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_____ _____

Allison C. Sylvetsky Date

Non-nutritive Sweeteners: Consumption Trends, Consumer Perceptions, and Metabolic Effects

By
Allison C. Sylvetsky
Doctor of Philosophy
Division of Biological and Biomedical Science
Nutrition and Health Sciences

_____
Kristina I. Rother MD, MHSc.
Advisor

_____
Miriam Vos, MD, MSPH
Advisor

_____
Nana Gletsu Miller, Ph.D.
Committee Member

_____
Dean Jones, Ph.D.
Committee Member

_____
Andrea Sharma, Ph.D.
Committee Member

Accepted:

_____
Dean of Graduate School

_____
Date

Non-nutritive Sweeteners: Consumption Trends, Consumers Perceptions, and Metabolic Effects

By
Allison C. Sylvetsky
B.A. The George Washington University, 2009

Advisor: Kristina I. Rother, MD, MHSc.

Advisor: Miriam B. Vos, MD, MSPH

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Abstract

Non-nutritive Sweeteners: Consumption Trends, Consumer Perceptions, and Metabolic Effects

By Allison C. Sylvetsky

We conducted several multi-disciplinary studies to 1) evaluate trends in the consumption of non-nutritive sweeteners over the last decade using national-level data from five 2-year cycles of the National Health and Nutrition Examination Survey (NHANES), 2) assess parental perceptions of non-nutritive sweeteners and parents' ability to recognize non-nutritive sweeteners in packaged foods and beverages, and 3) determine the acute effects of sucralose on glycemia, satiety, gut hormone responses, and levels of free fatty acids. We also conducted a review of existing research assessing the consumption trends, regulations, and recommendations for use of non-nutritive sweeteners in children, which provided an opportunity to synthesize available data assessing their effects on glycemia, energy intake, body weight, and taste preferences in pediatric populations.

We found that the consumption of non-nutritive sweeteners has increased dramatically in the United States over the last decade, among both children and adults. Furthermore, our data demonstrated that parents are largely unable to identify foods and beverages which contain non-nutritive sweeteners. Parents however expressed overall negative attitudes toward providing non-nutritive sweeteners to their children, despite these widespread increases in their consumption. Meanwhile, our metabolic data do not suggest harmful effects of short-term consumption of non-nutritive sweeteners on glycemia, satiety, or glucose kinetics. Future studies are needed to assess the metabolic

consequences of long term exposure to non-nutritive sweeteners and to determine the impact of non-nutritive sweeteners on dietary patterns and taste preferences. This knowledge will allow us to develop evidence-based recommendations for the use of non-nutritive sweeteners as a replacement for added sugars in the general population.

Non-nutritive Sweeteners: Consumption Trends, Consumer Perceptions, and Metabolic Effects

By

Allison Cara Sylvetsky
B.A. The George Washington University, 2009

Advisor: Miriam B. Vos, MD, MSPH
Advisor: Kristina I. Rother, MD, MHSc.

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Abbreviations Used

AAP……	American Academy for Pediatrics
ADA……...	American Diabetes Association
ADI…….	Acceptable daily intake
ASB……	Artificially sweetened beverages
BMI……	Body mass index (kg/m^2)
EDI…….	Estimated daily intake
FFQ……	Food Frequency Questionnaire
IOM……	Institute of Medicine
LCS……	Low-calorie sweeteners
NHA……..	Nutrition and Health Awareness
NNS……	Non-nutritive sweeteners
NNS-ACCEPT……..	Non-nutritive sweetener acceptance
PCA……..	Principle components analysis
SSB……	Sugar-sweetened beverages
VAS……..	Visual analogue scale

Chapter One: INTRODUCTION

Since the 1980s, the prevalence of obesity in the United States has dramatically increased. The trend is of concern because of obesity's association with the development of chronic diseases including type 2 diabetes, non-alcoholic fatty liver disease, hypertension, cardiovascular disease and stroke, which lead to costly health related and non-health related consequences [1]. Consumption of added sugars, particularly in the form of sugar-sweetened beverages is thought to be a significant contributor to the rapid rise in obesity worldwide [2].

As will be discussed in detail in Chapter 4, non-nutritive sweeteners (NNS) have become increasingly popular because they provide sweetness with little or no contribution to energy intake. Despite their widespread consumption, knowledge about the effects of NNS on glycemia, satiety, and food preferences is sparse [3], resulting in inconsistent guidelines for their consumption, especially for children. Given the lack of conclusive recommendations, many consumers are skeptical about the safety of NNS [4]. Previous research relating to NNS across a wide range of disciplines will be summarized in Chapter 2.

Despite the lack of convincing evidence for benefits or harm associated with the use of NNS, emotionally-charged attitudes about their benefits or detriments have formed [5]. Thus, rigorous intervention studies are imperative to inform policy recommendations and ultimately, consumer choices. With the use of NNS rapidly expanding, studying them in the context of society must approach the subject from an interdisciplinary perspective, taking into account not just mechanistic physiology, clinical and behavioral

research, but also epidemiology, biological anthropology, sensory science, and social psychology.

The body of research presented in this dissertation was designed to answer the following questions:

1. Has consumption of NNS increased in the last decade? If so, how much has consumption of NNS increased and among what socio-demographic subgroups? Which types of foods and/or beverages containing NNS are predominantly increasing?
2. What knowledge and attitudes do parents have regarding providing products sweetened with NNS to their children?
3. What effects do NNS have on glycemia, glucose kinetics, gastric emptying and satiety *compared to plain water*, and how might this affect food intake and weight control?

To address these questions, the following chapters will present an extensive body of research collected using a variety of multi-disciplinary techniques. Following a detailed description of the methods in Chapter 3, Chapter 4 will describe socio-demographic trends in the consumption of foods and beverages containing NNS using data from the National Health and Nutrition Examination survey (NHANES). Chapter 5 will then present data collected from questionnaires assessing parental attitudes towards providing foods and beverages containing NNS to their child. Data exploring whether parents can recognize NNS in commercially available foods and beverages will also be discussed.

Chapter 6 will next describe the testing of four concentrations of sucralose (e.g. Splenda™) as preloads to an oral glucose load. To conclude, Chapter 7 will summarize the findings of this dissertation and present the strengths, limitations and implications of this body of research. Chapter 7 will also present ideas for future studies to further elucidate the mechanisms by which consumption of NNSs may affect appetite, food intake, and body weight among healthy, free-living humans.

Literature Cited

1. Flegal KM, Carroll MD, Ogden CL, et al. Prevalence and trends in obesity among US adults, 1999-2008. JAMA 2010 Jan 20;303(3):235-241.

2. Drewnowski A, Bellisle F. Liquid calories, sugar, and body weight. Am J Clin Nutr 2007 Mar;85(3):651-661.

3. Sylvetsky A, Rother KI, Brown R. Artificial sweetener use among children: epidemiology, recommendations, metabolic outcomes, and future directions. Pediatr Clin North Am 2011 Dec;58(6):1467-1480.

4. Gardner C, Wylie-Rosett J, Gidding SS, et al. Nonnutritive sweeteners: current use and health perspectives: a scientific statement from the American Heart Association and the American Diabetes Association. Diabetes care 2012 Aug;35(8):1798-1808.

5. Gardner C, Wylie-Rosett J, Gidding SS, et al. Nonnutritive sweeteners: current use and health perspectives: a scientific statement from the American Heart Association and the American Diabetes Association. Circulation 2012 Jul 24;126(4):509-519.

Chapter 2: BACKGROUND

The history of non-nutritive sweeteners

Non-nutritive sweeteners (NNS) were discovered by accident in the late 19th century. After working with coal and neglecting to wash his hands, a chemist ate lunch and tasted sweetness on his bread. He later traced the sweetness to a specific compound in his laboratory, known today as saccharin [1]. Until the mid-20th century, saccharin was the only NNS available in the United States and it was consumed only by persons with diabetes, who restricted their sugar intake for medical reasons [2]. It was not until the First World War that saccharin use became widespread due to war-time sugar shortages [1]. Up until this time, saccharin use was entirely unrelated to metabolic or weight control benefits. It was not until the post-World War era in the 1950's that American consumers began to seek out saccharin for weight management [2].

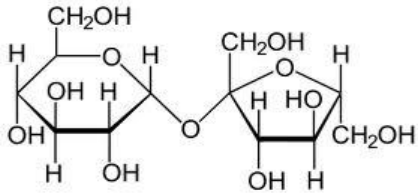
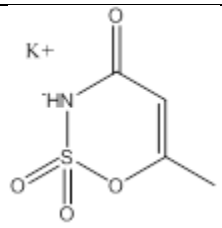
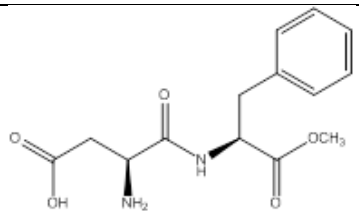
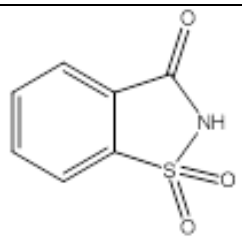
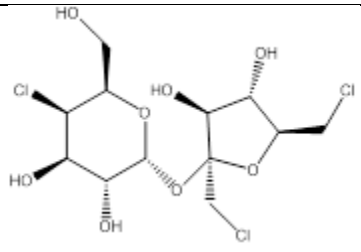
By the 1980's, a second generation of NNS, including cyclamates and aspartame, had emerged. Concurrently, dieting and diet programs became increasingly popular, creating a true market for NNS. Not only were NNS many times sweeter than sucrose, miniscule quantities generated the sweetness level desired, with minimal caloric contribution. These sweeteners became central in the lives of healthy non-diabetic Americans. In fact, when saccharin was banned by the Food and Drug Administration (FDA) in 1977 due to an association with bladder cancer, the public protested vehemently until the sweetener reappeared on the market with a warning label several years later[3]. Even years before the emergence of newer sweeteners such as sucralose, acesulfame-potassium, and neotame, NNS were a controversial health topic from the time of their discovery, and remain the subject of heated debate today.

Definition of non-nutritive sweeteners

As a group, NNS are dietary tools that provide the sweet taste and palatability of caloric sugars, yet do not substantially contribute to energy intake when ingested [4, 5]. NNS can also be referred to as "artificial sweeteners", although some NNS do occur naturally (e.g. stevioside) [6]. Other common names include low-calorie sweeteners, non-caloric sweeteners, as well as high intensity sweeteners, since their sweetness per unit of weight is several hundred times more potent than the sweetening power of sucrose (table sugar) [7].

There are currently six NNS approved for use in the general population in the United States. Their chemical name (e.g. aspartame), chemical formula, trade name (e.g. Equal™), and chemical structure are displayed in **Table 1** below. Aspartame, acesulfame-potassium, neotame (not shown), saccharin, and sucralose are approved by the FDA as food additives, while stevioside is classified as a dietary supplement [8]. The names, year discovered, acceptable daily intake (ADI), and sweetening potency[4] of each of the six NNS approved for use in the United States, are displayed in **Table 2**.

Table 1. Chemical Name, Chemical Formula, Trade Name and Structure of sucrose (for reference) and the five non-nutritive sweeteners approved for use in the United States

Chemical Name	Chemical Formula	Trade Name	Structure
Sucrose **(Nutritive Sweetener)**	($C_{12}H_{24}O_{12}$)	Table Sugar	
Acesulfame Potassium	($C_4H_4KNO_4S$)	Sunett™ SweetOne™	
Aspartame	($C_{14}H_{18}N_2O_5$)	Nutrasweet™ Equal™	
Saccharin	($C_7H_5NO_3S$)	Sweet N'Low™	
Sucralose	($C_{12}H_{19}Cl_3O_8$)	Splenda™	

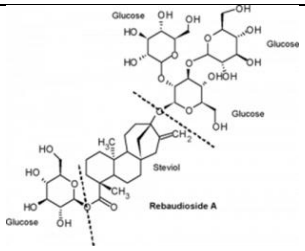
Stevioside (Stevia)	$(C_{38}H_{60}O_{18})$	Truvia™	

Table 2. Acceptable daily intake (ADI) and sweetness potency of six FDA approved non-nutritive sweeteners

Sweetener	Year of Discovery	Acceptable Daily Intake (ADI)[2]	Sweetness Relative to Sucrose
Acesulfame Potassium	1967	15 mg/kg	200X
Aspartame	1981	50 mg/kg	160-220X
Neotame	1965	2 mg/kg	7,000-13,000X
Saccharin	1879	5 mg/kg	300X
Stevia	N/A[1]	5 mg/kg	300X
Sucralose	1976	5 mg/kg	600X

[1]Stevia has been present in many parts of the world since ancient times which are not well documented

[2]Quantity of sweetener in specific foods and beverages vary, but 5 mg/kg of sucralose would correspond to approximately 6- 12 oz. cans of diet soda per day for a 150 lb. (70 kg) adult, whereas 50 mg/kg aspartame would correspond to approximately 30- 12 oz. cans of diet soda per day for a 150 lb. (70 kg) adult.

Recommendations for non-nutritive sweetener consumption among children and adults

Guidelines for the consumption of NNS in the general population are lacking. For example, the United States Department of Agriculture's (USDA) Dietary Guidelines for Americans (2010) do not contain details on the use of NNS, other than emphasizing lowering added sugar intake and practicing proper energy balance to maintain a healthy weight [9]. Similarly, there are few explicit recommendations for NNS consumption among children, and those that do exist are inconsistent and controversial. In 2009, the American Academy for Nutrition and Dietetics stated that NNS were "safe to use within the range of the acceptable daily intake (ADI)" for a specific sweetener which accounts for a child's body weight [3]. In marked contrast, the Institute of Medicine (IOM) does not support NNS use in children because more research is required to determine if NNS are effective for weight management [10]. The IOM also expressed concern regarding the safety of NNS when consumed over many years starting in childhood. Similarly, the American Academy for Pediatrics (AAP) stated that NNS have been inadequately studied for use in children and, thus, should not form a significant part of a child's diet [11]. Various guidelines for the consumption of NNS among children and adults are displayed in **Table 3**.

Table 3. Position statements for use of sweeteners from various scientific organizations

Scientific Organization	Year	Position statement	Population considered
American Dietetic Association	2004 2009	Consumers can safely use artificial sweeteners when consumed in a diet guided by current federal nutrition recommendations. The wide range of artificial sweeteners available in food supply should keep artificial sweeteners intake in children well below the acceptable daily intakes.	Children and adults
Institute of Medicine	2007	No recommendations are made regarding foods containing artificial sweeteners because 1) artificially sweetened beverages have been shown to displace milk and 100% juice at mealtimes 2) more research is needed on the effectiveness of artificial sweeteners in foods for weight management, and 3) more studies are needed on safety effects when artificial sweeteners are consumed over many years starting in childhood or adolescence	Specific to children
American Academy of Pediatrics	2010	The use of artificial sweeteners to provide health benefits for children and adolescents has been inadequately studied. As such, they should not form a significant part of a child's diet.	Specific to children
American Heart Association	2010	People with diabetes can use artificial sweeteners, as can people on a weight loss diet	General population
American Diabetes Association	2010	Foods and drinks that contain artificial sweeteners are an option for those with diabetes to consume fewer calories and carbohydrates when replaced for a food or drink containing sugar.	General population

Regulation of non-nutritive sweeteners

For each NNS, the FDA establishes an Acceptable Daily Intake (ADI) [12], in milligrams (mg) per kilogram (kg) body weight. The ADI is typically 100 times lower than the dose of the sweetener that caused toxicity in animal studies, and is the amount of sweetener thought to be safe for an individual to consume every day for a lifetime. To determine if a sweetener should be approved for use, the FDA then must establish that the typical human intake of the sweetener (Estimated Daily Intake, or EDI) will be below the ADI. If the estimated daily intake (EDI) is below the ADI, then the sweetener is considered safe for human use. Aspartame, saccharin, sucralose, and neotame are classified as food additives by the FDA, while stevia is classified as a dietary supplement which means that the manufacturers of stevia are responsible for its safety. Dietary supplements are regulated differently than food additives, as food additives undergo testing by the FDA before approval whereas dietary supplements are approved until adverse effects are demonstrated at which point the FDA will remove them from the market[13].

Assessing dietary intake of non-nutritive sweeteners

Because the FDA does not require manufacturers to report the amount of NNS contained in foods and beverages, quantification of the precise amount of sweeteners present in different types of food is difficult. Information about the total quantity of sweeteners in use is extracted from intake information for the foods that contain them, by using food composition tables and validated food databases [8]. It is important to understand that the sweetening power of the NNS listed above is hundreds of times greater than that of sucrose **(Table 2)**.

Coupled with the difficulty of assessing the specific amount of NNS in commercially-available foods and beverages, assessing dietary intake is challenging. Because most dietary intake data are based on self-report, intake is often under-estimated, due to social desirability bias (reporting less of something that is not perceived as "healthy"), limited knowledge of what ingredients are in a food (i.e., the person does not know that acesulfame-potassium is a NNS when they see it on a food label), and recall bias (where the item or quantity consumed is reported inaccurately, due to misperception or due to memory limitations). Despite these limitations, various methods relying on self-report exist and each has its advantages and disadvantages (discussed in more detail in Chapter 3). These methods include 24-hour dietary recall, food-frequency questionnaires, 3-day food records, and food diaries. While other methods exist, such as the use of food availability or disappearance data[14] the self-reported methods used in this dissertation tend to be more accurate and provide an opportunity to compare consumption across socio-demographic subgroups[14].

Possible associations between non-nutritive sweetener consumption, appetite, food intake, and body weight

NNS consumption, most commonly in the form of no-and low-calorie beverages, has been associated with adverse metabolic outcomes in cross-sectional studies, including weight gain [15], metabolic syndrome [16], and vascular dysfunction [17]. Due to the cross-sectional nature of the majority of these epidemiologic studies suggesting adverse metabolic effects of NNS, causality cannot be determined. Meanwhile, various physiological and behavioral mechanisms have been hypothesized to explain the observed correlation between NNS consumption and body weight. A few randomized controlled trials assessing the effects of NNS have demonstrated subtle beneficial effects, such as marginal weight loss, successful weight maintenance, or less weight gain [18, 19], but additional trials looking specifically at the effects of NNS consumption on body weight are required.

The most obvious explanation for the positive epidemiologic association between NNS consumption and body weight is reverse causality, in that those individuals who are already overweight or have difficulty controlling their weight will turn to NNS as a weight loss tool. Additional well-supported explanations have been proposed and are displayed in **Figure 1** below. It has also been suggested that consumption of NNS may lead to a disconnect between sweetness and calories and, ultimately, to changes in dietary patterns [20]. This explanation is based on a classical conditioning model where humans associate sweetness with a lack of calories when they are repeatedly exposed to NNS. As a result, they do not recognize or are not prepared for the energy load when sweetness and calories are administered together, as in sugar-sweetened beverages (SSBs), baked goods, or sweetened yogurts. This proposed mechanism has been supported by rodent

data showing that rats continually exposed to saccharin will increase food intake and gain more weight as compared to littermates who are exposed a caloric sweetener, such as glucose [21].

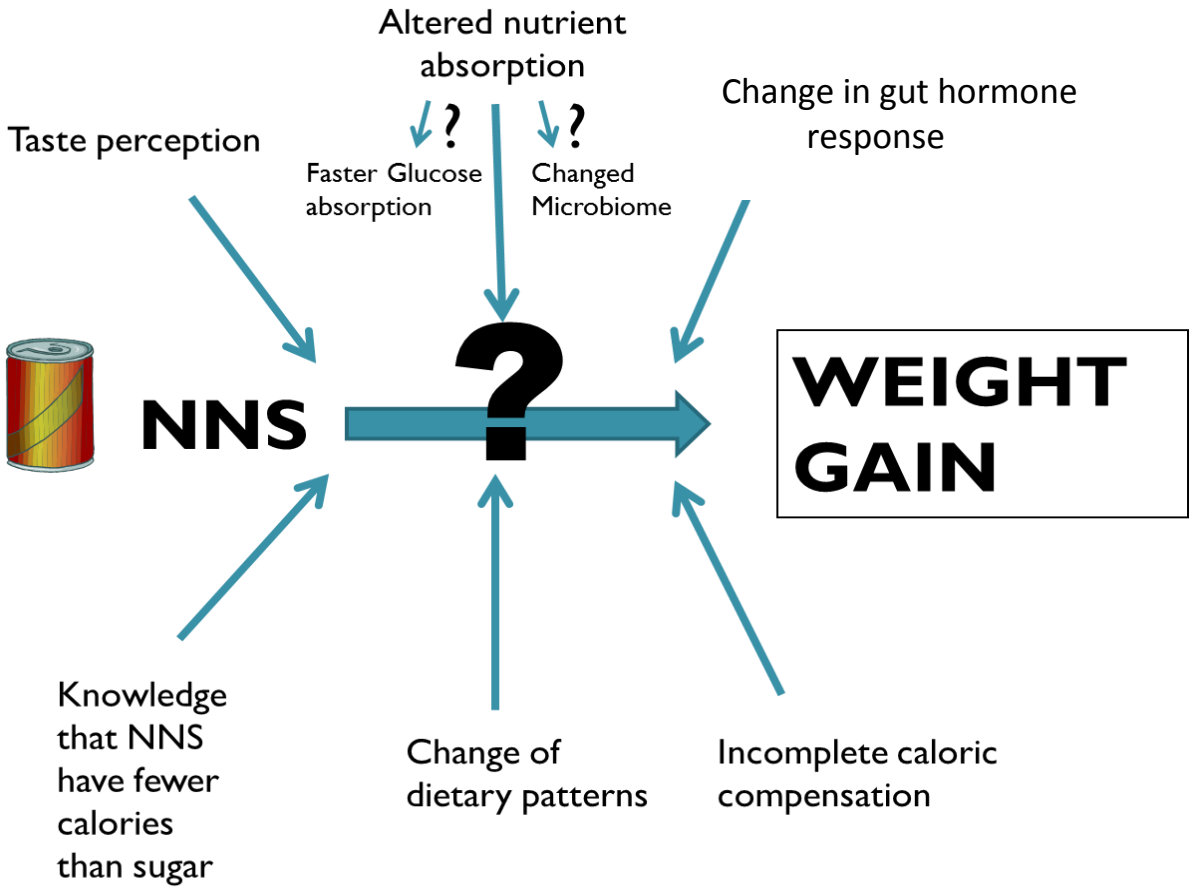
Another popular physiologic explanation of increased food intake is that consumption of NNS leads to alterations in gut hormone responses, which could theoretically reduce the feeling of satiety (e.g., via greater insulin secretion stimulated by incretins) and increase energy intake. Rodent data also exist to suggest that consumption of NNS, specifically sucralose, leads to an increase in the rate of intestinal glucose absorption, when NNS are consumed together with glucose, as is commonly the case in the human diet [22]. Moreover, a recent report suggests that NNS may alter the proportions of different bacterial species in the gut microbiome [23], which may also alter absorption rates.

Beyond potential alterations in gut physiology, the association between NNS and body weight may be explained by processes occurring in the brain [7]. A growing body of literature on sweet taste, reward, and tolerance has expanded to include NNS, as caloric sugars have been shown to cause the release of endogenous opioids, endorphins, and dopamine from the brain. Since the nutritive value of sweet-tasting food plays a sweet-taste independent role in inducing dopaminergic responses [24], NNS recapitulate some, but not all, of the reward response elicited by caloric sugars [25].

Two recent cross-sectional studies have demonstrated that consumption of NNS may blunt or alter the central reward response to caloric sugars [26, 27], suggesting that individuals may increase their consumption of sweet tasting calorie-containing foods and beverages to feel satisfied. Furthermore, repeated exposure to highly sweet foods and

beverages may lead to alterations in one's preference for sweet tasting substances, many of which are very high in energy [28, 29]. Finally, it is also possible that awareness of consuming foods or beverages containing NNS may lead to overconsumption, due to an individual's knowledge that they are ingesting a reduced-calorie item [30].

Figure 1. Proposed mechanisms for the association between non-nutritive sweetener consumption and body weight



Role of sweet taste receptors in glucoregulatory hormone secretion, appetite, and food intake

Recent evidence has shown that taste receptors are present not only in the oropharynx, but also in the intestine and the pancreas [31]. Nutrient responsive G-protein coupled taste receptors are found on the enteroendocrine cells in the gut, and respond to bitter (T2R's), umami (T1R1 and T1R3), and sweet taste (T1R2 and T1R3) [32, 33]. These receptors are activated by a wide variety of sweet tasting compounds including caloric sugars (i.e., glucose and fructose), NNS (i.e., sucralose and acesulfame-potassium), and sweet tasting proteins and amino acids (i.e., D-tryptophan), where they are co-localized with entero-endocrine L-cells expressing glucagon-like-peptide 1 (GLP-1) and PYY. Thus, the sweet taste receptors in the gut are thought to be involved in the regulation of insulin secretion and blood plasma glucose levels [34]. The importance of the sweet taste receptor in mediating glucoregulatory hormone responses has been clearly demonstrated using sweet taste receptor inhibitors (i.e., lactisole and gumarin) that at least partially, suppress gut peptide secretion in response to glucose and NNS [35, 36].

Role of sweet taste preference in shaping eating behaviors

Taste is an important factor in an individual's decision to consume or to avoid a certain food or beverage, and it is mediated by genetic and environmental factors [37]. People are born with an innate preference for sweet tasting substances, particularly those that also contain fat [38, 39], and express an innate aversion to bitter tasting compounds. Sweet substances may have analgesic effects in infants [40, 41], lead to addictive-like behaviors in rodents [40, 42], and augment dopamine response in a fashion similar to that of addictive drugs, such as cocaine [43]. While seeking highly caloric sweet foods and avoiding bitter and often poisonous substances were once advantageous survival traits, excessive intake of high sugar foods today often leads to increased energy intake and ultimately, weight gain.

A growing body of research provides evidence for individual differences in preference of sweet taste. Changes in sweet taste preference are observed in childhood and again among the elderly, and may vary greatly between individuals [38]. Preferences may also be altered by individual experience with sweet taste [44]. Greater liking of sweet taste in overweight and obese individuals has been demonstrated [45]. One reason for the correlation between higher weight and sweet preference may be a lower perceived intensity of sweet taste among heavier people [29], causing them to consume more of a sweet tasting substance in order to feel satisfied. Interestingly, sensitivity to sweet taste has been shown to increase dramatically among some post-operative Roux-en-Y gastric bypass patients [46-48], resulting in lower detection thresholds and increased reward responses. However, not all investigators report these observations. Limited evidence

also suggests that the association between sweet taste preferences, food and beverage intake, and body weight may vary by ethnicity [18] and by gender [49].

Beyond genetic and physiologic determinants of sweet taste preference mentioned above, psychosocial determinants of food preference play a pivotal role in determining an individual's food intake [50]. While food preferences are in part shaped by innate predisposition to seek sugar and fat, while avoiding bitter substances, taste preference is largely attributable to learning processes [51-53]. Among children in particular, the influence of parents and other family members in the development of eating behavior has been widely studied [50, 54]. Parents can build environments for their children that encourage healthy diets, not only by controlling what the child eats but, equally important, by modeling their own dietary practices [55-57].

While the biological and psychological determinants of taste preference are beyond the scope of this dissertation, NNS provide an interesting model for studying food preference. Since NNS provide the sweet sensation that is innately attractive to humans, while lacking the energy content that was once required for survival, it is possible that they may satisfy a person's need for sweet taste while reducing energy intake. On the contrary, consumption of NNS may force the body to compensate for the lack of calories associated with the sweet sensation by increasing sugar-seeking behavior, leading to increased energy intake and weight gain. Beyond a possible dissociation of sweet taste and calorie content, several of the previously mentioned mechanisms by which NNS may affect body weight are based on the complicated interplay between sweet taste, food selection, and calorie intake.

Purpose of research

As the prevalence of obesity continues to increase in the United States and globally, there is a great need for effective public health strategies to facilitate weight control among both children and adults. One of many potential approaches is to reduce the consumption of added sugars, which have been clearly linked to weight gain [58]. NNS offer a potential alternative to added sugars, providing sweet taste with no or low-contribution to caloric intake. Although NNS do show promise as a tool for weight management and obesity prevention, their metabolic effects are largely unknown. This has led to inconclusive recommendations for their use. The unclear benefits of NNS on weight control, coupled with their heavily debated safety among the public, has challenged the viability of substituting NNS for caloric sugars.

The purpose of this dissertation was to assess current national trends in the consumption of NNS, to explore the public perception and recognition of NNS, and to rigorously explore the metabolic effects of NNS. To do so, we used a wide range of methods including national level dietary consumption data from NHANES, attitudinal questionnaires administered among convenience samples of parents, and the recruitment and enrollment of healthy volunteers in clinical trial.

Literature cited

1. Bartoshuk LM. Artificial Sweeteners: Outwitting the Wisdom of the Body? Observer. 2009.

2. de la Pena C. Artificial sweetener as a historical window to culturally situated health. Annals of the New York Academy of Sciences 2010 Mar;1190:159-165.

3. United States Food and Drug Administration. Guidance for Industry and Other Stakeholders Toxicological Principles for the Safety Assessment of Food Ingredients. In: Department of Health and Human Services, ed., 2000.

4. Brown RJ, de Banate MA, Rother KI. Artificial sweeteners: a systematic review of metabolic effects in youth. Int J Pediatr Obes 2010 Aug;5(4):305-312.

5. Gardner C, Wylie-Rosett J, Gidding SS, et al. Nonnutritive sweeteners: current use and health perspectives: a scientific statement from the American Heart Association and the American Diabetes Association. Circulation 2012 Jul 24;126(4):509-519.

6. Prakash I, Dubois GE, Clos JF, et al. Development of rebiana, a natural, non-caloric sweetener. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 2008 Jul;46 Suppl 7:S75-82.

7. Sylvetsky A, Rother KI, Brown R. Artificial sweetener use among children: epidemiology, recommendations, metabolic outcomes, and future directions. Pediatr Clin North Am 2011 Dec;58(6):1467-1480.

8. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. The American journal of clinical nutrition 2009 Jan;89(1):1-14.

9. United States Department of Agriculture. Dietary Guidelines for Americans. Washington, DC: U.S. Government Printing Office, 2010.

10. Institute of Medicine FaNB. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids. Washington, DC: Institute of Medicine; 2002/2005.

11. American Academy of Pediatrics. Combating Obesity: Frequently Asked Questions about Food Choices. 2011 [cited January 9th, 2013]; Available from: http://www.pediatricassociatespa.com/pdf/Combating_Obesity_FAQs_About_Food_Choices.pdf

12. Adminstration USFaD. **Guidance for Industry and Other Stakeholders Toxicological Principles for the Safety Assessment of Food Ingredients**. In: Services DoHaH, ed., July 2000, Revised July 2007.

13. United States Food and Drug Administration. Dietary Supplements. In: Services USDoHaH, ed., 2013.

14. United States Food and Drug Administration. Guidance for Industry: Estimating Dietary Intake of Substances in Food. In: United States Department of Health and Human Services, ed., 2006.

15. Fowler SP, Williams K, Resendez RG, et al. Fueling the Obesity Epidemic[quest] Artificially Sweetened Beverage Use and Long-term Weight Gain. Obesity 2008.

16. Nettleton JA, Lutsey PL, Wang Y, et al. Diet Soda Intake and Risk of Incident Metabolic Syndrome and Type 2 Diabetes in the Multi-Ethnic Study of Atherosclerosis. Diabetes care 2009 Jan 16.

17. Gardener H, Rundek T, Markert M, et al. Diet Soft Drink Consumption is Associated with an Increased Risk of Vascular Events in the Northern Manhattan Study. J Gen Intern Med 2012 Jan 27.

18. Ebbeling CB, Feldman HA, Chomitz VR, et al. A randomized trial of sugar-sweetened beverages and adolescent body weight. The New England journal of medicine 2012 Oct 11;367(15):1407-1416.

19. de Ruyter JC, Olthof MR, Seidell JC, et al. A trial of sugar-free or sugar-sweetened beverages and body weight in children. The New England journal of medicine 2012 Oct 11;367(15):1397-1406.

20. Swithers SE, Martin AA, Davidson TL. High-intensity sweeteners and energy balance. Physiology & behavior 2010 Apr 26;100(1):55-62.

21. Swithers SE, Laboy AF, Clark K, et al. Experience with the high-intensity sweetener saccharin impairs glucose homeostasis and GLP-1 release in rats. Behavioural brain research 2012 Jul 15;233(1):1-14.

22. Mace OJ, Affleck J, Patel N, et al. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. J Physiol 2007 Jul 1;582(Pt 1):379-392.

23. Abou-Donia MB, El-Masry EM, Abdel-Rahman AA, et al. Splenda alters gut microflora and increases intestinal p-glycoprotein and cytochrome p-450 in male rats. Journal of toxicology and environmental health Part A 2008;71(21):1415-1429.

24. de Araujo IE, Oliveira-Maia AJ, Sotnikova TD, et al. Food reward in the absence of taste receptor signaling. Neuron 2008 Mar 27;57(6):930-941.

25. Smeets PA, Weijzen P, de Graaf C, et al. Consumption of caloric and non-caloric versions of a soft drink differentially affects brain activation during tasting. NeuroImage 2011 Jan 15;54(2):1367-1374.

26. Green E, Murphy C. Altered processing of sweet taste in the brain of diet soda drinkers. Physiology & behavior 2012 Nov 5;107(4):560-567.

27. Rudenga KJ, Small DM. Amygdala response to sucrose consumption is inversely related to artificial sweetener use. Appetite 2012 Apr;58(2):504-507.

28. Birch LL, Anzman-Frasca S. Learning to prefer the familiar in obesogenic environments. Nestle Nutr Workshop Ser Pediatr Program 2011;68:187-196; discussion 196-189.

29. Sartor F, Donaldson LF, Markland DA, et al. Taste perception and implicit attitude toward sweet related to body mass index and soft drink supplementation. Appetite 2011 Aug;57(1):237-246.

30. Shide DJ, Rolls BJ. Information about the fat content of preloads influences energy intake in healthy women. Journal of the American Dietetic Association 1995 Sep;95(9):993-998.

31. Kojima I, Nakagawa Y. The Role of the Sweet Taste Receptor in Enteroendocrine Cells and Pancreatic beta-Cells. Diabetes & metabolism journal 2011 Oct;35(5):451-457.

32. Egan JM, Margolskee RF. Taste cells of the gut and gastrointestinal chemosensation. Mol Interv 2008 Apr;8(2):78-81.

33. Margolskee RF, Dyer J, Kokrashvili Z, et al. T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. Proceedings of the National

Academy of Sciences of the United States of America 2007 Sep 18;104(38):15075-15080.

34. Jang HJ, Kokrashvili Z, Theodorakis MJ, et al. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. Proceedings of the National Academy of Sciences of the United States of America 2007 Sep 18;104(38):15069-15074.

35. Jiang P, Cui M, Zhao B, et al. Lactisole interacts with the transmembrane domains of human T1R3 to inhibit sweet taste. The Journal of biological chemistry 2005 Apr 15;280(15):15238-15246.

36. Steinert RE, Gerspach AC, Gutmann H, et al. The functional involvement of gut-expressed sweet taste receptors in glucose-stimulated secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). Clinical nutrition 2011 Feb 14.

37. Birch LL, Fisher JO. Development of eating behaviors among children and adolescents. Pediatrics 1998 Mar;101(3 Pt 2):539-549.

38. Drewnowski A, Mennella JA, Johnson SL, et al. Sweetness and food preference. The Journal of nutrition 2012 Jun;142(6):1142S-1148S.

39. Drewnowski A, Krahn DD, Demitrack MA, et al. Taste responses and preferences for sweet high-fat foods: evidence for opioid involvement. Physiology & behavior 1992 Feb;51(2):371-379.

40. Bucher HU, Baumgartner R, Bucher N, et al. Artificial sweetener reduces nociceptive reaction in term newborn infants. Early Hum Dev 2000 Jul;59(1):51-60.

41. Mennella JA, Pepino MY, Lehmann-Castor SM, et al. Sweet preferences and analgesia during childhood: effects of family history of alcoholism and depression. Addiction 2010 Apr;105(4):666-675.

42. Avena NM, Rada P, Hoebel BG. Sugar bingeing in rats. Curr Protoc Neurosci 2006 Aug;Chapter 9:Unit9 23C.

43. Rada P, Avena NM, Hoebel BG. Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience 2005;134(3):737-744.

44. Sclafani A. Oral, post-oral and genetic interactions in sweet appetite. Physiol Behav 2006 Nov 30;89(4):525-530.

45. Macdiarmid JI, Vail A, Cade JE, et al. The sugar-fat relationship revisited: differences in consumption between men and women of varying BMI. Int J Obes Relat Metab Disord 1998 Nov;22(11):1053-1061.

46. Bueter M, Miras AD, Chichger H, et al. Alterations of sucrose preference after Roux-en-Y gastric bypass. Physiol Behav 2011 Oct 24;104(5):709-721.

47. Burge JC, Schaumburg JZ, Choban PS, et al. Changes in patients' taste acuity after Roux-en-Y gastric bypass for clinically severe obesity. J Am Diet Assoc 1995 Jun;95(6):666-670.

48. Miras AD, Jackson RN, Jackson SN, et al. Gastric bypass surgery for obesity decreases the reward value of a sweet-fat stimulus as assessed in a progressive ratio task. Am J Clin Nutr 2012 Sep;96(3):467-473.

49. Lanfer A, Knof K, Barba G, et al. Taste preferences in association with dietary habits and weight status in European children: results from the IDEFICS study. Int J Obes (Lond) 2012 Jan;36(1):27-34.

50. Scaglioni S, Arrizza C, Vecchi F, et al. Determinants of children's eating behavior. Am J Clin Nutr 2011 Dec;94(6 Suppl):2006S-2011S.

51. Anzman-Frasca S, Savage JS, Marini ME, et al. Repeated exposure and associative conditioning promote preschool children's liking of vegetables. Appetite 2012 Apr;58(2):543-553.

52. Mennella JA, Castor SM. Sensitive period in flavor learning: Effects of duration of exposure to formula flavors on food likes during infancy. Clinical nutrition 2012 Dec;31(6):1022-1025.

53. Trabulsi JC, Mennella JA. Diet, sensitive periods in flavour learning, and growth. International review of psychiatry 2012 Jun;24(3):219-230.

54. Savage JS, Fisher JO, Birch LL. Parental influence on eating behavior: conception to adolescence. The Journal of law, medicine & ethics : a journal of the American Society of Law, Medicine & Ethics 2007 Spring;35(1):22-34.

55. Scaglioni S, Salvioni M, Galimberti C. Influence of parental attitudes in the development of children eating behaviour. The British journal of nutrition 2008 Feb;99 Suppl 1:S22-25.

56. Lopez NV, Ayala GX, Corder K, et al. Parent support and parent-mediated behaviors are associated with children's sugary beverage consumption. Journal of the Academy of Nutrition and Dietetics 2012 Apr;112(4):541-547.

57. Eisenberg CM, Ayala GX, Crespo NC, et al. Examining multiple parenting behaviors on young children's dietary fat consumption. Journal of nutrition education and behavior 2012 Jul-Aug;44(4):302-309.

58. Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. The American journal of clinical nutrition 2006 Aug;84(2):274-288.

Chapter 3: METHODS

This chapter will describe the general methodologies utilized in the studies contained in this dissertation to meet the objectives listed below. Because each of these objectives is addressed in a separate chapter, the detailed approach for each project is described in each respective chapter (Chapters 4-6). In this expanded methods section, we provide additional details on the justification for the major methodologies selected, and describe the concepts behind the statistical approaches employed.

Objectives and hypotheses

The primary objectives of this dissertation are 1) to assess recent trends in the consumption of NNS, 2) to evaluate parental perception and recognition of NNS, and 3) to investigate the metabolic effects of NNS, particularly as they relate to glycemia control, gut peptide secretion, and glucose kinetics.

The specific hypotheses are:

1. Given that consumption of added sugars has been declining [1], consumption of NNS will have increased among both children and adults.
2. Parents will view NNSs as unsafe for their child to consume, yet they will not be able to identify many NNS containing foods and beverages.
3. Administering a sucralose-sweetened beverage ten minutes prior to an oral glucose load will result in increased secretion of GLP-1 and insulin.

Summary of methods

Anthropometric measurements

In our first study which used NHANES data to estimate trends in the consumption of NNS (chapter 4), height and weight measurements were obtained by trained NHANES staff using calibrated stadiometers linked directly to a digital information system, and using calibrated digital scales, respectively [2]. In our second study, assessing parental perceptions of recognition of NNS (chapter 5), child height and weight were reported by parents to the nearest half inch and to the nearest pound, respectively in our study of parental recognition and perceptions of NNS. In our clinical study assessing the metabolic effects of sucralose (chapter 6), trained nurses measured height to the nearest centimeter using calibrated stadiometers and measured weight to the nearest 0.1 kilogram using digital scales. In all studies, body-mass-index (BMI) was calculated from the measured height and weight (kg/m^2) for adults. BMI percentile was calculated to determine weight status among children based on age- and sex- specific cut-offs from standardized CDC growth charts [3, 4]. While other measures exist to assess adiposity, including waist circumference and waist-to-hip ratio[5], the use of BMI and BMI percentile are valid and efficient ways to assess an individual's body weight for comparison on a population level [6].

24-hour dietary recalls

As will be described in more detail in the chapters to follow, 24-hour dietary recalls were the main source of dietary data in this dissertation. NHANES 24-hour recall data

were collected through an in-person interview using a computerized five step method [7]. All NHANES dietary data were collected in accordance with standard NHANES procedures, detailed in the Dietary Interviews Procedure Manual [8]. To obtain our own dietary data through 24-hour recalls in the clinical study, validated multiple pass procedures [9] were used in conjunction with the Nutrition Data System for Research (NDSR) [10], to obtain reliable estimates. Short food-frequency questionnaires (FFQ) were also used to assess habitual NNS consumption during the clinical study assessing the metabolic effects of sucralose [11].

Because most dietary intake data is based on self-report, social desirability bias (reporting less of something that is not perceived as "healthy"), limited knowledge of what ingredients are in a food (i.e., the person does not know that acesulfame-potassium is a NNS when they see it on a food label), and recall bias (where the item or quantity consumed is reported inaccurately, due to misperception or due to memory limitations) may influence estimates [12]. Despite the limitations of self-reported intake, 24-hour dietary recalls and the use of food frequency questionnaires are both validated and highly accepted methods [13], and provided us with the opportunity to compare consumption across subgroups.

Interview-assisted questionnaires (non-dietary)

In addition to the questionnaire data collected through NHANES [14], we used closed-ended questionnaires (described in detail in Chapter 5) to gather information about socio-demographic and anthropometric characteristics, and parental recognition of NNS during our grocery store study assessing parental attitudes and recognition of NNS. We also used closed-ended questionnaires, called visual analogue scales (described below) to

assess subjective ratings of hunger and satiety during our clinical study investigating the metabolic effects of sucralose. Due to the novelty of our research questions, the questionnaires utilized were not validated. However, questionnaires were designed in accordance with standards for question sequence, item consistency, and questionnaire appearance [15] and content was developed based on the expertise of the research team. The use of well-designed questionnaire instruments provided an efficient way for us to collect, analyze, and interpret a breadth of data [16].

The use of carefully created questionnaires also assisted the research team in maintaining objectivity and standardizing the process for each respondent [15], as different trained interviewers were surveying the respondent on different days and for different participants. One setback of using questionnaire data rather than a qualitative approach was that participant's answers could not be explored in greater detail and complex underlying concepts could not be elucidated [17]. Questionnaires, however, were well-suited in meeting our objectives, as we were able to study a relatively large sample in an efficient and straight-forward manner [18], whereas open-ended techniques such as interviews or focus group discussions are limited in that they require longer time periods and more expensive resources [19].

Each type of questionnaire utilized throughout this dissertation is described below.

1. *Demographic screener questionnaires*

   To assess socio-demographic characteristics, a sub-set of items from the validated demographic screener questionnaire used in NHANES were collected [14].

2. *Likert-scales*

Likert scales are a validated method for examining psychosocial questions[20] and were used to assess parental attitudes toward NNS use among children. Attitudes were evaluated based on participant responses to statements read out-loud by trained research assistants. Agreement with each statement was determined using a 5-point Likert scale, where "1" was strongly disagree and "5" was strongly agree. A response of 4 or 5 on the Likert scale indicated agreement with a given questionnaire item. The use of a five-point Likert scale was selected for usability purposes and to allow for collection of ordinal data could be reliably dichotomized for analysis purposes [21].

3. Visual analogue scales

We used similar well-accepted instruments called visual analogue scales (VAS) [22] to assess subjective ratings of hunger and satiety in our clinical study (see chapter 6). 100mm visual analog scales allowed participants to rate the intensity of their feelings on a spectrum from "not at all" to "extremely," by placing a mark on a line [22]. This allowed the research team to obtain a numeric estimate for the intensity of their response, by measuring with a ruler the length from the end of the scale ("not at all") to the mark that they placed on the line.

Methods specific to Chapter 4: evaluating recent trends in the consumption of non-nutritive sweeteners

Selecting a national level data set

The National Health and Nutrition Examination Survey (NHANES) is a set of studies carried out by the National Center for Health Statistics (NCHS) which combines individual interviews with physical examinations and laboratory measures to monitor the health of children and adults throughout the United States population [14]. NHANES interviews gather demographic, socio-economic, dietary, and health-related data while the physical examination measures dental, anthropometric, and physiologic measurements including laboratory analysis [14]. NHANES examines approximately 5,000 participants each year from counties across the nation allowing for the generation of nationally representative data. A complex, multistage, sampling design is used to randomly select participants for NHANES and certain population subgroups are oversampled to increase the reliability of the estimates for these groups [14]. Given the extensive amount of dietary and socio-demographic information available, we were able to assess consumption of low-calorie sweeteners (LCS) on a population level.

Categorizing dietary data

Quantitative dietary intake data are obtained for all NHANES participants using a 24-hour computer assisted dietary recall interview. The nutrient content of foods and beverages reported in NHANES is determined using the Food and Nutrient Database for Dietary Studies, which uses food-composition data from the USDA National Nutrient Database for Standard Reference [23]. We used food codes from the database to identify

and categorize foods and beverages that contained low-calorie sweeteners (LCS) that the participants reported consuming. A description of the use of USDA food codes can be found elsewhere [24].

We decided to broadly categorize items with NNS as foods containing NNS and beverages containing NNS. Under the umbrella of foods containing NNS, we sub-categorized items as condiments with NNS, desserts with NNS and other foods with NNS. We sub-categorized beverages with NNS as those containing no-calories (such as diet soda) and those which did contain calories (reduced-calorie) and were sweetened with NNS. We chose to categorize the items this way because the majority of NNS reported came either from beverage sources, or from condiments or desserts with NNS. Categorization of NNS containing foods and beverages based on either the amount of NNS or the specific sweetening agent was not possible because reliable quantitative data for NNS concentration are not available and the type of NNS used was not distinguished by the food codes.

Methods specific to Chapter 5: assessing parental perceptions and recognition of non-nutritive sweeteners

Selection of NNS sweetened foods and beverages

The purpose of this activity was to present participants with a variety of commercially-available packaged food and beverage items to assess if they were able to distinguish items that were sweetened with NNS from similar items which did not contain NNS. Since the activity was conducted outside of a grocery store, we used the store's online grocery shopping website to view all available products

(www.safeway.com). Research assistant's searched products aisle-by-aisle and identified foods and beverages with NNS by reading detailed product descriptions and ingredients lists.

Items that contained NNS, including aspartame, saccharin, acesulfame-potassium, stevia, rebaudiana (another sweet extract of the S. Rebaudiana plant from which stevia is extracted), or sucralose were selected. Once all grocery aisles had been examined for NNS containing products, duplicates of similar NNS containing items were removed. For example, though Diet Coke and Diet Pepsi were both NNS sweetened diet colas, only Diet Coke was selected. One exception was when similar products both contained NNS but had food claims or product descriptions which may influence a consumer's perception of the product. For example, though compositionally similar, both Quaker weight-control oatmeal and Quaker reduced-sugar oatmeal were selected. Finally, only one flavor of a given NNS sweetened item was selected when numerous were available. NNS-containing items with similar flavoring were duplicated, however, when they were compositionally different. For example, both "light cranberry juice" and "diet cranberry juice," both sweetened with NNS were presented. After excluding duplicate versions of the same or similar products, we selected 45 NNS containing foods and beverages to test recognition.

Selection of matched foods and beverages that did not contain NNS

Up to three analogous non-NNS containing matches were selected for each of the 45 NNS containing foods and beverages presented, depending on availability. Matches were selected to be as similar in terms of brand, size, and contents to the NNS containing item as possible. For example, lemon-lime flavored low-calorie Gatorade was matched

with regular (full-sugar) lemon-lime flavored Gatorade, rather than a different flavor of Gatorade or a different brand of sports drink, such as Powerade. This enabled us to eliminate confounding by these factors, and focus on the product description, front-of-package food claims and participant knowledge of the product's ingredients.

Presentation of food and beverage array

All NNS sweetened foods and beverages and their matches were presented on large tables outside of the grocery store. Items were organized by category so that foods and beverages that would normally be found in the same aisle of a grocery store were placed next to each other. For example, an NNS sweetened breakfast cereal would be placed next to similar breakfast cereals that did not contain NNS. Once a suitable arrangement of items was determined, the tables were photographed so that the research team could replicate the arrangement each day of the study. Photographs were also taken from different angles to ensure that all items on the table were easily visible. Numeric codes which corresponded to each item were then attached to the table in order to depict correct item placement.

Methods specific to Chapter 6: Investigating the metabolic effects of non-nutritive sweeteners

Outpatient Beverage Challenge Visits

Beverage challenge visits were conducted as part of a same-subject randomized crossover study where each participant served as their own control, which is a validated approach recommended for studying short-term effects [25]. Study visits were scheduled on separate days, between 2 days and 6 weeks apart, to avoid carryover effects from the prior visit [26] and to avoid any significant changes in body weight or metabolic

parameters. Block randomization, a scientifically valid randomization scheme based on a random number table [27], was used to assign each subject to a random sequence of test conditions.

In each test period, participants ingested either a NNS or an unsweetened control beverage, and ten minutes later, they consumed a glucose load (either as an oral glucose drink or as a small glucose-containing breakfast bar). Following consumption of the glucose load, blood samples were drawn at short intervals over a two hour time period. Blood samples were then analyzed using a variety of laboratory techniques for various compounds of interest which are listed below (see laboratory measurements).

This design was a reliable approach for assessing the metabolic effects over a short-time period since the use of the same subject design reduced inter-individual variability [28] and allowed us to focus on the effects of the NNS containing preload with repeated measurements at pre-determined time points [25]. An alternative way to conduct this study would be to use a parallel design where participants are randomized to consume either sucralose or the plain water control during the study visit and then compare the outcomes of interests between the two groups. We decided not to use this method, however, because variability between subjects in clinical studies is large, and thus, it is likely that any effects of the sucralose may have been confounded by differences not related to the treatment received.

Challenges of conducting short-term intervention studies using a preload design

The majority of studies looking at the metabolic effects of NNSs use a "preload" design where a test substance (food, beverage, capsule etc.) containing a NNS is provided and then blood samples are obtained at frequent time points over the next several hours

[29-32]. The timing of the preload, the length of follow-up, and the vehicle of sweetener administration depend on the mechanism under investigation [33, 34].

We chose to administer sucralose orally to ensure interaction with the sweet taste receptors both in the oropharynx and on the intestinal entero-endocrine cells [35, 36]. We used a beverage to administer the sweetener rather than a food because this is the form in which NNS are most commonly ingested in free living populations [37] and because the use of a beverage allowed the preload to be non-caloric. The NNS containing beverage was administered prior to a glucose-containing food or beverage to investigate a possible synergism between the NNS and the nutritive sweetener, as has been suggested in animal studies [38]. We took serial blood measurements over the course of 2-3 hours because changes in our outcomes of interest, including glucose, insulin, and GLP-1 occur rapidly following ingestion of glucose and return to baseline within this time frame [31].

Laboratory measures

Blood samples were collected at multiple time points to assess biomarkers relevant to glycemia control, satiety, gastric emptying, and glucose kinetics. Blood samples were obtained using an intravenous cannula which was inserted into the participant's upper arm at the start of each study visit. This was done to avoid having to repeatedly insert a needle since a relatively large amount of blood was drawn at serial time points. Blood was drawn repeatedly over the course of 2-3 hours following consumption of the test beverage so that we could estimate the physiological response of each analyte of interest. A brief description of the laboratory techniques used to measure each analyte of interest is found below.

1. GLP-1 and GIP

Total GLP-1 was measured using a radioimmunoassay (Millipore, Billerica MA, USA). Active GLP-1 and gastric inhibitory peptide (GIP) were measured by enzyme-linked immunosorbent assay (ELISA) (Millipore, Billerica MA, USA).

2. Glucose

Serum glucose was determined using the glucose oxidase method Free fatty acids (FFA) were measured were measured by colorimetric methods using reagents from Wako Chemicals (Richmond, VA).

3. Insulin

Insulin was measured using a chemiluminescence immunoassay with a normal fasting range of 42–188 pmol/l.

4. Free fatty acids

Free fatty acids (FFA) were measured were measured by colorimetric methods using reagents from Wako Chemicals (Richmond, VA).

5. Gastric emptying

Acetaminophen was used for measuring the rate of gastric emptying, and 1450 mg were administered together with the glucose load.

6. Glucose absorption

Glucose absorption was measured using 3-*O*-methyl-glucose (7.5 g administered with the glucose load), which is an inert, non-metabolizable glucose analog. The appearance of 3-*O*-methyl-glucose in blood can thus be used as a proxy measure of the rate of intestinal glucose absorption.

Statistical Considerations

Use of complex survey procedures

Statistical analysis software (SAS version 9.2; SAS Institute) was used for all analyses and specific SAS procedures that allowed for analysis of a complex survey were employed. The use of complex survey procedures was required to make statistically valid inferences from the sample to the study population, while accounting for the sample design [14]. All data were weighted using census data to produce nationally representative estimates and were adjusted for post-stratification and non-response bias in each of the survey cycles used [14]. Frequency procedures (PROCSURVEYFREQ) and univariate and multivariate regression for complex survey design (PROCSURVEYREG and PROCSURVEYLOGISTIC) were used to assess prevalence of consumption and linear trends in intake.

Conducting subgroup analyses

When possible, subgroup analyses by gender, self-reported weight status; BMI (calculated from self-reported weight and height), race/ethnicity, and age-group were performed. Subgroup comparisons in the small clinical studies were limited due to small

sample size[39]. P-values were calculated using t-tests for comparison of two means, ANOVA for comparison of means among 3 or more groups, and chi-square test for frequency analysis, where appropriate. A p-value < 0.05 was considered statistically significant.

Sample size calculations

Power and sample size calculations were not needed in our epidemiologic study using NHANES because the study was observational and we were conducting an analysis of data that was pre-existent [40]. We also did not conduct a power calculation in the questionnaire-based study because it was an exploratory, hypothesis generating experiment, for which recommendations for determining sample size exist [41]. In the same-subject crossover study, sample size was determined using the difference in the outcome of interest following sucralose and carbonated water found in a prior study [31]. Sample size calculations were done under the assumption that the washout phase is long enough to rule out carryover effects [25]. Using the difference observed in the outcome of interest during a prior study, a sample size of 60 participants was determined to have a probability of 80% of detecting a difference in the outcome at a two sided 5% significance level [31]. We conducted a planned interim analysis after the completion of 30 subjects.

Analysis of repeated time-course measures on the same-subject

Descriptive statistics were calculated for each outcome of interest during each test visit. Mean, standard deviation, and peak were calculated, as well as area under the curve

(AUC) using the trapezoidal method. Area under the curve is a useful technique to summarize the concentration of a given compound in the blood (i.e. blood glucose) over an extended time period. The trapezoidal method is a well-accepted approach which allows for calculation of the areas between each consecutive time point [42]. Differences between the mean peak and AUC in the four conditions were assessed using ANOVA, and post-hoc Tukey tests were used for pairwise comparisons, where necessary. Since we collected serial data on the same individual following four different treatments, we used linear mixed modeling to account for fixed and random effects, given the same-subject crossover design of the study [42].

Principle components analysis of survey data

All items from the attitude questionnaire were subjected to a principal components analysis (PCA). Principle components analysis is a validated mathematical technique used to convert a set of responses that may be correlated (responses to each questionnaire item) into a set of values that are not correlated [43]. Rather than analyzing the responses to each questionnaire item separately, conducting principle components analysis generated three factors rather which summarized responses from all 28 questionnaire items, while accounting for the maximum amount of variability in our data. Whereas the Likert responses that we collected from each participant for each of the survey items were ordinal data, principle components analysis transformed the responses to be numeric.

We determined the number of principle components using a scree plot (see chapter 5), which allowed us to see which principle components accounted for the

majority of the variance in our dataset. The scree plot was used in combination with our scientific knowledge of the survey items that made up the principle components to reduce the entire survey into 3 principle components accounting for over half of the variance. Each principle component (or factor) was given a name and was used as a predictor for our outcomes of interest, such as parental ability to recognize NNSs.

Literature Cited

1. Welsh JA, Sharma AJ, Grellinger L, Vos MB. Consumption of added sugars is decreasing in the United States. Am J Clin Nutr 2011;94(3):726-34.

2. Centers for Disease Control. National Health and Nutrition Examination Survey: Anthropometry Procedures Manual. In; 2007.

3. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. Adv Data 2000(314):1-27.

4. Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, et al. 2000 CDC Growth Charts for the United States: methods and development. Vital Health Stat 11 2002(246):1-190.

5. Prentice AM, Jebb SA. Beyond body mass index. Obes Rev 2001;2(3):141-7.

6. Centers for Disease Control. Healthy weight- its not a diet, it's a lifestyle! In; 2011.

7. Center for Nutrition Coordination. Nutrition Data System for Research (NDSR). In: Minnesota Uo, editor.; 2012.

8. Kristal AR SA, & Williams AE. Food Frequency Questionnaires for Diet Intervention Research. In.

9. Schoeller DA. How accurate is self-reported dietary energy intake? Nutr Rev 1990;48(10):373-9.

10. Burrows TL, Martin RJ, Collins CE. A systematic review of the validity of dietary assessment methods in children when compared with the method of doubly labeled water. J Am Diet Assoc 2010;110(10):1501-10.

11. National Center for Health Statistics. National Health and Nutrition Examination Survey. In: Centers for Disease Control and Prevention.

12. McColl E, Jacoby A, Thomas L, Soutter J, Bamford C, Steen N, et al. Design and use of questionnaires: a review of best practice applicable to surveys of health service staff and patients. Health Technol Assess 2001;5(31):1-256.

13. Hood K, Robling M, Ingledew D, Gillespie D, Greene G, Ivins R, et al. Mode of data elicitation, acquisition and response to surveys: a systematic review. Health Technol Assess 2012;16(27):1-162.

14. Christensen J. Educational research: quantitative, qualitative, and mixed approaches. Thousand Oaks, CA: Sage; 2008.

15. Boet S, Sharma S, Goldman J, Reeves S. Review article: medical education research: an overview of methods. Can J Anaesth 2012;59(2):159-70.

16. Monique Hennink IH, Ajay Bailey. Qualitiative Research Methods. Los Angeles: Sage; 2011.

17. Hasson D & Arnetz BB. Validation and Findings Comparing VAS vs. Likert Scales for Psychosocial Measurements. International Electronic Journal of Health Education 2005;8:178-192.

18. Boone HN & Boone DA. Analyzing Likert data. Journal of Extension 2012;50(2).

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

20. Statistics NCfH. National Health and Nutrition Examination Survey 1999–2012 Survey Content Brochure. In: Control CfD, editor.; 2012.

21. US Department of Agriculture ARS. National Nutrient Database for Standard Reference.

22. US Department of Agriculture. USDA Food and Nutrient Database for Dietary Studies, 3.0. In: Agricultural Research Service FSRG, editor. Beltsville, MD; 2008.

23. Mills EJ, Chan AW, Wu P, Vail A, Guyatt GH, Altman DG. Design, analysis, and presentation of crossover trials. Trials 2009;10:27.

24. Cleophas TJ, Zwinderman AH. Crossover studies with continuous variables: power analysis. Am J Ther 2002;9(1):69-73.

25. Pocock SJ. Allocation of patients to treatment in clinical trials. Biometrics 1979;35(1):183-97.

26. Wellek S, Blettner M. On the proper use of the crossover design in clinical trials: part 18 of a series on evaluation of scientific publications. Dtsch Arztebl Int 2012;109(15):276-81.

27. Birch LL, Deysher M. Caloric compensation and sensory specific satiety: evidence for self regulation of food intake by young children. Appetite 1986;7(4):323-31.

28. Birch LL, McPhee L, Sullivan S. Children's food intake following drinks sweetened with sucrose or aspartame: time course effects. Physiol Behav 1989;45(2):387-95.

29. Brown RJ, Walter M, Rother KI. Ingestion of diet soda before a glucose load augments glucagon-like peptide-1 secretion. Diabetes Care 2009;32(12):2184-6.

30. Anton SD, Martin CK, Han H, Coulon S, Cefalu WT, Geiselman P, et al. Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. Appetite 2010;55(1):37-43.

31. Sylvetsky A, Rother KI, Brown R. Artificial sweetener use among children: epidemiology, recommendations, metabolic outcomes, and future directions. Pediatr Clin North Am 2011;58(6):1467-80.

32. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. Am J Clin Nutr 2009;89(1):1-14.

33. Jang HJ, Kokrashvili Z, Theodorakis MJ, Carlson OD, Kim BJ, Zhou J, et al. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. Proc Natl Acad Sci U S A 2007;104(38):15069-74.

34. Margolskee RF, Dyer J, Kokrashvili Z, Salmon KS, Ilegems E, Daly K, et al. T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. Proc Natl Acad Sci U S A 2007;104(38):15075-80.

35. Sylvetsky AC, Welsh JA, Brown RJ, Vos MB. Low-calorie sweetener consumption is increasing in the United States. Am J Clin Nutr 2012;96(3):640-6.

36. Mace OJ, Affleck J, Patel N, Kellett GL. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. J Physiol 2007;582(Pt 1):379-92.

37. Brookes ST, Whitley E, Peters TJ, Mulheran PA, Egger M, Davey Smith G. Subgroup analyses in randomised controlled trials: quantifying the risks of false-positives and false-negatives. Health Technol Assess 2001;5(33):1-56.

38. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Epidemiology 2007;18(6):805-35.

39. Johanson GA, Brooks GP. Initial Scale Development: Sample Size for Pilot Studies. Educational and Psychological Measurement 2010;70(3):394-400.

40. Matthews JN. Multi-period crossover trials. Stat Methods Med Res 1994;3(4):383-405.

41. Le Floch JP, Escuyer P, Baudin E, Baudon D, Perlemuter L. Blood glucose area under the curve. Methodological aspects. Diabetes Care 1990;13(2):172-5.

42. Hayat MJ, Hedlin H. Modern statistical modeling approaches for analyzing repeated-measures data. Nurs Res 2012;61(3):188-94.

43. SAS. Principle Components Analysis. In.

44. Bentler PM, Yuan KH. Test of linear trend in eigenvalues of a covariance matrix with application to data analysis. Br J Math Stat Psychol 1996;49 (Pt 2):299-312.

CHAPTER 4: LOW-CALORIE SWEETENER CONSUMPTION IS INCREASING IN THE UNITED STATES

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Allison C. Sylvetsky: Nutrition and Health Science Program, Graduate Division of Biological and Biomedical Sciences, Laney Graduate School, Emory University & Section on Pediatric Diabetes, Diabetes Endocrinology and Obesity Branch, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health

Jean A. Welsh: Department of Pediatrics, School of Medicine, Emory University & Children's Healthcare of Atlanta at Egleston

Rebecca J. Brown MD, MHSc.: Section on Pediatric Diabetes, Diabetes Endocrinology and Obesity Branch, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health

Miriam B. Vos: Department of Pediatrics, School of Medicine, Emory University & Children's Healthcare of Atlanta at Egleston

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Running head: Low-calorie sweetener consumption

Abbreviations: USDA: United States Department of Agriculture, NHANES: National Health and Nutrition Examination Survey, LCS: Low-calorie sweetener, FDA: United States Food and Drug Administration, BMI: Body Mass Index (kg/m^2)

Abstract

Background: Low-calorie and no-calorie sweeteners (LCS) have emerged as alternatives to added sugars. Research suggests that consumption among all Americans is increasing, yet it is unknown if consumption trends differ among population subgroups.

Objective: Our study aimed to assess recent national trends in LCS consumption among children and other demographic subgroups in the U.S.

Design: We used National Health and Nutrition Examination Survey (NHANES) data collected in five 2-year cycles from 1999-2000 to 2007-2008. Consumption of foods and beverages with LCS was estimated using one 24-hour dietary recall. Estimates of the proportion of the population consuming foods and beverages containing LCS (prevalence of consumption) were weighted to obtain nationally representative results. Trends in prevalence of LCS consumption and mean intake of beverages sweetened with LCS were tested using chi-square tests for trend and F-tests.

Results: In 2007-2008, the percentage of children and adults consuming foods and beverages containing LCS increased. Prevalence of consuming beverages with LCS increased from 6.1% to 12.5% among children (p-trend < 0.0001), and from 18.7% to 24.1% among adults ($p<0.001$). Increases in calorie containing beverages with LCS were observed among all weight, age, socioeconomic, and race/ethnicity subgroups in both children and adults. However, there was little change in consumption of no-calorie beverages with LCS or LCS containing foods.

Conclusions: The consumption of LCS containing beverages has doubled among US children over the past decade. Further research is needed to understand the health impacts of this trend.

Introduction

The prevalence of obesity has increased dramatically in both children and adults [1, 2] and is associated with adverse health conditions including type 2 diabetes and cardiovascular disease [3]. Epidemiologic and experimental evidence demonstrates that intake of added sugars is strongly associated with weight gain and obesity [4]. Low- and no-calorie sweeteners offer an alternative to sugars, providing sweetness without significantly contributing to caloric intake [5].

The effects of these sugar alternatives have not been well studied and both short and long term effects have yet to be determined [6]. The FDA has approved five low- and no-calorie sweeteners for use in the US. These are collectively referred to as low calorie sweeteners (LCS) and include aspartame, acesulfame- potassium, neotame, saccharin, sucralose [7], and the dietary supplement stevia, an extract from the leaves of the S. Rebaudiana (Bertoni) plant [8]. In addition to widespread use in "diet" beverages, LCS are increasingly incorporated into foods [9].

Recent human and animal studies [10-12] have shown that LCS may impact glucose metabolism [11, 13, 14], satiety [15], and vascular function [12], despite their inherent lack of energy. A growing body of evidence suggests that repeated exposure to sweet substances may lead to the development of preferences for highly sweet foods and beverages [16]. This is particularly concerning in young children, among whom early exposure to highly sweet substances can lead to the development of dietary patterns replete with highly caloric foods, typically lacking in nutritional value [17].

Mattes & Popkin [18] reported that substantial increases in the consumption of foods with LCS and marginal increases in the consumption of beverages with LCS occurred among all Americans between 1989 and 2004. The purpose of this study was to assess recent trends in consumption of food and beverage products containing LCS in the U.S. by demographic subgroups over the last decade. We aimed to build on the findings of Mattes & Popkin [18], by evaluating recent trends among demographic subgroups and by stratifying our analyses by LCS source. As the consumption of sugar-sweetened beverages and other sources of calorie-containing sugars has been declining [19], we hypothesized that consumption foods and beverages containing LCS has risen in children as well as adults and among other demographic subgroups.

Subjects and Methods

We used data from the National Health and Nutrition Evaluation Survey (NHANES), which is a continuous, cross-sectional study of the US population with data released in 2-year cycles. A description of NHANES sampling methods is provided elsewhere[20]. Our sample consisted of persons 2 years or older, who agreed to participate in one of the five NHANES cycles from 1999-2000 through 2007-2008 (n=47,396). Only those subjects who provided reliable dietary information were included (n=42,453). Demographic information collected included the participant's age in years (categorized as 2–5, 6–11, 12–17, 18–34, 35–54, and above 55 years), sex, socioeconomic status (determined using tertiles of income to poverty ratio), and self-reported race-ethnicity (non-Hispanic white, non-Hispanic black, or Hispanic). Those who identified as Mexican

American or other Hispanic were combined into one race-ethnicity group entitled "Hispanic." Participants indicating identification with another race-ethnicity group were included in all analyses, but their estimated trends in LCS consumption were not displayed due to small sample size and heterogeneity within the "other" categorization. All NHANES protocols were approved by the Institutional Review Board at the National Center for Health Statistics. Adult participants and parents/guardians of child participants signed informed consent, and all child participants provided assent prior to enrollment in the study.

We used data collected from one 24-hour dietary recall to estimate the prevalence of consumption of LCS in the US population [21]. While two 24-hour dietary recalls have been collected from all NHANES participants since 2003, only one was collected in the earlier years (NHANES 1999-2000 and 2001-2002). To ensure consistency in methods across all time points we used data from only the first of the two recalls to assess dietary intake. Proxy respondents (parents/guardians) were used for survey examinees who were under 6 years of age, and children aged 6–11 years underwent assisted interviews[20]. The nutrient content of foods and beverages consumed was determined by NHANES by using the Food and Nutrient Database for Dietary Studies, which uses food-composition data from the USDA National Nutrient Database for Standard Reference [22]. The Standard Reference provides a product description which indicates if it contains LCS. These foods were identified by searching for all food items containing the terms "low-calorie" or "sugar-free". Because these low-calorie sweeteners are classified as food additives in accordance with Good Manufacturing Practices by the FDA, producers are

not required to provide information regarding the quantity of LCS contained in their products. As a result, no information on the specific type or quantity of LCS in foods or beverages is available in the USDA database.

A total of 6,113 unique food and beverage items were consumed by NHANES participants' between1999 and 2008. Of these food items, 168 contained LCS, including 81 different beverage items and 87 food items. We used food codes to group foods and beverages that contained LCS into the following subgroups: reduced-calorie drinks (i.e. light fruit juices, diet lemonade), no-calorie drinks (i.e. diet soda, sugar-free flavored water beverages), reduced-calorie desserts (i.e. sugar-free ice cream, sugar-free pudding), reduced-calorie condiments (i.e. reduced sugar ketchup, sugar-free pancake syrup), and other reduced-calorie foods (i.e. light yogurt, no sugar added canned peaches). Low-calorie was used in this study to represent the use of a low-calorie sweetener, not to suggest that the food or beverage is low in calories. As such, beverages which contained calories and were sweetened with LCS are referred to as reduced-calorie beverages to distinguish them from beverages sweetened with LCS that do not contain calories, referred to as no-calorie beverages.

Our key outcome was the trends in the percentage of U.S. children and adults who consumed ≥1 food and/or 1 beverage sweetened with a LCS daily (prevalence of consumption). Trends were assessed among all participants ≥ 2 years, and among demographic and weight status subgroups. A "consumer" was defined as an individual who consumed at least one food or beverage item containing LCS during the 24 hour

dietary recall period. Among consumers, trends in the mean intake of beverages containing LCS were assessed.

Statistics

Statistical analysis software (SAS version 9.3; SAS Institute) was used for all analyses and specific SAS procedures that allowed for analysis of a complex survey data were employed. Sample weights were used to generate nationally representative estimates of the United States population $\geq$ 2 years. Prevalence of LCS consumption was assessed using frequency procedures and subgroup comparisons were made using Rao's chi-squared test. Trends in the mean intake (g) of beverages containing LCS (among consumers only) were also estimated. Linear trends in intake were tested using chi-squared tests for trends and F-tests. All P values were 2-sided and $P < 0.05$ was considered statistically significant.

Results

Percentage of population consuming low-calorie sweeteners

Figure 1 demonstrates that the percentage of children consuming foods and beverages containing LCS nearly doubled from 8.7% in 1999-00 to 14.9% in 2007-08 (p-trend <0.0001), while the percentage of the adult population consuming items with LCS increased by 18%, from 26.9% to 32.0% (p-trend<0.001). During the same time period, there were no differences in mean caloric intake among children or adults (data not shown).

Intake of beverages containing low-calorie sweeteners

As shown in **Figure 2**, the prevalence of consumption of beverages with LCS has increased dramatically. The increases observed were largely attributable to increased consumption of reduced-calorie beverages rather than no-calorie beverages. Among children, prevalence of reduced-calorie beverage consumption increased from less than 1% to over 7% (p-trend <0.0001) (data not shown). Among adults, prevalence of reduced-calorie beverage consumption increased from 2% in 1999-2000 to over 8% in 2007-2008 (p-trend <0.0001) (data not shown).

Intake of foods containing low-calorie sweeteners

As shown in **Figure 2**, there was no difference in the prevalence of consumption of LCS containing food items between 1999-2000 and 2007-2008.

Subgroup analyses

Results presented in **Table 1 and Table 2** show the trends in the prevalence of LCS consumption by type and by age, race, weight, income, and gender subgroups. Though the degree of increase in LCS consumption differed across subgroups, increases in the consumption of reduced-calorie beverages, but not no-calorie beverages or LCS containing foods were observed in all subgroups.

Gender and age trends

The proportion of consumers of any food or beverage source of LCS increased significantly only among females (girls: p-trend=0.03, women: p-trend= 0.002) in both children and adults. Increases in the prevalence of reduced-calorie beverage consumption were observed among all males (boys: p-trend <0.01, men: p-trend <0.01) and females (girls: p-trend <0.0001, women: p-trend <0.0001). Stratified by age group, the prevalence

of consuming any LCS-containing food or beverage increased only among 6-<12 year old children (p-trend <0.05), and was not statistically significant in any adult age group. Dramatic increases in reduced-calorie beverage consumption were observed among all adult age groups, but only among 6-<12 year old children (p-trend <0.0001).

Race and socioeconomic trends

Increases in the prevalence of consuming LCS from any food or beverage source were observed among non-Hispanic black (p-trend=0.02) and Hispanic (p-trend=0.0006) adults, but not among non-Hispanic white adults. Increases in reduced-calorie beverage consumption, specifically, were observed in Hispanic and non-Hispanic white adults. Though increases in consumption of reduced-calorie beverages were significant in all child racial subgroups, there were no differences in the prevalence of consuming any LCS containing food or beverage. Increases in consumption of LCS from any food or beverage source were observed only among the highest income tertile among adults (p<0.05), yet heightened prevalence of reduced-calorie beverage consumption was observed in all income groups in both children and adults.

Weight-related trends

After stratifying by weight status, we observed a significant increase in the prevalence of consuming LCS from any food or beverage source in normal weight (p-trend < 0.05) and overweight children (p-trend = 0.03) and obese adults (p-trend < 0.05), but not in normal weight adults. Increases in the prevalence of reduced-calorie beverage consumption were observed in all weight subgroups among adults (p-trend <0.0001), but only among normal weight children (p-trend <0.0001).

Mean intake trends among LCS consumers only

The mean intake of LCS beverages (grams) among consumers remained stable in the entire cohort, but increased among non-Hispanic black (p-trend=0.03), and middle income children (p-trend=0.02) (data not shown). Among adult consumers, mean intake of beverages with LCS increased significantly only among older adults, ≥55 years (p-trend= 0.0004), but not among middle-aged or younger adults (data not shown).

Discussion

The results of our study demonstrate that consumption of LCS has increased substantially since 1999-2000 in both children and adults. Our findings indicate that increased consumption of reduced-calorie beverages, rather than no-calorie beverages or foods containing low-calorie sweeteners, is driving the overall increase in LCS use. Among consumers of beverages with LCS, mean intake of LCS containing beverages has remained stable overall. Building on the findings of Mattes and Popkin (18), our results demonstrate that consumption of foods and beverages with LCS is fairly common, with 28% of the total U.S. population reporting LCS consumption, and much higher prevalence among certain demographic subgroups. Our results also confirm the Mattes and Popkin (18) finding that consumption of foods with LCS is relatively rare in the general population.

The shift toward LCS could have come as a result of recent obesity prevention campaigns and the growing popularity of low- and reduced- carbohydrate diets for weight loss over the past decade. The increased prevalence of type 2 diabetes may have also fostered the growing trends in LCS consumption, particularly among older adults in

whom diabetes is much more prevalent. It is also possible that the increasing awareness of negative health associations with high added sugar consumption in recent years may have promoted a switch to beverages (and foods) with LCS. With recent discussions of taxing sugar-sweetened-beverages, banning regular sodas in school systems, and the growing popularity of differential pricing structures to promote healthier choices, it may be anticipated that LCS consumption will increase further. Most importantly, given the rapid increases in LCS consumption among children, their long-term effects, particularly when started in the early years, need to be studied.

Our data showed age, racial, income, weight, and gender differences in the percentage of the population consuming items containing LCS. We found that LCS consumption, specifically reduced-calorie beverage consumption, increased the most among females, non-Hispanic black children, and Hispanic adults, although non-Hispanic whites of all ages continued to have the highest prevalence of LCS containing food and beverage consumption. We also observed dramatic increases in the consumption of LCS among older adults, obese adults, and adults in the highest tertile of income.

Our study is the first known to evaluate national trends in consumption of LCS containing foods and in beverages among both children and adults, and among race/ethnic, gender, income, and weight status subgroups. The analysis of a large body of dietary and demographic information, collected over a10 year period, enabled us to make meaningful subgroup comparisons and analyze various sources of LCS in the diet.

Our study has several limitations. Importantly, the lack of information about the type and quantity of LCS contained in commercial foods and beverages precluded estimation of the absolute amounts of sweeteners consumed. Because we were unable to determine quantities of LCS, and because each of the five NHANES cycles was comprised of different participants, we were unable to examine trends in consumption on an individual level. Furthermore, given the self-reported nature of the dietary recall data used, our results may be subject to recall bias and information bias. For example, some participants may not remember consuming a food or beverage sweetened with an LCS or may not have been aware that the food or beverage that they consumed contained LCS; hence, our data may have underestimated true consumption levels. Since consumer perceptions of food and beverages containing LCS have not been studied, social desirability bias may have resulted in either over or under reporting of true intake and, as such, is expected to have had little overall effect on our estimates.

In summary, the prevalence of consumption of LCS in the U.S. has increased substantially since 1999-2000, with consumption predominantly in form of beverages. The largest observed increases were in reduced-calorie beverage consumption among both children and adults. Our findings emphasize the need for long-term controlled studies to determine the impact of this trend on energy balance and on indicators such as glucose metabolism that have been shown to be associated with LCS consumption.

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Table 1. Prevalence of low-calorie sweetener consumption by subgroup among children and adolescents

			Any item with LCS	Red-calorie beverage	No-calorie beverage	Condiment with LCS	Dessert with LCS
Total							
All							
	1999-2000	N=333	8.7±0.9	1.0±0.3	5.3 ±0.7	0.7±0.3	0.9±0.2
	2007-2008	n=283	15.0± 1.2*	7.4±0.08***	6.1±0.9	1.7 ±0.4	1.1±0.4
Male							
	1999-2000	n=170	6.8±0.7	0.7±0.3	3.5 ±0.5	0.6±0.2	0.9±0.3
	2007-2008	n=146	12.2±1.1	6.7±0.9**	3.7±0.5	1.37 ±0.3	1.2 ±0.3
Female							
	1999-2000	n=163	8.5±0.7	1.0±0.2	4.8±0.5	0.9±0.2	0.7±0.1
	2007-2008	n=137	13.6±0.8*	6.7±0.6****	4.8 ±0.5	1.7±0.4	1.3±0.4
Age							
2 ≤ 6 years							
	1999-2000	n=665	7.0±0.9	0.8 ±0.4	3.3±0.8	0.3±0.2	1.1±0.4
	2007-2008	n=832	11.9±2.0	6.5±1.1	2.9±0.8	1.6±0.5	1.4±0.6
6≤12 years							
	1999-2000	n=961	7.0±0.8	0.2±0.2	4.1±0.6	0.2±0.1	0.8±0.4
	2007-2008	n=112	13.7±0.9*	6.9±0.8****	4.6±0.5	1.5±0.3***	1.3±0.4
12≤ 18years							
	1999-2000	n=170	8.3±0.7	1.3±0.3	4.6±0.5	1.2±0.4	0.70±0.2
	2007-2008	n=886	12.9±1.6	6.7±1.01	5.1±1.2	1.5±0.5	1.0±0.3
Race							
White							
	1999-2000	n=730	11.9±1.4	1.5±0.5	7.3±1.0	1.1±0.5	1.0±0.3
	2007-2008	n=902	16.4 ±1.2	6.9 ±0.7**	7.4±1.0	1.9±0.4	1.6±0.6
Black							
	1999-2000	n=929	5.6±0.9	0.6±0.2	1.7±0.7	0.3±0.2	1.0±0.4
	2007-2008	n=721	11.1±1.3	7.8±0.9***	1.9±0.7	0.7±0.3***	1.0±0.4
Hispanic							
	1999-2000	n=152	6.6±0.5	0.7±0.2	4.1±0.4	0.9±0.2	0.7±0.2
	2007-2008	n=107	11.0±0.0	5.2±0.8**	3.3±0.7	2.0±0.3	1.1±0.5
Weight status							
Normal							
	1999-2000	n=181	7.0±0.8	0.8±0.2	3.9 ±0.5	0.7±0.2	0.6±0.3

	2007-2008	n=143	11.9±1.2*	6.2±0.6***	3.9±0.8	1.1±0.3	1.0 ±0.4
Overweight							
	1999-2000	n=469	8.1±1.3	1.5±0.7	3.2±0.7	1.1±0.4	1.3±0.5
	2007-2008	n=373	15.6±1.5*	7.8±1.3	5.4±1.0	2.4±0.7	1.3±0.6
Obese							
	1999-2000	n=592	10.3±1.4	1.2±0.5	6.8 ±1.1	0.8±0.4	1.0±0.6
	2007-2008	n=531	17.0±1.5	8.8±1.1	6.6±0.9	2.0±0.7***	1.6±0.5
Income							
Low							
	1999-2000	n=186	7.0±0.7	0.7±0.2	3.5±0.5	0.7±0.3	0.9±0.2
	2007-2008	n=142	11.0±0.8	6.0	2.8±0.5	1.3±0.3	1.3±0.4
Middle							
	1999-2000	n=861	6.7±0.9	0.8±0.3	3.7±0.8	0.7±0.2	0.6±0.2
	2007-2008	n=834	14.5±1.6	7.0±1.1*	4.9±0.9	1.8±0.4	1.6±0.6
High							
	1999-2000	n=574	11.2±1.8	1.6±0.7	7.0±1.20	1.1±0.4	0.9±0.2
	2007-2008	n=577	15.3±1.5	8.2 ±1.2*	6.8±1.07	1.7±0.5	0.5±0.

[1]All values are presented as percent ± standard error

[2]Data was collected from one 24 hour dietary recall

[3]Linear trends in prevalence of consumption of each source of low-calorie sweeteners were analyzed using logistic regression

[4]Prevalence of consumption of LCS was assessed using frequency procedures for complex survey design

[5]Reduced-calorie drinks (i.e. light fruit juices, diet lemonade), no-calorie drinks (i.e. diet soda, sugar-free flavored water beverages), low-calorie desserts (i.e. sugar-free ice cream, sugar-free pudding), low-calorie condiments (i.e. reduced sugar ketchup, sugar-free pancake syrup), and other low-calorie foods (i.e. light yogurt, no sugar added canned peaches)

* P-trend < 0.05 ** P-trend< 0.01 *** P-trend <0.001 **** P-trend<0.0001

Table 2. Linear trends in prevalence of consumption of sources of low-calorie sweeteners from 1999-00 to 2007-08 among adults by demographic and weight subgroup

			Any item with LCS	Red-calorie beverage	No-calorie beverage	Condiment with LCS	Dessert with LCS
Total							
All							
	1999-2000	n=4736	26.9±1.0	2.1±0.2	17.2±1.2	10.4±0.8	1.4±0.3
	2007-2008	n=5690	32.0±1.1	8.1±0.8***	18.6±0.9	12.4±0.6	2.0±0.3*
Male							
	1999-2000	n=2218	21.4±0.9	1.8±0.3	1.7±0.8	9.8±0.7	1.2±0.3
	2007-2008	n=2809	25.1±1.3	6.0±0.5**	13.8±0.9	10.8±0.9	1.9±0.2*
Female							
	1999-2000	n=2518	25.4±1.0	2.1±0.2	14.5±0.9	11.2±1.0	1.6±0.2
	2007-2008	n=2881	32.9±1.3	8.3±0.7***	15.6 1.1	15.2±0.7*	1.9±0.3
Age							
18 ≤ 35							
	1999-2000	n=1635	11.0± 0.8	1.0±0.2	7.2±0.6	2.6±0.6	0.4±0.1
	2007-2008	n=1530	15.5±1.3	5.0±0.8**	9.2±0.2	3.5±0.4	0.4±0.2
35≤55 years							
	1999-2000	n=1343	26.6±1.4	1.9±0.3	18.2±1.3	9.4±0.8	0.7±0.2
	2007-2008	n=1845	28.7±1.6	6.4±0.7**	17.1±1.5	11.8±0.8	0.8±0.2
≥55 years							
	1999-2000	n=1758	32.9±1.2	2.8±0.4	14.9±0.8	18.8±1.3	3.0±0.2
	2007-2008	n=2315	38.2±1.1	9.2±0.6**	16.3±1.0	20.3±1.1	3.7±0.4***
Race							
White							
	1999-2000	n=273	31.6±1.1	2.6±0.3	19.2±1.4	13.6±1.1	2.0±0.3
	2007-2008	n=353	35.7±0.9	8.4±0.8**	20.1±0.8	14.7±1.3	2.7±0.2*
Black							
	1999-2000	n=188	15.7±1.5	2.7±0.6	6.2±0.9	9.2±1.5	0.8±0.2
	2007-2008	n=192	22.4±1.4*	6.9±0.6	8.8±1.0*	10.4±0.7	1.3±0.3
Hispanic							
	1999-2000	n=320	18.7±1.6	1.0±0.3	10.3±0.9	7.7±1.0	1.3±0.3
	2007-2008	n=270	24.5±1.3**	5.5±0.5**	10.8±1.3	13.1±1.0**	1.1±0.4
Weight status							
Normal							
	1999-2000	n=280	17.3±1.4	1.4±0.3	9.2±1.2	7.6±1.0	1.0±0.2
	2007-2008	n=244	21.5±1.3	5.5±0.7***	10.0±0.9	9.3±0.8	1.3±0.3
Overweight							

	1999-2000	n=194	25.0±0.8	1.8±0.4	14.1±0.8	11.1±0.8	1.8±0.4
	2007-2008	n=216	29.5±1.6	7.0±0.5**	14.6±1.4	13.1±1.0	2.0±0.3
Obese							
	1999-2000	n=170	29.5±1.4	2.7±0.4	16.8±1.1	13.2±1.3	1.5±0.3
	2007-2008	n=216	35.9±1.4	9.0±0.9***	19.1±1.0	16.6±0.8	2.1±0.3***
Income							
Low							
	1999-2000	n=394	18.7±1.4	1.2±0.2	9.4±1.0	9.1±0.9	1.1±0.2
	2007-2008	n=363	22.9±1.4	5.8±0.5***	10.8±1.0	10.2±0.7	1.7±0.3**
Middle							
	1999-2000	n=227	23.0±1.4	2.2±0.3	12.6±0.8	10.4±1.1	1.5±0.4
	2007-2008	n=261	27.6±1.7	6.8±0.7**	12.7±1.2	12.1±1.0	2.2±0.3**
High							
	1999-2000	n=185	32.0±1.4	3.0±0.6	20.0±1.3	13.1±1.4	1.9±0.5
	2007-2008	n=228	38.3±1.4	9.4±1.2***	21.8±1.0	17.6±1.1	1.8±0.3

[1]All values are presented as percent ± standard error

[2]Data was collected from one 24 hour dietary recall

[3]Linear trends in prevalence of consumption of each source of low-calorie sweeteners were analyzed using logistic regression

[4]Prevalence of consumption of LCS was assessed using frequency procedures for complex survey design

[5]Reduced-calorie drinks (i.e. light fruit juices, diet lemonade), no-calorie drinks (i.e. diet soda, sugar-free flavored water beverages), low-calorie desserts (i.e. sugar-free ice cream, sugar-free pudding), low-calorie condiments (i.e. reduced sugar ketchup, sugar-free pancake syrup), and other low-calorie foods (i.e. light yogurt, no sugar added canned peaches)

* P-trend < 0.05 ** P-trend< 0.01 *** P-trend <0.001 **** P-trend<0.0001

Figure 1. Percentage (%) ± SE of children (n=16,716) and adults (n=26,737) who reported consuming ≥1 food or beverage containing low-calorie sweeteners in each NHANES cycle from 1999-2000 to 2007-2008. Linear trends were calculated using Wald's chi-squared test and all values are presented as % ± SE. Consumers are defined as those who reported consuming at least one low-calorie sweetened food or beverage during the 24-hour recall. ***** = p-trend<.001, ****=** p-trend<.0001.

Figure 2. Percentage (%) of children (n=16, 716) and adults (n=26, 737) who reported consuming food or beverage sources of low-calorie sweeteners in each NHANES cycle from 1999-2000 to 2007-2008. Linear trends were calculated using Wald's chi-squared test and all values are presented as % ± SE. Consumers are defined as those who reported consuming ≥1 food or beverage containing LCS during the 24-hour recall. ***** = p-trend<.001, ****=** p-trend<.0001.

Figure 1. Percentage (%) ± SE of children (n=16,716) and adults (n=26,737) who reported consuming ≥1 food or beverage containing low-calorie sweeteners in each NHANES cycle from 1999-2000 to 2007-2008.

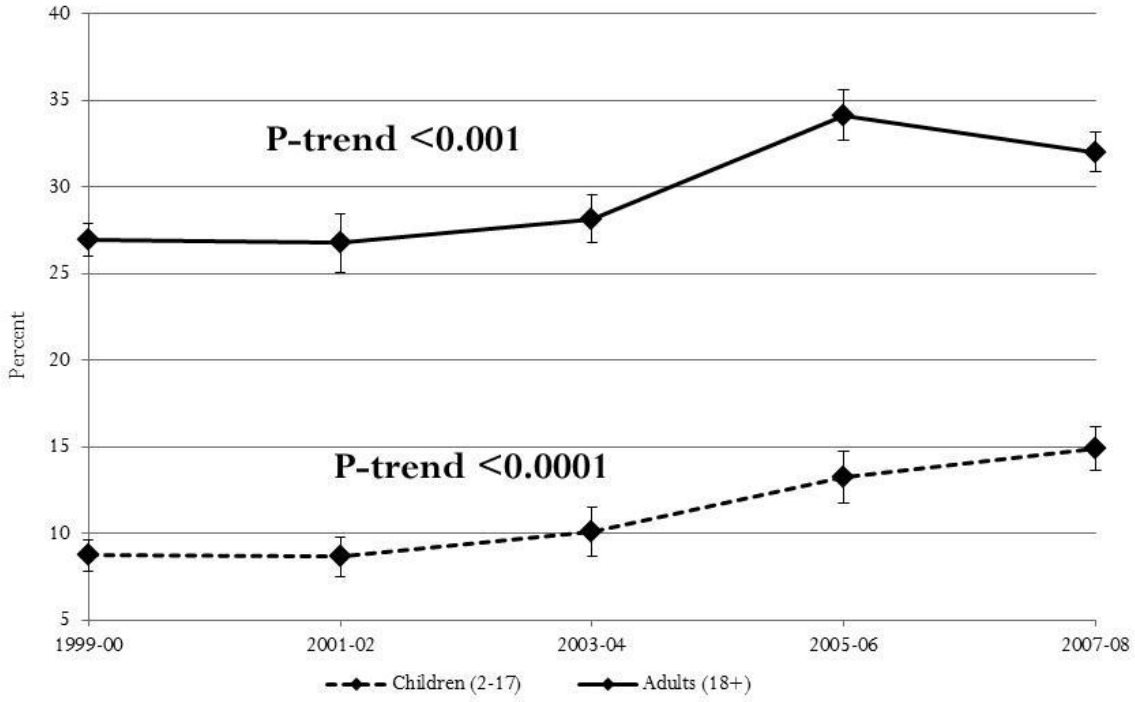
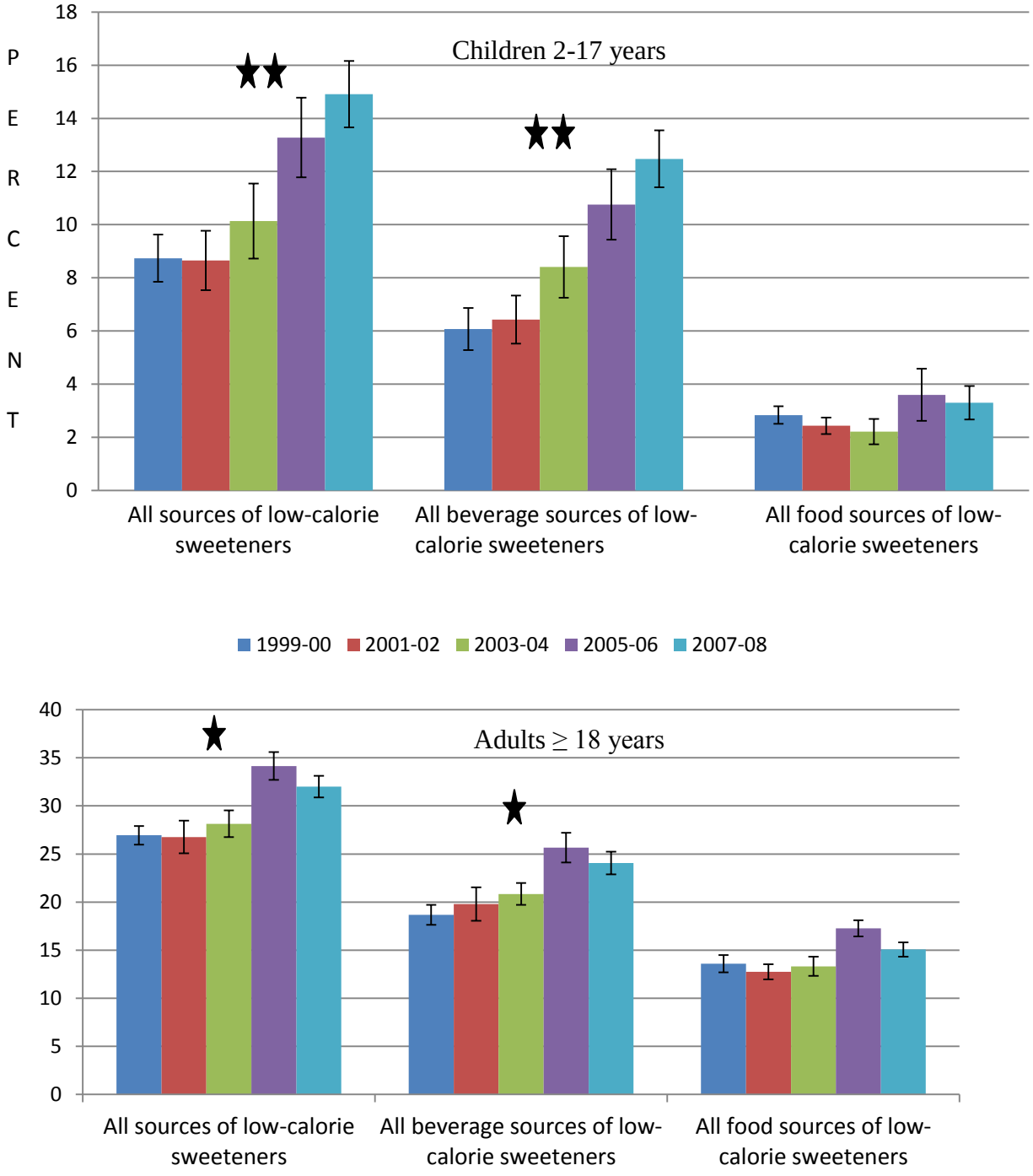


Figure 2. Prevalence of consuming[1] foods[2] and beverages items with low-calorie sweeteners from 1999-00 through 2007-08



Literature cited

1. Flegal KM, Carroll MD, Ogden CL, et al. Prevalence and trends in obesity among US adults, 1999-2008. JAMA 2010 Jan 20;303(3):235-241.

2. Ogden CL, Carroll MD, Curtin LR, et al. Prevalence of high body mass index in US children and adolescents, 2007-2008. JAMA : the journal of the American Medical Association 2010 Jan 20;303(3):242-249.

3. Vos MB, Welsh J. Childhood obesity: update on predisposing factors and prevention strategies. Curr Gastroenterol Rep 2010 Aug;12(4):280-287.

4. Hanson LA, Korotkova M, Hahn-Zoric M, et al. Perinatal PUFA intake affects leptin and oral tolerance in neonatal rats and possibly immunoreactivity in intrauterine growth retardation in man. Nestle Nutr Workshop Ser Pediatr Program 2006;57:223-233; discussion 233-224.

5. Blackburn GL, Kanders BS, Lavin PT, et al. The effect of aspartame as part of a multidisciplinary weight-control program on short- and long-term control of body weight. Am J Clin Nutr 1997 Feb;65(2):409-418.

6. Brown RJ, de Banate MA, Rother KI. Artificial sweeteners: a systematic review of metabolic effects in youth. Int J Pediatr Obes 2010 Aug;5(4):305-312.

7. Sylvetsky A, Rother KI, Brown R. Artificial sweetener use among children: epidemiology, recommendations, metabolic outcomes, and future directions. Pediatr Clin North Am 2011 Dec;58(6):1467-1480.

8. Gardana C, Scaglianti M, Simonetti P. Evaluation of steviol and its glycosides in Stevia rebaudiana leaves and commercial sweetener by ultra-high-performance liquid chromatography-mass spectrometry. J Chromatogr A 2010 Feb 26;1217(9):1463-1470.

9. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. Am J Clin Nutr 2009 Jan;89(1):1-14.

10. Mace OJ, Affleck J, Patel N, et al. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. J Physiol 2007 Jul 1;582(Pt 1):379-392.

11. Brown RJ, Walter M, Rother KI. Ingestion of diet soda before a glucose load augments glucagon-like peptide-1 secretion. Diabetes care 2009 Dec;32(12):2184-2186.

12. Gardener H, Rundek T, Markert M, et al. Diet Soft Drink Consumption is Associated with an Increased Risk of Vascular Events in the Northern Manhattan Study. J Gen Intern Med 2012 Jan 27.

13. Mace OJ, Affleck J, Patel N, et al. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. J Physiol 2007 Jul 1;582(Pt 1):379-392.

14. Brown RJ, Walter M, Rother KI. Diet soda increases early insulin and GLP-1 secretion in healthy volunteers. Horm Res 2009;72:159-159.

15. Pepino MY, Bourne C. Non-nutritive sweeteners, energy balance, and glucose homeostasis. Current opinion in clinical nutrition and metabolic care 2011 Jul;14(4):391-395.

16. Zhang GH, Chen ML, Liu SS, et al. Effects of mother's dietary exposure to acesulfame-K in Pregnancy or lactation on the adult offspring's sweet preference. Chem Senses 2011 Nov;36(9):763-770.

17. Birch LL, Anzman-Frasca S. Learning to prefer the familiar in obesogenic environments. Nestle Nutr Workshop Ser Pediatr Program 2011;68:187-196; discussion 196-189.

18. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. The American journal of clinical nutrition 2009 Jan;89(1):1-14.

19. Welsh JA, Sharma AJ, Grellinger L, et al. Consumption of added sugars is decreasing in the United States. The American journal of clinical nutrition 2011 Sep;94(3):726-734.

20. National Center for Health Statistics. National Health and Nutrition Examination Survey. [cited January 3rd, 2013; Available from: http://www.cdc.gov/nchs/nhanes.htm

21. Lechtig A, Yarbrough C, Martorell R, et al. The one-day recall dietary survey: a review of its usefulness to estimate protein and calorie intake. Arch Latinoam Nutr 1976 Sep;26(3):243-271.

22. US Department of Agriculture ARS. National Nutrient Database for Standard Reference.

CHAPTER 5: PARENTAL PERCEPTIONS AND RECOGNITION OF NON-NUTRITIVE SWEETENERS IN COMMERCIALLY AVAILABLE PRODUCTS

Allison C. Sylvetsky: Nutrition and Health Science Program, Graduate Division of Biological and Biomedical Sciences, Laney Graduate School, Emory University & Section on Pediatric Diabetes & Metabolism, Diabetes Endocrinology and Obesity Branch, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health

Mitchell Greenberg: Section on Pediatric Diabetes & Metabolism, Diabetes Endocrinology and Obesity Branch, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health

Xiongce Zhao Ph.D.: Diabetes Endocrinology and Obesity Branch, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health

Kristina I. Rother MD, MHSc.: Section on Pediatric Diabetes & Metabolism, Diabetes Endocrinology and Obesity Branch, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health

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Abstract

Introduction: Non-nutritive sweetener (NNS) consumption has increased among children and adults. Our study aimed to explore parental ability to recognize NNS in packaged foods and beverages and to examine parental attitudes toward providing NNS containing items to their children.

Methods: 120 parents (≥ 18 years old) of children (≥1 and ≤ 18 years old) completed brief questionnaires upon entering or exiting a local grocery store. Grocery selection and NNS recognition were assessed using a 142 item grocery shopping simulation activity, while demographic information and parental attitudes toward NNS were assessed using an interviewer-assisted survey.

Results: The parents' ability to recognize NNS was low (mean percentage of items correctly identified 23 ± 14%). The majority of parents (72 %) believed that NNS were not safe to provide to their children. Parents' ability to correctly identify NNS containing foods and beverages was inversely related to their attitude towards providing NNS to their children.

Conclusions: Food and beverage labeling should be simplified in order for consumers to easily identify NNS containing items. Prospective controlled studies on long-term health

effects of NNS consumption are needed in order to formulate and communicate evidence based recommendations regarding the use of NNS in children.

Introduction

Non-nutritive sweeteners (NNS) provide sweet taste with no or few calories [1-5] and may serve as a transitional step in decreasing caloric intake by reducing sugar in the diet [6-9]. It may appear counterintuitive that increased consumption of NNS has been associated with higher rates of obesity in several large-scale studies [10-12]. A likely explanation is that persons at risk for weight gain or already obese individuals may consume more NNS to reduce caloric intake. However, support for alternative explanations comes from behavioral studies in humans [13] and animal studies [14, 15]. The metabolic effects of NNS in humans have not been explored in great detail, and little evidence exists for conclusive recommendations regarding their consumption in children [16].

Parents influence a child's food choices and taste preferences [17], and especially in younger children, exert control over what children consume [18]. Dietary acceptance among young children is driven largely by social influences [19]. If NNS in foods and beverages are widely accepted by parents, it is therefore likely that further increases [20] in consumption of NNS-containing foods and beverages among children will result [21, 22]. Meanwhile, a recent survey conducted by the International Food Information Council Foundation (IFIC) found that 20% of their American adult participants reported consciously avoiding non-nutritive sweeteners, though specific concerns about their use were not detailed [23].

In conducting this pilot study, we aimed to assess parents' ability to identify common, commercially available products containing NNS and to evaluate parental attitudes toward providing NNS containing items to their children.

Methods

Sample

This survey-based pilot study was conducted in a convenience sample of 125 adults recruited upon entering or exiting a grocery store in Kensington, Maryland, a suburb of Washington, D.C. Individuals were eligible to participate if they were ≥18 years of age, had a child aged ≥1 year and ≤ 18 years, and if they spoke and understood English. Volunteers were excluded if they indicated that they were employees of a food or a beverage company, nutritionists, or health policy specialists.

Procedure

The Office of Human Subjects Research (OHSR) at the National Institutes of Health (NIH) approved the content of the questionnaires and the procedure, while the management of the national grocery chain granted permission to conduct the project on store premises.

Parents were surveyed throughout the summer of 2012 at varying times of the day and on various days of the week to avoid selection bias.

After providing verbal informed consent, participants were asked to partake in 4 components of our study (**Figure 1**): 1) to complete an 11-item interviewer-assisted demographic questionnaire. 2) to indicate which products they would like to purchase for their families, 3) to identify NNS in foods and beverages and 4) to share their attitude towards NNS in their children's diet. Common commercially available food and beverage items (n=142)[24] were displayed on a large table and were arranged in the same order

each day. Forty-four of the 142 items contained NNS. The NNS products were selected to include a wide range of low-calorie beverages, no-calorie beverages, low-calorie condiments, low-calorie desserts, and low-calorie grains and cereals. Non-NNS containing products (up to three per NNS containing item) were chosen which most closely resembled a NNS containing food or beverage. Fresh fruits, vegetables and other perishable, non-packaged foods were not displayed. Presentation of foods and beverages was such that the front of the package was easily visible and all items were numerically coded. The 142 foods and beverages are detailed in **Appendix 1**.

Parents were asked to indicate which items they would hypothetically purchase for their family by stating the numeric code of each item selected and were instructed to assume that price was not an issue. After a brief explanation of what constitutes a NNS, participants were asked to identify NNS containing items. Participants were instructed not to look at the nutrition facts panel or the ingredients list of any of the items presented. Study staff recorded their verbal responses and afterwards disclosed which items actually contained NNS. Participants next completed a 28-item questionnaire (**see Appendix 2**) to assess their attitudes toward providing foods and beverages with NNS to their child. Parents were asked to provide answers that applied to a single child (if they had more than 1 child in the home) and individuals were only eligible to complete the survey once. Participants received a $20 grocery store gift card as compensation for their time and willingness to participate in the study.

Measures

Socio-demographic characteristics

Socio-demographic variables (**Table 1**) included gender, age-group (18-25, 26-35, 36-45, 46-55, or > 55 years), race and ethnicity (non-Hispanic white, non-Hispanic black, Hispanic or other), self-reported weight and height, perceived weight category (underweight, normal weight, overweight, obese), number of children in the household, occupation, and educational attainment (≤high school, some college, bachelor's degree, master's degree, doctorate).

Non-nutritive Sweetener Containing Grocery Choices

The NNS grocery score (based on how many NNS containing items were included in the total number of groceries selected) was calculated for each participant and was compared with NNS recognition and NNS attitude measures. To determine a participant's NNS grocery score, the number of groceries selected which contained NNS was summed and divided by the number of items selected by the participant.

Non-nutritive Sweetener Recognition

Our primary outcome was the parental NNS recognition score, which was calculated based on how many of the 44 NNS containing items were identified among the 142 presented products**.** For example, if a participant correctly identified 15 NNS containing items, this participant's NNS recognition score would be 34% (15 out of 44 x 100). Recognition of NNS was also compared across sub-categories of NNS containing items.

Non-nutritive Sweetener Attitudes

NNS attitudes were measured using a 28-item questionnaire (**Appendix 2**). The wording and items in the NNS-attitude questionnaire were developed based upon the expertise of the authors. Trained research assistants read the statements out-loud and parents indicated their level of agreement with each statement using a 5-point Likert scale, where "1" was strongly disagree and "5" was strongly agree.

Statistical Analysis

Descriptive statistics were calculated for NNS-recognition and NNS-grocery scores. P-values were calculated using t-tests for comparison of two means, ANOVA for comparison of means among 3 or more groups, and chi-square test for frequency analysis, where appropriate. A p-value < 0.05 was considered statistically significant. Eighteen items from the 28-item parent NNS-survey (**Appendix 2**) were subjected to a principal component analysis (PCA) in order to evaluate correlated survey items. Items that assessed demographic information (questions 1-4) were not included in the PCA because we explored the relationship between parental responses and demographic characteristics. Items related to child NNS use (questions 9, 10, 22-25) were not included because only 23% of parents reported actual child NNS use.

Subgroup analyses by gender, BMI (calculated from self-reported weight and height), race/ethnicity, and age-group were performed. Proportions of individuals

demonstrating high (above the mean) and low (below the mean) NNS recognition were also compared between subgroups using Pearson's chi-square and t-tests.

Results

The socio-demographic characteristics of our sample are shown in **Table 1**. A total of 125 adults agreed to participate in the study, 120 of whom were included in the analyses. Two individuals were excluded because their child was less than 1 year old, while three parents were excluded due to lack of compliance with study procedures. Seventy-eight percent of participants were female and most self-identified as either non-Hispanic white (44%) or non-Hispanic black (34%). Fifty-one percent of participants characterized themselves as having a normal weight, and the mean parental BMI (based on self-reported weight and height) was 26.4 ± 5.5 kg/m^2.

Grocery Choices

The average number of groceries that the participants indicated they would hypothetically buy was 22 items (16%) out of the 142 presented foods and beverages. Honey flavored breakfast cereal (57%), natural spring water (53%), and ketchup (53%) were selected most frequently. On average, 22% of the groceries selected by parents contained NNS (NNS-GS). There were no statistically significant differences in total number of groceries selected or the NNS-grocery score based on parent BMI, education, race, number of children in the household, or parent gender.

NNS recognition

The mean NNS-recognition score was 23 ± 14%. As shown in **Figure 2**, recognition of NNS depended on the type of food and beverage presented (p=0.02). Participants generally recognized NNS with higher frequency in beverages, condiments, desserts and yogurts while NNS in grains, canned goods, and other foods were more frequently overlooked.

Participants with a lower BMI ($p < 0.002$) and those who self-identified as non-Hispanic white ($p<0.007$) demonstrated better recognition of foods and beverages with NNS compared to heavier and non-white parents. Neither parent gender nor parent age-group was significantly associated with NNS recognition.

Non-nutritive Sweetener Attitudes

Parental agreement with survey items assessing NNS acceptance are shown in **Table 3**. Seventy-two percent of parents disagreed with the statement "NNS are safe for my child to use" (16% agreed and 12% were neutral). Fifty-eight percent of participants indicated that they looked for NNS in the ingredients lists on foods and beverages because they wanted to avoid purchasing items that contained NNS. These parents were significantly more accurate in recognizing foods and beverages with NNS (p=0.003). However, the mean NNS-grocery score did not differ between parents who reported avoiding foods and beverages with NNS and those who did not.

Parents indicated a preference for items labeled "reduced sugar" and "no sugar added" (53% and 52%, respectively). Fewer indicated that they sought out items labeled "light", "low-carb", or "sugar-free" (37%, 33%, and 22%, respectively). Definitions of relevant food claims based on FDA guidance [25] are detailed in **Appendix 3.**

Principle Component Analysis

Three factors (principle components) were extracted that accounted for 53% of the variance in the parent NNS survey results: nutrition and health awareness, NNS acceptance and high sweetness liking (**Table 4)**.

We found that nutrition and health awareness was not associated with NNS recognition, but was positively associated with choosing NNS containing groceries (NNS-grocery score, ($p<0.02$)). No differences in nutrition and health awareness were observed across race/ethnicity, age, weight, or gender subgroups, while education tended to be positively associated with this factor ($p=0.10$).

Acceptance of NNS was inversely related to recognition of NNS ($p<0.0001$) but positively associated with selection of foods and beverages with NNS (reflected by the NNS-grocery score ($p<0.02$)). Non-Hispanic black and Hispanic participants, and those who did not complete college, indicated higher NNS acceptance compared to non-Hispanic whites ($p<0.0001$) and individuals with a bachelor's degree or higher ($p=0.0004$). There were no differences in NNS acceptance by gender, age, or weight subgroups. Child sweetness liking was positively associated with the total number of groceries selected ($p<0.05$), yet there were no differences in NNS-grocery scores or NNS recognition.

Discussion

Our study demonstrated that parental recognition of NNS in commercially available foods and beverages was limited. We also observed strikingly negative parental attitudes towards purchasing NNS containing foods and beverages for their children. NNS acceptance was significantly lower among parents who had achieved higher education levels and who self-identified as non-Hispanic white. However, even among these study participants NNS recognition was low. Thus, our findings raise questions as to why parents have an overall negative view of NNS and how parents avoid purchasing and providing items with NNS if they are unable to recognize them.

Furthermore, nutrition and health awareness was not associated with parental ability to recognize NNS. In fact, parents demonstrating higher nutrition and health awareness were more likely to select groceries containing NNS. This suggests that parents who read nutrition labels and report being concerned about their sugar consumption, may not realize that ingredients such as sucralose, aspartame, saccharin, and acesulfame-potassium are NNS, or may not be actively looking for NNS in foods and beverages. Alternatively, health conscious individuals may pay more attention to food claims such as “no-sugar added” resulting in inadvertent selection of NNS containing foods and beverages.

The widespread parental negativity toward NNS challenges the viability of replacing sugar-sweetened beverages with NNS as a weight management strategy [7]. Recent scrutiny over sugar-sweetened beverage consumption has prompted school districts throughout the United States to replace them in their vending machines and cafeterias [26-28] and higher taxation of sugar-sweetened beverages to reduce their

consumption has also been discussed [29]. These measures are expected to further increase availability and consumption of NNS.

The inability to identify NNS in 77% of the NNS containing products observed in our cohort calls attention to issues in food labeling. While 73% of parents indicated that NNS were not safe for their child, over half of them reported preferring foods and beverages labeled as "no-sugar added," most of which do in fact contain NNS. Meanwhile, "sugar-free," or "light" may more obviously convey the replacement of caloric sugars with NNS, and thus, were perceived less favorably. The finding that a "no-sugar added" food claim was often sought out whereas a "sugar-free" food claim was often avoided, highlights the necessity to clarify the meaning of food claims.

Strengths of the present study include the use of a relatively large and diverse population of parents, as well as the representation of various race/ethnicity groups and a wide range of educational attainment. Our participants were required to choose products in a setting which simulated grocery shopping in a free-living population. Limitations of our study include the inclusion of a convenience sample at a single grocery store and the use of non-validated survey-instruments. It is however unlikely that the questions were answered randomly, due to trained interviewers asking the questions and based on the coherence demonstrated among correlated survey items (see principle component analysis). Social desirability and recall bias might have also influenced our results.

Despite these limitations, our study clearly demonstrates that parents frequently do not recognize NNS containing foods and beverages, which they generally perceive as unsafe for their children. This emphasizes the need for rigorous clinical trials to study whether NNS use in children has long-term health effects. Our findings also support the

need for improved nutritional literacy and clearer food labeling to facilitate informed food and beverage choices among consumers.

Acknowledgements

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Figure Legends:

Figure 1. Participants completed a brief demographic questionnaire and then selected groceries that they would hypothetically purchase from an array of foods and beverages presented. Next, participants identified the items presented that they believed contained NNS and then completed an interviewer-assisted survey assessing their perceptions of NNS.

Figure 2. Recognition of NNS varied based on the type of food and beverage presented ($p=0.02$). Each dot corresponds to each NNS containing food or beverage in the category. Participants generally recognized NNS with higher frequency in beverages, condiments, desserts and yogurts, while NNS in grains, canned goods, and other foods were more frequently overlooked.

Table 1. ***Socio-demographic characteristics of sample based on NNS recognition***

	All (% of total)	High recognition of NNS[a] (% of subgroup)	Low recognition of NNS (% of subgroup)	P*
N	120 (100%)			
Gender, N (%)				
Male	26 (22%)	13 (50%)	13 (50%)	0.92
Female	94 (78%)	46 (49%)	48 (51%)	
Age Group, N (%)				
18-25	8 (7%)	2 (25%)	6 (75%)	0.44
26-35	23 (19%)	13 (57%)	10 (43%)	
36-45	44 (37%)	21 (48%)	23 (52%)	
46-55	34 (28%)	19 (56%)	15 (44%)	
55+	11 (9%)	7 (64%)	4 (36%)	
Race, N (%)				
Non-Hispanic White	53 (44%)	34 (64%)	19 (26%)	0.01^
Non-Hispanic Black	41 (34%)	13 (32%)	28 (68%)	
Hispanic	18 (15%)	7 (39%)	11 (61%)	
Other	8 (6%)	5 (63%)	3 (37%)	
BMI (kg/m^2), Mean±SD	26.4± 5.5	25.6 ± 4.60	27.19 ± 6.26	0.13
Education, N (%)				
≤ High school	20 (17%)	3 (15%)	17 (85%)	P<0.001^^
Some college	21 (18%)	8 (38%)	13 (62%)	
Bachelors	39 (33%)	19 (49%)	20 (51%)	
Masters/Doctorate	40 (33%)	29 (73%)	11 (27%)	

[a] High and low NNS recognition determined by whether participant scored above or below the mean recognition

*Pearson Chi-square test or student's t-test was used to compare proportion of high and low recognizers within each subgroup across subgroups

^ $p < 0.05$

^^$p<0.001$

Table 2. Percent agreement with questionnaire items evaluating NNS acceptance and general nutrition and health awareness

Statement	Percent agreement (%)
I seek out items labeled “reduced sugar”	53
I seek out items labeled “no sugar added”	52
I seek out items labeled light	37
I seek out items labeled low-carb	33
I seek out items labeled sugar-free	22
I read the ingredients in the packaged items that I purchase	64
I look for NNS in packaged foods and beverages because I want to avoid them	58
I am concerned with the calorie content of the items that I select	52
I am concerned with the sugar content of the items that I select	73
I am concerned with the fat content of the items that I select	68
Non-nutritive sweeteners (i.e. Splenda™, Sweet N Low™, Equal™) are safe for my child to use	16
I recommend that my child use diet (NNS) foods and beverages because I am concerned about his/her sugar intake	14
I recommend that my child use diet (NNS) foods and beverages because I am concerned about his/her weight	13

Table 3. Parent NNS survey items comprising each of the three principal components

Principal Component	Number of items included	% Variance	Item	Statement
Nutrition and Health Awareness	7	25%	12	I seek out items labeled "no-sugar added"
			14	I seek out items labeled "reduced-sugar"
			16	I read the ingredients in the packaged items that I purchase
			17	I read the nutrition facts panel on the packaged items that I purchase
			18	I am concerned with the calorie content of the items that I select
			19	I am concerned with the sugar content of the items that I purchase
			20	I am concerned with the fat content of the items that I purchase
NNS acceptance	8	18%	5	Non-nutritive sweeteners (such as Splenda, Sweet N Low, Equal) are safe for my child to use
			6*	My child is not old enough to have foods and beverages with non-nutritive sweeteners
			7	I recommend that my child use diet (NNS) foods and beverages because I am concerned about his or her sugar intake
			8	I recommend that my child use diet (NNS) foods and beverages because I am concerned about his or her weight
			11	I seek out items labeled "sugar-free"
			13	I seek out items labeled "light"
			15	I seek out items labeled "low-carb"
			21*	I look for non-nutritive sweeteners in packaged items because I want to avoid purchasing items that contain them

High Sweetness Liking	3	10%	26	When given the choice between a sweetened or unsweetened drink such as plain water, my child always chooses the sweetened option
			27	My child frequently adds sugar or sweeteners to food
			28	My child frequently adds sugar or sweeteners to beverages

*Likert responses to item were reverse coded

Figure 1. Sequence of study procedures

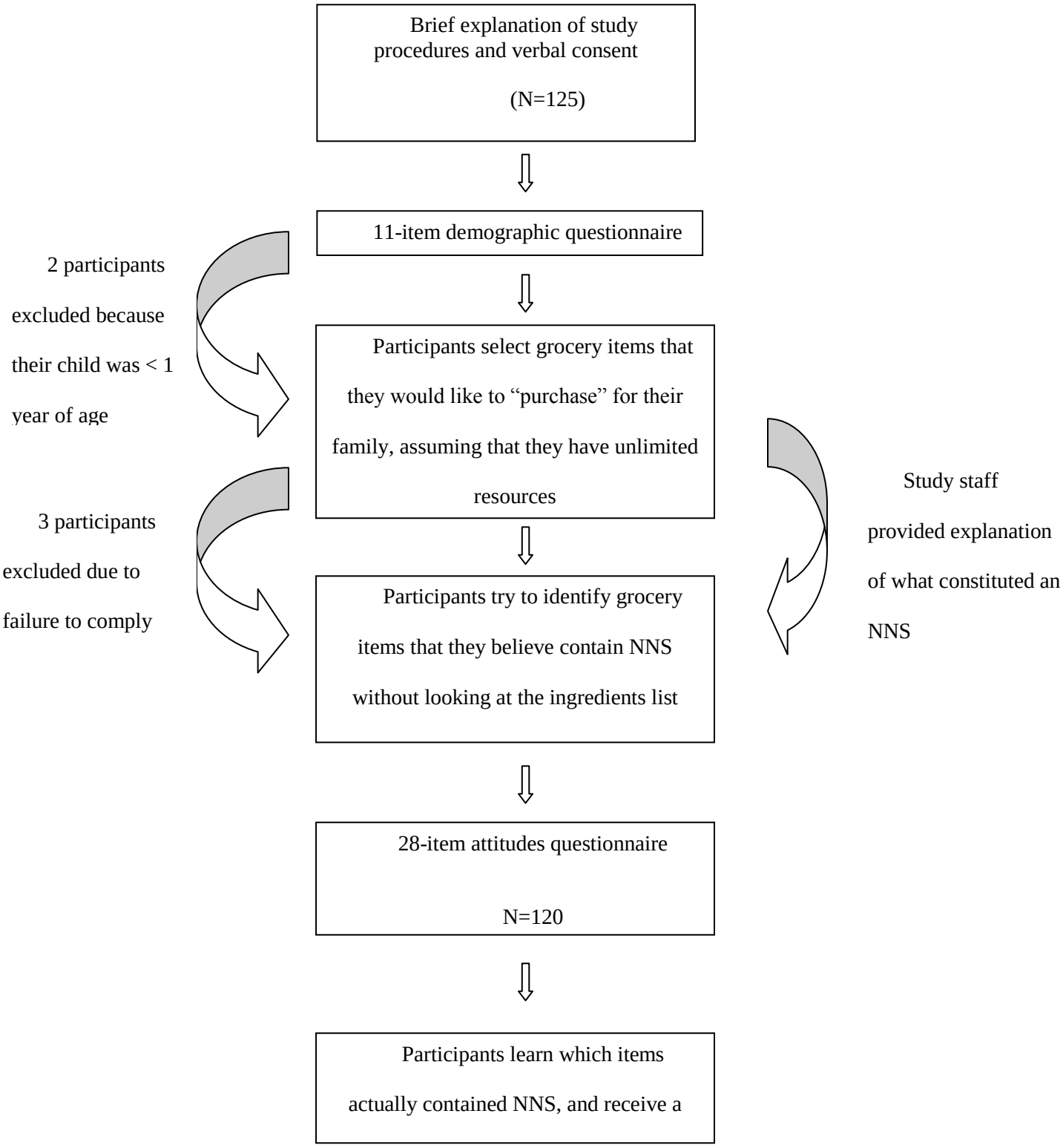
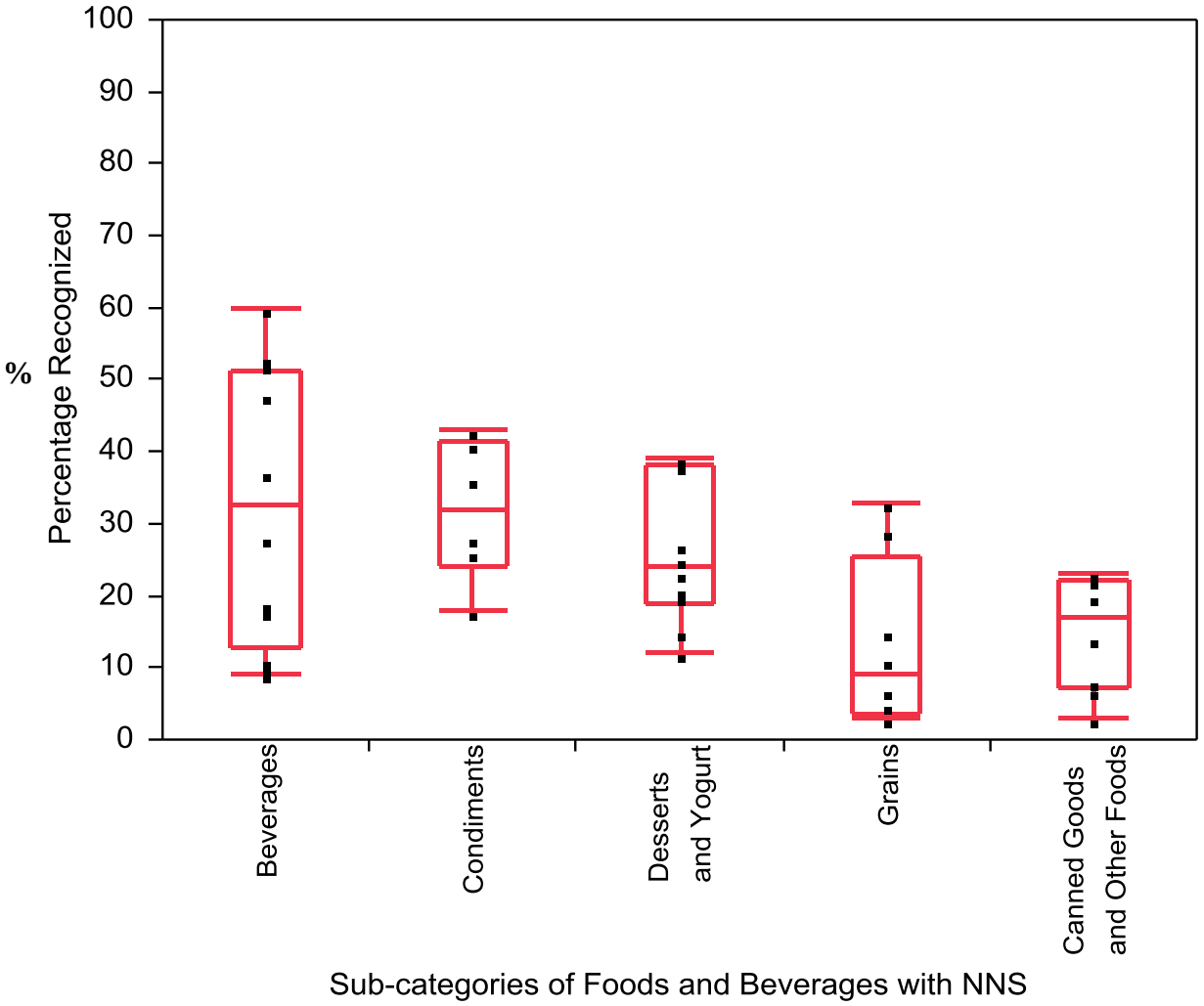


Figure 2. Box-and-whiskers plot illustrating distribution of parental recognition of NNS-sweetened foods and beverages by sub-category of NNS-containing items. Each dot corresponds to each NNS containing food or beverage in the category. Box shows interquartile range divided at the median.



Appendix 1: List of all food and beverages items presented to participants and percent recognition for items (n=45) containing NNS

Item	Non-nutritive sweetener	Percent recognition (%)
Beverages		
Lemon Lime sports drink	N	
Low-calorie sports drink	Y	37
Citrus Soda	Y	53
"Zero" Soda	Y	53
Diet Soda	Y	60
Peach Mango Juice Light	Y	38
100% Apple Juice	N	
Half & Half Lemonade Iced Tea	Y	28
Sweet Tea	N	
Diet Cranberry Juice Beverage	Y	52
Cranberry Juice No Sugar Added	Y	11
Vanilla Soymilk	N	
Fat Free Chocolate Milk	Y	10
100% Juice Apple Raspberry	N	
Cranberry Cocktail Juice	N	
Light Cranberry Juice	Y	48
Natural Spring Water	N	
Seltzer Water Mandarin Orange	N	
Fruit Flavored Water Beverage	Y	19
Reduced-calorie Orange Juice	Y	18
Peach Iced Tea Mix	N	
No Sugar added hot chocolate	Y	9
Condiments		
Teriyaki Sesame Marinade	N	
Lite Raspberry Salad Dressing	N	
Pasta Sauce Extra Chunky	N	
Heart Smart Pasta Sauce	N	
Light Pasta Sauce	N	
Sugar Free Pancake Syrup	Y	36
Original Pancake Syrup	N	
Pancake Syrup Butter Lite	N	
Mayonnaise	N	
Ketchup	N	
Grape Jelly	N	
Sugar Free Grape Jelly	Y	43
Orange Marmalade	N	
Low Sugar Orange Marmalade	N	
Crunchy Reduced Fat Peanut Butter	N	

Alfredo Sauce	N	
Fat Free Whipped Topping	N	
Sugar Free Chocolate Syrup	Y	26
Chocolate Syrup Bottle	N	
Lite Chocolate Syrup Bottle	Y	18
Chocolate Fudge Frosting	N	
Sugar Free French Vanilla Liquid Non	Y	28
Fat Free French Vanilla Liquid Non	N	
Strawberry reduced fat cream cheese	N	
Canned Fruit		
Sliced Pears	N	
Sliced No Sugar Added Pears	Y	8
Lite Pear Halves Extra Light Syrup	Y	22
Low Sugar Mandarin Orange	N	
Lite Fruit Cocktail Extra Low Sugar	Y	23
Sliced Yellow Cling Peaches In Light	N	
Diced Peaches In Light Syrup	N	
Diced Peaches No Sugar Added-	Y	22
Applesauce	N	
Desserts		
Chocolate Coconut Bar (Diet)	Y	23
Peach Smoothie	N	
Shortbread Cookie Crisps	N	
Chewy chocolate chip cookies	N	
No sugar added apple pie filling	Y	20
Frosted Donuts	N	
Sugar Free Chocolate Fudge Frosting	Y	25
Double Chocolate Pie Pudding Snacks	Y	12
Strawberry Cheesecake Pudding	N	
Chocolate Sandwich Cookie	N	
Fat Free Cranberry Orange Muffin	N	
Sugar Free Devil's Food Cake Mix	Y	39
Gelatin Dessert Raspberry	N	
Fat Free Raspberry Sorbet	N	
No Sugar Added Mint Chocolate Chip	Y	27
Light Caramel Ice Cream	Y	
Fat Free Vanilla Frozen Yogurt	N	
Fat Free Vanilla Ice Cream	N	
Whole Fruit Bar Variety Pack	N	
Carb Smart Ice Cream Bar	Y	38

Apple Toaster Strudel	Y	15
Fat Free Fudge Ice Cream Bar	N	
Cookies And Cream Ice Cream Cones	N	
Weight Control Ice Cream Bars	Y	
Toaster Pastries Frosted Cherry	N	
Grains & Sides		
High Fiber Flavored Instant Oatmeal	Y	15
Low Sugar Flavored Instant Oatmeal	Y	33
Weight Control Flavored Instant	Y	29
Oatmeal Variety Pack	N	
Butter Snaps Pretzels	N	
High Fiber Honey Clusters Breakfast	Y	7
High Fiber Bran Cereal	Y	11
Cereal with Red Berries	N	
Sweetened Cereal with marshmallows	N	
Cinnamon Toast flavored cereal	N	
Sweetened Honey flavored cereal	N	
Graham Cracker flavored cereal	N	
Frosted breakfast cereal	N	
Cereal bar with marshmallows	N	
Low Fat Chocolate Chunk granola bars	N	
Reduced Sugar Cookies N Cream	Y	20
Cereal Bars Fruit & Grain Strawberry	N	
Cereal Bars Blueberry	Y	
Hamburger Stroganoff	N	
Rice Pilaf	N	
Instant Mashed Potatoes	N	
Macaroni & Cheese	N	
Whole Grain Brown Ready Rice	N	
Buttermilk Bread	N	
Hearty White Bread	N	
Light 100% Multi Grain Bread	Y	5
100% Whole Wheat Bread -	N	
Cinnamon Raisin Bagels	N	
English Muffins Original	N	
English Muffins 100% whole wheat	N	
English Muffins light	Y	3
Frozen foods		
Frozen miniature pizza bagels	N	
Swedish Meatballs Frozen Dinner	N	

Frozen French Toast Sticks	N	
Frozen Waffles Blueberry	N	
Frozen dinner: Chicken Fettuccini	N	
Frozen dinner: Home-style Chicken In	N	
Frozen dinner: Chicken Teriyaki Stir	Y	7
Frozen Pizza	N	
Frozen “healthy weight” Assorted	N	
Raspberries Sweetened (frozen)	N	
Other foods		
Apple Cinnamon Mini Rice Cakes	Y	14
Microwave Popcorn Kettle Corn	Y	3
Microwave Popcorn Movie Theater	N	
Dry Honey Roasted Peanuts	N	
Multigrain Tortilla Chips	N	
Original Potato Chips	N	
Mixed Fruit Snacks	N	
Vanilla Yogurt Parfait with blueberries	N	
100 Calorie Cottage Cheese w. Peach	N	
Light blueberry yogurt	Y	21
Vanilla yogurt with probiotics	N	
Chocolate whipped yogurt	N	
French Vanilla Yogurt	N	
Light Strawberry Banana Yogurt	Y	38
Blueberry Fruit On The Bottom Yogurt	N	
Microwave Chicken Noodle Soup	N	

Appendix 2: Parent Non-Nutritive Sweetener Survey

One last thing that we want to learn from our study today is what types of foods and beverages your child commonly consumes and how you feel about his or her dietary habits. Below are statements for you to respond to with either strongly disagree, disagree, neutral, agree or strongly agree. If you have more than one child, we will have you pick a number out of a bag, which will correspond to the child's birth order for whom you will answer the questions. For example, if you choose the number "2," then you should respond with your second born child in mind. This should only take about 5 minutes and then you will get your gift card and be on your way!

1. What is this child's (the one who you are responding about) **age**? _____

2. How would you characterize your child's **gender**?

Male……..……1

Female……..….2

Refused……..3

3. Which of the following **best** describes this child's **weight**?

Underweight……...……1

Normal Weight……..2

Just above normal weight..……...……...…….3

Overweight…….4

Very overweight……...……..5

Obese……..……6

Refused……..…….7

4. What is this child's weight (in pounds) and height (in inches)?

   Weight _____ pounds

   Height _____ inches

	Strongly Disagree	**Disagree**	**Neutral**	**Agree**	**Strongly Agree**
5. Non-nutritive sweeteners (such as Splenda, Sweet N Low, Equal) are safe for my child to use					
6. My child is not old enough to have foods and beverages with non-nutritive sweeteners					
7. I recommend that my child use diet (NNS) foods and beverages because I am concerned about his or her sugar intake					
8. I recommend that my child use diet (NNS) foods and beverages because I am concerned about his or her weight					
9. My child uses diet (NNS) foods and beverages because someone in the household has diabetes					

10. My child uses diet (NNS) foods and beverages because he or she has diabetes					

When I am grocery shopping for my family….					
11. I seek out items labeled "sugar-free"					
12. I seek "no sugar added" items					
13. I seek out items labeled "light"					
14. I seek out "reduced sugar" items					
15. I seek out items labeled "low-carb"					
16. I read the ingredients in the packaged items that I purchase					
17. I read the nutrition facts panel on the packaged items that I purchase					
18. I am concerned with the calorie content of the items that I select					
19. I am concerned with the sugar content of the items that I purchase					
20. I am concerned with the fat content of the items that I purchase					
21. I look for non-nutritive sweeteners in packaged items because I want to avoid purchasing items that contain them					

My child drink diet sodas, diet fruit drinks, diet sports drinks, and other beverages with non-nutritive sweeteners because	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree

22. These beverages are always available at home					
23. These are the only drinks other than water to purchase at school					
24. He/she does not like the taste of plain water					
25. All of his/her friends drink diet beverages					
26. When given the choice between a sweetened or unsweetened drink such as plain water, my child always chooses the sweetened option					
27. My child frequently adds sugar or sweeteners to food					
28. My child frequently adds sugar or sweeteners to beverages					

Appendix 3. Definitions[1] of food claims included in the parental attitudes questionnaire

Food Claim	**Definition[1]**
Sugar-free	Less than 0.5 g sugar per reference amounts customarily consumed (RACC) and per labeled serving
No sugar added	No sugar or sugar containing ingredient is added during processing. Does not include sugar alcohols.
Reduced sugar	At least 25% less sugars per RACC than an appropriate reference food (or for meals and main dishes, at least 25% less sugar per 100g)
Light	If 50% or more of the calories are from fat, fat must be reduced by at least 50% per serving. If less than 50% of calories are from fat, fat must be reduced at least 50% or calories reduced at least 1/3 per serving.
Low-carbohydrate	No current FDA guidance for use of carbohydrate content claims

[1]Reference: US Food and Drug Administration (2009).

http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodLabelingNutrition/FoodLabelingGuide/ucm064911.htm

1. Williams CL, Strobino BA, Brotanek J. Weight control among obese adolescents: A pilot study. Int J Food Sci Nutr 2007;58(3):217-230.

2. Bellisle F, Altenburg de Assis MA, Fieux B, et al. Use of 'light' foods and drinks in French adults: biological, anthropometric and nutritional correlates. J Hum Nutr Diet 2001 Jun;14(3):191-206.

3. Husoy T, Mangschou B, Fotland TO, et al. Reducing added sugar intake in Norway by replacing sugar sweetened beverages with beverages containing intense sweeteners - A risk benefit assessment. Food and Chemical Toxicology 2008 Sep;46(9):3099-3105.

4. Guthrie JF, Morton JF. Food sources of added sweeteners in the diets of Americans. Journal of the American Dietetic Association 2000 Jan;100(1):43-51, quiz 49-50.

5. Bellisle F, Drewnowski A. Intense sweeteners, energy intake and the control of body weight. Eur J Clin Nutr 2007 Jun;61(6):691-700.

6. de Ruyter JC, Olthof MR, Seidell JC, et al. A trial of sugar-free or sugar-sweetened beverages and body weight in children. The New England journal of medicine 2012 Oct 11;367(15):1397-1406.

7. Ebbeling CB, Feldman HA, Chomitz VR, et al. A randomized trial of sugar-sweetened beverages and adolescent body weight. The New England journal of medicine 2012 Oct 11;367(15):1407-1416.

8. Morris PJ, Luthy W, Weber HP. High-intensity rectangular fiber-coupled diode laser array for solid-state laser pumping. Appl Opt 1993 Sep 20;32(27):5274-5279.

9. Caprio S. Calories from soft drinks--do they matter? N Engl J Med 2012 Oct 11;367(15):1462-1463.

10. Forshee RA, Storey ML. Total beverage consumption and beverage choices among children and adolescents. Int J Food Sci Nutr 2003 Jul;54(4):297-307.

11. Blum JW, Jacobsen DJ, Donnelly JE. Beverage consumption patterns in elementary school aged children across a two-year period. J Am Coll Nutr 2005 Apr;24(2):93-98.

12. Johnson L, Mander AP, Jones LR, et al. Is sugar-sweetened beverage consumption associated with increased fatness in children? Nutrition 2007 Jul-Aug;23(7-8):557-563.

13. Birch LL, McPhee L, Sullivan S. Children's food intake following drinks sweetened with sucrose or aspartame: time course effects. Physiology & behavior 1989 Feb;45(2):387-395.

14. Swithers SE, Davidson TL. A role for sweet taste: calorie predictive relations in energy regulation by rats. Behav Neurosci 2008 Feb;122(1):161-173.

15. Swithers SE, Laboy AF, Clark K, et al. Experience with the high-intensity sweetener saccharin impairs glucose homeostasis and GLP-1 release in rats. Behavioural brain research 2012 Jul 15;233(1):1-14.

16. Sylvetsky A, Rother KI, Brown R. Artificial sweetener use among children: epidemiology, recommendations, metabolic outcomes, and future directions. Pediatr Clin North Am 2011 Dec;58(6):1467-1480.

17. Scaglioni S, Salvioni M, Galimberti C. Influence of parental attitudes in the development of children eating behaviour. Br J Nutr 2008 Feb;99 Suppl 1:S22-25.

18. Scaglioni S, Arrizza C, Vecchi F, et al. Determinants of children's eating behavior. Am J Clin Nutr 2011 Dec;94(6 Suppl):2006S-2011S.

19. Addessi E, Galloway AT, Visalberghi E, et al. Specific social influences on the acceptance of novel foods in 2-5-year-old children. Appetite 2005 Dec;45(3):264-271.

20. Sylvetsky AC, Welsh JA, Brown RJ, et al. Low-calorie sweetener consumption is increasing in the United States. The American journal of clinical nutrition 2012 Sep;96(3):640-646.

21. Patch CS, Tapsell LC, Williams PG. Attitudes and intentions toward purchasing novel foods enriched with omega-3 fatty acids. J Nutr Educ Behav 2005 Sep-Oct;37(5):235-241.

22. Patch CS, Tapsell LC, Williams PG. Overweight consumers' salient beliefs on omega-3-enriched functional foods in Australia's Illawarra region. J Nutr Educ Behav 2005 Mar-Apr;37(2):83-89.

23. International Food Information Council Foundation. Food & Health Survey: Consumer Attitudes toward Food Safety, Nutrition and Health. 2012 [cited November 28th, 2012]; Available from: http://www.foodinsight.org/Resources/Detail.aspx?topic=2012_Food_Health_Survey_Consumer_Attitudes_toward_Food_Safety_Nutrition_and_Health

24. Slining MM, Ng SW, Popkin BM. Food companies' calorie-reduction pledges to improve u.s. Diet. American journal of preventive medicine 2013 Feb;44(2):174-184.

25. United States Food and Drug Administration. Guidance for Industry: A Food Labeling Guide. 2009.

26. Johnson DB, Bruemmer B, Lund AE, et al. Impact of school district sugar-sweetened beverage policies on student beverage exposure and consumption in middle schools. J Adolesc Health 2009 Sep;45(3 Suppl):S30-37.

27. Blum JE, Davee AM, Beaudoin CM, et al. Reduced availability of sugar-sweetened beverages and diet soda has a limited impact on beverage consumption patterns in Maine high school youth. J Nutr Educ Behav 2008 Nov-Dec;40(6):341-347.

28. Briefel RR, Crepinsek MK, Cabili C, et al. School food environments and practices affect dietary behaviors of US public school children. J Am Diet Assoc 2009 Feb;109(2 Suppl):S91-107.

29. Andreyeva T, Chaloupka FJ, Brownell KD. Estimating the potential of taxes on sugar-sweetened beverages to reduce consumption and generate revenue. Prev Med 2011 Jun 1;52(6):413-416.

CHAPTER 6: INGESTION OF SUCRALOSE PRIOR TO AN ORAL GLUCOSE LOAD DOES NOT ALTER GLYCEMIA, GUT HORMONES OR SATIETY IN HEALTHY ADULTS

Rebecca J. Brown MD, MHSc.: Clinical and Cellular Biology Section, Diabetes Endocrinology and Obesity Branch, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health

Allison C. Sylvetsky: Nutrition and Health Science Program, Graduate Division of Biological and Biomedical Sciences, Laney Graduate School, Emory University & Section on Pediatric Diabetes & Metabolism, Diabetes Endocrinology and Obesity Branch, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health

Kristina I. Rother MD, MHSc.: Section on Pediatric Diabetes & Metabolism, Diabetes Endocrinology and Obesity Branch, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health

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Abstract

Context: Consumption of non-nutritive sweeteners (NNS) is increasing among children and adults in the US. Our group has previously demonstrated that ingestion of Diet Rite Cola™ sweetened with sucralose and acesulfame-potassium, administered prior to an oral glucose tolerance test (OGTT), resulted in increased GLP-1 secretion compared to unsweetened carbonated water.

Objective: To test whether sucralose augmented GLP-1 release and altered gastric emptying, intestinal glucose absorption and subjective hunger and satiety ratings.

Design: Same subject subjective crossover study involving four outpatient visits.

Setting: Outpatient clinic in Bethesda, MD.

Participants: 30 healthy adults (aged 18-45 years).

Interventions: Subjects were assigned in randomized order to consume 0 mg, 68 mg, 170 mg, or 250 mg sucralose, each dissolved in 360 mL plain water, 10 minutes prior to an OGTT. Blood samples were drawn at -10, 0, 10, 20, 30, 60, 90, and 120 minutes following consumption of the glucose load.

Main outcome measures: Total GLP-1, glycemia, satiety, glucose kinetics

Results: There were no differences in concentrations of GLP-1, glucose, insulin, C-peptide, or free fatty acids. Intestinal glucose absorption, rate of gastric emptying, and hunger and satiety ratings remained unaffected in the 3 sucralose conditions compared to the control.

Conclusion: Likely explanations for the discrepant findings in the current study with sucralose and our prior study with Diet Rite Cola™ include: 1) the other NNS

(acesulfame-potassium), 2) synergism between the two NNS (acesulfame-K and sucralose), or 3) an entirely different substance in diet soda (e.g. caramel color) caused the observed GLP-1 increase. Future studies assessing various ingredient combinations in diet soda are underway.

Introduction

Non-nutritive sweeteners (NNS) are 100-1,000 times sweeter than sucrose and provide sweet taste with no or minimal contribution to caloric intake [1]. Consumption of NNS has been steadily increasing in both children and adults [2]. This trend will likely continue, since NNS are used to replace sugar, which has been linked with the development of obesity [3]. However, several large cross-sectional studies have also suggested adverse metabolic effects resulting from NNS consumption similar to sugar intake, including weight gain [4, 5], insulin resistance, and cardiovascular disease [6]. In contrast, randomized controlled trials with NNS have shown neutral effects or possible weight management benefits of NNS consumption [7, 8].

One proposed mechanism for the observed association between NNS use and weight gain is reverse causality; that is, individuals at risk for weight gain are more likely to attempt to limit caloric intake by using more NNS. An alternate explanation is that NNS binding to the sweet taste receptor complex TIR2/TIR3 may alter glucose metabolism and/or appetite. Sweet taste receptors, which are activated by both caloric sugars and NNS [9], are present in the oropharynx, in the gastrointestinal tract, in the pancreas [10] and also in glucose-sensing regions of the brain [11, 12]. Activation of oral sweet taste receptors conveys the cognitive sensation of sweetness, whereas activation of intestinal sweet taste receptors is involved in amplification of various gut hormone responses, including GLP-1 and peptide YY (PYY) secreted from enteroendocrine L-cells, gastric inhibitory peptide (GIP) secreted from enteroendocrine K-cells and cholecystokinin (CCK) secreted by enteroendocrine I-cells [13].

We previously demonstrated that ingestion of a commercially available diet soda (Diet Rite Cola™) sweetened with sucralose and acesulfame-potassium, administered prior to an oral glucose load, resulted in increased glucagon-like-peptide 1 (GLP-1) secretion in comparison to unflavored carbonated water [14, 15]. In the current study, we tested the hypothesis that sucralose was the active ingredient in the diet soda that caused increased glucose-dependent GLP-1 secretion. We also investigated the impact of sucralose on the rate of intestinal glucose absorption, on glucose, insulin, amylin, and free fatty acid levels, on the rate of gastric emptying, and on subjective hunger and satiety ratings.

Materials and Methods

Thirty healthy adults participated in the study. All subjects provided informed consent prior to participation. The protocol was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Subjects were aged between 18 and 45 years had no known active medical conditions, and were not using medication other than oral contraceptives. All subjects had normal glucose tolerance.

Subjects arrived at the Mark. O. Hatfield Clinical Center at approximately 8:30am following a ten hour fast. Subjects were instructed to avoid foods and beverages containing NNS, acetaminophen, and alcohol for 2 days prior to each study visit. Subjects were also asked to record their food consumption and physical activity during the 24 hours prior to each study visit, and were encouraged to maintain a similar diet and

physical activity level prior to each subsequent visit. Study visits were scheduled on 4 separate days, between 2 days and 6 weeks apart, to avoid carryover effects from the prior visit and to avoid any significant changes in body weight or metabolic parameters.

Block randomization (block size 24) based on a random number table was used to assign each subject to a random sequence of 4 test conditions: 360 ml water mixed with a sucralose dose of 0 mg (plain water control), 68 mg, 170 mg, or 250 mg. After a baseline blood sample was drawn at -10 minutes, subjects consumed the sucralose or water test beverage in 2-3 minutes. Ten minutes later, (at time 0 minutes) a second blood sample was obtained, after which subjects ingested a standard oral glucose load (75 g glucose) mixed with 1450 mg of acetaminophen and 7.5g 3-*O*-methylglucose. Blood samples were drawn 10, 20, 30, 60, 90, and 120 minutes following consumption of the glucose load. Satiety questionnaires were administered at baseline prior to ingesting the study drink (0 mg, 68 mg, 170 mg, 250 mg sucralose in 360 mL water), immediately following consumption of the 75 g oral glucose load (Glucola™), and after 30, 60, 90, and 120 minutes.

Study data were collected and managed using REDCap electronic data capture tools hosted at Vanderbilt University [16]. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

Measures

Insulin was measured using a chemiluminescence immunoassay with a normal fasting range of 42–188 pmol/l (interassay CV 11.5% at 69 pmol/l and 8.1% at 198 pmol/l; intraassay CV 6.2% at 56 pmol/l and 4.9% at 429 pmol/l). Serum glucose was determined using the glucose oxidase method (interassay CV 3.9% at 2.4 mmol/l and 1.2% at 22.1 mmol/l; intraassay CV 2.9% at 2.4 mmol/l and 0.4% at22.1 mmol/l). Free fatty acids (FFA) were measured were measured by colorimetric methods using reagents from Wako Chemicals (Richmond, VA). Total GLP-1 was measured using a radioimmunoassay (Millipore, Billerica MA, USA). The lowest detectable level of total GLP-1was 3 pmol/L, using a 300 μL extracted sample (inter-assay CV, 23%; intra-assay CV, 22%). Active GLP-1 and GIP were measured by enzyme-linked immunosorbent assay (Millipore, Billerica MA, USA). The lowest detectable level of active GLP-1 was 2 pmol/L (inter-assay CV 8% and intra-assay CV 7%). The lowest detectable level of GIP was 8.2 pg/mLinter-assay CV 1.8–6.1% and intra-assay CV 3.0–8.8 %).

Glucose absorption was measured using 3-*O*-methyl-glucose (7.5 g administered with the glucose load), which is an inert, non-metabolizable glucose analog. The appearance of 3-*O*-methyl-glucose in blood can thus be used as a proxy measure of the rate of intestinal glucose absorption[17]. Acetaminophen appearance in the blood was used as a proxy measure of the rate of gastric emptying; acetaminophen was administered as1450 mg mixed with the glucose load[18]. 3-OMG and acetaminophen were analyzed by GC-MS using a deuterated analyte as the internal standard. Plasma was deproteinated, dried,

and derivatized with methylboronic acid in pyridine for 3-OMG analysis. Acetaminophen was purified from acidified plasma by SPE, than silylated prior to GC-MS analysis. Hunger and satiety were measured using 100mm visual analog scales, validated for assessing hunger, satiety, and prospective food intake before and after a meal [19].

Statistical Analysis

Descriptive statistics were calculated for each outcome of interest during each of the four test visits. Mean, standard deviation, and peak were calculated, as well as total area under the curve (AUC) using the trapezoidal method [20]. Differences between the mean peak and AUC in the four conditions were assessed using ANOVA, and post-hoc Tukey tests were used for pairwise comparisons, where necessary. Linear mixed modeling was used to account for fixed and random effects, given the same-subject crossover design of the study.

Results

Baseline subject characteristics are shown in **Table 1.** Reported habitual consumption of NNS was rather low in our study cohort; 57% of participants reported never drinking beverages sweetened with NNS, and only 17% indicated consuming diet beverages on a daily basis. Seventy percent of participants reported never adding sweetener packets to their foods and beverages, whereas 7% reported using sweetener packets at least once a day.

As shown in **Figure 1**, no difference in the primary outcome of active GLP-1 AUC between the three sucralose conditions and the plain water control was observed (AUC 1268±171, 1218±175, 1328±157, 1352±189 for 0 mg, 68 mg, 170 mg, 250 mg respectively, p= 0.24). Similarly, there were no differences total GLP-1 AUC (AUC 2259 ± 194, 2354 ± 244, 2390±178, 2437 ±223 for 0 mg, 68 mg, 170 mg, 250 mg respectively, p=0.56) or total or active GLP-1 peak between the four test conditions. There were no differences for GIP (AUC 13478±1155, 13298±1073, 13647±1160, 13882±1227 for 0 mg, 68 mg, 170 mg, and 250 mg, respectively, p=0.85) or for amylin (AUC 4914±373, 5112 ±392, 5231±473, 5123± 355 for 0 mg, 68 mg, 170 mg, and 250 mg, respectively, p=0.89). As shown in **Figure 2**, glucose (AUC 13515±491, 13813±487, 13419±443, 13168±430 for 0 mg, 68 mg, 170 mg, and 250 mg, respectively, p=0.55), insulin (AUC 4830±345, 5228±365, 4929±401, 4806±327 for 0 mg, 68 mg, 170 mg, and 250 mg, respectively, p=0.33) and C-peptide (AUC 832±40, 843±39, 825±47, 817±34 for 0 mg, 68 mg, 170 mg, and 250 mg, respectively, p=0.89) and free fatty acid (FFA) (AUC 5652±236 vs. 5750± 209 for 0 mg vs. 170 mg respectively, p= 0.37) levels were similar between the four conditions. Intestinal glucose absorption and rate of gastric emptying were also no different following sucralose compared to water and are displayed in **Figure 3**. No differences were observed in or hunger and satiety ratings between the three sucralose conditions and the control (data not shown).

Discussion

The current study did not show any effects of a sucralose preload on incretin response, glucose metabolism, intestinal glucose absorption, gastric emptying, or hunger

and satiety in healthy adult volunteers. In contrast, we recently reported an augmentation in GLP-1 secretion in response to a diet soda sweetened with sucralose and acesulfame-potassium followed by a glucose load. The enhanced glucose-stimulated GLP-1 secretion in response to Diet Rite Cola™ was observed among both healthy 12 to 25 year olds [14] and similar aged patients with type 1 diabetes [15], but not those with type 2 diabetes [15]. Mace et al. had shown faster intestinal glucose absorption when rodents were exposed to glucose and sucralose combined [21]. Based on these results in human and animal studies, we hypothesized that sucralose was the active ingredient in the Diet Rite Cola™ causing higher GLP-1 secretion, and that altered glucose absorption kinetics would also be observed in humans. However, the current results did not support this hypothesis.

The lack of an effect of sucralose on metabolic parameters observed in this study is consistent with results of other human studies. In several reports sucralose and other NNS administered in isolation (without glucose) did not increase GLP-1 secretion [22-25] and thus, it appears that the sweet taste sensation and binding of sucralose to intestinal sweet taste receptors is not sufficient to effect gut hormone secretion *in vivo*. In addition, one study found no difference in plasma GLP-1 during intraduodenal infusion of sucralose versus saline in combination with glucose [26], supporting our finding that sucralose does not augment glucose-stimulated GLP-1 secretion in humans.

All NNS share the ability to stimulate sweet taste receptors, but each NNS has a different chemical structure. Because these structurally diverse sweet compounds bind to different domains of the sweet taste receptor [27], they may exert varying downstream effects. For example, acesulfame-potassium is a likely candidate to account for the

discrepancy between the current study and our prior findings. In addition to binding to sweet-taste receptors, acesulfame-potassium binds to bitter taste receptors [28], which are also expressed on intestinal enteroendocrine cells. It is also possible that our previous findings resulted from a synergism of the two NNS, sucralose and acesulfame-potassium, or that other ingredients in the diet soda interacted with sucralose and/or acesulfame-potassium to augment GLP-1 release. In addition to sucralose and acesulfame-potassium, Diet Rite Cola™ contains caramel color, gum acacia, natural flavors, citric acid, potassium benzoate, phosphoric acid, and potassium citrate (http://www.dietrite.com/textonly/cola.aspx). While none of these compounds has been reported to alter GLP-1 secretion, we cannot rule out the possibility that one or more of these compounds may have contributed to the enhanced GLP-1 secretion seen in our previous study.

It is unlikely that differences in subject characteristics were responsible for our present results as age, BMI and gender distribution was similar in the previous and present studies. However, polymorphisms in sweet taste receptor genes may lead to clinically relevant individual differences. This has been shown with regard to satiety responses to sweet tasting foods and beverages [29], and may be relevant for hormonal responses mediated by sweet taste receptors, as well. Preferences for sweetness may correlate with sweet taste receptor responses, and these preferences can change with age, with obesity, after surgical weight loss, and based on individual experience with sweet taste [30-35]. Limited evidence also suggests that the association between sweet taste preferences, food and beverage intake, and body weight may vary by ethnicity [7] and gender [33, 36].

Limitations of our study include a short (two day) washout period between the four test days, lack of standardization of meals and physical activity prior to each visit, and inclusion of individuals with a range of habitual NNS consumption**.** Measurements of hunger and satiety were done using visual analog scales, which have lower validity than the gold standard ad-libitum test meal [37]. Finally, administration of a glucose load, rather than a mixed macronutrient meal, limits the generalizability of our findings. The strengths of our study include the ability to test sucralose in isolation and at varying doses, eliminating confounding by other ingredients in diet soda. The use of a crossover design allowed for control of intra-individual differences.

In conclusion, the current study demonstrated that a wide range of sucralose concentrations, analogous to concentrations in approximately one (68 mg) to four (250 mg) commercial diet sodas (depending on the specific brand and composition), was not sufficient to affect the kinetics of enteral glucose absorption, glucose, insulin, free fatty acid levels or gut hormone secretion in response to a glucose load. These findings are important because our results support acute metabolic inactivity of sucralose alone. However, this study should be seen as a first step in the careful evaluation of one specific NNS in isolation and not indicate that NNS in general are metabolically inert. Prospective, controlled, prolonged exposure trials are needed to determine the role of NNS in metabolic health**.**

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Table 1. Characteristics of healthy adult volunteers

Variable	Mean (± se)
Age	29.72± 7.62
Gender	
Female	53%
Male	47%
Race	
White	60%
Black	37%
Other	3%
Body Mass Index (BMI)	25.83± 4.18
Glucose (mg/dL)	83.40± 5.24
Hemoglobin A1c (%)	5.30± 0.32
Triglycerides (mg/dL)	74.43± 29.39
HDL (mg/dL)	59.60± 12.89
LDL (mg/dL)	92.13± 26.66
Total cholesterol (mg/dL)	166.70± 29.84

Figure Legends

Figure 1. Serial data from OGTTs. Active glucagon-like-peptide 1 (GLP-1) (A), total GLP-1 (B), gastric inhibitory peptide (GIP) (C), and amylin (D) levels are shown after ingestion of either 68-mg sucralose (■ with dashed line), 170-mg sucralose (△ with dotted line), 250-mg sucralose (◊ with dashed and dotted line) or water (● with solid line) 10 minutes prior to a 75 gram oral glucose load. There were no differences in GLP-1, GIP, or amylin following the sucralose vs. the water condition, nor were there differences between sucralose doses.

Figure 2. Serial data from OGTTs. Glucose (A), insulin (B), and C-peptide (C), and free fatty acids (FFA) (D) are shown after ingestion of either 68-mg sucralose (■ with dashed line), 170-mg sucralose (△ with dotted line), 250-mg sucralose (◊ with dashed and dotted line) or water (● with solid line) 10 minutes prior to a 75 gram oral glucose load. There were no differences in glucose, insulin, C-peptide, or free fatty acids (FFA) following the sucralose vs. the water condition, nor were there differences between sucralose doses.

Figure 3. Serial data from OGTTs. Acetaminophen (A) and 3-OMG levels (B) are shown after ingestion of either 68-mg sucralose (■ with dashed line), 170-mg sucralose (△ with dotted line), 250-mg sucralose (◊ with dashed and dotted line) or water (● with solid line) 10 minutes prior to a 75 gram oral glucose load. There were no differences in

acetaminophen or 3-OMG following the sucralose vs. the water condition, nor were there differences between sucralose doses.

Figure 1. Serial data from OGTTs.

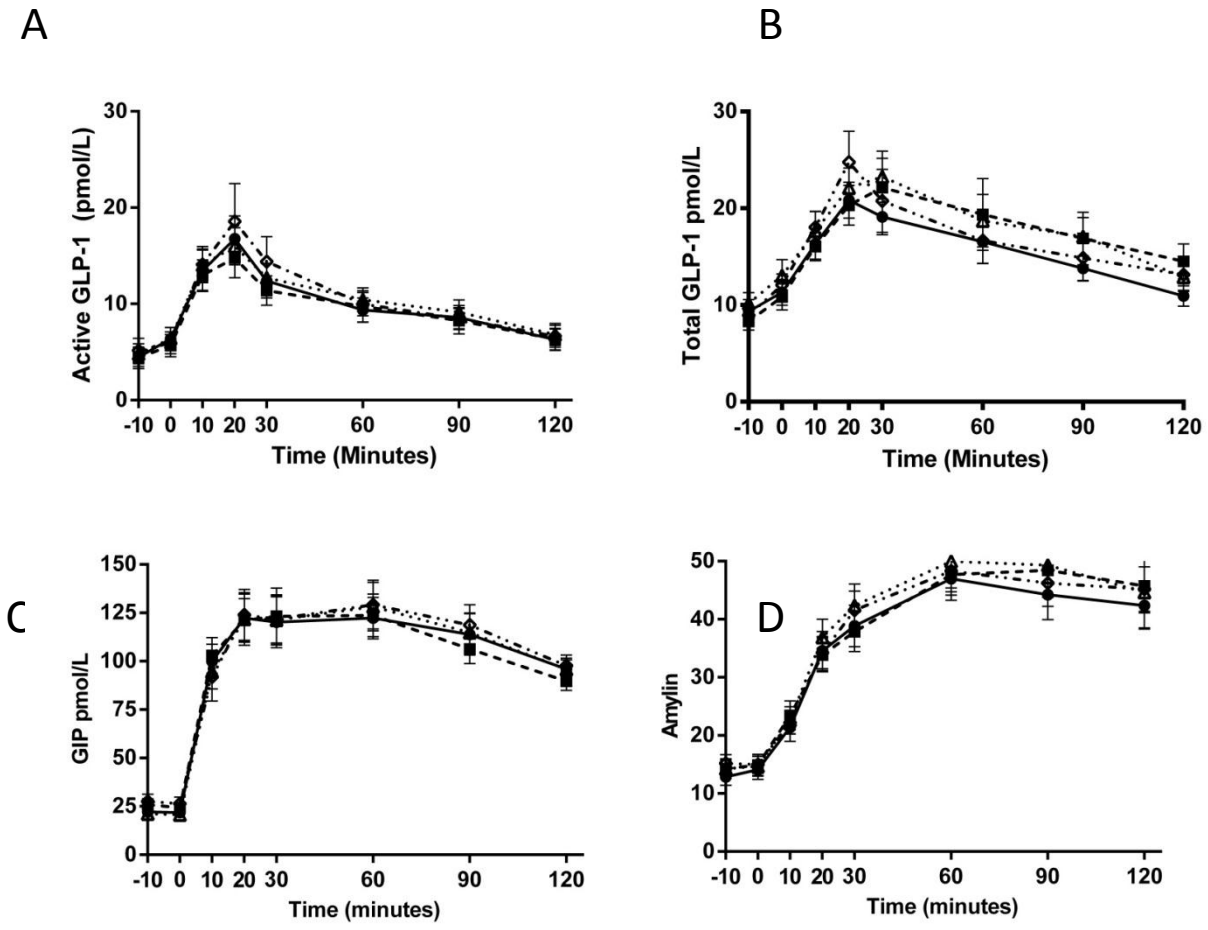


Figure 2. Serial data from OGTT's.

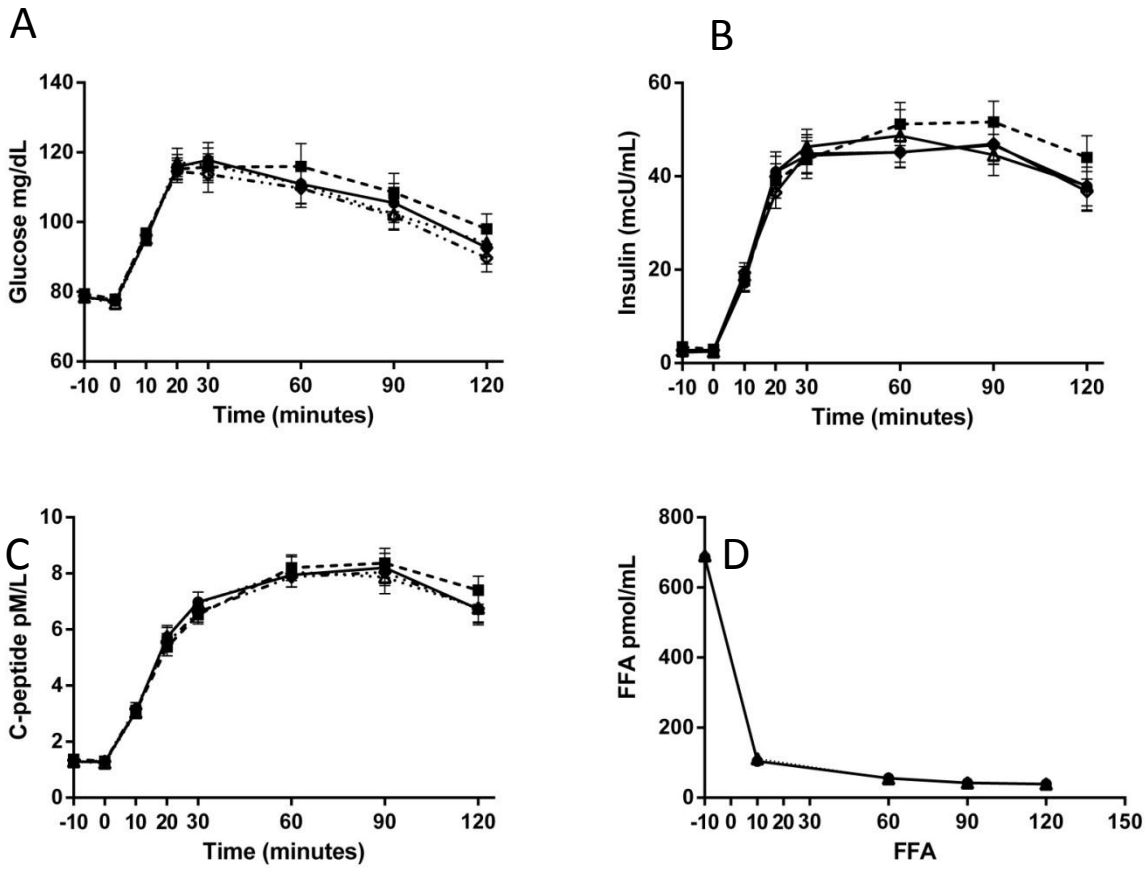
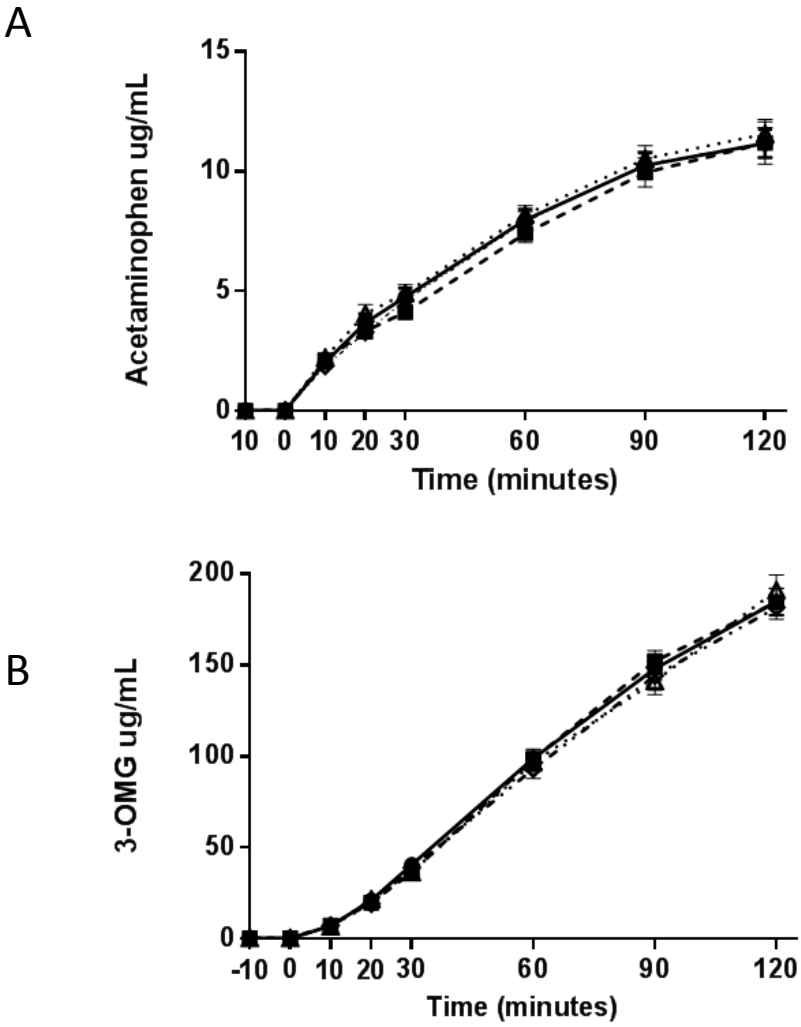


Figure 3. Serial data from OGTT's.



Literature Cited

1. Sylvetsky A, Rother KI, Brown R. Artificial sweetener use among children: epidemiology, recommendations, metabolic outcomes, and future directions. Pediatr Clin North Am 2011 Dec;58(6):1467-1480.

2. Sylvetsky AC, Welsh JA, Brown RJ, et al. Low-calorie sweetener consumption is increasing in the United States. The American journal of clinical nutrition 2012 Sep;96(3):640-646.

3. Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. The American journal of clinical nutrition 2006 Aug;84(2):274-288.

4. Stellman SD, Garfinkel L. Artificial sweetener use and one-year weight change among women. Preventive medicine 1986 Mar;15(2):195-202.

5. Berkey CS, Rockett HR, Field AE, et al. Sugar-added beverages and adolescent weight change. Obes Res 2004 May;12(5):778-788.

6. Fung TT, Malik V, Rexrode KM, et al. Sweetened beverage consumption and risk of coronary heart disease in women. The American journal of clinical nutrition 2009 Apr;89(4):1037-1042.

7. Ebbeling CB, Feldman HA, Chomitz VR, et al. A randomized trial of sugar-sweetened beverages and adolescent body weight. The New England journal of medicine 2012 Oct 11;367(15):1407-1416.

8. de Ruyter JC, Olthof MR, Seidell JC, et al. A trial of sugar-free or sugar-sweetened beverages and body weight in children. The New England journal of medicine 2012 Oct 11;367(15):1397-1406.

9. Margolskee RF, Dyer J, Kokrashvili Z, et al. T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. Proceedings of the National Academy of Sciences of the United States of America 2007 Sep 18;104(38):15075-15080.

10. Nakagawa Y, Nagasawa M, Yamada S, et al. Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. PloS one 2009;4(4):e5106.

11. Ren X, Zhou L, Terwilliger R, et al. Sweet taste signaling functions as a hypothalamic glucose sensor. Frontiers in integrative neuroscience 2009;3:12.

12. Kojima I, Nakagawa Y. The Role of the Sweet Taste Receptor in Enteroendocrine Cells and Pancreatic beta-Cells. Diabetes & metabolism journal 2011 Oct;35(5):451-457.

13. Jang HJ, Kokrashvili Z, Theodorakis MJ, et al. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. Proceedings of the National Academy of Sciences of the United States of America 2007 Sep 18;104(38):15069-15074.

14. Brown RJ, Walter M, Rother KI. Ingestion of diet soda before a glucose load augments glucagon-like peptide-1 secretion. Diabetes care 2009 Dec;32(12):2184-2186.

15. Brown RJ, Walter M, Rother KI. Effects of Diet Soda on Gut Hormones in Youths with Diabetes. Diabetes care 2012;In press.

16. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. Journal of Biomedical Informatics 2009 Apr;42(2):377-381.

17. Sigalet DL, Lam V, Boctor D. The assessment, and glucagon-like peptide-2 modulation, of intestinal absorption and function. Semin Pediatr Surg 2010 Feb;19(1):44-49.

18. Clements JA, Heading RC, Nimmo WS, et al. Kinetics of acetaminophen absorption and gastric emptying in man. Clinical pharmacology and therapeutics 1978 Oct;24(4):420-431.

19. Flint A, Raben A, Blundell JE, et al. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 2000 Jan;24(1):38-48.

20. Le Floch JP, Escuyer P, Baudin E, et al. Blood glucose area under the curve. Methodological aspects. Diabetes care 1990 Feb;13(2):172-175.

21. Mace OJ, Affleck J, Patel N, et al. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. J Physiol 2007 Jul 1;582(Pt 1):379-392.

22. Steinert RE, Frey F, Topfer A, et al. Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides. The British journal of nutrition 2011 May;105(9):1320-1328.

23. Ma J, Bellon M, Wishart JM, et al. Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects. Am J Physiol Gastrointest Liver Physiol 2009 Apr;296(4):G735-739.

24. Fujita Y, Wideman RD, Speck M, et al. Incretin release from gut is acutely enhanced by sugar but not by sweeteners in vivo. Am J Physiol Endocrinol Metab 2009 Mar;296(3):E473-479.

25. Brown AW, Bohan Brown MM, Onken KL, et al. Short-term consumption of sucralose, a nonnutritive sweetener, is similar to water with regard to select markers of hunger signaling and short-term glucose homeostasis in women. Nutr Res 2011 Dec;31(12):882-888.

26. Ma J, Chang J, Checklin HL, et al. Effect of the artificial sweetener, sucralose, on small intestinal glucose absorption in healthy human subjects. The British journal of nutrition 2010 Sep;104(6):803-806.

27. Cahill F, Shea JL, Randell E, et al. Serum peptide YY in response to short-term overfeeding in young men. Am J Clin Nutr 2011 Apr;93(4):741-747.

28. Dotson CD, Zhang L, Xu H, et al. Bitter taste receptors influence glucose homeostasis. PLoS One 2008;3(12):e3974.

29. Fushan AA, Simons CT, Slack JP, et al. Allelic polymorphism within the TAS1R3 promoter is associated with human taste sensitivity to sucrose. Curr Biol 2009 Aug 11;19(15):1288-1293.

30. Sclafani A. Enhanced sucrose and Polycose preference in sweet "sensitive" (C57BL/6J) and "subsensitive" (129P3/J) mice after experience with these saccharides. Physiol Behav 2006 Apr 15;87(4):745-756.

31. Bueter M, Miras AD, Chichger H, et al. Alterations of sucrose preference after Roux-en-Y gastric bypass. Physiol Behav 2011 Oct 24;104(5):709-721.

32. Burge JC, Schaumburg JZ, Choban PS, et al. Changes in patients' taste acuity after Roux-en-Y gastric bypass for clinically severe obesity. J Am Diet Assoc 1995 Jun;95(6):666-670.

33. Lanfer A, Knof K, Barba G, et al. Taste preferences in association with dietary habits and weight status in European children: results from the IDEFICS study. Int J Obes (Lond) 2012 Jan;36(1):27-34.

34. Miras AD, Jackson RN, Jackson SN, et al. Gastric bypass surgery for obesity decreases the reward value of a sweet-fat stimulus as assessed in a progressive ratio task. Am J Clin Nutr 2012 Sep;96(3):467-473.

35. Sartor F, Donaldson LF, Markland DA, et al. Taste perception and implicit attitude toward sweet related to body mass index and soft drink supplementation. Appetite 2011 Aug;57(1):237-246.

36. Macdiarmid JI, Vail A, Cade JE, et al. The sugar-fat relationship revisited: differences in consumption between men and women of varying BMI. Int J Obes Relat Metab Disord 1998 Nov;22(11):1053-1061.

37. Anton SD, Martin CK, Han H, et al. Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. Appetite 2010 Aug;55(1):37-43.

CHAPTER 7: SUMMARY, CONCLUSIONS, AND PUBLIC HEALTH IMPLICATIONS

Key findings

The main objectives of the projects presented in this dissertation were to expand our knowledge about the consumption of NNS and their metabolic effects. Though once thought to be metabolically inert, NNS are indeed metabolically active in rodents. Whether this can be confirmed in humans, however, remains unclear. Translating physiologic mechanisms revealed in rodents into the context of human behavior is challenging due to a myriad of psychosocial and intellectual elements which complicate the study of human behavior. Thus, studying consumption trends, perceptions, and metabolic effects of NNS required a multi-disciplinary approach to account for the contribution of societal influence in translating basic and clinical science to better human health.

Our analysis of trends and sources of NNS over the past decade is the first to show a step-wise increase in the prevalence of NNS consumption in the general population. We found that the consumption of NNS has increased dramatically over the last decade predominantly in beverage form, among both children and adults. Our observation that the proportion of children using NNS in the United States nearly doubled was particularly noteworthy as this may represent a shift in food manufacturing practices as a result of scrutiny of the calorie content of popular soft drinks. Upon looking further into the contribution of beverages to the rising prevalence of consumption, we found that it is the reduced-calorie drinks, such as diet lemonades and light juices that have

increased the most, while the consumption of non-caloric diet sodas and iced teas has not changed. This is particularly important because calories contained in beverages are not adequately recognized, and people therefore fail to subsequently adjust their intake in response to liquid calories, which may promote sustained positive energy balance [1].

Despite marked increases in consumption, conclusive information about safe levels of NNS consumption among children is lacking, as children may be introduced to NNS at any early age, after which exposure may continue throughout their lifetime. As such, dietary guidance is limited and contradictory. Given the lack of conclusive guidance in light of a doubling in the prevalence of child consumption of NNS, our next step was to assess how parents view their use among children.

Parents expressed overwhelmingly negative attitudes toward use of NNS by children. Parents did not believe that foods and beverages with NNS were a good substitute for added sugars, and most did not think that NNS were safe to provide to their children. However, parental ability to recognize commercially available foods and beverages that contained NNS was very low, suggesting that parents might provide their children with NNS inadvertently. Meanwhile, the negative attitudes of parents toward NNS use among children are not based on any conclusive scientific evidence, which supports the need for data documenting the benefits or harms of NNS.

Thus, we conducted a study to assess the effects of sucralose on glycemia, glucose kinetics, gut hormones, gastric emptying, and satiety, and found sucralose to have no- or neutral-effects compared to plain unsweetened water. Provision of sucralose in isolation prior to an oral glucose load also did not affect the subjective hunger or satiety ratings of our participants. Thus, it appears that the sweet taste sensation and binding of sucralose to

gut sweet taste receptors is not sufficient to effect gut hormone secretion *in vivo*. Our clinical study analyzing the acute metabolic effects of sucralose should be seen as a starting point in the investigation of a single NNS and supports the need for prospective, controlled, prolonged exposure trials to determine the relationship between NNS consumption and health.

Taken together, we have demonstrated overwhelming distrust of NNS among parents, which is incongruent with both the marked increases in NNS consumption observed, and with the lack of experimental evidence supporting short-term adverse effects of sucralose. The discrepancy between consumer behavior, consumer attitudes, and objective experimental evidence uncovered by this body of research further supports the need for effective communication and transparency between academia, industry, and the consumer and also exemplifies the need for widespread reliable nutrition education to allow consumers to make informed decisions about their dietary choices.

Strengths and limitations

Strengths of our studies include the use of both primary and secondary data that enhanced our ability to generalize our findings. The secondary data was efficient and cost-effective in providing national level consumption estimates, while the primary data collection allowed for well-controlled data collection and internal validity. The utilization of clinical, epidemiologic, and socio-behavioral methods allowed us to gain a unique insight into the possible role of NNS in weight management.

The analysis of ten years of NHANES data was particularly worthwhile, as this allowed us to analyze dietary intake from over 26,000 adults and over 16,000 children, as well as

analyze different sources of NNS, and conduct subgroup analyses assessing NNS consumption by age, weight status, income level, race/ethnicity, and gender. In the clinical aspects, the use of a same-subject, randomized, crossover design eliminated between-subjects variability. The use of escalating doses of sucralose in isolation provided the opportunity to evaluate the effects of varying concentrations and eliminate confounding results by other ingredients contained in the diet soda. The use of plain water as the comparison group, rather than a sugar-sweetened beverage, was also beneficial in our clinical trial, as the dramatic increases in post-prandial glucose and insulin concentrations following the consumption of caloric sugars have been well demonstrated[2]. Finally, in the questionnaire based studies, we were able to recruit a relatively large number of parents with a wide range of racial/ethnic backgrounds and highly variable levels of education. Particularly in the grocery store based survey study, participants were assessed in a real-life situation, and the findings are thus more representative of a "free-living" population.

One overarching limitation of our research was the use of self-reported dietary intake data that was likely subject to recall bias and interviewer bias. For example, participants may not remember consuming a certain food or beverage that was sweetened or may not be aware of the contents of a food or beverage consumed. Thus, it is likely that we underestimated the true levels of NNS consumption when analyzing the current trends. In the NHANES and questionnaire data, social desirability bias also may have impacted our findings, as participants may have responded in accordance with what they perceived to be "healthier." Our questionnaire-based studies also surveyed a convenience

sample and used non-validated survey instruments to assess parental attitudes toward child consumption of NNS. However, our questionnaire design was novel as information about the perception and recognition of NNS in commercial foods and beverages was previously lacking. Failure to standardize the diet and physical activity of our participants during our clinical study assessing sucralose may also have biased our results, while the use of a single NNS (sucralose) limits our ability to generalize to other NNS found in commercially-available foods and beverages.

Implications of study findings

The results of our studies underscore the importance of conducting long-term, randomized, well-controlled trials in free-living populations in order to determine if NNS have a role in promoting metabolic health and weight management. Given that NNS consumption is increasing, conclusive recommendations for their consumption must be formulated based on reliable scientific evidence. In light of discussions to implement taxation of sugar-sweetened beverages, and to remove sugar-sweetened beverages in school cafeterias and vending machines to reduce their consumption, the safety and acceptance of NNS are of paramount importance from a policy standpoint. Both of these measures targeting reduction in sugar-sweetened beverages are expected to further promote consumption of beverages sweetened with NNS.

Whether or not NNS demonstrate promise in aiding a sustained reduction in energy intake, the observed parental distrust of NNS is in contrast to the recent increases in the availability and consumption of the foods and beverages containing them. Furthermore, the disconnect between negative parental perceptions of NNS and low parental ability to identify the foods and beverages that contain them, further emphasize

the need for data supporting or dismissing the long term safety of NNS use in children. If NNS are to be recommended as a substitute for added sugars, our data suggests that parents will need to be convinced of their safety in children through widespread nutrition education. These findings also highlight the necessity of changing food labeling regulations to facilitate consumers in making informed food and beverage choices.

The current experimental evidence suggests that NNS will reduce total energy intake if used a substitute for added sugars, yet it is unclear whether or not they will be used in this manner. If NNS are used in addition to added sugars rather than as a replacement for added sugars, and thus lead to increases in food and beverage consumption, NNS may encourage positive energy balance and foster weight gain. Research investigating the determinants of sweet taste perception and preference has demonstrated that the addition of sugar-sweetened beverages to the diet leads to an increased preference for sweet taste among individuals with low sweet taste preference [3]. Given the highly sweet nature of NNS, it is likely that supplementation of the diet with NNS may lead to an increase in the liking of sweet-tasting foods and beverages, which are often also high in calories.

In addition to promoting liking and consumption of sweet items, sweet taste perception may be altered as well, requiring a higher concentration of the sweetener to perceive the sweet taste as palatable[3]. This represents a vicious cycle where consumption of sweet foods and beverages (with caloric sugars or non-nutritive sweeteners) leads to greater preference for sweet taste, further encouraging one to seek more intensely sweet substances, and therefore likely modifying eating behavior and increasing caloric intake. Thus, in the context of our findings, indirect effects of NNS

consumption must be considered, regardless of whether long-term consumption of NNS exerts direct effects on metabolic health.

Future directions

Several recent reviews have been published summarizing epidemiologic and interventional studies evaluating NNS consumption among children and adults [4-7]. Though these reviews were variable in scope, the main conclusions were that NNS do not directly promote increases in food intake or weight gain in the short-term, while the longer-term and indirect effects of consuming NNS are unclear. While short-term feeding studies and cross-sectional studies are relevant for hypothesis generation, well controlled, long term trials assessing NNS consumption are imperative to elucidate clinically significant effects of NNS on weight management.

In particular, conducting well-designed studies to answer the following questions are necessary in order to understand the more permanent effects of prolonged consumption of NNS on health:

1. Does repeated consumption of NNS lead to increases in sweet taste perception and preference, resulting in subsequent changes in food and beverage consumption?
2. Do the effects of NNS on food preferences and dietary intake differ based on the age at which NNS are introduced, the form (i.e. liquid vs. solid), the source (i.e.whether consumed with calories in a food, with calories in a beverage, or in isolation), and the combination (with caloric sweeteners, with other NNS, or in isolation) of the NNS?

3. Does the consumption of NNS over time lead to changes in the gut microbiome, affecting energy balance, body weight, and inflammation?
4. What are the best strategies to incorporate NNS in interventions targeting the reduction of added sugar consumption and how will this best be communicated to the public for implementation?

Literature Cited

1. DiMeglio DP, Mattes RD. Liquid versus solid carbohydrate: effects on food intake and body weight. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 2000 Jun;24(6):794-800.

2. Raben A, Moller BK, Flint A, et al. Increased postprandial glycaemia, insulinemia, and lipidemia after 10 weeks' sucrose-rich diet compared to an artificially sweetened diet: a randomised controlled trial. Food Nutr Res 2011;55.

3. Sartor F, Donaldson LF, Markland DA, et al. Taste perception and implicit attitude toward sweet related to body mass index and soft drink supplementation. Appetite 2011 Aug;57(1):237-246.

4. Brown RJ, de Banate MA, Rother KI. Artificial sweeteners: a systematic review of metabolic effects in youth. Int J Pediatr Obes 2010 Aug;5(4):305-312.

5. Sylvetsky A, Rother KI, Brown R. Artificial sweetener use among children: epidemiology, recommendations, metabolic outcomes, and future directions. Pediatr Clin North Am 2011 Dec;58(6):1467-1480.

6. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. The American journal of clinical nutrition 2009 Jan;89(1):1-14.

7. Benton D. Can artificial sweeteners help control body weight and prevent obesity? Nutr Res Rev 2005 Jun;18(1):63-76.

APPENDIX A: ARTIFICIAL SWEETENER USE AMONG CHILDREN: EPIDEMIOLOGY, RECOMMENDATIONS, METABOLIC OUTCOMES, AND FUTURE DIRECTIONS

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Allison Sylvetsky: Nutrition and Health Science Program, Graduate Division of Biological and Biomedical Sciences, Laney Graduate School, Emory University & Section on Pediatric Diabetes & Metabolism, National Institutes of Health, National Institute of Diabetes, Digestive, and Kidney Diseases

Kristina Rother, MD, MHSc: Section on Pediatric Diabetes & Metabolism, National Institutes of Health, National Institute of Diabetes, Digestive, and Kidney Diseases

Rebecca Brown, MD, MHSc: Section on Pediatric Diabetes & Metabolism, National Institutes of Health, National Institute of Diabetes, Digestive, and Kidney Diseases

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Keywords: artificial sweeteners, non-nutritive sweeteners, non-caloric sweeteners, low-calorie sweeteners, sugar substitutes, obesity, overweight

Synopsis: This review summarizes the existing literature pertaining to the epidemiology and current recommendations for pediatric artificial sweetener use and presents the results of studies investigating metabolic responses to artificial sweeteners among children. Observational and interventional studies testing the effects of artificial sweeteners on body weight, short-term satiety, glycemia, and glucoregulatory hormones are described. In addition, this review touches on the growing body of literature about taste, craving, and addiction to sweet taste. Gaining an understanding of the research previously conducted and the gaps that remain will inform future clinical and translational research, in order to develop evidence-based recommendations for artificial sweetener use in the prevention and treatment of pediatric obesity.

Introduction: Artificial sweeteners and obesity

Childhood obesity is associated with numerous unfavorable consequences including type 2 diabetes, non-alcoholic fatty liver disease, hypertension and psychosocial problems, and often results in obesity during adulthood[1]. Consumption of added sugars is positively associated with higher energy intakes, and is thought to be a significant contributor to the rapid rise in obesity worldwide[2]. Since the majority of added sugars are obtained from consumption of soft drinks [3], artificially sweetened beverages have emerged as an alternative, providing the desired sweetness and palatability without contributing to caloric intake[4]. In addition to their use in "diet" and "light" beverages, artificial sweeteners are often used to replace added sugars in various foods, including yogurts, puddings, baked goods, and ice cream, among many other items frequently consumed by children and adolescents[5]. Despite their widespread and increasing use[6], the effects of artificial sweeteners in children have not been well studied.

The purpose of this review is to summarize the existing literature pertaining to the epidemiology and current recommendations for artificial sweetener use in children, and to present the results of studies investigating metabolic responses to artificial sweeteners among children. In addition, this review will touch on the growing body of literature about taste, craving, and addiction to sweet taste. Artificial sweeteners have also been studied in relation to dental cavities, fetal outcomes, and carcinogenesis, but these issues will not be addressed in this review. In presenting and analyzing the current scientific evidence on the metabolic safety of artificial sweeteners and their potential effectiveness in promoting weight loss and weight management, this review aims to

provide clinicians with a comprehensive understanding of current knowledge about artificial sweetener usage in children.

Regulatory status of artificial sweeteners
FDA, ADI, EDI, aspartame, acesulfame-potassium, sucralose, stevia, neotame, saccharin

There are currently five artificial sweeteners approved by the Food and Drug Administration (FDA) for use in the United States (Table 1). These include aspartame, acesulfame-potassium, saccharin, sucralose, and neotame[7]. In addition, stevia, a natural sweetener made from extracts of the intensely sweet S. Rebaudiana (Bertoni) plant has been approved for limited use[8]. For each sweetener, the FDA establishes an Acceptable Daily Intake (ADI)[9], in mg per kg body weight, which is the amount of sweetener thought to be safe to consume every day for a lifetime. The ADI is typically 100 times lower than the dose of the sweetener that caused toxicity in animal studies. To determine if a sweetener should be approved for use, the FDA then must establish that typical human intake of the sweetener (Estimated Daily Intake, or EDI) will be below the ADI. If the estimated daily intake (EDI) is below the ADI, then the sweetener is considered safe for human use. Aspartame, saccharin, sucralose, and neotame are classified as food additives by the FDA, while stevia is classified as Generally Recognized as Safe (GRAS), meaning that similar data consistent with its safety exist as for food additives.

Key points:

- There are five artificial sweeteners currently approved for use in the United States as well as stevia, a natural non-caloric sweetener

- For each sweetener, the FDA establishes an acceptable daily intake (ADI) which is the amount of sweetener thought to be safe to consume every day for a lifetime.

Artificial sweetener consumption among children
Sweetness, intake, and consumption

Apparent consumption of artificial sweeteners (based on servings of foods and beverages containing these sweeteners) has increased with time across all age groups[10]. Because the FDA does not require manufacturers to report the actual amounts of sweeteners contained in foods and beverages[11]," quantification of the precise amount of sweeteners present in the food is difficult. Hence, information about the total quantity of sweeteners in use is extracted from intake information for the various foods that contain them, using food composition tables and validated food databases[12]. It is important to understand that the sweetening power of the artificial sweeteners listed above is hundreds of times greater than that of sucrose (Table 1)[13]. Therefore,, it takes a much smaller amount of an artificial sweetener relative to caloric sugars to produce the same level of sweetness in a product.

Due to their smaller size and relatively high intake of beverages, children consume the highest amount of artificial sweeteners relative to their body weight per day[5]. A recent systematic review estimated that between 4 and 18% of total carbonated beverage intake among children is from artificially sweetened beverages[14]. A second review determined that approximately 15% of the total United States population above the age of 2 years use artificial sweeteners[10]. A third study, comparing NHANES data from 1999-2000 to 2007-2008[15] and a recent study (unpublished data, courtesy of Jean Welsh, PhD) showed that consumption of artificially sweetened beverages has increased

in the general population, and has doubled among children over this time period. Artificial sweetener consumption in foods has increased to a greater extent than in beverages. Furthermore, of those who already consume artificially sweetened products, the amount of these products being consumed has increased[10]. Given the extent to which consumption of artificially sweetened products is rising , it is important that more intervention studies testing the effects are conducted, in order to develop evidence-based recommendations for artificial sweetener usage in the prevention and treatment of childhood obesity.

Key points:

- Artificial sweetener consumption is increasing in all age groups, particularly in children
- Because the FDA does not require manufacturers to report the actual amounts of sweeteners contained in foods and beverages[11], quantification of the precise amount of sweeteners in food is difficult

Current recommendations:

Guidelines, American Diabetes Association (ADA), American Dietetic Association, American Academy of Pediatrics (AAP), Institute of Medicine (IOM),

There are few explicit recommendations regarding consumption of artificially sweetened foods and beverages in children; however, the American Dietetic Association (ADA) states that both nutritive and artificial sweeteners may comprise part a of a diet that follows the Dietary Guidelines for Americans[5]. Specifically, a position statement from the ADA stated that artificial sweeteners can allow consumers to enjoy sweetness

while continuing to manage weight, diabetes, and other chronic illnesses. With regard to children specifically, the ADA stated that artificial sweeteners are safe to use within the range of the acceptable daily intake (ADI), which varies for each of the five FDA approved artificial sweeteners. Current intake levels of artificial sweeteners among children are believed to be well below the ADI, but range from around 10% of the ADI for current levels of aspartame consumption to as high as 60% of the ADI for acesulfame-potassium [5]. In contrast, the Institute of Medicine (IOM) does not support artificial sweetener use in children because artificially sweetened beverages have been shown to displace milk and 100% juice at mealtimes. In addition, the IOM stated that more research is needed on the effectiveness of artificial sweeteners for weight management and that more studies are needed on safety effects when artificial sweeteners are consumed over many years starting in childhood or adolescence. Similarly, the American Academy for Pediatrics stated that artificial sweeteners have been inadequately studied for use in children and that they should not form a significant part of a child's diet. Other medical societies have stated their positions on the use of artificial sweeteners, which are outlined in **Table 2** (below). However, these statements are not sweetener specific and many do not make recommendations for the use of these sweeteners in a pediatric population.

Key points:

- There are few explicit recommendations for artificial sweetener consumption in children
- Recommendations from medical societies are conflicting

Artificial Sweeteners and the control of body weight:

Calories, compensation, energy, BMI, weight

Although artificial sweeteners do not contribute significantly to energy intake, their effectiveness in promoting weight loss and weight control has been questioned [16], To date, eight observational studies have explored the relationship between consumption of artificial sweeteners and weight in children[17-22]. Of the three cross-sectional studies, including between 385 and 3311 children, the two conducted in school-age and adolescent children showed positive associations between artificial sweetener consumption and BMI[19, 21], while the one in 2-5 year olds did not find an association[20]. Similarly, four of the five longitudinal cohort studies, including between 166 and 11654 children, showed positive associations between artificially sweetened beverage consumption and weight related outcomes including BMI change (in boys, but not girls) [23], BMI z-score[17], energy intake (but not BMI)[24], and fat mass (no longer significant after adjustment for covariates)[25]. A single study showed no association between artificially sweetened beverage intake and BMI, but an inverse correlation with incident obesity, meaning that children who consumed more artificially sweetened beverages were less likely to become obese[26]. Given the observational nature of the above mentioned studies, these data cannot establish that consumption of artificially sweetened beverages was the cause of increased body weight or food intake. There are likely to be many differences, both genetic and cultural, between families that do versus do not offer their children artificially sweetened beverages. Children consuming artificial sweeteners may be those who are at risk of weight gain, thus reversing the direction of the causal relationship.

One proposed explanation for the association between artificial sweetener consumption and weight gain in epidemiologic studies is that knowledge of consuming a substance lower in energy could drive people to eat more [10]; this phenomenon has been best described in the context of low-fat foods, in which people overeat foods after receiving a food labeled as low-fat [27]. In addition, studies in animals (who have no cognitive awareness of the energy content of foods) have shown that the disconnect between sweetness and caloric content from use of artificial sweeteners may impair energy regulation and lead to positive energy balance [28, 29]. It has also been suggested that the observed paradoxical relationship between artificial sweetener intake and body weight may be due to alteration of gut microbiota [30]. Although these hypotheses are intriguing, few data exist to support them, especially in children, and future human studies are greatly needed.

While it is expected that substituting artificially sweetened beverages in place of sugar-sweetened beverages would lead to weight loss due the lower caloric intake, experimental studies have shown that the assumed calorie deficit is not maintained[31-34]. One reason for this is that people tend to compensate for the "missing calories" in an artificially sweetened food or drink by subsequently eating more. Compensation involves the ability to account for excess calorie consumption by reducing intake later, or in the case of an artificially sweetened beverage, to account for the "missing calories," by subsequently consuming more. Seven studies have evaluated how children compensate for changes in calorie density due to use of caloric versus artificial sweeteners. These studies involved between 14 and 262 participants, ages two to 14 years[32, 35-40]. The results of these studies are complex, and vary significantly based on study design. In

general, younger children seemed to compensate better for missing calories in artificially sweetened foods and drinks by increasing subsequent food intake, thus raising questions about the efficacy of these products for weight control in young children. It is important to realize, however, that this study design only provides insight into effects that occur within hours, while changes in body weight occur over much longer time frames[41]. In addition, these studies generally take place in laboratory settings and it may not be accurate to generalize their findings to 'real life'. It is therefore of great interest to evaluate changes in food intake and body weight that occur with chronic consumption of artificial sweeteners, over the course of weeks, months, or years.

There are very few randomized controlled trials evaluating the effects of artificial sweeteners on weight change in children. One study randomized 103 adolescents of varying BMI to either consume only non-caloric beverages (including both water and artificially sweetened beverages) or to maintain their normal beverage consumption habits. At the end of the 25-week intervention, no difference in BMI was found between groups; however, a post hoc subgroup analysis including only overweight participants did show lower BMI in the treatment group[42]. However, the effect of increased water versus artificially sweetened beverages cannot be determined. A confirmatory study enrolling only overweight adolescents is currently in progress[43]. A second trial randomized overweight adolescent girls to a restricted 1500 kcal per day diet that either permitted sugar-sweetened soda (within the 1500 kcal limit), or permitted only water or artificially sweetened beverages[44]. Both diets led to a modest amount of weight loss, but there were no significant differences between groups in this small pilot study. In a third study designed to prevent excess weight gain, children in the intervention group

were assigned to replace sugar with artificial sweeteners and increase physical activity. As in other studies, the primary outcome of change in BMI z-score was not different between groups, but fewer children in the intervention group increased their BMI z-score. However, the effect of the artificial sweetener and the physical activity intervention cannot be separated[45]. Finally, a study conducted shortly after the approval of aspartame randomized 55 children and young adults to consume an aspartame capsule three times daily or a placebo while on a calorie restricted diet. No significant difference in weight loss was observed at the end of the 13 week intervention[46].

Key points:

- The majority of observational studies show a positive association between artificial sweetener consumption and body weight.
- The results of short-term satiety studies are complex and vary significantly based on study design. In general, younger children seem to compensate better for lower calories in artificially sweetened drinks by increasing subsequent food intake.
- Unlike observational studies, randomized controlled trials of artificial sweeteners in children have not shown that artificial sweeteners cause weight gain. However, the current studies are not sufficient to show that these sweeteners aid in weight loss, either.

Effects artificial sweeteners on glycemia and glucoregulatory hormones

Glucose, insulin, glucagon-like peptide 1, gastric inhibitory peptide, GLUT2, metabolism

Because artificial sweeteners are frequently recommended for use by patients with diabetes, it is critical to understand their effects on glycemia. Early studies in adults with diabetes did not show acute or chronic effects artificial sweeteners on blood glucose or insulin levels[47, 48]. However, this topic has recently been readdressed as a result of new evidence that artificial sweeteners may be biologically active in the gastrointestinal tract, via binding to sweet taste receptors located on enteroendocrine L-cells [49, 50]. The biological relevance of intestinal sweet taste receptors in gut hormone secretion in humans has been nicely demonstrated in two recent studies. In both experiments, blockade of these receptors using the sweet taste antagonist lactisole reduced glucose-stimulated secretion of glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), both of which are made by L-cells. The effects of artificial sweeteners binding to intestinal sweet taste receptors are still being elucidated. *In vitro* studies demonstrated that artificial sweetener binding to intestinal sweet taste receptors increased secretion of the incretin hormones GLP-1 and gastric inhibitory peptide(GIP) , and, in rodents *in vivo*, increased the rate of intestinal glucose absorption by upregulating the apical glucose transporter, GLUT-2[51]. The relevance of these findings in humans is under active investigation, and this review will focus on studies conducted in humans.

In contrast to in vitro data, human studies do not support an effect of artificial sweeteners in isolation on gut hormone secretion. When artificial sweeteners were delivered in 240 mL solutions by intragastric infusion to adults, none had an effect on ghrelin, PYY, glucose, GLP-1 or insulin[52]. In a similar study, no changes in insulin, glucose, GLP-1, or gastric emptying were observed following intragastric infusions of sucralose solutions[53] A third study provided adults with equi-sweet solutions of

glucose, fructose, saccharin, or aspartame via intra-gastric infusion[54]. The artificial sweeteners alone did not slow gastric emptying to a greater extent than water, though the caloric glucose and fructose solutions did. In another study, eight healthy adults orally ingested 50 mL of sucralose solution versus water, and no differences in PYY, insulin, or GLP-1 were observed[55]. Finally, when aspartame was provided in a tablet form, no insulin or glucose response was observed[56].

A recent study conducted by Brown et al suggests that artificial sweeteners might affect gut hormone secretion when given in combination with caloric sugars. In this study, healthy adolescents and young adults drank 240 milliliters of diet soda, containing acesulfame potassium and sucralose prior to a 75g glucose load[57]. No significant changes in glucose or insulin were observed, but the diet soda led to a higher GLP-1 response when compared to carbonated water. This study suggests that artificial sweeteners do not affect glucoregulatory hormones when delivered alone, but might have an effect when administered in conjunction with an energy containing food item. In contrast, however, in a human study where intra-duodenal infusion of glucose was accompanied by an intra-duodenal infusion of sucralose or a saline control, sucralose had no effect on either GLP-1 secretion, or the absorption of glucose from the small intestine[58].

The conflicting data from available studies might be related to differences in sweetener dose, content (acesulfame-K plus sucralose, versus sucralose alone), mode of delivery (oral versus intraduodenal, or infusion rates. Artificial sweeteners each have a different chemical structure and may affect metabolic response differently. Similarly, the physiologic response to a sweetener ingested orally may be different from a sweetener

infused intra-gastrically, due to interactions with taste and reward pathways and with cephalic phase responses, in which small insulin responses are observed in response to gustatory stimulation, prior to the absorption of nutrients[59]. Cephalic phase insulin response has been observed before swallowing non-sweet nutritive substances and artificially sweetened energy containing substances in humans. However, sweet non-caloric stimuli alone have not been sufficient to generate an expectatory, cephalic phase response in humans[59]. Further studies are needed to determine whether artificial sweeteners can reliably elicit a gut hormone response in humans.

Key points:

- Human studies do not support an effect of artificial sweeteners in isolation on gut hormone secretion
- Recent studies suggest that artificial sweeteners might affect gut hormone secretion when given in combination with caloric sugars

Artificial sweeteners and their potential effects on taste, reward, and addiction pathways

Addiction, dependence, taste, reward, craving, dopamine, opioids

In an effort to further understand and explain the etiology behind the rising epidemic of obesity, a new research area exploring potentially addictive properties of sugar has emerged. The concept of addiction is hard to define, but is commonly characterized by compulsive and uncontrollable behaviors that are driven by cravings. Though most addiction research examines more common drugs of abuse, such as alcohol, cocaine, morphine, and nicotine, various studies have drawn parallels between drug

seeking behavior and food seeking behavior. This has led some to believe that sugar and other sweet substances could become physiologically addictive [60].

Both feeding patterns and drug use involve learned habits, intense reinforcement, and reward pathways, which persist despite the likelihood of negative consequences.[61] The neurobiological pathways that underlie drug addiction and proposed sugar addiction share the same neurotransmitters, the same receptors, and activate many of the same brain regions [62]. Interestingly, a recent study in children demonstrated that familial alcoholism and depressive symptoms were associated with a preference for more concentrated sucrose solutions and a greater liking of sweet foods[63]. Specifically, sugar has been shown to cause release of endogenous opioids, endorphins, and dopamine from the brain in an analogous manner to addictive drugs [64]. Furthermore, artificially sweetened solutions have, like sugar, been shown to be effective for pain reduction in infants, providing solid evidence that perception of sweet taste alters central responses[65]. In fact, in rats, gene expression for dopamine receptors and opioids is altered in sugar-dependent rats in a similar manner to morphine-dependent rats[66]. Sugar dependence, defined by indices of bingeing, withdrawal, and increased intake after deprivation arises when rats are maintained on a schedule of intermittent access to a sugar and chow which leads to behavioral and neurological changes[64, 66]. However, while animals can be conditioned to follow particular eating patterns, which may evoke a drug-like response, this cannot be replicated in humans, and the observed reaction may result from the specific feeding pattern rather than a physical addiction[67].

Limited data from humans and animal models suggest that some, but not all, effects of caloric sugars on brain reward systems are recapitulated by artificial

sweeteners. Although sweet-taste from either caloric or artificial sweeteners produce activation of dopaminergic reward systems in wild-type mice, rodents with an inability to sense sweet-taste only increase dopamine in response to caloric sugars, not artificial sweeteners[68]. In addition, in humans, drinking a caloricly versus artificially sweetened beverage led to greater activation of the amygdala in functional MRI studies [69]. Both of these studies suggest that, although artificial sweeteners can stimulate reward pathways, the nutritive value of sweet foods and drinks plays a role in brain reward signaling, independent of their sweetness.

It has also been proposed that frequent exposure to highly sweet items alters food preferences, rather than promoting tolerance; hence, humans develop an expectation that foods and beverages ingested will be sweet and increase their intake of sweet items in accordingly[67] Recent animal data suggest that artificial sweeteners can be ingested during infancy through breastmilk and prenatally through amniotic fluid, exerting changes in sweetness preferences of exposed offspring[70]. Further supporting this view, experimental studies in young children have shown that early and repeated exposure to sweet taste can shape preferences for sugar-rich food items[71]. The idea of habituation to consume a palatable, low energy substance leading to inability to compensate for higher energy variants with similar flavor was tested by randomizing healthy adults to consume a yogurt drink of low or high energy, and then reversing the energy content after a 9 week habituation period[72]. Participants who switched from the low-energy yogurt drink to the identically flavored high-energy yogurt drink were unable to compensate for the additional calories and overate at the subsequent ad libitum meal. Meanwhile, those who were habituated to the high energy variant did not alter their energy intake when

they were provided with the lower-energy yogurt[72]. Similar findings were observed in three to five year old children habituated to aspartame-sweetened versus maltodextrin-sweetened pudding [38]. This inability for both children and adults to adequately alter their energy intake after repeated experience with a specific pairing of flavor and calories supports the idea that regular consumption of artificial sweeteners which provide sweetness without calories might lead to overconsumption when presented with a sweet energy-containing food or beverage. Key points:

- Sugar has been shown to cause release of endogenous opioids, endorphins, and dopamine from the brain in an analogous manner to addictive drugs.
- More research is needed to further examine if consumption of artificial sweeteners can evoke similar brain responses that may lead to increased craving for sweet taste.

Conclusion:

This review aims to provide clinicians with current and comprehensive information regarding the effects of artificial sweeteners on food intake, body weight, glycemic control, and sweet liking, craving, and addiction in children. Understanding and critically evaluating past research will assist clinicians in making informed recommendations for use of artificial sweeteners as a means of combating pediatric obesity. Taking into consideration the evidence that exists, we can cautiously conclude that there are no benefits of artificial sweetener use in young children, though it is possible that consumption of artificial sweeteners may be beneficial in limiting weight gain in overweight adolescents. In order to recommend consuming or avoiding artificially sweetened products as a weight control strategy, more studies evaluating the effect of

artificially sweeteners on hormonal and metabolic response and on sweet craving must be conducted in children. It is also imperative that longer term studies be carried out in children, as metabolic and behavioral alterations that occur in response to artificial sweeteners introduced and conditioned during childhood may accumulate throughout adolescence and adulthood. Continued research about the various mechanisms that underlie energy compensation, satiety, sweet craving, food intake, and weight control will contribute to the growing body of literature examining the role of artificial sweeteners in combating childhood obesity.

Literature cited

1. FM Biro MW. Childhood obesity and adult morbidites. American Journal of Clinical Nutrition 2010;91:1499S-1505S.

2. Drewnowski A, Bellisle F. Liquid calories, sugar, and body weight. Am J Clin Nutr 2007 Mar;85(3):651-661.

3. Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. The American journal of clinical nutrition 2006 Aug;84(2):274-288.

4. Blackburn GL, Kanders BS, Lavin PT, et al. The effect of aspartame as part of a multidisciplinary weight-control program on short- and long-term control of body weight. The American journal of clinical nutrition 1997 Feb;65(2):409-418.

5. Association AD. Position of the American Dietetic Association: Use of Nutritive and Nonnutritive Sweeteners. Journal of the American Dietetic Association 2004;104:255-275.

6. Fowler SP, Williams K, Resendez RG, et al. Fueling the obesity epidemic? Artificially sweetened beverage use and long-term weight gain. Obesity (Silver Spring) 2008 Aug;16(8):1894-1900.

7. Artificial sweeteners: no calories...sweet! FDA Consum 2006 Jul-Aug;40(4):27-28.

8. Gardana C, Scaglianti M, Simonetti P. Evaluation of steviol and its glycosides in Stevia rebaudiana leaves and commercial sweetener by ultra-high-performance liquid chromatography-mass spectrometry. J Chromatogr A 2010 Feb 26;1217(9):1463-1470.

9. Adminstration USFaD. **Guidance for Industry and Other Stakeholders Toxicological Principles for the Safety Assessment of Food Ingredients**. In: Services DoHaH, ed., July 2000, Revised July 2007.

10. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. The American journal of clinical nutrition 2009 Jan;89(1):1-14.

11. Administration IFICaUSFaD. Food Ingredients and Colors. Washington, D.C., November 2004, Revised April 2010.

12. Magnuson BA, Burdock GA, Doull J, et al. Aspartame: a safety evaluation based on current use levels, regulations, and toxicological and epidemiological studies. Crit Rev Toxicol 2007;37(8):629-727.

13. Renwick AG, Molinary SV. Sweet-taste receptors, low-energy sweeteners, glucose absorption and insulin release. Br J Nutr 2010 Nov;104(10):1415-1420.

14. Brown RJ, de Banate MA, Rother KI. Artificial sweeteners: a systematic review of metabolic effects in youth. Int J Pediatr Obes 2010 Aug;5(4):305-312.

15. Welsh JA, Sharma AJ, Grellinger L, et al. Consumption of added sugars is decreasing in the United States. Am J Clin Nutr 2011 Jul 13.

16. Blundell JH, AJ. Paradoxical effects of an intense sweetener (aspartame) on appetite. Lancet 1986(May 10th):1092-1093.

17. Blum JW, Jacobsen DJ, Donnelly JE. Beverage consumption patterns in elementary school aged children across a two-year period. J Am Coll Nutr 2005 Apr;24(2):93-98.

18. Ludwig DS, Peterson KE, Gortmaker SL. Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. Lancet 2001 Feb 17;357(9255):505-508.

19. Forshee RA, Storey ML. Total beverage consumption and beverage choices among children and adolescents. Int J Food Sci Nutr 2003 Jul;54(4):297-307.

20. O'Connor TM, Yang SJ, Nicklas TA. Beverage intake among preschool children and its effect on weight status. Pediatrics 2006 Oct;118(4):e1010-1018.

21. Giammattei J, Blix G, Marshak HH, et al. Television watching and soft drink consumption: associations with obesity in 11- to 13-year-old schoolchildren. Archives of pediatrics & adolescent medicine 2003 Sep;157(9):882-886.

22. Kral TV, Stunkard AJ, Berkowitz RI, et al. Beverage consumption patterns of children born at different risk of obesity. Obesity 2008 Aug;16(8):1802-1808.

23. Berkey CS, Rockett HR, Field AE, et al. Sugar-added beverages and adolescent weight change. Obes Res 2004 May;12(5):778-788.

24. Striegel-Moore RH, Thompson D, Affenito SG, et al. Correlates of beverage intake in adolescent girls: the National Heart, Lung, and Blood Institute Growth and Health Study. The Journal of pediatrics 2006 Feb;148(2):183-187.

25. Johnson L, Mander AP, Jones LR, et al. Is sugar-sweetened beverage consumption associated with increased fatness in children? Nutrition 2007 Jul-Aug;23(7-8):557-563.

26. Ludwig DS. Relation between consumption of sugar sweetened drinks and childhood obesity: a prospective, observational analysis. Lancet 2001 17 February;357(9255):505-508.

27. Shide DJ, Rolls BJ. Information about the fat content of preloads influences energy intake in healthy women. Journal of the American Dietetic Association 1995 Sep;95(9):993-998.

28. Swithers SE, Baker CR, Davidson TL. General and persistent effects of high-intensity sweeteners on body weight gain and caloric compensation in rats. Behav Neurosci 2009 Aug;123(4):772-780.

29. Swithers SE, Doerflinger A, Davidson TL. Consistent relationships between sensory properties of savory snack foods and calories influence food intake in rats. Int J Obes (Lond) 2006 Nov;30(11):1685-1692.

30. Pepino MY, Bourne C. Non-nutritive sweeteners, energy balance, and glucose homeostasis. Current opinion in clinical nutrition and metabolic care 2011 Jul;14(4):391-395.

31. Rogers PJ, Blundell JE. Separating the actions of sweetness and calories: effects of saccharin and carbohydrates on hunger and food intake in human subjects. Physiology & behavior 1989 Jun;45(6):1093-1099.

32. Birch LL, McPhee L, Sullivan S. Children's food intake following drinks sweetened with sucrose or aspartame: time course effects. Physiology & behavior 1989 Feb;45(2):387-395.

33. Kim JY, Kissileff HR. The effect of social setting on response to a preloading manipulation in non-obese women and men. Appetite 1996 Aug;27(1):25-40.

34. Drewnowski A, Massien C, Louis-Sylvestre J, et al. Comparing the effects of aspartame and sucrose on motivational ratings, taste preferences, and energy intakes in humans. Am J Clin Nutr 1994 Feb;59(2):338-345.

35. Anderson GH, Saravis S, Schacher R, et al. Aspartame: effect on lunch-time food intake, appetite and hedonic response in children. Appetite 1989 Oct;13(2):93-103.

36. Bellissimo N, Pencharz PB, Thomas SG, et al. Effect of television viewing at mealtime on food intake after a glucose preload in boys. Pediatric research 2007 Jun;61(6):745-749.

37. Bellissimo N, Thomas SG, Goode RC, et al. Effect of short-duration physical activity and ventilation threshold on subjective appetite and short-term energy intake in boys. Appetite 2007 Nov;49(3):644-651.

38. Birch LL, Deysher M. Conditioned and Unconditioned Caloric Compensation: Evidence for Self-Regulation of Food Intake in Young Children. Learn Motiv 1985;16:341-355.

39. Birch LL, Deysher M. Caloric compensation and sensory specific satiety: evidence for self regulation of food intake by young children. Appetite 1986 Dec;7(4):323-331.

40. Johnson SL, Taylor-Holloway LA. Non-Hispanic white and Hispanic elementary school children's self-regulation of energy intake. The American journal of clinical nutrition 2006 Jun;83(6):1276-1282.

41. Swinburn BA, Sacks G, Lo SK, et al. Estimating the changes in energy flux that characterize the rise in obesity prevalence. Am J Clin Nutr 2009 Jun;89(6):1723-1728.

42. Ebbeling CB, Feldman HA, Osganian SK, et al. Effects of decreasing sugar-sweetened beverage consumption on body weight in adolescents: a randomized, controlled pilot study. Pediatrics 2006 Mar;117(3):673-680.

43. Reducing Sugar-sweetened Beverage Consumption in Overweight Adolescents (BASH).

44. Williams CL, Strobino BA, Brotanek J. Weight control among obese adolescents: A pilot study. Int J Food Sci Nutr 2007;58(3):217-230.

45. Rodearmel SJ, Wyatt HR, Stroebele N, et al. Small changes in dietary sugar and physical activity as an approach to preventing excessive weight gain: the America on the Move family study. Pediatrics 2007 Oct;120(4):e869-879.

46. Knopp RH, Brandt K, Arky RA. Effects of aspartame in young persons during weight reduction. J Toxicol Environ Health 1976 Nov;2(2):417-428.

47. Grotz VL, Henry RR, McGill JB, et al. Lack of effect of sucralose on glucose homeostasis in subjects with type 2 diabetes. Journal of the American Dietetic Association 2003 Dec;103(12):1607-1612.

48. Shigeta H, Yoshida T, Nakai M, et al. Effects of aspartame on diabetic rats and diabetic patients. J Nutr Sci Vitaminol (Tokyo) 1985 Oct;31(5):533-540.

49. Margolskee RF, Dyer J, Kokrashvili Z, et al. T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. Proceedings of the National Academy of Sciences of the United States of America 2007 Sep 18;104(38):15075-15080.

50. Jang HJ, Kokrashvili Z, Theodorakis MJ, et al. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. Proceedings of the National Academy of Sciences of the United States of America 2007 Sep 18;104(38):15069-15074.

51. Mace OJ, Affleck J, Patel N, et al. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. J Physiol 2007 Jul 1;582(Pt 1):379-392.

52. Steinert RE, Frey F, Topfer A, et al. Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides. Br J Nutr 2011 May;105(9):1320-1328.

53. Ma J, Bellon M, Wishart JM, et al. Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects. Am J Physiol Gastrointest Liver Physiol 2009 Apr;296(4):G735-739.

54. Little TJ, Gupta N, Case RM, et al. Sweetness and bitterness taste of meals per se does not mediate gastric emptying in humans. Am J Physiol Regul Integr Comp Physiol 2009 Sep;297(3):R632-639.

55. Ford HE, Peters V, Martin NM, et al. Effects of oral ingestion of sucralose on gut hormone response and appetite in healthy normal-weight subjects. Eur J Clin Nutr 2011 Apr;65(4):508-513.

56. Abdallah L, Chabert M, Louis-Sylvestre J. Cephalic phase responses to sweet taste. Am J Clin Nutr 1997 Mar;65(3):737-743.

57. Brown RJ, Walter M, Rother KI. Ingestion of diet soda before a glucose load augments glucagon-like peptide-1 secretion. Diabetes care 2009 Dec;32(12):2184-2186.

58. Ma J, Chang J, Checklin HL, et al. Effect of the artificial sweetener, sucralose, on small intestinal glucose absorption in healthy human subjects. Br J Nutr 2010 Sep;104(6):803-806.

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

60. Avena NM, Rada P, Hoebel BG. Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. Neurosci Biobehav Rev 2008;32(1):20-39.

61. Volkow ND, Wise RA. How can drug addiction help us understand obesity? Nat Neurosci 2005 May;8(5):555-560.

62. Fortuna JL. Sweet preference, sugar addiction and the familial history of alcohol dependence: shared neural pathways and genes. J Psychoactive Drugs 2010 Jun;42(2):147-151.

63. Mennella JA, Pepino MY, Lehmann-Castor SM, et al. Sweet preferences and analgesia during childhood: effects of family history of alcoholism and depression. Addiction 2010 Apr;105(4):666-675.

64. Rada P, Avena NM, Hoebel BG. Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience 2005;134(3):737-744.

65. Bucher HU, Baumgartner R, Bucher N, et al. Artificial sweetener reduces nociceptive reaction in term newborn infants. Early Hum Dev 2000 Jul;59(1):51-60.

66. Spangler R, Wittkowski KM, Goddard NL, et al. Opiate-like effects of sugar on gene expression in reward areas of the rat brain. Brain Res Mol Brain Res 2004 May 19;124(2):134-142.

67. Benton D. The plausibility of sugar addiction and its role in obesity and eating disorders. Clin Nutr 2010 Jun;29(3):288-303.

68. de Araujo IE, Oliveira-Maia AJ, Sotnikova TD, et al. Food reward in the absence of taste receptor signaling. Neuron 2008 Mar 27;57(6):930-941.

69. Smeets PA, Weijzen P, de Graaf C, et al. Consumption of caloric and non-caloric versions of a soft drink differentially affects brain activation during tasting. NeuroImage 2011 Jan 15;54(2):1367-1374.

70. Zhang GH, Chen ML, Liu SS, et al. Effects of Mother's Dietary Exposure to Acesulfame-K in Pregnancy or Lactation on the Adult Offspring's Sweet Preference. Chemical senses 2011 Jun 7.

71. Liem DG, Mars M, De Graaf C. Sweet preferences and sugar consumption of 4- and 5-year-old children: role of parents. Appetite 2004 Dec;43(3):235-245.

72. Zandstra EH, Stubenitsky K, De Graaf C, et al. Effects of learned flavour cues on short-term regulation of food intake in a realistic setting. Physiol Behav 2002 Feb 1-15;75(1-2):83-90.

Table 1. FDA approved artificial sweeteners

Sweetener	**FDA Status**	**Acceptable Daily Intake (ADI)**	**Sweetness Relative to Sucrose**
Acesulfame Potassium	NNS, REG	15 mg/kg (~ 30 cans of diet soda)	200X
Aspartame	NUTRS, REG, GMP	50 mg/kg (~ 18 cans of diet soda)	160-220X
Neotame	NNS, REG, GMP	2 mg/kg	7,000-13,000X
Saccharin	NNS, REG/ITEM	5 mg/kg	300X
Stevia	GRAS	5 mg/kg	300X
Sucralose	NNS, REG, GMP	5 mg/kg (~ 6 cans of diet soda)	600X

Table 1 describes the six sweeteners currently approved by the FDA, in terms of the approval status by the FDA, and the acceptable (ADI) and estimated (EDI) intake levels for adults of children. If ADI is greater than or equal to the EDI, the sweetener is approved for use. Aspartame has caloric value, and hence is defined as a nutritive sweetener; however, because it is so much sweeter than sucrose, its caloric value is negligible in the quantities typically consumed. NNS: Non-nutritive sweetener, NUTRS: Nutritive sweetener, GMP: Good manufacturing practices, REG: Food additives for which a petition has been filed and a regulation issued.

Table 2. Position statements for use of sweeteners from various scientific organizations

Scientific Organization	Year	Position statement	Population considered
American Dietetic Association	2004, 2009	Consumers can safely use artificial sweeteners when consumed in a diet guided by current federal nutrition recommendations. The wide range of artificial sweeteners available in food supply should keep artificial sweeteners intake in children well below the acceptable daily intakes.	Children and adults
American Academy of Pediatrics	2010	The use of artificial sweeteners to provide health benefits for children and adolescents has been inadequately studied. As such, they should not form a significant part of a child's diet.	Specific to children
American Heart Association	2010	People with diabetes can use artificial sweeteners, as can people on a weight loss diet	General population
American Diabetes Association	2010	Foods and drinks that contain artificial sweeteners are an option for those with diabetes to consume fewer calories and carbohydrates when replaced for a food or drink containing sugar.	General population
Institute of Medicine	2007	No recommendations are made regarding foods containing artificial sweeteners because 1) artificially sweetened beverages have been shown to displace milk	Specific to children

		and 100% juice at mealtimes 2) more research is needed on the effectiveness of artificial sweeteners in foods for weight management, and 3) more studies are needed on safety effects when artificial sweeteners are consumed over many years starting in childhood or adolescence	