

2022

Addressing the association between artificially sweetened beverages and obesity

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BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

**ADDRESSING THE ASSOCIATION BETWEEN ARTIFICIALLY
SWEETENED BEVERAGES AND OBESITY**

by

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B.S., Boston College, 2018

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science

2022

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ACKNOWLEDGMENTS

I would like to thank my thesis advisor, Dr. Stacey Zawacki, for all of her help and guidance during this writing process. I would also like to thank my incredible family, especially my mother and father, who made my educational journey possible and have always supported me and encouraged me to pursue my dreams. Special thanks as well to my fiancé, who has been my rock throughout my PA school journey and beyond.

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ABSTRACT

Obesity is a growing problem in the United States and excess sugar is thought to be a major contributor, leading to its growth over the previous decades. Artificial sweeteners began growing in popularity throughout recent decades, in part as a response to this. The inception of artificial sweeteners and their widespread use, however, has not led to a dramatic decrease in obesity rates. In fact, overweight and obesity rates continue to climb in the United States, despite the pervasive presence of these sweeteners in the food supply. This increase in overweight and obesity prevalence is likely due to many complex factors but some researchers believe that, although artificial sweeteners contribute little to no calories into the diet, they themselves could be promoting obesity in other ways, such as physiologically and behaviorally altering the way consumers respond to food. The first part of this thesis aims to explore the history of artificial sweetener use in the United States as well as the current research pertaining to the relationship between artificial sweetener use and obesity. The second part of this thesis proposes a study to further investigate the gaps in the literature surrounding artificial sweetener effects on the human body and brain and the mechanisms by which they may paradoxically promote overweight, obesity, and other adverse health effects.

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LIST OF ABBREVIATIONS

ABA.....	American Beverage Association
AC.....	Attention Control
ADI.....	Acceptable Daily Intake
AS.....	Artificial Sweeteners
ASB.....	Artificially Sweetened Beverage
BMI.....	Body Mass Index
BP.....	Blood Pressure
CDC.....	Center of Disease Control
CI.....	Confidence Interval
DB.....	Diet Beverage
DXA.....	Dual-Energy X-ray Absorptiometry
FDA.....	Food and Drug Administration
fMRI.....	Functional Magnetic Resonance Imaging
FU.....	Follow Up
GI.....	Gastrointestinal
GRAS.....	Generally Recognized as Safe
HDL.....	High Density Lipoprotein
IRB.....	Institutional Review Board
LCS.....	Low-Calorie Sweeteners
LDL.....	Low-Density Lipoprotein
NNS.....	Non-Nutritive Sweeteners

OW/OB_{inc}.....Overweight/Obesity Incidence
RCT.....Randomized Control Trial
SALSA.....San Antonio Longitudinal Study of Aging
SGFE.....*Siraitia grosvenorii* fruit extracts
SSB.....Sugar-Sweetened Beverage
TG.....Triglycerides
US.....United States
USB.....Unsweetened Beverage
WC.....Waist Circumference

INTRODUCTION

Background

Obesity is a growing problem in the United States, contributing to numerous negative health outcomes, including type II diabetes and cardiovascular diseases.^{1,2,3} Although the increase in obesity rates is multifactorial, one major contributor to the increased obesity prevalence in the United States is thought to be excess sugar intake.⁴ In response to this issue, artificial sweeteners have become popular in the U.S. in recent decades due to the increased public interest in dieting, as well as an increase in the marketing and production of these sweeteners.^{5,6} Artificial sweeteners provide a sweet taste with no or very few calories compared to sugar and are considered safe to consume within the daily intake levels set forth by the FDA.⁷ However, although no definitive links to toxicity of these sweeteners have been identified when consumed in accordance with FDA guidance, some research has found a link between artificial sweeteners and obesity, as well as other health complications.⁸⁻¹⁷ This link seems paradoxical - a substance with no or few calories that was meant to replace sugar and, thus, help combat the obesity epidemic potentially worsens the obesity epidemic. While some researchers have speculated that this apparent link could be due to reverse causality, since overweight or obese individuals already on a weight gain trajectory might gravitate toward using artificial sweeteners more so than non-obese individuals in an attempt to decrease caloric intake,^{13,18} some speculate that there may be more to this link than correlation alone.^{8,9,11} Many mechanisms have been proposed to try to explain this phenomenon in individuals who consume artificial sweeteners, such as altered behaviors towards food, altered

metabolism, increased appetite, and others.^{11,13-16,18-20} Although some observational studies have found a link between artificial sweetener use and obesity,^{8,9} other studies conclude that these artificial sweeteners may have the opposite effect and aid in weight loss²¹ while others find almost no effect at all.^{22,23} Therefore, there exists conflicting evidence in the literature for this topic, which requires clarity in order for consumers to make informed decisions on what they are consuming.

Statement of the Problem

There lacks sufficient proof to show a true link between artificial sweeteners and increased rates of overweight and obesity, as well as a definitive mechanism for this increase. Therefore, there is a need for more research to clarify this relationship. This thesis will explore this topic further and propose a study to investigate this issue, so that consumers can be more aware of what they are ingesting and make better, more informed decisions for their health. In addition, if these sweeteners are indeed harmful for humans to consume, public health and government agencies should be made aware so that they may properly regulate these substances.

Hypothesis

Although artificial sweeteners contribute a similarly sweet taste to foods as sugar with the benefit of having little or no calories, they cause changes in physiologic and behavioral responses to food, which over time promotes overweight and obesity.

Objectives and specific aims

The obesity epidemic in the United States is due, in part, to excess calories in the American diet, one of the main contributors being excess sugar. Artificial sweeteners are

currently being used widely as a sugar substitute but the actual health consequences of and relationship of these sweeteners to obesity are unclear.

This thesis aims to:

- Discuss the epidemiology of obesity in the United States, as well as the history of artificial sweetener use in the United States;
- Review the current research conducted on these sweeteners and their relationship to overweight and obesity, as well as discuss proposed mechanisms by which these sweeteners could contribute to obesity and their other potential effects on the human brain and body;
- Propose a study that could elucidate the relationship between artificial sweeteners on overweight and obesity and investigate mechanisms for their potential effects on the human brain and body.

REVIEW OF THE LITERATURE

Overview

Obesity is a growing problem in the United States. In 2017-2018, the prevalence of obesity among adults was 42.4%²⁴ and has been increasing over the last 50-60 years, up from 13.4% in 1960-1962.⁴ Obesity is associated with serious and costly health risks, such as type II diabetes, dyslipidemia, hypertension, cardiovascular disease, and even certain cancers.^{1,3} The United States alone spends about \$147-210 billion dollars every year on healthcare related to obesity.⁴ Although obesity is a multifactorial disease with many contributing factors, one major environmental and behavioral contributor in the United States has been postulated to be the abundance and overconsumption of sugar.⁴ Obesity and sugar consumption in the United States are interconnected; when sugar consumption increased drastically in the 1970's to the late 1990's, obesity rates skyrocketed in turn.⁴ In addition to this, there is a strong link between increased sugar intake and obesity in the literature.^{4,7,11}

Given the extreme toll of obesity on the health of individuals and its burden on the healthcare system, and how excess sugar consumption is likely one of its many causes, artificial sweeteners were considered a potential solution to this crisis. Artificial sweeteners have no or very few calories compared to sugar and produce a similarly sweet taste. Since the caloric content of foods sweetened with artificial sweeteners would be spared the calories from sugar, it seems intuitive that substituting these artificial sweeteners in place of sugar in some of the most commonly consumed sugar-sweetened

products, such as soft drinks (the leading source of excess sugar in everyday diet),⁴ would help solve the problem of excess calories from sugar and curtail obesity rates.

Artificial sweeteners, also called low calorie sweeteners (LCS), high-intensity sweeteners, or non-nutritive sweeteners (NNS), have been around since the late 19th century when the first LCS, saccharin, was discovered by accident in a laboratory.^{5,6,15} There are semantic differences in terminology when referring to these artificial sweeteners; per the FDA, non-nutritive approved high-intensity sweeteners (Acesulfame Potassium, Advantame, Neotame, Saccharin, SGFE, Steviol glycosides, Sucralose) contain less than 2% of the calories than an equivalent amount of sugar would have, whereas nutritive high-intensity sweeteners (Aspartame) contain at least 2% or more.²⁵ Two of the above mentioned sweeteners (SGFE and Steviol glycosides) are generally recognized as safe (GRAS) by the FDA, whereas the other sweeteners listed (Acesulfame Potassium, Advantame, Neotame, Saccharin, Sucralose, Aspartame) are considered food additives. The FDA has put forth acceptable daily intake (ADI) amounts in which these substances are considered safe to consume (Table 1).²⁵ For the purposes of this thesis, both nutritive and non-nutritive high intensity sweeteners will be grouped under the umbrella term, “artificial sweeteners”. Saccharin (found in Sweet’n Low)^{6,15} had previously only been used by diabetic patients and briefly in the general population during World War II due to sugar shortages and did not become widely used until newer artificial sweeteners such as aspartame (found in Equal and Nutrasweet),^{14,15} sucralose (found in Splenda),¹⁵ and acesulfame-potassium (found in Sunett and Sweet One)¹⁵ were discovered and approved as food additives by the FDA in the 1980s and 1990s.⁶ Due to

these new FDA approvals, increased marketing and availability of these sweeteners, and an increase in public interest in dieting at that time, these artificial sweeteners grew in popularity.^{5,6} Around the same time, sugar intake began to steadily drop among Americans, after hitting a peak in 1999.⁴ Artificial sweeteners became exceedingly popular in certain industries, such as the beverage industry. Diet soft drinks make up the largest percentage of artificial sweetener consumption in the world but artificial sweeteners can be found virtually everywhere, especially in food or beverage products branded sugar-free, lite, or diet.⁶ New artificial sweeteners are still being discovered today with varying degrees of sweetness compared to table sugar.²⁵ See Table 1 for a current list of artificial sweeteners approved by the FDA as food additives or GRAS, and their respective acceptable daily intake (ADI) values (Table 1).

Sugar consumption began to drop in the late 1990's to 2010's, after artificial sweeteners were approved and were gaining popularity. There was a slowdown in the growth of obesity during that time period, but it still increased steadily.^{4,14,15} The fact that artificial sweeteners are pervasive in the diet food industry and that obesity rates in the United States continued to climb raises some questions. First, are artificial sweeteners useful tools for weight loss? If so, why did obesity rates continue to grow when artificial sweeteners were gaining popularity, especially in the diet food market? And if artificial sweeteners are not effective tools for weight loss, could they actually be contributing to obesity in some way? Several studies have found a link between artificial sweeteners, obesity, and other adverse health outcomes,^{8-10,12,17} but these studies are observational studies. Observational studies may demonstrate correlation but they cannot prove

causation. In addition, some studies found the opposite effect - artificial sweeteners can actually lead to enhanced weight loss²¹ or have little to no effect on weight.^{22,23} So, the question remains- what are these chemicals doing to the bodies of their consumers, are they helpful for maintaining good health, or could they be harmful to consume? What are the potential mechanisms in which artificial sweeteners affect the health of their consumers? These questions become exceedingly more important today as artificial sweeteners seem to be penetrating the market in more places than just diet beverages; they can now be found virtually everywhere, including in frozen meals,¹⁴ yogurts,⁶ chewing gum,^{7,15} toothpaste,^{15,6} and even medicines.^{15,6}

There is certainly a gap in the literature to analyze the true effects of these substances on human health and obesity. This thesis will analyze the most prevalent literature on the topic, specifically the effect of diet beverages on health (since these are the most commonly consumed form of artificial sweeteners)^{14,26} and propose a study to provide insight in the areas where the science is lacking on this topic.

Existing research

What are some potential ways that artificial sweeteners could be effecting the health of consumers? First, it is important to note that different types of artificial sweeteners are processed differently when ingested. Some, like sucralose, are not metabolized at all and are excreted unchanged in feces or in urine, while others, like aspartame, are broken down in the small intestine.^{7,20} Given these differences among the various artificial sweeteners, each may affect the body differently and it may be inappropriate to generalize the consequences of some sweeteners to all sweeteners.

However, it is difficult to analyze the effects of individual artificial sweeteners as they often exist in combination in the foods that contain them and the combinations of artificial sweeteners found in products often change over time. Therefore, for the purposes of this literature analysis, studies were chosen which examined the effects of artificially sweetened soft drink consumption on health, since soft drinks are one of the most popularly consumed products that contain artificial sweeteners.^{14,26} See the appendix for search terms utilized to find the studies discussed in this thesis (Appendix 1).

Studies that Find an Association Between Artificial Sweetener Use and Obesity/Adverse Health Outcomes

Several observational studies have demonstrated a link between artificial sweeteners and obesity. In a study published in 2008, Fowler and colleagues observed a positive dose-response relationship between artificial sweetened beverage consumption and long-term weight gain in their large sample of San Antonio Heart Study participants, 25-64 years old.⁸ Participants in the study were grouped into quartiles depending on the amount of artificially sweetened beverages they consumed per week, BMIs were recorded at baseline and were re-measured when the cohort returned for follow-up visits after 7-8 years (Table 2). Incidence of overweight/obesity (OW/OB_{inc}), which the study defined as the percentage of participants who were normal weight at baseline who became overweight or obese by follow-up, and incidence of obesity (OB_{inc}), which the study defined as percentage of normal weight or overweight individuals who became

obese by their follow up visit, both demonstrated a dose-response relationship with artificial sweetener consumption (Table 3). A dose-response relationship between artificial sweetener consumption and increase in BMI was also observed after adjusting for baseline BMI and lifestyle/behavioral and demographic risk factors (Table 3). Overall, increases in BMI were 47% greater in participants who reported consuming any artificial sweetener compared to participants who didn't report consuming any (Table 3).⁸ Participants who reported consuming the most artificially sweetened beverages, 22 or more servings per week (quartile 4), had 78% greater increases in BMI than participants who did not consume artificial sweeteners. Participants who used artificial sweeteners but discontinued use during the study experienced a 59% lower increase in BMI than those who continued using artificial sweeteners throughout the study (1.03 kg/m² vs. 1.62kg/m², respectively, p =0.038).⁸ Limitations to this study include that the study is observational and, therefore, cannot definitively prove causation. Baseline data was self-reported and the amount of artificially sweetened beverages consumed was estimated based on this self-reported data and, therefore, the actual amount of artificial sweetened beverages consumed likely differed. In addition, since artificial sweeteners are so available and included in some foods and other products, those in the non-user group might have been unknowingly consuming some artificial sweeteners during the study. Strengths of this study were its large sample size and ability to follow participants over a long period of time (7-8 years). The dose-response results observed in this study, in addition to the adjustments done, strengthen the argument that these results are less likely to have happened by chance alone.⁸

In a separate study in 2015 of older adults, Fowler and colleagues observed another positive dose-response relationship between artificial sweetener use and increase in waist circumference.⁹ The cohort studied was recruited from the San Antonio Longitudinal Study of Aging (SALSA) and all participants were age 65 and older. Anthropometric measurements and beverage use were recorded at baseline and all follow up appointments over an average of 9.41 years (Table 2). Average diet soda intake was calculated from all participants and participants were then separated into 3 groups according to artificially sweetened beverage consumption frequency (Table 2). At baseline, diet beverage drinkers were more educated, more likely to live in suburban areas, and were more active.⁹ Despite all of these seemingly health-promoting advantages, these participants tended to have higher BMIs at baseline and larger waist circumferences. At the end of the study, interestingly, adjusted changes in BMI were minimal, yet statistically significant between each consumption group, and it was found that diet soda users had larger BMI increases compared to diet soda non-users (Table 3).⁹ However, change in waist circumference showed much more striking results and was higher for diet soda users even after adjustments, demonstrating a positive dose-response relationship (Table 3).⁹ Normal weight (BMI <25) individuals who drank diet soda experienced minimal BMI change compared to non-users. Overweight diet soda users (with BMI > 25) and obese diet soda users (BMI > 30) experienced a double and quadruple increase in waist circumference, respectively, compared to non-users (Table 3).⁹ This observation raises concerns for obese individuals who might gravitate more to diet sodas to decrease caloric intake, since for an unknown reason, obese diet soda users

seemed to experience the most dramatic gain in waist circumference in this study. This study raises other concerns as well, as increasing waist circumference, especially in older individuals (such as the population observed in this study), is linked to worse cardiovascular health and other morbidities.⁹ Limitations of this research include that it was strictly observational and cannot definitively prove causation. This study lacked a comprehensive collection of caloric intake data for participants and its data is, therefore, unadjusted for caloric intake. Diet soda intake/artificial sweetener consumption was estimated based on self-reported data and participant measurements and diet soda intake was only assessed at study visits (no daily beverage logs recorded); therefore, it is possible that participants drank more or less diet soda in-between visits than estimated. This study's population was 65 and older and, therefore, may not be generalizable to a younger population. Strengths of this study include its long-term follow up period of about 10 years and its representation of older individuals (over age 65) who potentially face greater health risks with increases in BMI and waist circumference.⁹

Based on the observational nature of the aforementioned studies, it is possible that residual confounding existed despite adjustments for some confounders (Table 3) and impacted the data interpretation. However, the dose-response increases in BMI and waist circumference that each study demonstrates strengthens the argument that artificial sweeteners may play a role in exacerbating the obesity epidemic, or at least that these sweeteners may not be aiding in combating this issue.^{8,9} These studies raise questions about the effects that artificial sweeteners may have on our health through an unexplained association between artificial sweeteners, obesity, and other health outcomes.

Studies That Show Positive or Neutral Health Outcomes from Artificial Sweetener Use

While the above observational studies demonstrated a potential link between artificially sweetened beverage intake and increasing obesity/waist circumference, some randomized clinical studies have reported data that contradict those results. In 2016, Peters and colleagues performed a randomized clinical trial (equivalence design) with 303 overweight and obese participants, randomly assigned to drink 24 oz. of bottled water or 24 oz. of artificially sweetened beverages daily for 1 year, all supplied by Coca-Cola, PepsiCo, and Dr. Pepper Snapple Group.²¹ All participants that were enrolled were regular consumers of diet sodas (3 or more per week) prior to the study. Participants in the water group were asked to abstain from drinking artificially sweetened beverages but were allowed to eat foods that contained artificial sweeteners. For the first 12 weeks of the study, all participants (including the water group) received weekly 60-minute weight loss cognitive-behavioral classes. For the latter 40 weeks of the study, participants attended monthly 60-minute group meetings led by psychologists or dieticians and weight measurements were taken. Participants kept food and activity diaries throughout the study. Waist circumference and other measurements were taken 3 times throughout the study (baseline, 12-weeks, 52 weeks; Table 2). At the end of the trial, those participants who completed the trial in the artificially sweetened beverage group lost more weight than the water group (-5.01 +/- 7.12 kg difference, $p < 0.001$) (Table 3).²¹ In addition, differences in waist circumference were seen between groups upon completion of the study, with the artificially sweetened beverage group having a greater reduction in

circumference than the water group (-4.50 +/- 1.16 cm difference, $p < 0.001$)²¹ (Table 3). The conclusion of this study was that artificially sweetened beverages were superior for weight loss than water, both after 12 weeks and 52 weeks.²¹ This study has several limitations. Two authors of the study received consulting fees from Coca-Cola, which raises the question of a conflict of interest, and the American Beverage Association (ABA) fully funded this study. It was stated that the ABA was not involved in study design, preparation, rollout, or interpretation.²¹ In addition, participants in the water group were not forbidden to avoid artificial sweeteners outright and could have still consumed these sweeteners elsewhere while participating in the study, potentially obscuring the results of the study. This study provided dietary counseling and guidance for participants, which may not be truly reflective of real-life scenarios, as many individuals in the real world may not have access to these resources and might render this study less generalizable. Advantages of the study included that participants kept daily logs to track beverage consumption and were also reported to keep logs of diet and exercise, which in theory could have helped maintain adherence to the study protocol.²¹

In 2012, Tate and colleagues performed a 3-arm, single-blind randomized clinical trial, which compared the replacement of sugar-sweetened beverages with artificially sweetened beverages or water over the course of 6 months.²³ Obese participants (N=318), aged 18 to 65, who routinely drank about 280 kilocalories of sugar sweetened beverages per day but were willing to substitute these beverages for water or sugar-free drinks during the study, if needed, were enrolled. The three arms of the study consisted of the diet beverage group, the water group, and the attention control (AC) group. All three

arms of the study received monthly dietary group counseling and monthly weigh-ins. All counseling session leaders were instructed to give the same information to all groups, except the substituted beverage arms were recommended to replace 2 servings of caloric beverages per day with either diet soda or water, depending on the arm of the study that was attending the meeting. Beverages for each study arm, except for the AC group who received no beverages or beverage substitution recommendations, were distributed at the monthly meetings. Anthropometric measurements were collected 3 times throughout the study, at baseline, 3 months, and 6 months (Table 2). All groups showed about the same amount of weight loss at 6 months and were not significantly different from each other (Table 3).²³ Waist circumference differences from the baseline to 6-month visit were statistically significant for each group but the losses were greater for the diet beverage and water group (Table 3).²³ Essentially, this study found an almost equivalent amount of weight loss for overweight/obese individuals in a weight loss program, regardless of the beverage group (the AC group reduced their caloric beverage consumption during the study, although not explicitly told to do so). However, the study found a more significant reduction in fasting blood glucose levels in the water group compared to the AC group which was not true for the diet soda group, even though weight loss was approximately equivalent.²³ The study additionally showed that participants in the diet beverage group had a higher likelihood of achieving 5% or greater weight loss from their baseline weight compared to the AC group, whereas the water group's odds for achieving 5% or greater weight loss was not significantly different from that of the AC group (Table 3).²³ Limitations of this study include that the data on diet, exercise, and beverage intake were

self-reported and infrequent (based on two 24-hour recalls during each assessment period) and, therefore, subject to being misreported. In addition, the study was a short duration (6 months) and, therefore, not able to demonstrate longer-term weight loss or health effects of its interventions. The study also recruited regular SSB drinkers and, therefore, the switch for these participants from soda to water, a drink with much less sweetness, may have been harder to adhere to during the study, possibly prompting non-adherence and potentially causing these participants to seek out other sweets in their diet to compensate. This study also provided dietary counseling and guidance for participants, which might render this study less generalizable as not all individuals in the real world have access to these resources.²³

In 2020, Ebbeling and colleagues performed a 12-month, randomized control trial where participants ages 18.5-40 who regularly consumed SSBs (at least one 12-ounce serving per day) were placed into 3 different groups: sugar sweetened beverage (SSB), artificially sweetened beverage (ASB), or unsweetened beverage (USB) groups.²² Participants' weights ranged from normal weight to obese (BMI 18.5 to 40 kg/m²) at baseline. Anthropometric measurements, as well as blood tests and telephone interviews to assess physical activity and dietary intake, were taken at baseline and at 12 months (Table 2). Biweekly telephone calls to encourage adherence to participant's specific study group intervention were also conducted. In contrast to the studies done by Tate and colleagues and Peters and colleagues, no group counseling/nutrition sessions were offered to participants throughout the study.²¹⁻²³ At the end of the study, it was found that there were no significant changes in weight or fat mass among the three study groups

(Table 3).²² There was also no significant changes in TG:HDL (the primary outcome of the study), LDL, or insulin sensitivity among groups.²² One interesting finding, however, occurred during data analysis when the cohorts were divided into tertiles based on trunk fat. Tertile 3, the tertile comprised of participants with the most trunk fat, gained more weight and fat mass when exposed to sugar sweetened beverages compared to diet beverages or unsweetened beverages (Table 3).²² However, the sample size of participants assigned to the SSB group in tertile 3 was small (n=17) and, therefore, it is possible this finding is spurious.²² Limitations of this study include its dependence on infrequent self-reported data (3 telephone call check-ins per assessment period) to assess dietary intake and physical activity and a relatively small sample size (around 60) included in each study group of SSB, ASB, or USB drinkers (Table 2).²²

The three randomized clinical studies listed above, all differing in some ways in terms of methods, have all come to the same basic conclusion: diet soda (a main vehicle in which artificial sweeteners are consumed)^{14,26} either has a beneficial effect in terms of weight loss or virtually no effect at all compared to water or calorie-free non-artificially sweetened beverages.²¹⁻²³ This is in sharp contrast to the two observational studies listed above, which both point in the direction that diet sodas may be harmful to individuals, especially those who are overweight and obese, as they are correlated in a positive dose-response relationship with weight gain and increase in waist circumference.^{8,9} The reasons for this disagreement in the data are unclear. As stated previously, observational studies cannot definitively prove causation and cannot, therefore, cannot definitively link diet soda/artificial sweetener consumption to weight gain or overweight/obesity. The

randomized clinical trial data above does not support the conclusions of the observational studies listed in this thesis; however, the randomized clinical trials have problems of their own. The length of the RCTs was limited, 6 months²³ to 1 year,^{21,22} whereas the observational studies by Fowler et al were nearly 10 years in duration.^{8,9} Therefore, it is possible that the effects of diet soda on overweight and obesity may take more time to witness. Two of the above RCTs, Peters et al and Tate et al, also provided dietary counseling and guidance for participants, which might render these studies less generalizable as not all individuals have access to these resources.^{21,23} The randomized clinical trial by Peters et al could be criticized for being fully funded by the ABA, which would benefit if their diet products were found to be superior than other drink choices for weight loss, although Peters and colleagues state that the ABA was not involved in the design, preparation, rollout, or interpretation of the study. Two of the authors of that study did, however, receive consulting fees from the Coca-Cola Company outside of the study (as stated in the disclosure section of the study) and these two authors were involved in the study's data analysis and data interpretation.²¹ All of the participants enrolled in studies by Tate and colleagues and Ebbeling and colleagues were regular consumers of sugar-sweetened beverages and, thus, might have had a difficult time adjusting to changing their caloric consumption habits during the study and may have increased caloric intake elsewhere in their diet to compensate.^{22,23} All of these issues make it difficult to decipher the real effect that diet soda and, more broadly, artificial sweeteners overall, have on weight gain, obesity, and other health problems. In addition,

the mechanisms behind which diet soda could be contributing to weight gain were not fully investigated in these studies.

Potential Mechanisms for Artificial Sweeteners to Contribute to Obesity and Poor Health

Outcomes

There are various theories of how artificial sweeteners may be contributing to obesity (or appear to contribute, see “Reverse Causality” below). Listed below are some of the most common. These mechanisms are not necessarily isolated and could exist in parallel with other mechanisms.

Reverse Causality

Some researchers have theorized that artificial sweeteners only appear to be linked to obesity because individuals who are overweight or obese, or otherwise on a weight-gain trajectory, may gravitate towards using artificial sweeteners more so than normal weight individuals as a means to limit calorie consumption and prevent further weight gain.^{8,10,13,21} However, contradictions to this claim cite data that adjusts for baseline BMI among other obesity markers and the results still find an association between weight gain⁸/increased waist circumference⁹ and artificial sweetener use.¹¹ Despite controlling for some of these factors, observational studies may still experience residual confounding and reverse causality is still a possible explanation for these findings.

Appetite Stimulation/Increased Palatability of Food

Another proposed mechanism as to how artificial sweeteners contribute to weight gain is that they stimulate appetite. One theory is that, since artificial sweeteners decouple sweet taste from caloric intake, this could trigger compensatory overeating to offset the low-to-no calories in artificially sweetened products.^{14,15} It has also been found that eating any kind of palatable food, such as salty soups, can also stimulate appetite, so this phenomenon may not be unique to artificial sweetened foods but rather to palatable substances with reduced caloric concentrations.¹⁸ Results of studies such as Peters and colleagues, where the water group reported greater subjective hunger than the artificial sweetener group, also refute claims that artificially sweetened products increase appetite.²¹

In addition, it has been postulated that just the sweet taste alone of artificial sweeteners can condition consumers to become accustomed to diets consisting of sweeter flavors and, therefore, encourage consumption of more sweet-tasting foods (which are often calorie-dense) to satisfy these cravings.¹⁴ However, cross-sectional studies investigating artificial sweeteners influencing dietary preferences have reported mixed results on this issue.¹³

There is some evidence that artificial sweeteners enhance the sensation of hunger when consumed alone but not when consumed alongside energy-containing foods.¹⁸ In addition, increased subjective appetite does not necessarily lead to increased caloric intake, as participants in several studies have demonstrated lower energy intake in artificial sweetener groups.^{18,20}

Overcompensating for “Saved Calories” and Resultant Overconsumption

In short-term studies, individuals who were aware that they were consuming artificial sweeteners increased their food intake but this occurred less in individuals who had been long-term users of artificial sweeteners.¹⁸ Interestingly, it has been found in some studies that individuals who consume artificial sweeteners unknowingly do not overcompensate with excess food intake and consume less calories overall.^{14,15,18} Therefore, it seems that individuals who are aware that they are consuming artificial sweeteners may have altered behaviors towards food, perhaps overestimating their caloric savings from these artificial sweeteners and overcompensating elsewhere in their diets, although some studies have cited lower energy intakes for these individuals.^{18,20}

Neurobiology of food reward

In a study by Green and Murphy, an artificial sweetener (saccharin) and a nutritive sweetener (sucrose) were administered to diet soda drinkers and non-diet soda drinkers and an fMRI scan was conducted to analyze brain activity. It was shown that diet soda drinkers had an altered reward response relative to the non-diet soda drinkers for both the nutritive and artificial sweeteners, specifically a decrease in caudate head activity in the brain (which helps modulate food intake), which can potentially lead to food overconsumption and obesity.¹⁹ Other researchers suggest that sweet taste, without the calories to accompany it, only partially activates the food reward pathways in the brain and leaves our bodies unsatisfied, increasing food-seeking behavior.^{14,20}

Altered Metabolic Signaling

Some researchers hypothesize that chronic consumption of artificial sweeteners essentially confuses the brain, making it less able to anticipate calories from sweet tastes,

and, therefore, leads to impaired metabolic signaling.^{11,13} In theory, this could also potentially lead to altered satiety signaling. This hypothesis remains to be widely tested and requires further studies to elucidate. Another theory is that artificial sweeteners potentially promote adipogenesis and impede lipolysis, leading to greater fat accumulation in the body. This theory is controversial, as other studies have shown contradictory results, therefore rendering this theory inconclusive.²⁷

Alterations in Gut Microbiota

Some theorize that alterations in the gut microbiota is to blame for the potential negative health effects that artificial sweeteners pose, since it is known that gut microbiome health plays a role in metabolism.²⁰ However, some commonly used artificial sweeteners in diet beverages such as Aspartame (currently used in Diet Coke, the most popular diet soda in the US in 2018,²⁸ as well as other present-day diet soda products) are not thought to directly alter gut microbiota because it does not interact with the lower GI system. Aspartame is metabolized in the small intestine into aspartic acid, phenylalanine, and methanol.²⁰ Methanol is metabolized by the liver and aspartic acid and phenylalanine are taken into the free amino acid pool to be used by bodily tissues for protein synthesis and metabolism and are later excreted. Therefore, aspartame is theorized not to alter gut microbiota, since its components are metabolized/utilized and later excreted before reaching the colon.²⁰ On an unrelated yet striking note, however, it has been recently confirmed that aspartame is a chemical carcinogen in rodents, leading to concerns for public health given its current widespread use.²⁹

Another popular artificial sweetener, Acesulfame Potassium, is not thought to alter the gut microbiome as it does not interact with the GI system. Following its consumption, it is almost immediately absorbed into the general circulation and within 24 hours is excreted by the kidney (over 99%), while less than 1% of it enters the GI tract to be excreted via feces.²⁰ Since it is absorbed so quickly into circulation, and since such a negligible amount of this sweetener is able to pass through the GI tract, it is thought to have no effect on the gut microbiota while consumed within FDA ADI parameters.²⁰

Other artificial sweeteners such as saccharin, which prior to 1985 was used to sweeten Diet Coke,³⁰ can potentially affect gut microbiota as about 5-15% of it is passed through the GI tract and excreted through feces unchanged, while the other 85-95% is absorbed in the systemic circulation and excreted in urine. Therefore, the small percentage that reaches the colon could theoretically interact and alter gut microbiota.²⁰

Sucralose can also potentially affect the gut microbiome and is thought to have bacteriostatic effects. Sucralose is not digested by the body and the majority of it passes through the GI system to be eliminated in the feces, while a smaller percentage (about 11-27%) is eliminated through the kidneys into urine. Therefore, since the majority of this sweetener passes through the GI tract to be excreted, it can potentially interact with and alter the gut microbiome composition.²⁰ One study by Thomson and colleagues investigated the short-term effects of high doses of sucralose (75% of ADI) on the human gut microbiome in 34 subjects (17 given sucralose, 17 given placebo) over 7 days. This study did not find any significant changes in the microbiomes of subjects in the sucralose group in that time frame in the healthy participants in the study. Interestingly, however,

the study found that participants who had higher insulinaemic responses had a higher Firmicutes:Bacteroidetes ratio, independent of which group they belonged to in the study.³¹

Another popular sweetener, steviol glycosides, is metabolized by colonic bacteria (primarily Bacteroides) and this direct contact with the gut microbiome can potentially lead to potential gut microbiome alteration.²⁰

Further research is needed to elucidate the full extent and significance of alterations of the gut microbiota by artificial sweeteners; however, many commonly used non-caloric sweeteners used in diet beverages in the United States may not have a strong potential to alter gut microbiota based upon the way they are processed by the human body.

Summary of Gaps in the Literature and Need for Future Research

The proposed study in the second part of this thesis will aim to fill those above gaps that exist in the literature. By studying a population that is overweight and obese that consumes 3 or more diet beverages per day, the proposed study will utilize the results shown from observational studies by Fowler et al, in which participants who drank the most diet soda experienced the greatest changes in BMI and waist circumference throughout the study.^{8,9} Therefore, if diet soda were to be having an effect on the study population in question, it would theoretically be easiest to see this effect in this population of high-volume consumers. In addition, a stool sample collection (modeled after the study by Thomson and colleagues),³¹ appetite questionnaire (modeled after

Peters et al),²¹ sweet taste preference test (modeled after the test performed in Ebbeling et al),²² fMRI (modeled after Green and Murphy's study),¹⁹ and DXA scan (modeled after the test performed in Ebbeling et al),²² will be collected. In doing so, the following proposed RCT will be investigating not only the occurrence of weight gain and waist circumference increase in the study population due to diet soda consumption but also the potential effects on the gut microbiome, hunger, sweet taste preference, food-reward brain response, and body composition/change in adiposity that participants may experience as a result of ceasing to consume diet soda over the course of 1 year. Therefore, the following proposed study might help to fill the gaps in the literature surrounding the effects that artificially sweetened beverages may have on their consumers. In other words, although two of the RCTs mentioned in this did not show a large change in weight between artificial sweetener beverage consumers and non-artificial sweetener beverage consumers over their short study duration (6 months to 1 year),²¹⁻²³ there exist other mechanisms that could cause physiologic and behavioral changes in consumers surrounding food over time that were not fully investigated in these studies and could help explain the differences in data between the long-term observational studies in the literature and short-term RCTs. These other mechanisms, once elucidated, may help explain the observed weight changes that were seen in studies by Fowler et al, which were longer in duration (~10 years),^{8,9} and help consumers of these beverages, and consumers of artificial sweeteners in general, become more aware of what they are consuming and how it may affect their bodies.

In this newly proposed RCT, individuals who are habitual, high-volume diet beverage consumers will be divided into two groups. One group will continue to drink diet beverages and will thus serve as a control group. The second group will cease to consume diet beverages and will instead be asked to replace diet beverages with water (may be flavored and carbonated, if desired, but will be calorie-free and not artificially sweetened). The study design will be modeled after Peters et al, since this study similarly compared the effects of diet soda to water on weight in their RCT in an equivalence design²¹ (in contrast, the other RCTs discussed in this thesis, Ebbeling et al and Tate et al, compared caloric beverages to artificially sweetened beverages and water).^{22,23} However, there will be several differences between the Peters et al study and this proposed study. First, there will be no weight loss intervention in the proposed study. Participants will not necessarily be trying to lose weight throughout the course of this study, and instead will only be altering their beverage choices (diet soda vs. water group). Therefore, it is likely that there will not be as dramatic of a weight change for participants during this study, as they will only be replacing one calorie-free beverage for another calorie-free beverage, unless the diet beverages themselves contribute to a weight change in some way (such as increased food-seeking behavior/altered metabolic signaling/altered reward response to food, overcompensation for saved calories, increased hunger, alterations in the gut microbiome, etc). Four 24-hour food recalls will be conducted throughout the study in order to estimate each study group's average daily caloric intake, and a physical activity questionnaire will also be obtained from participants to get an idea of each group's average calories burned from activity on an average week. This increase in frequency of

24-hour recalls and activity estimates compared to the other studies discussed in this thesis will work to reduce bias, as it will help account for changes in diet and activity throughout different seasons of the year.³² The proposed study will be monitoring BMI and waist circumference throughout its duration. However, this study will be most interested in investigating the physiologic and behavioral mechanisms behind which diet beverages and the artificial sweeteners within them could be effecting consumers, more so than weight gain and increased waist circumference alone, as the potential physiological and behavioral mechanisms in question may have more long-lasting effects on weight and health over time than short-term weight gain. This proposed study is looking to investigate several potential mechanisms in order to further elucidate the effects that artificial sweeteners may have on their consumers, and may help bridge the gap between the differences between the observational study data and the RCT data that currently exists in the literature on this topic. Since this study will not be funded by industry, it will also be free from any potential bias (publication bias or otherwise) that other studies could be criticized for having.

Table 1: List of Currently Approved Sweeteners by the FDA, Their Brand Names, Sweetness Compared to Table Sugar (sucrose), and ADIs. Adapted from table on FDA.gov website.²⁵

Sweetener	FDA Regulatory Status	Brand Names Containing Sweetener	Sweetness Compared to Sucrose	Acceptable Daily Intake Per FDA (mg/kg of bodyweight per day)	Number Of Sweetener Packets Equivalent to ADI (For a 60kg/132lb Person)
Acesulfame Potassium	Approved as a sweetener and flavor enhancer in foods generally (except in meat and poultry)	Sweet One® Sunett®	200x	15	23
Advantame	Approved as a sweetener and flavor enhancer in foods generally (except in meat and poultry)		20,000x	32.8	4,920
Aspartame	Approved as a sweetener and flavor enhancer in foods generally	NutraSweet® Equal® Sugar Twin®	200x	50	75
Neotame	Approved as a sweetener and flavor enhancer in foods generally (except in meat and poultry)	Newtame®	7,000-13,000x	0.3	23 (at sweetness intensity 10,000x sucrose)
Saccharin	Approved as a sweetener only in certain special dietary foods and as an additive used for certain technological purposes	Sweet and Low® Sweet Twin® Sweet'N Low® Necta Sweet®	200-700x		45 (at sweetness intensity 400x sucrose)
<i>Siraitia grosvenorii</i> Swingle (Luo Han Guo) fruit extracts (SGFE)	SFGE containing 25%, 45% or 55% Mogroside V is the subject of GRAS notices for specific conditions of use	Nectresse® Monk Fruit in the Raw® PureLo®	100-250x	Not specified	Not determined
Certain high purity Steviol Glycosides purified from leaves of <i>Stevia rebaudiana</i> (Bertoni)	≥95% pure glycosides Subject of GRAS notices for specific conditions of use	Truvia® PureVia® Enliten®	200-400x	4 (ADI established by the Joint FAO/WHO Expert Committee on Food Additives)	9 (at sweetness 300x sucrose)
Sucralose	Approved as a sweetener in foods generally	Splenda®	600x	5	23

Author	Type of study	Interventions	Population	Measurements
Fowler et. al (2008) ⁸	Prospective cohort study	N/A	San Antonio Heart Study participants age 25-64 (n= 3,371) in 2 cohorts: - Cohort 1 measured from 1979-1982 - Cohort 2 measured from 1984-1988 Follow up visits occurred 7-8 years later Mean Baseline BMIs (s.d.): - Non-AS users: 26.9 (5.3) - AS users: 27.9 (5.6)	- Change in BMI recorded at baseline and 7-8 year follow up - Exercising frequency recorded at baseline and 7-8 year follow up - In cohort 1 only, 24hr dietary recalls were recorded -In cohort 2 only, follow up artificial sweetener use was recorded -Participants grouped into quartiles depending on the amount of artificial sweetened (AS) beverages consumed per week (none, <3, 3-10, 11-21, 22+)
Fowler et. al (2015) ⁹	Prospective cohort study	N/A	San Antonio Longitudinal Study of Aging (SALSA) participants age 65+ (n= 466 included in at least FU1, n=375 included all 3 follow-ups) Mean Baseline BMIs: Non-users: 28.0 +/- 5.1 <1/day users: 29.0 +/- 5.3 >1/day users: 30.0 +/- 5.1	-Waist circumference -Fasting plasma glucose -kcal/week of leisure time activity -Dietary surveys -Height and weight measured at baseline (1992-1996), FU1 (2000-2001), FU2 (2001-2003), FU3 (2003-2004) -Diet soda intake separated into 3 categories (non-users, <1 per day, >1 per day)
Peters et. al (2016) ²¹	Randomized Clinical Trial (Equivalence design) Intent-to-treat analysis	Non- nutritive sweetened (NNS) beverage group vs. water group in 52-week study - Participants consumed 24oz NNS beverage or water per day for entire study duration - NNS beverages and bottled water provided free-of-charge to participants by The Coca-Cola company, Pepsi Co, and Dr. Pepper Snapple Group via manufacturer coupons - Water group instructed to refrain from all NNS beverages and artificial sweetener packets 12-week weight loss intervention: - Cognitive behavioral weight loss classes (The Colorado Weigh)	- Age 21-65 - Regular consumers of NNS (drinking 3+ NNS beverages/ week) but willing to stop if in water group - BMI 27-40 (N= 303 treated, 222 participants completed the study)	-Height measured at screening visit only -Weight, waist circumference x2, blood pressure x2, venipuncture for fasting blood sugar and lipids, and urine osmolality measurements conducted at baseline, 12-weeks, and 52 weeks -Hunger questionnaires at baseline and 12, 24, 36, and 52 weeks -Daily beverage logs

		<ul style="list-style-type: none"> - Weekly, 60-min sessions 40-week weight maintenance intervention: - The Colorado Weigh Monthly 60-min meetings led by dietitians /psychologists with weight measurements, dietary recommendations (25-35% cal from fat, personalized daily energy intake targets calculated), 6 days unsupervised exercise sessions (60 min daily recommendation, arm bands worn to track this)- same curriculum for NNS and water groups besides beverage recommendation 		
Tate et. al (2012)²³	<p>Randomized clinical trial</p> <p>3-arm, single blind, single center</p> <p>Intent-to-treat analysis</p>	<ul style="list-style-type: none"> - Participants substituted 2 servings/day (≥ 200 kcal) of caloric beverages for either water or diet beverages, or participants made their own dietary choices (attention control group) for 6 months - Monthly behavioral counseling meetings for diet beverage and water groups to encourage beverage adherence and to provide monthly supplies of beverages free-of-charge - AC group attended similar monthly behavioral counseling meetings/weigh-ins, and were given similar general weight-loss information as other groups, but were not given weight-loss calorie reduction goals, nor instructed to modify their beverage choices/consumption 	<ul style="list-style-type: none"> - Overweight and obese adults (n=318) - BMI 36.3 +/- 5.9 - Age 18-65 years - Regular consumers of sugar sweetened beverages (≥ 280 kcal/day) who were willing to substitute this with a different beverage during the study Diet beverage group (n=105), water group (n=108), AC group (n=105) 	<p>Taken at baseline after a 12-hr fast, at 3 months, and at 6 months:</p> <ul style="list-style-type: none"> - Body weight, height, waist circumference, resting BP x2, fasting blood samples, urine samples for osmolality - 2 unannounced 24hr dietary recalls performed via telephone at each assessment period - Energy expenditure measured by 7-day recall conducted after the first 24hr dietary recall

<p>Ebbeling et al (2020)²²</p>	<p>RCT</p>	<p>12-month intervention, 3 groups: Sugar Sweetened beverage (SSB), Artificially Sweetened Beverage (ASB), and Unsweetened beverage (USB) groups - Beverages were free of charge and delivered to participants with instructions to replace their usual SSB intake with these beverages - SSB group was instructed to not drink ASBs, ASB group was instructed not to drink SSBs, and USB group was instructed not to drink SSBs or ASBs</p>	<p>- Adults age 18-40 who habitually consume SSBs ≥ 1 12oz serving/day - BMIs 18.5 to 40 N=203 randomized; N=186 completed 12 month study: SSB (n= 60), ASB (n= 60), USB (n= 66)</p>	<p>Measured after a 12-hr fast at 2 visits at each time point (baseline, 12 mo): - Serum triglyceride to high-density lipoprotein cholesterol concentration - Blood levels of LDL, c-reactive protein, fibrinogen, uric acid, alanine aminotransferase, glucose, and insulin - Bodyweight and height - Whole body fat mass and trunk fat mass - Sweet taste preference -Unannounced check-in telephone calls (3 at baseline, 3 at 12 months) to assess physical activity and dietary intake pertaining to the day before the call - Biweekly check-in calls to encourage adherence to study group intervention</p>
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Table 2. Selection of Existing Research Regarding Artificial Sweetened Beverages and Obesity Discussed in Existing Research Section- Type of Study, Interventions, Population, and Measurements

Author	Results	Adjustments
Fowler et al (2008) ⁸	<p>Positive dose response relationship between Artificial Sweetener (AS) use and incidence of overweight/obesity (OW/OB_{inc})</p> <ul style="list-style-type: none"> For AS quartiles 1-4, respectively: 1.56 (1.02, 2.40, P = 0.041), 1.74 (1.10, 2.77, P = 0.018), 1.75 (1.09, 2.82, P = 0.021), and 1.93 (1.20, 3.11, P = 0.007) <p>Positive dose response relationship between Artificial Sweetener (AS) use and incidence of obesity (OB_{inc})</p> <ul style="list-style-type: none"> For AS quartiles 1-4, respectively: 1.34 (0.86, 2.08), 1.46 (0.96, 2.22, P = 0.075), 1.73 (1.13, 2.63, P = 0.011), and 2.03 (1.36, 3.03, P = 0.0005) <p>Positive dose response relationship between Artificial Sweetener (AS) use and increase in BMI</p> <ul style="list-style-type: none"> Average change in BMI: 1.01 (0.88, 1.14), 1.11 (0.85, 1.38), 1.46 (1.20, 1.73, P = 0.003), 1.50 (1.23, 1.78, P = 0.002), and 1.78 (1.51, 2.06, P < 0.0001) kg/m² for non-AS users and AS user quartiles 1–4, respectively AS users had 47% higher changes in BMIs than AS non-users (+1.48 vs. +1.01 kg/m², respectively, P < 0.0001) <p>No increases in BMI seen in participants who drank sugar-sweetened beverages</p>	BMI at baseline, age, ethnicity, gender, education level, socioeconomic index, baseline/interim change in exercise frequency, baseline/interim smoking status/cessation
Fowler et al (2015) ⁹	<p>Positive dose-response relationship between increasing diet soda intake and increasing abdominal obesity</p> <p>Change in waist circumference was significantly higher in diet beverage users (>1/day users experienced a ~4x increase in waist circumference from that of non-users)</p> <ul style="list-style-type: none"> Change in waist circumference (95% CI) for non-users, <1/day users, and >1/day users were 0.77 (0.29-1.23), 1.76 (0.96-2.57), and 3.04 (1.82-4.26) cm, respectively Normal weight (BMI <25) individuals who drank diet soda experienced minimal BMI change compared to non-users, 1.70 (0.68 to 2.72) and 1.92 (0.10 to 3.74), respectively, 0.22 difference P=0.833. Overweight diet soda users (with BMI > 25) had ~2x increase in waist circumference compared to non-users (BMI < 25), 1.19 (0.55 to 1.84) to 2.24 (1.38 to 3.10), respectively, a difference of 1.05 p=0.067 In obese diet soda users (BMI > 30), the increase in waist circumference was ~4x for diet soda users compared to non-users, -0.53 (-1.68 to 0.62) and 1.53 (0.19 to 2.87) respectively, a difference of 2.06 (0.20 to 3.93) p=0.031 <p>No consistent relationship seen between increase in waist circumference and regular-sugar beverage drinkers</p> <p>Point estimates for changes in BMI (95% CI) were minimal overall but least amongst non-users [-0.41 (-0.57 to -0.25) kg/m²], slightly higher amongst <1/day users [-0.11 (-0.38 to 0.16) kg/m²], and largest for >1/day users [0.05 (-0.35 to 0.45) kg/m²; p=0.043 for >1/day vs. non-users; p=0.049 for trend]</p>	At baseline: interval-change analyses were adjusted for sex, ethnicity, years of education, and residential neighborhood <u>At each follow up interval:</u> age, WC/BMI, diabetes status, kcal/wk of leisure-time activity, smoking status, as well as length of follow-up interval were recorded
Peters et al (2016) ²¹	<p>Water vs. NNS treatments found to be non-equivalent, NNS being superior for weight loss (Satterwaite two-sample t-test)</p> <ul style="list-style-type: none"> Participants who completed the trial in the artificially sweetened beverage group lost more weight than the water group, -8.39 +/- 7.79 kg, p<0.001 compared to -3.39 +/- 6.63 kg, p<0.001, respectively 44.2% of NNS participants lost at least 5% of their starting weight, compared to only 25.5% of water group participants (P<0.001) NNS group experienced a larger difference in waist circumference at week 52 (-8.67 +/- 0.80, P<0.001) compared to water group (-4.17 +/- 0.83, P<0.001) 	N/A

	<ul style="list-style-type: none"> • Difference in urine osmolality and mean caffeine consumption not statistically significant between NNS and water groups • Both NNS and water groups had a decrease in total cholesterol, LDL cholesterol, and triglycerides; both groups had increases in HDL cholesterol • Total amount of moderate/vigorous activity increase not significantly different between NNS and water groups <p>Water group reported more subjective hunger at week 52 vs. NNS group</p>	
Tate et. al (2012) ²³	<p>All 3 groups lost weight at 3 and 6 mo (all P < 0.01); the DB group and the Water group did not differ from the AC group at either time point measurement</p> <p>Percentage Weight Loss:</p> <ul style="list-style-type: none"> • -1.34% +/- 0.27 in AC group, -1.87% +/- 0.32 in diet beverage group, and -1.31% +/- 0.27 in water group at 3 mo • -1.76% +/- 0.35 in AC group, -2.54% +/- 0.45 in diet beverage group, and -2.03% +/- 0.40 in water group at 6 months <p>Participants in the diet beverage group had a higher likelihood of attaining a 5% weight loss than did the AC group (OR: 2.29; 95% CI: 1.05, 5.01; P = 0.04), but the water group did not have a significantly different OR compared to the AC group (OR: 1.87; 95% CI: 0.84, 4.14; P = 0.13).</p> <p>19.5% (n = 42) in the diet beverage group and water group combined achieved a 5% loss compared with 10.5% (n = 11) in the AC group</p> <ul style="list-style-type: none"> • Participants in the diet beverage group and water group attended significantly more monthly meetings compared to AC group • Diet beverage group and water group had significantly more reductions in caloric intake from beverages compared to AC group • All groups showed significant reductions in energy intake from food from 0-6 months (P < 0.0001) <p>Waist circumference loss from the baseline to 6-month visit of the AC group, diet beverage group, and the water group went from 116.5 to 115.9 (p=0.0107); 115.5 to 113.4 (p=0.0103); and 115.1 to 113.1 (p=0.0143), respectively</p>	Exclude for blood pressure and diabetes medication use
Ebbeling et al (2020) ²²	<p>Change in bodyweight and fat mass did not differ among groups:</p> <ul style="list-style-type: none"> • Mean change in body weight from baseline to 12 months was +1.2 +/- 0.6kg for SSB group, +0.6 +/- 0.6kg for ASB group, and 0.7 +/- 0.5kg for USB group, P for difference between groups = 0.66 • Mean change in whole-body fat mass from baseline to 12 months was +1.0 +/- 0.5kg for SSB group, +0.1 +/- 0.5kg for ASB group, and 0.1 +/- 0.4kg for USB group, P for difference between groups = 0.27 <p>Individuals with central adiposity gained more weight drinking SSBs than others:</p> <p><u>Effect Modification by Baseline Trunk Fat:</u></p> <ul style="list-style-type: none"> • Change in body weight (kg) in Trunk Fat Tertile 3 (most trunk fat): SSB group 4.4 +/- 1.0 P<0.001, ASB group 0.5 +/- 0.9 P=0.60, USB group -0.2 +/- 0.9 P=0.84 • Change in whole-Body fat mass (kg) in Trunk Fat Tertile 3 (most trunk fat): SSB group 2.8 +/- 0.8 P=0.001, ASB group -0.1 +/- 0.8 P=0.88, USB group -1.1 +/- 0.8 P= 0.16 <p>Replacing SSBs with ASBs or USBs had no significant cardio metabolic effects in this trial (no statistically significant changes in TG:HDL-C, LDL-C, insulin sensitivity, weight, or whole-body fat mass between study groups)</p> <p>USBs > ASBs for decreasing sweet taste preference</p>	Sex, ethnicity, race, age

Table 3. Selection of Existing Research Regarding Artificial Sweetened Beverages and Obesity Discussed in Existing Research Section- Results and Adjustments

METHODS

Study design

The proposed study will be a single-blind randomized control trial among individuals who are habitual, high-volume diet beverage consumers to compare 2 treatment arms: a diet beverage group who will be instructed to drink at least 3 study-provided diet beverages per day and a water group who will be instructed to drink at least 3 servings of bottled water per day provided by the study (may be flavored and carbonated, if desired, but will be calorie-free and not artificially sweetened) and refrain from diet beverage consumption.

Study population and sampling

The study population for this trial will be composed of consenting participants age 18 to 65, who will be recruited from primary care clinics in the Boston area. The participants will be “overweight” or “obese”, as defined by the CDC as having a BMI of 25 to $<30 \text{ kg/m}^2$, or $>30 \text{ kg/m}^2$, respectively. Exclusion criteria include if participants are not regular artificially-sweetened beverage consumers (for the purposes of this study, defined as consuming 3 or more diet beverages daily); if they have any medical comorbidities such as diabetes, heart disease, cancers, or other chronic illness; if the participant is unwilling to consume their assigned beverage in the assigned volume per day; or if the participants have had any recent significant weight changes ($>10 \text{ lbs}$) in the last year. The design of this study will be modeled after Peters et al, since this study showed the most dramatic response of participants to artificially sweetened beverages in all of the above analyzed studies. Sample size appropriate for this study will be

approximately 24 patients in each group for a total of 48 participants, using 80% power, effect size of 4.84, an S of 5.8, and an alpha of 0.05. The S value for this calculation was taken from a meta-analysis by Graham et al, looking at 21 studies where a small change approach was taken to achieve weight loss.³³ The S value used was the largest and SD seen in the study (5.8) and therefore will make for the most conservative S value for this study.³³ The effect size for this calculation was taken from the RCT by Peters et. al, which used a cut off value of 2.2kg (4.84 lbs.) to assess for equivalence of diet beverages and water for weight loss.²¹

Intervention

The intervention in this study will consist of replacing diet beverages with water, in a population that drinks diet soda regularly (>3 servings per day), or continuing to drink diet sodas habitually without change. This intervention will take place over the course of 12 months. Subjects will not be blinded to their beverage groups but researchers will be blinded to which intervention group each subject belongs to. Compliance with beverage assignment will be assessed via daily text questionnaire, sent each evening to subjects in both diet beverage and water groups, where participants will log whatever beverages they consumed on that day. The study will provide bottled water to water group participants, as well as diet beverages to the diet beverage group (choices of flavors will be given to participants in both groups and water group participants can elect to drink carbonated water). Participants will participate in weigh-ins, waist circumference measurements, blood pressure measurements, and urinalyses every 4 months. Participants will also be called on a random day every 4 months to provide a 24-

hour food recall and a 7-day physical activity recall, in order to capture dietary intake and activity for participants throughout the different seasons of the year.³² Participants will be asked at the first study visit and last study visit to have bloodwork drawn to measure a lipid panel and A1c, and will be asked to provide a stool sample at these visits (to assess gut microbiota). Participants will be asked to undergo sweet taste preference testing, fill out an appetite questionnaire, undergo a DXA body composition scan, and have an fMRI completed at the first and last study visits as well.

Study variables and measures

The independent variable of the study will be the amount of diet beverages or water consumed by participants in both groups. The dependent variables that will be examined will be weight, waist circumference, blood pressure, urinalysis (to assess for hydration status, as this can affect weight), lipid panel (to assess TG:HDL ratio, as this has been shown to be an independent predictor of adverse cardiovascular events and all-cause mortality),²² A1c (to measure overall glycemic control and screen for diabetes), stool sample (specifically measuring Firmicutes:Bacteroidetes ratio, as a higher ratio of these bacteria has been seen to be associated with obesity and type II diabetes),^{20,31} sweet taste preference testing (see “Data collection” section for details), appetite questionnaire (see “Data collection” section for details), DXA scan data (to measure body composition and adiposity), and fMRI data (specifically activity of the orbitofrontal cortex, lentiform nucleus, dopaminergic midbrain, right amygdala, and caudate head) to a 0.64M solution of sucrose (modeled after a study by Green and Murphy).¹⁹ The primary end point of the study is to see if ceasing regular diet beverage consumption in a habitual diet beverage

consuming individual will decrease incidence of overweight and obesity and waist circumference. Secondary endpoints will be to measure if lipid panel, A1c, sweet taste preference, appetite, gut microbiota (measured via stool sample), DXA body composition scan, and brain response to food reward (fMRI data) change in response to change in beverage consumption from baseline visit to end visit. These secondary endpoints have been chosen to elaborate on the potential mechanisms in which artificial sweeteners may impact the human body (see “Potential Mechanisms for Artificial Sweeteners to Contribute to Obesity and Poor Health Outcomes” section for details).

Recruitment

Subjects will be recruited from primary care clinics in the Boston area via fliers and provider referrals. If a potential participant is interested, they will then be screened for eligibility by members of the clinical research team (see “Study Population and Sampling” for eligibility criteria)

Data collection

Upon successful verification of eligibility for the study, participants will be consented to the study and will undergo an initial baseline visit consisting of a weigh in, waist circumference measurement, blood pressure measurement, urinalysis, and fasting blood draw to assess lipid panel²² and A1c. The participants will also be mailed a stool sample collection kit with instructions prior to this visit, which they will be asked to complete and bring to the baseline visit, in order to measure gut microbiota.^{20,31} The participants will also be given a sweet taste preference assessment, where they will be asked to taste 10 different samples of sucrose in water ranging from 0% to 18% sucrose

concentration, and use a 10cm visual analog scale to rate the samples from 0, “not sweet at all” to 10, “extremely sweet,” and rate their favorite sample concentration (modeled after the test performed in Ebbeling et al, to assess sweet taste perception/preference).²² Participants will also undergo a DXA body composition scan to measure adiposity (modeled after the test performed in Ebbeling et al),²² complete an appetite questionnaire while fasting where participants will be asked to rate their subjective hunger from “not hungry at all” to “extremely hungry” using a 100cm visual analog scale (modeled after Peters et al),²¹ family medical history questionnaire, and an fMRI scan to assess brain-reward response to a 0.64M solution of sucrose (modeled after a study by Green and Murphy).¹⁹

After this initial baseline visit, participants will attend weigh-ins and waist circumference measurements, BP measurements, and urinalyses every 4 months, as well as randomly be contacted every 4 months for a 24-hour food recall and 7-day physical activity recall (modeled after those conducted in Tate et al).²³

At the 12-month exit visit, participants will also be asked to provide another stool sample to assess for microbiome changes from baseline, and fasting blood sample to test lipids and A1c. They will also answer an appetite questionnaire, undergo another sweet taste preference test, and a second fMRI and DXA scan in order to compare data from the 12-month visit to baseline data.

Data analysis

The primary outcome of the study will be to determine changes in weight and waist circumference between the two study groups (diet beverage and water). This study will

be designed similarly to Peters et al, but with a hypothesis that there will be a significant weight difference between the two study groups. The bounds of equivalence was specified in Peters et al as (+/- 2.2 kg) and this will be used as the expected effect size. Therefore, the mean difference for weight gain would need to be significantly greater than 2.2kg in order for the diet beverage group and the water group to be considered different. An independent two sided t-test with 95% confidence intervals will be used to analyze data. To determine the impact that diet beverages have, if any, on secondary outcomes of blood pressure, lipid panel, A1c, gut microbiome, body composition, sweet taste preference, appetite, and reward centers of the brain, baseline tests will be compared to tests measured at the exit visit in 12 months with linear mixed effect models. Data will be stratified based on age, baseline BMI, sex, beverage type, and amount of beverage consumed during this study.

Timeline and resources

The duration of intervention for this study will be 12 months. Prior to beginning this study, time will be needed to recruit subjects, research team staff, and for approval by the IRB. Due to these requirements, it is likely that an extra 4-6 months should be built into the study timeline. Because of the large number of participants in this study and the suspected limited amount of available fMRI machines and DXA scanners in the Boston area, start times for participants will likely need to be spread out over the course of the study. The total time it will likely take to collect data for this study from start to finish will be 2-3 years. An additional 1-3 years will likely be needed for data analysis.

Clinical research staff will consist of one primary investigator and 5-7 other support personnel (physicians, physician assistants, nurse practitioners, nurses, and/or research assistants) who are capable of recruiting participants and meeting with participants each month to collect the necessary data from participants (weight, waist measurements, urinalyses, blood draws, BP measurements, stool sample collections, questionnaires, sweet taste preference tests). A registered dietician will also be present during the study to perform 24-hour food recalls and 7-day physical activity recalls, in order to increase the validity of the data collected. This study will also require fMRI machinery and technicians to perform scans for each participant at baseline and after 12 months of study intervention, and a medical professional (physician, nurse practitioner, or physician assistant) to interpret the results. In addition, laboratory personnel who are able to analyze blood samples, stool samples, and urinalyses will be required.

Institutional Review Board

The proposed study requires the participation of human subjects, and therefore requires review and approval from the Institutional Review Board of Boston University Medical Center. A full board application will be filed, as some participants in this study are being asked to continue to consume a dietary additive with unknown risks. Once approved, participants may be recruited and randomized into the study in accordance with IRB guidelines.

Strengths and Limitations of Study

The proposed study, although its purpose is to attempt to fill the gaps that currently exist in the literature, has a few limitations of its own. First, it uses weight as its

primary outcome, in order to calculate the sample size. This is because, although the study is more interested in other outcomes besides weight, one primary outcome had to be selected in order to calculate sample size, and this outcome was in line with other RCTs discussed in this thesis (Peters et al and Tate et al) which focused on weight as their primary objective. Despite this, the sample size calculated should be enough to detect other outcomes such as change in gut microbiome changes and fMRI differences between groups, as Green and Murphy and Thomson and colleagues used smaller sample sizes than what is proposed in this study (24 and 34, respectively).^{19,31} Therefore, this larger sample size would theoretically be sufficient to detect changes, if any occur, between the two groups. However, the sample size calculated in Thomson and colleagues' study was based on difference of glycaemia after administration of an oral glucose tolerance test, whereas the proposed study in this thesis will not conduct a glucose tolerance test and will instead measure hemoglobin A1c as this is less burdensome on participants. It is unclear if this would alter the sample size needed and render differences less detectable or not.

Other potential limitations of this study include adherence of the study population to the beverage intervention. The population being studied in this RCT are habitual, high-volume consumers of diet soda (>3 per day) and, therefore, may find it difficult to adhere to a sudden change in this regimen. This will be attempted to be mitigated by offering flavored, carbonated, non-caloric, non-artificially sweetened water to the water group. However, caffeine content may not be present in this beverage substitution, which could potentially lead to compliance issues as well.

Another potential limitation is that participants in the water group will not be forbidden to consume artificial sweeteners in their foods (often labeled as lite, diet, sugar-free, etc.) since forbidding these foods may cause participants to choose calorie-dense alternatives and may in turn cause weight gain in one group compared to the other, skewing the results of the study. Because of this, the water group may consume some artificial sweeteners in their foods; however, their dosage of artificial sweeteners relative to the artificially sweetened beverage group should still be low, since the artificially sweetened beverage group consists of high-volume diet beverage consumers (>3 per day) and they are also likely to be consuming artificial sweeteners in their foods, whereas the water group will be abstaining from artificially sweetened beverages.

Another limitation of this study is its length; as a 1-year RCT, it may not be possible to detect a change in weight, waist circumference, gut microbiota via stool sample collection, hunger, sweet taste preference, fMRI data, or DXA scan body composition from individuals who cease drinking diet soda throughout the duration of the study. However, the burden of the study on participants, as well as the cost of the study, might be too great and compliance with the study may suffer even more if the study were to be made any longer. In addition, the other studies examining many of the same variables lasted a shorter duration. For example, Thomson and colleagues' study of sucralose effects on gut microbiota lasted 7 days (but did not show a difference in gut microbiota in this timeframe)³¹ and the other RCTs discussed in detail in this thesis (Ebbeling et al, Peters et al, and Tate et al) lasted 6 months to 1 year.²¹⁻²³

Another limitation of this study is that it will rely on self-reported data for information such as 24-hour food recalls and 7-day physical activity recalls, which are subject to participant recall and social desirability bias. However, in an effort to reduce bias, these two questionnaires will be conducted randomly every 4 months over the course of the study. Having these recalls 4 times throughout the year will theoretically reduce bias from dietary and activity differences throughout different seasons of the year.

CONCLUSION

Discussion

Artificial sweeteners, although providing little-to-no calories, may promote overweight and obesity by causing physiologic and behavioral changes that can alter the way our bodies and brains respond to food. The first part of this thesis aimed to give the history of artificial sweetener use in the United States, as well as reviewed the literature on artificial sweeteners and their relationship to overweight and obesity. A few observational studies show a dose-response increase in weight gain and waist circumference in populations that consume artificial sweeteners.^{8,9} On the other hand, several randomized control trials fail to show that artificial sweeteners cause weight gain compared to non-artificially sweetened beverages²¹⁻²³; however, they do not investigate many of the other physiologic and behavioral changes that artificial sweeteners could have on consumers, and were shorter in duration (6 months to 1 year) compared to the observational studies conducted (about 7-10 years). The study proposed in this thesis aims to fill some of the gaps that exist in the literature surrounding the effects that artificially sweetened beverages may have on their consumers, by investigating not only the occurrence of weight gain and waist circumference increase in the study population due to diet soda consumption, but also the potential effects on the gut microbiome, hunger, sweet taste preference, food-reward brain response, and body composition/change in adiposity that participants may experience as a result of ceasing to consume diet soda over the course of 1 year. These effects, once elucidated, would

hopefully help to explain the incongruity in data between the long-term observational studies and the short term RCTs that exist in the literature.

Summary

Artificial sweeteners have been rising in popularity over the last few decades and are used widely in the food supply as a replacement for sugar. Although they are used in many “diet” and “light” products, they may be paradoxically promoting overweight and obesity over time by causing physiologic and behavioral changes in those who consume them. Several observational studies have shown that individuals who consume artificial sweeteners in high volumes have a dose-response increase in BMI and waist circumference over time but observational studies cannot conclusively prove causation.^{8,9} Although several RCTs have failed to replicate these findings,²¹⁻²³ and one RCT actually shows that consumers of diet beverages may have greater weight loss over a 1 year duration than water consumers,²¹ the RCTs in the literature have not investigated many of the other mechanisms by which diet beverages, and the artificial sweeteners within them, could be altering their consumers behavior towards food and potentially even the way their bodies could be metabolizing and processing the food they eat. This thesis proposed a study to help fill in these gaps in the literature in hopes that one day there will be a clearer understanding of the effect, if any, that artificial sweeteners have on the human body.

Clinical and/or public health significance

Artificial sweeteners are pervasive in the food supply, especially in foods that are branded “diet,” or “light,” which are marketed towards individuals who are conscious

about their weight and wish to make efforts to lose weight. Although these sweeteners are low-to-no calorie, it is important to fully understand if they are altering the behavioral and physiologic responses that the human body has towards food, ultimately promoting overweight and obesity over time. There are several gaps in the literature and current research to draw definitive conclusions about this at the current moment, however this thesis proposes a study to help investigate some of these potential mechanisms so that the public, as well as the FDA and CDC, and other worldwide government agencies can become more informed about the true effects that these sweeteners have on those who consume them. This information is critical, as the rates of overweight and obesity in the US continue to climb, potentially causing the health issues that are associated with them to climb as well.

APPENDICIES

APPENDIX 1

Search terms for studies and papers included in the literature analysis section:

("Sweetening Agents"[Mesh] OR Agent, Sweetening OR Agents, Sweetening OR Sweetening Agent OR Sweeteners OR Sweetener OR Sugar Substitutes OR Substitute, Sugar OR Substitutes, Sugar OR Sugar Substitute OR Artificial Sweeteners OR Artificial Sweetener OR Sweetener, Artificial OR Sweeteners, Artificial) AND ("Obesity"[Mesh] OR obesity) Filters: from 2006 – 2022

Additional studies and papers were found using a combination of keywords and MeSH terms for artificial sweeteners AND obesity from 2006 to 2022

LIST OF JOURNAL ABBREVIATIONS

Am J Clin Nutr	The American Journal of Clinical Nutrition
Ann N Y Acad Sci	Annals of the New York Academy of Sciences
Br J Nutr	British Journal of Nutrition
Curr Gastroenterol Rep	Current Gastroenterology Reports
Environ Health	Environmental Health
Front Nutr	Frontiers in Nutrition
J Acad Nutr Diet	The Journal of the Academy of Nutrition and Deitetics
J Am Geriatr Soc	Journal of the American Geriatrics Society
J Am Heart Assoc	Journal of the American Heart Association
J Gen Intern Med	Journal of General Internal Medicine
Obes Rev	Obesity Reviews
Obes Silver Spring Md	Obesity (Silver Spring)
Physiol Behav	Physiology and Behavior
Pol J Food Nutr Sci	Polish Journal of Food and Nutritional Sciences
Trends Endocrinol Metab	Trends in Endocrinology and Metabolism
Yale J Biol Med	Yale Journal of Biology and Medicine

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