insulin when consumed orally during an intraperitoneal glucose tolerance test in rats (Fujita et al., 2009). Evidence from one trialsuggests that rats fed 5-7mg/kg body of aspartame per day had impaired glucose tolerance due to changes in gut microbiota populations and metabolite production. However, this finding requires further investigation, because aspartame is metabolized in the proximal small intestine and does not reach the colon (discussed in Chapter 3).

Multiple RCT have measured the glycemic response of aspartame and consistently find no effect. Aspartame consumption had no effect on GLP-1 (Steinert et al., 2011), post-prandial blood

glucose (C. E. Bryant et al., 2014; Steinert et al., 2011), or insulin (W. L. Hall et al., 2003; Tey et al., 2016) in pre-load design trials and no effect on glycemic response to an OGTT after 6 months of aspartame consumption (Engel et al., 2017). Select trials have documented higher glucose AUC with aspartame consumption compared to an unsweetened control (D. L. Horwitz et al., 1988) and lower GLP-1 levels with aspartame consumption compared to a corn flour control (W. L. Hall et al., 2003). Aspartame contributes 4 kcal per gram, which was not accounted when aspartame was compared to the unsweetened control. Corn flour may not be the appropriate control for aspartame because of the differences in sensory properties and digestibility. Overall, aspartame is inert in the GI tract and has limited effect on glycemic response consumed alone or with a glucose load.

Sucralose

Evidence of increased glucose absorption and GLP-1 expression from enteroendocrine cells has led to extensive experimentation of sucralose exposure in animal and human *in vivo* trials. Sucralose exposure was shown to bind to T1R2/T1R3 receptors in mice enteroendocrine cells, increase transcription of SGLT1 mRNA, expression of SGLT1 protein, glucose uptake, and GLP-1 expression (H. J. Jang et al., 2007; Margolskee et al., 2007). This response was also documented in an animal trial in which jejunal infusions of glucose with sucralose increased glucose absorption dose dependently with increasing sucralose levels (Mace et al., 2007). Sucralose exposure *in vitro* also led to insulin release from pancreatic beta cells in another trial (Nakagawa et al., 2009). However, blood glucose following sucralose consumption was no different than when no sweetener was administered orally during an intraperitoneal glucose tolerance test in rats (Fujita et al., 2009).

With the exception of two trials among populations with overweight or obesity, sucralose consumption does not have an acute or chronic effect on glucose tolerance. Glucose AUC was

significantly reduced and GLP-1 was significantly elevated when sucralose was consumed as a preload during an OGTT compared to a water control among healthy individuals only; the effect was not significant among individuals with T2D (Temizkan et al., 2015). In addition, subjects with obesity that were naïve to LCS had significantly higher peak insulin and insulin incremental AUC when sucralose was consumed prior to an extended OGTT compared to water. Glucose incremental AUC, GLP-1, and GIP were not different between water and sucralose treatments (Pepino et al., 2013). Blood glucose and insulin levels were not affected by sucralose consumption orally compared to water (A. W. Brown et al., 2011; Steinert et al., 2011; A. C. Sylvetsky et al., 2016; T. Wu et al., 2012) or intragastrically (Ma et al., 2009). Similarly, sucralose consumed as an exclusive LCS also had no effect on GLP-1 or GIP excretion (Ma et al., 2009; A. C. Sylvetsky et al., 2016; Tongzhi Wu et al., 2012) in multiple RCT. The null effect of sucralose consumption on glycemia is consistent with chronic consumption. Twelve-week consumption of 1000mg of encapsulated sucralose per day had no effect on fasting insulin, glucose, HbA1c, or blood glucose or insulin responses during an OGTT among healthy male participants (Grotz et al., 2017). While sucralose may increase levels of insulin and GLP-1 in select populations, the evidence is not robust and produces predominately null effects among healthy populations.

Steviol Glycosides, Rebaudioside A

Both steviosides (Jeppesen et al., 2000) and rebaudiosides (Abudula et al., 2008) increase secretion of insulin from mouse pancreatic cells, but an enteroendocrine response has not been evaluated *in vitro*. Doses of stevia ranging from 5mg/kg to 1g/kg body weight had no effect on blood glucose during a two-hour intraperitoneal glucose tolerance test in rats (Fujita et al., 2009) or levels of plasma GIP or GLP-1 when administered at the upper 1g/kg dose. In humans, evidence of differential responses to stevia compared to non-energetic or energetic controls is mixed. In one

trial, isosweet beverages sweetened with steviol glycoside and rebA, monk fruit extract, or aspartame had no effect on blood glucose or insulin during the three hours post-prandial period while sucrose beverages elevated both glucose and insulin levels (Tey et al., 2016). However, in another trial, stevia consumption as part of an energetic preload led to significantly lower insulin (but not glucose) levels compared to aspartame (Anton et al., 2010). An isoenergetic carbohydrate or water control was not available for comparison within this trial. Consumption of 1 g encapsulated stevioside also reduced blood glucose during a meal glucose tolerance test compared to a corn starch control among adults with T2D, but had no effect on post-prandial insulin or GLP-1 (Gregersen et al., 2004). Longer trials to determine steviol tolerance have been conducted and find no effect of sixteen weeks of consumption of 1g of rebA daily on glucose tolerance or HbA1c among individuals with T2D (Maki, Curry, Reeves, et al., 2008). While evidence is mixed whether steviol glycosides or rebaudiosides reduce postprandial blood glucose or insulin, there is no evidence that they increase them.

Explanation of Differential Findings between *In Vitro* and *In Vivo* Human and Animal Trials

Trials investigating the endocrine and glycemic response to LCS in humans consistently find no effect with the exception of select trials, yet this is not consistent with the evidence from select *in vitro* and *in vivo* animal trials. Methodological differences in *in vitro*, animal, and human trials may account for these different results. These differences include sweetener responsiveness, location of exposure, sweetener combinations, and the sweetener vehicle.

In *in vitro* trials, pancreatic β-cells and enteroendocrine cells are exposed directly to LCS, often at doses that exceed a typical dose consumed in the diet. However, direct exposure to LCS is not physiologically possible for all LCS. For example, rebA and steviosides are converted to

steviol in the intestinal lumen before they are absorbed. Therefore, direct exposure of rebA to pancreatic β-cells would not occur if administered orally or intragastrically. In addition, only a minimal amount of sucralose is absorbed (2-15%) (Magnuson et al., 2016). The implication of this reduced dose in circulation may explain the insulin response with direct exposure to β-cells and lack of response in animal and human trials. Aspartame exposure to enteroendocrine or pancreatic β-cells also lacks ecological validity, because aspartame is hydrolyzed prior to absorption. Select animal and human trials administer LCS as capsules, intragastrically, or intestinally. This bypass of the oral cavity could have an effect of its own due to the elimination of sweetness and palatability of the LCS stimuli. Even if endocrine responses to LCS occur *in vitro*, physiological barriers may prevent an *in vivo* endocrine response to select LCS.

Sweetener responsiveness varies widely between species, and stimuli that are perceived as sweet to humans are not necessarily perceived by other mammals (described in "Energy Uncoupling with LCS Consumption on Appetite and Energy Intake"). Rodents demonstrate no preference for aspartame, and mice (Bachmanov et al., 2001) but not rats (Sclafani et al., 2004) demonstrate preference for sucralose. Saccharin is commonly used in rodent feeding models because its preference is similar to that of low concentrations of sucrose. Genetic and structural variation in the *Tas1r2/Tas1r3* genes and T1R2/T1R3 may alter sweetness perception and sweetener receptor binding affinity (Alexander et al., 2011; Li et al., 2002). These differences in T1R2/T1R3 binding have implications for glycemia, because the T1R2/T1R3 receptor is expressed throughout the GI tract and in the pancreas. Using rodent models to determine the effect of LCS on glycemia may only be effective for select LCS that exhibit similar receptor binding as humans.

The LCS delivery vehicle also has implications for glycemic responses, primarily related to cephalic phase responses initiated in the oral cavity. Administration of LCS via capsules

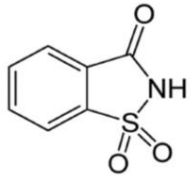
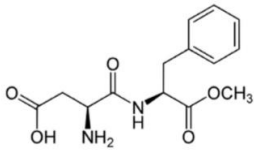
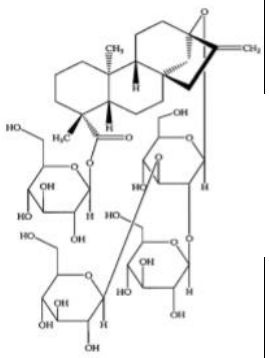
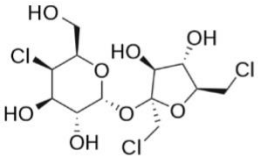
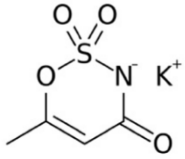
bypasses these responses and may lack ecological relevance, because LCS are consumed for their sweetness. Solid and liquid LCS loads may have differential glycemic responses because solid foods require chewing. While chewing and sweet taste alone do not lead to CPIR for all LCS, the combination of chewing aspartame-sweetened gum increased serum insulin within five minutes of consumption (Bruce et al., 1987). LCS are commonly combined in foods and beverages, including diet sodas, because they can synergistically enhance sweetness intensity and reduce the unfavorable sensory characteristics of some sweeteners. However, utilizing LCS in combination raises multiple methodological issues for measurement of glycemic response. First, glycemic or enteroendocrine responses of individual LCS cannot be differentiated when they are consumed concurrently. Researchers make the assumption all LCS exhibit similar responses despite different chemical structures, receptor binding, and biological fates. Secondly, diet sodas contain many flavorings, thickeners, and ingredients to prolong shelf-life which could affect incretin release if comparing to a water or carbonated water control. The presence of other ingredients (both nutritive and non-nutritive) may also affect glycemic response by increasing palatability or initiating other glycemic responses independent of the LCS.

In a systematic review of RCT investigating the effect of LCS consumption on glycemic and enteroendocrine response (Tucker et al., 2017), only four of fourteen trials documented increased GLP-1 with LCS consumption. Three of the four trials with positive results administered LCS as diet soda containing sucralose and ace-K. GLP-1 AUC was higher with consumption of an ace-K/sucralose sweetened cola beverage in adults (R. J. Brown et al., 2009) and youths with and without T1D but not T2D (R. J. Brown et al., 2012). There was no effect on blood glucose or insulin compared to a carbonated water control in either trial. The increase in GLP-1 is likely an artifact of increased palatability from the soda and not the individual or combination of LCS.

Subsequent trials document increases in GLP-1 with diet soda consumption compared to seltzer water, while sucralose alone at varying concentrations had no effect on GLP-1 (A. C. Sylvetsky et al., 2016). A replication of the trial found no difference in GLP-1 with administration of sucralose, ace-K, and sucralose plus ace-K solutions compared to water (Wu et al., 2013).

In summary, select LCS increase intestinal glucose absorption, enteroendocrine GLP-1 and GIP secretion, and pancreatic β-cell insulin secretion in select *in vitro* and rodent *in vivo* trials. However, these endocrine responses are not replicated in humans. All LCS consistently have no effect on glycemia, with the exception of select trials among populations with T2D or obesity. A majority of the RCT conducted examining the effect of individual LCS on glycemia are pre-load designed trials. Investigation of chronic LCS consumption are limited, yet consistently fail to find an effect of LCS consumption on glycemia (Grotz et al., 2017; Maki, Curry, Reeves, et al., 2008). The LCS delivery vehicle and location of delivery may affect glycemic and incretin response, but acute and chronic effects of consumption of any LCS do not appear to significantly affect glycemia.

Table 1 Summary of LCS Structure, Sensory, and Physiological Effects

	Saccharin	**Aspartame**	**Reb A (Stevioside)**	**Sucralose**	**Ace K**
Structure [0 6]					
T1R2/T1R3 Receptor Affinity [1 2]	T1R2 ATD Residue: R383, D142, and E882	T1R2 ATD Residue: E302, S144, D142, Y103, and D278	T1R2/T1R3 Residues: S40, K65, Y103, D142, D278, E302, P277, and R383	T1R2/T1R3 ATD Residue: D278 and Y103	T1R2 ATD Residue: R383, D142, and E882
Sweetness Potency (Relative to Sucrose)	300	180-200	400	600	200
Other sensory properties [3 4]	Bitter, metallic, licorice-like, slow onset sweetness	Slow onset sweetness, sweet aftertaste	Medicinal, bitter, licorice	Sweet aftertaste	Bitter, metallic
Digestibility and Metabolism [5 6 7]	No detectable digestion; Excreted primarily in the urine, some in feces [3]	Hydrolyzed to aspartate, phenylalanine, and methanol [3]	Conversion of steviosides to steviol to glucuronide for excretion in the urine; some excreted in the feces [4]	Primarily undigested in feces [3]; 2.6% metabolized and excreted in urine in humans [5]	No detectable digestion; Excreted primarily in the urine, some in feces [3]

	Saccharin	**Aspartame**	**Reb A (Stevioside)**	**Sucralose**	**Ace K**
Cephalic Phase Insulin Response	Response (Just, 2008); No response (Teff, 1995)	No Response (Teff, 1995; Abdallah, 1997)	-	Response (Dhillon, 2017); No Response (Ford, 2011)	-
Entero-endocirine Response *(in vitro)*	-	-	-	GLP-1, GIP secretion from Enteroendocrine Cells (Jang, 2007)	No effect on GLP-1 (Remann, 2008)
Entero-endocrine Response (animal trials)	Increased glucose absorption (Mace et al., 2007); Increased SGLT1 expression (Margolskee, 2007); No effect on GIP, GLP-1 (Fugita, 2008)	No effect on SGLT1 expression (Margolskee, 2007); No effect on GIP, GLP-1 (Fugita, 2008)	No effect on GIP, GLP-1 (Fugita, 2008)	Increased SGLT1 expression and glucose absorption (Margolskee, 2007); Increased GLP-1 secretion (Jang, 2007); No effect on GIP, GLP-1 (Fugita, 2008)	Increased glucose absorption (Mace et al., 2007); Increased SGLT1 expression (Margolskee, 2007); No effect on GIP, GLP-1 (Fugita, 2008)
Entero-endocrine Response (human trials)	-	No effect (Steinert, 2011)	-	Increased GLP-1 during an OGTT (Temizkan, 2015); No effect (Steinert, 2011; Ma, 2009; Sylvetsky, 2016; Wu, 2012)	No Effect on PYY or GLP-1 (Steinert, 2011); No effect on CCK (Meyer-Gerspach, 2018)
Insulin Response *(in vitro)*	Insulin Release from Pancreatic β-Cells (Nakagawa, 2009)	-	Insulin Release from Pancreatic β-Cells (Jeppesen, 2000; Abudula, 2008)	Insulin Release from Pancreatic Beta Cells (Nakagawa, 2009)	Insulin Release from Pancreatic β-Cells (Nakagawa, 2009)

	Saccharin	Aspartame	Reb A (Stevioside)	Sucralose	Ace K
Insulin Response (human trials)	No effect on insulin or glucose (D.L. Horwitz, 1988); no effect on glucose during OGTT (Bryant, 2014)	No Effect (Tey, 2017; Hall, 2003)	No Effect (Gregersen et al., 2004) (Tey, 2016)	Increased Insulin during OGTT among individuals with obesity (Pepino, 2013); No effect (Ma, 2009; Brown, 2011; Steinert, 2011; Sylvetsky, 2016; Wu, 2012)	No effect (Steinert, 2011)

- No evidence
0 (Roberts, 2016)
1 (Masuda et al., 2012)
2 (Kim et al., 2017)
3 (O'Donnell et al., 2012)
4 (Reyes et al., 2017)
5 (Magnuson et al., 2016)
6 (Carakostas et al., 2008)
7 (Roberts et al., 2000)

CHAPTER 3. PROPOSED MECHANISMS LOW CALORIE SWEETENERS AFFECT BODY WEIGHT, ENERGY INTAKE, APPETITE, AND GLYCEMIA

Multiple mechanisms have been proposed to explain the positive and negative associations between LCS consumption and body weight. Historically, LCS were believed to be inert past the oral cavity. The energy reduction when LCS replaced sugars would reduce energy intake and body weight. However, the mechanism of energy reduction with LCS consumption is over-simplified. The T1R12/T1R3 sweet taste receptor is present throughout the gastrointestinal tract, pancreas, adipose tissue, hypothalamus, and elsewhere. Activation of receptors on these cells elicits responses characteristic of the tissue, raising new questions about whether LCS are inert after swallowing. Energy dilution strictly focuses on differences in dietary energy and fails to account for sensory, pre-ingestive, post-ingestive, and cognitive consequences of LCS on appetite, energy intake, and body weight.

Mechanisms for how LCS reduce or increase body weight have been proposed. A single mechanism is often proposed to explain both positive and negative effects of LCS consumption. For example, dietary sweetness exposure may reduce energy intake through sensory specific satiety or increase energy intake because of increased dietary palatability. Some proposed mechanisms are generalized to all sweeteners, based on the positive or negative effects of providing sweetness without energy; while other hypotheses are specific to the LCS's chemical structures. LCS vary in chemical structure, T1R2/T1R3 binding domains, sensory profiles, digestibility, and biological fates, which can lead to differential physiological responses between LCS (see Table **1**). The presence or absence of various physiological responses that accompany

different LCS may contribute to different metabolic or behavioral changes that could positively or negatively impact body weight.

Hypothesized mechanisms to explain how LCS may affect body weight and glycemia are explained below. Proposed mechanisms by which LCS decrease body weight include energy dilution, sensory and pre-ingestive effects, gastric effects, and gut peptide release with exposure to select LCS. Proposed mechanisms by which LCS may increase body weight include sensory and preingestive effects, energy uncoupling, gut peptide release, brain reward effects, informed compensation, and gut microbiotic changes.

Energy Dilution

LCS contribute little to no dietary energy. Some LCS are not metabolizable (saccharin, sucralose, and ace-K) and yield no energy; others are energetic (aspartame yields 4 kcal/g and rebA yields 2.7 kcal/g). Yet all are highly potent sweeteners, ranging from 180-600 times sweeter than sucrose depending on the sweetener and desired sweetness equivalent (see **Table 1**). LCS that are nutritive (i.e. aspartame) are still low calorie because of the reduced amount needed to achieve the same sweetness of sucrose, that contributes 4 kcal/g.

The addition of a low or no energy LCS beverage or food to the diet would have no effect on energy intake while replacement of an energetic sugar-sweetened beverage or food with a low or no energy LCS alternative would result in a decrease in energy intake and body weight when consumed chronically. This assumes that the energy displaced with substitution of a LCS for sugar is not compensated. Evidence largely suggests that sugars are only partially compensated (DellaValle et al., 2005; Gadah et al., 2016; M. Reid et al., 2007; Soenen et al., 2007) with the exception of select trials that report no (Panahi et al., 2013) or complete (Holt, 2000) compensation of energy from sugar. The effect of energy reduction with LCS is most robust in liquids and to a

lesser extend in semi-solid foods, because beverage energy is less satiating than energy in more viscous or solid form (Almiron-Roig et al., 2013; Bellisle et al., 2007; Mourao et al., 2007). For example, supplementation of 449 kcal/day of solid carbohydrates (jelly beans) for four weeks resulted in complete dietary compensation (118%) while liquid carbohydrates (soda) resulted in no displacement of other dietary energy (-17%), increasing total energy intake and body weight in one RCT (DiMeglio et al., 2000). Reducing SSB consumption also reduces diet palatability and sweetness, potentially explaining the lack of BMI change with reduction of SSB intake (R. Mattes et al., 2011). Replacement of SSB with LCS beverages may be a technique to reduce energy intake greater than reduction of SSB alone. Therefore, because SSB are poorly compensated, replacement with LCS beverages should reduce energy intake without noticeable changes in appetite.

The effect of energy dilution by substituting sugars with LCS has been studied extensively in short-term, pre-load design interventions. A meta-analysis of 68 short-term (<1 day) interventions comparing energy intake following consumption of a LCS versus a sugar sweetened control concluded LCS consumption significantly reduced subsequent energy intake by 94 kcals (95% CI: -122, -66) (P. J. Rogers, Hogenkamp, Graaf, et al., 2016). Among the trials assessed in this meta-analysis, eighteen trials used single LCS and reported cumulative energy intake. Five trials found significant reductions in cumulative energy intake with consumption of aspartame compared to sugar sweetened controls (Anton et al., 2010; Beridot-Therond et al., 1998; Drewnowski et al., 1994; Lavin et al., 1997; Monsivais et al., 2007; Wilson, 2000); only one reported a significant increase in cumulative energy intake (Lavin et al., 1997). Other trials found no differences in cumulative energy intake with consumption of aspartame and sugar sweetened preloads (Rolls et al., 1989) or inconsistent results between treatments (Hetherington et al., 2000; Rodin, 1990; Rolls et al., 1990). Four trials used sucralose as the LCS. Two trials found sucralose

preloads significantly reduced cumulative energy intake compared to sugar sweetened controls (Tina Akhavan et al., 2007; T. Akhavan et al., 2010) and two found no significant differences between treatments (Carvalho et al., 2013; P. J. Rogers et al., 2012). Cumulative energy intake was not significantly different following saccharin and glucose sweetened yogurt preloads in the one trial assessing saccharin (P. J. Rogers et al., 1989). Cumulative energy intake was significantly reduced following stevia preloads compared to a sucrose sweetened preloads in the one trial assessing stevia (Anton et al., 2010). Energy intake with the stevia preload was not significantly different than an aspartame preload in this trial. Few trials assessed multiple LCS, and there were not enough trials to conduct meta-analyses of individual sweeteners on energy intake.

The reduction in energy is maintained in longer-term trials measuring energy intake, with reductions in energy intake ranging from -75 to -514 kcal/day with LCS consumption compared to sugar or water (P. J. Rogers, Hogenkamp, Graaf, et al., 2016). Three of these trials using a single LCS for sustained duration monitored energy intake; only one of these compared energy intake between individual LCS. In a two-arm, four-week parallel design trial investigating compensation of sucrose and aspartame, sucrose supplements of 430 kcal/day significantly increased energy intake by approximately 273 kcal at week one and 190 kcal at week four, while aspartame consumption reduced energy intake by 86 and 94 kcal/day at week one and four, respectively (M. Reid et al., 2007). Another trial reported no difference in energy intake between aspartame consumers and non-consumers during a sixteen week weight loss intervention or during a twelve month weight maintenance period (Blackburn et al., 1997). Despite no difference in energy intake, the aspartame treatment had significantly lower weight regain compared to the no-aspartame control. Energy intake in response to aspartame, saccharin, or sucrose consumption in a cross-over design trial among preschool (3-5years) and school age (6-10 years) children found energy intake

was significantly higher while on the sucrose sweetened diets for both age groups with no significant differences in energy intake between the aspartame and saccharin treatment groups (Wolraich et al., 1994). Again, there were not enough trials to determine differences in energy intake in response to different LCS consumed long-term.

Despite the abundance of trials monitoring energy intake and compensation between LCS and sugar, only a few trials monitor sustained energy intake longer than 24 hours. Longer-term trials are necessary to determine whether repeated exposure leads to learned satiety and long-term compensation when LCS are substituted for sugars. Trials utilizing an individual LCS in human feeding studies commonly use aspartame, and their results lean towards reductions or no effect on energy intake compared to sugar sweetened controls (reviewed in **Chapter 2**). Considering all LCS together, the energy displaced when LCS replace sugars is only partially compensated resulting in decreased energy intake. While the mechanism of energy dilution explains the reduced energy intake and body weight when LCS are substituted for sugar, it does not explain the difference in energy intake and body weight when LCS are compared to water. LCS have additional sensory, preingestive, and postingestive consequences that may affect body weight beyond energetics.

Sensory and Pre-ingestive Effects of LCS on Appetite and Energy Intake

Sweetness

Sweet is a primary taste quality, is innately liked, and is sought for its pleasant taste. The addition of LCS or sugars can increase the sweetness, mask unfavorable taste qualities, and increase the palatability of a food or beverage. It has been proposed that heightened sweetness (separate from energy) increases hunger and/or desire to eat (DTE), resulting in increased energy intake. Preliminary trials by one research group documented increased hunger and DTE after

aspartame consumption and increased preference for commercially available foods following aspartame and saccharin consumption compared to a water preload (P. J. Rogers et al., 1988). While no difference in energy intake was reported sixty minutes after consumption of LCS sweetened and unsweetened preloads, subsequent trials reported increased energy intake following a saccharin sweetened yogurt preload compared to plain yogurt over a twelve hour post-preload period (P. J. Rogers et al., 1989). However, this heightened appetitive response is not consistent across all trials (Canty et al., 1991; Drewnowski et al., 1994; Rolls et al., 1990). For example, twenty participants reported similar appetitive sensations and energy intake between aspartame, saccharin, water, and sucrose preloads with differences observed only in DTE between sucrose and water preloads (Canty et al., 1991). This trial utilized a similar paradigm as Rogers *et al.* (P. J. Rogers et al., 1988) but was double blinded, included a larger sample size, and held participants in the laboratory between breakfast and preload delivery, making the results from this trial more robust.

Conversely, sweetness may decrease hunger and/or DTE through sensory specific satiety (SSS). SSS is the change in hedonic response (typically the decline in pleasantness) to a particular food as it is consumed (Rolls, 1986). SSS is specific to the taste quality (e.g., sweetness) but not the sweetener, demonstrated by decreased liking of 10-40% sucrose solutions following ingestion of both glucose and cyclamate solutions (O. W. Wooley et al., 1972). In a study investigating the motivational ratings and energy intake following sweet and non-sweet lunch preloads, isoenergetic preloads had similar effects on appetite for a meal, but decreased DTE sweet following a sweet lunch and decreased DTE savory following the savory lunch (de Graaf et al., 1993). This translated to decreased sweet foods and savory foods consumption at the subsequent meal after sweet and savory preloads, respectively. These results contradict the findings that sweetness exposure

increases hunger and DTE as well as the null findings of sweetness from LCS on appetite referenced above (Canty et al., 1991; Drewnowski et al., 1994; Rolls et al., 1990). The effect of sweetness on appetitive sensations and energy intake has been studied extensively in preload design trials (Sorensen et al., 2003); yet, report contradictory findings and provide little information about the effect of chronic sweetness exposure on appetite and energy intake.

Chronic and frequent dietary exposure to sweetness may lead to the development of a "sweet tooth," a heightened preference for dietary sweetness (Reed et al., 2006). This is often measured in cross-sectional studies, such as in one trial that reported the preferred concentration of sucrose in coffee and oatmeal was positively associated with percent of dietary energy from sweet foods and from carbohydrates (R. D. Mattes et al., 1986). Results from studies assessing the effect of the manipulation of dietary sugar on preference for sweet is less clear. Increased sucrose consumption resulted in increased preference for a sweeter beverage among children but not adults (Liem, 2004), and reduced dietary sugar intake for three months was associated with greater perceived sweetness without changes in sweetness preference (Wise et al., 2016). A systematic review of RCTs exploring sweetness exposure on acceptance, preference, food choice, and sweet food choice revealed that higher sweetness exposure tended to reduce sweet preference, but the effects are inconsistent from the limited number of longer trials and cohort studies (Appleton et al., 2018). The developed preference for sweetness only has implications for body weight if the increased sugar intake is not compensated via reductions in other dietary energy. While an increase in dietary sugar intake was positively associated with greater body weight in a meta-analysis of ten trials, isoenergetic exchanges of sugar with other macronutrients had no effect on body weight among results compiled from twelve trials (Te Morenga et al., 2013). Therefore,

implications of a developed sweet preference from dietary exposures on body weight must be considered in the context of total energy intake.

The impact of dietary sweetness on sugar and energy intake varies based on the sweetness delivery vehicle, either a LCS or nutritive sweetener. If increased sweetness enhances palatability and alters appetite and energy intake, then it would be predicted that all LCS and nutritive sweeteners would contribute similar ingestive effects, though with some variability. LCS have different sensory profiles and other chemosensory sensations, such as bitter taste, metallic flavors, and temporal differences (Antenucci et al., 2015; DuBois et al., 2012; O'Donnell et al., 2012). For example, saccharin and ace-K are perceived as bitter (Helgren et al., 1955; Reyes et al., 2017), binding to the T2R bitter receptors (C. Kuhn et al., 2004). Other LCS exhibit rapid sweetness appearance (aspartame) and/or delayed sweet extinction (saccharin, aspartame, and sucralose) (O'Donnell et al., 2012; Reyes et al., 2017). If these additional sensory characteristics increase or decrease palatability or prolong sweetness exposure, then individual LCS may have differente effects on appetite. Conversely, if dietary sweetness exposure leads to SSS, then LCS and nutritive sweeteners would reduce subsequent sugar intake. However, if dietary sweetness is held constant, then cumulative sugar intake would be lower with LCS consumption, making LCS superior to nutritive sugars for dietary sugar and energy reduction. If dietary sweetener exposure increases preference for dietary sweetness, then LCS and nutritive sweeteners would both increase dietary sweetness intake, either in the form of dietary sugars or additional LCS. Whether a developed sweet preference increases body weight should be considered in the context of total energy balance.

Cephalic Phase Insulin Response

The cephalic phase responses are brought on by sensory and cognitive stimulation by factors including smell, taste, appearance, oral mechanical processes (i.e. chewing and swallowing), and thoughts of food (T. Powley et al., 1985). Cephalic phase responses potentially exist as an anticipatory response to prepare the body to digest, absorb, metabolize, and store nutrients (Power et al., 2008) and serve as a neural regulation of food intake prior to ingestion (Nicolaïdis, 1969). The role of the cephalic phase insulin response (CPIR) is to lessen the impact of a rapid post-prandial load of glucose to the blood stream. The CPIR precedes an increase in blood glucose accompanying carbohydrate consumption, occurring within four minutes of oral exposure, and contributing up to 25% of total insulin release (K. Teff, 2000). While taste is enough to elicit cephalic phase salivary and gastric acid responses, there is conflicting evidence that sweetness alone is enough to trigger a CPIR. It was originally believed that the CPIR was attributed to binding of sweet compounds to the T1R2/T1R3 receptor. However, a CPIR is maintained in T1R3 knockout mice (Glendinning et al., 2015), suggesting the existence of another sweet taste receptor or a receptor specifically responsible for the CPIR. A CPIR has been documented for saccharin (Just et al., 2008) and potentially sucralose (Dhillon et al., 2017; Ford et al., 2011), but not for other LCS (Abdallah et al., 1997; K. L. Teff et al., 1995).

The implications of the CPIR in response to LCS on glycemia and energy intake have not been determined. If select LCS can activate the CPIR, then it is important to investigate the effects of an insulin response, particularly when LCS are consumed in the absence of energy. If a CPIR does occur, then the associated increase in insulin could reduce plasma glucose, disrupting glucose homeostasis. On the other hand, if an increase in blood insulin concentrations serves as a satiety signal in the brain (Woods et al., 2006), then saccharin and potentially sucralose could induce

satiety. There is speculation that the CPIR can be disrupted with intermittent consumption of regular and low- energy substitutes (P. Smeets et al., 2010), diminishing the learned association between taste and energy, and contributing to increased energy intake (S. E. Swithers et al., 2010). However, rats repeatedly exposed to saccharin do not exhibit a diminished CPIR in over 15 trials (T. Powley et al., 1985). The implication of LCS on the presence of absence of a CPIR requires further investigation.

Energy Uncoupling with LCS Consumption on Appetite and Energy Intake

The uncoupling of sweet taste and energy with LCS consumption has been hypothesized to be a mechanism by which LCS disrupt energy balance. Based on classical Pavlovian conditioning, sweetness is associated with intake of energy from carbohydrates and the post-ingestive consequences of carbohydrate intake. Consumption of sweetness with energy reinforces this conditioned relationship, while sweetness without energy weakens this association and results in loss of ability to predict energetic consequences of energy intake (Davidson et al., 2004). This hypothesis has been tested extensively in rodent trials that report rats increased energy intake when the diet was intermittently supplemented with saccharin or glucose sweetened liquids (sweet nonpredictive) compared to a diet sweetened with sucrose or glucose (sweet predictive) (Davidson et al., 2004). Additional experiments by this group using the sweet predictive/nonpredictive paradigm with different sweetened foods (i.e. yogurt, refried beans) and different LCS (i.e. ace-K, stevia) yielded similar results of energy overcompensation and elevated body weight with LCS exposure (S. E. Swithers et al., 2009; S. E. Swithers et al., 2008).

Results from a trial in humans testing the hypothesis of sweetness and energy uncoupling with LCS consumption are not consistent with these animal trials (Appleton et al., 2007). Instead of training participants with sweet predictive and non-predictive diets utilized in these animal

models, high consumers of LCS beverages, who were exposed to LCS and nutritive sweeteners in a free-living environment (sweet non-predictive), were compared to non-consumers (sweet predictive). Participants consumed either water, LCS beverages, or SSBs throughout one waking day in a cross-over design. There was no difference in energy intake with consumption of water, LCS beverages, or SSB among high LCS beverage consumers. Conversely, LCS non-consumers consumed more energy with LCS beverage loads compared to water loads, but there was no difference in energy intake between LCS beverage and SSB treatments. This suggests that LCS consumers did not use sweetness as a signal for energy, implying energy and sweetness was uncoupled, but this did not contribute to an increase in energy intake as demonstrated in rodent studies.

Although animal models often serve as an adequate model for mechanistic studies, differences in dietary exposures, preferences, carbohydrate metabolism, and sweetness detection make rodent models questionable for studies pertaining to associative learning of chemosensory stimuli and predictive energy. Learned associations between sensory cues and energy may more easily form in rodents, because their diets lack the variety of a human diet (P. J. Rogers, Hogenkamp, Graaf, et al., 2016). The duration of exposure is substantially different between rodent and human models. Conversion of 10 days of conditioning with 50 mL of a sweetened supplement to a 10 day old rat is equivalent to feeding 1.8L to a 2.2 year old for 265 days (Sengupta, 2013). While dietary carbohydrates are converted to fatty acids during carbohydrate and energy excess via *de novo* lipogenesis in rodents and other species, the extent *de novo* lipogenesis occurs in humans is limited, with only ~9 g of carbohydrates converted to fat with a 50% dietary carbohydrate surplus (Hellerstein et al., 1996). There are also considerable differences in LCS sensory perception between rodents and humans. Saccharin is the preferred LCS in rat and mice models; 0.2-0.4%

saccharin is iso-preferred to low sucrose concentrations (Smith et al., 2002). Concentrations of saccharin used in the Swithers and Davidson models (0.3% saccharin) not only exceed the acceptable daily intake level for humans but are unpalatable, characterized with a bitter or metallic off-taste detected by humans at 0.07% saccharin (Helgren et al., 1955). Primates, specifically chimpanzees, are the ideal animal model of human sweet taste because of their genetic similarity and neural and behavioral response similarity to sweeteners (Hellekant et al., 1996). However, there are no trials investigating the effect of LCS consumption on food intake and body weight among primates. Pig models have also been used to monitor feed intake in response to saccharin, which document an increased feed intake when low levels of saccharin were incorporated into the diet (Aldinger et al., 1959). Whether this increase in intake is in response to dietary sweetness and palatability or saccharin requires further investigation, because pigs do not detect saccharin as sweet (i.e. no response from the chorda tympani nerve with saccharin administration (Hellekant et al., 1996)) and exhibit either preference, avoidance, or indifference for saccharin in other trials (Baldwin, 2007; Clouard et al., 2012; Kare et al., 1965).

Therefore, the direct conversion of LCS conditioning in rats to humans is not ethical or feasible. The effect of intermittent LCS and sugar exposure is consistently associated with increased body weight in rodent models, with increases in body weight documented in 14 of 22 rodent learning studies in a recent meta-analysis (P. J. Rogers, Hogenkamp, Graaf, et al., 2016). However, the limited ability to translate learned eating behavior paradigms demonstrated in animals to humans (Brunstrom, 2004) and no loss of energy signal fidelity among habitual LCS beverage consumers (Appleton et al., 2007) questions not only whether sweetness-energy learning regulates energy intake but also whether LCS disrupt the learned association between sweetness and energy in humans.

Gastric Effects of LCS on Appetite and Energy Intake

Ingested substances reaching the stomach result in gastric distension. Mechanoreceptors on the hepatic branch of the vagus nerve detect changes in stomach volume and send neural signals to the brain that induce satiation. The distension of the stomach alone in the absence of energy can affect energy intake in the short term (Phillips et al., 1996). In fact, increasing volume (but not energy) resulted in decreased energy intake at the subsequent meal when 200 ml/200 kcal, 400 ml/200 kcal, and 400 ml/400 kcal preloads were administered intragastrically (Rolls et al., 2002). If gastric distension alone determines satiation, then theoretically isovolumetric SSB and LCS beverages will have similar effects on satiation despite the differences in energy. However, the extent of gastric distension is determined by rate of gastric emptying, which is regulated by nutrient-sensitive neural and endocrine signaling from the intestines. Gastric emptying rate is determined by the viscosity, nutritive density, initial gastric volume, and osmolarity of the gastric load (Hunt et al., 1975; T. Powley et al., 2004; Steinert et al., 2017; Vist et al., 1995). CCK and GLP-1 released from enteroendocrine cells in response to nutrients in the intestinal lumen slow gastric emptying via vagal or endocrine signaling and act synergistically with gastric distension to induce satiation (T. Powley et al., 2004; Steinert et al., 2017). The delay in gastric emptying is hexose sugar dependent, but not responsive to sweetness alone (i.e. sweet taste receptor binding) (C. Bryant et al., 2016; Little et al., 2010). If the duration of gastric distension (determined by gastric emptying rate) alone determines satiety, then the decreased osmolality and carbohydrate content would result in a faster gastric emptying rate and reduced satiety compared to sugar sweetened controls. Indeed, iso-sweet solutions of aspartame, saccharin, fructose, and water delivered intragastrically were not significantly different from each other but promoted faster emptying rates than iso-sweet glucose solutions (Little et al., 2010). Intragastric sucralose

administration also does not slow gastric emptying or increase GLP-1 or GIP compared to a water or iso-osmotic saline control and did not have a significantly different appetite profile compared to water, glucose, and fructose (Ma et al., 2009; Steinert et al., 2011; Wu et al., 2013). Gastric emptying is different between LCS and sugar, but its regulation of energy intake is limited. The effects are diminished when considered in the context of numerous cognitive, sensory, endocrine, metabolic, and cultural factors affecting ingestive behavior in a free-living environment.

Gut Peptide Release with LCS Exposure on Appetite and Glycemia

The presence of sweet taste receptors throughout the gastrointestinal tract provide opportunities for potential responses to LCS past the oral cavity. Specifically, gut peptides that are commonly believed to be satiety biomarkers are secreted from enteroendocrine L and K cells that express T1R2/T1R3 receptors. Binding of LCS to T1R2/T1R3 receptors on these cells could increase levels of incretins such as GLP-1 and GIP (Egan et al., 2008), resulting in increased release of insulin from pancreatic β-cells. The rise of insulin in the absence of energy has been proposed to disrupt glucose homeostasis and increase appetite. Conversely, GLP-1 is one of many proposed gut "satiety" peptides. GLP-1 acts as an anorexogenic hormone via vagal afferent neuron signaling or in circulation, binding to receptors on the arcuate nucleus. This leads to decreased expression of NPY/AgRP neuropeptides and increased expression of proopiomelanocortin (POMC), which has an anorexigenic effects. If GLP-1 concentrations increase in response to LCS exposure, then LCS consumption could increase satiety and decrease energy intake.

The theory that LCS consumption disrupts energy and glycemic regulation is not supported by the literature. Select LCS do bind to T1R2/T1R3 receptors on endocrine and absorptive cells; resulting in increased intestinal glucose absorption, increased pancreatic insulin secretion, and increased GIP and GLP-1 secretion in *in vitro* and select animal trials (see **Table 1**). However,

these incretin and glycemic responses have not been consistently replicated in humans for any of the LCS administered orally or intragastrically (summarized in **Chapter 2**). Many trials measure gastric emptying and gut peptide release as predictors of appetite, yet surprisingly few monitor appetitive sensations concurrently. Intragastric loads of aspartame, sucralose, and ace-K result in similar concentrations of GLP-1, PYY, or ghrelin secretion as water; while glucose (but not fructose) lead to significantly greater releases of all peptides (Steinert et al., 2011). Despite these differences in gut peptide release, fullness and hunger ratings were not different between any of the treatment groups, though ratings tended to follow the pattern of carbohydrate sugars having greater ratings of fullness and lower ratings of hunger, followed by LCS treatments with intermediate appetitive changes, and water having the least change. Oral sucralose administered with a carbohydrate load led to reduced plasma GLP-1 and GIP compared to glucose and a non-metabolizable, 3-O-methylglucose, yet there were no significant differences in appetitive sensations (Tongzhi Wu et al., 2012). One trial documented an increase in GLP-1 release with sucralose and ace-K sweetened cola consumption but reported no changes in appetitive response (R. J. Brown et al., 2009). The hypothesized appetitive changes in response to elevations in GLP-1 and GIP levels are moot, because there is little evidence these peptides are released with LCS exposure.

Brain Reward Effects of LCS on Ingestive Behavior

Sweet taste exposure leads to activation of dopamine and opioid systems in the brain responsible for reward processing. LCS and sugars exhibit differential brain activation, suggesting that LCS and sugars have different rewarding values. The “hijacking” of brain reward systems with dietary sweetness exposure has been postulated as a mechanism to promote cravings, addictive-like behavior, and energy overconsumption (Aoyama et al., 2014; Murray et al., 2016).

Both heightened and reduced activation of feeding regions in the brain have been hypothesized as explanations for the positive associations between LCS use and body weight in epidemiological trials. If LCS are supranormal stimuli and exposure leads to heightened responses in the brain, then LCS could stimulate reward driven feeding. If LCS consumption leads to decreased activation of reward regions of the brain, then LCS could promote overconsumption to achieve the same level of reward.

LCS and sugar exposures differentially recruit food reward-related brain regions in both animals (Ostlund et al., 2013; Tellez et al., 2016) and humans (Frank et al., 2008; P. A. Smeets et al., 2011). Dopamine and opioid reward systems differentiate sweetness with and without energy. Dopamine is released in the dorsal striatum in response to energy-yielding sweeteners; dopamine is released in the ventral striatum in response to sweet taste (Tellez et al., 2016). Sucrose (but not sucralose) taste stimulation elicits greater dopaminergic midbrain activation and greater activation of anterior insula, frontal operculum, striatum, and anterior cingulate brain regions (Frank et al., 2008). The opioid food reward systems are related to the palatability of the food (Olszewski et al., 2007; Peciña et al., 2010). Mu opioid receptor knockout mice lick sucralose solutions at half the rate compared to wildtype mice (Ostlund et al., 2013). Knockout mice were only responsive to sucrose solutions during food deprivation, suggesting that the post-ingestive effects of carbohydrate sweetener consumption were still maintained. The opioid reward system is responsive to the palatability of increased dietary sweetness not just the energy from nutritive sweeteners.

Trials investigating neural activation in response to sweetness report different responses with exposure to LCS and sucrose. Most studies investigating brain activation with sweetness exposure in humans are cross-sectional. They compare diet-soda consumers to non-consumers, do

not concurrently measure ingestive behaviors, have high within/between subject variability, and arrive at conflicting results. For example, activation of the amygdala (a region of the brain associated with stimulus-reward learning (Baxter et al., 2002)) was reduced among high LCS consumers in one trial (Rudenga et al., 2012) and greater with sucrose and saccharin exposure among diet soda consumers compared to non-consumers in another trial (Green et al., 2012). Despite opposing data, these two trials draw the same conclusion that LCS consumption results in changes in neural responses and could influence eating behavior, yet neither trial measures ingestive behavior beyond perceived pleasantness of the sweet stimuli.

Few trials have investigated brain activation and ingestive behaviors in response to LCS and sugars. One trial measured greater activation of the amygdala and reduced activation of the striatum with LCS beverage consumption compared to sucrose beverage consumption (P. A. Smeets et al., 2011). However, appetitive sensations following sucrose or LCS consumption were not significantly different. Research studies on long-term changes in reward processing with sweetness exposure in humans are few. In one trial, repeated exposure to SSB over a twenty day period resulted in increased activation of the right precuneus compared to consumption of LCS beverages (S. Griffioen-Roose et al., 2013). Although two other regions of the brain related to food cues and learning (the middle cingulum and left precentral gyrus) had greater activation with SSB consumption versus LCS consumption, these responses did not change with repeated exposure to either treatment. Beverage intake, liking, and expected satiety increased from baseline among both treatment groups; however, reward value (measured as implicit intake, explicit wanting, implicate association task, expected satiety) of LCS- and SSB did not differentially change over the trial. Whether differential activation of select food reward brain regions between LCS and sugars translates into difference in ingestive behaviors requires further investigation, but results from

these trials do not suggest the LCS exposure leads to heighted activation of brain reward regions compared to sugars.

More research on the mechanisms by which LCS affect brain reward activation are necessary to determine if LCS differentially affect brain reward. If differential responses in brain reward occur at the sensory level, then all LCS could exhibit distinct responses. If LCS in circulation activate brain reward processes at the blood brain barrier, then saccharin and sucralose could lead to heightened or reduced responses. The current state of available research is too limited to draw conclusions about the consequences of LCS consumption, let alone differences between LCS, on reward driven feeding.

Informed Compensation and Energy Intake

LCS sweetened products typically accompany diet, sugar-free/reduced sugar, or low-calorie health claims. Knowledge of reduced energy content of LCS products has been theorized to lead to overcompensation at subsequent meals (O. W. Wooley et al., 1972). While knowledge of the energy content of a meal and "expected satiety" altered appetitive sensations and energy intake in select trials (Brunstrom et al., 2011; Shide et al., 1995; O. W. Wooley et al., 1972), others found no effect of energy information on satiety and energy intake (Chambers et al., 2013; R. Mattes, 1990; Rolls et al., 1989; Wardle, 1987; Yeomans et al., 2001). In studies exploring energy information manipulations with the use of LCS, knowledge of energy content had no effect on total energy intake (R. Mattes, 1990; M. Reid et al., 2007; Rolls et al., 1989). Daily energy intake was not significantly different when healthy, non-dieting adults were informed of the energy content of high and low energy, sugar and LCS sweetened preloads compared to uninformed participants (Rolls et al., 1989). Similarly, normal weight women actively watching their diet exhibited no difference in energy intake or body weight over a four-week timeframe when they

were correctly and incorrectly informed about the energy content of a sucrose or aspartame sweetened beverage (M. Reid et al., 2007). In another trial, normal weight individuals informed they were consuming an aspartame sweetened cereal breakfast tended to consume more energy at the subsequent meal and more energy daily over a five day intervention than uninformed participants or participants who consumed an unsweetened or sucrose sweetened cereal, though this difference was not statistically significant (R. Mattes, 1990). Data from trials providing accurate and misinformation about energy content of sugar and LCS products do not support the hypothesized risk of overcompensation that would occur in a free-living environment.

Gut Microbiota Effects of LCS on Body Weight and Glycemia

The ecosystem of microorganisms that live in the human gastrointestinal tract have a spectrum of beneficial and harmful effects on health, including energy balance, glycemia, gut motility, and cardiovascular health (H. J. Flint et al., 2012). The microbial populations vary between individuals who are lean and have obesity (Ley et al., 2006). In addition, colonization of germ-free mice with the microbiota from obese mice results in an obese phenotype compared to when colonized with the microbiota from lean mice (Turnbaugh et al., 2006). Fecal microbiotic transplantation studies in humans have also documented increased insulin sensitivity six weeks after transplantation from a lean donor to a recipient with metabolic syndrome despite no changes in body weight (Kootte et al., 2017). The gut microbiota is sensitive to dietary changes, but changes in microbiotic populations and levels of fermentation do not necessarily result in dysbiosis or obesity. For example, despite increased levels of fermentation of resistant starch and energy harvesting, body weight changes were not different between obesity-prone and obesity-resistant rats when fed the same diet (Diana et al., 2018). There are hypothesized mechanisms that the gut

microbiota affects body weight and glycemic control, yet a known causal relationship is not clear (H. J. Flint et al., 2012; Shen et al., 2013).

The influence of acute and chronic diet on changes in microbiome species composition, metabolic products, and/or energy harvesting efficiency raises questions on how gut microbiota populations respond to LCS in the colon (Nettleton et al., 2016). Sucralose, steviol, and a fraction of saccharin reach the colon and have the potential to induce changes in the colonic microbiota that may then increase or decrease energy harvesting efficiency, which could affect body weight (Magnuson et al., 2016; Jotham Suez et al., 2015). Preliminary studies suggest LCS consumption leads to glucose intolerance via compositional and functional alterations of the gut microbiota in mice and humans but result in limited alterations in body weight. Mice fed a high-fat diet supplemented with saccharin had impaired glucose tolerance, which corresponded with changes in microbioal population, metagenomics, and increased short chain fatty acid production (J. Suez et al., 2014). However, saccharin-fed mice and germ-free mice receiving fecal transplantations from saccharin-fed mice experienced no significant changes in body weight or chow consumption (J. Suez et al., 2014). In another trial, rats provided a low dose of aspartame (5-7mg/kg/d) had elevated fasting glucose and impaired insulin tolerance, higher total bacteria, and increased *Enterobacteriaceae* and *Clostridium leptum* (Palmnas et al., 2014). However, the aspartame treatment attenuated body weight and was associated with a *Firmicutes* to *Bacteriodetes* ratio characteristic of non-obese populations when rats were on a high fat diet. Similar null effects on body weight despite changes in gut microbial populations and metabolite production have been reported with sucralose (Abou-Donia et al., 2008; Uebanso et al., 2017). Based on these preliminary trials, gut microbiotic changes with LCS consumption may alter glucose tolerance but have limited effect on body weight.

Understanding the role of the microbiome in glycemic and body weight regulation is in the early stages of exploration, with no causal mechanism known. Trials conducted to determine the effect of LCS consumption on microbiotic changes associated with glycemia and obesity have limitations. Variations in sampling techniques, differences in LCS biological fates, dietary manipulations independent of LCS, and small sample sizes can lead to different results. Fermentation in the gut is largely anaerobic (H. J. Flint et al., 2012); however, analysis of microbiotic changes are commonly conducted using fecal collections instead of cecal collections, which are poor representations of colonic microbial populations (Daly et al., 2016). Only sucralose, a fraction of saccharin, and products of steviol glycoside digestion reach the colon and have opportunities to interact with the gut microbiota (see **Table 1**). Documentation of microbiotic and phenotypic changes with aspartame consumption requires additional investigation, because aspartame is metabolized in the proximal small intestine. The addition of a LCS to the diet typically includes displacement of sugar or carbohydrates, resulting in a higher percentage of dietary fat and/or protein if the energy of the diet is held constant. Therefore, it is possible the LCS may alter the gut microbiota indirectly through dietary changes made to the diet. Finally, elevated blood glucose and microbiotic changes associated with saccharin consumption were only documented in a subset of the sample: a total of 4 participants out of 7 involved in a 7 day trial (J. Suez et al., 2014). Larger sample sizes are needed because of wide between- and within-subject variability in the gut microbiota. While alteration of microbiota diversity, metabolite production, and/or energy harvesting efficiency are linked to obesity and glucose tolerance, more research is needed to determine the mechanism specific LCS that reach the colon may play in this relationship.

Summary of Mechanisms

LCS are used to reduce the sugar and energy content of the diet while maintaining sweetness and palatability. While this dilution of sugar and energy theoretically facilitates weight and glycemic control; additional sensory, physiological, and behavioral responses to LCS may enhance or compromise the beneficial effects of LCS use. Some hypothesized mechanisms are generalized to all LCS: mechanisms related to energy dilution, dietary sweetness, sweetness and energy uncoupling, gastric effects, and informed compensation. Other mechanisms are specific to the chemical properties of the LCS that lead to differential physiological effects such as the CPIR, gut peptide release, brain reward, and gut microbiotic changes. Differences in taste and enteroendocrine T1R2/T1R3 receptor affinity, digestibility, and biological fate contribute to these differential responses. The following trials were conducted to test the effect of LCS consumption on body weight and glycemia. Mechanisms were not explored, but inferences can be drawn from these hypothesized mechanisms when interpreting results from the trials.

CHAPTER 4. DIFFERENTIAL EFFECTS OF LOW CALORIE SWEETENER CONSUMPTION ON BODY WEIGHT, ENERGY INTAKE, APPETITE, AND GLYCEMIA IN INDIVIDUALS WITH OVERWEIGHT OR OBESITY

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Abbreviations: LCS, Low Calorie Sweeteners; RCT, Randomized Control Trial; CPIR, Cephalic Phase Insulin Response; Ace-K, Acesulfame-potassium; GLP-1, Glucagon-like Peptide-1; GIP, Gastric Inhibitory Polypeptide; RebA, Rebaudioside A; BMI, Body Mass Index; TFEQ, Three Factor Eating Questionnaire; PABA, Para-aminobenzoic acid; VAS, Visual Analog Scale; OGTT, Oral Glucose Tolerance Test; DEXA, Dual-Energy X-ray Absorptiometry; FMC, Fine Motor Control; TBW, Total Body Water; ASA24, Automated Self-Administered 24-hour Dietary

Recall; TAG, Serum triacylglycerol; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; HbA1c, Hemoglobin A1c; EE, Total Energy Expenditure; DTE, Desire to eat; mBEV, Beverage Consumption Habits Questionnaire; AUC, incremental area under the curve; SSB, Sugar-sweetened beverages

Abstract

Background: Low calorie sweeteners (LCS) provide sweetness with little or no energy. However, each LCS's unique chemical structure has potential for different sensory, physiological, and behavioral responses that affect body weight.

Objective: The purpose of this trial was to compare the effects of consumption of four LCS and sucrose on body weight, ingestive behaviors, and glucose tolerance over a 12-week intervention in adults (18-60 y/o) with overweight or obesity (BMI 25-40kg/m^2).

Design: In a parallel-arm design, 154 participants were randomly assigned to consume 1.25 to 1.75L of beverage sweetened with sucrose (n=39), aspartame (n=30), saccharin (n=29), sucralose (n=28), or rebaudioside A (rebA)) (n=28) daily for 12 weeks. The beverages contained 400-560kcal/day (sucrose treatments) or less than 5kcal/day (LCS treatments). Anthropometric indices, energy intake, energy expenditure, appetite, and glucose tolerance were measured at baseline. Body weight was measured every two weeks with energy intake, expenditure, and appetite assessed every 4 weeks. 24-hour urine collections were completed every 4 weeks to determine study compliance via PABA excretion.

Results: Of the participants enrolled in the trial, 123 completed the 12-week intervention. Sucrose and saccharin consumption lead to increased body weight across the 12-week intervention (Δweight =+1.85kg ± 0.36 and +1.18kg ± 0.36, $p \leq 0.02$) and did not differ from each other. There was no significant change in body weight with consumption of the other LCS treatments compared to baseline, but change in body weight for sucralose was negative and significantly lower compared to all other LCS at week 12 (weight difference ≥ -1.37kg ± 0.52, $p \leq 0.008$). Energy intake decreased with sucralose consumption (p=0.02), and eating frequency was lower for

sucralose than saccharin (p=0.045). Glucose tolerance was not significantly affected by any of the sweetener treatments.

Conclusions: Sucrose and saccharin consumption significantly increase body weight compared to aspartame, rebA, and sucralose while weight change was directionally negative and lower for sucralose compared to saccharin, aspartame, and rebA consumption. LCS should be categorized as distinct entities because of their differing effects on body weight.

Introduction

Low calorie sweeteners (LCS) are ubiquitous in the US food supply. They are consumed most commonly in beverages (by 30.8% of American adults), but also in foods (10.3%), and added to products via LCS packets (14.1%) (A. C. Sylvetsky et al., 2017). Results from meta-analyses of randomized controlled trials (RCT) (Azad et al., 2017; P. E. Miller et al., 2014; P. J. Rogers, Hogenkamp, de Graaf, et al., 2016) conclude there is beneficial or no effect of LCS consumption on body weight compared to sugar sweetened and water alternatives. Yet results from select epidemiological (Fowler et al., 2008), *in vitro* (Egan et al., 2008), and animal trials (S. E. Swithers et al., 2008) continue to raise consumer concerns about the efficacy of LCS for weight management and glucose tolerance. Clinical and policy recommendations generally aggregate LCS. This reflects a view that their common mode of action is to provide sweetness while contributing little or no energy to the diet. However, each LCS has a unique chemical structure that elicits different sensory properties and digestibility, which may differentially effect appetite, brain reward activation, intestinal microbiota populations, metabolism, and body weight. If the latter is true, it may explain the variability in responses to single or combinations of LCS in the literature.

At the sensory level, LCS bind to different regions of the T1R2/T1R3 G-protein coupled heterodimer sweet taste receptor (Kim et al., 2017; Masuda et al., 2012; Nie et al., 2005). This results in differences in cellular biophysical processes (Nakagawa et al., 2013) that likely contribute to their unique sensory profiles (DuBois et al., 2012), preingestive responses (e.g., cephalic phase insulin response (CPIR)), and downstream effects. A CPIR has been documented for saccharin (Just et al., 2008) and potentially sucralose (Dhillon et al., 2017; Ford et al., 2011), but not for other LCS (Abdallah et al., 1997; K. L. Teff et al., 1995). There is speculation that the CPIR can be disrupted with intermittent consumption of nutritive and LCS sweeteners (P. Smeets

et al., 2010), uncoupling the association between taste and energy, and contributing to increased energy intake (S. E. Swithers et al., 2010).

Although the T1R2/T1R3 receptor was originally identified in the oral cavity, this receptor is present throughout the gastrointestinal tract, pancreas, adipose tissue, hypothalamus, and elsewhere. Activation of receptors on cells in these tissues elicits responses characteristic of the tissue, raising new questions about whether LCS are inert after swallowing. Differences in LCS digestibility can also contribute to disparate physiological outcomes. Aspartame, a dipeptide, is hydrolyzed to aspartate, phenylalanine, and methanol in the proximal intestinal lumen and has no documented effects on gastric emptying, gut peptide release, or glucose absorption (Steinert et al., 2011). Steviol glycosides also undergo metabolism to glucuronide in the liver. Other LCS are partially digested (approximately 2.6% of sucralose) or remain intact (saccharin and acesulfame-potassium (ace-K)) (Magnuson et al., 2016; Roberts et al., 2000). These fates result in different opportunities to bind to the T1R2/T1R3 receptors on enteroendocrine cells and stimulate release of gut-peptides glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), such has been reported with sucralose (Jang et al., 2007). Select LCS are absorbed into the blood (saccharin, ace-K, a fraction of sucralose, and steviol) and are excreted in the urine. These sweeteners may interact with sweet receptors in the brain and periphery. Pancreatic β-cell exposure to saccharin, sucralose, and steviol elicits insulin release *in vitro* (Jeppesen et al., 2000; Nakagawa et al., 2009) but such responses are not consistent across trials (Fujita et al., 2009). If true, a LCS mediated insulin release could increase hunger and energy intake (Ludwig et al., 1999). The post-ingestive consequences of different LCS on reward-driven feeding remains unclear as well. Sweetness is a pleasant sensation; LCS and carbohydrate sweetener exposures differentially recruit food reward-related brain regions in both animals (Tellez et al., 2016) and humans (P. A.

Smeets et al., 2011). Despite suggestions that sugar and LCS promote cravings or addictive like behavior (Murray et al., 2016), the available evidence is limited and these claims have not been substantiated (S. Griffioen-Roose et al., 2013). Some LCS are excreted in the feces (sucralose, steviol glycosides, and a fraction of saccharin), with opportunities to alter the microbiota, metabolite production, and/or energy harvesting efficiency (Magnuson et al., 2016).

Before further investigation into the mechanisms by which LCS may influence body weight, it is prudent to first establish that they do lead to differential weight outcomes when chronically consumed. Therefore, we conducted a five-parallel arm, RCT to compare the effects of consumption of four commonly consumed LCS (aspartame, saccharin, rebaudioside A (rebA), and sucralose) and sucrose on body weight, body composition, energy intake, energy expenditure, appetite, and glycemia over 12 weeks in adults with overweight or obesity (BMI 25-40 kg/m^2). We hypothesized that the unique profile of physiological responses each LCS elicits will result in differential changes in body weight both between the tested LCS and between LCS and sucrose.

Methods

Study Overview

In a parallel-arm design, one hundred fifty-four participants were randomly assigned to consume beverages sweetened with one of five sweeteners (sucrose, saccharin, aspartame, rebA, or sucralose) daily for 12 weeks. Saccharin, aspartame, rebA, and sucralose were selected for study, because they are the most widely consumed LCS in the US and approved for commercial use (FDA, 2018). Primary outcomes measured in this trial were body weight, body composition, dietary intake, energy expenditure, appetite, and glycemia. Secondary outcomes were serum lipids, sweetness perception, and overall liking of the delivery beverage. Urinary biomarker para-aminobenzoic acid (PABA) was used to monitor participant compliance with the beverage

intervention. Participants were informed the study was an investigation of sweetener consumption on fine motor control (FMC). This was intended to minimize participant modification of diet or lifestyle that could affect body weight outside of the sweetener intervention. The research protocol was reviewed and approved by Purdue University's Institutional Review Board (IRB #: 1510016667) and is registered at Clinicaltrials.gov (Identifier: NCT02928653).

Participants

Participants were recruited from the Greater Lafayette, Indiana area from January 2016 to March 2018. Two hundred and ninety-three individuals completed the screening process and qualified for the trial. One hundred fifty-four participants completed baseline procedures and were randomly assigned to a sweetener treatment. One hundred twenty-three participants completed the entire twelve-week trial (see CONSORT Flow Diagram in **Figure 1**). Participants completed a pre-screening questionnaire eliciting health and lifestyle information and had height and weight measured. Eligibility criteria included 1) 18-60 years of age, 2) BMI between 25-40 kg/m^2, 3) healthy (i.e. no history of diabetes, hypertension, etc.), 4) low consumers of LCS (reported consumption $\leq$ 1 time/week, 5) weight stable (weight change $\leq$ 3kg in the past 3 months), and 6) Three Factor Eating Questionnaire (TFEQ) dietary restraint score < 14.

Protocol

Participants completed a series of five testing days at baseline. Three of these days included measurements of baseline energy intake and energy expenditure. On one of the three days, participants completed an appetite log for one 24-hour period and one 24-hour urine collection. The latter was to obtain a baseline level of the urinary biomarker, para-aminobenzoic acid (PABA), to track compliance throughout the study. On the fourth baseline day, a dual-energy x-ray

absorptiometry (DXA) scan and an oral glucose tolerance test (OGTT) were completed. On the final baseline day, participants reported for FMC testing, body weight measurements, and beverage sensory/hedonic evaluations. Measurements of FMC included hand steadiness, rotary tracking, and a questionnaire regarding FMC in daily life. Body weight and beverage sensory/hedonic evaluations were measured every two weeks; assessment of energy intake, energy expenditure, appetite, and a 24-hour urine collection were repeated during weeks 4, 8, and 12; a DXA scan and OGTT were repeated at week 12.

Intervention

Following baseline participants were randomly assigned to a sweetener treatment (assignments generated from GraphPad Software (www.graphpad.com)). Due to the high attrition rate in the sucrose treatment group, randomization was regenerated after 100 participants to assign two participants to the sucrose treatment for every one assigned to the LCS treatments. Participants consumed between 1.25-1.75L of a colored, fruit flavored beverage (Kool-Aid, Kraft Foods) per day. Volume consumed was dependent on body weight at baseline: body weight 60-75kg consumed 1.25L/day, 76-90kg consumed 1.50L/day, and >91kg consumed 1.75L/day. Participants were blinded to the sweetener treatment and were provided no additional dietary recommendations besides required consumption of the intervention beverage. Participants choose their beverage flavor (lemon, orange, or mixed berry) on a weekly basis.

Sucrose beverages contained 100, 120, or 140 g of sucrose in 1.25, 1.50, or 1.75L of water (400, 480, 560 kcal), respectively. To put this into perspective of typical beverage consumption, mean water from beverages other than plain water is approximately 1.5L/day (Kant et al., 2009). Mean energy from sugar-sweetened beverages (SSB) ranges from 236-338 kcal/day among US

adults, with up to 20% of young adults consume greater than 500 kcal/day from SSB (Han et al., 2013).

LCS beverages contained 0.73, 0.58, 0.66, or 0.16g of saccharin, aspartame, rebA, and sucralose, respectively. Sweetener concentrations of the beverages were designed to fall within acceptable daily intake ranges set by the FDA and Joint FAO/WHO Expert Committee on Food Additives (JEDFA) (FDA, 2018) and to match the sweetness intensity of an 8% weight/volume sucrose beverage based on predicted iso-sweet concentrations from results of a pre-trial sensory test. Because sweetness potency varies by sweetener and concentration, LCS concentrations were not the same across treatments. In the sensory test, one hundred thirty-five participants sampled five quarter-log dilutions of sucrose (2.5-25%) and five quarter-log dilutions of one of four LCS (0.014-0.14% saccharin, 0.017-0.17% aspartame, 0.016-0.16% rebA, and 0.0044-0.044% sucralose). Participants tasted and expectorated each sample and rated sweetness intensity on a visual analog scale (VAS) with endpoints of "not sweet" to "extremely sweet" via Qualtrics Survey Software. Linear models were used to interpolate the necessary concentration of each LCS to match the sweetness of 8% sucrose, (the desired concentration of sucrose of our beverage formulation).

Anthropometrics

Body weight and total body water (TBW) were measured using a Tanita Body Composition Analyzer (Model TBF-410GS, Tanita Inc., Arlington Heights, IL). Participants removed their shoes, socks, and excess clothing (i.e. coats, sweater, jewelry, etc.) prior to weighing. Height was measured at baseline with a Holtain stadiometer. DXA (GE/Lunar Prodigy DXA) was used to measure body composition at baseline and week 12. Specific body composition measures of

interest included total fat mass, total fat-free mass (FFM), total tissue percent fat, android fat mass, and gynoid fat mass.

Sensory and Hedonic Beverage Assessment

Participants were told not to consume any food or drink other than water for least two hours prior to the beverage evaluation. They were asked to take one sip of the beverage and rate its sensory characteristics of intensity (sweet, salty, sour, bitter, off-flavor, mouth-drying, sweet aftertaste, bitter aftertaste, off-flavor aftertaste) and overall liking on 100-point VAS with endpoints of "not at all" to "extremely" via a Qualtrics Survey. Participants were also asked to report what time they typically consumed the study beverages and if they consumed the beverages with other foods or drinks.

FMC Testing

After participants finished consuming the beverage, they completed a survey on their subjective FMC and had hand steadiness (Impulse Counter, Lafayette Instrument Co.) and rotary tracking (Standard Rotary Pursuit, Model 30010A; Somatco) measured.

Oral Glucose Tolerance Test (OGTT)

A two-hour, seven sample OGTT was administered at baseline and week 12 (Dalla Man et al., 2005). Upon arrival after an overnight fast, an indwelling catheter was placed (time = -10min). A 2 mL whole blood sample with EDTA and a 4mL sample for serum were obtained in vacutainer tubes at time 0 immediately prior to consumption of the 75g glucose drink (Azer Scientific; Morgantown, PA). Subsequent blood samples were collected at minutes 10, 20, 30, 60, 90, and 120. Samples were centrifuged for 10 minutes at 4,300 rpm and the serum aliquoted and frozen at -80^{o}C for batch analysis. A single fasting blood draw was collected at week 6. Whole blood

HbA1c and serum glucose, insulin, triacylglycerol (TAG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol were determined on a Roche COBAS 400 Plus analyzer.

Dietary Intake

Food and energy intake were measured on three days (two non-consecutive week days and one weekend day) during baseline and weeks 4, 8, and 12 using the web-based NIH "Automated Self-Administered 24-hour Dietary Recall" (ASA24), version 2014 system, developed by the National Cancer Institute (Bethesda, MD). If the participant failed to complete a recall, the testing day was rescheduled within a week of the missed recall. The Goldberg Formula was used to determine whether recorded dietary intake was physiologically plausible (Black, 2000).

Energy Expenditure and Physical Activity

Free-living energy expenditure (EE) was measured using RT6 triaxial accelerometers (Higi LLC, Monrovia, CA). Measurements were taken at baseline and weeks 4, 8, and 12 on the same three testing days that dietary intake was recorded. EE was captured every 60 seconds and calculated using the Katch-McArdle Equation (McArdle, 2007). Habitual work, sport, and leisure physical activity were assessed using the Baecke Questionnaire (Baecke et al., 1982).

Appetitive Sensations

Appetite logs were completed using a smartphone and/or web-based Qualtrics survey on one of the days participants completed a dietary recall every four weeks. The appetite log asked participants to rate their hunger, fullness, desire to eat (DTE), prospective consumption, preoccupation with food, and thirst on 100-point VAS (A. Flint et al., 2000) on the hour, every hour while awake for a 24-hour period.

Compliance

Eighty milligrams of PABA were incorporated into the beverage powders daily to serve as a compliance biomarker. While urinary LCS can be used to measure LCS intake (Logue et al., 2017), not all LCS are excreted in the urine. Twenty-four hour urine samples were collected in 3 L opaque collection bottles at baseline as well as weeks 4, 8, and 12. Urinary PABA recovery was analyzed using spectrophotometric methods outlined by Sharma *et al.* (Sharma et al., 2014). Completeness of PABA collection was calculated as the measure of compliance using the following equation:

$$\left(\frac{(Measured\ PABA - Baseline\ PABA)}{Observed{:}Predicted\ Creatinine}\right).$$

Beverage Consumption Habits Questionnaire (mBEV)

A modified version of the Brief Questionnaire to Assess Habitual Beverage Intake (Hedrick et al., 2012) (referred to as mBEV) measured habitual beverage intake over the past month. The mBEV questionnaire was modified to include questions assessing consumption of flavored water; vegetable juice; diet sweet tea; diet energy drinks; diet sports drinks; subdivision of "Soft Drinks, Regular" and "Diet Soft Drinks/Artificially Sweetened Drinks" into "Regular Soda, soft drink," "Diet Soda, Soft Drinks," "Sweetened Juice beverage/drink," and "Diet sweetened drinks;" and subdivision of "tea or coffee, black, with/without artificial sweetener" into "tea or coffee with cream or non-dairy creamer," "tea or coffee, black with LCS," and "tea or coffee, with cream or non-dairy creamer with LCS." mBEV was completed at baseline, and weeks 4, 8, and 12 to track if and how participants responded to the intervention by adding or substituting the intervention beverage into their diet. An estimate of daily energy provided from discretionary beverages (not beverages required for consumption) was calculated based on ingestion frequency,

typical volume of beverage, and average energy content of the beverage (determined based on nutrient content obtained from the USDA Food Composition Database standard reference (ARS, 2018)).

Week 12 Questionnaire

At the end of the 12-week trial, participants completed an additional questionnaire. Questions included what type of sweetener they thought they consumed (sugar, LCS, no sweetener, both sugar and LCS, alternating LCS and sugar) and if the study altered their desire to eat sweet foods and beverages. If they indicated that they had a change in desire for sweet foods/beverages, they then indicated how strong the change in their desire for sweet was on a 100-point VAS with equi-spaced descriptors of "desire decreased extremely," "desire decreased slightly," "no change in desire," "desire increased slightly," and "desire increased extremely."

Statistical Analysis

Power calculations were based on a significant change in body weight from a report by Raben *et al.* (A. Raben et al., 2002), that compared two groups (one LCS (n=21) and one sucrose (n=20)) who adhered to a LCS intervention for 10 weeks. The present experiment was two weeks longer and sampled >25 individuals per treatment group. We considered a variance slightly larger (approx. 4%) than observed (standard deviation =1.78 kgs) in the Raben *et al*. report to reflect its expected increase given the additional two weeks of intervention and used a significance level of $\alpha<0.05$. With this variance and stated sample size, we had 80% power to detect differences in average weight gain of 2.8kg.

One-way ANOVA was used to compare baseline characteristics between treatment groups; Pearson's chi-squared was used to compare gender distributions and attrition between treatments.

Linear mixed models were used to determine the effect of time, treatment, and the treatment-by-time interaction on change in body weight, absolute body weight, and BMI. Additional linear mixed models controlling for baseline body weight were used to determine the effect of time, treatment, and time-by-treatment interaction on body weight and body composition. Linear mixed models were used to determine the effect of time, treatment, and treatment-by-time interaction on dietary intake, energy expenditure, appetitive sensations, beverage sensory attributes, and hedonics as well. Sweetener treatment and time were fixed- effects. Participants were treated as random effects repeated over time using a Toeplitz repeated covariance matrix. Statistical significance was determined by $\alpha < 0.05$. LSD was used for post-hoc comparisons.

The median PABA recovery from each participant during week 4, 8, and 12 was used to assess compliance. A Kruskal-Wallis H test was used to determine if median urinary PABA recovery was different between treatments. Statistical significance was determined by $\alpha < 0.05$.

One outlier (defined as outside the 25^{th} and 75^{th} percentile ± 3.1*IQR) in the aspartame treatment group was removed prior to analysis of EE. Values for the appetite logs were reported as mean appetitive sensation from the 24-hour appetite logs. Logs with fewer than four entries were excluded from analysis (data from four participants were excluded). Baseline urinary PABA data were missing for one participant due to a missed collection. Mean PABA at baseline of the total sample was used to replace the missing value.

Only participants who completed the OGTT at week 0 and week 12 were included in the statistical analysis of blood metabolites (n=123). Repeated measures ANOVA was used to compare blood glucose and insulin concentrations from the OGTTs and values of incremental area under the curve (AUC), HbA1c, total cholesterol, HDL, LDL, and TAG. Participants were treated as random effects. For blood glucose and insulin, main effects were time point during the two-

hour test (time), sweetener treatment (treatment), session (baseline and week 12), treatment-by-time, treatment-by-session, and treatment-by-session-by-time. When a sample value was missing, the value was replaced with the mean value for all treatment groups at the missing time point. Significant main effects of treatment, time, and the interaction of treatment-by-time were tested for HbA1c, total cholesterol, HDL, LDL, and TAG.

Data are expressed as mean ± standard error (SE) unless otherwise stated. IBM SPSS Statistics 24 was used to complete the statistical analysis with the exception of the blood glucose/insulin AUC calculation where SAS 9.4 was used.

Results

Baseline Characteristics

Baseline characteristics for the 154 participants who enrolled in the trial are in **Table 3**. There were no significant group differences for baseline anthropometric measures, age, gender, TFEQ sub-scores, and BPAQ Indices ($p \geq 0.14$). There were significantly more participants who discontinued participation in the study from the sucrose group compared to the other sweetener groups ($p=0.006$).

Compliance

Median urinary PABA recovery corrected for urinary creatinine was 63.3%, 71.5%, 71.5%, 70.0%, and 82.6% for sucrose, saccharin, aspartame, rebA, and sucralose treatment groups, respectively. PABA recovery was not significantly different between treatments ($p=0.37$). Follow up experiments indicated that incomplete PABA recovery was likely due to a lack of preservative (such as boric acid (Sharma et al., 2014)) during urine collection.

Sensory Properties of the Beverages and Overall Liking

The beverages used in this trial were not significantly different in perceived intensity of sweet, salty, sour, bitter, off-flavor, drying, bitter aftertaste, and off aftertaste between treatments ($p \geq 0.10$) or between treatments over time ($p \geq 0.10$). Overall liking was not significantly different between beverage treatments or between treatments over time ($p \geq 0.52$).

Body Weight Change

Change in body weight was significantly different among the five sweetener groups (treatment-by-time effect p=0.01). There was a statistically significant increase in body weight over the 12-week trial for participants in the sucrose and saccharin treatments (weight change = 1.85 ± 0.36 kg and 1.18 kg ± 0.36; p=<0.001, 0.02; respectively) (**Figure 2**). The non-significant changes in body weight for aspartame, rebA, and sucralose were 0.73kg ± 0.35, 0.60kg ± 0.37, and -0.78 ±0.36kg, respectively ($p \geq 0.07$). Body weight at baseline among the sucrose group tended to be greater than the sucralose treatment (p=0.05). An analysis of BMI and body weight controlling for baseline body weight followed a similar pattern of increased BMI and body weight with sucrose and saccharin consumption ($p \leq 0.04$) and no significant change in BMI with aspartame, rebA, and sucralose consumption ($p \geq 0.07$) (**Table 2**). Therefore, changes in body weight were not simply an artifact of individuals with higher initial weight gaining more weight. Mean change in body weight was not significantly different between sucrose and saccharin (p=0.21) and both were significantly greater than sucralose (+1.51kg, p<0.001 for sucrose and +1.05kg, p=0.01 for saccharin). Mean body weight change was also significantly higher for sucrose compared to aspartame and rebA ($p \leq 0.02$). Mean change in body weight was not significantly different between aspartame, rebA, and sucralose ($p \geq 0.09$). Between the LCS treatments, change in body weight at week 12 was statistically lower among the sucralose

treatment compared to the saccharin (difference = -1.96, p<0.001), aspartame (-1.50, p=0.003), and rebA (-1.37, p=0.008) groups.

Body Composition

Total fat mass (not controlling for baseline body weight) increased significantly during the intervention among participants in the sucrose treatment (Δ=+1.35kg ± 0.25, p<0.001). At week 12, change in total fat mass was significantly higher for the sucrose group compared to all LCS (p≤0.01). Among the LCS groups, change in total fat mass was negative and significantly lower for the sucralose group than the saccharin and aspartame groups (-0.79kg ± 0.33 and -0.80kg ± 0.33, respectively; p ≤ 0.02). Change in total fat mass controlling for baseline body weight **(Table 4**) was significantly higher for the sucrose group compared to the sucralose group (p=0.045) with no difference in total fat mass between the other sweetener groups.

Total fat-free mass significantly increased among the sucrose, saccharin, and aspartame groups (+0.84kg ± 0.20, p=0.001 for sucrose; +0.70 ± 0.18, p=0.006 for saccharin; +0.63kg ± 0.18, p=0.01 for aspartame). At week 12, change in FFM was significantly greater among the sucrose group compared to the rebA and sucralose groups (+0.60kg ± 0.27, p=0.03 compared to rebA; +1.17kg ± 0.27, p<0.001 compared to sucralose). Change in FFM among the sucralose group was negative and significantly lower than other LCS groups (p≤0.03). Controlling for baseline body weight , total FFM increased significantly among the sucrose, saccharin and aspartame groups (+0.85kg ± 0.30, p=0.004 for sucrose; +0.71 ± 0.28, p=0.01 for saccharin; +0.63kg ± 0.27, p=0.02 for aspartame).

Android and Gynoid Fat Mass increased significantly during the intervention among participants in the sucrose treatment (+0.16 ± 0.03 and +0.28kg ± 0.05, p<0.001). At week 12, changes in android fat mass and gynoid fat mass in the sucrose group were significantly greater

than all LCS groups ($p \leq 0.03$, $p \leq 0.01$, respectively). There were no significant differences in change in android fat mass between the LCS groups. Change in gynoid fat mass was significantly higher among the saccharin group compared to the rebA and sucralose treatment groups (+0.18kg ± 0.06 for both rebA ($p=0.006$) and sucralose ($p=0.005$)). After controlling for baseline body weight, gynoid fat mass significantly increased in the sucrose group ($p<0.001$) with no significant difference among any of the LCS treatment groups ($p \geq 0.18$). The treatment, time, and treatment-by-time effects were not significant for android fat mass ($p \geq 0.06$).

Total body water was not significantly between treatments, over time, or between treatments over time ($p \geq 0.13$) with and without controlling for baseline body weight.

Energy Intake

Self-reported energy intake data from the ASA24 is in **Figure 3**. Energy intake at baseline was not significantly different between treatments ($p \geq 0.11$). Reported energy intake increased with sucrose consumption and decreased with sucralose consumption throughout the 12-week trial ($p = 0.007, 0.02$). Mean reported energy intake for the sucrose treatment group was significantly higher than saccharin, aspartame, rebA, and sucralose treatment groups by 584 kcal ± 162, 336 kcal ± 160, 587 kcal ± 164, and 553 kcal ± 164, respectively ($p \leq 0.04$). At week 12, energy intake was significantly higher for the sucrose group compared the saccharin, rebA, and sucralose treatment groups ($p \leq 0.004$) and only tended to be greater than the aspartame treatment group ($p=0.07$). The difference in energy intake between the sucrose and LCS groups is attributed to the energy provided from the beverage. When the energy provided by the beverage is removed from the reported energy intake, the treatment and treatment-by-time main and interaction effects are not statistically significant ($p=0.37, 0.96$). There was no significant difference in energy intake

between the LCS groups at any time point. Notably, only 14.2% of the mean reported energy intakes fell within the Goldberg cut-offs.

mBEV Results

Mean energy provided from discretionary beverages estimated from the mBEV beverage frequency questionnaire was 249 kcal ± 20.7, 205 kcal ± 21.1, 183 kcal ± 22.3, and 208 kcal ± 21.6 at week 0, 4, 8, and 12 respectively (p=0.003). Treatment and time by treatment effects were not statistically significant (p=0.64, 0.76).

Ingestive Frequency

Ingestive frequency and portion size data from the ASA24 are displayed in **Table 5**. Self-reported number of eating events ≥100kcal was used to determine ingestive frequency. Ingestive frequency increased significantly in the sucrose group and significantly decreased in the sucralose group ($p<0.001$, p=0.005). Ingestive frequency was significantly greater among the sucrose group compared to the LCS groups at week 4, 8, and 12 ($p\leq0.001$). Ingestive frequency was higher for the aspartame group compared to sucrose at baseline (p=0.03). Ingestive frequency among the sucralose group was significantly lower than saccharin at week 12 (difference = -0.70 events/d, p=0.04), but not differ from other groups at any other weeks.

Portion Size

When energy provided from the sucrose beverages was included, mean portion size was 628 ± 17.9, 605 ± 18.7, 617 ± 19.4, 566 ± 19.5 kcal at weeks 0, 4, 8, and 12, respectively (p=0.02). Treatment and treatment-by-time effects were not statistically significant (p=0.09, p=0.14). Analyses of portion size determined by energy provided from foods and beverages other than the study beverages revealed the sucrose treatment was associated with significantly greater portions

than the saccharin, aspartame, and rebA treatments ($p < 0.05$) and tended to be greater than the sucralose treatment ($p=0.09$). Participants who consumed saccharin reported significantly lower portion sizes than the aspartame and sucralose groups ($p \leq 0.04$) and tended to be lower than the rebA treatment ($p=0.08$). The treatment-by-time interaction effect was not statistically significant ($p=0.58$).

Glycemic Response

Results from the 123 participants who completed the OGTT at baseline and week 12 are presented in **Figure 4 and 5.** There was no significant treatment effect ($p=0.08$), though mean glucose levels during the OGTT increased from baseline levels to week 12 ($p=0.02$). Treatment-by-time, treatment-by-session, session-by-time, and treatment-by-session-by-time interaction effects were not statistically significant ($p>0.34$). The main effects of sweetener, time, and the interaction of sweetener-by-time on glucose AUC were not statistically significant ($p=0.06, 0.18, 0.74$). Insulin concentrations did not differ between baseline and week 12 during the OGTT, and there was no significant treatment effect ($p=0.85$, $p=0.27$, respectively). The main effects of sweetener, time, and the interaction of sweetener-by-time on insulin AUC were not statistically significant ($p=0.62, 0.28, 0.10$). Fasting serum glucose and insulin were not different between treatment groups at baseline, week 6, or week 12 ($p \geq 0.21$, $p \geq 0.47$).

Total cholesterol and LDL cholesterol at week 12 significantly decreased from baseline ($p<0.05$) with no difference between treatments or between treatments over time ($p \geq 0.54$). HbA1c, HDL, and TAG were not affected by the intervention.

Energy Expenditure and Physical Activity

There was no significant treatment effect or treatment-by-time interaction effect on EE (p=0.56, 0.17). There was no difference in the Baecke's indices for work, sport, or leisure physical activity between treatments, over time, or between treatments overtime ($p \geq 0.05$).

Appetite

Appetitive ratings throughout the trial are in **Figure 6**. Hunger ratings among the saccharin sweetener group were significantly greater than all other treatment groups ($p \leq 0.03$). The main effect of treatment was not significant for any other appetitive sensation ($p \geq 0.12$). DTE and prospective consumption ratings increased significantly from baseline only for the saccharin treatment group. The time by treatment interaction was not statistically significant for any other appetitive sensation. ($p \geq 0.09$).

Week 12 Questionnaire

Fifty-seven percent, 69%, 52%, and 56% of participants correctly identified whether their beverage contained a LCS in the saccharin, aspartame, rebA, and sucralose groups respectively; 42% of participants assigned to consume beverages sweetened with sucrose correctly reported their beverage was sugar sweetened. The likelihood of correctly identifying the beverage sweetener was not different between treatment groups (p=0.44). Only individuals in the aspartame treatment were able to correctly identify their beverage contained a LCS at greater than chance probability ($p<0.05$). Fifty-three percent of participants reported no change in DTE sweet foods, while 31% and 17% reported a decrease and increase in the DTE sweet foods, respectively. Forty-seven percent, 41%, and 12% of individuals reported no change, decreased, and increased DTE

sweet beverages. Changes in the DTE sweet items were not significantly different across sweetener groups and was not associated with change in body weight (p=0.28).

Discussion

Numerous RCT have investigated the effects of combinations of LCS on body weight. The most comprehensive meta-analyses of these RCT indicate body weight is slightly, but significantly lower with LCS use. Conversely, cohort studies reveal no association or a slight positive relationship (P. Miller et al., 2014; P. J. Rogers, Hogenkamp, de Graaf, et al., 2016). These weak and inconsistent findings could reflect dissimilar effects of individual LCS, with the beneficial effects of one LCS offset by other LCS. Studies contrasting the long-term effects of individual LCS on body weight are lacking. This trial required healthy, non-dieting adults with overweight or obesity to consume a beverage containing sucrose or one of four LCS for 12 weeks. Sucrose and saccharin consumption significantly increased body weight compared to aspartame, rebA, and sucralose and weight change was directionally negative and lower for sucralose compared to the saccharin, aspartame, and rebA. This differential response among LCS indicates their consumption likely exerts effects beyond the contribution of sweetness with negligible energy. The present findings raise questions about viewing the diverse molecules that serve as LCS in aggregate.

Evidence for the effect of saccharin consumption on energy intake and body weight is mixed. Prospective cohort trials conclude saccharin consumption is associated with weight gain (Colditz et al., 1990; Parker et al., 1997) though a causal association cannot be determined in these trials. In these reports, no other LCS were evaluated exclusively because other LCS were not commercially available or were minimally consumed at the time of data collection. Consequently, these trials do not permit determination of whether an association between saccharin intake and weight gain is unique to this sweetener. Saccharin supplemented diets increase energy intake and

body weight in select rodent studies while others find no difference compared to water (reviewed in (P. J. Rogers, Hogenkamp, de Graaf, et al., 2016)). The present findings are consistent with the positive association in epidemiological and rodent trials. Collectively, the evidence suggests saccharin stands apart from the other popular LCS in that it may actually contribute to weight gain. A treatment-independent weight gain among the study population, estimated as 0.5-0.6kg annually for adults (0.12-0.15kg/3 months) (Malhotra et al., 2013) and 1.5kg annually among college students (0.38kg/3 months) (Fedewa et al., 2014) could account for a portion of the change. However, the gain noted here was 3 to 10-fold higher than this background trend. While saccharin consumption increased total body weight in this trial, indices of fat mass (total, android, and gynoid fat mass) were positive but not statistically significant. Effects on other health outcomes often related to adiposity, such as glucose tolerance or serum lipid levels, were also not significant, but interpretation of this should be tempered by the short duration of this trial. Replication of the present findings and identification of the underlying mechanisms will be required to accept the hypothesis that saccharin specifically is problematic for weight management.

Sucralose consumption did not significantly alter body weight compared to baseline, but consumers' weights were trending downward and were significantly lower than all other treatments at week 12. Sucralose is the most commonly consumed LCS within the US (Research, 2013); yet, to our knowledge, only one trial examined the effect of sucralose consumption as an exclusive LCS on body weight (Baird et al., 2000). They concluded that 12 weeks of consumption of up to 500mg of sucralose per day did not lead to a change in body weight. The trial was designed to measure sucralose tolerance (not body weight), the dose was approximately five times the estimated daily intake, and was delivered as a 35g/L solution. The extent to which sucralose consumption facilitates weight loss requires additional documentation.

The effect of aspartame consumption on body weight has been tested in multiple human feeding trials. They consistently document no effect compared to water or a no treatment control (Blackburn et al., 1997; Higgins et al., 2018; Maersk et al., 2012). In the present trial, weight change with aspartame consumption was not statistically significant. The slight increase in body weight observed (+0.73kg) may reflect the background trend in body weight but is still about 2-5 times that rate. Unfortunately, the present trial did not include a water control to test this hypothesis.

RebA consumption had no effect on body weight. Few RCTs have investigated consumption of rebA or other steviol glycosides on health outcomes, yet the trials that do monitor body weight find no significant change (Hsieh et al., 2003; Maki, Curry, Carakostas, et al., 2008). Like aspartame, the slight increase in body weight (+0.60kg) may be partially attributed to a rebA independent trend for weight gain in the general population.

Sucrose consumption, particularly in the form of SSB is positively associated with weight gain in RCTs and epidemiological trials (reviewed in (Te Morenga et al., 2013)). Our trial is consistent with these findings. Using the NIH Body Weight Planner (K. D. Hall et al., 2011), the estimated increase in body weight based on baseline characteristics assuming 0% dietary compensation and 100% compliance was +5.0kg and +4.4kg for males and females, respectively. Accounting for the compliance from the PABA collection (63% among the sucrose treatment) the predicted change in body weight is +3.1kg and +2.8kg. This predicted weight change is higher than the observed change in body weight among the sucrose group (1.85kg). The difference may be attributable to partial dietary compensation. The partial compensation for energy derived from sugars in beverages is well documented (Gadah et al., 2016; M. Reid et al., 2007). This may stem from weaker satiety effects of beverage energy relative to more viscous or solid foods (Almiron-

Roig et al., 2013; DiMeglio et al., 2000). A recent meta-analysis indicates that the mandatory *addition* of SSB to the diet is associated with increased body weight, but the recommended *reduction* of SSB is not associated with BMI in effectiveness studies (R. D. Mattes et al., 2011). Reduction of SSB alone decreases dietary energy but also palatability. This may result in lower diet adherence and a sub-optimal weight management outcome. Thus, select LCS beverages may hold advantages for energy restriction over reduction of SSB alone by maintaining diet acceptance and improving compliance.

The present study was designed to document effects of selected sweeteners on body weight, not identify mechanisms. Nevertheless, one notable mechanism relates to energy balance; i.e. nutritive sweeteners provide energy directly while LCS displace energy sources in the diet. There was no change in estimated energy provided from discretionary beverages throughout the trial, suggesting that the beverages were added to the diet or displaced energy from foods, not beverages. In this trial, increased reported energy intake and ingestive frequency corresponded with increased body weight with sucrose consumption, and the decreased energy intake and ingestive frequency with sucralose consumption corresponded with weight reduction, albeit only a trend. However, no association between energy intake and saccharin use was observed despite increases in hunger, DTE, and prospective consumption. Whether the increase in body weight among the saccharin group is attributed to metabolic changes unrelated to energy intake (i.e. changes in metabolic rate (S. McGregor et al., 1995), gut microbiota energy harvesting efficiency) requires further investigation. Thus, a reduction of energy intake elicited by sucralose with resulting weight reductions remains an open question. A lack of effect of sucralose on appetite or energy intake has been reported previously, but only from short-term feeding trials (Ford et al., 2011; Steinert et al., 2011).

Theoretically, if sweetness alone promotes reward driven feeding, then it may be expected that all sweeteners would exhibit increased DTE and energy intake. However, DTE ratings increased only among the saccharin group and only at selected timepoints. Further, energy intake did not differ across treatments excluding the energy contributed by sucrose. Sweetness exposure also did not alter perceived intensity of multiple sensory attributes and overall liking among any treatment group throughout the trial. This suggests that sweetness alone does not account for the weight gain and that additional mechanisms may be involved. Possibilities include effects of LCS on the CPIR, incretin release, glycemic response, and/or gut microbiota changes. Elucidation of these mechanisms was not the aim of the trial, but inferences can be drawn based on the observed differences in body weight.

There is speculation that the CPIR can be disrupted with intermittent consumption of nutritive sweeteners and LCS. This is claimed to dissociate sweetness from the metabolic consequences associated with eating, thereby corrupting a normal control of feeding (Paul AM Smeets et al., 2010). Saccharin (Just et al., 2008) and potentially sucralose (Dhillon et al., 2017; Ford et al., 2011) are the only LCS with documented CPIR, yet they had significantly different effects on body weight. Thus, this explanation seems unlikely.

An increase in insulin release in the absence of energy due to LCS binding to T1R2/T1R3 receptors on taste and enteroendocrine cells is hypothesized to disrupt glucose homeostasis and increase appetite (Egan et al., 2008). However, daily sweetener consumption for 12 weeks did not alter insulin concentrations during the OGTT in this trial. Additionally, LCS consumed orally or via intragastric administration have no effect on incretin release in humans (Tucker et al., 2017) with the exception of an increase in GLP-1 with consumption of soda sweetened with ace-K and sucralose (A. C. Sylvetsky et al., 2016). There, the increase in GLP-1 was attributed to the cola

rather than the sweetener and not replicated when the sweeteners were delivered in water (A. C. Sylvetsky et al., 2016; Wu et al., 2013). Even if sucralose consumption does lead to increased concentrations of GLP-1, the effect of GLP-1 on satiety may be limited, because supra-physiological doses of GLP-1 are necessary to promote satiety (Mars et al., 2012). The glycemic response during the OGTT was not significantly altered with 12 weeks of sucrose consumption despite evidence of increased risk of type 2 diabetes with SSB intake (Malik et al., 2010). This may be because there was some compensation for the sucrose load resulting in a lower challenge or the duration of exposure was not sufficient for other metabolic changes to manifest. Another RCT that examined the effect of consumption of 1 L/day of SSB for six months among individuals with overweight/obesity also found no effect on glycemic response compared to water or isoenergetic milk controls (Engel, 2017). Still, we cannot conclude that sucrose does not affect glycemia, as effects may just not be observable in trials of only 12-week or 6-month duration.

A fraction of saccharin, rebA, and sucralose reaches the colon and have the potential to induce changes in the colonic microbiota that may then increase or decrease energy harvesting efficiency and body weight (Magnuson et al., 2016; Jotham Suez et al., 2015). However, mice fed a high fat, saccharin supplemented diet or germ-free mice receiving fecal transplantations from saccharin-feed mice experienced no significant changes in body weight or chow consumption (J. Suez et al., 2014). Similar null effects have been reported with sucralose (Abou-Donia et al., 2008; Uebanso et al., 2017) and body weight reduction with aspartame consumption (Palmnas et al., 2014). Aspartame is digested in the proximal small intestine, making changes in gut microbial populations difficult to interpret. Changes to the gut microbiota were not monitored in this trial, but the pattern of weight change is not consistent with hypothetical changes based on LCS effects on the microbiota.

This trial has limitations. Because it was designed to address differences between sweeteners, we did not include an unsweetened, water control group. Data from such a group would have helped clarify the effects of fluid intake alone on the study outcomes. Eight participants in the sucrose treatment group withdrew prior to week 4, so it is unlikely the high attrition rate of the sucrose treatment stemmed from detection of undesired weight gain. Additionally, this attrition rate was not observed in the saccharin group who gained a comparable amount of weight. Based on anecdotal comments, we hypothesize that the additional energy from the sucrose beverage somewhat increased satiety (M. Reid et al., 2007) and caused greater disruptions to regular meal patterns than the LCS beverages. We are unable to test this hypothesis, because the dropouts occurred prior to week 4 when dietary recalls and appetite logs were collected. Participants consumed beverage volumes ranging from 1.25-1.75L/day, contributing 400-560kcal/day. While this is a substantial volume, mean water from beverages other than plain water is approximately 1.5L/day and therefore within a customary intake range (Kant et al., 2009). Participants were blinded to the treatment and primary aim of measuring body weight in this trial, while in the free-living environment, individuals are usually aware of when products contain LCS. Such knowledge may alter ingestive behaviors (Shide et al., 1995; Orland W. Wooley et al., 1972). EE was measured with tri-axial accelerometers; while they are validated to predict EE and indicated that physical activity was not different in response to the beverage intervention, they provide imperfect measurements of EE. The same holds for diet intake data. Dietary energy is generally underreported, with a greater risk of underreporting among individuals with obesity (R. J. Hill et al., 2001). The critical issue for the present purpose is whether under-reporting might be expected to have disproportionately occurred in one or more treatment groups. There is no evidence of this. LCS concentrations in the beverages were designed to match the sweetness intensity of 8%

sucrose. While the intake of all LCS fell within respective ADIs, saccharin and rebA intake exceeded typical estimated intake of high saccharin and rebA consumers (based on estimates of a 70kg person) (FDA, 2018; Fernstrom, 2015; Renwick, 2008). While LCS are most commonly consumed as beverages (as in the current trial), they are also increasingly incorporated into food products. Factors such as mastication, food and beverage viscosity, consumption with other nutrients, carbonation, and palatability may all affect ingestive responses to LCS differently depending on the delivery vehicle. Other LCS not tested in this trial may have differential effects on body weight. Ace-K, commonly used synergistically with other LCS, has a similar structure, receptor affinity, and digestibility as saccharin (C. Kuhn et al., 2004; Magnuson et al., 2016; Masuda et al., 2012). Ace-K was not included in this trial, because it is rarely consumed in isolation. Whether it leads to similar effects on body weight and whether commercial mixtures of LCS can be optimized by more detailed knowledge of their effects warrants testing.

If substantiated through additional testing and confirmed through mechanistic studies, findings from this trial have implications for consumers, clinicians, policy makers, and the food industry. Some LCS may not hold the anticipated beneficial effects on body weight (e.g., saccharin), and beneficial effects of one LCS (e.g., sucralose) may be attenuated if combined with selected other LCS. Going forward it will be important to consider each LCS as a distinct entity with respect to its potential health effects.

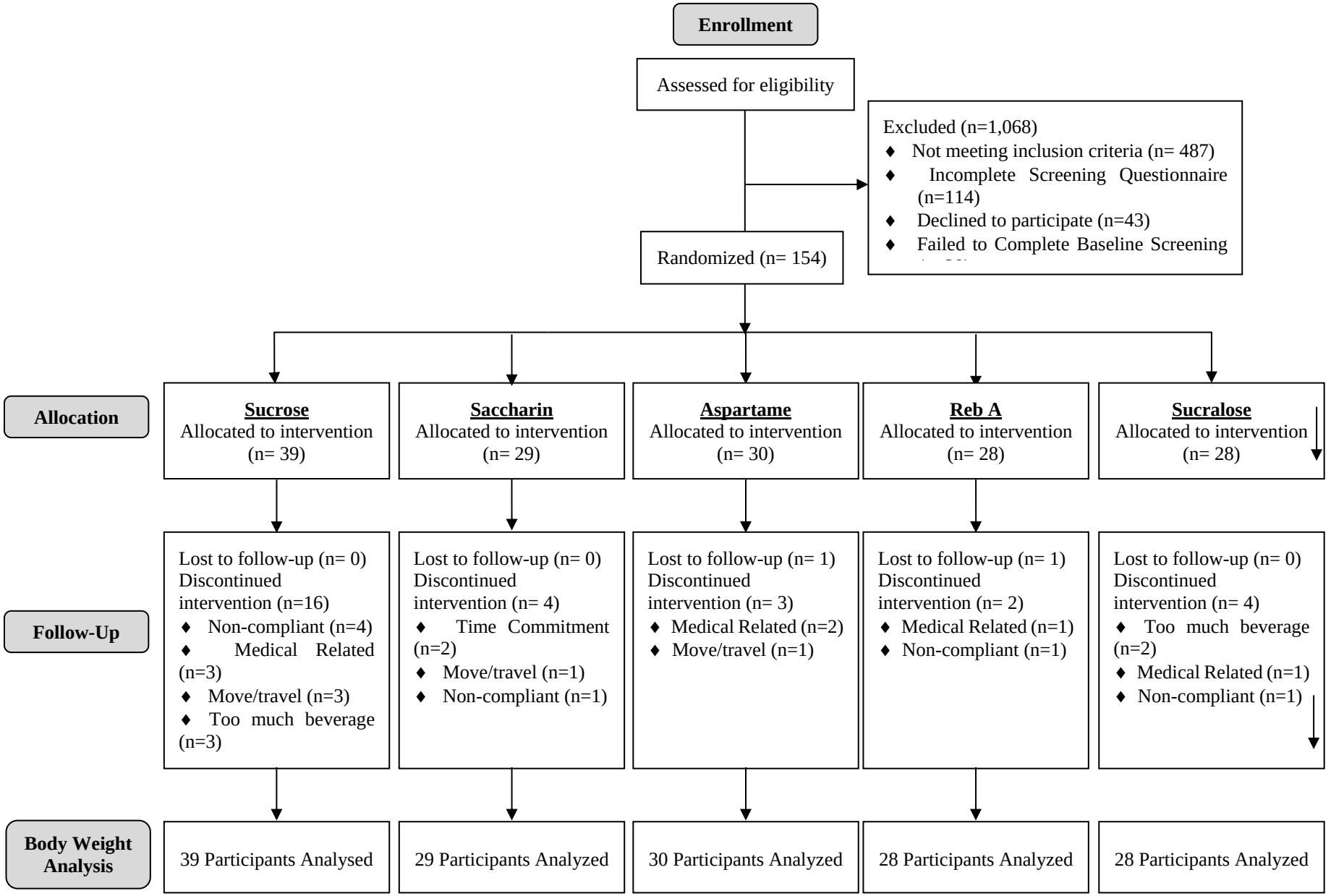


Figure 1 CONSORT Flow Diagram of Participants through Beverage Intervention

Table 2 Composition of LCS and Sucrose Beverages Consumed Daily

	Sweetener (g)	PABA (mg)	Unsweetened Kool-Aid (g)	Energy (kcal)	Overall Liking at Baseline (VAS Rating)	Sweetness Intensity at Baseline (VAS Rating)
LCS (1.25L-1.75L)						
Aspartame	0.584	80	4.46	< 5	55.63 ± 4.34	46.32 ± 4.47
Reb A	0.655	80	4.46	< 5	57.10 ± 4.18	58.79 ± 4.39
Saccharin	0.733	80	4.46	< 5	50.33 ± 4.60	42.12 ± 4.73
Sucralose	0.160	80	4.46	< 5	49.46 ± 4.26	52.86 ± 4.47
Sucrose						
1.25L	100	80	4.46	400	50.90 ± 7.21	39.90 ± 7.65
1.50L	120	80	4.46	480	56.17 ± 6.59	50.00 ± 6.98
1.75L	140	80	4.46	560	56.65 ± 5.53	41.59 ± 5.87

Table 3 Baseline Characteristics of Participants Enrolled in the Sweetener Intervention

		Sucrose	Saccharin	Aspartame	Reb A	Sucralose	p-value
n		39	29	30	28	28	
Age (years)		28.2 ± 9.5	25.8 ± 6.9	29.5 ± 12.0	27.1 ± 9.6	25.9 ± 9.0	0.538
Weight (kg)		90.2 ± 18.1	84.4 ± 16.9	87.6 ± 15.7	84.4 ± 12.3	82.9 ± 10.6	0.278
Height (cm)		171.6 ± 10.2	170.8 ± 9.9	170.2 ± 10.8	168.1 ± 9.1	170.2 ± 10.1	0.720
BMI (kg/m^2)		30.4 ± 4.1	28.8 ± 4.4	30.3 ± 5.3	29.9 ± 3.8	28.7 ± 4.0	0.368
Female (%)		53.8%	58.6%	50.0%	64.3%	57.1%	0.848
Drops (count)		16	4	4	3	4	0.006*
TFEQ Scores	Restraint	6.4 ± 3.3	7.8 ± 3.7	6.9 ± 3.2	6.8 ± 4.1	6.7 ± 3.6	0.661
	Disinhibition	6.8 ± 3.8	5.5 ± 3.3	6.1 ± 2.7	6.8 ± 3.6	6.4 ± 3.2	0.565
	Hunger	5.5 ± 3.3	4.6 ± 3.5	4.5 ± 3.1	5.3 ± 3.9	4.3 ± 2.9	0.564
Baecke	Work Index	2.3 ± 0.4	2.4 ± 0.5	2.2 ± 0.6	2.4 ± 0.6	2.1 ± 0.6	0.136
	Sport Index	2.4 ± 0.7	2.5 ± 0.7	2.5 ± 1.1	2.5 ± 0.8	2.7 ± 0.9	0.545
	Leisure Index	2.8 ± 0.6	2.8 ± 0.5	2.6 ± 0.6	2.7 ± 0.5	2.7 ± 0.8	0.645

Data presented as mean ± SD.

TFEQ: Three Factor Eating Questionnaire which includes scores for dietary restraint, disinhibition, and hunger. Baecke: Baecke Physical Activity Questionnaire which included indices of work, sport, and leisure physical activity.

One-way ANOVA was used to compare baseline characteristics between treatment groups; Pearson's chi-squared was used to compare gender distributions and attrition between treatments.

*Significant difference between treatments ($p<0.05$)

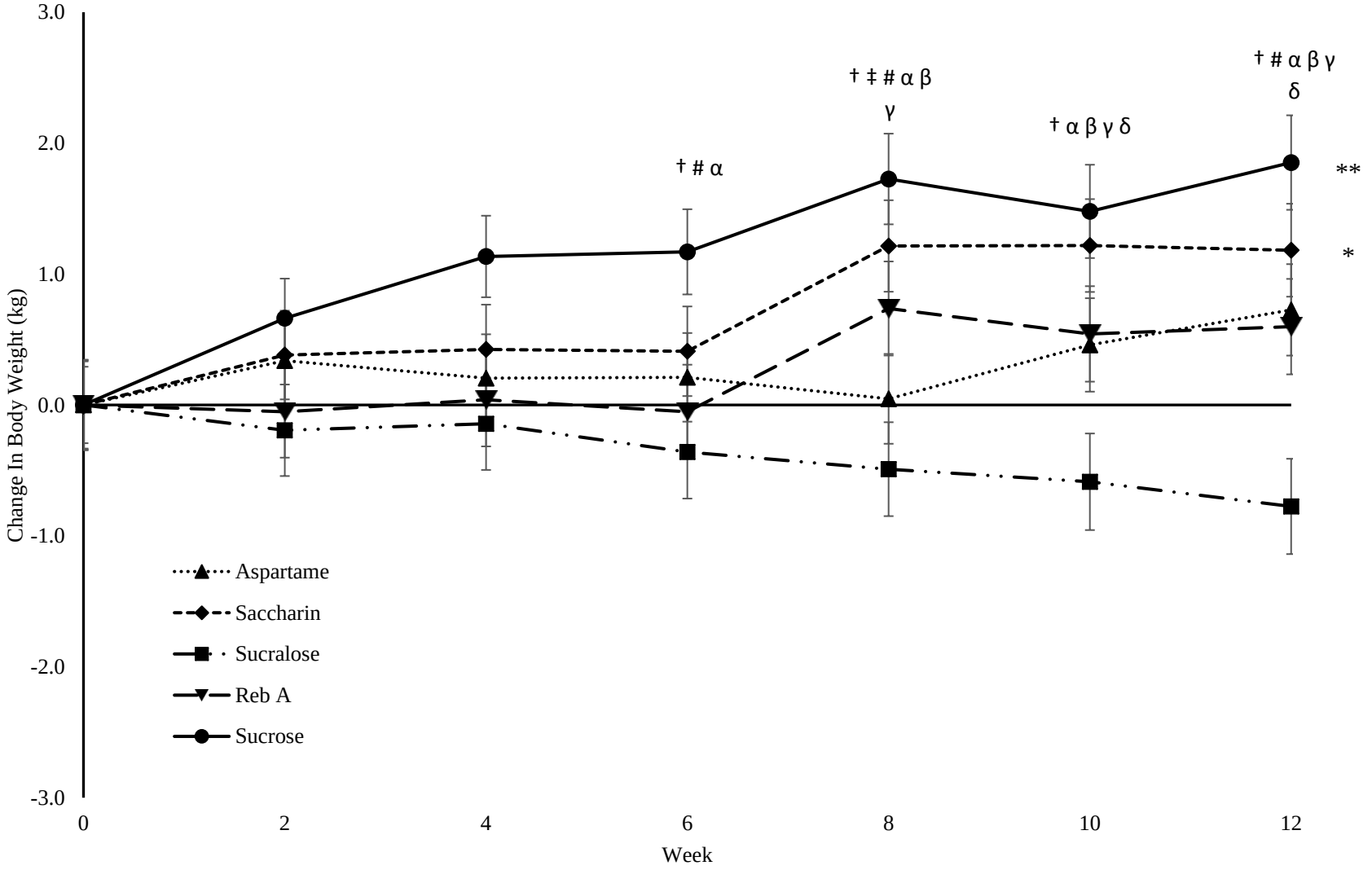


Figure 2 Change in Body Weight with 12 Weeks of LCS and Sucrose Consumption

Data presented as mean ± SE.

n=39, 29, 30, 28, 28 for sucrose, saccharin, aspartame, rebA, and sucralose groups, respectively.

Linear mixed model was used to determine the main effects of treatment and time and the interaction of treatment-by-time on change in body weight. Treatment, time, and treatment-by-time interaction were statistically significant ($p \leq 0.01$). Change in body weight over time statistically significant for sucrose (** = $p<0.001$) and saccharin (* = $p=0.02$) treatments.

The following symbols above the data points denotes significant differences between treatments within a week:

† aspartame significantly different than sucrose ($p<0.05$); ‡aspartame significantly different than saccharin ($p<0.05$)

rebA sucralose significantly different than sucrose ($p<0.05$)

α sucralose significantly different than sucrose ($p<0.05$); β sucralose significantly different than saccharin ($p<0.05$);

γ sucralose significantly different than rebA; δ sucralose significantly different than aspartame

Table 4 Body Composition at Baseline and after 12 Weeks of Sweetener Consumption

							p-values		
		Sucrose	Saccharin	Aspartame	Reb A	Sucralose	Treatment	Time	Treatment x Time
Body Weight (kg)	Week 0	90.24 ± 2.46	84.38 ± 2.85	87.57 ± 2.81	84.36 ± 2.90	82.86 ± 2.90	0.157	0..005	0.012
	Week 12	92.04 ± 2.48 *1	85.55 ± 2.86 *1,2	88.23 ± 2.81 2	84.92 ± 2.91 2	82.10 ± 2.91 3			
Δ Body Weight (kg)	Week 12	1.85 ± 0.36 *1	1.18 ± 0.36 *1,2	0.73 ± 0.35 2	0.60 ± 0.36 2	-0.78 ± 0.36 3	0.002	0.003	0.010
Body Weight (kg) Adjusted †	Week 0	86.24 ± 0.29	86.24 ± 0.34	86.24 ± 0.33	86.24 ± 0.35	86.25 ± 0.35	0.002	0.003	0.010
	Week 12	88.09 ± 0.36 *1	87.43 ± 0.36 *1,2	86.97 ± 0.35 2	86.84 ± 0.37 2	85.47 ± 0.37 3			
BMI (kg/m2)	Week 0	30.44 ± 0.70	28.83 ± 0.81	30.32 ± 0.80	29.86 ± 0.83	28.72 ± 0.83	0.237	0.006	0.021
	Week 12	31.04 ± 0.71 *1	29.23 ± 0.81 *1,2	30.52 ± 0.80 1,2	30.04 ± 0.83 1,2	28.46 ± 0.83 2			
Δ in BMI (kg/m2)		0.62 ± 0.12 *1	0.41 0.12 *1,2	0.22 ± 0.12 2	0.20 ± 0.12 2	-0.27 ± 0.12 3	0.002	0.003	0.015
BMI (kg/m2) Adjusted †	Week 0	29.60 ± 0.48	29.22 ± 0.55	30.04 ± 0.54	30.25 ± 0.56	29.44 ± 0.56	0.653	0.007	0.022
	Week 12	30.18 ± 0.49 *	29.63 ± 0.55 *	30.24 ± 0.54	30.44 ± 0.56	29.18 ± 0.57			
Total Fat Mass (kg)	Week 0	34.35 ± 1.79 1	30.70 ± 2.07 1,2	32.24 ± 2.04 1,2	32.10 ± 2.11 1,2	28.10 ± 2.11 2	0.149	0.001	0.032
	Week 12	35.68 ± 1.81 *1	31.16 ± 2.08 1,2	32.72 ± 2.04 1,2	32.19 ± 2.11 1,2	27.80 ± 2.11 2			
Δ Total Fat Mass (kg)		1.35 ± 0.25 *1	0.48 ± 0.23 *2	0.49 ± 0.23 2	0.09 ± 0.23 2,3	-0.31 ± 0.24 3	0.006	0.004	0.006
Total Fat Mass (kg) Adjusted †	Week 0	32.26 ± 1.29	31.62 ± 1.49	31.52 ± 1.46	33.03 ± 1.52	29.80 ± 1.52	0.488	0.012	0.033
	Week 12	33.58 ± 1.32 *1	32.08 ± 1.49 1,2	32.01 ± 1.47 1,2	33.11 ± 1.52 1,2	29.49 ± 1.53 2			
Total Fat-Free Mass (kg)	Week 0	55.62 ± 1.73	53.72 ± 2.01	55.04 ± 1.97	52.08 ± 2.04	54.21 ± 2.04	0.673	0.001	0.030
	Week 12	56.47 ± 1.74 *1	54.43 ± 2.01 *1,2	55.67 ± 1.97 *1,2	52.33 ± 2.04 2	53.89 ± 2.04 3			
Δ Total Fat-Free Mass (kg)		0.84 ± 0.20 *	0.70 ± 0.18 *	0.63 ± 0.18 *	0.25 ± 0.18	-0.33 ± 0.19	0.009	<0.001	0.009

Total Fat-Free Mass (kg) Adjusted †	Week 0	53.68 ± 1.30	54.58 ± 1.49	54.38 ± 1.47	52.95 ± 1.52	55.80 ± 1.52	0.802	0.001	0.028
	Week 12	54.54 ± 1.31 *	55.29 ± 1.50 *	55.01 ± 1.47 *	53.20 ± 1.52	55.46 ± 1.53			
Total Tissue % Fat	Week 0	39.08 ± 1.55	37.22 ± 1.80	37.57 ± 1.77	39.11 ± 1.83	35.18 ± 1.83	0.434	0.558	0.476
	Week 12	39.65 ± 1.57	37.20 ± 1.80	37.62 ± 1.77	39.07 ± 1.83	35.01 ± 1.84			
Δ Tissue % Fat		0.57 ± 0.20	-0.01 ± 0.19	0.06 ± 0.19	-0.04 ± 0.19	-0.17 ± 0.19	0.289	0.482	0.289
Total Tissue % Fat Adjusted †	Week 0	38.54 ± 1.54	37.46 ± 1.77	37.38 ± 1.74	39.36 ± 1.80	35.63 ± 1.81	0.587	0.562	0.478
	Week 12	39.10 ± 1.55	37.44 ± 1.77	37.44 ± 1.74	39.31 ± 1.80	35.46 ± 1.81			
Android Fat Mass (kg)	Week 0	3.22 ± 0.22	2.69 ± 0.26	3.01 ± 0.26	2.91 ± 0.26	2.33 ± 0.26	0.076	0.075	0.054
	Week 12	3.38 ± 0.23	2.75 ± 0.26	3.03 ± 0.26	2.89 ± 0.27	2.31 ± 0.27			
Δ Android Fat Mass (kg)		0.16 ± 0.03 *1	0.06 ± 0.03 2	0.02 ± 0.03 2	-0.03 ± 0.03 2	-0.02 ± 0.03 2	0.012	0.047	0.012
Android Fat Mass (kg) Adjusted †	Week 0	2.94 ± 0.15	2.82 ± 0.17	2.92 ± 0.17	3.04 ± 0.18	2.56 ± 0.18	0.278	0.080	0.058
	Week 12	3.10 ± 0.15	2.87 ± 0.17	2.94 ± 0.17	3.01 ± 0.18	2.54 ± 0.18			
Gynoid Fat Mass (kg)	Week 0	5.75 ± 0.32	5.43 ± 0.37	5.38 ± 0.36	5.66 ± 0.38	4.96 ± 0.38	0.411	0.044	0.004
	Week 12	6.03 ± 0.32 *1	5.54 ± 0.37 1,2	5.41 ± 0.36 1,2	5.60 ± 0.38 1,2	4.90 ± 0.38 2			
Δ Gynoid Fat Mass (kg)		0.28 ± 0.05 *1	0.12 ± 0.04 2	0.04 ± 0.04 2,3	-0.06 ± 0.04 3	-0.06 ± 0.05 3	<0.001	0.021	<0.001
Gynoid Fat Mass (kg) Adjusted †	Week 0	5.45 ± 0.27	5.56 ± 0.31	5.27 ± 0.30	5.79 ± 0.31	5.21 ± 0.31	0.637	0.048	0.004
	Week 12	5.73 ± 0.27 *	5.67 ± 0.31	5.31 ± 0.30	5.73 ± 0.31	5.14 ± 0.31			
TBW (kg)	Week 0	44.39 ± 1.42	42.06 ± 1.58	43.66 ± 1.55	40.94 ± 1.58	41.94 ± 1.63	0.491	0.133	0.863
	Week 12	44.32 ± 1.43	42.24 ± 1.58	43.08 ± 1.55	40.73 ± 1.58	41.52 ± 1.64			
Δ TBW (kg)		-0.07 ± 0.35	0.18 ± 0.36	-0.60 ± 0.36	-0.22 ± 0.36	-0.41 ± 0.37	0.164	0.186	0.910
TBW (kg) Adjusted †	Week 0	42.59 ± 1.05	42.78 ± 1.16	43.18 ± 1.14	41.71 ± 1.16	43.26 ± 1.21	0.920	0.131	0.860
	Week 12	42.52 ± 1.06	42.97 ± 1.16	42.60 ± 1.14	41.50 ± 1.16	42.84 ± 1.21			

Data represented as mean ± SE.

n= 39, 29, 30, 28, 28 for sucrose, saccharin, aspartame, rebA, and sucralose groups, respectively.

Linear mixed model of main effects of treatment, time, and the interaction of treatment-by-time on anthropometrics and change in anthropometrics.

† Linear mixed models of main effects of treatment, time, and the interaction of treatment-by-time on anthropometrics controlling for body weight at baseline.

* Denote significant difference ($p<0.05$) across weeks within each treatment

Different numbers denote significant difference ($p<0.05$) between treatments within a week

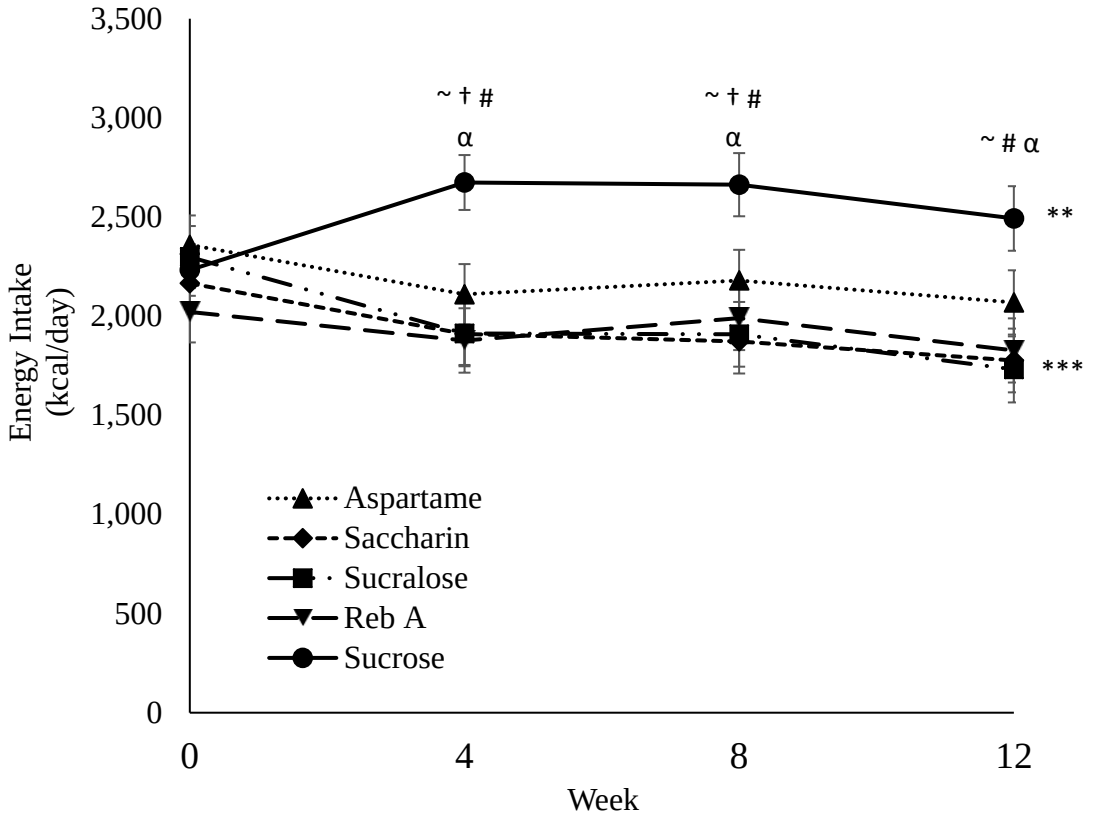


Figure 3 Change in Energy Intake with Sweetener Consumption for 12 Weeks

Data presented as mean ± SE.

n=39, 29, 30, 28, 27 for sucrose, saccharin, aspartame, rebA, and sucralose groups, respectively.

Linear mixed model of main effects of treatment and time and the interaction of treatment-by-time on change in energy intake. Treatment, time, and treatment-by-time statistically significant ($p \leq 0.04$). Change in energy intake over time statistically significant for sucrose (** = $p=0.007$) and sucralose (*** = $p=0.02$) treatments.

The following symbols above the data points denotes significant differences between treatments within a week:

~ saccharin significantly different than sucrose

† aspartame significantly different than sucrose ($p<0.05$)

rebA sucralose significantly different than sucrose ($p<0.05$)

α sucralose significantly different than sucrose ($p<0.05$)

Table 5 Ingestive Frequency and Portion Size with Sweetener Consumption for 12 Weeks

		Sucrose	Saccharin	Aspartame	Reb A	Sucralose	p-values		
							Treatment	Time	Treatment x Time
Ingestive Frequency (occasions/day)	Week 0	3.4 ± 0.2 [1]	3.7 ± 0.2 [1,2]	4.0 ± 0.2 [2]	3.4 ± 0.2 [1,2]	3.6 ± 0.2 [1,2]	<0.001	0.993	<0.001
	Week 4	4.8 ± 0.2 [*1]	3.5 ± 0.2 [2,3]	3.6 ± 0.2 [2]	3.1 ± 0.2 [2,3]	3.0 ± 0.2 [*3]			
	Week 8	4.9 ± 0.2 [*1]	3.4 ± 0.2 [2]	3.5 ± 0.2 [*2]	3.3 ± 0.2 [2]	2.9 ± 0.2 [*2]			
	Week 12	4.8 ± 0.2 [*1]	3.6 ± 0.2 [2]	3.5 ± 0.2 [2,3]	3.2 ± 0.2 [2,3]	2.9 ± 0.2 [*3]			
Portion Size Per Ingestive Event With Beverage (kcal/event)	Week 0 [a]	676.6 ± 35.2	596.5 ± 40.8	613.5 ± 40.1	620.5 ± 41.5	633.9 ± 42.3	0.093	0.021	0.138
	Week 4 [a,b]	566.3 ± 37.2	521.8 ± 42.6	627.8 ± 41.3	651.3 ± 44.2	659.2 ± 42.9			
	Week 8 [a]	550.0 ± 43.5	533.4 ± 44.0	688.6 ± 41.9	627.5 ± 43.5	687.2 ± 44.2			
	Week 12 [b]	519.1 ± 43.5	505.8 ± 43.3	614.9 ± 43.0	586.8 ± 43.5	605.3 ± 44.9			
Portion Size Per Eating Event Without Beverage (kcal/event)	Week 0 [a,b]	676.6 ± 36.4	596.5 ± 42.2	613.5 ± 41.5	620.5 ± 43.0	633.9 ± 43.8	0.002	0.048	0.577
	Week 4 [a,b]	738.6 ± 38.5	522.1 ± 44.1	627.8 ± 42.7	651.6 ± 45.7	659.2 ± 44.4			
	Week 8 [a]	782.0 ± 44.9	532.9 ± 45.4	688.2 ± 43.3	627.3 ± 45.0	686.7 ± 45.8			
	Week 12 [b]	699.5 ± 44.7	505.9 ± 44.7	615.1 ± 44.4	586.5 ± 44.9	604.1 ± 46.4			

90

Data presented as mean ± SE.

n=39, 29, 30, 28, 27 for sucrose, saccharin, aspartame, rebA, and sucralose groups, respectively.

Linear mixed model of main effects of treatment, main effects of treatment and time and the interaction of treatment-by-time on change in ingestive frequency and portion size.

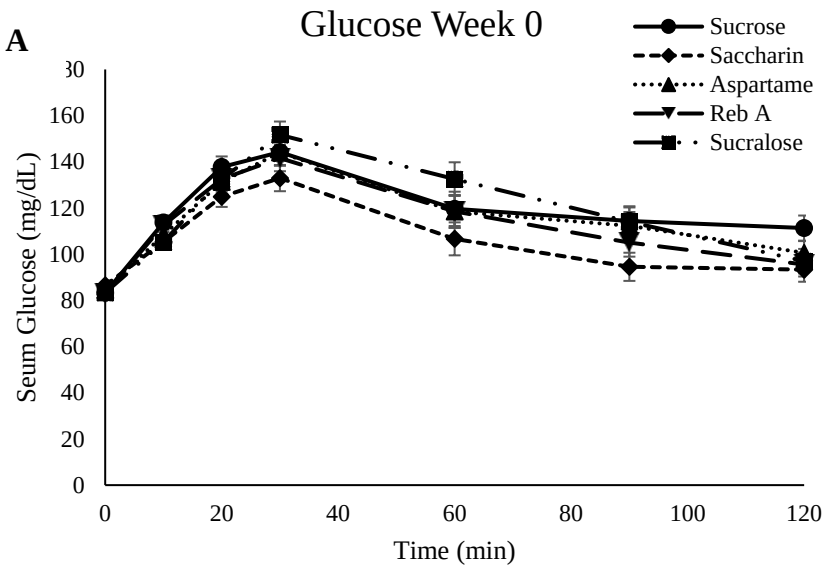
IF: main effect of treatment statistically significant. IF of sucrose treatment greater than all LCS groups ($p<0.01$); aspartame groups significantly higher than sucralose ($p=0.03$).

PS without beverage: main effect of treatment statistically significant. PS without beverages of sucrose group significantly greater than saccharin, aspartame, and rebA groups ($p<0.05$); aspartame significantly higher than saccharin ($p=0.04$); rebA significantly higher than saccharin ($p=0.02$); sucralose significantly higher than saccharin ($p=0.03$).

Different letters denote significant difference ($p<0.05$) across weeks

Different numbers denote significant difference ($p<0.05$) between treatments within a week

*Denotes significantly different ($p<0.05$) than values at Week 0 within each treatment.



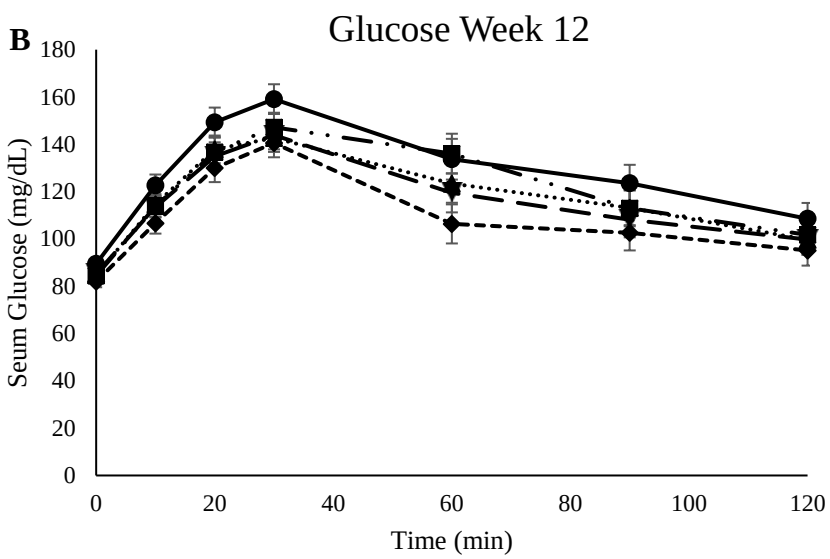
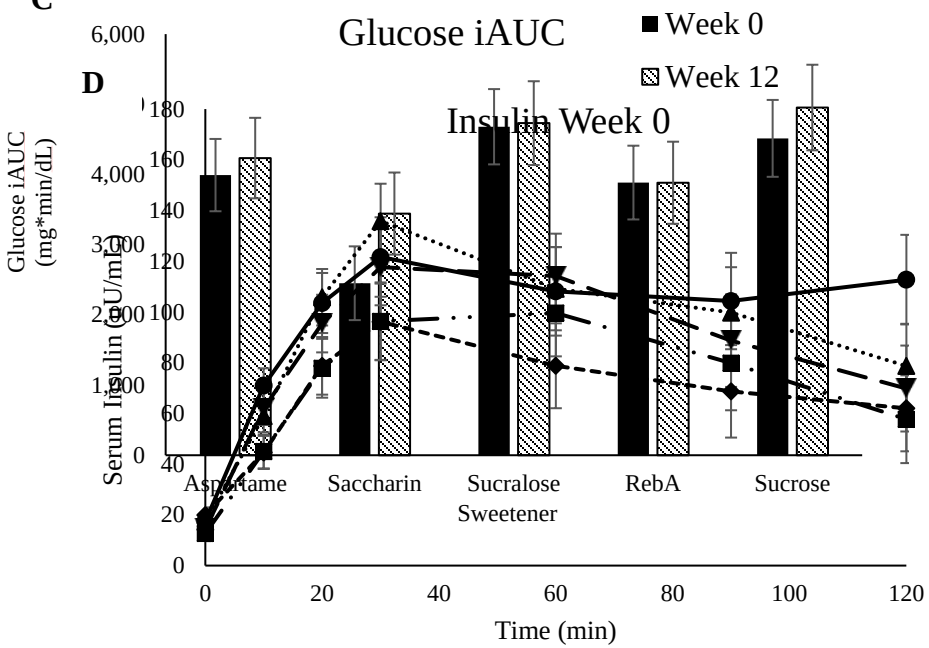


Figure 4 Serum Glucose (A, B) Concentrations and incremental Area Under the Curve (AUC) (C) during OGTT at Baseline and After 12 Weeks of Sweetener Consumption

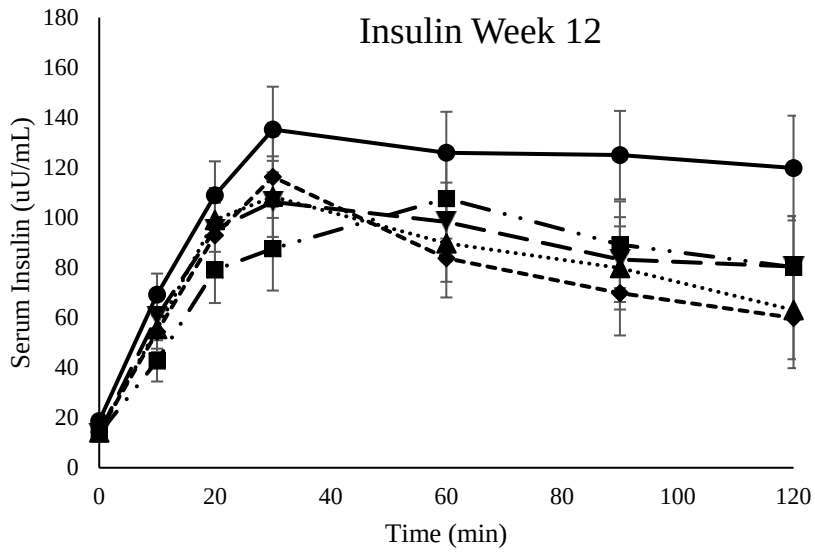
C


Glucose iAUC
Week 0
Week 12
Glucose iAUC (mg*min/dL)
6,000
4,000
2,000
0
Aspartame
Saccharin
Sucralose
RebA
Sucrose
Sweetener

D

Insulin Week 0
Serum Insulin (uU/mL)
180
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (min)

E


Insulin Week 12
Serum Insulin (uU/mL)
180
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (min)

F

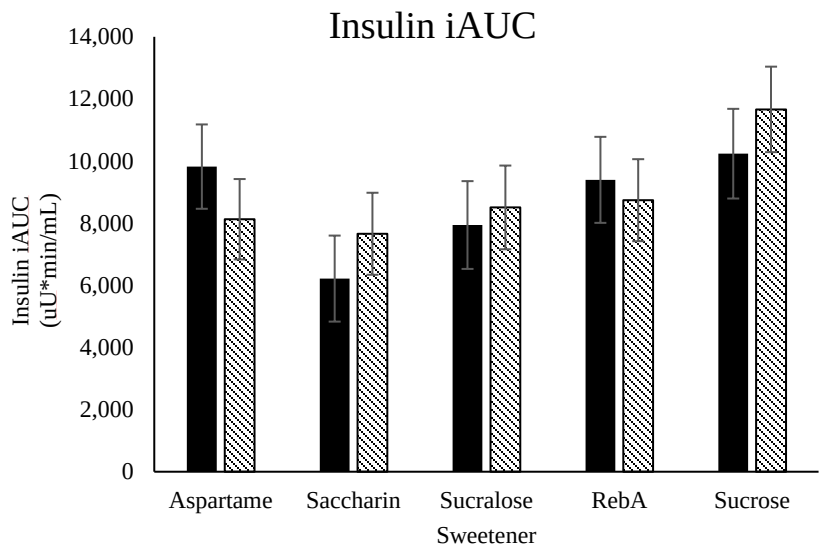


Figure 5 Serum Insulin (D, E) Concentrations and incremental Area Under the Curve (AUC) (F) during OGTT at Baseline and After 12 Weeks of Sweetener Consumption

Data equals mean ± SE.

Repeated measures ANOVA was used to compare blood glucose and insulin concentrations from the OGTTs

Treatment, treatment-by-time, treatment-by-session, session-by-time, treatment-by-session-by-time interaction effects were not statistically significant for glucose or insulin values.

Main effect of time on glucose values statistically significant (p=0.02).

Treatment, session, and treatment-by-session effects were not statistically significant for glucose or insulin incremental area under the curve (iAUC).

Table 6 Fasting Blood Metabolites and AUC for Glucose and Insulin During OGTT at Baseline and after 12 Weeks of Sweetener Consumption

		Aspartame	Saccharin	Sucralose	Reb A	Sucrose	p-values		
							Treatment	Session	Treatment-by-Session
Glucose iAUC (mg*min/dL)	Baseline	3991.44 ± 515.22	2450.55 ± 525.42	4680.23 ± 536.26	3885.01 ± 525.42	4514.66 ± 547.79	0.06	0.18	0.74
	Week 12	4233.75 ± 573.50	3443.23 ± 584.86	4731.74 ± 596.92	3882.40 ± 584.86	4954.13 ± 609.76			
Insulin iAUC (uU*min/mL)	Baseline	9821.09 ± 1357.45	6216.62 ± 1384.33	7941.09 ± 1412.87	9395.21 ± 1384.33	10237.50 ± 1443.26	0.28	0.62	0.10
	Week 12	8127.01 ± 1294.31	7660.08 ± 1319.94	8508.08 ± 1347.16	8740.29 ± 1319.94	11662.88 ± 1376.13			
HbA1c (%)	Baseline	5.37 ± 0.05	5.32 ± 0.06	5.45 ± 0.06	5.36 ± 0.05	5.43 ± 0.06	0.70	0.07	0.25
	Week 12	5.34 ± 0.05	5.34 ± 0.06	5.42 ± 0.05	5.36 ± 0.05	5.31 ± 0.06			
Total Cholesterol (mg/dL)	Baseline	145.71 ± 5.70	146.67 ± 5.94	148.35 ± 6.07	140.60 ± 5.82	155.27 ± 6.20	0.54	0.05	0.92
	Week 12*	140.67 ± 5.79	142.90 ± 6.03	142.61 ± 6.16	139.74 ± 5.91	152.30 ± 6.30			
HDL Cholesterol (mg/dL)	Baseline	41.66 ± 2.36	42.62 ± 2.46	46.77 ± 2.51	42.16 ± 2.41	43.22 ± 2.57	0.84	0.71	0.27
	Week 12	40.87 ± 2.64	44.34 ± 2.75	43.91 ± 2.81	43.30 ± 2.69	42.55 ± 2.87			
LDL Cholesterol (mg/dL)	Baseline	82.02 ± 5.20	78.06 ± 5.42	84.61 ± 5.53	80.00 ± 5.31	89.36 ± 5.66	0.68	0.04	0.98
	Week 12*	78.98 ± 4.73	76.04 ± 4.93	80.74 ± 5.03	77.54 ± 4.83	84.50 ± 5.15			
Triglycerides (mg/dL)	Baseline	109.71 ± 12.28	130.08 ± 12.78	87.89 ± 13.05	92.20 ± 12.52	113.59 ± 13.35	0.19	0.49	0.21
	Week 12	103.33 ± 11.73	104.77 ± 12.21	89.43 ± 12.47	93.64 ± 11.96	126.18 ± 12.75			

Data presented as mean ± SE.

All analytes measured in serum except HbA1c which was measure in whole blood.

*statistically significant time trend ($p<0.05$)

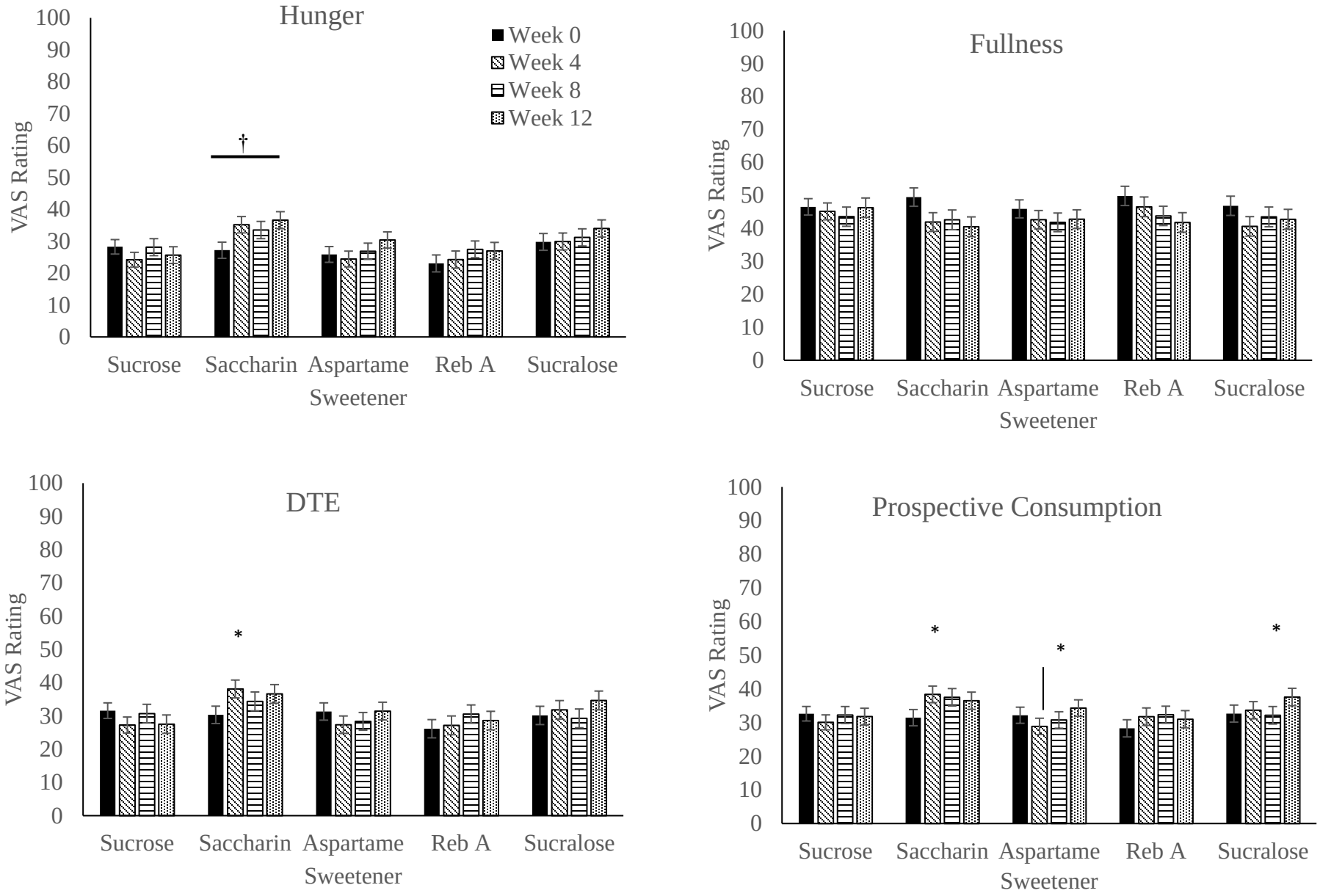


Figure 6 Appetitive Sensations with Sweetener Consumption for 12 Weeks

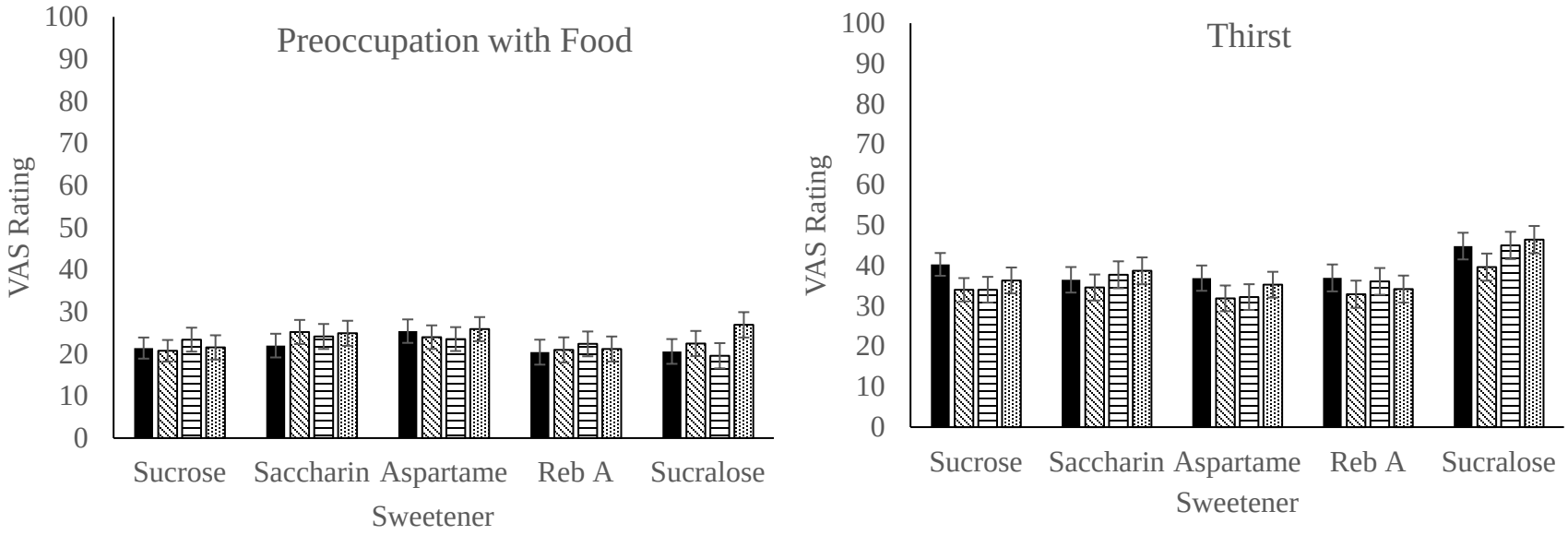


Figure 6 (continued) Appetitive Sensations with Sweetener Consumption for 12 Weeks

Data equals mean ± SE.

Linear mixed model was used to determine the main effects of treatment and time and the interaction of treatment-by-time on change in body weight.

Main effect of time statistically significant for hunger, fullness, and thirst VAS ratings ($p<0.05$).

† denotes statistical significant treatment main effect ($p<0.05$).

* denotes statistical differences within treatments ($p<0.05$).

CHAPTER 5. TWELVE-WEEK CONSUMPTION EFFECTS OF ASPARTAME ON GLYCEMIA, APPETITE, AND BODY WEIGHT AMONG HEALTHY, LEAN ADULTS

Higgins K., Considine R., and Mattes R. 2018. Aspartame consumption for 12 weeks does not affect glycemia, appetite, or body weight of healthy, lean adults in a randomized controlled trial. *The Journal of Nutrition*, 148(4), 650-657. doi:10.1093/jn/nxy021

Footnotes

This study was funded by the Ajinomoto Co., Inc. It was designed, conducted, analyzed and this report of the findings were all executed independently of the funding source.

Abbreviations used: ace-K, acesulfame potassium; ASP group, aspartame group; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1; HbA1c, glycated hemoglobin; LCS, low-calorie sweetener; OGTT, oral glucose tolerance test; PABA, para-amino benzoic acid.

Abstract

Background: Low-calorie sweeteners are often used to moderate energy intake and postprandial glycemia, but some evidence indicates that they may exacerbate these aims.

Objective: The trial's primary aim was to assess the effect of daily aspartame ingestion for 12 wk on glycemia. Effects on appetite and body weight were secondary aims.

Methods: One hundred lean [body mass index (kg/m2): 18–25] adults aged 18–60 y were randomly assigned to consume 0, 350, or 1050 mg aspartame/d (ASP groups) in a beverage for 12 wk in a parallel-arm design. At baseline, body weight and composition were determined, a 240-min oral-glucose-tolerance test (OGTT) was administered, and measurements were made of appetite and selected hormones. Participants also collected a 24-h urine sample. During the intervention, the 0-mg/d ASP group consumed capsules containing 680 mg dextrose and 80 mg

para-amino benzoic acid. For the 350-mg/d ASP group, the beverage contained 350 mg aspartame and the 1050-mg/d ASP group consumed the same beverage plus capsules containing 680 mg dextrose and 700 mg aspartame. Body weight, blood pressure, heart rate, and waist circumference were measured weekly. At weeks 4, 8, and 12, participants collected 24-h urine samples and kept appetite logs. Baseline measurements were repeated at week 12.

Results: With the exception of the baseline OGTT glucose concentration at 60 min (and resulting area under the curve value), there were no group differences for glucose, insulin, resting leptin, glucagon-like peptide 1, or gastric inhibitory peptide at baseline or week 12. There also were no effects of aspartame ingestion on appetite, body weight, or body composition. Compliance with the beverage intervention was ~95%.

Conclusions: Aspartame ingested at 2 doses for 12 wk had no effect on glycemia, appetite, or body weight among healthy, lean adults. These data do not support the view that aspartame is problematic for the management of glycemia, appetite, or body weight. This trial was registered at www.clinicaltrials.gov as NCT02999321.

Introduction

The high global prevalence of overweight and obesity in children and adults is well documented (Ng et al., 2012). One of the many complications associated with high adiposity is impaired glucose tolerance and diabetes (American Diabetes, 2016; Bhupathiraju et al., 2016). Low calorie sweeteners (LCS) are added to foods and beverages by the food industry and consumers for multiple economic, sensory and health purposes. A key driver for LCS use is to displace sugar intake and thereby reduce total energy intake and the glycemic response to sweet food ingestion. LCS consumption by children and adults is high and increasing (Ng et al., 2012; C. Piernas et al., 2013; A. C. Sylvetsky et al., 2016; A. C. Sylvetsky, Y. Jin, et al., 2017; A. C. Sylvetsky et al., 2012). Approximately 25% of children and 41% of adults report using these products (A. C. Sylvetsky, Y. Jin, et al., 2017) and many more consume them unwittingly, because they have become pervasive in the food supply (A. C. Sylvetsky, P. J. Walter, et al., 2017).

Recent large meta-analyses indicate that use of LCS is associated with lower body mass index (BMI) (Paige E. Miller et al., 2014; P. J. Rogers, Hogenkamp, de Graaf, et al., 2016), and LCS are commonly used by individuals with sustained weight loss in the National Weight Control Registry (Catenacci et al., 2014). Several professional societies including The American Heart Association (Gardner et al., 2012), Academy of Nutrition and Dietetics (Fitch et al., 2012) and Canadian Diabetes Association (Gougeon R, 2004) have noted that LCS either moderate (Anton et al., 2010; Curi et al., 1986) or have no effect on post-prandial glycemia, but none has concluded they exacerbate blood sugar excursions. Their use has also been associated with a higher Healthy Eating index (Drewnowski et al., 2014). With this evidence base, over 25% of consumers report they would pay a premium price for sugar-free products (M. E. Kuhn, 2017). However, some researchers maintain that LCS undermine attempts to manage body weight and glycemia

(Davidson et al., 2014; Fowler, 2016; S. E. Hill et al., 2014; Palmnas et al., 2014; Pepino et al., 2011; A. E. Reid et al., 2016; Schiffman, 2012; J. Suez et al., 2014; S. E. Swithers, 2013; Yang, 2010). Thus, the issue remains contentious.

Questions related to the safety of LCS have been extensively reviewed by the United States Food and Drug Administration and multiple international bodies (Roberts, 2016). These products have consistently been deemed safe by these agencies, though this conclusion is still challenged by some investigators (e.g., (Schiffman, 2012)). This issue aside, mechanisms that have been posited for the adverse effects of LCS on body weight and/or glycemia include "hijacking" of sweet receptors with resulting hyper-stimulation of brain reward systems; activation of a cephalic phase insulin response; disassociation of sweetness and energy yield, training the palate to prefer very sweet foods and beverages; increased hunger and desire to eat; as well as enhanced energy absorption from the GI tract via augmented glucose transport; and/or shifts in gut microflora leading to more efficient energy harvesting. Evidence that LCS are associated with lower BMI (P. E. Miller et al., 2014; P. J. Rogers, Hogenkamp, de Graaf, et al., 2016) and don't promote hyperglycemia (Anton et al., 2010; C. E. Bryant et al., 2014) not with-standing, the plausibility of these mechanisms still provides grounds for questioning the health effects of LCS.

ASP is one widely used LCS implicated in the claim that LCSs are problematic with respect to energy balance and glycemia (Yang, 2010; Fowler et al., 2008). However, there is reason to question this view as each of the proposed mechanisms is of uncertain validity. First, ASP and other LCS do not elicit greater sweet intensity than sucrose (Antenucci et al., 2015). They may achieve their maximal effect at a lower concentration compared to sucrose, but do not elicit greater perceived sweetness and may not be as strong a stimulus for dopamine release as nutritive sweeteners (Anton et al., 2010; Bergstrom et al., 2007; McCutcheon et al., 2012). Second, several

investigators report oral sweet exposure is not a sufficient stimulus for insulin release (Abdallah et al., 1997; Morricone et al., 2000; K. L. Teff et al., 1995). While there is documentation that some LCS have supported cephalic phase insulin responses (Just et al., 2008) (or biphasic responses in beta cells (Ace-K (Liang et al., 1987)), ASP has not proven to be an effective stimulus for insulin release (Duskova et al., 2013; Hartel B, 1993). Such a lack of effect could be argued equally to lead to a reduced, instead of a larger, meal size (Power et al., 2008). Other work indicates oral exposure to a LCS (e.g., stevioside, sucralose) reduces, rather than increases post-prandial glycemia (Gregersen et al., 2004). Third, there is very limited evidence of a hedonic training effect. In one trial, adults exposed to intensely sweet foods showed no hedonic shift (Liem et al., 2004), although a very small effect was noted in children. The intervention entailed added sucrose so it was reinforcing through both its sensory and energy-yielding properties. ASP would contribute negligible energy, and energy may play an under-appreciated role in supporting a learned preference effect (de Araujo, 2016; de Araujo et al., 2008). In other work, no difference in food choice, implicit intake, explicit liking or expected satiety was observed following consumption of soft drinks and yogurt drinks containing a nutritive sweetener and LCS (S. Griffioen-Roose et al., 2013). Further, even if ASP enhanced the appeal of highly sweetened foods, this is not a reliable predictor of food choice or energy intake in individuals who are lean or have high adiposity (Witherly et al., 1980). Fourth, with respect to augmentation of appetitive sensations, the preponderance of the literature fails to support this effect (e.g., (Little et al., 2009; P. J. Rogers et al., 1988)). Fifth, a mechanism involving enhanced glucose transport has been supported by cell culture studies (Mace et al., 2007), but not *in vivo* human trials (R. J. Brown et al., 2009; Ma et al., 2010; T. Wu et al., 2012). Moreover, even if the time course of glucose absorption was altered by oral ASP exposure, an effect on energy balance is doubtful since the

efficiency of glucose absorption is extremely high. Thus, there would be a ceiling effect. Finally, because ASP is a dipeptide, it is efficiently hydrolyzed in the small intestine so would not be expected to reach the colon and exert an effect on the local microbiome.

Given the ongoing controversy and lack of a likely mechanism, a larger, longer term and more definitive study of ASP's effect on post-prandial glycemia was undertaken. Acute effects were assessed as well as the effect of daily consumption of three doses (0, 350 and 1050 mg/d) for three months by healthy adults with normal BMI. The primary outcome was the glycemic response. Secondary outcomes were appetite, body weight and body composition.

Methods

Study Design

This was a randomized, three, parallel-arm design trial. The randomization schedule was obtain from the internet. Participant assignment was sequential based on order of recruitment with no adjustments.

Participants

A total of 100 individuals 214 individuals responded to advertisements for the study and 100 were randomized to treatments between August 10, 2016 and February 7, 2017 **(Figure 7).** During a pre-screening assessment, participants completed a battery of questionnaires to obtain baseline health and demographic information and had measurements taken of body weight, waist circumference, blood pressure and heart rate. Those meeting eligibility criteria and choosing to participate signed a consent form approved by the Purdue University Institutional Review Board.

The eligibility criteria were: male or female, age 18-60 years, BMI of 18 – 25 kgm-2, not taking medications that affect metabolism or appetite, fasting serum glucose between 4.0 and 6.0

mmol/L (72 to 108 mg/dL) via capillary finger-stick, willing to comply with the study protocol, no reported ASP sensitivity and non- or low-user of LCSs, including ASP. The latter was determined during the pre-screening assessment where individuals reported low (<1x/week) or no discretionary use of LCS and no purposeful selection of products containing LCS. They also indicated non-use of items on a food frequency questionnaire comprised of commercial products containing LCS that are reduced in energy density by greater than 50% through substitution of a LCS for a nutritive sweetener.

Participants were sequentially randomized based on a generated random order (https://www.randomizer.org/) to one of three groups resulting in samples of 33 in the group ingesting no ASP, 33 in the group ingesting 350mg/d of ASP and 34 in the group ingesting 1050 mg/d ASP. A total of 93 individuals completed the trial; 31 in each of the groups. Thus, attrition was 2, 2 and 3 in the groups ingesting 0, 350 or 1050 mg/d of ASP respectively. Withdrawals were attributable to conflicts of time management or loss of interest, but not because of known study-related complications (i.e., adverse side-effects).

Protocol

During baseline, participants were instructed on methods for 24-hour urine collection and for recording appetitive sensations over the waking part of 24-hour periods. Appetite ratings were self-reported on smartphones via Qualtrics software under free-living conditions. Participants were instructed to record their sensation for each quality (hunger, fullness, desire to eat, thirst, prospective consumption, preoccupation with food) on an hourly basis for each waking hour of the day. Following completion of the urine collection and appetite log, participants reported to the laboratory in a fasted state. An indwelling catheter was placed in an arm vein and a baseline blood sample was obtained 10 minutes after catheter insertion. A blood sample was collected for

assessment of fasting glucose, HbA1c, the incretin hormones GIP and GLP-1, insulin, leptin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, Gamma-GTP, ALT and AST. Next participants ingested a 296 ml bottle of glucola (75g glucose) within 10 minutes and additional blood draws were taken at 15, 30, 60, 90, 120,180 and 240 minutes. This was followed by a body composition measurement via Plysmography (BOD POD).

During the intervention phase, participants consumed an assigned beverage and capsules daily for 12 weeks. They reported to the laboratory weekly to pick-up their next week's supply of beverage powder and capsules, measurement of body weight, blood pressure, heart rate, and waist circumference as well as assessment of study progress. At weeks 4 and 8, they collected a 24-hour urine sample and kept a 24-hour appetite log. At the end of 12 weeks, participants repeated the baseline measurements.

Intervention

Participants consumed 500ml of water or fruit-flavored beverages sweetened with aspartame (preferred flavors self-selected from three alternatives) and 4 capsules each day. This provided 80mg of PABA, 680mg of dextrose and either 350 mg or 1050 mg of ASP (the equivalent of 0, 5mg/kg or 15mg/kg of ASP based on a 70 kg reference individual). The specific group interventions were:

0 mg/d Group – 500 ml of water containing 0% mg ASP; 2 capsules collectively containing 680 mg dextrose and 80 mg para-amino benzoic acid (PABA) and 2 empty capsules.

350 mg/d Group – Premeasured sachets of lemon, orange or fruit punch flavored dry powder beverage mixture reconstituted by participants to yield 500 ml containing 350mg ASP and 80mg PABA; 2 capsules collectively containing 680 mg dextrose and 2 empty capsules.

1050 mg/d Group – Premeasured sachets of lemon, orange or fruit punch flavored dry powder beverage mixture reconstituted by participants to yield 500 ml containing 350mg ASP and 80mg PABA; 4 capsules collectively containing 700 mg ASP and 680 mg dextrose.

Analyte Assays

Blood samples were drawn from a catheter into vacutainer tubes for preparation of serum or EDTA plasma. Dipeptidyl peptidase-4 inhibitor (Millipore Corp, Billerica, MA) was immediately added to the plasma tube, samples were spun at 4oC, aliquoted and frozen in a -80oC freezer within 15 min of the draw. Serum insulin, glucose, cholesterol (total, HDL, LDL), triglycerides, gamma-glutamyltranspeptidase (GGTP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured on a Roche COBAS 400 Plus analyzer. Hemoglobin A1c (HbA1c) was determined in whole blood using the COBAS analyzer. GLP-1 active (7-36) and GIP were quantitated in plasma using ELISA (Millipore Corporation, Billerica, MA) with minimum sensitivities of 0.14 pM and 4.2 pg/ml, respectively. All samples for a participant (pre- and post-intervention) were run on the same ELISA plate. Serum leptin was measured using a radioimmunoassay (Millipore). All samples for an individual were run in the same assay.

Compliance

PABA was inextricably mixed with the beverage powder. Analysis of 24-h urine samples for PABA recovery followed the method of Sharma et al. (Sharma et al., 2014). Urine samples were collected at baseline to establish the PABA composition of each individual's customary diet and again at weeks 4, 8 and 12. Creatinine was measured on a COBAS 400 Plus analyzer to ensure

complete 24-hour samples were collected and if not, then corrections were applied as previously published (K. Murakami, et al., 2008).

Statistical Analysis

A mixed model repeated measures analysis of variance was used to assess glucose and insulin responses, appetite, body weight and body composition. Session (baseline, end of week 12) and time (0-240 minutes) were within-subjects factors and treatment group was the between subjects factor. Analyses were conducted using the IBM SPSS Statistics software package (version 23). The alpha level was set at $p<0.05$, two tailed. The Bonferroni correction was used for post-hoc analysis.

Results

Baseline Characteristics

There were no differences in baseline characteristics between groups based on the total recruited and among completers. Baseline characteristics of those completing the trial are presented in Table 8. There were no significant differences between treatment groups for age, BMI, waist circumference, systolic or diastolic blood pressure, HbA1c or fasting glucose at baseline.

Biochemical outcomes

The effects of daily ASP consumption over 12 weeks on the hematological indices monitored are presented in Table 8. There was no significant main effect of treatment or session, nor a treatment by session interaction for HbA1c, lipids or liver function indices. The estimated

compliance with beverage consumption based on PABA and creatinine excretion was 95.4 ± 11.8% in ASP consuming treatment groups.

Baseline and week 12 serum glucose values from the OGTT tests are plotted in **Figure 8A and 8B**, respectively. Data are reported on 27 and 30 participants in the groups ingesting 350 and 1050mg/d of ASP, respectively, due to complications in sample collection. There was no significant main effect of treatment or session. There was a significant time (0-240 minutes) by treatment (0, 350, 1050 mg/d) by session (baseline, week 12) interaction ($p<0.03$). The 350 mg/d treatment group had lower serum glucose concentrations at 60 minutes than the 0 mg/d ($p<0.01$) or 1050 mg/d ($p<0.05$) treatment groups at baseline, but there were no significant treatment group differences at week 12. Overall glucose values were significantly higher at week 12 compared to baseline ($p<0.05$). There was also a significant main effect of treatment where the 350 mg/d treatment group had a lower glycemic response (7.6 mg/dl) than the 0 mg/d treatment group ($p<0.05$). Area-under-the-curve (AUC) values were also computed and repeated measures analysis of variance revealed a significant ($p<0.03$) treatment effect (350 mg/d group < 0 mg/d or 1050 mg/d groups), but no treatment X time interaction (data not shown). Baseline and week 12 insulin values from the OGTT tests are also plotted in **Figure 8C and 8D**, respectively. There was no main effect of treatment nor was there a significant treatment by session interaction.

GLP-1 and GIP concentrations during the OGTT at baseline and week 12 are presented in **Figure 10**. No significant main effect of treatment or treatment by session interaction was observed for either of the incretins. Resting leptin concentrations also did not differ by treatment or over time **(Figure 11).**

Appetite

Ratings of hunger, desire to eat, fullness and thirst are presented in **Figure 9**. Values represent the mean of the totality of data available for each group and time point (i.e., N's differed). Because of differences in the timing and duration of each person's waking time and numerous instances where hourly ratings were missed, the analysis of changes over time was restricted to individuals who captured at least 6 hourly ratings at baseline and week 12 (N =16, 12, 16 for the 0, 350 and 1050 mg/d groups). No significant differences were observed for hunger, desire to eat, fullness or thirst in this subset of appetite ratings. These ratings did not differ from the smaller subset (N = 5, 6, 9) who recorded >12 hourly ratings each day.

Body weight

Participants were not placed on an energy restricted diet. The only guidance provided was that they consume the daily beverage portion which contained no, or a trivial amount of energy. Thus, no weight change was expected in any treatment group. There was no main effect of treatment nor a treatment by session interaction for body weight. There also were no significant main effects of treatment or session nor an interaction between treatment and session for percent fat mass, percent fat-free mass, fat mass or fat-free mass (Table 9).

Discussion

The primary outcome of this trial was the glycemic response to acute and chronic ASP ingestion. The 350mg/d/day dose corresponds to an intake of about one can of low calorie soda per day and the mean of intake for ASP consumers, whereas the 1050mg/d dose approximates the 95th percentile for consumers (Magnuson et al., 2007). The glycemic response to each treatment (including a no ASP control) was measured at baseline and again following 12 weeks of daily

beverage ingestion. The data reveal that responses after ingestion of the 350 or 1050mg/d doses were not significantly different from the no ASP treatment either at baseline or after the 12-week daily consumption period. The two random single time point differences are not viewed as meaningful. A true lack of effect is supported by urinary biomarker excretion findings of approximately 95% compliance with the ASP ingestion intervention. Similarly, the insulin, GLP-1 and GIP responses after both doses of ASP were not significantly different from the control condition. Body composition and fasting leptin was also unaffected by ASP ingestion.

These findings confirm and extend current understanding of the effects of ASP and other LCS on glycemia. Early interventional studies indicated that LCS did not alter the glycemic response in healthy individuals, individuals with obesity or patients with Type 1 or Type 2 diabetes (Anton et al., 2010; C. E. Bryant et al., 2014; Magnuson et al., 2007; Renwick, 1994). However, with the discovery of sweet receptors in the GI tract, new interest arose regarding the implications of sweetener signaling on incretin secretion, glucose absorption and peripheral glycemia (Egan et al., 2008). However, neither increased incretin secretion nor glucose absorption were observed in response to ASP in in vitro trials with endocrine and absorptive cells or in in vivo animal or human trials. The same cannot be said for other LCS. Cell culture work suggested sucralose enhances GLP-1 secretion (Jang et al., 2007) and a variety of LCS (except ASP) activate the glucose transporters SGLT1 and GLUT2 and glucose absorption (Mace et al., 2007; Margolskee et al., 2007).

Enhanced absorption of glucose was observed in animal trials with exposure to ace-K, saccharin, and sucralose (Mace et al., 2007), but this was not supported by other studies involving an array of LCS (Fujita et al., 2009). An early trial in humans noted consumption of “Diet Rite Soda” sweetened with sucralose and ace-K prior to an OGTT led to a greater rise of GLP-1 than

carbonated water, though glucose and insulin concentrations were unaffected (R. J. Brown et al., 2009). To isolate sweetener effects in the GI tract, sucralose was infused into the duodenum with no effect on incretin secretion, glucose absorption or glycemia (Ma et al., 2009), and ASP, ace-K and sucralose were administered intragastrically without effect on gut peptides (Steinert et al., 2011). In contrast, effects were noted with nutritive sweeteners. The preponderance (not totality (Pepino, et al., 2011)) of human trials have since confirmed the lack of LCS on incretin release and no effect, or a lowering of glycemia and/or insulinemia (Anton et al., 2010; Temizkan et al., 2015; T. Wu et al., 2012; R.J. Brown et al.; 2012; Tey, 2017). Interestingly, several trials report an augmentation by diet cola (A. C. Sylvetsky et al., 2016; C. E. Bryant et al., 2014; 53, R.J. Brown et al.; 2012), but the effect appears to be related to the cola rather than the sweetener (A. C. Sylvetsky et al., 2016). The present findings with a sample size three to nine times greater than prior similar trials, are consistent with this body of evidence and extend understanding by documenting that daily ASP consumption for 12 weeks has no effect on incretin release, glycemia or insulinemia.

An *a priori* identified secondary outcome pursued in this work related to the effects of ASP on appetite. Some early work suggested that oral exposure to ASP in a medium that did not provide energy (e.g., water or chewing gum) could augment hunger (Black et al., 1993; Blundell et al., 1986; Rogers et al., 1988; Tordoff et al., 1990). However, others did not replicate these findings (Black et al., 1993; Black et al., 1991; Canty et al., 1991) and subsequent reviews and later clinical trials of LCS, including ASP, indicate LCS do not augment hunger (Anderson GH, 1996; Anton et al., 2010; Bellisle et al., 2007; Benton, 2005; Blundell et al., 1996; de la Hunty A, 2006; Drewnowski, 1995; Renwick, 1994; Rogers P, 1989; Rolls, 1991; Steinert et al., 2011; Vermunt et al., 2003). One group reported ingestion of ASP in a capsule decrebolased hunger (Blundell et

al., 1986; Rogers et al., 1991; Rogers et al., 1990) though this finding has not been replicated. We observed no effect of ASP exposure at either the moderate or high dose relative to the water control at any time point and no shift in hunger, fullness or desire to eat ratings over time in any treatment group. Participants were free-living and not instructed as to when and how to consume their daily beverage. Additionally, acute effects were not monitored precluding a direct test of hypotheses related to either hunger stimulation or suppression following single ingestive events. Nevertheless, it may be noted that despite many participants indicating they frequently consumed the non-energy-yielding beverages alone, no augmentation of hunger was detected and in the 1050 mg/d treatment group, where two-thirds of the dose was delivered in capsules, no reduction of hunger was detected. Our appetite findings with normal weight individuals are also consistent with a 10-week trial of individuals who were overweight and consumed a beverage with ASP as the primary sweetener (Raben et al., 2002).

The effects of ASP on body composition was an additional secondary outcome. Recent meta-analyses have yielded conflicting conclusions about the role of LCS (type not segregated) on BMI. In two reports, cohort studies revealed LCS use is associated with higher (Azad et al., 2017; Miller et al., 2014) or no change (P. J. Rogers, Hogenkamp, de Graaf, et al., 2016) of BMI while randomized controlled trials indicate they are associated with (Miller et al., 2014) or no effect (Azad et al., 2017) on BMI. Both cohort studies and randomized controlled trials indicate reported energy intake is lower with LCS use (P. J. Rogers, Hogenkamp, de Graaf, et al., 2016; Zheng et al. 2015), except where comparisons are made to treatments with water, nothing or unsweetened products. In the later cases, findings are that LCS lead to lower (Zheng et al. 2015) or comparable effects (P. J. Rogers, Hogenkamp, de Graaf, et al., 2016) on energy intake. This seeming inconsistency likely reflects multiple subtle analytical differences. Among these are differences

in study inclusion criteria (all trials base their analysis on different studies, some published in close temporal contiguity actually have no overlap in trials), the nature of the comparisons made (LCS versus different treatments), dissimilar study participants (lean, obese, minors, adults, etc.), duration of study, and types of LCS. Resolution of the issue will likely require additional data to build the database to a point where there is adequate power to tease out these nuances.

In summary, the present trial found daily ingestion of ASP for 12 weeks in a fruit-flavored beverage did not elicit any change of glycemia, incretin release, appetite, body weight or body composition in healthy, lean adults. The present study was not intended to evaluate ASP's efficacy for purposeful weight loss, but the findings do not indicate its use as a substitute for energy-yielding sweeteners would be counter-productive.

Acknowledgements

The authors responsibilities were as follows: RDM conceived the study, conducted statistical analyses and wrote the first draft of the manuscript. KAH assisted with study execution, analyzed the dietary compliance data and reviewed the manuscript. RVC helped design the protocol, oversaw the incretin hormone analyses and reviewed the manuscript. All authors approved the final manuscript. Testing was conducted in the Bionutrition facilities (CTSI grant # UL1RR025761) at Purdue University. Incretin assays were performed in the Translation Core of the Center for Diabetes and Metabolic Diseases (P30 DK097512) at the Indiana University School of Medicine.

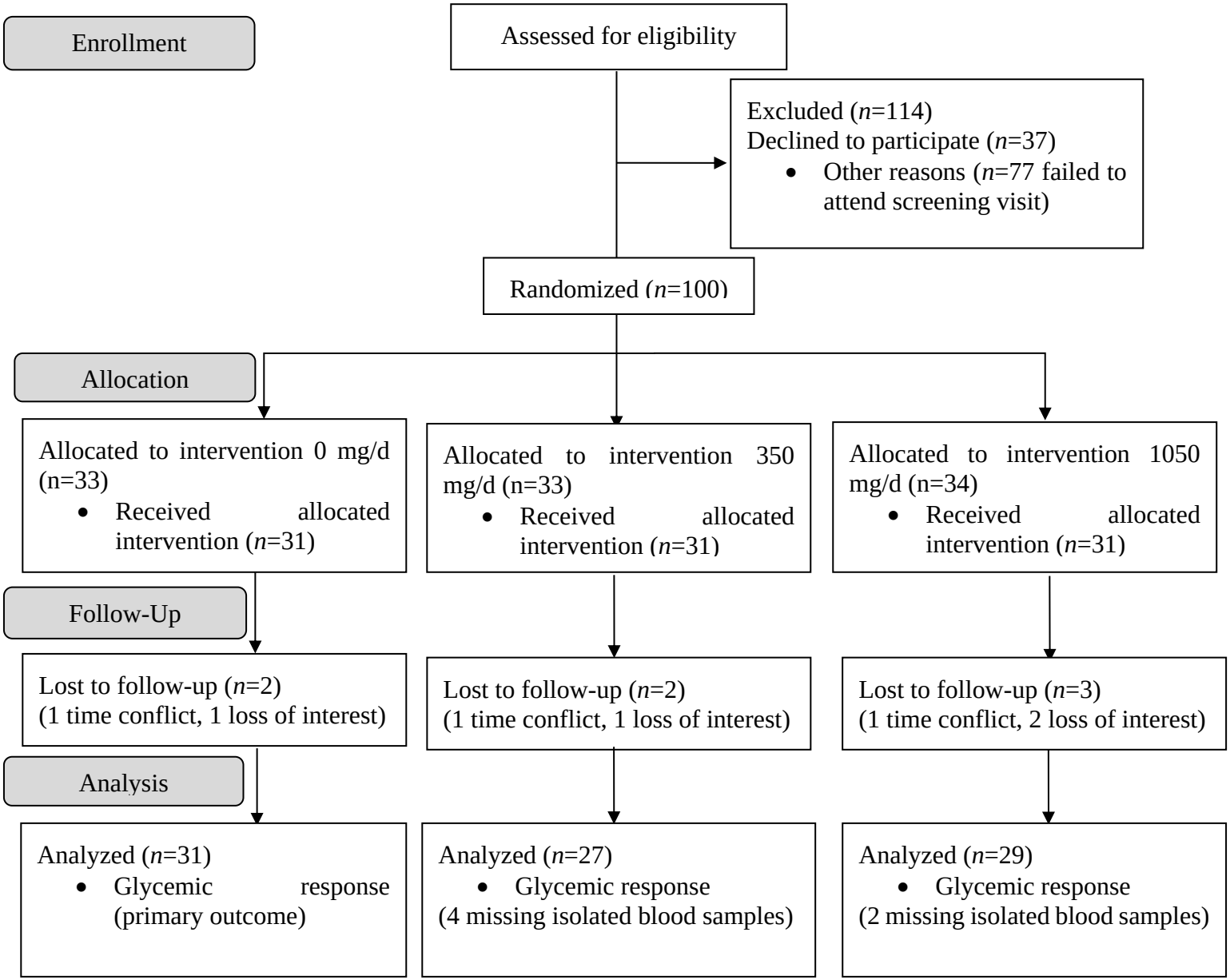


Figure 7 Participant Flow Chart through Aspartame Intervention

Table 7 Baseline Characteristics of Study Participants Enrolled in the Aspartame Randomized Control Trial

ASP Group (mg/d)	Sex (M/F)	Age (years)	BMI (kg/m^2)	WC (cm)	SBP (mmHg)	DBP (mmHg)	HbA1c (%) (whole blood)	Fasting Serum Glucose (mg/dl)
0	13/18	24.2±1.3	21.7±0.3	74.1±1.3	118.8±2.1	73.4±1.4	5.13±0.04	83.5±1.4
350	17/14	22.8±1.0	22.3±0.3	74.5±1.1	123.8±2.4	76.1±1.7	5.12±0.04	81.5±1.5
1050	13/18	21.8±0.6	22.1±0.3	71.4±2.9	124.0±2.8	77.2±1.4	5.15±0.04	81.6±1.5

Values are means ± SDs, n = 31/group. There were no significant differences between treatment groups for for age, BMI, waist circumference, systolic or diastolic blood pressure, HbA1c, or fasting glucose at baseline. ASP group, aspartame group; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; SBP, systolic blood pressure; WC, waist circumference.

Table 8 HbA1c and serum biochemistry of participants at baseline and after 12 wk of daily consumption of 0, 350, or 1050 mg aspartame in a beverage

	0 mg/d	350 mg/d	1050 mg/d
HbA1c baseline (%)	5.13±0.04	5.12±0.04	5.15±0.04
HbA1c week 12 (%)	5.20±0.04	5.22±0.04	5.23±0.04
Total Cholesterol baseline (mg/dl)	157.2±4.8	149.4±5.0	148.7±5.0
Total Cholesterol week 12 (mg/dl)	158.4±4.6	151.6±4.8	153.7±4.8
HDL Cholesterol baseline (mg/dl)	53.6±2.3	54.0±2.4	59.0±2.4
HDL Cholesterol week 12 (mg/dl)	56.7±2.5	56.7±2.6	61.0±2.6
LDL Cholesterol baseline (mg/dl)	88.5±4.2	79.3±4.4	72.7±4.4
LDL Cholesterol week 12 (mg/dl)	85.8±3.8	78.6±4.0	76.2±4.0
Triglycerides baseline (mg/dl)	75.7±6.1	79.4±6.3	85.1±6.3
Triglycerides week 12 (mg/dl)	80.4±6.4	81.4±6.6	85.5±6.6
GGTP baseline (U/l)	14.2±1.5	12.9±1.5	13.3±1.6
GGTP week 12 (U/l)	14.9±1.6	13.9±1.7	15.0±1.7
ALT baseline (U/l)	13.3±1.3	13.5±1.3	12.7±1.3
ALT week 12 (U/l)	14.5±1.6	14.3±1.6	16.4±1.7
AST baseline (U/l)	19.6±1.5	19.5±1.6	19.1±1.6
AST week 12 (U/l)	20.6±1.9	21.2±2.0	19.8±2.0

Values are means ± SEs, n = 31/group. Variables did not differ between groups at baselineorchangeovertime.ALT,alaninetransaminase;AST,aspartatetransaminase; GGTP, γ-glutamyltranspeptidase; HbA1c, glycated hemoglobin.

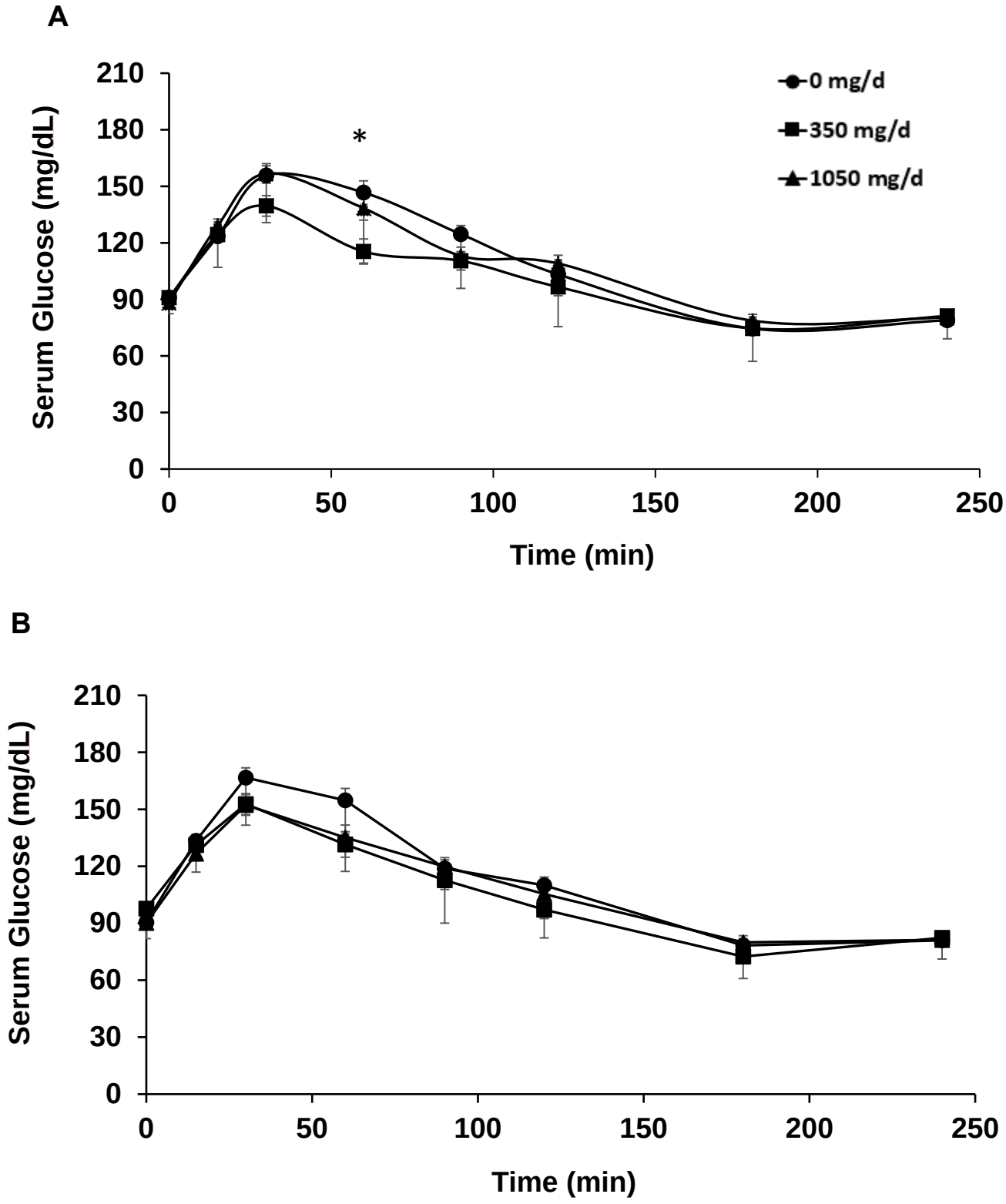


Figure 8 Serum glucose (A, B) and insulin (C, D) concentrations during the oral-glucose-tolerance test for participants consuming 0mg (n=31), 350 mg (n=27), or 1050 mg (n=30) aspartame/d in a beverage at baseline (A, C) and after 12 wk (B, D) of daily consumption.

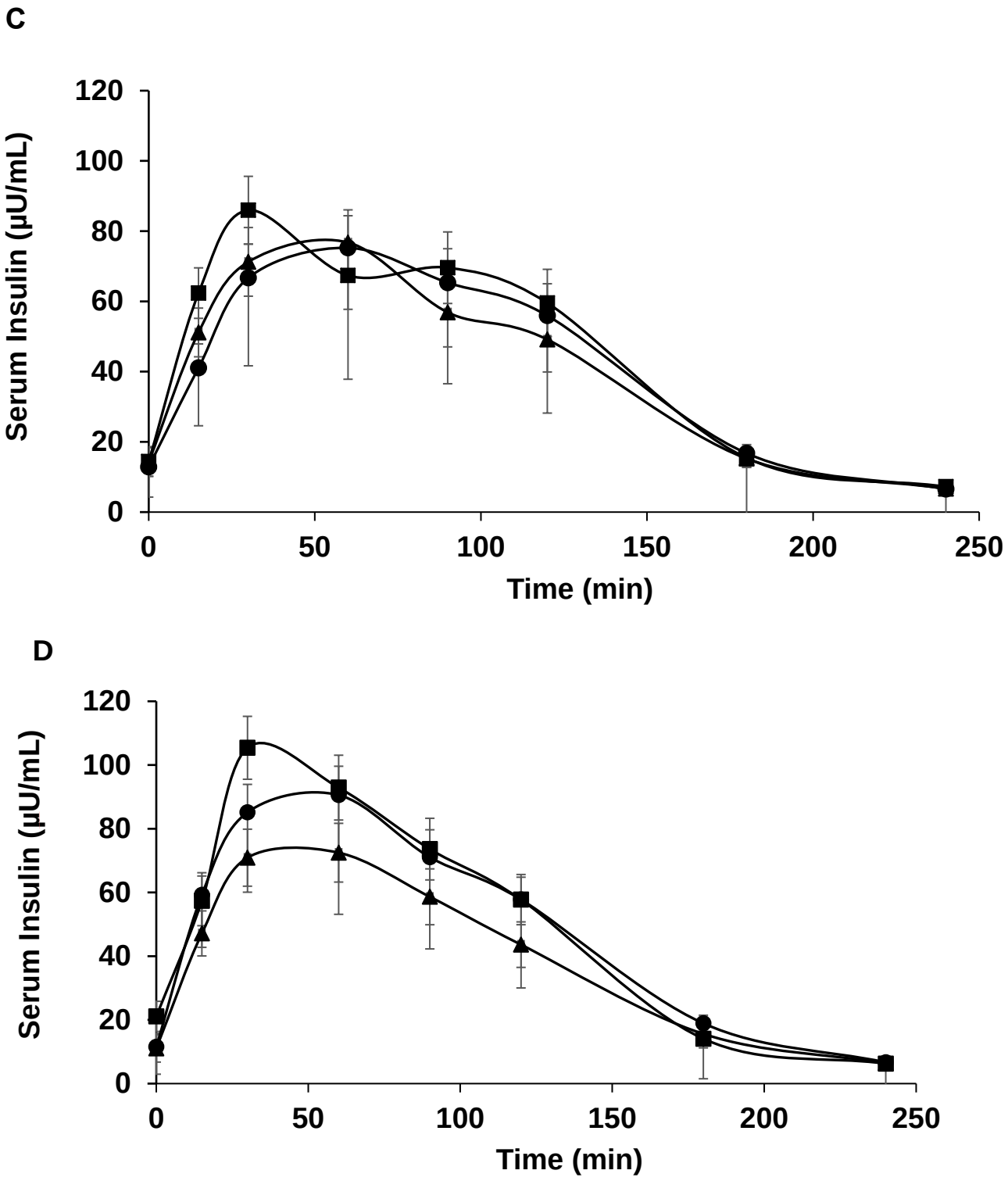


Figure 8 (continued) Serum glucose (A, B) and insulin (C, D) concentrations during the oral-glucose-tolerance test for participants consuming 0mg (n=31), 350 mg (n=27), or 1050 mg (n=30) aspartame/d in a beverage at baseline (A, C) and after 12 wk (B, D) of daily consumption

Values are means ± SEs. *Different between 0- and 350-mg/d treatments, $P < 0.05$. There were no significant differences between 0and 1050-mg/d treatments or 350- and 1050-mg/d treatments.

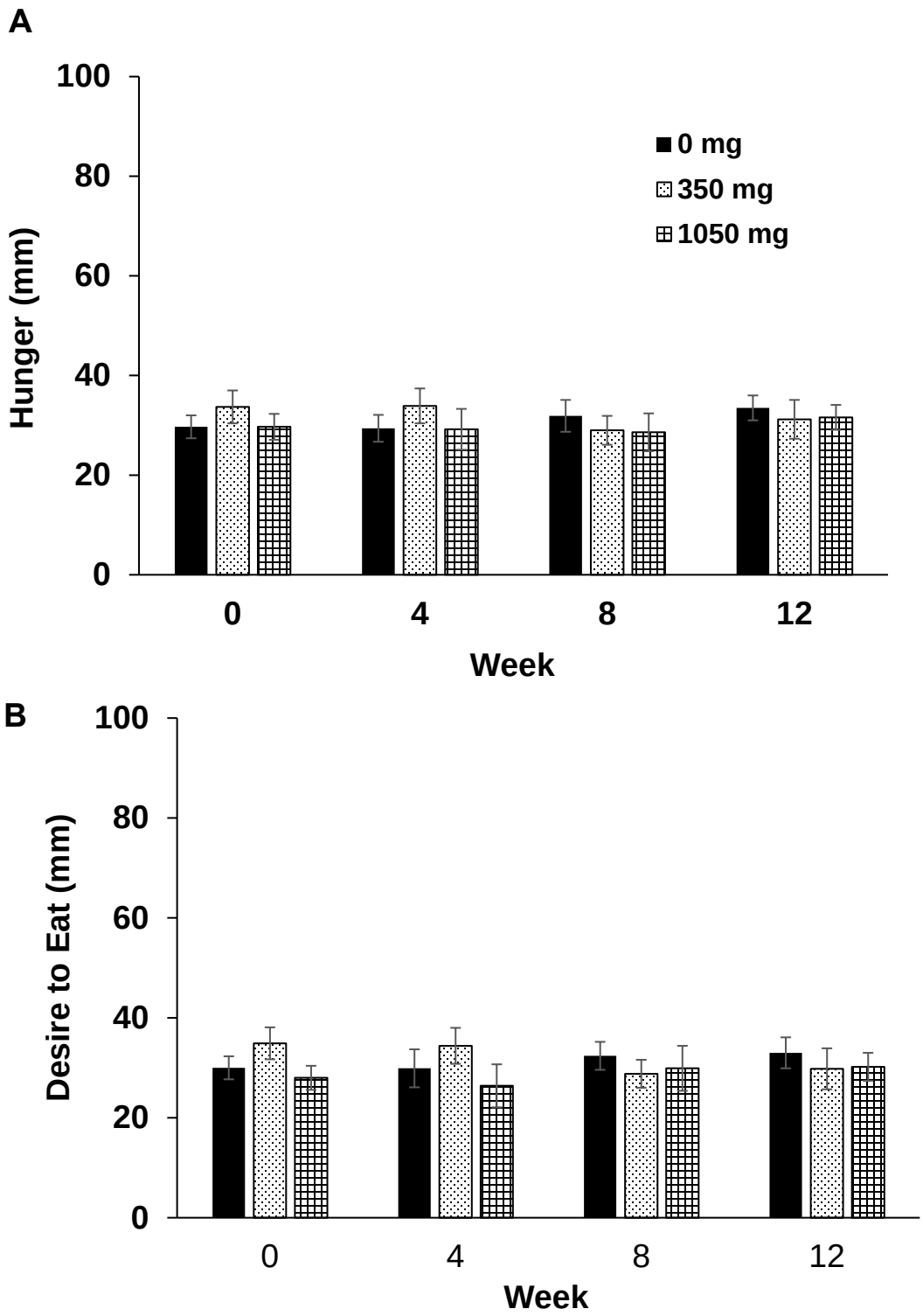


Figure 9 Appetite ratings for hunger (A), desire to eat (B), fullness (C), and thirst (D) by participants consuming 0 mg (n = 16), 350 mg (n = 12), or 1050 mg (n = 16) aspartame/d in a beverage at baseline and after 12 wk of daily consumption.

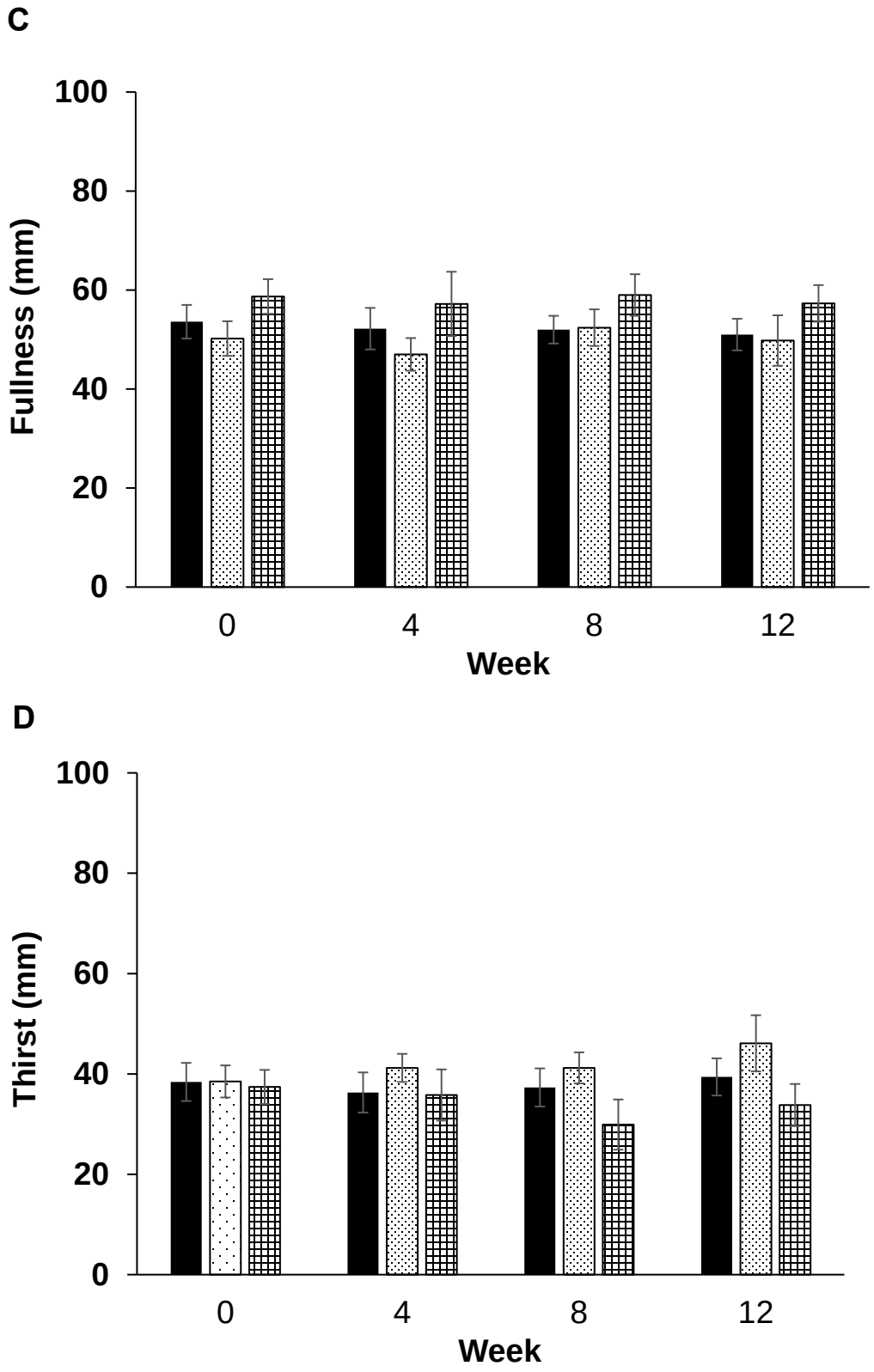


Figure 9 (continued) Appetite ratings for hunger (A), desire to eat (B), fullness (C), and thirst (D) by participants consuming 0 mg (n = 16), 350 mg (n = 12), or 1050 mg (n = 16) aspartame/d in a beverage at baseline and after 12 wk of daily consumption

Values are means ± SEs. No significant differences were observed for hunger, desire to eat, fullness, or thirst.

Table 9 Body-composition indexes of participants at baseline and after consumption of 0, 350, or 1050 mg aspartame/d in a beverage for 12 weeks

Group	Baseline Body Weight (kg)	Week 12 Body Weight (kg)	Baseline % Fat Mass	Week 12 % Fat Mass	Baseline % Fat-Free Mass	Week 12 % Fat-Free Mass	Baseline Fat Mass (kg)	Week 12 Fat Mass (kg)	Baseline Fat-Free Mass (kg)	Week 12 Fat-Free Mass (kg)
0 mg	63.5±1.8	63.0±1.8	22.8±1.6	22.6±1.6	75.2±2.0	77.4±1.6	14.1±1.6	13.7±0.9	49.0±2.0	49.2±2.1
350 mg	65.3±1.8	65.6±1.8	19.6±1.6	21.0±1.6	79.4±2.0	79.0±1.6	13.3±1.6	13.7±0.9	52.3±2.1	52.6±2.2
1050 mg	63.6±1.8	63.8+1.8	22.4±1.6	21.8±1.6	77.5±2.0	78.2±1.6	14.0±1.6	13.5±0.9	49.7±2.0	50.3±2.1

Values are means ± SEs, n=31/group. There were no significant differences between treatment groups or over time. ASP group, aspartame group

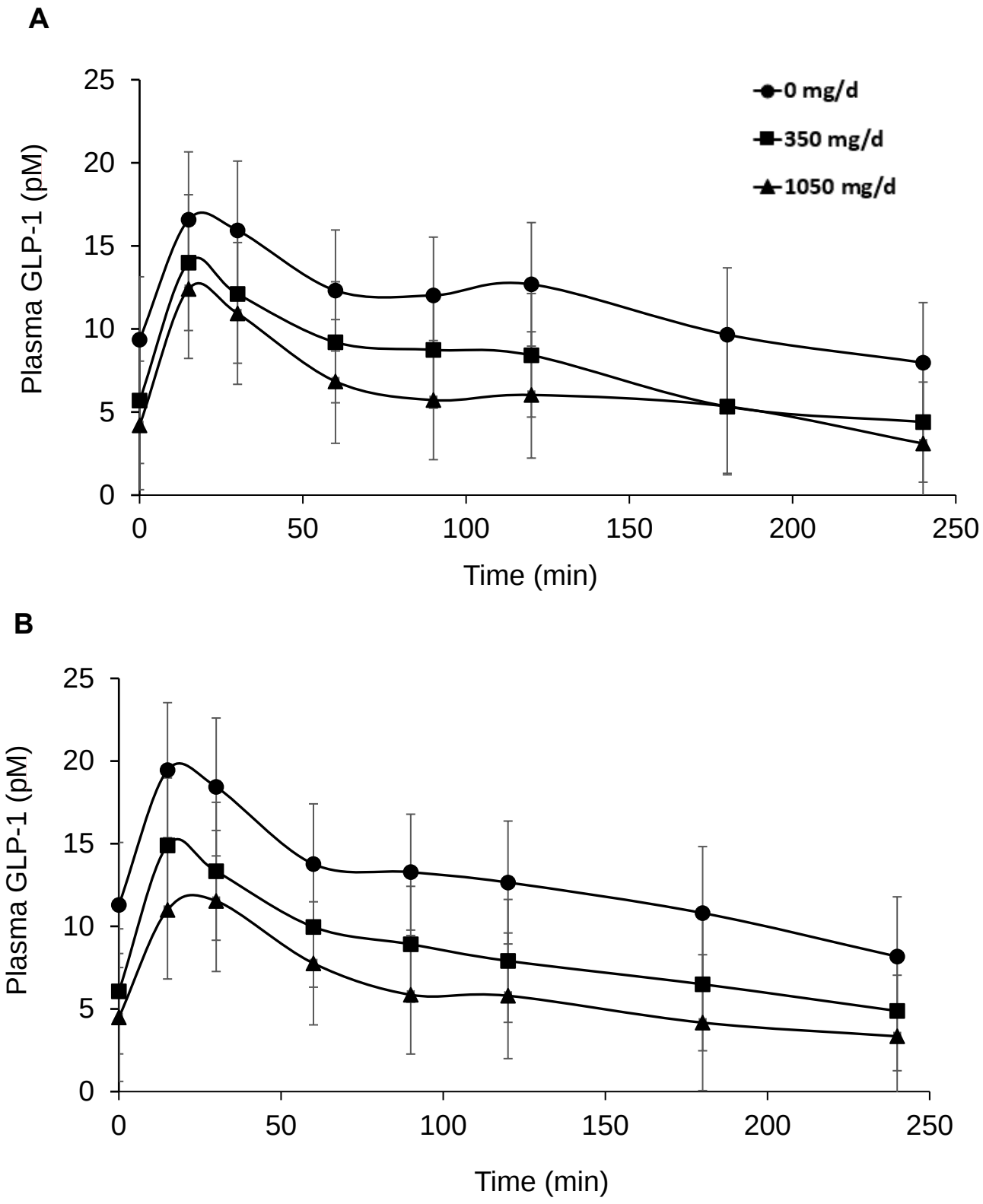


Figure 10 Plasma GLP-1 and GIP concentrations (Mean ± SE) during OGTT of participants consuming 0 mg/d (N=23, 31 respectively), 350 mg/d (N=23, 26 respectively) or 1050 mg/d (N=21, 29 respectively) of ASP in a beverage at baseline and after 12 weeks of daily consumption.

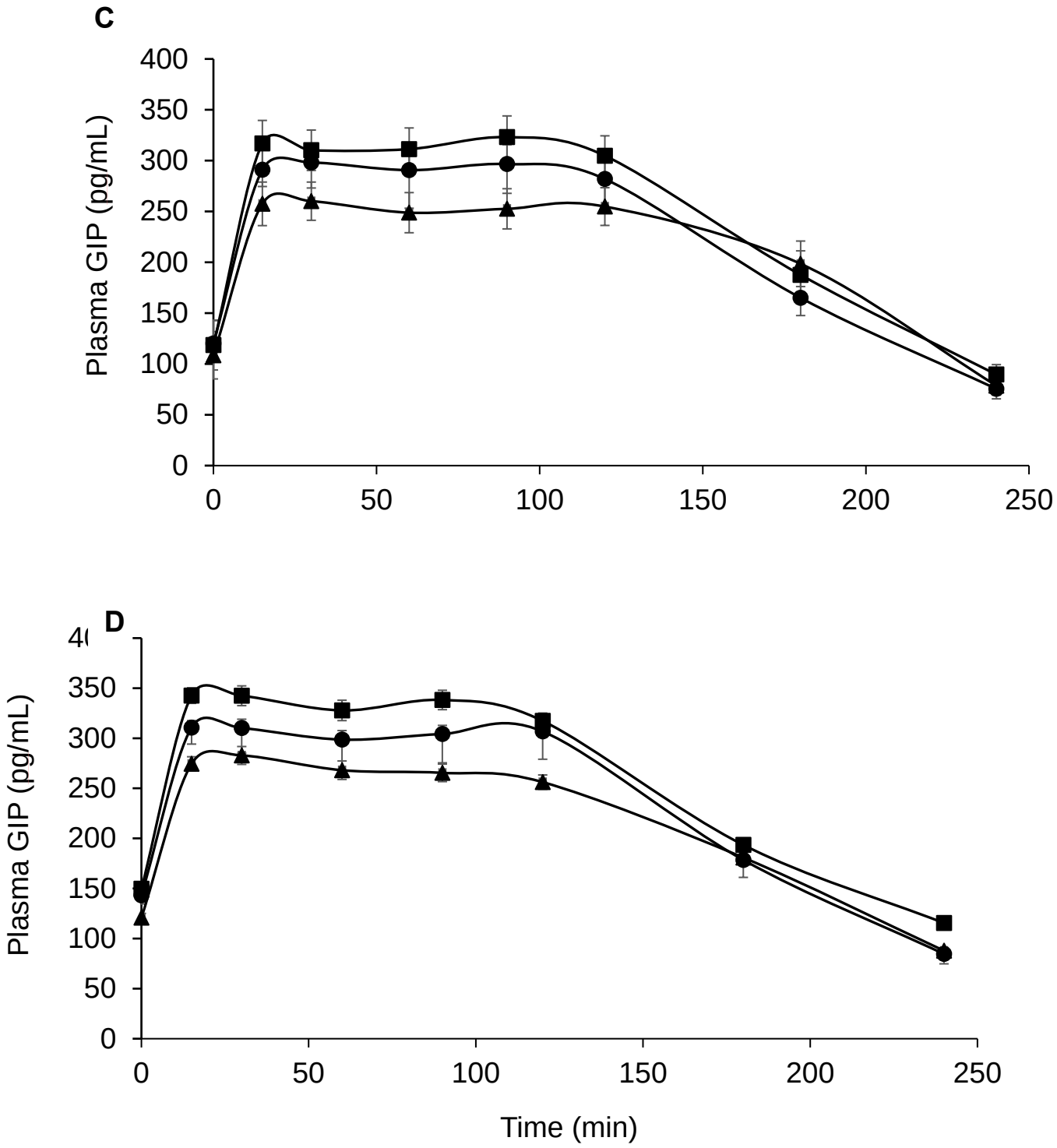


Figure 10 (continued) Plasma GLP-1 and GIP concentrations (Mean ± SE) during OGTT of participants consuming 0 mg/d (N=23, 31 respectively), 350 mg/d (N=23, 26 respectively) or 1050 mg/d (N=21, 29 respectively) of ASP in a beverage at baseline and after 12 weeks of daily

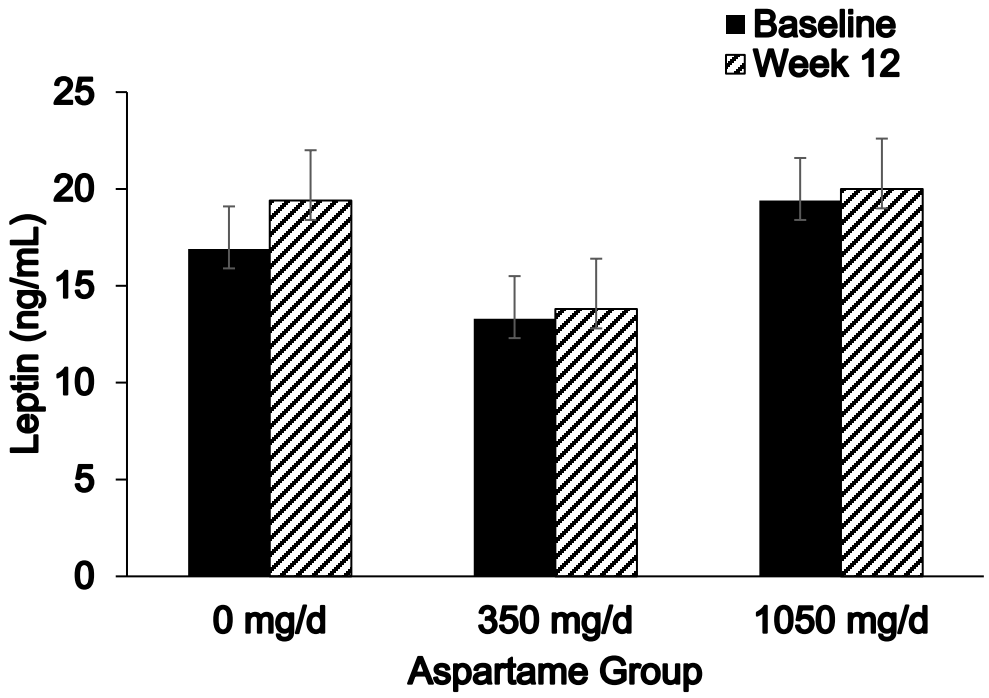


Figure 11 Fasting serum leptin concentrations (Mean ± SE) of participants (n=31/group) consuming 0 mg/d, 350 mg/d or 1050 mg/d of ASP in a beverage at baseline and after 12 weeks of daily consumption.

No significant differences in time or treatment were observed.

CHAPTER 6. SUMMARY AND FUTURE DIRECTIONS

Summary

LCS provide sweet taste and minimal energy yet exhibit striking differences in their structure, receptor binding, and biological fate. These differences have sensory, metabolic, and behavioral ramifications that can affect body weight, glycemia, and ingestive behavior. Numerous preload trials have investigated acute effects of LCS consumption on energy intake, appetite, and glycemia; and some RCT and epidemiological trials have investigated long-term effect of LCS consumption on body weight and glycemia. Meta-analyses of these trials come to conflicting conclusions regarding LCS use for weight management, with recommendations ranging from replacement of SSB with LCS (P. J. Rogers, Hogenkamp, de Graaf, et al., 2016) to LCS avoidance (Azad et al., 2017). The variability in findings between these trials is potentially attributed to the type of LCS used in the intervention. Few trials examine ingestive behaviors to specific LCS or compare responses between LCS. Therefore, this research was conducted to determine the effect of specific LCS and varying doses of aspartame on body weight and glycemia in two, twelve-week, RCT (in chapters 4 and 5, respectively).

Major Findings

Differential Effects of LCS on Body Weight

This dissertation provides evidence that the different chemical structures of LCS contribute to differential body weight change when consumed chronically among individuals with overweight but otherwise healthy. Therefore, the mechanisms LCS affect body weight go beyond energy dilution or dietary sweetness. The major findings of this trial are as follows:

- LCS differentially affect body weight compared to sucrose and other LCS when consumed for 12 weeks among healthy individuals with overweight and obesity. Sucrose and saccharin consumption lead to increased body weight across the 12-week intervention and did not differ from each other. While there was no significant change in body weight with consumption of the other LCS treatments compared to baseline, changes in weight compared to sucrose were significantly reduced for aspartame, rebA, and sucralose. In addition, change in body weight was significantly lower between sucralose and all other LCS at the end of the 12-week trial.
- Increased reported energy intake and eating frequency was associated with increased body weight with sucrose consumption and decreased energy intake and eating frequency corresponded with negative, but not significant weight reduction with sucralose consumption. However, a lack of change over the 12-week trial among saccharin consumers does not correspond with the increase in body weight and warrants further investigation.
- DTE increased among the saccharin group but not the other LCS or sucrose groups, and prospective consumption increased among saccharin consumers and at select time points among sucralose consumers but not the other LCS or sucrose groups. This suggests that sweetness alone does not stimulate appetite and attribute to weight gain.
- Glycemic response during an OGTT was not significantly altered allowing 12 weeks of sweetener consumption. There was no difference in glycemic response between LCS and sucrose treatment groups.

No Effect of Aspartame Consumption on Glycemia

Consumption of aspartame at levels equivalent to one can of diet soda or at levels equivalent to the 95th percentile of consumption had no effect on glycemia, incretin release, appetite, body weight, or body composition among normal weight, healthy adults. The major findings of this trial are as follows:

- With the exception of the baseline OGTT glucose concentration at 60 min (and resulting area under the curve value), there were no differences between aspartame doses for glucose, insulin, resting leptin, glucagon-like peptide 1, or gastric inhibitory peptide at baseline or week 12.
- There also were no effects of daily ingestion of 350 and 1050mg aspartame for 12 weeks on appetite, body weight, or body composition.

Future Directions

Despite numerous trials investigating the effect of LCS consumption on body weight, glycemia, and ingestive behavior, many questions about their efficacy for weight loss or maintenance and glycose tolerance remain. While short-term, preload design trials are necessary to measure physiological and appetitive response under tightly controlled environments, they do not allow for learned or adapted responses to LCS and lack ecological validity. Future trials should monitor weight and glycemic changes with chronic consumption of LCS. The trials presented in this dissertation were 12 weeks in duration. While we were able to detect changes in body weight for select LCS, longer trials are necessary to determine if weight would continue to change with chronic consumption or reach a plateau. RCTs in humans typically use aspartame; long-term RCT

examining body weight and glycemia with saccharin, rebA, and sucralose consumption are lacking.

We tested three levels of aspartame (two levels in normal weight individuals and one level among individuals with overweight/obesity), all of which had no effect on body weight or glycemia. Whether similar responses among individuals with normal weight and individuals with overweight occurs for all LCS requires further investigation. Additional trials measuring multiple doses of other LCS, including those not tested in this series of experiments (i.e. ace-K, neotame, advantame), are necessary to replicate the results from this trial. In addition, multiple LCS are commonly combined in food and beverage products, because of the enhanced and improved quality of their sweetness profile (Schiffman et al., 1995; Zhao et al., 2007). Whether the sensory effects of binary mixtures of LCS affect metabolic or behavioral responses differently than individual LCS is unknown.

If LCS chemical structure is directly related to how a LCS will affect body weight, then ace-K could exhibit similar increases in body weight as saccharin. Ace-K (commonly used synergistically with other LCS) has similar structure, receptor affinity, and digestibility as saccharin (C. Kuhn et al., 2004; Magnuson et al., 2016; Masuda et al., 2012). Whether it leads to similar effects on body weight as saccharin warrants testing.

Participants in these trials were not actively attempting to lose weight and were asked to maintain their current diet and lifestyle. However, LCS are commonly used as a diet tool. The efficacy of use of LCS, specifically sucralose, during a weight loss intervention will provide insight into whether LCS are effective for weight loss.

Changes in energy intake do not explain the increase in body weight among the saccharin group. This may be due to poor dietary intake self-report. However, numerous other hypothesized

mechanisms not explored in these trials may explain the difference in body weight between LCS treatments. These include a CPIR, brain reward, and gut microbiotic changes.

The CPIR is sweetener specific and mediated independent of the T1R2/T1R3 receptor (Glendinning et al., 2015). The implications of a CPIR elicited without energy or a diminished CPIR with repeated LCS exposure have been proposed to increase energy intake (Paul AM Smeets et al., 2010), but has not been tested in humans. A CPIR occurs with saccharin oral exposure (Just et al., 2008). A CPIR was documented with sucralose exposures in one trial (Dhillon et al., 2017); however, the response was only documented for a subset of the sampled participants. Another trial reported no evidence for a sucralose CPIR (Ford et al., 2011). Whether sucralose exposure causes a CPIR needs to be confirmed. If a CPIR does not occur with oral exposure to sucralose, then a CPIR with saccharin exposure may explain the significant increase in body weight with saccharin consumption and not the other LCS. If a CPIR does occur with oral exposure to sucralose, then a LCS mediated CPIR effect on body weight can be rejected because of the differences in body weight with saccharin and sucralose consumption.

Brain reward response to LCS is in the preliminary phase of exploration. Available cross-sectional trials provide limited insight because of high within- and between-subject variability in fMRI brain imagining. Only one trial monitored the effect of LCS on reward value with repeated exposure to sucralose sweetened beverages and sucralose and ace-K sweetened yogurt drinks (S. Griffioen-Roose et al., 2013). A single measured response to oral exposure only provides information about the rewarding values of LCS at the sensory level. Saccharin, ace-K, and a fraction of sucralose enter circulation and can affect brain reward post-absorptively. Therefore, trials should employ procedures to measure brain reward during oral exposure and again during the post-absorptive phase. It is important to measure appetitive and ingestive responses to LCS

with brain imaging to determine if activation or inhibited activation of brain reward centers translate to measurable changes in the reward value of foods.

Implication of LCS that reach the colon (saccharin, sucralose, and steviol) on gut microbiotic changes that may affect body weight and glycemia needs to be determined. Changes to colonic microbiota population that may alter energy harvesting efficiency have been proposed to affect body weight (Magnuson et al., 2016; Jotham Suez et al., 2015), but a causal mechanism is not known. Future experiments in animals and humans should use a standard sampling technique to ensure they are obtaining an accurate sample of gut microbiotic populations (Daly et al., 2016). Because LCS are used to displace dietary sugars, incorporation of LCS into the diet typically results in a higher percentage of dietary fat and/or protein if the energy of the diet is held constant. Adequate control groups should include a sucrose-sweetened treatment and a macronutrient and energy matched control to determine if microbiotic changes are due to the sweetener or the diet. Finally, larger sample sizes are needed to draw conclusions regarding the effect of LCS on body weight and glycemia because of wide between- and within- subject gut microbiota variability. While alterations to microbiota diversity, metabolite production, and/or energy harvesting efficiency are linked to obesity and glucose regulation, more research is needed to determine whether LCS exert different effects and the mechanisms these microbiotic changes affect body weight and glycemia.

LCS consumption has increased among American adults and children (A. C. Sylvetsky et al., 2017). A better understanding of LCS consumption patterns is necessary to determine the relationship between consumption of specific LCS and body weight in free-living conditions. LCS intake levels are difficult to measure because ingredient labels only require a label that a product contains a LCS but not how much. In addition, produce formulations continually change and

ingredient contents are only available for a fraction of the available food supply. While LCS are most commonly consumed as beverages, they are also increasingly incorporated into food products. Factors such as mastication, food and beverage viscosity, consumption with other nutrients, and palatability may all affect ingestive responses to LCS differently depending on the delivery vehicle. Whether LCS have differential effects when consumed as a food versus incorporated into a beverage requires further investigation.

In general, LCS should not be aggregated, and it should not be assumed that they have similar glycemic and body weight responses. The lack of differentiation between LCS may explain the positive and negative association between LCS and body weight. Future trails monitoring effects of sweetness and energy should define what sweeteners are used, compare multiple sweeteners, and interpret results in the context of the type of LCS used. A better understanding of the weight and glycemic responses to specific LCS will enable consumers, clinicians, policy makers, and the food industry to make informed decisions and develop products that emphasize the beneficial effects of select LCS and limit potential adverse health consequences.

REFERENCES

Abdallah, L., Chabert, M., & Louis-Sylvestre, J. (1997). Cephalic phase responses to sweet taste. *The American Journal of Clinical Nutrition, 65*(3), 737-743.

Abou-Donia, M. B., El-Masry, E. M., Abdel-Rahman, A. A., McLendon, R. E., & Schiffman, S. S. (2008). Splenda alters gut microflora and increases intestinal p-glycoprotein and cytochrome p-450 in male rats. *J Toxicol Environ Health A, 71*. doi:10.1080/15287390802328630

Abudula, R., Matchkov, V. V., Jeppesen, P. B., Nilsson, H., Aalkjaer, C., & Hermansen, K. (2008). Rebaudioside A directly stimulates insulin secretion from pancreatic beta cells: a glucose-dependent action via inhibition of ATP-sensitive K-channels. *Diabetes Obes Metab, 10*(11), 1074-1085. doi:10.1111/j.1463-1326.2008.00864.x

Akhavan, T., & Anderson, G. H. (2007). Effects of glucose-to-fructose ratios in solutions on subjective satiety, food intake, and satiety hormones in young men. *The American Journal of Clinical Nutrition, 86*(5), 1354-1363. doi:10.1093/ajcn/86.5.1354

Akhavan, T., Luhovyy, B. L., & Anderson, G. H. (2010). Effect of drinking compared with eating sugars or whey protein on short-term appetite and food intake. *International Journal Of Obesity, 35*, 562. doi:10.1038/ijo.2010.163

Aldinger, S. M., Speer, V. C., Hays, V. W., & Catron, D. V. (1959). Effect of Saccharin on Consumption of Starter Rations by Baby Pigs. *Journal of Animal Science, 18*(4), 1350-1355. doi:10.2527/jas1959.1841350x

Alexander, B., Natalia, B., Wely, F., Masashi, I., Xia, L., Cailu, L., . . . Gary, B. (2011). Genetics of sweet taste preferences. *Flavour and Fragrance Journal, 26*(4), 286-294. doi:doi:10.1002/ffj.2074

Almiron-Roig, E., Palla, L., Guest, K., Ricchiuti, C., Vint, N., Jebb, S. A., & Drewnowski, A. (2013). Factors that determine energy compensation: a systematic review of preload studies. *Nutr Rev, 71*(7), 458-473. doi:10.1111/nure.12048

American Diabetes, A. (2016). 6. Obesity Management for the Treatment of Type 2 Diabetes. *Diabetes Care, 39 Suppl 1*, S47-51. doi:10.2337/dc16-S009

Antenucci, R. G., & Hayes, J. E. (2015). Nonnutritive sweeteners are not supernormal stimuli. *Int J Obes (Lond), 39*(2), 254-259. doi:10.1038/ijo.2014.109

Anderson GH, L. L. (1996). Sweeteners and food intake: relevance to obesity. . In A. H. Angel A, Bouchard C, Lau D, Leiter LA, Mendelson R (Ed.), *Progress in obesity research.* New York, NY: John Libbey & Company, LTD.

Anton, S. D., Martin, C. K., Han, H., Coulon, S., Cefalu, W. T., Geiselman, P., & Williamson, D. A. (2010). Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. *Appetite, 55*(1), 37-43. doi:10.1016/j.appet.2010.03.009

Aoyama, K., Barnes, J., & Grimm, J. W. (2014). INCUBATION OF SACCHARIN CRAVING AND WITHIN-SESSION CHANGES IN RESPONDING FOR A CUE PREVIOUSLY ASSOCIATED WITH SACCHARIN. *Appetite, 72*, 114-122. doi:10.1016/j.appet.2013.10.003

Appleton, K. M., & Blundell, J. E. (2007). Habitual high and low consumers of artificially-sweetened beverages: effects of sweet taste and energy on short-term appetite. *Physiol Behav, 92*(3), 479-486. doi:10.1016/j.physbeh.2007.04.027

Appleton, K. M., Tuorila, H., Bertenshaw, E. J., de Graaf, C., & Mela, D. J. (2018). Sweet taste exposure and the subsequent acceptance and preference for sweet taste in the diet: systematic review of the published literature. *The American Journal of Clinical Nutrition, 107*(3), 405-419. doi:10.1093/ajcn/nqx031

ARS. (2018). USDA National Nutrient Database for Standard Reference, Legacy. Retrieved from http://www.ars.usda.gov/nutrientdata

Azad, M. B., Abou-Setta, A. M., Chauhan, B. F., Rabbani, R., Lys, J., Copstein, L., . . . Zarychanski, R. (2017). Nonnutritive sweeteners and cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials and prospective cohort studies. *Canadian Medical Association Journal, 189*(28), E929-E939. doi:10.1503/cmaj.161390

Bachmanov, A. A., Tordoff, M. G., & Beauchamp, G. K. (2001). Sweetener preference of C57BL/6ByJ and 129P3/J mice. *Chemical senses, 26*(7), 905-913.

Baecke, J. A., Burema, J., & Frijters, J. E. (1982). A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *American Journal of Clinical Nutrition, 36*(5), 936-942.

Baird, I. M., Shephard, N. W., Merritt, R. J., & Hildick-Smith, G. (2000). Repeated dose study of sucralose tolerance in human subjects. *Food and Chemical Toxicology, 38, Supplement 2*, 123-129. doi:https://doi.org/10.1016/S0278-6915(00)00035-1

Baldwin, B. A. (2007). Quantitative studies on taste preference in pigs. *Proceedings of the Nutrition Society, 35*(1), 69-73. doi:10.1079/PNS19760011

Baxter, M. G., & Murray, E. A. (2002). The amygdala and reward. *Nature Reviews Neuroscience, 3*, 563. doi:10.1038/nrn875

Bellisle, F., & Drewnowski, A. (2007). Intense sweeteners, energy intake and the control of body weight. *Eur J Clin Nutr, 61*(6), 691-700. doi:10.1038/sj.ejcn.1602649

Benton, D. (2005). Can artificial sweeteners help control body weight and prevent obesity? *Nutr Res Rev, 18*(1), 63-76. doi:10.1079/NRR200494

Bergstrom, B. P., Cummings, D. R., & Skaggs, T. A. (2007). Aspartame decreases evoked extracellular dopamine levels in the rat brain: an in vivo voltammetry study. *Neuropharmacology, 53*(8), 967-974. doi:10.1016/j.neuropharm.2007.09.009

Beridot-Therond, M. E., Arts, I., Fantino, M., & De La Gueronniere, V. (1998). Short-term Effects of the Flavour of Drinks on Ingestive Behaviours in Man. *Appetite, 31*(1), 67-81. doi:https://doi.org/10.1006/appe.1997.0153

Berkey Catherine, S., Rockett Helaine, R. H., Field Alison, E., Gillman Matthew, W., & Colditz Graham, A. (2012). Sugar-Added Beverages and Adolescent Weight Change. *Obesity Research, 12*(5), 778-788. doi:10.1038/oby.2004.94

Bhupathiraju, S. N., & Hu, F. B. (2016). Epidemiology of Obesity and Diabetes and Their Cardiovascular Complications. *Circ Res, 118*(11), 1723-1735. doi:10.1161/CIRCRESAHA.115.306825

Bingham, S., & Cummings, J. (1983). The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 h urine collections in man. *Clin Sci, 64*(6), 629-635.

Black, AE. (2000). Critical evaluation of energy intake using the Goldberg cut- off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *International Journal of Obesity, 24*(9), 1119. doi:10.1038/sj.ijo.0801376

Black, R. M., Leiter, L. A., & Anderson, G. H. (1993). Consuming aspartame with and without taste: differential effects on appetite and food intake of young adult males. *Physiol Behav, 53*(3), 459-466.

Black, R. M., Tanaka, P., Leiter, L. A., & Anderson, G. H. (1991). Soft drinks with aspartame: effect on subjective hunger, food selection, and food intake of young adult males. *Physiol Behav, 49*(4), 803-810.

Blackburn, G. L., Kanders, B. S., Lavin, P. T., Keller, S. D., & Whatley, J. (1997). The effect of aspartame as part of a multidisciplinary weight-control program on short- and long-term control of body weight. *The American Journal of Clinical Nutrition, 65*(2), 409-418.

Blundell, J. E., & Hill, A. J. (1986). Paradoxical effects of an intense sweetener (aspartame) on appetite. *Lancet, 1*(8489), 1092-1093.

Blundell, J. E., & King, N. A. (1996). Overconsumption as a cause of weight gain: behavioural-physiological interactions in the control of food intake (appetite). *Ciba Found Symp, 201*, 138-154; discussion 154-138, 188-193.

Brown, A. W., Bohan Brown, M. M., Onken, K. L., & Beitz, D. C. (2011). Short-term consumption of sucralose, a nonnutritive sweetener, is similar to water with regard to select markers of hunger signaling and short-term glucose homeostasis in women. *Nutrition Research, 31*(12), 882-888. doi:http://dx.doi.org/10.1016/j.nutres.2011.10.004

Brown, R. J., Walter, M., & Rother, K. I. (2009). Ingestion of diet soda before a glucose load augments glucagon-like peptide-1 secretion. *Diabetes Care, 32*. doi:10.2337/dc09-1185

Brown, R. J., Walter, M., & Rother, K. I. (2012). Effects of Diet Soda on Gut Hormones in Youths With Diabetes. *Diabetes Care, 35*(5), 959.

Bruce, D. G., Storlien, L. H., Furler, S. M., & Chisholm, D. J. (1987). Cephalic phase metabolic responses in normal weight adults. *Metabolism, 36*(8), 721-725. doi:https://doi.org/10.1016/0026-0495(87)90106-5

Brunstrom, J. M. (2004). Does dietary learning occur outside awareness? *Consciousness and Cognition, 13*(3), 453-470. doi:https://doi.org/10.1016/j.concog.2004.05.004

Brunstrom, J. M., Brown, S., Hinton, E. C., Rogers, P. J., & Fay, S. H. (2011). 'Expected satiety' changes hunger and fullness in the inter-meal interval. *Appetite, 56*(2), 310-315. doi:https://doi.org/10.1016/j.appet.2011.01.002

Bryant, C., & McLaughlin, J. (2016). Low calorie sweeteners: Evidence remains lacking for effects on human gut function. *Physiology & Behavior, 164*, 482-485. doi:http://dx.doi.org/10.1016/j.physbeh.2016.04.026

Bryant, C. E., Wasse, L. K., Astbury, N., Nandra, G., & McLaughlin, J. T. (2014). Non-nutritive sweeteners: no class effect on the glycaemic or appetite responses to ingested glucose. *Eur J Clin Nutr, 68*(5), 629-631. doi:10.1038/ejcn.2014.19

Canty, D. J., & Chan, M. M. (1991). Effects of consumption of caloric vs noncaloric sweet drinks on indices of hunger and food consumption in normal adults. *The American Journal of Clinical Nutrition, 53*(5), 1159-1164. doi:10.1093/ajcn/53.5.1159

Carakostas, M. C., Curry, L. L., Boileau, A. C., & Brusick, D. J. (2008). Overview: The history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. *Food and Chemical Toxicology, 46*(7, Supplement), S1-S10. doi:https://doi.org/10.1016/j.fct.2008.05.003

Carels, R. A., Harper, J., & Konrad, K. (2006). Qualitative perceptions and caloric estimations of healthy and unhealthy foods by behavioral weight loss participants. *Appetite, 46*(2), 199-206.

Carvalho, P., Sousa, M., Barros, R., Padrao, P., Moreira, P., & Teixeira, V. (2013). impact Of Morning Ingestion Of Sugary And Sweetened Beverages On Energy And Fluid Intake Throughout Day: po2450. *Annals of Nutrition and Metabolism, 63*, 1448.

Catenacci, V. A., Pan, Z., Thomas, J. G., Ogden, L. G., Roberts, S. A., Wyatt, H. R., . . . Hill, J. O. (2014). Low/No calorie sweetened beverage consumption in the National Weight Control Registry. *Obesity, 22*(10), 2244-2251. doi:10.1002/oby.20834

Chambers, L., Ells, H., & Yeomans, M. R. (2013). Can the satiating power of a high energy beverage be improved by manipulating sensory characteristics and label information? *Food Quality and Preference, 28*(1), 271-278. doi:https://doi.org/10.1016/j.foodqual.2012.08.008

Clouard, C., Chataignier, M., Meunier-Salaün, M.-C., & Val-Laillet, D. (2012). Flavour preference acquired via a beverage-induced conditioning and its transposition to solid food: Sucrose but not maltodextrin or saccharin induced significant flavour preferences in pigs. *Applied Animal Behaviour Science, 136*(1), 26-36. doi:https://doi.org/10.1016/j.applanim.2011.11.007

Colditz, G. A., Willett, W. C., Stampfer, M. J., London, S. J., Segal, M. R., & Speizer, F. E. (1990). Patterns of weight change and their relation to diet in a cohort of healthy women. *The American Journal of Clinical Nutrition, 51*(6), 1100-1105.

Committee, D. G. A. (2015). *Scientific Report of the 2015 Dietary Guidelines Advisory Committee*.

Curi, R., Alvarez, M., Bazotte, R. B., Botion, L. M., Godoy, J. L., & Bracht, A. (1986). Effect of Stevia rebaudiana on glucose tolerance in normal adult humans. *Braz J Med Biol Res, 19*(6), 771-774.

Dalla Man, C., Campioni, M., Polonsky, K. S., Basu, R., Rizza, R. A., Toffolo, G., & Cobelli, C. (2005). Two-hour seven-sample oral glucose tolerance test and meal protocol: minimal model assessment of beta-cell responsivity and insulin sensitivity in nondiabetic individuals. *Diabetes, 54*(11), 3265-3273.

Daly, K., Darby, A. C., & Shirazi-Beechey, S. P. (2016). Low calorie sweeteners and gut microbiota. *Physiology & Behavior, 164*, 494-500. doi:https://doi.org/10.1016/j.physbeh.2016.03.014

Davidson, T. L., Sample, C. H., & Swithers, S. E. (2014). An application of Pavlovian principles to the problems of obesity and cognitive decline. *Neurobiol Learn Mem, 108*, 172-184. doi:10.1016/j.nlm.2013.07.014

Davidson, T. L., & Swithers, S. E. (2004). A Pavlovian approach to the problem of obesity. *Int J Obes Relat Metab Disord, 28*(7), 933-935. doi:10.1038/sj.ijo.0802660

de Araujo, I. E. (2016). Circuit organization of sugar reinforcement. *Physiol Behav, 164*(Pt B), 473-477. doi:10.1016/j.physbeh.2016.04.041

de Araujo, I. E., Oliveira-Maia, A. J., Sotnikova, T. D., Gainetdinov, R. R., Caron, M. G., Nicolelis, M. A., & Simon, S. A. (2008). Food reward in the absence of taste receptor signaling. *Neuron, 57*(6), 930-941. doi:10.1016/j.neuron.2008.01.032

de Graaf, C., Schreurs, A., & Blauw, Y. H. (1993). Short-term effects of different amounts of sweet and nonsweet carbohydrates on satiety and energy intake. *Physiology & Behavior, 54*(5), 833-843. doi:https://doi.org/10.1016/0031-9384(93)90290-V

de la Hunty A, G. S., Ashwell M. (2006). A review of the effectiveness of aspartame in helping with weight control. *Nutr Bull, 31*, 115-128.

de Ruyter, J. C., Katan, M. B., Kuijper, L. D. J., Liem, D. G., & Olthof, M. R. (2013). The Effect of Sugar-Free Versus Sugar-Sweetened Beverages on Satiety, Liking and Wanting: An 18 Month Randomized Double-Blind Trial in Children. *PLoS ONE, 8*(10), e78039. doi:10.1371/journal.pone.0078039

DellaValle, D. M., Roe, L. S., & Rolls, B. J. (2005). Does the consumption of caloric and non-caloric beverages with a meal affect energy intake? *Appetite, 44*(2), 187-193. doi:https://doi.org/10.1016/j.appet.2004.11.003

Dhillon, J., Lee, J. Y., & Mattes, R. D. (2017). The cephalic phase insulin response to nutritive and low-calorie sweeteners in solid and beverage form. *Physiol Behav, 181*, 100-109. doi:10.1016/j.physbeh.2017.09.009

Diana, O., Ryan, P., Justin, G., M., R. A., Claudia, H., Brian, M., . . . J., K. M. (2018). CD Obesity-Prone Rats, but not Obesity-Resistant Rats, Robustly Ferment Resistant Starch Without Increased Weight or Fat Accretion. *Obesity, 26*(3), 570-577. doi:doi:10.1002/oby.22120

DiMeglio, D. P., & Mattes, R. D. (2000). Liquid versus solid carbohydrate: effects on food intake and body weight. *Int J Obes Relat Metab Disord, 24*(6), 794-800.

Drewnowski, A., Massien, C., Louis-Sylvestre, J., Fricker, J., Chapelot, D., & Apfelbaum, M. (1994). Comparing the effects of aspartame and sucrose on motivational ratings, taste preferences, and energy intakes in humans. *The American Journal of Clinical Nutrition, 59*(2), 338-345. doi:10.1093/ajcn/59.2.338

Drewnowski, A., & Rehm, C. D. (2014). Consumption of low-calorie sweeteners among U.S. adults is associated with higher Healthy Eating Index (HEI 2005) scores and more physical activity. *Nutrients, 6*(10), 4389-4403. doi:10.3390/nu6104389

Drewnowski, A., & Rehm, C. D. (2016). The use of low-calorie sweeteners is associated with self-reported prior intent to lose weight in a representative sample of US adults. *Nutrition & Diabetes, 6*(3), e202. doi:10.1038/nutd.2016.9

DuBois, G. E., & Prakash, I. (2012). Non-caloric sweeteners, sweetness modulators, and sweetener enhancers. *Annu Rev Food Sci Technol, 3*, 353-380. doi:10.1146/annurev-food-022811-101236

Dunford, E., Taillie, L., Miles, D., Eyles, H., Tolentino-Mayo, L., & Ng, S. (2018). Non-Nutritive Sweeteners in the Packaged Food Supply—An Assessment across 4 Countries. *Nutrients, 10*(2), 257.

Duskova, M., Macourek, M., Sramkova, M., Hill, M., & Starka, L. (2013). The role of taste in cephalic phase of insulin secretion. *Prague Med Rep, 114*(4), 222-230. doi:10.14712/23362936.2014.11

D'Anci, K. E. (1999). Tolerance to Morphine-Induced Antinociception Is Decreased by Chronic Sucrose or Polycose Intake. *Pharmacology Biochemistry and Behavior, 63*(1), 1-11. doi:https://doi.org/10.1016/S0091-3057(98)00254-8

Egan, J. M., & Margolskee, R. F. (2008). Taste cells of the gut and gastrointestinal chemosensation. *Mol Interv, 8*(2), 78-81. doi:10.1124/mi.8.2.5

Engel, S., Tholstrup, T., Bruun, J. M., Astrup, A., Richelsen, B., & Raben, A. (2017). Effect of high milk and sugar-sweetened and non-caloric soft drink intake on insulin sensitivity after 6 months in overweight and obese adults: a randomized controlled trial. *European journal of clinical nutrition*, 1.

FDA. (2018). Additional information about high-intensity sweeteners permitted for use in food in the United States. Retrieved from https://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm397725.htm

Fedewa, M. V., Das, B. M., Evans, E. M., & Dishman, R. K. (2014). Change in Weight and Adiposity in College Students: A Systematic Review and Meta-Analysis. *American Journal of Preventive Medicine, 47*(5), 641-652. doi:https://doi.org/10.1016/j.amepre.2014.07.035

Feijó, F. d. M., Ballard, C. R., Foletto, K. C., Batista, B. A. M., Neves, A. M., Ribeiro, M. F. M., & Bertoluci, M. C. (2013). Saccharin and aspartame, compared with sucrose, induce greater weight gain in adult Wistar rats, at similar total caloric intake levels. *Appetite, 60*, 203-207. doi:https://doi.org/10.1016/j.appet.2012.10.009

Fernstrom, J. D. (2015). Non-nutritive sweeteners and obesity. *Annu Rev Food Sci Technol, 6*, 119-136. doi:10.1146/annurev-food-022814-015635

Fitch, C., Keim, K. S., Academy of, N., & Dietetics. (2012). Position of the Academy of Nutrition and Dietetics: use of nutritive and nonnutritive sweeteners. *J Acad Nutr Diet, 112*(5), 739-758. doi:10.1016/j.jand.2012.03.009

Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord, 24*(1), 38-48.

Flint, H. J., Scott, K. P., Louis, P., & Duncan, S. H. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology and Hepatology, 9*(10), 577.

Ford, H. E., Peters, V., Martin, N. M., Sleeth, M. L., Ghatei, M. A., Frost, G. S., & Bloom, S. R. (2011). Effects of oral ingestion of sucralose on gut hormone response and appetite in healthy normal-weight subjects. *Eur J Clin Nutr, 65*(4), 508-513.

Fowler, S. P. (2016). Low-calorie sweetener use and energy balance: Results from experimental studies in animals, and large-scale prospective studies in humans. *Physiol Behav, 164*(Pt B), 517-523. doi:10.1016/j.physbeh.2016.04.047

Fowler, S. P., Williams, K., Resendez, R. G., Hunt, K. J., Hazuda, H. P., & Stern, M. P. (2008). Fueling the Obesity Epidemic? Artificially Sweetened Beverage Use and Long-term Weight Gain. *Obesity, 16*(8), 1894-1900. doi:10.1038/oby.2008.284

Frank, G. K. W., Oberndorfer, T. A., Simmons, A. N., Paulus, M. P., Fudge, J. L., Yang, T. T., & Kaye, W. H. (2008). Sucrose activates human taste pathways differently from artificial sweetener. *NeuroImage, 39*(4), 1559-1569. doi:https://doi.org/10.1016/j.neuroimage.2007.10.061

Fujita, Y., Wideman, R. D., Speck, M., Asadi, A., King, D. S., Webber, T. D., . . . Kieffer, T. J. (2009). Incretin release from gut is acutely enhanced by sugar but not by sweeteners in vivo. *American Journal of Physiology - Endocrinology And Metabolism, 296*(3), E473.

Gadah, N. S., Brunstrom, J. M., & Rogers, P. J. (2016). Cross-over studies underestimate energy compensation: The example of sucrose-versus sucralose-containing drinks. *Appetite, 107*, 398-405. doi:https://doi.org/10.1016/j.appet.2016.08.113

Gardner, C., Wylie-Rosett, J., Gidding, S. S., Steffen, L. M., Johnson, R. K., Reader, D., . . . the American, D. (2012). Nonnutritive sweeteners: current use and health perspectives: a scientific statement from the American Heart Association and the American Diabetes Association. *Circulation, 126*(4), 509-519. doi:10.1161/CIR.0b013e31825c42ee

Garner, D. M., Olmsted, M. P., Bohr, Y., & Garfinkel, P. E. (1982). The Eating Attitudes Test: psychometric features and clinical correlates. *Psychol Med, 12*(04), 871-878. doi:doi:10.1017/S0033291700049163

Glendinning, J. I., Stano, S., Holter, M., Azenkot, T., Goldman, O., Margolskee, R. F., . . . Sclafani, A. (2015). Sugar-induced cephalic-phase insulin release is mediated by a T1r2+T1r3-independent taste transduction pathway in mice. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 309*(5), R552-R560. doi:10.1152/ajpregu.00056.2015

Gougeon R, S. M., Lee K, Field CJ. (2004). Canadian diabetes association national nutrition committee technical review: non-nutritive intense sweeteners in diabetes management. *Canadian J Diabetes, 28*(4), 385-399.

Green, E., & Murphy, C. (2012). Altered processing of sweet taste in the brain of diet soda drinkers. *Physiology & Behavior, 107*(4), 560-567. doi:https://doi.org/10.1016/j.physbeh.2012.05.006

Gregersen, S., Jeppesen, P. B., Holst, J. J., & Hermansen, K. (2004). Antihyperglycemic effects of stevioside in type 2 diabetic subjects. *Metabolism, 53*(1), 73-76.

Griffioen-Roose, S., Smeets, P. A., Weijzen, P. L., van Rijn, I., van den Bosch, I., & de Graaf, C. (2013). Effect of replacing sugar with non-caloric sweeteners in beverages on the reward value after repeated exposure. *PLoS One, 8*(11), e81924. doi:10.1371/journal.pone.0081924

Grotz, V. L., Pi-Sunyer, X., Porte Jr, D., Roberts, A., & Richard Trout, J. (2017). A 12-week randomized clinical trial investigating the potential for sucralose to affect glucose homeostasis. *Regulatory Toxicology and Pharmacology, 88*, 22-33. doi:https://doi.org/10.1016/j.yrtph.2017.05.011

Hall, K. D., Sacks, G., Chandramohan, D., Chow, C. C., Wang, Y. C., Gortmaker, S. L., & Swinburn, B. A. (2011). Quantification of the effect of energy imbalance on bodyweight. *Lancet, 378*(9793), 10.1016/S0140-6736(1011)60812-X. doi:10.1016/S0140-6736(11)60812-X

Hall, W. L., Millward, D. J., Rogers, P. J., & Morgan, L. M. (2003). Physiological mechanisms mediating aspartame-induced satiety. *Physiology & Behavior, 78*(4–5), 557-562. doi:http://dx.doi.org/10.1016/S0031-9384(03)00034-9

Han, E., & Powell, L. M. (2013). Consumption Patterns of Sugar-Sweetened Beverages in the United States. *Journal of the Academy of Nutrition and Dietetics, 113*(1), 43-53. doi:https://doi.org/10.1016/j.jand.2012.09.016

Hartel B, G. H. J., Schneider B. (1993). The influenece of sweetener solutions on the secretion of insulin and the blood glucose level. *Ernahrungsumschau, 40*(4), 152-155.

Hedrick, V. E., Comber, D. L., Estabrooks, P. A., Savla, J., & Davy, B. M. (2010). The beverage intake questionnaire: determining initial validity and reliability. *J Am Diet Assoc, 110*(8), 1227-1232. doi:10.1016/j.jada.2010.05.005

Hedrick, V. E., Savla, J., Comber, D. L., Flack, K. D., Estabrooks, P. A., Nsiah-Kumi, P. A., Davy, B. M. (2012). Development of a brief questionnaire to assess habitual beverage intake (BEVQ-15): sugar-sweetened beverages and total beverage energy intake. *J Acad Nutr Diet, 112*(6), 840-849. doi:10.1016/j.jand.2012.01.023

Helgren, F. J., Lynch, M. J., & Kirchmeyer, F. J. (1955). A taste panel study of the saccharin "off-taste". *Journal of the American Pharmaceutical Association, 44*(6), 353-355. doi:doi:10.1002/jps.3030440613

Hellekant, G., & Danilova, V. (1996). Species differences toward sweeteners. *Food Chemistry, 56*(3), 323-328. doi:https://doi.org/10.1016/0308-8146(96)00033-7

Hellerstein, M. K., Schwarz, J.-M., & Neese, R. A. (1996). Regulation of hepatic de novo lipogenesis in humans. *Annual review of nutrition, 16*(1), 523-557.

Hetherington, M. M., Wood, C., & Lyburn, S. C. (2000). Response to Energy Dilution in the Short Term: Evidence of Nutritional Wisdom in Young Children? *Nutritional Neuroscience, 3*(5), 321-329. doi:10.1080/1028415X.2000.11747329

Higgins, K. A., Considine, R. V., & Mattes, R. D. (2018). Aspartame Consumption for 12 Weeks Does Not Affect Glycemia, Appetite, or Body Weight of Healthy, Lean Adults in a Randomized Controlled Trial. *The Journal of Nutrition, 148*(4), 650-657. doi:10.1093/jn/nxy021

Hill, R. J., & Davies, P. S. W. (2001). The validity of self-reported energy intake as determined using the doubly labelled water technique. *British Journal of Nutrition, 85*(4), 415-430. doi:10.1079/BJN2000281

Hill, S. E., Prokosch, M. L., Morin, A., & Rodeheffer, C. D. (2014). The effect of non-caloric sweeteners on cognition, choice, and post-consumption satisfaction. *Appetite, 83*, 82-88. doi:10.1016/j.appet.2014.08.003

Holt, N. S. J. C. B.-M. S. H. A. (2000). The effects of sugar-free vs sugar-rich beverages on feelings of fullness and subsequent food intake. *International Journal of Food Sciences and Nutrition, 51*(1), 59-71. doi:10.1080/096374800100912

Horwitz, D. L., McLane, M., & Kobe, P. (1988). Response to Single Dose of Aspartame or Saccharin by NIDDM Patients. *Diabetes Care, 11*(3), 230-234. doi:10.2337/diacare.11.3.230

Hsieh, M. H., Chan, P., Sue, Y. M., Liu, J. C., Liang, T. H., Huang, T. Y., Chen, Y. J. (2003). Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: a two-year, randomized, placebo-controlled study. *Clin Ther, 25*(11), 2797-2808.

Hunt, J. N., & Stubbs, D. F. (1975). The volume and energy content of meals as determinants of gastric emptying. *The Journal of Physiology, 245*(1), 209-225.

Jang, H.J., Kokrashvili, Z., Theodorakis, M. J., Carlson, O. D., Kim, B.-J., Zhou, J., Egan, J. M. (2007). Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proceedings of the National Academy of Sciences, 104*(38), 15069-15074. doi:10.1073/pnas.0706890104

Jang, H. J., Kokrashvili, Z., Theodorakis, M. J., Carlson, O. D., Kim, B. J., Zhou, J., Juhaszova, M. (2007). Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc Natl Acad Sci U S A, 104*. doi:10.1073/pnas.0706890104

Jeppesen, P. B., Gregersen, S., Poulsen, C. R., & Hermansen, K. (2000). Stevioside acts directly on pancreatic β cells to secrete insulin: Actions independent of cyclic adenosine monophosphate and adenosine triphosphate—sensitivie K+-channel activity. *Metabolism, 49*(2), 208-214. doi:https://doi.org/10.1016/S0026-0495(00)91325-8

Just, T., Pau, H. W., Engel, U., & Hummel, T. (2008). Cephalic phase insulin release in healthy humans after taste stimulation? *Appetite, 51*(3), 622-627. doi:10.1016/j.appet.2008.04.271

Kanders, B. S., Lavin, P. T., Kowalchuk, M. B., Greenberg, I., & Blackburn, G. L. (1988). An evaluation of the effect of aspartame on weight loss. *Appetite, 11, Supplement 1*, 73-84. doi:http://dx.doi.org/10.1016/S0195-6663(88)80050-3

Kant, A. K., Graubard, B. I., & Atchison, E. A. (2009). Intakes of plain water, moisture in foods and beverages, and total water in the adult US population--nutritional, meal pattern, and body weight correlates: National Health and Nutrition Examination Surveys 1999-2006. *Am J Clin Nutr, 90*(3), 655-663. doi:10.3945/ajcn.2009.27749

Kare, M. R., Pond, W. C., & Campbell, J. (1965). Observations on the taste reactions in pigs. *Animal Behaviour, 13*(2), 265-269. doi:https://doi.org/10.1016/0003-3472(65)90045-X

Kim, S.-K., Chen, Y., Abrol, R., Goddard, W. A., & Guthrie, B. (2017). Activation mechanism of the G protein-coupled sweet receptor heterodimer with sweeteners and allosteric agonists. *Proceedings of the National Academy of Sciences, 114*(10), 2568-2573. doi:10.1073/pnas.1700001114

Kootte, R. S., Levin, E., Salojärvi, J., Smits, L. P., Hartstra, A. V., Udayappan, S. D., Nieuwdorp, M. (2017). Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition. *Cell Metabolism, 26*(4), 611-619.e616. doi:https://doi.org/10.1016/j.cmet.2017.09.008

Kuhn, C., Bufe, B., Winnig, M., Hofmann, T., Frank, O., Behrens, M., . . . Meyerhof, W. (2004). Bitter Taste Receptors for Saccharin and Acesulfame K. *The Journal of Neuroscience, 24*(45), 10260-10265. doi:10.1523/jneurosci.1225-04.2004

Kuhn, M. E. (2017). Hitting the Health and Wellness Target. *Food Technology, 71*(2), 38-49.

Laska, M. N., Murray, D. M., Lytle, L. A., & Harnack, L. J. (2012). Longitudinal associations between key dietary behaviors and weight gain over time: Transitions through the adolescent years. *Obesity (Silver Spring, Md.), 20*(1), 118-125. doi:10.1038/oby.2011.179

Lavin, J., French, S., & Read, N. (1997). The effect of sucrose-and aspartame-sweetened drinks on energy intake, hunger and food choice of female, moderately restrained eaters. *International journal of obesity, 21*(1), 37.

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Human gut microbes associated with obesity. *Nature, 444*, 1022. doi:10.1038/4441022a https://www.nature.com/articles/4441022a#supplementary-information

Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M., & Adler, E. (2002). Human receptors for sweet and umami taste. *Proc Natl Acad Sci U S A, 99*(7), 4692-4696. doi:10.1073/pnas.072090199

Liang, Y., Steinbach, G., Maier, V., & Pfeiffer, E. F. (1987). The effect of artificial sweetener on insulin secretion. 1. The effect of acesulfame K on insulin secretion in the rat (studies in vivo). *Horm Metab Res, 19*(6), 233-238. doi:10.1055/s-2007-1011788

Liem, D. G., & de Graaf, C. (2004). Sweet and sour preferences in young children and adults: role of repeated exposure. *Physiol Behav, 83*(3), 421-429. doi:10.1016/j.physbeh.2004.08.028

Little, T. J., Gopinath, A., Patel, E., Mcglone, A., Lassman, D. J., D'amato, M., . . . Thompson, D. G. (2010). Gastric emptying of hexose sugars: role of osmolality, molecular structure and the CCK1 receptor. *Neurogastroenterology & Motility, 22*(11), 1183-e1314. doi:doi:10.1111/j.1365-2982.2010.01552.x

Little, T. J., Gupta, N., Case, R. M., Thompson, D. G., & McLaughlin, J. T. (2009). Sweetness and bitterness taste of meals per se does not mediate gastric emptying in humans. *Am J Physiol Regul Integr Comp Physiol, 297*(3), R632-639. doi:10.1152/ajpregu.00090.2009

Logue, C., Dowey, L. R. C., Strain, J. J., Verhagen, H., McClean, S., & Gallagher, A. M. (2017). Application of Liquid Chromatography–Tandem Mass Spectrometry To Determine Urinary Concentrations of Five Commonly Used Low-Calorie Sweeteners: A Novel Biomarker Approach for Assessing Recent Intakes? *Journal of Agricultural and Food Chemistry, 65*(22), 4516-4525. doi:10.1021/acs.jafc.7b00404

Ludwig, D. S., Majzoub, J. A., Al-Zahrani, A., Dallal, G. E., Blanco, I., & Roberts, S. B. (1999). High Glycemic Index Foods, Overeating, and Obesity. *Pediatrics, 103*(3), e26-e26. doi:10.1542/peds.103.3.e26

Ma, J., Bellon, M., Wishart, J. M., Young, R., Blackshaw, L. A., Jones, K. L., Rayner, C. K. (2009). Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects. *Am J Physiol Gastrointest Liver Physiol, 296*(4), G735-739. doi:10.1152/ajpgi.90708.2008

Ma, J., Chang, J., Checklin, H. L., Young, R. L., Jones, K. L., Horowitz, M., & Rayner, C. K. (2010). Effect of the artificial sweetener, sucralose, on small intestinal glucose absorption in healthy human subjects. *Br J Nutr, 104*(6), 803-806. doi:10.1017/S0007114510001327

Mace, O. J., Affleck, J., Patel, N., & Kellett, G. L. (2007). Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. *J Physiol, 582*(Pt 1), 379-392. doi:10.1113/jphysiol.2007.130906

Macintyre, A. K., Marryat, L., & Chambers, S. (2018). Exposure to liquid sweetness in early childhood: artificially-sweetened and sugar-sweetened beverage consumption at 4–5 years and risk of overweight and obesity at 7–8 years. *Pediatric Obesity, 0*(0). doi:10.1111/ijpo.12284

Maersk, M., Belza, A., Stodkilde-Jorgensen, H., Ringgaard, S., Chabanova, E., Thomsen, H., . . . Richelsen, B. (2012). Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: a 6-mo randomized intervention study. *Am J Clin Nutr, 95*(2), 283-289. doi:10.3945/ajcn.111.022533

Magnuson BA, Burdock GA, Doull J, Kroes RM, Marsh GM, Pariza MW, Spencer PS, Waddell WJ, Walker R, Williams GM. (2007). Aspartame: a safety evaluation based on current use levels, regulations, and toxicological and epidemiological studies. *Crit Rev Toxicol*, 37, 629-727.

Magnuson, B. A., Carakostas, M. C., Moore, N. H., Poulos, S. P., & Renwick, A. G. (2016). Biological fate of low-calorie sweeteners. *Nutrition Reviews, 74*(11), 670-689. doi:10.1093/nutrit/nuw032

Maki, K. C., Curry, L. L., Carakostas, M. C., Tarka, S. M., Reeves, M. S., Farmer, M. V., Bisognano, J. D. (2008). The hemodynamic effects of rebaudioside A in healthy adults with normal and low-normal blood pressure. *Food and Chemical Toxicology, 46*(7, Supplement), S40-S46. doi:https://doi.org/10.1016/j.fct.2008.04.040

Maki, K. C., Curry, L. L., Reeves, M. S., Toth, P. D., McKenney, J. M., Farmer, M. V., Tarka, S. M. (2008). Chronic consumption of rebaudioside A, a steviol glycoside, in men and women with type 2 diabetes mellitus. *Food and Chemical Toxicology, 46*(7, Supplement), S47-S53. doi:https://doi.org/10.1016/j.fct.2008.05.007

Malhotra, R., Ostbye, T., Riley, C. M., & Finkelstein, E. A. (2013). Young adult weight trajectories through midlife by body mass category. *Obesity (Silver Spring), 21*(9), 1923-1934. doi:10.1002/oby.20318

Malik, V. S., Popkin, B. M., Bray, G. A., Després, J.-P., Willett, W. C., & Hu, F. B. (2010). Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes care, 33*(11), 2477-2483. doi:10.2337/dc10-1079

Margolskee, R. F., Dyer, J., Kokrashvili, Z., Salmon, K. S. H., Ilegems, E., Daly, K., Shirazi-Beechey, S. P. (2007). T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. *Proceedings of the National Academy of Sciences, 104*(38), 15075-15080. doi:10.1073/pnas.0706678104

Mars, M., Stafleu, A., & de Graaf, C. (2012). Use of satiety peptides in assessing the satiating capacity of foods. *Physiology & Behavior, 105*(2), 483-488. doi:http://dx.doi.org/10.1016/j.physbeh.2011.08.033

Masuda, K., Koizumi, A., Nakajima, K.-i., Tanaka, T., Abe, K., Misaka, T., & Ishiguro, M. (2012). Characterization of the Modes of Binding between Human Sweet Taste Receptor and Low-Molecular-Weight Sweet Compounds. *PLoS ONE, 7*(4), e35380. doi:10.1371/journal.pone.0035380

Mattes, R. (1990). Effects of aspartame and sucrose on hunger and energy intake in humans. *Physiol Behav, 47*(6), 1037-1044.

Mattes, R., Shikany, J., Kaiser, K., & Allison, D. (2011). Nutritively sweetened beverage consumption and body weight: a systematic review and meta- analysis of randomized experiments.(Report). *Obesity Reviews, 12*(5), 346.

Mattes, R. D., & Mela, D. J. (1986). Relationships between and among selected measures of sweet-taste preference and dietary intake. *Chemical Senses, 11*(4), 523-539. doi:10.1093/chemse/11.4.523

Mattes, R. D., & Popkin, B. M. (2009). Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. *The American Journal of Clinical Nutrition, 89*(1), 1-14. doi:10.3945/ajcn.2008.26792

Mattes, R. D., Shikany, J. M., Kaiser, K. A., & Allison, D. B. (2011). Nutritively sweetened beverage consumption and body weight: a systematic review and meta- analysis of randomized experiments.(Report). *Obesity Reviews, 12*(5), 346.

McArdle, W. D. (2007). *Exercise physiology : energy, nutrition, and human performance* (6th ed.. ed.). Philadelphia: Philadelphia : Lippincott Williams & Wilkins.

McCutcheon, J. E., Beeler, J. A., & Roitman, M. F. (2012). Sucrose-predictive cues evoke greater phasic dopamine release than saccharin-predictive cues. *Synapse, 66*(4), 346-351. doi:10.1002/syn.21519

Mezitis, N. H., Maggio, C. A., Koch, P., Quddoos, A., Allison, D. B., & Pi-Sunyer, F. X. (1996). Glycemic effect of a single high oral dose of the novel sweetener sucralose in patients with diabetes. *Diabetes Care, 19*(9), 1004-1005.

Miller, P. E., & Perez, V. (2014). Low-calorie sweeteners and body weight and composition: a meta-analysis of randomized controlled trials and prospective cohort studies. *Am J Clin Nutr, 100*(3), 765-777. doi:10.3945/ajcn.113.082826

Monsivais, P., Perrigue, M. M., & Drewnowski, A. (2007). Sugars and satiety: does the type of sweetener make a difference? *The American Journal of Clinical Nutrition, 86*(1), 116-123. doi:10.1093/ajcn/86.1.116

Morricone, L., Bombonato, M., Cattaneo, A. G., Enrini, R., Lugari, R., Zandomenighi, R., & Caviezel, F. (2000). Food-related sensory stimuli are able to promote pancreatic polypeptide elevation without evident cephalic phase insulin secretion in human obesity. *Horm Metab Res, 32*(6), 240-245. doi:10.1055/s-2007-978628

Mourao, D. M., Bressan, J., Campbell, W. W., & Mattes, R. D. (2007). Effects of food form on appetite and energy intake in lean and obese young adults. *Int J Obes, 31*(11), 1688-1695.

Murakami K, Sasaki S, Takahashi Y, Uenishi K, Watanabe T, Kohri T,Yamasaki M, Watanabe R, Baba K, Shibata K, et al. (2008). Sensitivity and specificity of published strategies using urinary creatinine to identify incomplete 24-h urine collection. *Nutrition*, 24, 16–22.

Murray, S., Tulloch, A., Criscitelli, K., & Avena, N. M. (2016). Recent studies of the effects of sugars on brain systems involved in energy balance and reward: Relevance to low calorie sweeteners. *Physiology & Behavior, 164*, 504-508. doi:https://doi.org/10.1016/j.physbeh.2016.04.004

Nakagawa, Y., Nagasawa, M., Mogami, H., Lohse, M., Ninomiya, Y., & Kojima, I. (2013). Multimodal function of the sweet taste receptor expressed in pancreatic β-cells: generation of diverse patterns of intracellular signals by sweet agonists. *Endocrine Journal, 60*(10), 1191-1206. doi:10.1507/endocrj.EJ13-0282

Nakagawa, Y., Nagasawa, M., Yamada, S., Hara, A., Mogami, H., Nikolaev, V. O., . . . Kojima, I. (2009). Sweet Taste Receptor Expressed in Pancreatic β-Cells Activates the Calcium and Cyclic AMP Signaling Systems and Stimulates Insulin Secretion. *PLOS ONE, 4*(4), e5106. doi:10.1371/journal.pone.0005106

Nettleton, J. E., Reimer, R. A., & Shearer, J. (2016). Reshaping the gut microbiota: Impact of low calorie sweeteners and the link to insulin resistance? *Physiology & behavior, 164*, 488-493.

Ng, S. W., Slining, M. M., & Popkin, B. M. (2012). Use of caloric and noncaloric sweeteners in US consumer packaged foods, 2005-2009. *J Acad Nutr Diet, 112*(11), 1828-1834.e1821-1826. doi:10.1016/j.jand.2012.07.009

Nicolaïdis, S. (1969). Early systemic responses to orogastric stimulation in the regulation of food and water balance: functional and electrophysiological data. *Annals of the New York Academy of Sciences, 157*(2), 1176-1203. doi:10.1111/j.1749-6632.1969.tb12942.x

Nie, Y., Vigues, S., Hobbs, J. R., Conn, G. L., & Munger, S. D. (2005). Distinct contributions of T1R2 and T1R3 taste receptor subunits to the detection of sweet stimuli. *Curr Biol, 15*(21), 1948-1952. doi:10.1016/j.cub.2005.09.037

O'Donnell, K., & Kearsley, M. (2012). *Sweeteners and sugar alternatives in food technology*: John Wiley & Sons.

Olszewski, P. K., & Levine, A. S. (2007). Central opioids and consumption of sweet tastants: When reward outweighs homeostasis. *Physiology & Behavior, 91*(5), 506-512. doi:https://doi.org/10.1016/j.physbeh.2007.01.011

Ostlund, S. B., Kosheleff, A., Maidment, N. T., & Murphy, N. P. (2013). Decreased consumption of sweet fluids in mu opioid receptor knockout mice: a microstructural analysis of licking behavior. *Psychopharmacology, 229*(1), 105-113. doi:10.1007/s00213-013-3077-x

Palmnas, M. S., Cowan, T. E., Bomhof, M. R., Su, J., Reimer, R. A., Vogel, H. J., . . . Shearer, J. (2014). Low-dose aspartame consumption differentially affects gut microbiota-host metabolic interactions in the diet-induced obese rat. *PLoS One, 9*. doi:10.1371/journal.pone.0109841

Pan, A., Malik, V. S., Hao, T., Willett, W. C., Mozaffarian, D., & Hu, F. B. (2013). Changes in water and beverage intake and long-term weight changes: results from three prospective cohort studies. *International journal of obesity (2005), 37*(10), 1378-1385. doi:10.1038/ijo.2012.225

Panahi, S., El Khoury, D., Luhovyy, B. L., Goff, H. D., & Anderson, G. H. (2013). Caloric beverages consumed freely at meal-time add calories to an ad libitum meal. *Appetite, 65*, 75-82. doi:https://doi.org/10.1016/j.appet.2013.01.023

Parker, D. R., Gonzalez, S., Derby, C. A., Gans, K. M., Lasater, T. M., & Carleton, R. A. (1997). Dietary factors in relation to weight change among men and women from two southeastern New England communities. *Int J Obes Relat Metab Disord, 21*(2), 103-109.

Peciña, S., & Smith, K. S. (2010). Hedonic and motivational roles of opioids in food reward: Implications for overeating disorders. *Pharmacology Biochemistry and Behavior, 97*(1), 34-46. doi:https://doi.org/10.1016/j.pbb.2010.05.016

Pepino, M. Y., & Bourne, C. (2011). Non-nutritive sweeteners, energy balance, and glucose homeostasis. *Curr Opin Clin Nutr Metab Care, 14*(4), 391-395. doi:10.1097/MCO.0b013e3283468e7e

Pepino, M. Y., Tiemann, C. D., Patterson, B. W., Wice, B. M., & Klein, S. (2013). Sucralose affects glycemic and hormonal responses to an oral glucose load. *Diabetes Care, 36*. doi:10.2337/dc12-2221

Peters, J. C., Beck, J., Cardel, M., Wyatt, H. R., Foster, G. D., Pan, Z., Hill, J. O. (2016). The effects of water and non-nutritive sweetened beverages on weight loss and weight maintenance: A randomized clinical trial. *Obesity, 24*(2), 297-304. doi:10.1002/oby.21327

Peters, J. C., Wyatt, H. R., Foster, G. D., Pan, Z., Wojtanowski, A. C., Vander Veur, S. S., Hill, J. O. (2014). The effects of water and non-nutritive sweetened beverages on weight loss during a 12-week weight loss treatment program. *Obesity (Silver Spring), 22*(6), 1415-1421. doi:10.1002/oby.20737

Phillips, R. J., & Powley, T. L. (1996). Gastric volume rather than nutrient content inhibits food intake. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 271*(3), R766-R769. doi:10.1152/ajpregu.1996.271.3.R766

Piernas, C., Mendez, M. A., Ng, S. W., Gordon-Larsen, P., & Popkin, B. M. (2014). Low-calorie- and calorie-sweetened beverages: diet quality, food intake, and purchase patterns of US household consumers. *The American Journal of Clinical Nutrition*. doi:10.3945/ajcn.113.072132

Piernas, C., Ng, S. W., & Popkin, B. (2013). Trends in purchases and intake of foods and beverages containing caloric and low-calorie sweeteners over the last decade in the United States. *Pediatric Obesity, 8*(4), 294-306. doi:10.1111/j.2047-6310.2013.00153.x

Power, M. L., & Schulkin, J. (2008). Anticipatory physiological regulation in feeding biology: cephalic phase responses. *Appetite, 50*(2-3), 194-206. doi:10.1016/j.appet.2007.10.006

Powley, T., & Berthoud, H.-R. (1985). Diet and cephalic phase insulin responses. *The American Journal of Clinical Nutrition, 42*(5), 991-1002.

Powley, T. L., & Phillips, R. J. (2004). Gastric satiation is volumetric, intestinal satiation is nutritive. *Physiology & Behavior, 82*(1), 69-74. doi:https://doi.org/10.1016/j.physbeh.2004.04.037

Raben, A., Vasilaras, T. H., Moller, A. C., & Astrup, A. (2002). Sucrose compared with artificial sweeteners: different effects on ad libitum food intake and body weight after 10 wk of supplementation in overweight subjects. *Am J Clin Nutr, 76*(4), 721-729.

Ramirez, I. (1990). Stimulation of Energy Intake and Growth by Saccharin in Rats. *The Journal of Nutrition, 120*(1), 123-133. doi:10.1093/jn/120.1.123

Reed, D. R., & McDaniel, A. H. (2006). The Human Sweet Tooth. *BMC Oral Health, 6*(Suppl 1), S17-S17. doi:10.1186/1472-6831-6-S1-S17

Reid, A. E., Chauhan, B. F., Rabbani, R., Lys, J., Copstein, L., Mann, A., Azad, M. B. (2016). Early Exposure to Nonnutritive Sweeteners and Long-term Metabolic Health: A Systematic Review. *Pediatrics, 137*(3), e20153603. doi:10.1542/peds.2015-3603

Reid, M., Hammersley, R., Hill, A. J., & Skidmore, P. (2007). Long-term dietary compensation for added sugar: effects of supplementary sucrose drinks over a 4-week period. *Br J Nutr, 97*(1), 193-203. doi:10.1017/s0007114507252705

Renwick AG. (1994). Intense sweeteners, food intake, and the weight of a body of evidence. *Physiol Behav*, 55,139-143.

Renwick, A. G. (2008). The use of a sweetener substitution method to predict dietary exposures for the intense sweetener rebaudioside A. *Food and Chemical Toxicology, 46*(7, Supplement), S61-S69. doi:https://doi.org/10.1016/j.fct.2008.05.009

Research, B. (2013). The Market For High-Intensity Sweeteners Is Expected To Reach Nearly $1.9 Billion In 2017. Retrieved from https://www.bccresearch.com/pressroom/fod/market-high-intensity-sweeteners-expected-reach-nearly-$1.9-billion-2017

Reyes, M. M., Castura, J. C., & Hayes, J. E. (2017). Characterizing dynamic sensory properties of nutritive and nonnutritive sweeteners with temporal check-all-that-apply. *Journal of Sensory Studies, 32*(3), e12270. doi:10.1111/joss.12270

Roberts, A. (2016). The safety and regulatory process for low calorie sweeteners in the United States. *Physiol Behav, 164*(Pt B), 439-444. doi:10.1016/j.physbeh.2016.02.039

Roberts, A., Renwick, A. G., Sims, J., & Snodin, D. J. (2000). Sucralose metabolism and pharmacokinetics in man. *Food and Chemical Toxicology, 38, Supplement 2*(0), 31-41. doi:http://dx.doi.org/10.1016/S0278-6915(00)00026-0

Rodearmel, S. J., Wyatt, H. R., Stroebele, N., Smith, S. M., Ogden, L. G., & Hill, J. O. (2007). Small Changes in Dietary Sugar and Physical Activity as an Approach to Preventing Excessive Weight Gain: The America on the Move Family Study. *Pediatrics, 120*(4), e869.

Rodin, J. (1990). Comparative effects of fructose, aspartame, glucose, and water preloads on calorie and macronutrient intake. *The American Journal of Clinical Nutrition, 51*(3), 428-435. doi:10.1093/ajcn/51.3.428

Rogers P, J. (1989). Evaluation of the influence of intense sweeteners on the short-term control of appetite and caloric intake: a psychobiological approach. In G. T (Ed.), *Progress in sweeteners*. New York, NY: Elsevier Applied Science.

Rogers, P. J., & Blundell, J. E. (1989). Separating the actions of sweetness and calories: Effects of saccharin and carbohydrates on hunger and food intake in human subjects. *Physiology & Behavior, 45*(6), 1093-1099. doi:https://doi.org/10.1016/0031-9384(89)90093-0

Rogers, P. J., Carlyle, J. A., Hill, A. J., & Blundell, J. E. (1988). Uncoupling sweet taste and calories: comparison of the effects of glucose and three intense sweeteners on hunger and food intake. *Physiol Behav, 43*(5), 547-552.

Rogers, P. J., Gadah, N. S., Kyle, L. A., & Brunstrom, J. M. (2012). Standard preload-test meal study designs may underestimate satiety effects: sugar containing drinks as an example. *Proceedings of the Nutrition Society, 70*(OCE6), E401. doi:10.1017/S0029665111004861

Rogers, P. J., Hogenkamp, P. S., de Graaf, C., Higgs, S., Lluch, A., Ness, A. R., . . . Mela, D. J. (2016). Does low-energy sweetener consumption affect energy intake and body weight? A systematic review, including meta-analyses, of the evidence from human and animal studies. *Int J Obes (Lond), 40*(3), 381-394. doi:10.1038/ijo.2015.177

Rogers, P. J., Keedwell, P., & Blundell, J. E. (1991). Further analysis of the short-term inhibition of food intake in humans by the dipeptide L-aspartyl-L-phenylalanine methyl ester (aspartame). *Physiol Behav, 49*(4), 739-743.

Rogers, P. J., Pleming, H. C., & Blundell, J. E. (1990). Aspartame ingested without tasting inhibits hunger and food intake. *Physiol Behav, 47*(6), 1239-1243.

Rolls, B. J. (1986). Sensory-specific Satiety. *Nutrition Reviews, 44*(3), 93-101. doi:10.1111/j.1753-4887.1986.tb07593.x

Rolls, B. J. (1991). Effects of intense sweeteners on hunger, food intake, and body weight: a review. *Am J Clin Nutr, 53*(4), 872-878.

Rolls, B. J., Kim, S., & Fedoroff, I. C. (1990). Effects of drinks sweetened with sucrose or aspartame on hunger, thirst and food intake in men. *Physiology & Behavior, 48*(1), 19-26. doi:https://doi.org/10.1016/0031-9384(90)90254-2

Rolls, B. J., Laster, L. J., & Summerfelt, A. (1989). Hunger and food intake following consumption of low-calorie foods. *Appetite, 13*(2), 115-127. doi:http://dx.doi.org/10.1016/0195-6663(89)90109-8

Rolls, B. J., & Roe, L. S. (2002). Effect of the volume of liquid food infused intragastrically on satiety in women. *Physiology & Behavior, 76*(4), 623-631. doi:https://doi.org/10.1016/S0031-9384(02)00801-6

Rudenga, K. J., & Small, D. M. (2012). Amygdala response to sucrose consumption is inversely related to artificial sweetener use. *Appetite, 58*(2), 504-507. doi:https://doi.org/10.1016/j.appet.2011.12.001

S. McGregor, I., & Lee, A. M. (1995). Metabolic changes associated with ingestion of different macronutrients and different meal sizes in rats. *Physiology & Behavior, 57*(2), 277-286. doi:https://doi.org/10.1016/0031-9384(94)00221-P

Schiffman, S. S. (2012). Rationale for Further Medical and Health Research on High-Potency Sweeteners. *Chemical Senses, 37*(8), 671-679. doi:10.1093/chemse/bjs053

Schiffman, S. S., Booth, B. J., Carr, B. T., Losee, M. L., Sattely-Miller, E. A., & Graham, B. G. (1995). Investigation of synergism in binary mixtures of sweeteners. *Brain Research Bulletin, 38*(2), 105-120. doi:https://doi.org/10.1016/0361-9230(95)00062-J

Sclafani, A., & Clare, R. A. (2004). Female Rats show a Bimodal Preference Response to the Artificial Sweetener Sucralose. *Chemical Senses, 29*(6), 523-528. doi:10.1093/chemse/bjh055

Sengupta, P. (2013). The Labratory Rat: Relating its Age With Human's. *Int J Prev Med, 4*(6), 624-630.

Sharma, R. S., Joy, R. C., Boushey, C. J., Ferruzzi, M. G., Leonov, A. P., & McCrory, M. A. (2014). Effects of para-aminobenzoic acid (PABA) form and administration mode on PABA recovery in 24-hour urine collections. *J Acad Nutr Diet, 114*(3), 457-463. doi:10.1016/j.jand.2013.07.045

Shen, J., Obin, M. S., & Zhao, L. (2013). The gut microbiota, obesity and insulin resistance. *Molecular Aspects of Medicine, 34*(1), 39-58. doi:https://doi.org/10.1016/j.mam.2012.11.001

Shide, D. J., & Rolls, B. J. (1995). Information About the Fat Content of Preloads Influences Energy Intake in Healthy Women. *Journal of the American Dietetic Association, 95*(9), 993-998. doi:https://doi.org/10.1016/S0002-8223(95)00273-1

Sicher, J. (2015). Special Issue: U.S. Beverage Business Results for 2014. *Beverage Digest, 66*(7).

Smeets, P. A., Erkner, A., & De Graaf, C. (2010). Cephalic phase responses and appetite. *Nutrition Reviews, 68*(11), 643-655.

Smeets, P. A., Weijzen, P., de Graaf, C., & Viergever, M. A. (2011). Consumption of caloric and non-caloric versions of a soft drink differentially affects brain activation during tasting. *Neuroimage, 54*(2), 1367-1374. doi:10.1016/j.neuroimage.2010.08.054

Smith, J. C., & Sclafani, A. (2002). Saccharin as a Sugar Surrogate Revisited. *Appetite, 38*(2), 155-160. doi:https://doi.org/10.1006/appe.2001.0467

Soenen, S., & Westerterp-Plantenga, M. S. (2007). No differences in satiety or energy intake after high-fructose corn syrup, sucrose, or milk preloads. *The American Journal of Clinical Nutrition, 86*(6), 1586-1594. doi:10.1093/ajcn/86.5.1586

Sorensen, L. B., Moller, P., Flint, A., Martens, M., & Raben, A. (2003). Effect of sensory perception of foods on appetite and food intake: a review of studies on humans. *Int J Obes Relat Metab Disord, 27*(10), 1152-1166. doi:10.1038/sj.ijo.0802391

Steinert, R. E., Feinle-Bisset, C., Asarian, L., Horowitz, M., Beglinger, C., & Geary, N. (2017). Ghrelin, CCK, GLP-1, and PYY(3–36): Secretory Controls and Physiological Roles in Eating and Glycemia in Health, Obesity, and After RYGB. *Physiological Reviews, 97*(1), 411-463. doi:10.1152/physrev.00031.2014

Steinert, R. E., Frey, F., Töpfer, A., Drewe, J., & Beglinger, C. (2011). Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides. *British Journal of Nutrition, 105*(09), 1320-1328. doi:doi:10.1017/S000711451000512X

Stellman, S. D., & Garfinkel, L. (1986). Artificial sweetener use and one-year weight change among women. *Prev Med, 15*(2), 195-202.

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of Psychosomatic Research, 29*(1), 71-83. doi:http://dx.doi.org/10.1016/0022-3999(85)90010-8

Subar, A. F., Crafts, J., Zimmerman, T. P., Wilson, M., Mittl, B., Islam, N. G.,Thompson, F. E. (2010). Assessment of the accuracy of portion size reports using computer-based food photographs aids in the development of an automated self-administered 24-hour recall. *Journal of the American Dietetic Association, 110*(1), 55-64.

Subar, A. F., Kipnis, V., Troiano, R. P., Midthune, D., Schoeller, D. A., Bingham, S., Schatzkin, A. (2003). Using Intake Biomarkers to Evaluate the Extent of Dietary Misreporting in a Large Sample of Adults: The OPEN Study. *Am J Epidemiol, 158*(1), 1-13. doi:10.1093/aje/kwg092

Subar, A. F., Thompson, F. E., Potischman, N., Forsyth, B. H., Buday, R., Richards, D., Baranowski, T. (2007). Formative research of a quick list for an automated self-administered 24-hour dietary recall. *Journal of the American Dietetic Association, 107*, 1002-1007.

Suez, J., Korem, T., Zeevi, D., Zilberman-Schapira, G., Thaiss, C., Maza, O., Weinberger, A. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature, 514*.

Suez, J., Korem, T., Zilberman-Schapira, G., Segal, E., & Elinav, E. (2015). Non-caloric artificial sweeteners and the microbiome: findings and challenges. *Gut Microbes, 6*(2), 149-155. doi:10.1080/19490976.2015.1017700

Swithers, S. E. (2013). Artificial sweeteners produce the counterintuitive effect of inducing metabolic derangements. *Trends Endocrinol Metab, 24*(9), 431-441. doi:10.1016/j.tem.2013.05.005

Swithers, S. E., Baker, C. R., & Davidson, T. L. (2009). General and persistent effects of high-intensity sweeteners on body weight gain and caloric compensation in rats. *Behavioral neuroscience, 123*(4), 772-780. doi:10.1037/a0016139

Swithers, S. E., & Davidson, T. L. (2008). A role for sweet taste: calorie predictive relations in energy regulation by rats. *Behav Neurosci, 122*(1), 161-173. doi:10.1037/0735-7044.122.1.161

Swithers, S. E., Martin, A. A., & Davidson, T. L. (2010). High-Intensity Sweeteners and Energy Balance. *Physiology & behavior, 100*(1), 55-62. doi:10.1016/j.physbeh.2009.12.021

Sylvetsky, A. C., Brown, R. J., Blau, J. E., Walter, M., & Rother, K. I. (2016). Hormonal responses to non-nutritive sweeteners in water and diet soda. *Nutrition & Metabolism, 13*(1), 71. doi:10.1186/s12986-016-0129-3

Sylvetsky, A. C., Jin, Y., Clark, E. J., Welsh, J. A., Rother, K. I., & Talegawkar, S. A. (2017). Consumption of Low-Calorie Sweeteners among Children and Adults in the United States. *Journal of the Academy of Nutrition and Dietetics, 117*(3), 441-448.e442. doi:https://doi.org/10.1016/j.jand.2016.11.004

Sylvetsky, A. C., & Rother, K. I. (2018). Nonnutritive Sweeteners in Weight Management and Chronic Disease: A Review. *Obesity, 26*(4), 635-640. doi:doi:10.1002/oby.22139

Sylvetsky, A. C., Walter, P. J., Garraffo, H. M., Robien, K., & Rother, K. I. (2017). Widespread sucralose exposure in a randomized clinical trial in healthy young adults. *Am J Clin Nutr, 105*(4), 820-823. doi:10.3945/ajcn.116.144402

Sylvetsky, A. C., Welsh, J. A., Brown, R. J., & Vos, M. B. (2012). Low-calorie sweetener consumption is increasing in the United States. *Am J Clin Nutr, 96*(3), 640-646. doi:10.3945/ajcn.112.034751

Tate, D. F., Turner-McGrievy, G., Lyons, E., Stevens, J., Erickson, K., Polzien, K., Popkin, B. (2012). Replacing caloric beverages with water or diet beverages for weight loss in adults: main results of the Choose Healthy Options Consciously Everyday (CHOICE) randomized clinical trial. *Am J Clin Nutr, 95*(3), 555-563. doi:10.3945/ajcn.111.026278

Te Morenga, L., Mallard, S., & Mann, J. (2013). Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. *BMJ : British Medical Journal, 346*. doi:10.1136/bmj.e7492

Teff, K. (2000). Nutritional implications of the cephalic- phase reflexes: endocrine responses (Vol. 34, pp. 206-213).

Teff, K. L., Devine, J., & Engelman, K. (1995). Sweet taste: effect on cephalic phase insulin release in men. *Physiol Behav, 57*(6), 1089-1095.

Tellez, L. A., Han, W., Zhang, X., Ferreira, T. L., Perez, I. O., Shammah-Lagnado, S. J., de Araujo, I. E. (2016). Separate Circuitries Encode the Hedonic and Nutritional Values of Sugar. *Nature neuroscience, 19*(3), 465-470. doi:10.1038/nn.4224

Temizkan, S., Deyneli, O., Yasar, M., Arpa, M., Gunes, M., Yazici, D., . . . Yavuz, D. G. (2015). Sucralose enhances GLP-1 release and lowers blood glucose in the presence of carbohydrate in healthy subjects but not in patients with type 2 diabetes. *Eur J Clin Nutr, 69*(2), 162-166. doi:10.1038/ejcn.2014.208

Tey, S. L., Salleh, N. B., Henry, J., & Forde, C. G. (2016). Effects of aspartame-, monk fruit-, stevia- and sucrose-sweetened beverages on postprandial glucose, insulin and energy intake. *International Journal Of Obesity, 41*, 450. doi:10.1038/ijo.2016.225 https://www.nature.com/articles/ijo2016225#supplementary-information

Tordoff, M. G., & Alleva, A. M. (1990). Oral stimulation with aspartame increases hunger. *Physiol Behav, 47*(3), 555-559.

Tucker, R. M., & Tan, S.-Y. (2017). Do non-nutritive sweeteners influence acute glucose homeostasis in humans? A systematic review. *Physiology & Behavior, 182*, 17-26. doi:https://doi.org/10.1016/j.physbeh.2017.09.016

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature, 444*, 1027. doi:10.1038/nature05414 https://www.nature.com/articles/nature05414#supplementary-information

Uebanso, T., Ohnishi, A., Kitayama, R., Yoshimoto, A., Nakahashi, M., Shimohata, T., Takahashi, A. (2017). Effects of Low-Dose Non-Caloric Sweetener Consumption on Gut Microbiota in Mice. *Nutrients, 9*(6), 560. doi:10.3390/nu9060560

Vermunt, S. H., Pasman, W. J., Schaafsma, G., & Kardinaal, A. F. (2003). Effects of sugar intake on body weight: a review. *Obes Rev, 4*(2), 91-99.

Vist, G. E., & Maughan, R. J. (1995). The effect of osmolality and carbohydrate content on the rate of gastric emptying of liquids in man. *The Journal of Physiology, 486*(Pt 2), 523-531.

Wardle, J. (1987). Hunger and satiety: a multidimensional assessment of responses to caloric loads. *Physiology & behavior, 40*(5), 577-582.

Wilson, J. F. (2000). Lunch eating behavior of preschool children: Effects of age, gender, and type of beverage served. *Physiology & Behavior, 70*(1), 27-33. doi:https://doi.org/10.1016/S0031-9384(00)00230-4

Wise, P. M., Nattress, L., Flammer, L. J., & Beauchamp, G. K. (2016). Reduced dietary intake of simple sugars alters perceived sweet taste intensity but not perceived pleasantness. *The American Journal of Clinical Nutrition, 103*(1), 50-60. doi:10.3945/ajcn.115.112300

Witherly, S. A., Pangborn, R. M., & Stern, J. S. (1980). Gustatory Responses and Eating Duration of Obese and Lean Adults. *Appetite, 1*(1), 53-63.

Wolraich, M. L., Lindgren, S. D., Stumbo, P. J., Stegink, L. D., Appelbaum, M. I., & Kiritsy, M. C. (1994). Effects of Diets High in Sucrose or Aspartame on The Behavior and Cognitive Performance of Children. *New England Journal of Medicine, 330*(5), 301-307. doi:doi:10.1056/NEJM199402033300501

Woods, S. C., Lutz, T. A., Geary, N., & Langhans, W. (2006). Pancreatic signals controlling food intake; insulin, glucagon and amylin. *Philosophical Transactions of the Royal Society B: Biological Sciences, 361*(1471), 1219-1235. doi:10.1098/rstb.2006.1858

Wooley, O. W., Wooley, S. C., & Dunham, R. B. (1972). Calories and sweet taste: Effects on sucrose preference in the obese and nonobese. *Physiology & Behavior, 9*(5), 765-768. doi:https://doi.org/10.1016/0031-9384(72)90048-0

Wooley, O. W., Wooley, S. C., & Dunham, R. B. (1972). Can calories be perceived and do they affect hunger in obese and nonobese humans? *Journal of Comparative and Physiological Psychology, 80*(2), 250-258. doi:10.1037/h0033069

Wu, T., Bound, M. J., Standfield, S. D., Bellon, M., Young, R. L., Jones, K. L., Rayner, C. K. (2013). Artificial Sweeteners Have No Effect on Gastric Emptying, Glucagon-Like Peptide-1, or Glycemia After Oral Glucose in Healthy Humans. *Diabetes Care, 36*(12), e202.

Wu, T., Zhao, B. R., Bound, M. J., Checklin, H. L., Bellon, M., Little, T. J., Rayner, C. K. (2012). Effects of different sweet preloads on incretin hormone secretion, gastric emptying, and postprandial glycemia in healthy humans. *Am J Clin Nutr, 95*(1), 78-83. doi:10.3945/ajcn.111.021543

Yang, Q. (2010). Gain weight by "going diet?" Artificial sweeteners and the neurobiology of sugar cravings: Neuroscience 2010. *Yale J Biol Med, 83*(2), 101-108.

Yeomans, M. R., & Clifton, P. G. (1997). Exposure to Sweetened Solutions Enhances the Anorectic Effect of Naloxone But Not d-Fenfluramine. *Physiology & Behavior, 62*(2), 255-262. doi:https://doi.org/10.1016/S0031-9384(97)00112-1

Yeomans, M. R., Lartamo, S., Procter, E. L., Lee, M. D., & Gray, R. W. (2001). The actual, but not labelled, fat content of a soup preload alters short-term appetite in healthy men. *Physiology & Behavior, 73*(4), 533-540. doi:https://doi.org/10.1016/S0031-9384(01)00502-9

Zhao, L., & Tepper, B. J. (2007). Perception and acceptance of selected high-intensity sweeteners and blends in model soft drinks by propylthiouracil (PROP) non-tasters and super-tasters. *Food Quality and Preference, 18*(3), 531-540. doi:https://doi.org/10.1016/j.foodqual.2006.07.004

Zheng M, Allman-Farinelli M, Heitmann BL, Rangan A. (2015). Substitution of sugar-sweetened beverages with other beverage alternatives: a review of long-term health outcomes. *J Acad Nutr Diet,* 115, 767-779.

APPENDIX A INSTITUTIONAL REVIEW BOARD DOCUMENTS

LCS and Body Weight

Health Effects of Revised 10/10 Ref. # _____

APPLICATION TO USE HUMAN RESEARCH SUBJECTS

Purdue University

Institutional Review Board

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1. Project Title: Beverage Consumption and Fine Motor Control
2. Full Review ☒ Expedited Review ☐
3. Anticipated Funding Source: Internal funds
4. Principal Investigator *[See Policy on Eligibility to serve as a Principal Investigator for Research Involving Human Subjects]*:

Name and Title	Department, Building, Phone, FAX, E-mail
Richard Mattes, PhD, M PH, RD Distinguished Professor	Nutrition Science Stone Hall, 113 Phone: 765-494-0662 Fax: 765-494-0674 mattes@purdue.edu

5. Co-investigators and key personnel *[See Education Policy for Conducting Human Subjects Research]*:

Name and Title	Department, Building, Phone, FAX, E-mail
Kelly Higgins Graduate Research Assistant Fax: 765-494-0674 higgin20@purdue.edu	Food Science Stone Hall, G4 Mobile: 573-694-0403
Name and Title	Department, Building, Phone, FAX, E-mail
Judy George	Nutrition Science

Laboratory Manager	Stone Hall, G4 Phone: 765-494-6192 Fax: 765-494-0674 georgej@purdue.edu
Robin Rhine Laboratory Technician	Nutrition Science Stone Hall, G4 Phone: 494-6192 Fax: 765-494-0674

7. The principal investigator agrees to carry out the proposed project as stated in the application and to promptly report to the Institutional Review Board any proposed changes and/or unanticipated problems involving risks to subjects or others participating in the approved project in accordance with the HRPP Guideline 207 Researcher Responsibilities, Purdue Research Foundation-Purdue University Statement of Principles and the Confidentiality Statement. The principal investigator has received a copy of the Federal-Wide Assurance (FWA) and has access to copies of 45 CFR 46 and the Belmont Report. The principal investigator agrees to inform the Institutional Review Board and complete all necessary reports should the principal investigator terminate University association.

_____
_____
Principal Investigator Signature Date

8. The Department Head (or authorized agent) has read and approved the application. S/he affirms that the use of human subjects in this project is relevant to answer the research question being asked and has scientific or scholarly merit. Additionally s/he agrees to maintain research records in accordance with the IRB's research records retention requirement should the principal investigator terminate association with the University.

_____
_____
Department Head *(printed)* Department Name

_____
_____
Department Head Signature Date

APPLICATION TO USE HUMAN RESEARCH SUBJECTS

9. This project will be conducted at the following location(s): (please indicate city & state)
   - ☒ Purdue West Lafayette Campus
   - ☐ Purdue Regional Campus (Specify): _____
   - ☐ Other (Specify): _____

10. If this project will involve potentially vulnerable subject populations, please check all that apply.
   - ☐ Minors under age 18
   - ☐ Pregnant Women
   - ☐ Fetus/fetal tissue
   - ☐ Prisoners Or Incarcerated Individuals
   - ☒ University Students (PSYC Dept. subject pool ___)
   - ☐ Elderly Persons
   - ☐ Economically/Educationally Disadvantaged Persons
   - ☐ Mentally/Emotionally/Developmentally Disabled Persons
   - ☐ Minority Groups and/or Non-English Speakers
   - ☐ Intervention(s) that include medical or psychological treatment

11. Indicate the anticipated maximum number of subjects to be enrolled in this protocol as justified by the hypothesis and study procedures: ___200_____

12. This project involves the use of an **Investigational New Drug** (IND) or an **Approved Drug For An Unapproved Use**.

☐ YES ☒ NO

Drug name, IND number and company: _____

13. This project involves the use of an **Investigational Medical Device** or an **Approved Medical Device For An Unapproved Use**.

☐ YES ☒ NO

Device name, IDE number and company: _____

14. The project involves the use of **Radiation or Radioisotopes**: DXA

☒ YES ☐ NO

15. Does this project call for: (check-mark all that apply to this study)
   - ☐ Use of Voice, Video, Digital, or Image Recordings?
   - ☒ Subject Compensation? Please indicate the maximum payment amount to subjects. $ 300
     Purdue's Human Subjects Payment Policy Participant Payment Disclosure Form
   - ☐ VO2 Max Exercise?
   - ☐ More Than Minimal Risk?
   - ☐ Waiver of Informed Consent?
   - ☐ Extra Costs To Subjects?
   - ☒ The Use of Blood? Total Amount of Blood 8ml x 7 x2 + 8 = 120 mL
     Over Time Period (days) 12 weeks
   - ☐ The Use of rDNA or Biohazardous materials?

☐ The Use of Human Tissue or Cell Lines?
☒ The Use of Other Fluids that Could Mask the Presence of Blood (Including Urine and Feces)?
☐ The Use of Protected Health Information (Obtained from Healthcare Practitioners or Institutions)?
☐ The Use of academic records?

16. Does investigator or key personnel have a potential financial or other conflict of interest in this study?

☐ YES ☒ NO

APPLICATION NARRATIVE

A. PROPOSED RESEARCH RATIONALE

As the prevalence of obesity continues to rise, it is essential to search for the cause and effective management approaches for this global epidemic. Of recent interest has been the role of low calorie sweeteners (LCS) on body weight, energy intake, and appetite. The literature on LCS use and body weight is rigorously debated and current evidence provides a basis for opposing views. Several epidemiological studies report a positive association between LCS use and BMI (Stellman et al., 1986). In some instances this may be attributable to reverse causality (heavy people choose to use LCS), but some reports note the positive association after controlling for BMI (Sharon P. Fowler et al., 2008). Some rodent feeding trials observe greater weight gain with LCS consumption (Davidson et al., 2004; S. E. Swithers et al., 2008); and a recent meta-analysis concluded that prospective cohort studies show LCS use is associated with a small, but statistically significant higher BMI (Paige E. Miller et al., 2014). In contrast to this, the same meta-analysis found LCS use in randomized controlled trials (a higher caliber of evidence) is associated with a small, but statistically significant lower BMI (Paige E. Miller et al., 2014). Additionally, another RCT published after the meta-analysis reported LCS use during an energy-restricted diet among 303 adults led to greater weight loss than an equal volume of water (J. C. Peters et al., 2014). The 2015 Dietary Guidelines Committee notes the inconsistency in long term observational studies and states there is "moderate and generally consistent evidence" that substitution of energy containing sweeteners with LCS reduces body weight, but concluded the evidence base was insufficient to make a recommendation regarding the utility of LCS in a weight management regimen (Committee, 2015).

We believe the lack of a clear answer regarding the efficacy of LCS on body weight stems, in part, from methodological differences; including the type of LCS used, that obscure effects. Commercially available LCS differ markedly in chemical structure. This property likely holds implications for LCS binding site and affinity for sweet taste receptors, taste quality (sweet and bitter sensory notes), digestibility, absorption, stimulation of gut peptide release, and endocrine response with consequent effects on appetite, energy intake, body weight, and body composition.

LCS used by the food industry are regulated by the Food and Drug Administration (FDA) and undergo extensive examination to determine their safety as food additives. However, the public's recently heightened concern regarding LCS has moved numerous companies to announce elimination of specific LCS from products. The concern regarding the safety of LCS

may be the cause of the a downward trend of LCS beverage consumption (Sicher, 2015), the largest medium of LCS consumption. The preponderance of evidence indicates LCS may be a valuable tool for weight loss and weight maintenance. Most frequently, commercially available mixtures of LCS are used in interventions (J. C. Peters et al., 2014) (A. Raben et al., 2002) (A. C. Sylvetsky et al., 2012), so it is possible the optimal individual or combination of LCS for weight management is not known. Varying properties of commercially approved sweeteners may differentially alter their efficacy for weight management, but this has not been systematically tested. *The aim of this study will be to compare the effects of daily consumption of sucralose, aspartame, saccharin, rebaudioside a (a stevia compound), and sucrose on body weight and composition in a standardized protocol, modeling the approach used by Raben et al. (A. Raben et al., 2002). We hypothesize that individual LCS (consumed in a beverage) differ from each other in promoting weight loss and decreased fat mass (without energy restriction or dietary guidance) based on their chemical properties.*

B. SPECIFIC PROCEDURES TO BE FOLLOWED

Recruitment: Potential participants will be asked to participate in a study to assess the effect of beverage consumption on fine motor control. Participants will be informed the aim is to assess sweetener consumption on fine motor control. While this will be measured, we also want to deemphasize our interest in the body weight outcome. It is believed knowledge of this outcome would alter behavior and confound an important goal of the work. Before baseline testing, potential participants will be pre-screened to verify they meet the inclusion criteria for the study. Inclusion and exclusion criteria are discussed below. Individuals who meet these requirements will participate in the study protocol outlined below in Table X.

Table 1 – Matrix of study procedures:

	B	1	2	3	4	5	6	7	8	9	10	11	12
Height	X												
Weight	X		X		X		X		X		X		X
% Body Fat	X												X
Fine motor control measurements	X		X		X		X		X		X		X
Energy Expenditure	X				X				X				X
Energy Intake	X				X				X				X
Appetite	X				X				X				X
OGTT (X) Single blood draw (S)	X						S						X
Urine Collection	X				X				X				X
Questionnaires	X						X						X

Measurements

Anthropometry*:* Height will be measured at baseline with a Holtain stadiometer fixed to the wall with participants in socks. Body weight will be measured weekly with the same calibrated clinical scale. Body composition will be measured at baseline and week 12 by DEXA.

Fine motor control measurements:

Grip Strength: Participants will grip the dynamometer device, applying as much pressure by squeezing as possible. Participants will repeat this action three times with both hands, recording the maximum reading for each hand.

Hand Steadiness: This task requires participants hold a metal stylus in 9 progressively smaller holes without touching the sides. The number of times the stylus touches the sides of the hole will be recorded.

Rotary Pursuit: Participants will follow a target spot around a rotating anodized disk for 20 second periods with a metal wand. Time spent in contact with the target spot will be measured.

Blood Draw: Participants will report to the Laboratory for Sensory and Ingestive Studies after an overnight fast. A blood sample will be obtained by venipuncture of an antecubital vein standard procedures. The sample will be analyzed for lipid profile, glucose, and insulin.

Oral Glucose Tolerance Test (OGTT): OGTT will be conducted at baseline and week 12. Participants will arrive at the facility in a fasted state. Blood will be collected into tubes containing 0.5 M EDTA at times 0, 10, 20, 30, 60, 90, and 120 minutes (Dalla Man et al., 2005). Upon arrival, they will have a catheter placed in an antecubital vein and sit quietly. Participants will consume a 75g glucose solution (standard OGTT test). 8 milliliters of blood will be collected in a red top vacutainer. Samples will be centrifuged and the serum aliquoted and frozen at -80^{o}C for batch analysis. Triacylglycerol, high density and low density cholesterol, and glucose concentrations will be determined on a Roche COBAS MIRA Analyzer. Insulin will be measured using the Elecsys® 2010 Immunoassay System (Roche Diagnostic Systems, Indianapolis, IN).

Questionnaires:

Modified Beverage Intake Questionnaire (BEVQ-15) and Sweetened Food Questionnaire: Participants will complete beverage intake questionnaire to assess habitual consumption frequency and quantity of both NS and LCS beverages. This questionnaire has been adapted from the BEVQ-15, validated as a reliable and rapid self-administered dietary assessment tool (Hedrick et al., 2010; Hedrick et al., 2012). The Sweetened Food Questionnaire models the format used in the BEVQ-15; participants will indicate frequency of consumption of common foods containing NS and LCS reported by Piernas et al. (Carmen Piernas et al., 2014) and Ng et al. (Ng et al., 2012).

Baecke Physical Activity Questionnaire: Habitual physical activity will also be assessed using the Baecke questionnaire, which has been validated to measure physical activity at work, sport activity during leisure time, and physical activity during leisure time excluding sport activity (Baecke et al., 1982).

Eating Attitudes Questionnaire: The Eating Attitudes Questionnaire is a validated assessment of symptoms of an eating disorder (Garner et al., 1982). This questionnaire will be used as a means to determine eligibility of the study during prescreening. Participants will be excluded if they exhibit symptoms of an eating disorder.

Three Factor Eating Questionnaire: This questionnaire is surveys three dimensions of eating behavior: dietary restraint, disinhibition, and hunger (A. Stunkard et al., 1985). This questionnaire will be used as a means to determine eligibility of the study during prescreening. Participants will be excluded if they exhibit signs of eating restraint. The questionnaire will be administered again at week 6 and 12 to determine change in eating behavior over the course of the study.

Modified Food Healthfulness Questionnaire: The Food Healthfulness Questionnaire assesses the perceived healthiness, ability to induce weight loss or gain, and energy content of various food items (Carels et al., 2006). The questionnaire utilized in this study has been adapted to include various LCS containing foods and beverages to assess participants' perception of these products.

Fine Motor Control Questionnaire: Participants will complete a questionnaire fine motor control in everyday life. The questionnaire also includes pattern replication in which participants will have to click on different areas of a graphic.

Beverage Evaluation Questionnaire: On the day of beverage pick-up, participants will consume one beverage in the laboratory and answer questions to evaluate the beverage flavor profile and appetite sensation. Participants will also be asked questions regarding how they typically consume the beverage throughout the intervention weeks.

Week 12 Follow-up Questionnaire: During the last appointment, participants will complete a questionnaire regarding how they perceive the beverages affected their fine motor control, body weight, and sweetness appetite. Participants will also be asked what kind of sweetener they consumed (LCS, sucrose, both, or neither). Participants will have the opportunity to provide feedback regarding the study protocol and any complications they may have experienced throughout the study they have not already reported to the study personnel.

Participants will also receive instruction about the following study procedures conducted outside of the laboratory setting:

Dietary intake: Food and energy intake will be assessed at baseline and weeks 4, 8 and 12 using the web-based, "Automated Self-Administered 24-hour Dietary Recall" (ASA24) system. Participants will be asked to record dietary intake for three non-consecutive days that include two weekdays and one weekend day for better representation of habitual intake. The respondent interface is modeled on an interviewer-administered Automated Multi-Pass Method (AMPM) developed by the US Department of Agriculture (USDA). It includes electronic notifications to prompt participants to record at designated times. It uses multi-level food probes to accurately assess food types and amounts. Participants will be reminded to complete their dietary recall using a unique username and password given to them. The system is linked to a nutrient database that provides researchers with an estimation of food and nutrient intakes (A. F. Subar et al., 2010; A. F. Subar et al., 2007).

Appetite: Ratings of hunger, fullness, desire to eat, desire to eat something sweet, desire to eat something salty, prospective consumption and thirst will be recorded at baseline and weeks 4, 8, and 12 (on the hour, every hour while awake for one day) using validated visual analog scales

(A. Flint et al., 2000) that are programmed into the participants smartphone using Qualtrics. These ratings will be obtained over one 24-hour period during one day they record food intake. Pattern, peak, nadir, and AUC values will be computed.

Energy Expenditure:
They will also be provided an accelerometer to monitor their daily physical activity for three days during the baseline week. Free-living physical activity levels and energy expenditure will be predicted using RT6 triaxial accelerometers (StayHealthy, Monrovia, CA) that provide activity counts on 3 axes. Free-living physical activity levels will be measured at baseline, week 4, 8, and 12. Each measurement will be taken over three consecutive days that include two weekdays and one weekend day.

Urine Collection: Para-aminobenzoic acid (PABA) is a compound found in foods (including grains and meat) as well as a dietary supplement, and is excreted in urine. Although PABA can be used as a supplement, the small quantity (240mg/day) of PABA added to beverages will only serve as a marker to measure compliance to beverage consumption. Urine samples will be collected at baseline to establish the PABA composition of each individual's customary diet and, again, at weeks 4, 8, and 12 to assess beverage consumption compliance. Spot urine collections may also be requested at unannounced times to ensure compliance. Participants will be provided urine containers with boric acid added as a preservative for a 24 hour collection to determine their baseline and intervention PABA and urinary nitrogen excretion. Urine will be collected the final day of food intake recording and no more than 48 hours prior to the participant's scheduled first intervention visit. Urine samples will be stored at -80^{o}C prior to analysis. Urinary PABA will be analyzed colorimetrically using a spectrophotometer as outlined in Sharma et al (Sharma et al., 2014).

At the end of the baseline week, participants will return to the laboratory. They will return the accelerometer and their urine samples, have weight and height measured, and be randomly assigned to a group that receives beverages sweetened with one of the four LCS or sucrose. They will then be instructed to initiate consumption of their prescribed beverage daily. No other dietary guidance will be provided. Participants will be instructed not to change any dietary or physical activities or begin any new medications during the study. If any of these changes are necessary, the PI should be informed as soon as possible and potential exclusion from the trial may follow. Participants will be informed of available consultation with a registered dietician post-study.

Intervention
Beverages: Sucrose and LCS will be presented in powdered beverage packets consisting of unsweetened Kool-Aid and PABA. The amount of sucrose will be set at a portion that provides ~1.8 g of sucrose per kilogram body weight. Based on body weight, participants will be assigned to one of three intake levels presented in a 8% sucrose solution: 100g, 120g, or 140g of sucrose. This equates to 1.25, 1.50, and 1.75 L of beverage a day. The dose of LCS in the remaining beverage treatments will be determined by the concentrations required to match the sweetness of the sucrose containing beverage. All LCS levels will be below the acceptable daily intake (ADI) set by the FDA, ranging approximately 0.16-0.76 g/day. Beverages will be presented as flavored dry mixes that participants will add to water. Participants will choose between 5 beverage

flavors. This will facilitate transport and convenience of the large quantities of beverage to be consumed throughout the day. It will also facilitate determination of compliance (noted below).

C. SUBJECTS TO BE INCLUDED

Five groups of 28 individuals (140 participants total) 18-60 years of age with a BMI >25kg/M^2 will be recruited. Participants will consist of approximately half male and half female with no weight change>3kg in the last year, no plan to initiate a new diet plan in the next 6 months, and not exhibit eating restraint (assessed via pre-study questionnaire). They will agree to adhere to their customary level of physical activity for the duration of the trial. Use of medications known to affect appetite and/or metabolism will not be permitted. Other currently used supplements and medications must have been taken for at least 6 months with no plan to alter their use. Participants must be healthy (e.g., no diabetes, hypertension), not pregnant or lactating, and willing to consume a large volume of beverage daily. Participants will not be excluded based on ethnic, cultural background.

Potential participants will be non- or rare users of LCS for > 6 months. Only approximately 10.8% (R. D. Mattes et al., 2009) to 24.1% (A. C. Sylvetsky et al., 2012) of the population consumes soda with LCS , the primary source of LCS in the diet. Diet soda drinkers will be excluded. Potential participants will complete a Modified Beverage Intake Questionnaire (BEVQ-15) and Sweetened Food Questionnaire comprised of commercial products containing LCS and are reduced in energy density by greater than 50% through substitution of an LCS for a NS (Carmen Piernas et al., 2014) (Ng et al., 2012). Use of these products will also be grounds for exclusion. Other exposures (e.g., through toothpaste which is not even swallowed) will be considered incidental and not likely to have disrupted sensory-nutrient conditioning.

D. RECRUITMENT OF SUBJECTS AND OBTAINING INFORMED CONSENT

Participants will respond to public advertisements (including *Purdue Today*, flyers, and newspaper ads) and/or be directly contacted through the laboratory's database who have already provided consent for notification of research opportunities such as this. They will report to the laboratory for a screening visit where they will complete a very extensive battery of questionnaires. Among the questionnaires in the screening battery are the Laboratory for Sensory and Ingestive Behavior Questionnaire (IRB #504002017) to assess diet, lifestyle, health, and demographics; Eating Inventory/Three-Factor Eating Questionnaire (A. Stunkard et al., 1985) to assess dietary restraint; and modified Beverage Intake Questionnaire BEVQ-15 (Hedrick et al., 2012) and Sweetened Food Questionnaire. Participants will also answer questions regarding beverage flavor preference and palatability. Height and weight will be measured to determine BMI. Individuals meeting pre-specified eligibility criteria and interested in volunteering for the trial will complete human subjects protection procedures, sign consent forms, and be scheduled for a baseline test day.

Following the completion of the 12 week intervention, participants will be offered consultation with a registered dietitian to develop a plan that will be matched to the individuals' lifestyle and

preferences if he or she desires to loss or regain any weight change that may have occurred in the study.

Although we will collect information on fine motor control throughout the study, this is not the primary outcome of our research. The grip strength, hand steadiness, rotary tracking, and motor control questionnaires will be used to distract the participant from possibly altering their ingestive behaviors to meet perceived researcher expectations. This study is recruiting participants on a rolling enrollment basis; therefore, we will not be providing a post-study explanation of the primary aim of the study. If the primary aim of body weight change becomes common knowledge among participants, then study outcomes may be compromised.

E. PROCEDURES FOR PAYMENT OF SUBJECTS

Participants will receive a total payment of $300 as compensation for the time and burden imposed by their participation.
For participants who complete the baseline week visits but do not meet eligibility criteria, a payment of $50 will be made to compensate for their time. Additionally, participants who do not wish to complete the study at weeks 4, 6, 8 weeks will be compensated $100, $150, $200, respectively. A payment of $300 will be made upon completion of the full study.

F. CONFIDENTIALITY

The project's research records may be reviewed by the Office for Human Research Protections and by departments at Purdue University responsible for regulatory and research oversight. All information collected in this study will be stored in a locked filing cabinet in a secure location in close proximity to the principal investigator. A copy of this consent form will be retained for three years after termination of the study at which time it will be destroyed. If any publication results from this research, you will not be identified by name. Participants' identities will not be released to any party outside the research team with the exception that your name, address, and social security number will be provided to the business office to enable processing of your financial compensation. Only Professor Mattes or his research team will have access to the data. All blood and urine samples will be stored in a freezer with study identification number. If the samples have not been used within two years following collection, they will be destroyed.

G. POTENTIAL RISKS TO SUBJECTS

<u>Risk of breach of confidentiality:</u>
There is a risk of breach of confidentiality that will be minimized by the steps described in section F.

<u>Beverages:</u>
All sweeteners and beverage products utilized in this study are commercially available and present in common foods and beverages. These ingredients undergo extensive examination by

the Food and Drug Administration (FDA) and will be used at levels within the acceptable daily intake (ADI) set by the FDA. Therefore, there is no foreseeable risk with HIS consumption.

Based on the literature and, more specifically, the findings by Raben et al. (A. Raben et al., 2002) it is possible participants will gain weight during this trial. If no dietary compensation occurs, participants could gain 7-12lbs across 12 weeks. All participants who gain weight will be offered follow-up counselling to reduce the small amount of weight they might have gained. Advice will be provided by a registered dietitian and the plan will be matched to the individuals' lifestyle and preferences.

PABA will be added to beverages and consumed at a rate of 240 mg per day. There are few side effects associated with oral consumption of PABA (including upset stomach, nausea, and loss of appetite); however, these are usually experienced at higher doses, around 400 mg per day. PABA has been utilized in other studies at this dose without any side effects (Bingham et al., 1983) (Amy F. Subar et al., 2003).

Blood collection:
The blood collections may result in pain, bruising and/or infection at the site of collection. Some participants might become lightheaded during blood collections and may faint. Appropriate techniques will be used and trained personnel will be responsible for all blood collection and will be present in the room for participant monitoring. The total amount of blood to be drawn will be no more than 120 ml during the 12 week study.

DXA:
DXA uses X-rays to determine body composition. The amount of radiation participants will receive is less than the dose that would be experienced during a roundtrip airplane flight between California and New York. Therefore, there is no foreseeable risk.

H. BENEFITS TO BE GAINED BY THE INDIVIDUAL AND/OR SOCIETY

No direct benefits can be guaranteed. Several recent RCT's document that LCS are equivalent or superior to water alone (a negative control) for weight loss (J. C. Peters et al., 2014; Deborah F Tate et al., 2012); therefore, there is potential for weight loss in the LCS groups. This study may yield useful information for curbing the incidence of obesity—a major public health issue.

I. INVESTIGATOR'S EVALUATION OF THE RISK-BENEFIT RATIO

Overall, findings of weight gain with LCS use would raise questions about recommendations to substitute LCS for sugar sweetened products for weight management while findings of weight loss for one, some, or all of the tested LCS would provide a basis for the use of that one, subset, or all of the tested forms for weight management.

Risks as outlined above, while real, are minimized by requiring participants to meet strict health criteria to be eligible and using appropriate precautions for each procedure. The findings may provide the basis for future approaches to curb the obesity epidemic. Therefore, the benefits outweigh the risks.

J. WRITTEN INFORMED CONSENT FORM

See attached consent form

K. WAIVER OF INFORMED CONSENT OR SIGNED CONSENT

N/A

L. INTERNATIONAL RESEARCH

N/A

M. SUPPORTING DOCUMENTS *(to be attached to the Application Narrative)*

- Recruitment flyer
- *Exponent, Purdue Today* Advertisement
- Prescreening survey
- Human Subject Research Application (accessible at http://www.cfs.purdue.edu/lsis)
- Baecke's Physical Activity Questionnaire
- Eating Attitudes Test
- Beverage, Sweetened Food Questionnaire (modified BEVQ-15)
- Food Healthfulness Questionnaire
- Appetite Questionnaire
- Fine Motor Control Questionnaire
- Beverage Evaluation Questionnaire
- Week 12 Questionnaire

References

1. Stellman SD, Garfinkel L. Artificial sweetener use and one-year weight change among women. Preventive medicine. 1986;15(2):195-202. Epub 1986/03/01. PubMed PMID: 3714671.

2. Fowler SP, Williams K, Resendez RG, Hunt KJ, Hazuda HP, Stern MP. Fueling the Obesity Epidemic? Artificially Sweetened Beverage Use and Long-term Weight Gain. Obesity. 2008;16(8):1894-900. doi: 10.1038/oby.2008.284.

3. Davidson TL, Swithers SE. A Pavlovian approach to the problem of obesity. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2004;28(7):933-5. Epub 2004/04/28. doi: 10.1038/sj.ijo.0802660. PubMed PMID: 15111986.

4. Swithers SE, Davidson TL. A role for sweet taste: calorie predictive relations in energy regulation by rats. Behavioral neuroscience. 2008;122(1):161-73. Epub 2008/02/27. doi: 10.1037/0735-7044.122.1.161. PubMed PMID: 18298259.

5. Miller PE, Perez V. Low-calorie sweeteners and body weight and composition: a meta-analysis of randomized controlled trials and prospective cohort studies. The American Journal of Clinical Nutrition. 2014. doi: 10.3945/ajcn.113.082826.

6. Peters JC, Wyatt HR, Foster GD, Pan Z, Wojtanowski AC, Vander Veur SS, Herring SJ, Brill C, Hill JO. The effects of water and non-nutritive sweetened beverages on weight loss during a 12-week weight loss treatment program. Obesity (Silver Spring). 2014;22(6):1415-21. doi: 10.1002/oby.20737. PubMed PMID: 24862170.

7. Committee DGA. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. In: USDA, editor. 2015.

8. Sicher J. Special Issue: U.S. Beverage Business Results for 2014. Beverage Digest. 2015;66(7).

9. Raben A, Vasilaras TH, Moller AC, Astrup A. Sucrose compared with artificial sweeteners: different effects on ad libitum food intake and body weight after 10 wk of supplementation in overweight subjects. The American journal of clinical nutrition. 2002;76(4):721-9. Epub 2002/09/27. PubMed PMID: 12324283.

10. Sylvetsky AC, Welsh JA, Brown RJ, Vos MB. Low-calorie sweetener consumption is increasing in the United States. Am J Clin Nutr. 2012;96(3):640-6. Epub 2012/08/03. doi: 10.3945/ajcn.112.034751. PubMed PMID: 22854409; PubMed Central PMCID: PMCPmc3417218.

11. Dalla Man C, Campioni M, Polonsky KS, Basu R, Rizza RA, Toffolo G, Cobelli C. Two-hour seven-sample oral glucose tolerance test and meal protocol: minimal model assessment of beta-cell responsivity and insulin sensitivity in nondiabetic individuals. Diabetes. 2005;54(11):3265-73. Epub 2005/10/27. PubMed PMID: 16249454.

12. Hedrick VE, Comber DL, Estabrooks PA, Savla J, Davy BM. The beverage intake questionnaire: determining initial validity and reliability. Journal of the American Dietetic Association. 2010;110(8):1227-32. doi: 10.1016/j.jada.2010.05.005. PubMed PMID: 20656099; PubMed Central PMCID: PMC2911642.

13. Hedrick VE, Savla J, Comber DL, Flack KD, Estabrooks PA, Nsiah-Kumi PA, Ortmeier S, Davy BM. Development of a brief questionnaire to assess habitual beverage intake (BEVQ-15): sugar-sweetened beverages and total beverage energy intake. Journal of the Academy of Nutrition and Dietetics. 2012;112(6):840-9. doi: 10.1016/j.jand.2012.01.023. PubMed PMID: 22709811; PubMed Central PMCID: PMC3379009.

14. Piernas C, Mendez MA, Ng SW, Gordon-Larsen P, Popkin BM. Low-calorie- and calorie-sweetened beverages: diet quality, food intake, and purchase patterns of US household consumers. The American Journal of Clinical Nutrition. 2014. doi: 10.3945/ajcn.113.072132.

15. Ng SW, Slining MM, Popkin BM. Use of caloric and noncaloric sweeteners in US consumer packaged foods, 2005-2009. Journal of the Academy of Nutrition and Dietetics. 2012;112(11):1828-34.e1-6. Epub 2012/10/30. doi: 10.1016/j.jand.2012.07.009. PubMed PMID: 23102182; PubMed Central PMCID: PMCPmc3490437.

16. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr. 1982;36(5):936-42.

17. Garner DM, Olmsted MP, Bohr Y, Garfinkel PE. The Eating Attitudes Test: psychometric features and clinical correlates. Psychological medicine. 1982;12(04):871-8. doi: doi:10.1017/S0033291700049163.

18. Stunkard A, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res. 1985;29:71 - 83. PubMed PMID: doi:10.1016/0022-3999(85)90010-8.

19. Carels RA, Harper J, Konrad K. Qualitative perceptions and caloric estimations of healthy and unhealthy foods by behavioral weight loss participants. Appetite. 2006;46(2):199-206.

20. Subar AF, Crafts J, Zimmerman TP, Wilson M, Mittl B, Islam NG, McNutt S, Potischman N, Buday R, Hull SG, Baranowski T, Guenther PM, Willis G, Tapia R, Thompson FE. Assessment of the accuracy of portion size reports using computer-based food photographs aids in the development of an automated self-administered 24-hour recall. J Am Diet Assoc. 2010;110(1):55-64.

21. Subar AF, Thompson FE, Potischman N, Forsyth BH, Buday R, Richards D, McNutt S, Hull SG, Guenther PM, Schatzkin A, Baranowski T. Formative research of a quick list for an automated self-administered 24-hour dietary recall. J Am Diet Assoc. 2007;107:1002-7.

22. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes. 2000;24(1):38-48.

23. Sharma RS, Joy RC, Boushey CJ, Ferruzzi MG, Leonov AP, McCrory MA. Effects of para-aminobenzoic acid (PABA) form and administration mode on PABA recovery in 24-hour urine collections. Journal of the Academy of Nutrition and Dietetics. 2014;114(3):457-63. doi: 10.1016/j.jand.2013.07.045. PubMed PMID: 24183994.

24. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. The American Journal of Clinical Nutrition. 2009;89(1):1-14. doi: 10.3945/ajcn.2008.26792. PubMed PMID: PMC2650084.

25. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. Journal of Psychosomatic Research. 1985;29(1):71-83. doi: http://dx.doi.org/10.1016/0022-3999(85)90010-8.

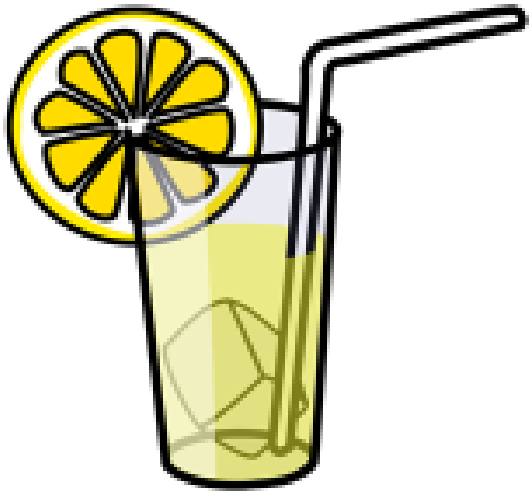
26. Bingham S, Cummings J. The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 h urine collections in man. Clin Sci. 1983;64(6):629-35.

27. Subar AF, Kipnis V, Troiano RP, Midthune D, Schoeller DA, Bingham S, Sharbaugh CO, Trabulsi J, Runswick S, Ballard-Barbash R, Sunshine J, Schatzkin A. Using Intake Biomarkers to Evaluate the Extent of Dietary Misreporting in a Large Sample of Adults: The OPEN Study. American journal of epidemiology. 2003;158(1):1-13. doi: 10.1093/aje/kwg092.

28. Tate DF, Turner-McGrievy G, Lyons E, Stevens J, Erickson K, Polzien K, Diamond M, Wang X, Popkin B. Replacing caloric beverages with water or diet beverages for weight loss in adults: main results of the Choose Healthy Options Consciously Everyday (CHOICE) randomized clinical trial. The American Journal of Clinical Nutrition. 2012;95(3):555-63. doi: 10.3945/ajcn.111.026278.

Beverage Consumption and Fine Motor Control

The purpose of this research study is to evaluate the effect of beverage consumption on fine motor control. Participants will consume beverages for 12 weeks, taking measurements every other week to assess change over time. Participants must be willing to consume up to 1.8 liters of non-carbonated beverages daily to participate in this study. Participants will be compensated $300.



Eligibility

- Age 18 to 60
- Overweight or obese
- Healthy
- Non user of diet beverages for > 6 months

Contact Kelly at beveragestudy@purdue.edu or visit http://cfs.purdue.edu/lsis.

Principal Investigator— Richard Mattes PhD RD MPH

Beverage Study
beveragestudy@purdue.edu
http://cfs.purdue.edu/lsis

***Exponent, Purdue Today* Advertisement:**

Beverage Consumption and Fine Motor Control: The purpose of this research study is to evaluate the effect of beverage consumption on fine motor control. Participants will consume beverages for 12 weeks, taking measurements every other week to assess change over time. Participants must be willing to consume up to 1.8 liters of non-carbonated beverages daily to participate in this study. Participants will be compensated $300.

Eligibility:

- Age 18-60
- Overweight or obese
- Healthy
- Non-user of diet beverages > 6 months
- Participants will be compensated

Contact Kelly by email at beveragestudy@purdue.edu

Principle Investigator: Richard Mattes, PhD RD MPH, Purdue University, Department of Nutrition Science

Aspartame and Glycemia

Revised 10/10 Ref. # _____

APPLICATION TO USE HUMAN RESEARCH SUBJECTS

Purdue University
Institutional Review Board

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1. Project Title: The effects of aspartame on appetite, body composition and oral glucose tolerance

2. Full Review ☒ Expedited Review

3. Anticipated Funding Source: Ajinomoto

4. Principal Investigator *[See Policy on Eligibility to serve as a Principal Investigator for Research Involving Human Subjects]*:

Name and Title	Department, Building, Phone, FAX, E-mail
Richard Mattes, PhD, M PH, RD	Nutrition Science
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	Phone: 765-494-0662
	Fax: 765-494-0674
	mattes@purdue.edu

5. Co-investigators and key personnel *[See Education Policy for Conducting Human Subjects Research]*:

Name and Title address	Department, Building, Phone, FAX, E-mail
Judy George, B.S., EMT	Nutrition Science
	Stone Hall, G4
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	georgej@purdue.edu
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7. The principal investigator agrees to carry out the proposed project as stated in the application and to promptly report to the Institutional Review Board any proposed changes and/or unanticipated problems involving risks to subjects or others participating in the approved project in accordance with the I-IRPP Guideline 207 Researcher Responsibilities, Purdue Research Foundation-Purdue University Statement of Principles and the Confidentiality Statement. The principal investigator has received a copy of the Federal-Wide Assurance (FWA) and has access to copies of 45 CFR 46 and the Belmont Report. The principal investigator agrees to inform the Institutional Review Board and complete all necessary reports should the principal investigator terminate University association.

Richard Matthys — 12/4/15

Principal lnvestigator Signature — Date

8. The Department Head (or authorized agent) has read and approved the application. S/he affirms that the use of human subjects in this project is relevant to answer the research question being asked and has scientific or scholarly merit. Additionally s/he agrees to maintain research records in accordance with the IRB's research records retention requirement should the principal investigator terminate association with the University.

Connie Weaver — NUTR SCI

Department Head *(printed)* — Department Name

Connie Weaver — 12/4/15

Department Head Signature — Date

APPLICATION TO USE HUMAN RESEARCH SUBJECTS

9. This project will be conducted at the following location(s): (please indicate city & state)
☒ Purdue West Lafayette Campus
☐ Purdue Regional Campus (Specify): _____
☐ Other (Specify): _____

10. If this project will involve potentially vulnerable subject populations, please check all that apply.
☐ Minors under age 18
☐ Pregnant Women
☐ Fetus/fetal tissue
☐ Prisoners Or Incarcerated Individuals
☐ University Students (PSYC Dept. subject pool ___)
☐ Elderly Persons
☐ Economically/Educationally Disadvantaged Persons
☐ Mentally/Emotionally/Developmentally Disabled Persons
☐ Minority Groups and/or Non-English Speakers
☐ Intervention(s) that include medical or psychological treatment

11. Indicate the anticipated maximum number of subjects to be enrolled in this protocol as justified by the hypothesis and study procedures: ___200_____

12. This project involves the use of an **Investigational New Drug** (IND) or an **Approved Drug For An Unapproved Use**.
☐ YES ☒ NO
Drug name, IND number and company: _____

13. This project involves the use of an **Investigational Medical Device** or an **Approved Medical Device For An Unapproved Use**.
☐ YES ☒ NO
Device name, IDE number and company: _____

14. The project involves the use of **Radiation or Radioisotopes**: DXA
☐ YES ☒ NO

15. Does this project call for: (check-mark all that apply to this study)
☐ Use of Voice, Video, Digital, or Image Recordings?
☒ Subject Compensation? Please indicate the maximum payment amount to subjects. $ 300
Purdue's Human Subjects Payment Policy Participant Payment Disclosure Form

☐ VO2 Max Exercise?
☐ More Than Minimal Risk?
☐ Waiver of Informed Consent?
☐ Extra Costs To Subjects?

☒ The Use of Blood? Total Amount of Blood 260 mL
Over Time Period (days) 12 weeks

☐ The Use of rDNA or Biohazardous materials?

☐ The Use of Human Tissue or Cell Lines?

☒ The Use of Other Fluids that Could Mask the Presence of Blood (Including Urine and Feces)?

☐ The Use of Protected Health Information (Obtained from Healthcare Practitioners or Institutions)?

☐ The Use of academic records?

16. Does investigator or key personnel have a potential financial or other conflict of interest in this study?

☐ YES ☒ NO

APPLICATION NARRATIVE

A. PROPOSED RESEARCH RATIONALE

Overweight and obesity are prevalent and nutritive sweetener ingestion has been implicated with weight gain and impaired glucose tolerance. Recent rodent studies have raised questions about the physiological effects of aspartame. This, in turn, has prompted calls by some to discourage aspartame ingestion and possibly reduced use by consumers. If the preliminary findings in rodents are true, the role of aspartame in a healthful diet will require reassessment. If the findings are not valid in humans, this should be made known to researchers, clinicians, policy makers and consumers who are attempting to moderate energy and sugar intake through the use of aspartame. With so few tools to aid weight loss and reduce nutritive sweetener intake, limiting use of a product that may be beneficial would not be appropriate.

B. SPECIFIC PROCEDURES TO BE FOLLOWED

- Screening Procedures

A total of 180 (60 per treatment) participants will be recruited through the Laboratory database and public advertisements. All will complete an online screening questionnaire http://www.cfs.purdue.edu/lsis/, (IRB approval #504002017). This electronic questionnaire will allow the researchers to assess if preset health and dietary eligibility criteria are met. Next, body weight, waist circumference, blood pressure and heart rate will be measured. Those meeting eligibility criteria and choosing to participate will sign an IRB approved consent form.

- Testing and Intervention

Participants will report to the laboratory in a fasted state. They will have an indwelling catheter placed in an arm vein and after a 10 minute rest period, a baseline blood draw will be taken for assessment of: Fasting glucose, HbA12c, GIP, GLP-I, Ghrelin,

Insulin, Leptin, Total cholesterol, LDL cholesterol, HDL cholesterol, Triglycerides, Gama-GTP, ALT, AST. Next, participants will ingest a 100ml bottle of glucola within 10 minutes and additional blood draws will be taken at 15, 30, 60, 90, 120,180 and 240 minutes. This will be followed by a body composition measurement (BodPodCl@, Life Instrument, Inc., Concord, CA). At the end of the session, participants will be instructed on the method for recording appetitive sensations over the waking part of a 24-hour period and collection of a 24-hour urine sample. The appetite ratings and urine collection will be conducted on the following day and returned to the laboratory the following day. All of these measurements will also be done at the end of the 12 week intervention. 24-hour urine sample and 24-hour appetite logs will also be completed at weeks 4 and 8.

Participants will be randomly assigned to one of the three parallel arms:

a) 0% (water) (N=60)
b) 5 mg/kg aspartame in a beverage (N=60)
c) 15mg/kg aspartame, 5mg/kg as a beverage and the balance in capsules (N=60).

Participants will consume their intervention product daily for 12 weeks. They will report to the laboratory weekly to pick-up their next week's supply of beverage powder, capsules if in that group) measurement of body weight and assessment of study progress.

PABA will be inextricably mixed with the beverage powder and added to the capsules. Analysis of 24-h urine samples for PABA recovery will allow documentation of intervention compliance. Urea nitrogen and creatinine will be measured on a COBAS 400 Plus analyzer. Urine samples will be collected at baseline to establish the PABA composition of each individual's customary diet and again at weeks 4, 8 and 12. Creatinine will be measured to ensure complete 24-hour samples are collected.

The beverage powder will be comprised of aspartame, PABA and Kool-Aid. The capsules will contain the same dry ingredients as the beverage. Each capsule will weigh about 1 gram. Four doses will be prepared and administered to participants according to their closest body weight. Doses will be:

Weight (KG)	Weight (LBS)	Height Range	ASP for 5mg/kg	ASP for 15mg/kg	ASP in Capsules	PABA (mg)
50	110	5ft Oin - 5ft 4in	250	750	500	240
60	132	5ft lin— 5ft 10in	300	900	600	240
70	154	5ft 7in — 6ft 4in	350	1050	700	240
80	176	6ft On — 6ft 4in	400	1200	800	240

c. SUBJECTS TO BE INCLUDED

- Males and females in roughly equal numbers
- Age 18-60 years
- BMI 18 -25 kg/m ^{2}
- Not taking medications that affect metabolism or appetite
- Non- or low-user of low calorie sweeteners, including aspartame • No reported aspartame sensitivity
- Non or light aspartame users
- Willing to comply with the study protocol
- Fasting blood glucose between 4.0 and 6.0 mmol/L (72 to 108 mg/dL) via capillary finger-stick

D. RECRUITMENT OF SUBJECTS AND OBTAINING INFORMED CONSENT

- Participants will be recruited through public advertisements on the Laboratory for Sensory and Ingestive Studies website: www.cfs.purdue.edu/lsis (IRB approval #504002017 and posted flyers (see attached). Advertisement in electronic and paper media may also be used (see attached). Those meeting the preset criteria described above will be contacted via their indicated preferred method (i.e., phone or email) to schedule a screening visit.

E. PROCEDURES FOR PAYMENT OF SUBJECTS

- Participants will receive a payment of $300.00 as compensation for any inconvenience caused by participating in this study. A partial payment of $50.00 will be made to participants should they withdraw or be withdrawn after baseline. They will receive payments of $100, $150 or $200 for withdrawal at 4, 6 or 8 weeks respectively.

F. CONFIDENTIALITY

- The records of participant progress in the study will be kept in a confidential file in a locked filing cabinet. The confidentiality of any computer record will also be carefully guarded by never including the participant's name on any data file. The information will be stored electronically in a password-protected file. A copy of the written consent form will be retained for three years after termination of the study at which time it will be destroyed. No information by which participants can be identified will be released or published. However, participants will be informed that to process their payments, it will be necessary to provide their name, social security number, and address to the university business office. In addition, participants will be notified that their research records may be reviewed by the National Institutes of Health and by Departments at Purdue University responsible for regulatory and research oversight.

G. POTENTIAL RISKS TO SUBJECTS

- The intervention compounds that participants will ingest are made of commercially available products and pose little foreseeable risk. PABA will be added to the beverages and consumed at a rate of 80 mg per day. There are few side effects associated with oral consumption of PABA (including upset stomach, nausea, and loss of appetite); however, these are usually experienced at higher doses, around 400mg per day. PABA has been utilized in other studies at this dose without side effects. All blood will be collected by an experienced technician using sterile techniques, but the procedure may result in a bruise, soreness and infection at the site of collection. The total amount ofblood collected over all test sessions will be less than the amount normally given at a blood donation.

H. BENEFITS TO BE GAINED BY THE INDIVIDUAL AND/OR SOCIETY

- There are no foreseeable direct benefits to participants. The knowledge gained from this study may provide new insights for the management of obesity and diabetes — some of the nation's most pressing public health problems.

1. INVESTIGATOR'S EVALUATION OF THE RISK-BENEFIT RATIO

J. WRITTEN INFORMED CONSENT FORM (to be attached to the Application Narrative)

See attached consent form

K. WAIVER OF INFORMED CONSENT OR SIGNED CONSENT

Not applicable

L. INTERNATIONAL RESEARCH

Not applicable

M. SUPPORTING DOCUMENTS (to be attached to the Application Narrative)

Recruitment Flyer
Multi-media advertisement

Aspartame Study

PI: Dr. Richard Mattes

Participants needed to study the effects of aspartame on appetite, body composition and oral glucose tolerance in adults.

Earn $300.00

Eligibility

Age 18 – 60

BMI 18-25

No reported aspartame sensitivity

Non or light aspartame users

Contact: Judy George at georgej@purdue.edu

IRB #: 1512016860

Aspartame Study georgej@purdue.edu
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APPENDIX B QUESTIONNAIRES

The following questionnaires were delivered through Qualtrics Survey Software.

Human Subject Research Application

Questions from the "Human Subject Research Application" that were utilized to screen participants and other demographic information are found below.

Thank you for your interest in research being conducted by the Laboratory for Sensory and Ingestive Studies.

1. Name:

_____

2. Gender:

- ○ Male (1)
- ○ Female (2)
- ○ Choose not to answer (3)

7. Date of birth:
(mm/dd/yyyy)

_____

8. Ethnic / racial background

- ○ Caucasian (Non-Hispanic) (1)
- ○ Asian or Pacific Islander (2)
- ○ Hispanic (3)
- ○ African American (4)
- ○ American Indian (5)
- ○ Other (enter below) (6) _____

9. Height:
(whole numbers only)

- ○ Height (feet) + (1) _____
- ○ Inches (2) _____

OR

- ○ Centimeters (1) _____

10. Weight:
(whole numbers only)

- ○ Pounds (1) _____

OR

- ○ Kilograms (1) _____

Do you plan on initiating a diet or exercise routine in order to loss weight in the next 12 weeks?

- ○ Yes, please specify (1) _____
- ○ No (2)

Do you currently use any of the following?

- [ ] Splenda (1)
- [ ] Sweet 'n Low (2)
- [ ] Equal (3)
- [ ] Stevia (4)
- [ ] No, I do not use these products (5)

Do you currently consume diet beverages (including diet soda, Crystal Lite)?

- ○ Yes (1)
- ○ No (2)

If so, how often do you consume diet beverages?

- ○ Never or less than 1x per week (1)
- ○ 1x per week (2)
- ○ 2-3x per week (3)
- ○ 4-6x per week (4)
- ○ 1x per day (5)
- ○ 2+ x per day (6)
- ○ 3+ x per day (7)

May we contact you for future studies?

- ○ Yes (1)
- ○ No (2)

If

If If so, how often do you consume diet beverages? 1x per day Is Selected
Or If so, how often do you consume diet beverages? 2+ x per day Is Selected
Or If so, how often do you consume diet beverages? 3+ x per day Is Selected

EndSurvey:

If yes, please indicate which of the following procedures may be acceptable or unacceptable:
ADMINISTRATION OF:

	Acceptable (1)	Unacceptable (2)	Choose not to answer (3)
Food/Diets (1)	○	○	○
Medication (2)	○	○	○
Exercise Programs (3)	○	○	○

QUESTIONNAIRES ON:

	Acceptable (1)	Unacceptable (2)	Choose not to answer (3)
Health (1)	○	○	○
Psychology (2)	○	○	○
Behavior (3)	○	○	○

MEASUREMENTS OF:

	Acceptable (1)	Unacceptable (2)	Choose not to answer (3)
Taste/smell (1)	○	○	○
Diets (2)	○	○	○
Height/weight (3)	○	○	○
Motor skills (4)	○	○	○
Body composition (5)	○	○	○
Energy expenditure (6)	○	○	○

COLLECTION OF:

	Acceptable (1)	Unacceptable (2)	Choose not to answer (3)
Blood (1)	○	○	○
Urine (2)	○	○	○
Saliva (3)	○	○	○
Feces (4)	○	○	○
Hair (5)	○	○	○
Breath (6)	○	○	○

12. Do you have or have you had any of the following?

	Never	Poorly-controlled	Well-controlled	Cured	Choose not to answer
a. Heart Attack	○	○	○	○	○
b. Stroke	○	○	○	○	○
c. Diabetes	○	○	○	○	○
d. Hypoglycemia	○	○	○	○	○
e. High blood pressure	○	○	○	○	○
f. Cancer	○	○	○	○	○
g. Anorexia	○	○	○	○	○
h. Bulimia	○	○	○	○	○
i. Psychiatric illness	○	○	○	○	○
j. Sickle cell anemia	○	○	○	○	○
k. Osteoporosis (Low bone density)	○	○	○	○	○
l. Hypothyroid	○	○	○	○	○
m. Hyperthyroid	○	○	○	○	○
n. Celiac disease	○	○	○	○	○
o. Other major illness (specify)	○	○	○	○	○

14. Are you currently pregnant and/or lactating?:

- neither (males, please choose this option) (1)
- Pregnant (2)
- Lactating (3)
- Both (4)
- Choose not to answer (5)

15. Are you using any forms of drugs or medications? (prescription, over-the-counter or the contraceptive pill)

- Yes (1)
- No (2)
- Choose not to answer (3)

Q18 If yes, please describe:

	Medication (e.g. Lanoxin)	Dosage & Frequency (e.g. 1 mg/ 2 times a day)	For how long (e.g. 4 years)	Reason for use (e.g. Fast heart rate)
(1)				
(2)				
(3)				

16. Do you have any allergies to medications, foods, or other substances?

- Yes (1)
- No (2)
- Choose not to answer (3)

If you answered "Yes", please provide list which items you are allergic to and describe your symptoms:
Which items are you allergic to?

_____

_____

What are the symptoms of your allergies?

_____

_____

19. Do you have any food sensitivities? (i.e. milk intolerance, heartburn)

- Yes (1)
- No (2)
- Choose not to answer (3)

If yes, please list the foods and the likely symptoms:

- Food (1) _____
- Symptoms (2) _____

20. Are you currently involved in any other research protocols?

- ○ Yes (1)
- ○ No (2)
- ○ Choose not to answer (3)

If yes, please list:

_____

_____

26. Do you regularly participate in physical activities?

- ○ Yes (1)
- ○ No (2)
- ○ Choose not to answer (3)

If yes, please specify:

	Type of activity	Typical times per week	Typical minutes per session	Since how many months ago?
(1)				
(2)				
(3)				
(4)				

27. Do you plan to start an exercise program in the near future?

- ○ Yes, within the next week (1)
- ○ Yes, within the next month (2)
- ○ No (3)
- ○ Choose not to answer (4)

33. Have you lost or gained more than 5 pounds in the last six months?

- ○ Yes (1)
- ○ No (2)
- ○ Choose not to answer (3)

If Yes:

- Lost (pounds): (1) _____
- Gained (pounds): (2) _____

35. Are you currently on a special diet? (low sodium, low cholesterol, low fat...)

- Yes (1)
- No (2)
- Choose not to answer (3)

If yes, what type of diet?

_____

- Self-prescribed (1)
- Healthcare Professional prescribed (2)

36. Do you currently practice any religious dietary restrictions?

- Yes (1)
- No (2)
- Choose not to answer (3)

If yes:
Which food(s) do you reject?

_____

_____

What is your reason for rejecting these food(s)?

_____

_____

37. Do you use any form of dietary supplements?
(Vitamins, minerals, protein powders...)

- ○ Yes (1)
- ○ No (2)
- ○ Choose not to answer (3)

If yes, please list below:

	Brand Name	Type of supplement	Dose	Frequency of use
(1)				
(2)				
(3)				

38. Do you have any abnormality of taste or smell?

- ○ Yes (1) _____
- ○ No (2)
- ○ Choose not to answer (3)

Three Factor Eating Questionnaire completed at this section of the survey. See "Three Factor Eating Questionnaire."

Beverage Consumption Habits Questionnaire completed at this section of the survey. See "Beverage Consumption Habits Questionnaire."

Three Factor Eating Questionnaire Please answer the following questions:	True	False
41. When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal. (1)	○	○
42. I usually eat too much at social occasions, like parties and picnics. (2)	○	○
43. I am usually so hungry that I eat more than three times a day. (3)	○	○
44. When I have eaten my quota of calories, I am usually good about not eating may more. (4)	○	○
45. Dieting is so hard for me because I just get too hungry. (5)	○	○
46. I deliberately take small helpings as a means of controlling my weight. (6)	○	○
47. Sometimes things just taste so good that I keep on eating even when I am no longer hungry. (7)	○	○
48. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat. (8)	○	○
49. When I feel anxious, I find myself eating. (9)	○	○
50. Life is too short to worry about dieting (10)	○	○
51. Since my weight goes up and down, I have gone on reducing diets more than once. (11)	○	○

	True	False
52. I often feel so hungry that I just have to eat something. (12)	○	○
53. When I am with someone who is overeating, I usually overeat too. (13)	○	○
54. I have a pretty good idea of the number of calories in common food. (14)	○	○
55. Sometimes when I start eating, I just can't seem to stop. (15)	○	○
56. It is not difficult for me to leave something on my plate. (16)	○	○
57. At certain times of the day, I get hungry because I have gotten used to eating then. (17)	○	○
58. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it. (18)	○	○
59. Being with someone who is eating often makes me hungry enough to eat also. (19)	○	○
60. When I feel blue, I often overeat. (20)	○	○
61. I enjoy eating too much to spoil it by counting calories or watching my weight. (21)	○	○
62. When I see a real delicacy, I often get so hungry that I have to eat right away. (22)	○	○

	True	False
63. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat. (23)	○	○
64. I get so hungry that my stomach often seems like a bottomless pit. (24)	○	○
65. My weight has hardly changed at all in the last ten years. (25)	○	○
66. I am always hungry so it is hard for me to stop eating before I finish the food on my plate. (26)	○	○
67. When I feel lonely, I console myself by eating. (27)	○	○
68. I consciously hold back at meals in order not to gain weight. (28)	○	○
69. I sometimes get very hungry late in the evening or at night. (29)	○	○
70. I eat anything I want, any time I want. (30)	○	○
71. Without even thinking about it, I take a long time to eat. (31)	○	○
72. I count calories as a conscious means of controlling my weight. (32)	○	○
73. I do not eat some foods because they make me fat. (33)	○	○

	True	False
74. I am always hungry enough to eat at any time. (34)	○	○
75. I pay a great deal of attention to changes in my figure. (35)	○	○
76. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods. (36)	○	○

Q53 77. How often are you dieting in a conscious effort to control your weight?

- Rarely (1)
- Sometimes (2)
- Usually (3)
- Always (4)

Q54 78. Would a weight fluctuation of 5 lbs affect the way you live your life?

- Not at all (1)
- Slightly (2)
- Moderately (3)
- Very much (4)

Q55 79. How often do you feel hungry?

- Only at mealtimes (1)
- Sometimes between meals (2)
- Often between meals (3)
- Almost always (4)

Q56 80. Do your feelings of guilt about overeating help you to control your food intake?

- Never (1)
- Rarely (2)
- Often (3)
- Always (4)

Q57 81. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?

- Easy (1)
- Slightly Difficult (2)
- Moderately Difficult (3)
- Very Difficult (4)

Q58 82. How conscious are you of what you are eating?

- Not at all (1)
- Slightly (2)
- Moderately (3)
- Extremely (4)

Q59 83. How frequently do you avoid "stocking up" on tempting foods?

- Almost never (1)
- Seldomly (2)
- Usually (3)
- Almost always (4)

Q60 84. How likely are you to shop for low calorie foods?

- Unlikely (1)
- Slightly unlikely (2)
- Likely (3)
- Very likely (4)

Q61 85. Do you eat sensibly in front of others and splurge alone?

- Never (1)
- Rarely (2)
- Often (3)
- Always (4)

Q62 86. How likely are you to consciously eat slowly in order to cut down on how much you eat?

- Unlikely (1)
- Slightly unlikely (2)
- Moderately likely (3)
- Very likely (4)

Q63 87. How frequently do you skip dessert because you are no longer hungry?

- Almost never (1)
- Seldomly (2)
- At least once a week (3)
- Almost every day (4)

Q64 88. How likely are you to consciously eat less than you want?

- Unlikely (1)
- Slightly Unlikely (2)
- Moderately likely (3)
- Very likely (4)

Q65 89. Do you go on eating binges though you are not hungry?

- Never (1)
- Rarely (2)
- Sometimes (3)
- At least once a week (4)

Q66 90. On a scale of 1 to 6, where 1 means no restraint in eating (eating whatever you want whenever you want it) and 6 means total restraint (constantly limiting food intake and never "giving in"), what number would you give yourself?

- 1. Eat whatever you want, whenever you want it (1)
- 2. Usually eat whatever you want, whenever you want it (2)
- 3. Often eat whatever you want, whenever you want it (3)
- 4. Often limit food intake, but often "give in" (4)
- 5. Usually limit food intake, rarely "give in" (5)
- 6. Constantly limiting food intake, never "giving in" (6)

91. To what extent does this statement describe your eating behavior?
"I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow."

- Not at all like me (1)
- A little like me (2)
- Pretty good description of me (3)
- Describes me perfectly (4)

Beverage Consumption Habits Questionnaire

In the past month, please indicate your response for each beverage type for "how often" and "how much each time."

1. Indicate how often you drank the following beverages. For example, if you drank 5 glasses of water per week, park 4-6 times per week.
2. Indicate the approximate amount of beverage you drank each time. For example, if you drank 1 cup of water each time, mark 1 cup under "how much each time."
3. Do not count beverages used in cooking or other preparations, such as milk in cereal.
4. Count milk added to tea and coffee in the tea/coffee with cream beverage category NOT in the milk category.

	How often?									How much each time?				
	Never or less than 1x per month (go to next beverage	1 x per month	2-3 x per month	1 x per week	2-3 x per week	4-6 x per week	1 x per day	2 x per day	3+ x per day	Less than 6 fl oz (3/4 cup)	8 fl oz (1 cup)	12 fl oz (1 1/2 cups)	16 fl oz (2 cups)	More than 20 fl oz (2 1/2 cups)
Water	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Water, flavored	○	○	○	○	○	○	○	○	○	○	○	○	○	○
100% fruit juice	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Vegetable Juice	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Regular Soda, soft drinks	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Diet Soda, soft drinks	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Whole Milk	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Reduced Fat Milk (2%)	○	○	○	○	○	○	○	○	○	○	○	○	○	○

	Never or less than 1x per month (go to next beverage	1 x per month	2-3 x per month	1 x per week	2-3 x per week	4-6 x per week	1 x per day	2 x per day	3+ x per day	Less than 6 fl oz (3/4 cup)	8 fl oz (1 cup)	12 fl oz (1 1/2 cups)	16 fl oz (2 cups)	More than 20 fl oz (2 1/2 cups)
Low fat/fat free milk (skim, 1%, buttermilk, soymilk)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Sweetened juice beverage/drink (fruit ades, lemonade, punch, Sunny Delight)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Diet sweetened drinks (fruit ades, lemonade, punch, Crystal Lite)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Sweet Tea	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Diet Sweet Tea	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Tea or Coffee, black (no cream or sugar)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Tea or Coffee, with cream or non-dairy creamer	○	○	○	○	○	○	○	○	○	○	○	○	○	○

	Never or less than 1x per month (go to next beverage	1 x per month	2-3 x per month	1 x per week	2-3 x per week	4-6 x per week	1 x per day	2 x per day	3+ x per day	Less than 6 fl oz (3/4 cup)	8 fl oz (1 cup)	12 fl oz (1 1/2 cups)	16 fl oz (2 cups)	More than 20 fl oz (2 1/2 cups)
Tea or Coffee, black, with low calorie sweetener (Splenda, Sweet 'n Low, Equal, Stevia)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Tea or Coffee, with cream or non-dariy creamer, with low calorie sweetener (Splenda, Sweet 'n Low, Equal, Stevia)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Beer, Ales, Wine Coolers (18)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Hard Liquor (shots, rum, tequila, etc.) (19)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Wine (red or white) (20)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Energy Drinks (Red Bull, Rockstar, etc.), regular (21)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Energy Drinks (Red Bull, Rockstar, etc.), diet (22)	○	○	○	○	○	○	○	○	○	○	○	○	○	○

	Never or less than 1x per month (go to next beverage	1 x per month	2-3 x per month	1 x per week	2-3 x per week	4-6 x per week	1 x per day	2 x per day	3+ x per day	Less than 6 fl oz (3/4 cup)	8 fl oz (1 cup)	12 fl oz (1 1/2 cups)	16 fl oz (2 cups)	More than 20 fl oz (2 1/2 cups)
Sports Drinks (Gatorade, Powerade, etc.) (23)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Sports Drinks (Gatorade, Powerade, etc.), diet (24)	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Other (25)	○	○	○	○	○	○	○	○	○	○	○	○	○	○

Check to ensure an amount is indicated for each beverage consumed

In the past month, please indicate your response for "how often" you consume each food type.

1. Indicate how often you ate the following foods. For example, if you ate 5 granola bars per week, mark 4-6 times per week.

2. If you typically eat a specific type of the particular food, please indicate by providing details in the text entry. Be as specific as possible (i.e. brand name, flavor, etc.)

	How often?								
	Never or less than 1 x per month	1 x per month	2-3 x per month	1 x per week	2-3 x per week	4-6 x per week	1 x per day	2 x per day	3+ x per day
Cheese	○	○	○	○	○	○	○	○	○
Desserts: cookies, cakes, pies, etc.	○	○	○	○	○	○	○	○	○
Fast food meals	○	○	○	○	○	○	○	○	○
Fruit, fresh	○	○	○	○	○	○	○	○	○
Fruit, canned/dried	○	○	○	○	○	○	○	○	○
Grains, pasta, rice	○	○	○	○	○	○	○	○	○

	Never or less than 1 x per month	1 x per month	2-3 x per month	1 x per week	2-3 x per week	4-6 x per week	1 x per day	2 x per day	3+ x per day
Granola, protein bars	○	○	○	○	○	○	○	○	○
Meat, fish,eggs, etc.	○	○	○	○	○	○	○	○	○
Nuts	○	○	○	○	○	○	○	○	○
Potatoes	○	○	○	○	○	○	○	○	○
Ready-to-eat cereal	○	○	○	○	○	○	○	○	○
Ready-to-eat mixed/frozen meals	○	○	○	○	○	○	○	○	○
Salad dressings and dips	○	○	○	○	○	○	○	○	○
Salty Snacks	○	○	○	○	○	○	○	○	○

	Never or less than 1 x per month	1 x per month	2-3 x per month	1 x per week	2-3 x per week	4-6 x per week	1 x per day	2 x per day	3+ x per day
Savory Snacks	○	○	○	○	○	○	○	○	○
Sugar free/low calorie sweetened foods	○	○	○	○	○	○	○	○	○
Sweet breads and pasteries	○	○	○	○	○	○	○	○	○
Sweet snacks	○	○	○	○	○	○	○	○	○
Vegetables	○	○	○	○	○	○	○	○	○
Yogurt, plain/unsweetened	○	○	○	○	○	○	○	○	○
Yogurt, sweetened	○	○	○	○	○	○	○	○	○

Baecke's Physical Activity Questionnaire
Please answer all questions as completely and honestly as possible.

Enter your participant number

_____

Q1 What is your main occupation?

- ☐ carpentry (1)
- ☐ clerical work (2)
- ☐ construction work (3)
- ☐ driving (4)
- ☐ factory worker (5)
- ☐ farming (6)
- ☐ housework (7)
- ☐ medical practice (8)
- ☐ plumbing (9)
- ☐ professional athlete (10)
- ☐ shopkeeping (11)
- ☐ studying (12)
- ☐ teaching (13)
- ☐ university student (14)
- ☐ other (15) _____

Q2 At work I sit

- never (1)
- seldom (2)
- Sometimes (3)
- Often (4)
- always (5)

Q3 At work I stand

- never (1)
- seldom (2)
- Sometimes (3)
- Often (4)
- always (5)

Q4 At work I walk

- never (1)
- seldom (2)
- Sometimes (3)
- Often (4)
- always (5)

Q5 At work I lift heavy loads

- never (1)
- seldom (2)
- Sometimes (3)
- Often (4)
- always (5)

Q6 After work I am tired

- very often (1)
- often (2)
- sometimes (3)
- seldom (4)
- never (5)

Q7 At work I sweat

- very often (1)
- often (2)
- sometimes (3)
- seldom (4)
- never (5)

Q8 In comparison of others of my own age, I think my work is physically

- ○ much heavier (1)
- ○ heavier (2)
- ○ as heavy (3)
- ○ lighter (4)
- ○ much lighter (5)

Q9 Do you play sports or participate in a form physical activity?

- ○ Yes (1)
- ○ No (2)

Q10 In comparison with others of my own age, I think my physical activity during leisure time is

- ○ much more (1)
- ○ more (2)
- ○ the same (3)
- ○ less (4)
- ○ much less (5)

Q11 During leisure time I sweat

- ○ very often (1)
- ○ often (2)
- ○ sometimes (3)
- ○ seldom (4)
- ○ never (5)

Q12 During leisure time I play sports

- ○ never (1)
- ○ seldom (2)
- ○ Sometimes (3)
- ○ Often (4)
- ○ very often (5)

Q13 What physical activity or sport do you play most frequently?

- aerobics (22)
- badminton (1)
- basketball (2)
- billiards (3)
- bowling (4)
- boxing (5)
- cycling (6)
- dancing (7)
- football (8)
- golf (9)
- high intensity interval training (HIIT) (21)
- rowing (10)
- rugby (11)
- running (12)
- sailing (13)
- soccer (14)
- swimming (15)
- tennis (16)
- weight training (17)
- yoga (20)
- I don't play sports (18)
- other (19) _____

Skip To: End of Block If What physical activity or sport do you play most frequently? = I don't play sports

Q26 Answer the following question regarding the sport you play most frequently from the previous question.

Q28{Q13/ChoiceGroup/SelectedChoices}

Q14 How many hours do you play a week?

- < 1 hour (1)
- 1-2 hours (2)
- 2-3 hours (3)
- 3-4 hours (4)
- > 4 hours (5)

Q15 How many months do you play in a year?

- < 1 month (1)
- 1-3 months (2)
- 4-6 months (3)
- 7-9 months (4)
- > 9 months (5)

Q16 What is your second most frequently played sport or physical activity?

- ○ aerobics (22)
- ○ badminton (1)
- ○ basketball (2)
- ○ billiards (3)
- ○ bowling (4)
- ○ boxing (5)
- ○ cycling (6)
- ○ dancing (7)
- ○ football (8)
- ○ golf (9)
- ○ high intensity interval training (HIIT) (21)
- ○ rowing (10)
- ○ rugby (11)
- ○ running (12)
- ○ sailing (13)
- ○ soccer (14)
- ○ swimming (15)
- ○ tennis (16)
- ○ weight training (17)
- ○ yoga (20)
- ○ I don't play sports (18)
- ○ other (19) _____

Skip To: End of Block If What is your second most frequently played sport or physical activity? = I don't play a second sport

Q17 Answer the following question regarding the sport you play second most frequently from the previous question.

Q29 {Q16/ChoiceGroup/SelectedChoices}

Q18 How many hours do you play a week?

- ○ < 1 hour (1)
- ○ 1-2 hours (2)
- ○ 2-3 hours (3)
- ○ 3-4 hours (4)
- ○ > 4 hours (5)

Q19 How many months do you play in a year?

- ○ < 1 month (1)
- ○ 1-3 months (2)
- ○ 4-6 months (3)
- ○ 7-9 months (4)
- ○ > 9 months (5)

Q20 During leisure time I watch television

- never (1)
- seldom (2)
- Sometimes (3)
- Often (4)
- very often (5)

Q21 During leisure time I walk

- never (1)
- seldom (2)
- Sometimes (3)
- Often (4)
- very often (5)

Q22 During leisure time I cycle

- never (1)
- seldom (2)
- Sometimes (3)
- Often (4)
- very often (5)

Q23 How many minutes do you walk and/or cycle per day to and from work, school, and shopping?

- < 5 minutes (1)
- 5-15 minutes (2)
- 15-30 minutes (3)
- 30-45 minutes (4)
- > 45 minutes (5)

Appetite Questionnaire
Participant Number

_____

How strong is your feeling of hunger?

Not at all Extremely

() 

How strong is your feeling of fullness?

Not at all Extremely

() 

How strong is your desire to eat?

Not at all Extremely

() 

How much food could you eat right now?

Not at all An extremely large amount

() 

How strong is your preoccupation with food?

Not at all Extremely

() 

How strong is your feeling of thirst?

Not at all Extremely

()	

How strong is your desire to eat something salty?

Not at all Extremely

()	

How strong is your desire to eat something fatty?

Not at all Extremely

()	

How strong is your desire to eat something sweet?

Not at all Extremely

()	

The shakiness of your hand is.....

Not at all Extremely

()	

How strong is your grip?

Not at all Extremely

()	

How itchy is your scalp?

Not at all Extremely

()	

Fine Motor Control Questionnaire
Please enter your participant number:

_____

Week

- ○ Baseline (1)
- ○ Week 2 (2)
- ○ Week4 (3)
- ○ Week 6 (4)
- ○ Week 8 (5)
- ○ Week 10 (6)
- ○ Week 12 (7)

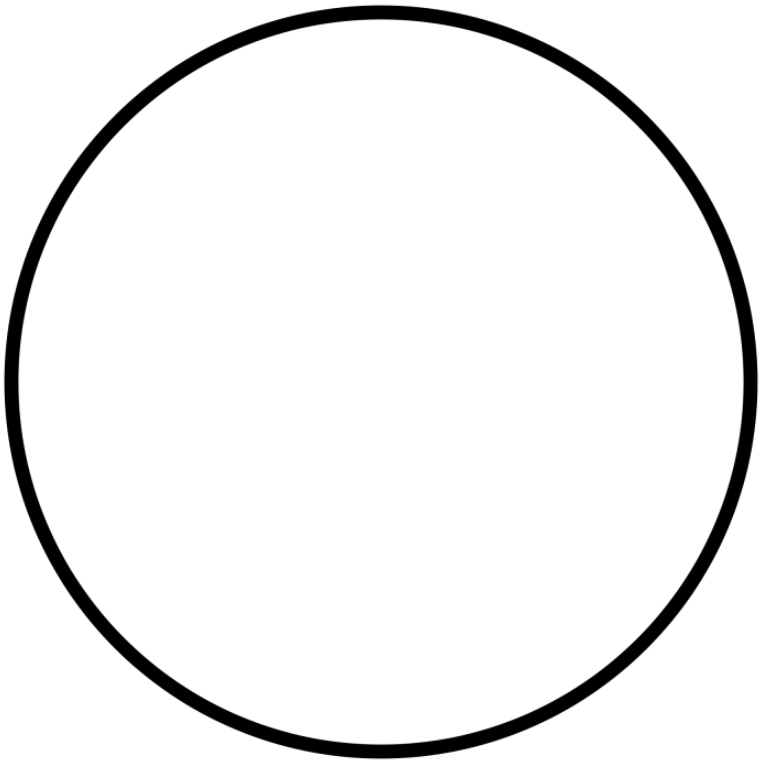
Q2 Please check all words that describe how you are currently feeling.

- ☐ Interested (1)
- ☐ Distressed (2)
- ☐ Excited (3)
- ☐ Upset (4)
- ☐ Strong (5)
- ☐ Guilty (6)
- ☐ Scared (7)
- ☐ Hostile (8)
- ☐ Enthusiastic (9)
- ☐ Proud (10)
- ☐ Irritable (11)
- ☐ Alert (12)
- ☐ Ashamed (13)
- ☐ Inspired (14)
- ☐ Nervous (15)
- ☐ Determined (16)
- ☐ Attentive (17)
- ☐ Jittery (18)
- ☐ Active (19)
- ☐ Afraid (20)
- ☐ None of the above (21)

Q14 Please check all words that best describe how you have felt the past 6 weeks.

- ☐ Interested (1)
- ☐ Distressed (2)
- ☐ Excited (3)
- ☐ Upset (4)
- ☐ Strong (5)
- ☐ Guilty (6)
- ☐ Scared (7)
- ☐ Hostile (8)
- ☐ Enthusiastic (9)
- ☐ Proud (10)
- ☐ Irritable (11)
- ☐ Alert (12)
- ☐ Ashamed (13)
- ☐ Inspired (14)
- ☐ Nervous (15)
- ☐ Determined (16)
- ☐ Attentive (17)
- ☐ Jittery (18)
- ☐ Active (19)
- ☐ Afraid (20)
- ☐ None of the above (21)

Q3 Please click 10 equally spaced points around the edge of the circle. Move around the circle in a **clockwise direction.**



Q27 Please click 10 equally spaced points around the edge of the circle. Move around the circle in a **counter-c**lockwise direction.

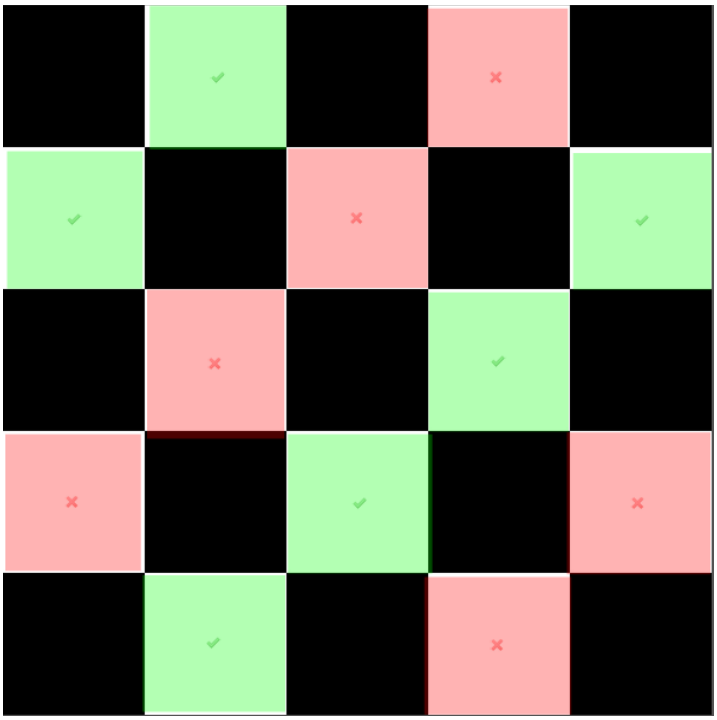
Q5 Please indicate how much you agree with the following statements regarding the past 6 weeks:

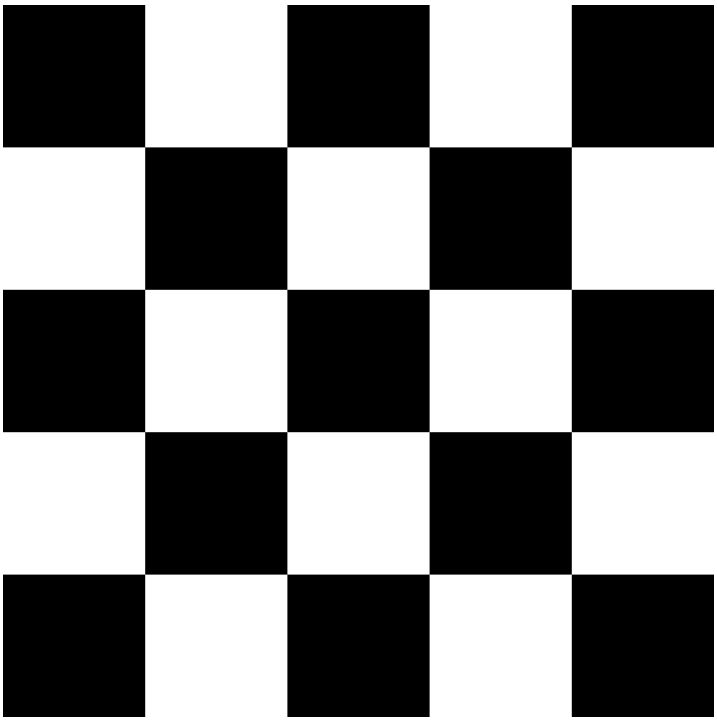
	Never	Rarely	Sometimes	Often	All of the Time
Have trouble maintaining balance (1)	○	○	○	○	○
Stumble will walking (2)	○	○	○	○	○
Cannot grasp door knobs (3)	○	○	○	○	○
Frequently drop pencils while writing (4)	○	○	○	○	○
Have no trouble opening jars (5)	○	○	○	○	○

Q13 Please answer the following questions regarding how you have felt over the past 6 weeks.

	Never	Rarely	Sometimes	Often	All of the Time
How often do you fidget or squirm with your hands or feet when you have to sit for a long time? (1)	○	○	○	○	○
How often do you feel overly active and compelled to do things, like you were driven by a motor? (2)	○	○	○	○	○
How often do you make careless mistakes wehn you have to work on a boring or difficult project? (3)	○	○	○	○	○

Q18 Replicate the image below by clicking once on the white box to make it appear green and twice to make the white box appear red. Complete this taste as quickly as possible with as few errors as possible.





Beverage Evaluation Questionnaire

Please begin drinking the beverage. You will answer 13 preliminary questions while consuming the beverage. Once you see the message:
"Answer the following questions after you have finished completely consuming the beverage," make sure you have finished drinking all of your beverage before moving on. Return the empty cup to the research personnel .

Please enter your participant number:
_____

Did you eat or drink anything within the past 2 hours of before your scheduled appointment?

- Yes (1)
- No (2)

If yes, please describe what you ate or drank. Was the previous meal...

- Breakfast (1)
- Lunch (2)
- Dinner (3)
- Snack (4)
- I did not eat 2 hours prior (5)

Describe what you ate or drank in as much detail as possible. Indicate brands, flavor, size of portions (i.e. 1 cup, 1 tablespoon). If you did not eat 2 hours prior, write NA

_____

Rate the beverage for the following characteristics

	Not at all — Extremely
Sweet	
Salty	
Sour	
Bitter	
Off flavor	
Mouth-drying	
Sweet aftertaste	
Bitter aftertaste	
Off flavor aftertaste	
Overall liking	

Answer the following questions after you have finished completely consuming the beverage. Return the empty cup to the research personnel.

Appetite Questionnaire completed at this section of the survey. See "Appetite Questionnaire."

Answer the following questions regarding how you have typically consumed the **first beverage of the day** over the past 2 weeks.

What time do you begin consuming the first beverage of the day?

_____

Do you consume the beverage with other foods or drinks? If yes, describe.

- Yes (1) _____
- No (2)

How long does it take to consume the beverage?

- < 1 minute (1)
- 1 to 3 minutes (2)
- 3.5 to 5 minutes (3)
- 5.5 to 7 minutes (4)
- 7.5 to 9 minutes (5)
- 9.5 to 11 minutes (6)
- 11.5 to 15 minutes (7)
- 15,5 to 20 minutes (8)
- 20.5 to 30 minutes (9)
- > 30 minutes (10)

When drinking the first beverage of the day...

	Not at all Extremely
Do you enjoy drinking this beverage? ()	
Do you have trouble consuming the entire beverage? ()	

Answer the following questions regarding how you have typically consumed the **second beverage of the day** over the past 2 weeks.

What time do you begin consuming the second beverage of the day?

_____

Do you consume the beverage with other foods or drinks? If yes, describe.

- Yes (1) _____
- No (2)

How long does it take to consume the beverage?

- < 1 minute (1)
- 1 to 3 minutes (2)
- 3.5 to 5 minutes (3)
- 5.5 to 7 minutes (4)
- 7.5 to 9 minutes (5)
- 9.5 to 11 minutes (6)
- 11.5 to 15 minutes (7)
- 15,5 to 20 minutes (8)
- 20.5 to 30 minutes (9)
- > 30 minutes (10)

When drinking the second beverage of the day...

	Not at all	Extremely
Do you enjoy drinking this beverage? ()		
Do you have trouble consuming the entire beverage? ()		

Answer the following questions regarding how you have typically consumed the **third beverage of the day** over the past 2 weeks.

What time do you begin consuming the third beverage of the day?

_____

Do you consume the beverage with other foods or drinks? If yes, describe.

- Yes (1) _____
- No (2)

How long does it take to consume the beverage?

- < 1 minute (1)
- 1 to 3 minutes (2)
- 3.5 to 5 minutes (3)
- 5.5 to 7 minutes (4)
- 7.5 to 9 minutes (5)
- 9.5 to 11 minutes (6)
- 11.5 to 15 minutes (7)
- 15,5 to 20 minutes (8)
- 20.5 to 30 minutes (9)
- > 30 minutes (10)

When drinking the third beverage of the day...

	Not at all — Extremely
Do you enjoy drinking this beverage? ()	
Do you have trouble consuming the entire beverage? ()	

What flavor of beverage would you like for not this week but the following week?

- ○ Lemonade (3)
- ○ Orange (4)
- ○ Mixed Berry (5)

Week 12 Questionnaire

Please enter your participant number:

_____

Three Factor Eating Questionnaire completed at this section of the survey. See "Three Factor Eating Questionnaire."

Beverage Consumption Habits Questionnaire completed at this section of the survey. See "Beverage Consumption Habits Questionnaire."

How do you think drinking these beverages influenced your fine motor control?

- ○ Improved fine motor control (1)
- ○ No change in fine motor control (3)
- ○ Decreased fine motor control (2)

How do you think drinking these beverages influenced your weight?

- ○ Caused me to gain weight (1)
- ○ No change in weight (2)
- ○ Caused me to lose weight (3)

What do you think your beverages were sweetened with?

- ○ Sugar (1)
- ○ Low calorie sweetener (2)
- ○ No sweetener (4)
- ○ Both sugar and low calorie sweetener in every beverage (3)
- ○ Some beverages were sweetened with sugar, some were sweetened with a low calorie sweetener (5)
- ○ Other (6) _____

Did you make any changes to your diet or exercise throughout the study besides the addition of the study's beverages? If yes, please explain. You may select more than one answer.

- ☐ Yes, I changed my diet (1) _____
- ☐ Yes, I changed my exercise routine (2) _____
- ☐ No, I did not change my diet or exercise (3)

Did this study alter your desire to eat sweet foods and/or beverages? You may select more than one answer.

- ☐ I had no change in my desire for sweet foods or beverages (5)
- ☐ I had an increased desire for sweet foods (2)
- ☐ I had an increased desire for sweet beverages (3)
- ☐ I had a decreased desire for sweet foods (4)
- ☐ I had a decreased desire for sweet beverages (6)

How strong was your change in desire for sweet foods/beverages? Drag the slider to indicate your response.

Desire decreased extremely	Desire decreased slightly	No change in desire	Desire increased slightly	Desire increased extremely

Q149 Please provide any additional comments regarding the beverages, measurements, side effects, study protocol, or complications completing the study. This section may be left blank.

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