A Comparison of the Effect of Consuming a Fructose-, Glucose-, or Aspartame-Sweetened Beverage on *Ad Libitum* Caloric Intake

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Abstract

Background: The per capita consumption of sugar-sweetened beverages (SSB) has increased in tandem with the obesity epidemic. While many epidemiological and clinical studies have shown a positive association between consumption of SSB and increased risk of weight gain, the exact mechanism by which the consumption of SSB leads to weight gain remains unclear.

Objectives: To determine whether *ad libitum* caloric intake is differentially affected by consumption of a fructose-, glucose-, or aspartame-sweetened beverage and to determine whether subjects are able to compensate for energy taken up through SSB by reducing their *ad libitum* intake of solid food.

Design: Nine subjects were randomly assigned to each of the three, eight-day isocaloric dietary periods, which were separated by 20-day washout periods. Order was determined by block randomization. The diet periods differed only in the type of SSB administered to the subjects. Solid food and the SSB were administered to subjects at the beginning of each dietary period. Subjects were asked to consume solid food *ad libitum*, but were required to consume all four servings of the SSB each day. *Ad libitum* caloric intake was assessed by weighing and measuring subjects' uneaten food at the end of each dietary period.

Results: Nine healthy, young, normal-weight subjects (4 male, 5 female) completed all three dietary periods. Subjects consumed an average of 2698 ± 607 kcal/day, 2629 ± 682 kcal/day, and 2307 ± 651 kcal/day during the fructose-, glucose-, and aspartame-diet periods, respectively. Subjects consumed significantly more calories during the fructose- and glucose-diet periods compared to the aspartame-diet period ($p < 0.001$). However, energy intake did not differ to a significant degree between the fructose- and glucose-diet periods ($p = 0.462$). Four subjects in the fructose-diet period and seven subjects in the

glucose-diet period were able to partially compensate for the energy intake from the SSB by reducing their intake of solid food. The degree to which subjects were able to compensate for energy in the SSB did not differ between the fructose- and glucose-diet periods ($p = 0.136$).

Conclusion: The consumption of SSB placed subjects in a state of increased energy intake as many of them were unable to reduce their intake of solid food to compensate for energy taken up through the SSB. This effect was present in both the fructose and glucose arms, thus suggesting that liquid calories from SSB promote overconsumption of energy independent of the type of sugar used to sweeten the beverage. Our study lacked the power to determine conclusively whether the degree of compensation for liquid calories taken up as fructose differed relative to liquid calories from glucose.

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INTRODUCTION

THE OBESITY EPIDEMIC

The obesity epidemic has taken an extreme economical, physical, and emotional toll on our nation. For example, the medical care costs associated with obesity in the U.S. alone amounted to $147 billion in 2008.[1] Furthermore, obese individuals can expect to pay $1,429 more per year in medical costs than normal weight individuals.[2] Besides accruing higher annual health care costs, obese individuals are at an increased risk of developing certain chronic diseases compared to normal weight individuals; it is well accepted that obesity is associated with a host of chronic diseases including type 2 diabetes, cardiovascular disease, and some forms of cancer.[3] The aforementioned chronic diseases are some of the leading causes of preventable deaths in the U.S., meaning that simple lifestyle interventions and modifications could make a sizeable impact on alleviating some of the health consequences associated with obesity.[2] Even though the obesity rate among adults hit an all-time high of 36% in 2010, there is some evidence that the obesity epidemic is finally starting to plateau.[4,5] The obesity epidemic still remains a public health crisis, however, as the proportion of adults who are extremely obese (at least 30 pounds overweight) has increased from 1.4% to 6.3% over the past three decades.[5] Sadly, children and adolescents are not immune to this epidemic, either, as obesity rates have doubled and tripled among these populations, respectively, since 1980.[4] The 2009-2010 NHANES survey concluded that nearly 17% of U.S. children and adolescents are obese.[6] If these trends continue, Kelly et al.'s projection estimates that, worldwide, nearly 573 million individuals will be obese in 2030.[3] The implication of this analysis is quite profound, as if these numbers hold to be true, the obesity epidemic will place an ever-increasing burden on clinical and public health resources worldwide.

CAUSES OF THE OBESITY EPIDEMIC

Socioeconomic Factors Contributing to the Obesity Epidemic

One of the hardest issues we face when attempting to solve the obesity epidemic is that there is no one specific cause to target. Instead, the etiology of the obesity epidemic is multifaceted and can be attributed to many things, from the changing food environment, to the transition away from traditional meal schedules, to the technological advancements that promote sedentary lifestyles, and to the types of food that are readily - and cheaply - available to the majority of Americans. We live in an era where it is cheaper to buy a bag of potato chips than a bag of apples, where it is easier to feed a family of four from a fast food restaurant than from a homemade meal, where pizza is considered a vegetable in school cafeterias, and where it is nearly impossible to purchase fresh fruits and vegetables in some areas of our country known as "food deserts." According to a 2009 study by Monsivais et al., not only do the nutrient-dense foods that are associated with healthy lifestyles tend to cost more per kilocalorie than refined grains, sweets, and fats, but the price disparities between healthful and less healthful foods appears to be growing.[7] Monsivais et al.'s study found that the mean cost of food in the top quintile of micronutrient density was $27.20/1000 kilocalories (kcal), whereas the food in the lowest quintile of micronutrient density was a mere $3.32/1000 kcal.[7] When people are faced with financial constraints, the high cost of healthier, nutrient-dense foods may be a likely barrier to a healthy diet.[7]

"Secondary Eating" as a Contributing Factor in the Obesity Epidemic

One of the factors that is thought to play a role in the obesity epidemic is the alteration in traditional food consumption patterns. The long-standing tradition of eating dinner together, as a family, at the kitchen table has since become largely extinct. Individuals, as well as families, now choose to eat in front of the television, while checking emails or blogs, in the car, on the bus, etc. Michael Pollan, the *New York Times* best selling author of multiple food-

based books, defines this shift away from having meals at the kitchen table as "secondary eating"; secondary eating is the consumption of food and beverages that occurs while driving, eating, watching TV, browsing the internet, etc.[8] The U.S. Department of Agriculture's Economic Research Service's 2011 report entitled, "How Much Time Do Americans Spend on Food?" found that the average American, aged 15 and older, spends 78 minutes per day participating in secondary eating![9] This report also found that those individuals who engaged in secondary eating while watching television had higher-than-average BMIs.[9] By focusing on the TV, the computer, or a magazine rather than our food, we pay less attention to the taste and the overall amount of food that we consume, which may cause us to eat beyond satiation. Thus, over the long term, secondary eating could contribute to excess caloric intake and weight gain.

Increased Snacking as a Contributing Factor to the Obesity Epidemic

One of the other factors that has contributed to the obesity epidemic is the disappearance of traditional meal times and routines. Eating three square meals per day has been replaced with grazing on small, snack-like meals throughout the day. The processed food industry has capitalized on this phenomenon, specifically designing food and food packaging to make it even easier for us eat on the go. Foods are strategically made into hand-held forms so that we are able to eat as we head out the door to our next destination or as we sit at the computer or in front of the television. Although these foods may be convenient, they tend to be high in salt, sugar, and fat since these foods are highly processed.

Addictive Qualities of Processed Foods as a Contributing Factor in the Obesity Epidemic

One cannot address the obesity epidemic without mentioning the dramatic shift that has occurred in the last 50 years in regards to the way that food is produced here in America. We have moved away from relying on our local farmer to provide us with meat, vegetables,

and milk, and instead now rely on factories and machines to provide us with processed foods that are high in salt, sugar, fat, calories, and refined seed oils. Not only does the majority of the American food supply come packaged in the form of boxes, sleeves, or bags, but this food is also physically manipulated to contain the exact amount of sugar needed to leave us constantly craving more and more of these unhealthy foods. Michael Moss's 2013 book, *Salt, Sugar, Fat*, explores how the food industry giants – such as Kraft and Coca-Cola - use technology to calculate the "bliss point" of processed foods in order to provoke the pleasure centers in our brains.[10] Processed foods are designed to elicit a response from the same areas of the brain that are stimulated by drugs like crack-cocaine.[10] Sugar-sweetened beverages, in particular, are especially efficient at hitting the brain's pleasure center, as sugar-sweetened beverages are an extremely concentrated form of refined sugar.[10] A 12-ounce can of Coca-Cola, for example, contains ten teaspoons of sugar, whereas the seemly innocuous cranberry juice contains 12 teaspoons of sugar in a 12-ounce glass.[11] Adding insult to injury is the fact that these sugary beverages are a source of "empty-calories," as they provide little to no nutritional benefit; compared to a fresh, whole piece of fruit, fruit juice provides next to nothing in terms of vitamins, minerals, or fiber. Similarly, soda, energy drinks, enhanced water beverages, and other sugary beverages are a source of empty-calories. Due to the addictive and non-nutritive nature of sugar-sweetened beverages, it's no wonder that these beverages are a top priority when implementing targeted behavior modifications for obesity prevention.

Increased Consumption of Sugary Beverages as a Contributing Factor to the Obesity Epidemic

The per capita consumption of sugary beverages has increased in tandem with the obesity epidemic; between 1977 and 2002, the per capita intake of caloric beverages doubled across all age groups in the United States.[12] The increase in consumption of caloric beverages can partly be attributed to the creation of high fructose corn syrup (HFCS), a

man-made sugar that is extremely cheap to produce and is created in abundance thanks to the fact that corn is highly subsidized in our country. HFCS, which contains either 55% fructose/45% glucose or 42% fructose/58% glucose, is readily found in most processed food products, including sugary beverages.[13] In fact, the per capita consumption of HFCS – the sugar responsible for sweetening more than 40% of the nation's food and beverages – increased more than 1,000% between 1970 and 1990, which far exceeds the consumption trends of any other food or food group.[12] Sugar is so ubiquitous that adults, on average, consume approximately 13% of their average daily calorie intake in the form of added sugar, whereas children and adolescents consume approximately 16% of their daily caloric intake in the form of added sugar.[14] The current trends and consumption patterns of HFCS and sugar, in general, further supports the crucial role that the consumption of sugary beverages has had on the obesity epidemic.[15]

Sugary Beverages and Energy Homeostasis: Evidence from Observational Studies

While there may be a myriad of factors that have contributed to the obesity epidemic, a multitude of observational, epidemiological, and clinical data has shown that the consumption of sugary beverages – which includes beverages containing added sugars (e.g., HFCS and sucrose), as well as those beverages sweetened by natural means (e.g., fruit juice) - is associated with an increased risk of being overweight or obese. Malik et al.'s systematic review of 30 publications showed a positive association between greater intakes of sugary beverages and weight gain in both adults and children.[16] While Malik et al.'s review mainly focused on soft drinks, Ludwig et al.'s 2001 prospective observational analysis took into account the consumption of sweetened fruit drinks (e.g., Koolaid, Hawaiian punch, lemonade) and iced tea, in addition to soft drinks.[17] The purpose of Ludwig et al.'s study was to examine the association between baseline and change in consumption of sugar-sweetened drinks and difference in measures of obesity.[17] Ludwig et al. concluded

that for each additional serving of sugar-sweetened drink consumed, both body mass index (BMI) and prevalence of obesity increased, even after adjusting for anthropometric, demographic, dietary, and lifestyle variables.[17] The aforementioned observational studies that have looked at the association between consumption of sugary beverages and obesity have helped formulate hypotheses that have been tested in subsequent experimental trials. However, these observational studies have not been able to prove that consumption of sugary beverages leads to obesity.

Sugary Beverages and Energy Homeostasis: Experimental Evidence

Several clinical trials have further supported the findings from these observational studies, indicating that long-term consumption of sugary beverages leads to weight gain and increases in adiposity.[18,19,20,21,22,23] Among these, Tordoff and Alleva's 1990 crossover study aimed to determine whether artificial sweeteners aid in the control of long-term food intake and body weight.[21] Normal weight subjects went through three different dietary periods of three weeks each. For each three-week period, subjects were required to consume 1150 grams of soda sweetened with aspartame or HFCS, or no experimental drink per day.[21] Subjects were required to keep a dietary log during the entire nine-week period. Tordoff and Alleva found that subjects gained significantly more weight after drinking the HFCS-sweetened soda for three weeks compared to the other two dietary conditions.[21] Similarly, Raben et al. instructed 41 overweight individuals to consume a specific minimum amount of either sucrose-sweetened or artificially sweetened food and drinks every day during a ten-week intervention period.[23] Even though this study included both food and beverages, approximately 70% of the subjects' sucrose consumption came from beverages (e.g., soda and fruit juices), while only 30% of the sucrose came from solid foods. In fact, the average intake of sweetened drinks was 1,285 g/day, which exceeds the amount given to subjects in Tordoff and Alleva's study.[23] After ten weeks, the sucrose group had significant increases in

body weight and fat mass compared to the artificial sweetener group.[23] Although this study showed that drinking sucrose-containing beverages led to weight gain, one of the major limitations to this study was that the authors observed the effects of sucrose, as a whole, on the outcome measures. The authors were unable to take into account the impact that sucrose's components – glucose and fructose – had on weight gain individually. In fact, many of the studies that have been done regarding the consumption of sugary beverages and energy homeostasis have not addressed whether the individual components of the sugars most frequently found in sugary beverages differentially affect energy homeostasis. By having both a glucose and a fructose dietary arm in our study, we will address how these two sugars differentially affect energy homeostasis. The other studies that have been done comparing the impact of fructose and glucose on energy homeostasis will be discussed below.[19,39,42,43]

OBJECTIVE OF THE PRESENT STUDY

Despite all of the observational and clinical data associating the consumption of sugary beverages with weight gain and obesity, the exact mechanism by which sugary beverages increase one's risk of obesity still remains unclear. The two main hypotheses include the liquid calories hypothesis and the fructose hypothesis. Both of these hypotheses are partly based upon the idea that the consumption of sugary beverages alters the body's energy homeostatic mechanisms, which ultimately leads one to consume more calories than needed to maintain energy balance. The objective of our study is to determine whether *ad libitum* caloric consumption is differentially affected by the consumption of fructose-, glucose-, or aspartame-sweetened beverages. The primary specific aim of this study is to test whether calorie intake is higher during the fructose-sweetened beverage diet period, as compared to the glucose- and aspartame-sweetened beverage diet periods. We hypothesize that, due to the lack of hormonal response elicited by the consumption of fructose, the total calorie

consumption will be greater during the fructose-containing diet period compared to the glucose- and aspartame-containing diet periods. The experimental design of our study is unique in that it will allow us to dissociate liquid calories from fructose intake, both of which are strongly associated with each other in free-living individuals consuming sugary beverages. Our double blind, crossover, randomized control trial will allow us to test the hypothesis that increasing fructose intake will be associated with greater calorie intake while standardizing liquid calorie intake.

MECHANISMS OF ENERGY HOMEOSTASIS - OVERVIEW

The human body has an intricate hormonal system in place that regulates one's food intake and energy balance in an attempt to maintain energy homeostasis; there are both short- and long-term regulators in place to mediate food intake and energy homeostasis. Whereas the short-term signals act primarily as determinants of satiety to limit the size of individual meals, the long-term signals ensure that homeostasis is maintained and that body weight remains relatively constant over time.[24]

Mechanisms of Energy Homeostasis: Short-Term Mechanisms

The short-term regulation of food intake is mainly driven by gastrointestinal peptides that are released in direct response to food intake.[24] The hormones either directly or indirectly interact with an area at the base of the brainstem known as the nucleus tractus solitarius (NTS).[25] The NTS is the major satiety center of the brain; it interprets all of the peripheral signals coming from the liver, stomach, and small intestines to regulate satiety.[25] Through the interaction with the NTS, the majority of these hormones, with the exception of ghrelin, act to inhibit food intake and to induce satiety. Ghrelin is an orexigenic hormone that is produced by the stomach and the hypothalamus.[24] Plasma concentrations of ghrelin peak immediately before a meal and drop precipitously after food intake.[25] Conversely, the main

anorexic hormones associated with the short-term regulation of food intake include cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), gastrin-releasing polypeptide (GRP), and peptide YY (PYY).[24] CCK is primarily released from endocrine cells located in the proximal small intestine in response to the consumption of dietary fat and proteins.[24] CCK is also produced in the central nervous system (CNS), specifically in regions of the brain that are involved in food intake regulation.[24] The mechanisms by which CCK promotes satiety are through delaying gastric emptying and activating the vagal afferent nerves that innervate the NTS.[26] GLP-1 is secreted by the endocrine L cells, which are located largely in the ileum.[24] GLP-1 induces satiety through two different mechanisms. First, GLP-1 has been shown to slow gastric emptying, thus prolonging the time that food stays in the stomach.[24] Secondly, GLP-1 binds to receptors on afferent nerves in the liver and GI tract, thereby relaying satiety signals to the NTS.[24] GRP promotes satiety by stimulating the release of CCK, by potentially delaying gastric emptying, and by reducing appetite and food intake in humans.[24,27] In addition to being produced in the brainstem, PYY is also released from the L cells in the ileum and colon.[24] PYY is released in response to a meal and reduces appetite by slowing gastric emptying.[24] Glucose and fructose consumption have been shown to significantly alter plasma levels of some of these short-term regulators of appetite (such as GLP-1, PYY, and ghrelin), albeit to different degrees.[28] In comparison to fructose, glucose metabolism produces a greater insulin response. Insulin is a modulator of plasma ghrelin; there is an inverse relationship between plasma insulin and plasma ghrelin levels.[29] Therefore, since glucose metabolisms elicits a higher insulin response compared to fructose metabolism, one can expect plasma ghrelin levels to be lower following glucose consumption compared to fructose consumption.

In addition to the gastrointestinal peptides, mechano- and chemoreceptors in the stomach and small intestines also play a role in the short-term regulation of food intake.[24] These receptors respond to the presence of food in the stomach and small intestines by transmitting signals via the vagal nerves to the hindbrain to initiate meal termination.[24] Although these short-term signals play important role in eliciting satiety, they are not capable of producing sustained alterations in energy intake and adiposity.

Mechanisms of Energy Homeostasis: Long-Term Mechanisms

In contrast to the short-term regulation of energy intake, the long-term regulation of energy homeostasis and food intake is an intricate system that involves the complex interplay between the hypothalamus and the circulating adiposity signals, leptin and insulin. Whereas the short-term satiety signals primarily act upon the NTS in the brainstem, leptin and insulin primarily interact with the various nuclei of the hypothalamus, including the arcuate, dorsomedial, ventromedial, lateral hypothalamic area, and ventral premammillary nuclei.[30] In the 1950's, G.C. Kennedy was the first person to hypothesize that there was a substance in the body that acted as one of the key regulators of body weight.[30] Kennedy proposed that the long-term regulation of body weight is mediated by a humoral factor that is produced by adipocytes in proportion to the amount of lipid stored in adipose tissue.[24] In 1994, this factor was identified as leptin. Since leptin is primarily made by adipose tissue, plasma leptin concentrations decrease after weight loss and increase with an increase in adiposity.[26] The importance of leptin in regulating weight maintenance over the long-term is highlighted by studies that have shown that leptin deficiencies or defects in the leptin receptors in the brain cause hyperphagia and severe obesity.[31,32,33] The overall mechanism for how leptin regulates energy balance and neuroendocrine function is quite complex; leptin acts via its brain receptor, LRb, in order to elicit satiety.[30] The hypothalamus is a one of the sites with the highest expression of LRb, hence why the hypothalamus is leptin's primary site of

action.[30] In the arcuate hypothalamus, leptin's attachment to LRb inhibits neurons that synthesize neuropeptide Y (NPY) and agouti-related peptide (AgRP) and stimulates the neurons that synthesize pro-opiomelanocortin (POMC).[30] NPY and AgRP are both appetite-stimulating peptides, whereas POMC produces a hormone (α-melanocyte-stimulating hormone) that decreases appetite.[30] Thus, leptin acts via LRb to decrease the production of orexigenic peptides and to increase the production of anorectic peptides. As previously mentioned, leptin is produced in proportion to body fat stores; plasma leptin levels tend to be higher in obese individuals compared to normal-weight individuals. Even though plasma leptin levels are mainly regulated by adiposity, insulin and glucose can also modulate leptin secretion.[24] In some *in vitro* and *in vivo* studies, insulin has been shown to increase leptin gene expression and secretion.[24] Results of Mueller et al.'s 1997 study demonstrated that leptin secretion is directly proportional to the amount of glucose taken up by adipocytes; thus, leptin secretion partly relies on insulin-mediated glucose uptake into adipocytes.[34] Compared to a high-carbohydrate meal, a high-fat meal results in significant reductions in plasma leptin and insulin levels, which could lead to alterations in satiety signals. The fact that leptin secretion can partly be influenced by the nutrient composition of a meal has significant implications when discussing the association between diet and obesity.

Besides simulating the secretion of leptin from adipocytes, insulin also acts as one of the long-term regulators of energy balance through several additional mechanisms. Insulin is secreted from the beta cells of the pancreas in response to the ingestion of food, specifically glucose and amino acids. Just as leptin levels are related to levels of adiposity, fasting plasma insulin levels and insulin responses to meal ingestion are correlated with adiposity.[24] Insulin is transported into the brain via saturable receptors.[24] Once in the hypothalamus, insulin takes a similar course of action as leptin as insulin binds to its specific receptors, which inhibits the production of the appetite-stimulating neuropeptides.[24] Insulin not only acts to inhibit food intake, but it also acts to increase energy expenditure as well by

increasing sympathetic neural activity.[24] Therefore, insulin impacts both sides of the energy balance equation, both the energy intake and energy expenditure sides.

There is good evidence that the short- and long-term regulators of appetite work together to regulate appetite and food intake. Leptin increases the brain's sensitivity to the short-term satiety signals that are released from the GI tract.[24,35] Morton et al.'s research article concluded that leptin-generated signals from the arcuate nucleus in the hypothalamus in the forebrain heighten the hindbrain's response to satiety signals, such as CCK.[35]

EFFECT OF SUGAR-SWEETENED BEVERAGES ON ENERGY HOMEOSTASIS

Both the short- and the long-term energy homeostatic mechanisms are affected by the consumption of sugary beverages. As previously mentioned, the exact mechanisms by which sugary beverages increase one's risk of obesity and weight gain remains unclear. However, the two main emerging hypotheses include the liquid calories hypothesis and the fructose hypothesis.

The Liquid Calorie Hypothesis

The liquid calories hypothesis stipulates that liquid calories in any form may produce an incomplete satiation response in the body. Compared to the consumption of an isocaloric amount of solid food, liquid calories seem to have less of an impact on the short-term energy homeostatic systems.[18,36] Since one may not be able to perceive liquid calories as well as those from solid foods, individuals are unable to adjust their overall caloric consumption to adjust for these liquid calories, leading to the consumption of more calories than needed to maintain energy balance.[36] In the long term, the chronic consumption of liquid calories may therefore lead to weight gain and eventually obesity because it creates a chronic positive energy balance.

The results of DiMeglio and Mattes's 2000 study support the liquid calories hypothesis.[37] The study participants were divided up into two groups – one group consumed 450 calories per day in the form of soft drinks, while the other group consumed an isocaloric amount of jelly beans.[37] Whereas the jelly bean group reduced their overall daily caloric consumption by slightly more than the amount contained in the jelly beans, the soft drink group failed to compensate for the liquid calories and ended up in a caloric surplus.[37] The results of this study suggest that the subjects were able to adjust their overall daily caloric intake when they consumed calories from solid foods, but not from liquid calories. In their discussion, DiMeglio and Mattes offer several explanations as to why subjects were unable to adjust their overall calorie intake in response to liquid calories. First, the physical act of chewing food may elicit an internal satiety signal that is not triggered by swallowing liquid calories.[37] DiMeglio and Mattes also suggest that, compared to liquids, solid foods release more of the satiety-promoting peptides, such as CCK.[37] The volume, energy density, and osmotic properties of solids versus liquids could also come into play; liquid foods empty from the stomach quicker than solid foods, thereby possibly making one feel hungrier sooner.[37]

Even though the mechanism for why liquid calories are not perceived by the body as well as calories from solid foods is biologically plausible, some studies have shown that liquid calories and solid foods have identical effects on hunger ratings and energy intakes.[18] In Drewnowski and Bellisle's 2007 review article, they conclude that they literature comparing the effects of liquid versus solid calories on satiety is inconclusive.[18] Their main criticism of the studies that have been done on liquid calories and satiety is that they are short-term and thus, may not be applicable in the long-term.[18] Often times, these studies examine the effect of a caloric preload on hunger and satiety ratings and on energy intake at the next meal; the authors argue that the mechanisms affecting short-term caloric intake may not affect dietary patterns the same way in the long-term.[18] Furthermore, factors such as

subject characteristics, volume of preload, nutrient composition of the preload, and the time interval between the consumption of preload and determination of satiety, could have impacted the results from these studies.[18]

The Fructose Hypothesis

The second hypothesis detailing how the consumption of sugary beverages increase one's risk of obesity relates to how fructose and glucose are metabolized differently in the body. The secretion of leptin, one of the long-term regulators of appetite, is regulated by insulin-mediated glucose uptake in adipose tissue.[38] Fructose, unlike glucose, does not stimulate an insulin response from the pancreatic beta cells.[38] Compared to glucose, the consumption and subsequent metabolism of fructose leads to a less pronounced plasma leptin, insulin, and ghrelin response.[38] Karen Teff and her colleagues published many studies examining the relationship between fructose consumption and plasma leptin, insulin, and ghrelin levels.[38,39] Teff et al. have found that fructose consumption leads to a lower area under the curve for insulin and leptin compared to glucose consumption.[39] Furthermore, they also discovered that the postprandial suppression of ghrelin was less pronounced following a high-fructose meal compared to a high-glucose meal.[39] Because the leptin and insulin responses are blunted with fructose consumption, one's total caloric consumption would be expected to be higher in individuals consuming diets rich in fructose compared to glucose.

Other than eliciting a different hormonal response, there is some new evidence that fructose consumption may alter the body's energy homeostatic mechanisms in an additional way – compared to glucose, fructose consumption results in a different activation pattern in the areas of the hypothalamus that are responsible for regulating appetite and reward processing. In a groundbreaking new study, Kathleen A. Page and colleagues from Yale University School of Medicine compared the effects of glucose and fructose consumption on

cerebral blood flow to the areas of the brain associated with appetite and reward pathways.[40] The researchers gave twenty healthy men and women a cherry-flavored drink sweetened with either 75 grams (300 kcal) of pure glucose or pure fructose.[40] Using magnetic resonance imaging, researchers were able to observe the alterations in blood flow to the appetite-regulating areas of the brain immediately after the subjects consumed the beverages. As soon as 15 minutes after ingesting the beverage, researchers were able to see that there was a greater reduction in hypothalamic cerebral blood flow with glucose ingestion compared to fructose ingestion; glucose consumption reduced activation of the brain regions that regulate appetite, motivation, and reward processing.[41] Furthermore, compared to fructose, glucose ingestion led to higher levels of satiety-producing hormones.[40] Thus, the glucose-containing beverage was able to slow brain activity in the hypothalamus – one of the regions of the brain that regulates appetite - and it was able to elicit a greater difference in predrink-postdrink changes in fullness and satiety.[40] The results from this study suggest that the consumption of fructose does not trigger changes in key areas of the CNS that are involved in the perception of fullness and satiety.

Although these two competing hypotheses may appear to be very similar, they differ in that the liquid calories hypothesis argues that the consumption of sugary beverages – regardless of the type of sweetener used – may produce an incomplete satiation sequence. On the other hand, the fructose hypothesis argues that the consumption of fructose, in particular, elicits a different hormonal response in the body compared to other sweeteners, which, in turn, leads to increased caloric consumption and a positive energy balance.

Commercially Available Sugary Beverages are Characterized by Both Liquid Calories and a High Fructose Content

HFCS and sucrose, both of which are composed of approximately 50% fructose and 50% glucose, are the two types of sugars that are found most often in commercially made beverages. Since sugary beverages have been associated with weight gain and obesity, the food industry has become very ardent about creating low- or zero-calorie beverages by using non-nutritive sweeteners (ex. aspartame, saccharin, sucralose, etc.). Thus, the three sugars of interest in our study are fructose, glucose, and aspartame.

The objective of this study is to determine whether *ad libitum* caloric consumption is differentially affected by the consumption of fructose-, glucose-, or aspartame-sweetened beverages. The primary specific aim of this study is to test whether calorie intake increases with increasing fructose content in the fructose-sweetened beverage, as compared to the glucose- and aspartame-sweetened beverages. The experimental design of our study is unique in that it will allow us to dissociate liquid calories from fructose intake, both of which are strongly associated with each other in free-living individuals consuming commercially available sugary beverages. Comparing aspartame-sweetened beverages as well as beverages sweetened with the major sugars found most readily in the American food supply - fructose and glucose - will allow us to assess whether caloric excess in individuals drinking sugary beverages is related to its high fructose content, or the ingestion of liquid calories per se. Our double blind, crossover, randomized control trial will allow us to test the hypothesis that, due to the lack of hormonal response elicited by the consumption of fructose, the total calorie consumption will be greater during the fructose-containing diet period compared to the glucose- and aspartame-containing diet periods.

DO FRUCTOSE AND GLUCOSE DIFFERENTIALLY AFFECT ENERGY HOMEOSTASIS: SUMMARY OF THE EVIDENCE

Some of the previous studies that have examined the effects of sugary beverages on obesity have used commercially made beverages, which makes it difficult to dissociate between the two competing hypotheses; it is hard to tell whether the adverse effects of sugary beverages are attributed to the ingestion of liquid calories, per se, or to the high fructose content of the beverages. However, the studies that have examined the effects of fructose and glucose consumption on energy homeostasis have had mixed conclusions. Dolan et al.'s 2010 review article examined the literature for evidence of a causal relationship between the ingestion of fructose and weight gain in overweight or obese individuals.[42] Dolan et al. analyzed 45 research studies that had subjects consuming "normal amounts" of fructose, which the authors define as <95th percentile as determined by NHANES data from 1999-2004. According to NHANES data, the 95th percentile fructose consumption values are 136.1 g/day (in 19-30 year old males), 18.8% of total energy intake (in 19-30 year old females), and 29.2% of total carbohydrate intake (in 19-30 year old females).[42] The authors concluded that intakes of normal amounts of fructose have the same effect on body weight in overweight or obese individuals as glucose or sucrose.[42] Dolan et al. argue that the studies that have shown an association between fructose intake and weight gain have been performed with abnormal levels of fructose ingestion (>95th percentile, as determined by NHANES data between 1999-2004).[42] Conversely, Teff and colleague's 2009 study concluded that, compared with a glucose-sweetened beverage, obese subjects who consumed a fructose-sweetened beverage had a lower area under the curve for insulin and leptin and had an increased area under the curve for triglycerides.[39] Since leptin and insulin are both involved in the long-term regulation of energy homeostasis, the results of Teff et al.'s study support the hypothesis that diets high in fructose may lead to increased energy intake and weight gain due to reductions in insulin secretion and leptin production.[39]

Sievenpiper et al.'s 2012 review article examined 41 controlled feeding trials for the effects of free fructose consumption, in its unbound, monosaccharide form, on body weight.[43] Controlled feeding trials were considered "isocaloric" if the amount of calories coming from fructose in the experimental group was equivalent to the amount of calories coming from non-fructose carbohydrate in the control group.[43] Controlled feeding trials were considered "hypercaloric" if fructose in the experimental group was added to the usual or control diet so that fructose provided excess energy relative to the diet alone.[43] Sievenpiper et al. concluded that the isocaloric trials did not consistently show evidence for a body weight-increasing effect of fructose, but the hypercaloric trials did, in fact, show an association between free fructose consumption and weight gain.[43] However, the weight gain seen in the hypercaloric trials could simply be attributed to excess caloric consumption rather than fructose consumption, per se.[43] The authors noted many limitations to the studies included in their review article; many of the studies had a small sample size (less than 15 subjects) and were often less than 12 weeks in duration.[43] Furthermore, not all of the studies in Sievenpiper et al.'s review directly compared the effects of free fructose consumption versus free glucose consumption; the meta-analysis included studies comparing fructose to sucrose, starch, glucose, HFCS, dextromaltose, and galactose consumption.[43] Our present study, on the other hand, will compare the effects of free glucose, free fructose, and aspartame consumption on caloric intake.

In Stanhope and colleague's 2009 study, overweight and obese subjects consumed glucose- or fructose-sweetened beverages at 25% of their estimated energy requirements with self-selected *ad libitum* diets for eight weeks.[19] Prior to the eight-week outpatient period, subjects participated in a two-week inpatient period where they consumed an energy-balanced, high-complex carbohydrate diet. At the end of the outpatient period, subjects in both the glucose- and fructose-sweetened beverage groups significantly increased their

body mass, fat mass, and weight circumference.[19] Dietary assessment – performed by 24-hour dietary recalls – indicated that both the fructose and glucose groups reported consuming significantly more calories than their calculated energy requirements.[19] In addition, compared to those drinking glucose-sweetened beverages, subjects who consumed fructose-sweetened beverages had a larger increase in visceral adiposity, higher fasting plasma glucose and insulin levels, decreased insulin sensitivity, and increased hepatic de novo lipogenesis.[19] Although subjects in the fructose and glucose groups had similar increases in weight gain, the differences in visceral and subcutaneous adiposity suggest that glucose and fructose differentially effect regional adipose distribution.[19]

Even though many studies have been done examining the effects of sugary beverages on weight gain and energy homeostasis, these studies have certain limitations, which we believe will be addressed in our present study. While Stanhope et al.'s 2009 study was ten weeks in duration, many of the other aforementioned studies have been short in duration, typically only lasting one to two days. Our study, on the other hand, consists of three dietary periods lasting eight days each. Furthermore, previous studies have used imprecise assessments when analyzing *ad libitum* food intake, such as 24-hour dietary recalls. However, in our study, we will be able to accurately measure *ad libitum* food intake as we are providing subjects with food during all three dietary periods and asking that they return all uneaten food back to the Nutrition Research Kitchen. Once we receive the uneaten food, we will weigh and measure it with ProNessy Software, which will enable us to accurately assess the type and amount of food eaten by the subjects.

Lastly, many of the studies done in the past have failed to screen for - what we believe to be – a crucial characteristic: fructose malabsorption. In study designs that include a pure fructose dietary intervention, screening for fructose malabsorption is necessary. When taken

up in a molar ratio to glucose that exceeds one, a significant portion of the population exhibits signs of fructose malabsorption.44 In fact, it is hypothesized that the threshold for fructose absorption in most healthy individuals is between 25 and 50 grams.44 One of the limitations to Stanhope et al.'s 2009 study was that they did not screen for fructose malabsorption prior to enrolling subjects in their study. Therefore, Stanhope et al. may have underestimated the impact that fructose consumption has on energy homeostatic mechanisms. Our study, on the other hand, is unique in that all of our subjects were screened for fructose malabsorption; subjects were excluded from our study if they were fructose malabsorbers. If we did not screen for fructose malabsorption prior to enrolling subjects in the study, we would be unable to account for the physiological impact that fructose metabolism has on the body's energy homeostatic mechanisms, thus making it impossible to dissociate between the two hypotheses.

PUBLIC HEALTH IMPACT

The consumption of fructose in the population is extremely ubiquitous; Americans consumed approximately 45 gallons of sugary beverages per capita in 2009.[45] Although recent data shows that Americans have decreased their consumption of added sugars since 2000, the average intake of added sugars still exceeds recommended limits set by health professionals; furthermore, sugary beverages still remain the predominant source of added sugars in the American diet.[46] Even though fructose consumption is modifiable on both an individual and a population level, recent initiatives to curb the public's intake of sugary beverages have faced fierce criticism. Most famously, former New York City Mayor Michael R. Bloomberg proposed a law that would ban the sale of large sodas and other sugary drinks at restaurants, street carts, and movie theaters, but it was ruled unconstitutional by the courts.[47] If the results from our study support our hypothesis, the public health impact of this project on the obesity epidemic might be considerable given that fructose is abundantly

found in many different foods in our society, and for many years it has been purported to contribute to weight gain and the obesity epidemic.[48,49]

METHODS

RECRUITMENT AND SCREENING

Ten subjects were enrolled in this study. Male and female volunteers were recruited via newspaper ads and posters that were placed on and around the University of Washington (UW) in Seattle, Washington. Eligibility for the study, as determined by the following criteria, was assessed during a screening visit at the UW Clinical Research Center (CRC) (**Table 1**).

Table 1. Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
• Age: 18-25 years; • BMI: 20-25 kg/m^2; • Weight stable to within five pounds for six months prior to entering the study, and at their lifetime maximum weight (or within ten pounds of it); • Ability to be admitted for five hours to the CRC at UW on three occasions; • Ability to provide informed written consent; • Willingness to consume only food provided by the Nutrition Research Kitchen (NRK) of the UW CRC for three periods of eight days each.	• History of cardiovascular disease; • Presence of diabetes mellitus or impaired glucose tolerance (fasting glucose > 100 mg/dL); • Presence of hypertension (blood pressure systolic/diastolic higher than 140/90 mm Hg); • Presence of phenylketonuria (PKU); • Presence of fructose malabsorption or hereditary fructose intolerance; • Presence of another chronic or psychiatric illness; • Use of anabolic steroids, glucocorticoids, warfarin, beta-blockers, antidepressants, or lipid-lowering agents; • Use of antibiotic drugs within three months of enrollment into the study; • Use of tobacco products; • Pregnancy or female subject not using contraception; • Regular intense exercise (more than three hours per week); • Vegetarian or extreme dietary preferences; • Alcohol consumption of more than two drinks per day; • Presence of eating disorder; • History of frequent attempts at weight loss; • Currently dieting or in a weight control program; • Recent blood donation or enrolled in other research which requires blood sampling; • Presence or history of anemia.

At the screening visit, subjects also completed an exercise questionnaire and answered questions regarding their past medical and nutrition history. Subjects were also screened for fructose malabsorption utilizing a hydrogen breath test.[50] As part of this test, subjects were given one serving of a fructose-sweetened beverage that contained approximately 6.25% of their daily calorie needs from fructose. The hydrogen content of the subjects' exhaled breath was measured at 0, 30, 60, 90, 120, 150 and 180 minutes after consuming the beverage. An increase of 20 parts per million over baseline on at least two occasions was indicative of fructose malabsorption.[50] Female subjects were given a pregnancy test to ensure that they were not pregnant. Lastly, subjects were given a stool sample collection kit with instructions on collecting a sample within a day or two of the screening visit.

STUDY DESIGN

Subjects who successfully passed the screening exam were enrolled to complete each of the three dietary periods in a randomized order. The order was determined utilizing a block randomization procedure. Each of the three, eight-day isocaloric diets differed only in the type of sweetened beverage administered to the subjects. Each of the diet periods was separated by a 20-day washout period. The length of the washout period was chosen to be 20 days to increase the likelihood that female subjects were in the same days of their menstrual cycle while they completed each dietary period.

The subjects received their food for each diet period from the NRK at the UW CRC. The subjects received 125% of their estimated caloric intake, as determined by the Mifflin formula. The Mifflin formula uses height, weight, age, and an activity factor to estimate one's caloric needs.[51] The nutrient composition of the subjects' diets resembles that of the average American diet. Table 2 describes the approximate macro- and micronutrient distribution of the subjects' diets during each of the three dietary periods.

Table 2. Planned composition of the study diets.

	Fructose-diet period	**Glucose-diet period**	**Aspartame-diet period**
Solid foods (consumed *ad libitum*)			
- Carbohydrates (% of total energy)	50	50	50
- Protein (% of total energy)	16	16	16
- Fat (% of total energy)	34	34	34
Sweetened beverages (consumption mandatory)			
- Fructose	25	0	0
- Glucose	0	25	0

In addition to the solid food, subjects were required to consume four servings of a sweetened beverage each day. This was a double-blinded study, so neither the subjects nor the investigators or staff who were in contact with subjects were aware of the order in which the subjects received the three beverages. The sweeteners of interest in this study

were fructose (period A), glucose (period B), and aspartame (period C). In periods A and B, the beverages contained 12 grams of sugar per 100 g, and the volume of this beverage was adjusted to provide 25% of the subjects' estimated total calorie requirements. In period C, subjects were asked to drink an isovolumetric amount of a beverage that was sweetened with aspartame, a non-caloric sweetener. Aspartame was also used during period B in order to try to match the perceived sweetness of the glucose-sweetened beverage to that of the fructose-sweetened beverage. The subjects were instructed to consume the beverages periodically throughout the day; at least two hours between each serving was preferred in order to minimize GI upset associated with excess fructose consumption.

Although subjects were asked to not consume any additional solid food other than what was provided for them at the NRK, subjects were allowed to drink non-calorie beverages such as water, coffee, or tea (without milk and sugar) *ad libitum*. However, we asked that these beverages not be sweetened by any caloric- or non-caloric sweeteners. The consumption of any other non-nutritive sweeteners (ex. sorbitol, mannitol, sucralose, saccharin, etc.) was discouraged due to their possible impact on appetite and food intake. Subjects were instructed to eat only as much of the solid food as they needed to feel comfortably satiated. Any uneaten food was returned to the NRK of the UW CRC and weighed using ProNessy Software, which enabled us to assess precisely the type and amount of all food consumed during each diet period. Subjects were encouraged to maintain their normal exercise routine and asked not to make any drastic changes to their training regimen for the entire time they were enrolled in the study.

At the end of each day during the three dietary periods, subjects were asked to rate their appetite and fullness. Additionally, they were also asked to rate the taste of the beverage and food they had consumed during that day.

OUTCOME MEASURES

The **primary outcome measures** include: total energy intake in each of the three diet phases and reduction in calorie intake from solid foods (compensation for calories contained in beverages).

STATISTICAL ANALYSES

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, version 16.0).

Distribution of the data was tested for normalcy prior to statistical analysis. Due to our small sample size, the Shaprio-Wilk Test was used to assess normality. We also checked for normality by analyzing histograms and residual plots of the data. Non-normally distributed variables were log-transformed prior to statistical analyses.

No power calculation was done as this study was designed to be a pilot study.

To determine the degree to which subjects were able to compensate for energy taken up as liquid calories, we used the following equation:

(Average energy intake from solid food during aspartame phase - average energy intake from solid food during fructose OR glucose phase) / (Average energy intake from fructose- OR glucose-sweetened beverage - average energy intake from aspartame-sweetened beverage) * 100%

A repeated measures ANOVA was used to determine if the type of sugar used to sweeten the beverages could explain the variance in the total eight-day calorie intakes. A paired t-

test was used to compare the reduction in solid foods (i.e. compensation for liquid calories) between the fructose- and glucose-diet periods, as compared to the aspartame-diet period. The *post hoc* paired t-test with Bonferroni adjustment was used to compare caloric intakes between the fructose versus glucose diet periods, the fructose versus aspartame diet periods, and the aspartame versus glucose diet periods. A *post hoc* paired t-test with Bonferroni adjustment was also used to compare energy intakes during the fructose- and glucose-diet periods, when expressed as percentages relative to energy intake during the aspartame-diet period.

RESULTS

SUBJECT POPULATION

Ten subjects were enrolled in the study. However, one female was excluded after completing the first dietary period due to noncompliance with the study protocol. Thus, data from nine subjects (four men, five women, **Table 3**) who completed all three dietary periods were included in the statistical analysis.

Table 3. Baseline characteristics of the study population*.

Age (years)	20.9 ± 2.0
Height (cm)	170.5 ± 10.6
Weight (kg)	66.0 ± 6.5
BMI (kg/m^2)	22.7 ± 1.3
Baseline fasting glucose (mg/dL)	83.1 ± 6.9
Baseline fasting triglycerides (mg/dL)	76.4 ± 34.2
Baseline fasting total cholesterol (mg/dL)	150.9 ± 22.8
Baseline fasting HDL cholesterol (mg/dL)	46.9 ± 12.1
Baseline fasting LDL cholesterol (mg/dL)	88.6 ± 21.4

* Data are means ± standard deviation.

DIET COMPOSITION

By design, the solid food administered to the participants in the three dietary periods was identical. However, due to the fact that the fructose- and glucose-sweetened beverages, but not the aspartame-sweetened beverage, provided additional carbohydrates, carbohydrate content of the fructose- and glucose-diet periods was higher than that of the aspartame diet periods ($p < 0.001$, **Table 4**). Conversely, the fat and protein content of the subjects' diets was statistically significantly lower during the fructose- and glucose-diet periods compared to the aspartame-diet period ($p < 0.001$ for both fat and protein intake, **Table 4**).

Table 4. Composition of the diets (solid food + sweetened beverages) consumed by participants during the three dietary periods*.

	Fructose-diet period	**Glucose-diet period**	**Aspartame-diet period**	**p-value**
Carbohydrates (% of total energy)	62.1 ± 2.7[a]	62.0 ± 3.1[a]	54.3 ± 3.3[b]	RM-ANOVA $p < 0.001$
Fat (% of energy)	24.4 ± 2.8[c]	24.2 ± 2.7[c]	29.3 ± 3.2[d]	RM-ANOVA $p < 0.001$
Protein (% of energy)	13.4 ± 0.73[e]	13.8 ± 0.83[e]	16.3 ± 0.50[f]	RM-ANOVA $p < 0.001$

* Data are means ± standard deviations.
**Variables that are statistically different from one another have different superscripts

ENERGY INTAKE

Total calorie intakes from solid food and sweetened beverages differed to a statistically significant degree between the three dietary periods ($p < 0.001$ for overall comparison of all three dietary periods by RM-ANOVA, **Table 5**). *Post hoc* paired t-tests with Bonferroni adjustment for multiple testing revealed that energy intakes during the fructose- and glucose-diet periods were significantly higher than during the aspartame diet period ($p < 0.001$ for both comparisons), but did not differ from each other ($p = 0.462$).

Most participants had the highest total energy intakes during the fructose-diet period and the lowest energy intakes during the aspartame-diet period (**Figure 1**).

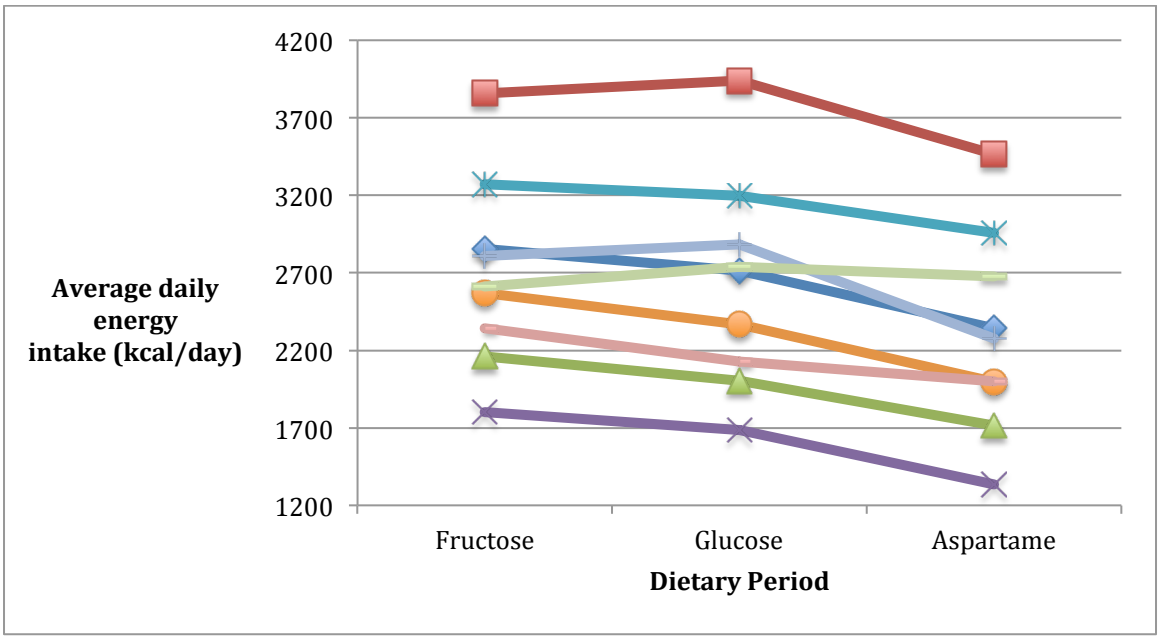

Figure 1. Subjects' average daily energy intake during each of the three dietary periods

When expressing energy intake in the fructose- and glucose-sweetened diet periods relative to energy intake during the aspartame diet period, participants consumed 391 calories per day (19%) more during the fructose dietary period and 322 calories per day (15%) more during the glucose dietary period ($p < 0.001$ for overall comparison of all three dietary periods by RM-ANOVA, **Table 5**). *Post hoc* paired t-tests with Bonferroni adjustment for

multiple testing revealed that energy intakes during the fructose- and glucose-diet periods, when expressed as percentages relative to energy intake during the aspartame-diet period, were significantly higher than during the aspartame diet period (p = 0.003 for fructose, p = 0.002 for glucose), but did not significantly differ from each other (p = 0.254).

The sweetened beverages accounted for 515 ± 91 calories per day during the fructose dietary period, 509 ± 92 calories per day during the glucose dietary period, and 85 ± 15 calories per day during the aspartame dietary period. The subjects' weights and BMIs remained stable throughout the entire study; there was not a statistically significant difference between the subjects' weights or BMIs across the three dietary periods (p = 0.243 for body weight, p = 0.247 for BMI, **Table 5**).

Table 5. Comparison of energy intake during each dietary period.*

	Fructose-diet period	Glucose-diet period	Aspartame-diet period	p-value
Total energy intake (kcal/day)	2698 ± 607[a]	2629 ± 682 [a]	2307 ± 651[b]	RM-ANOVA: $p < 0.001$
Total energy intake (% of energy consumed during aspartame diet phase)	119 ± 11[c]	115 ± 8[c]	100 ± 0[d]	RM-ANOVA: $p < 0.001$
Compensation for calories consumed as sugar-sweetened beverage (%)	6.0 ± 49.0	24.0 ± 37.1	N/A	Paired t-test: $p = 0.136$
Weight (kg)	65.3 ± 6.7[e]	66.0 ± 7.1[e]	65.3 ± 7.2[e]	RM-ANOVA: $p = 0.243$
BMI (kg/m^2)	22.5 ± 1.3[f]	22.7 ± 1.2[f]	22.5 ± 1.2[f]	RM-ANOVA: $p = 0.247$

*Data are means ± standard deviations.
**Variables that are statistically different from one another have different superscripts

A key question of this study was whether individuals are able to fully compensate for calories taken up as sugar-sweetened beverages by reducing their intake of solid food.

The majority of subjects were unable to compensate for the calories that they had consumed from the sugary beverages. Table 6 shows the difference in energy taken up as solid food between the fructose and glucose phases, respectively, and the aspartame phase, in relation to the energy consumed as fructose- or glucose-sweetened beverage, respectively. In table 6, the numbers relate to the degree of compensation for calories taken up through the sugar-sweetened beverages. A positive number indicates a reduction in energy intake from solid food when subjects consumed the fructose- or glucose-sweetened beverage, as compared to the aspartame-sweetened beverage. A compensation of 100% would indicate that the energy intake from solid food was reduced by exactly the caloric content of the fructose- or glucose-sweetened beverage. A negative number indicates an increase in solid food intake in the respective sugar-sweetened beverage phase, compared

to the aspartame phase, in spite of the substantial energy intake from the sugar-sweetened beverage. Overall, during the glucose dietary period, seven out of the nine subjects were able to partially compensate for the liquid calories, whereas only four out of the nine subjects were able to partially compensate for the liquid calories during the fructose phase. This means that five of the nine subjects consumed more solid food during the fructose-diet period compared to the aspartame-diet period, although they also consumed 515 ± 91 kcal/day from the fructose-sweetened beverage. Even though a greater number of subjects were able to partially compensate for the energy content of the sugar-sweetened beverage during the glucose phase, the results of the paired t-test show that there was not a significant statistical difference between the degree to which subjects, on average, compensated for these beverages between the fructose- and glucose-diet periods (p = 0.136, **Table 5**).

Table 6. Degree of compensation for energy taken up as beverage.

Subject	Fructose-diet period (%)	Glucose-diet period (%)
1	-7.56	20.90
2	29.44	13.48
3	-31.86	12.91
4	-36.84	-4.15
5	37.52	52.29
6	-35.22	10.36
7	-18.36	-37.39
8	1.44	62.76
9	115.05	84.91

DISCUSSION

Between 1977 and 2002, the per capita intake of caloric beverages doubled across all age groups in the United States.[12] The increased consumption of sugar-sweetened beverages (SSB) has occurred in tandem with the rise of the obesity epidemic in the United States.[12] Even though current trends show that Americans are decreasing their consumption of SSB, average intakes are still exceeding recommended amounts set by health professionals.[46] Adults, on average, consume approximately 13% of their average daily calorie intake in the form of added sugar (in both food and beverages), whereas children and adolescents consume approximately 16% of their daily caloric intake in the form of added sugar.[14] While there are several contributing factors to the obesity epidemic, many epidemiological and clinical studies have shown that consumption of SSB are associated with an increased risk of weight gain and obesity.[16,17,18,19,20,21,22,23,46,52]

The exact mechanism by which consumption of SSB increases one's risk of obesity is unclear. The two main hypotheses include the liquid calories hypothesis and the fructose hypothesis. Both of these hypotheses are partly based upon the idea that the consumption of SSB alters the body's energy homeostatic mechanisms, which ultimately leads one to consume more calories than needed to maintain energy balance. One of the aims of our study was to dissociate the liquid calories hypothesis from the fructose hypothesis. There were two main findings from this study, both of which support the liquid calories hypothesis. First, subjects had significantly higher daily energy intakes, on average, during the fructose- and glucose-diet periods compared to the aspartame-diet period. However, there was not a statistically significant difference between average energy intakes during the fructose- and glucose-diet periods. Secondly, many of the subjects were unable to reduce their intake of solid foods to compensate for the energy consumed in the fructose- and glucose-sweetened beverages. Both of these findings support the liquid calories hypothesis, in that consumption

of calories as liquid, irrespective of the type of sugar used, contributes to weight gain and obesity. However, our findings do not conclusively rule out the fructose hypothesis. Even though there was not a statistically significant difference between energy intakes during the fructose- and glucose-diet periods (p = 0.462), Figure 1 shows a trend that most subjects tended to have higher energy intakes during the fructose-diet period compared to the glucose-diet period. Furthermore, our data shows a trend that, compared to the fructose-diet period, subjects were able to reduce their consumption of solid food to a greater extent during the glucose-diet period. Our data may be sufficient, however, to motivate and design further, larger studies to test the fructose hypothesis specifically.

PROPOSED MECHANISM BY WHICH SUGAR-SWEETENED BEVERAGES CONTRIBUTE TO WEIGHT GAIN

Liquid Calorie Hypothesis

The liquid calories hypothesis stipulates that liquid calories in any form may produce an incomplete satiation response in the body. Compared to the consumption of an isocaloric amount of solid food, liquid calories seem to have less of an impact on the short-term energy homeostatic systems.[18,36,54] Since one may not be able to perceive liquid calories as well as those calories from solid foods, individuals are unable to adjust their overall caloric consumption to compensate for these liquid calories, leading to the consumption of more calories than needed to maintain energy balance.[36] In the long term, the chronic consumption of liquid calories may therefore lead to weight gain and eventually obesity because it creates a chronic positive energy balance.

The findings from our study are consistent with the liquid calories hypothesis. Most of the subjects in our study were unable to reduce their consumption of solid foods to fully compensate for the energy in the fructose- and glucose-sweetened beverages. During the glucose-diet period, seven out of the nine subjects were able to partially compensate for the

liquid calories, whereas only four out of the nine subjects were able to partially compensate for the liquid calories during the fructose-diet period. Thus, many subjects ended up actually consuming more solid food during the fructose- and glucose-diet periods compared to the aspartame-diet period, despite consuming additional calories in the form of a sweetened-beverage. The results of our study suggest that the chronic consumption of liquid calories – without a concomitant decrease in solid foods - would lead to weight gain because it creates a chronic positive energy balance. Many observational and clinical studies have also found that drinking SSB leads to weight gain. Although Tordoff and Alleva used different sweeteners in their study (HFCS and aspartame), subjects in their study gained significantly more weight after drinking HFCS-sweetened soda compared to the aspartame- and no-beverage diet-periods.[21] Similarly, Raben et al. found that subjects who consumed sucrose-sweetened, but not artificially sweetened, food and beverages gained a significant amount of weight after a ten-week intervention period.[23]

The results of our project also support the findings in Stanhope et al.'s 2009 study.[19] For eight weeks, overweight and obese subjects consumed fructose- or glucose-sweetened beverages at 25% of their estimated energy requirements with self-selected *ad libitum* diets. At the end of the intervention, subjects in both the fructose- and glucose-sweetened beverage groups had comparable increases in body mass, fat mass, and waist circumference.[19] Similarly, in our study, subjects had increased energy intakes during the fructose- and glucose-diet periods compared to the aspartame-diet period. Although subjects' weights remained stable since our intervention period was only eight days long, subjects' weights would have likely increased if they continued to ingest the SSB. Consumption of the fructose- and glucose-sweetened beverages caused the subjects to consume significantly more calories, on average, per day than during the aspartame-diet

period. Over time, chronic consumption of SSB – without a concomitant decrease in intake of solid foods – would lead to weight gain.

Many other studies have shown that people are unable to adjust their intake of solid foods to compensate for liquid calories.[18,37,53,54,55] Compared to an isocaloric amount of solid foods, liquid calories are associated with decreased satiety and increased *ad libitum* energy intakes.[37,53,54,55] In DiMeglio and Mattes's 2000 study, subjects who consumed 450 calories per day in the form of jelly beans were able to reduce their intake of solid food to compensate for the energy in the jelly beans.[37] However, subjects who consumed 450 calories in the form of soft drinks failed to compensate for the liquid calories, thus resulting in a caloric surplus.[37] Furthermore, other studies have shown that subjects are unable to compensate for liquid calories coming from beverages besides SSB, such as alcohol, milk, and juice.[53,55] In Mattes' 1996 study, subjects consumed either 1.08 liters of beer, light beer, non-alcoholic beer, soda, or carbonated water every three to four days with a midday meal. Subjects recorded *ad libitum* food intake during the day prior-to and the day-of beverage ingestion. Mattes discovered that energy intakes were significantly higher on days when subjects consumed the energy-containing beverages compared to the preceding day when the beverages were not consumed.[53] Similarly, DellaValle et al. also found that drinking a caloric, but not a non-caloric, beverage caused subjects to have higher *ad libitum* energy intakes during a mid-day meal.[55] Once a week for six weeks, 44 women were given either 360 grams of water, diet soda, regular soda, orange juice, 1% milk, or no beverage with lunch, which was consumed *ad libitum*. *Ad libitum* energy intakes did not differ between the no beverage and non-caloric beverage conditions. However, women consumed significantly more calories (104 ± 16 kcal) when they had a caloric beverage with lunch compared to no beverage or a non-caloric beverage.[55] While the exact mechanism for why liquid calories are less satiating than energy from solid foods is unknown, researchers

believe that rapid transit of liquids through the stomach and intestines may dampen the response of our bodies' short-term satiety signals.[54] Furthermore, the absence of mastication may result in decreased pancreatic exocrine and endocrine response when compared to solids, which can impact hunger and satiety.[37,56] It is important to note that the nutrient composition of the liquid calories can play a role in how satiating they are. For example, a liquid meal replacement that contained a mix of sugar, fat, and protein was found to be more satiating than an isocaloric beverage containing only sugar.[55] Thus, liquids calories in certain forms, such as soup – which may contain a mixture of fats, protein, and carbohydrates – may in fact be just as satiating as an isocaloric amount of solid food.

The Fructose Hypothesis

The second hypothesis detailing how the consumption of SSB may increase one's risk of obesity relates to how fructose and glucose are metabolized differently in the body. The secretion of leptin, one of the long-term regulators of energy homeostasis, is regulated by insulin-mediated glucose uptake in adipocytes.[38] Fructose, unlike glucose, does not stimulate an insulin response from the pancreatic beta cells.[38] Compared to glucose, fructose metabolism leads to a less pronounced plasma leptin, insulin, and ghrelin response.[38] Because the leptin, insulin, and ghrelin responses are blunted with fructose consumption, and because leptin, insulin, and ghrelin play crucial roles in energy homeostasis, one's total caloric consumption may be higher in individuals consuming diets rich in fructose compared to glucose.

In order to confirm the fructose hypothesis, we would have expected to see two different findings with our data. Firstly, we would have expected subjects to have significantly higher energy intakes during the fructose-diet period compared to the glucose-diet period. Even

though there was not a statistically significant difference between energy intakes during the fructose- and glucose-diet periods (p = 0.462), Figure 1 shows a trend that most subjects tended to have higher energy intakes during the fructose-diet period compared to the glucose-diet period. Secondly, in order to confirm the fructose hypothesis, subjects would have had to compensate for the energy taken up through the glucose-sweetened beverage to a greater extent compared to the fructose-sweetened beverage. Even though a greater number of subjects were able to partially compensate for the energy taken up through the glucose-sweetened beverage, the results of the paired t-test showed that there was not a statistically significant difference between the degree to which subjects, on average, compensated for these beverages between the fructose- and glucose-diet periods (p = 0.136, **Table 5**). Although this difference is not statistically significant, our data shows a trend that, compared to the fructose-diet period, subjects were able to reduce their consumption of solid food to a greater extent during the glucose-diet period. Thus, taken together, our data supports the liquid calories hypothesis, but does not provide conclusive evidence regarding the fructose hypothesis. Our data may be sufficient, however, to motivate and design further, larger studies to test the fructose hypothesis specifically.

Even though the findings from our study were unable to support the fructose hypothesis, other researchers have shown that the consumption and subsequent metabolism of fructose alters our body's energy homeostatic mechanisms in a way that could promote weight gain.[19,38,39,40,58,57] After an eight-week outpatient intervention period, Stanhope et al. demonstrated that subjects who consumed fructose-, but not glucose-sweetened, beverages had decreases in energy expenditure and fat oxidation during an eight-week outpatient period.[19] Furthermore, regional adipose tissue deposition significantly differed between the two dietary periods.[19] Subjects in the glucose-diet period had significant increases in subcutaneous adipose tissue, whereas subjects in the fructose-diet period had significant

increases in both total abdominal fat and visceral adipose tissue volume.[19] Therefore, these results suggest that fructose and glucose consumption may differentially affect regional adipose tissue deposition.[19] Interestingly, Stanhope et al. found that subjects in the fructose- and glucose-diet periods both had significant increases in body weight, fat mass, and weight circumference during the eight-week outpatient intervention period.[19] While there was not a significant difference in energy intakes during the fructose- and glucose-diet periods in our study, many of the subjects had higher energy intakes during the fructose-diet period compared to the glucose-diet period (**Figure 1**). If these trends continued over time, we would expect those drinking fructose-sweetened beverages to gain more weight than those drinking glucose-sweetened beverages. The 8-week *ad libitum* study by Stanhope et al. could provide information on whether the degree of energy overconsumption is greater when subjects consume fructose- as compared to glucose-sweetened beverages. Unfortunately, however, Stanhope et al. did not screen for fructose malabsorption prior to enrolling subjects in the study. When fructose is consumed in a molar ratio to glucose that exceeds one, a significant portion of the population exhibits signs of fructose malabsorption.[44] In the artificial setting of studies such as ours and the one by Stanhope et al., subjects were given doses of fructose that exceed the amount of fructose typically found in commercially made beverages. Thus, due to the volume of fructose consumed by subjects, testing for fructose malabsorption is necessary in studies that contain a pure fructose dietary intervention. Therefore, fructose malabsorption could have been a possible confounder in Stanhope et al.'s study, as they were not able to account for the true impact that fructose metabolism has on the body's energy homeostatic mechanisms.[58]

One of the studies that looked at the effects of long-term fructose consumption on body weight and caloric intake was performed on rhesus monkeys.[59] For 12 months, adult male

monkeys were fed an *ad libitum* chow diet supplemented with either glucose- or fructose-sweetened beverages.[58] The beverages provided the monkeys with 100 grams of sugar per day; the monkeys consumed, on average, 43.8 ± 4.1% and 41.5 ± 2.7% of total energy as glucose and fructose, respectively, during the 12-month trial.[58] Monkeys who consumed the fructose-sweetened beverage gained a significant amount of weight at the three and six month mark compared to their baseline weight, whereas the monkeys who consumed the glucose-sweetened beverage did not see a significant increase in their weight at these time periods.[58] At the end of the 12-month intervention period, there was not a significant difference in weight gain between the two groups. Interestingly, the researchers attribute the difference in weight gain during the first six months to differences in energy expenditure, rather than energy intake.[58] Indirect calorimetry was used to measure the monkeys' energy expenditures for 24-hours at baseline, and at the three-, six-, and 12-month marks. In the fructose-fed monkeys, energy expenditure during the post-prandial period was significantly lower at the three- and six-month marks compared to the baseline measurement.[58] Energy expenditure was unchanged at the three- and six-month marks in the glucose-fed monkeys. However, by the end of the 12-month intervention period, energy expenditure in the glucose-fed monkeys decreased to a point that was comparable to the fructose-fed monkeys.[58] Although this study was performed on monkeys, the results of this study show one additional mechanism for how fructose can possibly alter our energy homeostatic mechanisms in a way that promotes weight gain. Stanhope et al. support our conclusion that additional well-controlled, long-term human studies are needed in order to determine whether fructose consumption preferentially promotes positive energy balance compared with consumption of glucose.[58]

STRENGTHS OF THE PRESENT STUDY

There were numerous strengths to our study design. First, subjects consumed beverages that contained pure fructose and pure glucose, rather than HFCS or sucrose. Many of the previous studies that investigated the effects of SSB on weight gain and caloric intake have used commercially available beverages, which are typically sweetened with HFCS or sucrose, i.e. mixtures of fructose and glucose. By providing subjects with beverages sweetened with either pure fructose or pure glucose, we were able to examine the effects that the individual components of HFCS and sucrose (i.e., glucose and fructose) had on energy intake. Secondly, even though subjects were not living in a metabolic ward during the diet periods, the subjects' diets were very well controlled. Thus, we were able to accurately assess the subjects' energy intakes. We provided subjects with food during each of the three dietary periods and asked them to return all of the uneaten food to the Nutrition Research Kitchen. By weighing and measuring the subjects' uneaten food, we were able to accurately assess the type and amount of food eaten by the subjects. Previous studies have relied on methods such as a 24-hour dietary recall to determine subjects' energy intakes. However, dietary recalls require subjects to accurately remember the type and amount of food they consumed in days past. The studies that have been done analyzing the validity and accuracy of 24-hour recalls have had conflicting results.[60,61,62,63] Therefore, measurement of energy intake by 24-hour dietary recall may not be very accurate. Third, even though people typically chronically consume SSB, we feel that the length of our dietary periods was long enough to measure the impact of SSB consumption on *ad libitum* energy intake. Fourth, our study, by design, was a crossover study, which allowed subjects to act as their own control. The length of our dietary periods was not long enough to cause a significant increase in weight, thus making the crossover design possible. The crossover design also allowed us to see the intrapersonal differences in caloric intake during the three dietary periods. Lastly, we screened for fructose malabsorption, a characteristic that must be considered when utilizing a study design that includes a pure fructose dietary

intervention. When taken up in a molar ratio to glucose that exceeds one, a significant portion of the population exhibits signs of fructose malabsorption, primarily gastrointestinal symptoms.[44] In fact, it has been hypothesized that the threshold for fructose absorption in a single meal for most healthy individuals is between 25 and 50 grams.[44] By excluding fructose malabsorbers from our study, we were able to assess the physiological impact that fructose metabolism has on the body's energy homeostatic mechanisms. Furthermore, the gastrointestinal upset that occurs as a result of fructose malabsorption may have reduced subjects' *ad libitum* consumption of solid foods in other studies, which could have impacted their overall caloric intake. Thus, we screened all eligible subjects for this crucial characteristic prior to enrollment in the study.

LIMITATIONS OF THE PRESENT STUDY

Our study had several important limitations. First, we only enrolled ten subjects in the study, nine of whom completed all three dietary periods. Secondly, our small sample size consisted of healthy young men and women. In order to be able to generalize our results to the majority of the population, we would need to have a larger, more diverse sample. Furthermore, due to the small sample size, our study had a significant lack of power, particularly for the fructose-glucose comparison. Since this was a pilot study, we did not conduct a sample size calculation. However, future studies will need a larger sample size in order to be appropriately powered to detect clinically meaningful differences between the fructose- and glucose-dietary arms. The fourth limitation of this study was that our subjects were free-living individuals; they were not in a metabolic ward during the dietary periods. Although the study diets were well controlled, we had to trust subjects not to eat or drink anything other than what we provided for them from the Nutrition Research Kitchen. We tried to minimize the chance of our subjects eating and/or drinking outside food by asking them not to consume any foods or drinks other than what we gave them. However, it is still possible that the subjects consumed additional food and/or beverages without our

knowledge. Lastly, our study only looked at fructose-containing beverages, not fructose-containing foods. Additional studies will need to be done to determine whether solid foods sweetened with fructose, sucrose, or HFCS or foods that naturally contain fructose (i.e. fruit) have the same effects on appetite, caloric intake, and weight gain as fructose-sweetened beverages.

PUBLIC HEALTH IMPACT

Our study provides further support for the liquid calories hypothesis; the results of our study suggest that consumption of SSB leads to weight gain and obesity as they cause people to be in a chronic state of increased energy intake. Even though the American Heart Association and the U.S. dietary guidelines advise against the regular consumption of SSB, the consumption of SSB in the U.S. has increased over the last 30 years among both children and adults.[64] In the 1970's, SSB accounted for four percent of the US daily calorie intake, but accounted for nine percent of the US daily calorie intake by 2001.[65] The consumption of SSB has been associated with poor diet quality, weight gain, diabetes, heart disease, and gout.[65] Furthermore, as the obesity epidemic costs our country millions of dollars every year in health-care related costs, our study provides further evidence that reducing the per capita consumption of SSB could have a significant impact on the obesity epidemic, as well as public health.

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