

Characterization of Non-Nutritive Sweetener Intake Patterns in a Sample of Rural  
Southwest Virginian Adults

Erin Marie Passaro

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Valisa E. Hedrick, chair  
Brenda M. Davy  
Wen You  
Jamie M. Zoellner

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## ABSTRACT

Controversy surrounds the use of artificial sweeteners (non-nutritive sweeteners [NNS]) as an effective weight-loss and/or maintenance strategy. This controversy is especially important as obesity is an epidemic in the United States. Excessive added sugar intake, primarily from sugar-sweetened beverages, has been linked to increased risk of overweight and obesity, as well as type 2 diabetes and cardiovascular disease. NNS provide minimal to no calories and thus, they have been suggested as a method to reduce added sugar intake, and consequently decrease energy intake, weight, and cardiometabolic risk. However, NNS intake has been associated with various health outcomes in observational studies and randomized controlled trials, including cancer, weight gain and loss, physiological and intestinal changes, cardiovascular disease, and diabetes. The uncertainties around the effect of NNS on health outcomes stem from a variety of limitations, one of which is inadequate dietary assessment methodology. Accuracy of dietary intake assessment methods is limited by the inability to distinguish between different types of NNS and lack of information about consumer use of NNS in a variety of beverages and foods. The purpose of this investigation is to explore NNS consumer characteristics and to characterize NNS intake in a sample of rural Southwest Virginian adults. This characterization is especially important for rural populations, as they are known to be high sugar-sweetened beverage consumers and are at an increased risk of obesity and chronic disease; thus, NNS could serve as a replacement method to facilitate cardiometabolic health. Cross-sectional data from a large randomized controlled trial, Talking Health (n=301), was utilized in this investigation to compare demographic characteristics, anthropometrics, biochemical markers, dietary quality, and dietary factors between NNS consumers and NNS non-consumers. This data was also used to characterize NNS intake (frequency, type, and source of sweetener). Of this rural sample, 33% consumed NNS, with sucralose being the most prevalent type of NNS and diet soda being the most frequently consumed source of NNS. NNS consumers had a higher BMI status than NNS non-consumers. However, NNS consumers had better overall dietary quality than NNS non-consumers. The characteristics of these NNS consumers and their intake patterns can be used to develop well-designed dietary intake assessment tools that accurately measure NNS intake, which can facilitate a better understanding of the associations of NNS with health outcomes.

# Characterization of Non-Nutritive Sweetener Intake Patterns in a Sample of Rural Southwest Virginian Adults

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## General Audience ABSTRACT

Uncertainty still surrounds the use of artificial sweeteners (non-nutritive sweeteners [NNS]) as a way to lose or control weight. This uncertainty deserves attention because obesity is a growing problem in the United States. High sugar intake, primarily from sugar-sweetened beverages, has been linked to increased risk of overweight and obesity, as well as type 2 diabetes and heart disease. NNS provide very little to no calories and have been suggested as a method to lower sugar and energy intake, weight, and disease risk. However, positive and negative health outcomes related to cancer, weight, heart disease, and diabetes have been associated with NNS intake. The uncertainty of the effects of NNS on health are caused by methodological limitations of the studies. One major obstacle is the limited accuracy of tools that measure dietary intake. These tools cannot identify different types or sources of NNS and the majority only collect information about NNS beverages, specifically diet sodas. It is important to collect intake information on different types of NNS beverages and also foods. The goal of the current investigation is to identify trends in characteristics of NNS consumers and consumption patterns. Data from a larger study, Talking Health (n=301), was utilized in this investigation to compare demographic characteristics, weight status, disease markers, dietary quality, and dietary intake of NNS consumers and NNS non-consumers. It was found that 33% of the population consumed NNS, with sucralose being the most prevalent type of NNS and diet soda being the most frequently consumed source of NNS. NNS consumers had a higher weight status than NNS non-consumers. However, NNS consumers had better overall dietary quality than NNS non-consumers. The characteristics of these NNS consumers and their intake patterns can be used to develop better dietary assessment tools that accurately measure NNS intake. Improved tools could facilitate a better understanding of the relationship between health outcomes and NNS.

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## **Chapter 1: Review of Literature**

### **Abstract**

Reduction in added sugar intake is becoming increasingly important in the United States with rising rates of overweight and obesity among adults and children. Many health concerns are associated with overweight and obesity, which necessitate the complete and thorough investigation of a useful alternative. Non-nutritive sweeteners (NNS) have been suggested as an alternative, as they provide minimal calories and thus have the potential to facilitate the reduction of weight and improvement of disease status. However, controversy surrounds associations of NNS with cancer, weight status, physiological and intestinal changes, cardiovascular disease, diabetes, and other conditions. Currently, many difficulties exist in measuring NNS intake due to small sample sizes and short study durations of randomized controlled trials, many confounders in observational studies, differing metabolic pathways between animal and human models, and inadequate dietary intake assessment methodologies. To understand the relationship between NNS and the plethora of reported health conditions, it is important to become familiar with the current literature and explore the many limitations to determining causality and measuring NNS intake accurately.

## **Obesity**

Obesity is an epidemic among United States (U.S.) adults  $\geq 20$  years old, with 71.1% of men and 65.5% of women classified as overweight (Body Mass Index [BMI] 25-29.9 kg/m<sup>2</sup>) or obese (BMI  $\geq 30$  kg/m<sup>2</sup>)<sup>1</sup>. Overweight and obese individuals are more likely to face negative health outcomes including cardiovascular disease, hypertension, diabetes mellitus, and certain cancers, as well as increased mortality<sup>2</sup>. While the possible causal factors of obesity are multifaceted, there is substantial evidence linking excessive added sugar intake, primarily from sugar-sweetened beverages, to increased risk of overweight and obesity, type 2 diabetes, and cardiovascular disease<sup>3-8</sup>.

## **Nutritive Sweeteners**

Nutritive sweeteners, also called caloric sweeteners or added sugars, provide four calories per gram and contain carbohydrates and energy<sup>9</sup>. Nutritive sweeteners can occur naturally in fruits, vegetables, and dairy products, or they can be added to foods and beverages during processing or preparation, which is then known as added sugar<sup>9</sup>. The two most commonly consumed forms of added sugar in the U.S. diet are sucrose<sup>9</sup> and high-fructose corn syrup<sup>10</sup>. These forms of added sugar provide the calories in energy-containing drinks known as sugar-sweetened beverages (SSB)<sup>11</sup>. SSB are the primary source of added sugar in the U.S. diet and include regular soft drinks, fruit drinks, energy/sport drinks, and ice tea and coffee sweetened with sugar<sup>11,12</sup>.

Added sugar and SSB intake is associated with increases in body weight<sup>5</sup> and risk for cardiovascular disease<sup>3,4</sup>, as well as with a decrease in overall diet quality<sup>13-15</sup>. Overtime, consumption of liquid calories from SSB can lead to weight gain if consumed beyond an individual's recommended daily caloric intake<sup>16</sup>. This weight gain is believed



to occur due to a lack of reduction in solid food, as well as the high added sugar concentration and low satiation effect of liquid beverages (SSB), which contribute to a positive energy balance<sup>7,8</sup>. Furthermore, the readily absorbable sugars and uncontrolled portion sizes of SSB provide a high glycemic load, causing inflammation and insulin resistance, which increases the risk for cardiovascular disease and its risk factors<sup>5,6</sup>. SSB were also linked to low diet quality in men, with reports of high intake of red and processed meats, carbohydrates, fat, and energy among SSB consumers<sup>13</sup>. This link is supported by Hedrick et al. who reported that Healthy Eating Index scores in a sample of high SSB consumers were poor (<51), indicating low adherence to the 2010 Dietary Guidelines for Americans<sup>15</sup>.

Using National Health and Nutrition Examination Survey (NHANES) data, Welsh et al. found that consumption of added sugar in the U.S. has changed drastically over the previous 40 years<sup>10</sup>. U.S. adults increased their added sugar consumption by 35% between 1977 and 1996<sup>10</sup>. Although added sugar intake decreased by 24% between 1999 and 2008, from 401 to 307 kcal/d<sup>10</sup>, added sugar still contributed 14.6% of total energy intake per day (18.3±0.6 teaspoons or 307±10 kcal) in 2008<sup>10</sup>. This consumption level of added sugar is greater than the amount recommended by the Scientific Report of the 2015 Dietary Guidelines Advisory Committee, which states that less than 10% of energy should come from added sugar<sup>17</sup>. Between 1999 and 2008, regular sodas contributed the highest amount of added sugar to U.S. diets among all age groups ≥ 2 years old (n=42,316) (37.4% in 2000, 22.8% in 2008), proceeded by cakes and cookies, fruitades and sports drinks, sugars and syrups, and candies and gums<sup>10</sup>. To achieve a healthier level

of added sugar intake in the U.S., replacement with water and/or non-nutritive sweeteners has been suggested by the 2010 Dietary Guidelines<sup>18</sup>.

### **Non-nutritive Sweeteners**

The American Diabetes Association, the American Heart Association, and the Academy of Nutrition and Dietetics suggest consumption of non-nutritive sweeteners as a possible method to reduce added sugar intake and consequently, decrease energy intake and weight while promoting cardiometabolic health<sup>18,19</sup>. Non-nutritive sweeteners (NNS), also known as artificial sweeteners, low-calorie sweeteners, or high-intensity sweeteners, provide a sweet taste while contributing minimally to energy intake<sup>9</sup>. Currently there are seven approved NNS in the U.S. which include acesulfame-potassium, aspartame, saccharin, stevia, sucralose, Luo Han Guo, and neotame<sup>9</sup>. However, because there is a lack of well-designed research that adequately explores the roles of NNS in decreasing weight and cardiometabolic risk, replacement of added sugar with NNS is controversial<sup>19</sup>. In fact, although acknowledging the role of NNS in short-term weight loss, the Scientific Report of the 2015 Dietary Guidelines Advisory Committee suggests using water as the primary replacement method of added sugar in beverages, due to inadequate evidence and uncertainty of the long-term health outcomes for NNS<sup>17</sup>.

### **Regulation and Safety Standards of Non-nutritive Sweeteners**

The regulations regarding the safety of NNS are enforced by the Food and Drug Administration (FDA) through the U.S. Food Additives Amendment of 1958<sup>20</sup>. This mandate requires any new food additive to be approved prior to entering the market unless it is deemed generally recognized as safe (GRAS)<sup>21</sup>. A GRAS exemption is determined by qualified experts who can ensure the substance is safe under its specified

conditions of use<sup>21</sup>. Once a company determines a substance to be GRAS, the FDA is contacted and approval or denial of the request is based on the provided evidence<sup>22</sup>. If a substance does not have a GRAS exemption, then premarket approval is required which involves a safety evaluation of the food additive<sup>21</sup>. This evaluation takes into account probable intake, health effects, and toxicological data and safety factors<sup>23</sup>. Toxicological data includes the extent and rate of absorption, distribution, metabolism, and excretion<sup>24,25</sup>. The FDA must also determine the highest no effect level, estimated daily intake (EDI), and acceptable daily intake (ADI)<sup>23</sup>. Determination of these levels allows comparison to ensure that the ADI is significantly higher than the normal exposure (EDI) to the food additive<sup>23</sup>. Both market approval for food additives and GRAS listings require the same level of safety, ensuring with “reasonable certainty” that the substance or food additive is not harmful<sup>23</sup>. The difference between GRAS substances and food additives is the level of widespread knowledge about the safety and acceptability of the ingredient<sup>26</sup>. The safety of a GRAS substance is determined using data that is widely available and easily accessible in the scientific literature; with a GRAS substance, there is a strong consensus among experts about their safety<sup>26</sup>. Safety information on food additives is private and determined through tests conducted by the FDA at the request of the additive’s sponsor<sup>26</sup>. **Table 1** summarizes basic information about the seven currently approved NNS in the U.S. food supply. Chemical and common names are provided, in addition to the FDA approval date. Most NNS are classified as food additives, while a few are GRAS. The table also includes sweetness levels compared to sucrose, ADI and EDI levels, and the number of sodas and sweetener packets equivalent to these values.

The seven types of NNS currently available in the U.S. consist of different components and are processed by the body and used in food products in varying ways. Acesulfame-potassium is composed of organic acid and potassium and because it is excreted mostly unchanged in the urine, it does not provide a significant amount of energy<sup>27</sup>. Acesulfame-potassium is present in many foods, including frozen desserts, candies, beverages, and baked goods, and is often combined with other NNS<sup>28</sup>.

Aspartame, a methyl ester of aspartic acid and phenylalanine dipeptide<sup>29</sup>, is one of the most thoroughly reviewed substances in the human diet, with over 100 studies approving it as safe<sup>28</sup>. Although referred to as a NNS, aspartame actually provides the same amount of calories as nutritive sweeteners (4 kcal/g)<sup>29</sup>; however due to the intensity of sweetness, only minimal amounts are needed<sup>29</sup>. Aspartame is often used in chewing gum, cereals, and dry bases for beverages, gelatins, and puddings<sup>28</sup>. In the gastrointestinal lumen, aspartame metabolizes into aspartic acid, methanol, and phenylalanine<sup>29</sup>, so individuals with phenylketonuria should use caution<sup>9,28</sup>. Once metabolized, these components are absorbed into the general circulation<sup>29</sup>.

Discovered in 1878, saccharin is the oldest NNS<sup>30</sup>, and is approved for use in beverages, fruit juice drinks, processed foods, bases or mixes, and as a sugar replacement for both cooking and table use<sup>28</sup>. Similar to acesulfame-potassium, saccharin is not metabolized and is excreted unchanged, providing no calories<sup>30</sup>.

Stevia or steviol glycoside, comes from the leaves of *Stevia rebaudiana* Bertoni plant. High-purity ( $\geq 95\%$  purity) steviol glycosides, including Rebaudioside A, Stevioside, Rebaudioside D, and steviol glycoside mixtures, are considered GRAS for use as sweeteners under specified conditions. However, stevia leaf and crude stevia extracts,

which are sold as dietary supplements, are not considered GRAS<sup>9,28</sup>. Stevia glycosides pass through the stomach and small intestine unchanged but are hydrolyzed by gut bacteria in the colon into steviol<sup>31</sup>. Then, steviol is metabolized by the liver to form steviol glucuronide, which is mostly excreted in the urine<sup>31</sup>.

Sucralose, which is sucrose with three chlorine molecules instead of three hydroxyl groups, is used as a general purpose sweetener in many foods, including baked goods, beverages, chewing gum, gelatins, and frozen desserts<sup>9,28</sup>. Like acesulfame-potassium and saccharin, the majority of sucralose is not absorbed, and excreted unchanged in the feces<sup>32</sup>; any absorbed sucralose is excreted mostly unchanged in the urine<sup>32</sup>.

A lesser known type of NNS, luo han guo or monk fruit extract, is composed of many different cucurbitane glycosides called mogrosides, predominantly mogroside V (>30% of product). Depending on the concentration of mogrosides, sweetness levels can vary for this substance<sup>9,28</sup>.

Neotame, another lesser known NNS, is a derivative of the dipeptide phenylalanine and aspartic acid<sup>30</sup>. Neotame is incompletely absorbed in the small intestine and then quickly metabolized to form a negligible amount of methanol along with esterified neotame, which is excreted in the urine and feces<sup>33</sup>.

### **Consumer Demographics and Trends of Non-nutritive Sweeteners**

Consumer aesthetics, economic benefit, and health-related reasons have been the driving forces behind the use of NNS for decades<sup>9</sup>. The first reported use of NNS was in the late 1800s, when saccharin was utilized to attain a sweet taste with less product; it also helped control blood glucose levels in diabetics and facilitated the reduction of

energy intake<sup>9</sup>. In 2012, the *Food and Health Survey: Consumer Attitudes toward Food Safety, Nutrition & Health*<sup>34</sup>, commissioned by the International Food Information Council Foundation, found that among 1,057 U.S. adults aged 18-80 years, over half (55%) were trying to lose weight<sup>34</sup>. Among those, 46% were purchasing foods based on the presence or absence of NNS. Consumers participating in this survey viewed NNS as a way to control blood glucose and reduce caloric intake, thus facilitating weight loss or weight management efforts<sup>34</sup>. Consumers also reported that NNS use “can be part of an overall healthful diet”<sup>34</sup>. One-third (30%) of consumers reported consumption of NNS (actively or passively) and most (73%) indicated this consumption for calorie control<sup>34</sup>. In observational studies, NNS consumers tended to have higher levels of education<sup>35-37</sup> and socioeconomic status<sup>35,37</sup>, and were more likely to engage in physical activity<sup>13,37</sup> and dieting behavior<sup>13,37</sup> than non-consumers. Usually their diets were lower in overall calories, as well as lower in calories from carbohydrates, sugar and SSB, alcohol, and milk<sup>13,36,37</sup>. These findings were also corroborated by Sylvetsky et al., who reported on national consumption trends of NNS foods and beverages using NHANES data; the results showed that 33% of females and 25% of males consumed NNS<sup>38</sup>. Of these consumers (both male and female), 38% were  $\geq 55$  years old (29% 39-54 and 15% 18-34 years old), 36% were Caucasian (22% Black and 25% Hispanic), 36% were obese (30% overweight and 22% normal), and 38% were in the highest income tertile (28% middle and 22% low)<sup>38</sup>. Differences between NNS consumers and non-consumers in observational studies can be reviewed in **Table 3**.

Between 1999 and 2004, NNS consumption increased, with > 6,000 new NNS products in the U.S.<sup>39</sup>. The most prevalent NNS in products was sucralose (2,500),

followed by acesulfame-potassium (1,103), and aspartame (974)<sup>39</sup>. Similar to added sugar, NNS is most commonly consumed in the form of carbonated beverages<sup>40</sup>. Mattes and Popkin utilized the U.S. Department of Agriculture's (USDA) Nationwide Food Consumption Surveys and NHANES data to analyze consumption patterns between 1965 and 2004<sup>40</sup>. The survey data revealed an increase in NNS consumption between 1989 and 2004, in both food and beverage products<sup>40</sup>. In 2007-2008, 19% of U.S. adults were consuming NNS through no-calorie beverages (diet soda and flavored sugar-free water), followed by condiments and other low-calorie foods (12% [reduced-sugar ketchup, sugar-free pancake syrup, jellies/jams, light yogurt, reduced-calorie beverages (8% [light fruit juices and lemonades]), and no-sugar-added canned fruit]), and desserts (2% [sugar-free ice cream and pudding])<sup>38</sup>. Despite this increase in NNS consumption, a decrease in products with added sugar was not reported<sup>38,40</sup>. This perhaps indicates that NNS is not being used as an added sugar replacement<sup>40</sup> and may be used in combination with added sugar in reduced-calorie beverages, which have been reported to be the driving force behind the recent increase in NNS consumption<sup>38</sup>. These findings necessitate further investigations of dietary intake patterns to understand how NNS is used<sup>38</sup>.

**Table 1. Non-nutritive Sweeteners<sup>a</sup>**

Sweetener (Chemical Name)	Common Brand Names	Times sweeter than sucrose	FDA Approval Date	Acceptable Daily Intake per kg of body weight	Estimated Daily Intake per kg of body weight	Number of 12 fl oz soda cans=ADI <sup>b</sup>	Number of sweetener packets=ADI <sup>c</sup>
Acesulfame-K (5,6-dimethyl-1,2,3-oxathiazine-4(3H)-1,2,2-dioxide)	Sweet One, Sunett	200	1988	15 mg/kg	0.2 to 1.7 mg/kg	25	20
Aspartame (L-aspartyl)-L-phenylalanine methyl ester)	Equal, NutraSweet, Sugar Twin	160-220	1981	50 mg/kg	0.2 to 4.1 mg/kg	14	68
Saccharin (1,1-dioxo-1,2-benzothiazol-3-one)	Sweet 'N Low, Sweet Twin, Sweet and Low, Necta Sweet	300	Before 1958	5 mg/kg	0.1 to 2 mg/kg	42	8.5
Stevia (Steviol glycosides, rebaudioside A, stevioside)	Truvia, PureVia, Sweet Leaf, Enliten	250	GRAS <sup>d</sup> 2008	JECFA <sup>e</sup> 4 mg/kg	1.3 to 3.4 mg/kg	16	30
Sucralose (Trichlorogalactosucrose)	Splenda	600	1999	5 mg/kg	0.1 to 2 mg/kg	15	30
Luo han guo extract (Siraitia grosvenorii Swingle fruit extract [SGFE])	Nectresse, Monk Fruit in the Raw, PureLo	150-300	GRAS 2010	ADI: ND <sup>h</sup>	6.8 mg/kg	NS <sup>f</sup>	None determined
Neotame (N-[N-(3,3-dimethylbutyl)-L- $\alpha$ -aspartyl]-L-phenylalanine 1-methyl ester)	Newtame	7,000-13,000	2002	18 mg/kg	0.05 to 0.17 mg/kg	Not present in sodas	Not present in packet form

<sup>a</sup> Table modified from FDA's Additional Information about High-Intensity Sweeteners Permitted for use in Food in the U.S.<sup>28</sup> and additional information from Gardner et al.<sup>19</sup> and Fitch et al.<sup>9</sup>

<sup>b</sup> Number of 12 fluid ounce soda cans that equal ADI for a 150 lb (68 kg) person

<sup>c</sup> Number of sweetener packets that equal ADI for a 150 lb (68 kg) person

<sup>d</sup> GRAS = generally recognized as safe

<sup>e</sup> JECFA – Joint Expert Committee on Food Additives (determined ADI for Stevia)

<sup>f</sup> NS = not specified because a numerical ADI may not be necessary for reasons such as evidence of ingredient's safety at levels well above the amount needed to achieve the desired effect in food



## **Health Concerns of Non-nutritive Sweeteners**

Replacing added sugar with NNS in the diet to reduce appetite, body weight, and/or cardiometabolic risk factors is a controversial topic<sup>19</sup>. With 30% of U.S. adults consuming NNS on any given day<sup>34</sup>, it is important to consider all the potential health effects of NNS consumption, such as cancer<sup>41-44</sup>, weight loss<sup>45-51</sup>, weight gain<sup>35,37,52,53</sup>, physiological and intestinal changes<sup>54-66</sup>, cardiovascular disease<sup>35,36,53,67-70</sup>, diabetes<sup>13,53,71</sup>, and migraines<sup>96,97</sup>.

Further information pertaining to cardiometabolic health concerns (weight, cardiovascular disease, and diabetes) in human populations is available in **Table 2** and **Table 3**, with details for each study's population, purpose and intervention, dietary assessment tool, variables controlled, confounding variables and limitations, and differences between NNS consumers and non-consumers and/or outcomes.

### Cancer

According to the National Cancer Institute, a clear evidence-based association has not been demonstrated between NNS consumption and cancer<sup>72</sup>. Although earlier studies on rats linked high doses of saccharin to cancer<sup>41,42</sup>, the cancer-producing mechanism was found to be species-specific to rats<sup>72,73</sup>. Aspartame has not been shown to cause cancer in animal studies even at very high doses<sup>74,75</sup>; however speculations were made on an association between increased cancer cases and entrance of aspartame into the food supply<sup>43</sup>. These conclusions were later refuted due to lack of supporting evidence<sup>76</sup>. Furthermore, examination of 473,984 adults in the National Institutes of Health American Association of Retired Persons (NIH-AARP) Diet and Health Study showed no association between high aspartame intake and risk of cancer<sup>77</sup>. Case-control studies are

important to assess this relationship further<sup>73</sup> and most do not show a significant increase in risk for bladder cancer with NNS consumption<sup>78-82</sup>; however one study showed heavy NNS consumption (> 1680 mg per day) among 31 bladder cancer patients was associated with an increased relative risk of 1.3 for bladder cancer<sup>44</sup>. Determination of the exact causal agent was impossible though, due to the combination of NNS found in current products<sup>44</sup>. Other types of NNS, including acesulfame-potassium, sucralose, and neotame have not been suspected to cause cancer<sup>73</sup> and the National Cancer Institute emphasizes the safety results of extensive safety testing conducted by the FDA, which have revealed no evidence linking cancer to NNS consumption<sup>72</sup>.

### Weight Loss

Although consuming NNS products have been suggested as a weight loss method, this suggestion remains controversial in both randomized controlled trials and observational studies<sup>19</sup>. In consideration of the effect of NNS consumption on weight, it is important to also look at energy intake. Participants in a 6-month randomized controlled trial replaced  $\geq 2$  servings of SSB per day with NNS beverages and were more likely to lose up to 5% of their body weight<sup>49</sup>, as well as significantly decrease total energy intake<sup>50</sup>. These findings support the results of an extensive review by De la Hunty et al., which looked at sixteen randomized controlled trials investigating the effects of aspartame on energy intake and weight loss<sup>48</sup>. Significant reductions in energy intake occurred with aspartame consumption when compared to a variety of controls (except non-sucrose controls like water)<sup>48</sup>. Overall, aspartame consumers had a mean reduction of approximately 10% of energy intake which corresponds to 222 kcal per day or 0.2 kg per week<sup>48</sup>. Of the reviewed studies with significant weight loss results, differences were

observed between aspartame and nutritive sweetener consumers in the short-term, with a weight change range of -0.47 to -1.0 kg (-1.0 to -2.2 lb) for aspartame and +0.52 to +1.6 kg (+1.1 to +3.52 lb) for sucrose<sup>45,46,83</sup>. Another study reviewed by De la Hunty et al. was a large cohort of obese women who were followed throughout a weight loss program and maintenance period. Both groups (aspartame and nutritive sweetener) lost 10±6.3 kg (22±13.9 lb) during the active weight loss program, however after two years, the aspartame group maintained a 5.1 kg (11.2 lb) weight loss while the nutritive sweetener group did not maintain any weight loss<sup>47</sup>. In other words, the weight regained for the aspartame group was significantly less than the nutritive sweetener group (+5.4 kg [11.9 lb] vs. +9.4 [20.7 lb] kg respectively)<sup>47</sup>. Furthermore, adolescent NNS consumers of higher weight statuses were also more successful in achieving a significant decrease in BMI (-0.63±0.23 kg/m<sup>2</sup>) compared to non-consumers (+0.12±0.26 kg/m<sup>2</sup>) over a twenty-five week period in a randomized controlled pilot study<sup>51</sup>. These studies provide support for the recommendation that replacing nutritive sweeteners with NNS can be a useful strategy to lose weight<sup>45-51,83</sup>.

### Weight Gain

Despite results that support the weight loss capabilities of NNS<sup>45-51</sup>, increases in weight and/or waist circumference with NNS consumption were observed in long-term prospective studies<sup>35,37,53</sup>. In the San Antonio Heart Study, a significant relationship existed between NNS beverage consumption, risk for overweight and obesity, and increased BMI (47% higher for NNS consumers)<sup>37</sup>. A dose-response relationship was also observed in the San Antonio Longitudinal Study of Aging, between NNS consumers and waist circumference, with increases in abdominal obesity ranging from 0.80 inches

for non-consumers to 3.16 inches for consumers<sup>35</sup>. Another study revealed overweight or weight-gaining children had higher NNS consumption over a two-year period than normal weight children<sup>52</sup>.

The current literature review demonstrates the challenges in reaching conclusions about NNS and weight. In this review, there are many short-term randomized controlled trials that observe associations between NNS intake and weight loss, perhaps due to their inability to observe long-term weight changes and the continuous motivational support from research staff<sup>19</sup>. There are also many long-term observational studies in this review that observe associations between NNS intake and weight gain, which could be the result of inaccurate assessment of NNS intake, confounders, and/or reverse causality<sup>19</sup>. The differing findings of randomized controlled trials and observational studies can be reviewed in Tables 2 and 3. These differences in weight-related outcomes and the limitations of studies investigating the effects of NNS consumption indicate a need for more of both study designs, intervention trials to determine causation, and observational studies to understand long-term changes<sup>19</sup>. Additionally, it is crucial to understand the proposed underlying mechanisms for weight gain, specifically pertaining to NNS-driven physiological and intestinal changes.

#### Physiological and Intestinal Changes

NNS may modify dietary patterns by altering calorie prediction of energy dense foods and changing the gut microflora, potentially even interacting with intestinal sweet-taste receptors<sup>84</sup>. Conducted on the concept that animals use the intensity of sweet taste to determine the caloric content of food, Swithers et al. used rat models to assess this relationship during NNS consumption<sup>55,56</sup>. This predictive energy mechanism was

degraded with consumption of NNS, with subsequent weight gain and fat accumulation due to increased energy intake<sup>55-57</sup>. Furthermore, an enhanced preference for sweet taste has also been associated with NNS consumption<sup>57</sup>; however it is unclear if this preference outweighs energy-reducing efforts in humans<sup>40</sup>. Another potential physiologic change is the potential for compensatory behavior after informed NNS consumption<sup>54</sup>; this occurs when an individual replaces added sugar with NNS and justifies consumption of a higher calorie food, negating the calorie savings<sup>40</sup>. De la Hunty reports that compensation is only about one-third of replaced calories which means a calorie savings exists, however these values come from short-term studies; long-term studies are necessary to assess if the savings are maintained<sup>48</sup>. Bacterial changes in the intestine, which can promote a state of inflammation, leading to insulin resistance, fat accumulation, and weight gain, are also of concern<sup>85,86</sup>. NNS consumption has been linked to altered microflora in both humans and rats<sup>60,61</sup>, with some studies linking this modified microflora following consumption of NNS to weight gain in rats and glucose intolerance in both rats and humans<sup>58,59</sup>. Furthermore, a recent hypothesis is that sweet-taste receptors in the gut are activated by NNS<sup>62-64</sup>. Sweet-taste gut receptors regulate release of glucose and gut hormones, which affect insulin release, appetite control, and gut motility<sup>62,87</sup>. In human and animal studies, NNS stimulated the release of gut hormones by interacting with sweet-taste receptors similarly to glucose<sup>62-66</sup>. However in both fasted rats and humans consuming NNS, these responses were not observed<sup>88-92</sup>. Differing results may be related to the concentration of NNS, the varying sensitivity of sweet taste within the gut, and the concurrent consumption of glucose<sup>84</sup>. Theoretically, the response of these receptors and hormones could lead to decreased satiety and increased energy intake relative to nutritive

sweeteners but further studies are needed<sup>40</sup>. These potential changes in physiological and intestinal mechanisms provide insight as to how NNS is linked to metabolic derangements and weight gain, however more research in free-living human populations is required to fully understand these relationships<sup>57</sup>.

### Cardiovascular Disease

Another potential health outcome of NNS consumption is cardiovascular disease (CVD). As previously mentioned, a dose-response relationship was observed among NNS consumers in the ten-year San Antonio Longitudinal Study of Aging, with increases in abdominal obesity ranging from 0.80 inches for NNS non-consumers to 3.16 inches for daily NNS consumers<sup>35</sup>. This relationship reveals a potential pathway through which daily NNS intake leads to accrual of cardiometabolic risk factors<sup>35</sup>, as abdominal obesity is associated with high glucose concentration<sup>93</sup>, dyslipidemia<sup>93</sup>, high C-reactive protein<sup>94</sup>, loss of physical function with metabolic syndrome<sup>95</sup>, coronary heart disease<sup>96,97</sup>, and cardiovascular events<sup>98,99</sup>. Most of these factors are determinants for metabolic syndrome, a condition that multiplies risk for CVD<sup>100</sup>. Metabolic syndrome and NNS have been associated in multiple other observational studies<sup>36,53,67,68</sup>. These findings are consistent with longitudinal studies associating the risk for vascular events with NNS consumption<sup>69,70</sup>. Participants consuming  $\geq 1$  serving of NNS beverage per day in the Nurses' Health Study had a relative risk of 1.16 for a stroke<sup>70</sup>. It is important to note that in most of these studies, individuals were free of baseline metabolic syndrome and other CVD risk factors which eliminates potential confounds, however observational, self-reported data limits conclusive statements<sup>36,67,69,70</sup>. The clinical implications of these studies are pertinent to those individuals who already have or are at high risk for

cardiovascular disease, as consumption of NNS may be considered as a healthy alternative to reduce health risk factors.

### Diabetes

NNS consumption has also been shown to have both positive and no-effect associations with diabetes risk. Observational studies reveal positive associations between NNS beverage consumption and incident type 2 diabetes (high fasting glucose [ $>126\text{mg/dL}$ ])<sup>13,53,71</sup>. Most of these associations, although weakened, remained strong after multivariate adjustments for confounders like demographic and lifestyle factors and baseline BMI and health status<sup>53,71</sup>. The associations in the Health Professionals Follow-Up Study did not remain significant after multivariate adjustments for confounders, indicating the association between NNS consumption and diabetes was mostly explained by pre-existing health, weight, and dieting statuses<sup>13</sup>. A study utilizing NHANES data found an association between NNS beverage consumption and poor blood glucose control among adults with diabetes<sup>101</sup>. However, these findings are not supported in the existing literature and the association may be mediated by the increased likelihood of poor blood glucose control among diabetics consuming NNS beverages<sup>101</sup>. This study, as well as the Health Professionals Follow-Up Study, show how confounding variables, if overlooked during adjustment and analyses of data, could contribute to misunderstood associations between health outcomes and NNS consumption. Furthermore, multiple randomized controlled trials lasting between one- and 16-weeks reported no significant effect of NNS consumption on measures of glycemic response (plasma glucose, insulin, hemoglobin A1C) indicating a lack of association between NNS intake and diabetes<sup>102-105</sup>. These findings are in line with conclusions from the Academy of Nutrition and

Dietetics, which state glycemic response is unaffected by NNS in individuals with diabetes mellitus<sup>106</sup>.

#### Other Concerns: Migraines and Pregnancy/Lactation

Reports of associations between intake of aspartame and incidence of migraines<sup>107,108</sup> have been a controversial concern as well. Studies suggest that aspartame triggers migraines through its degradation to formaldehyde and formic acid and accumulation of chemical substances<sup>107</sup>. In patients experiencing migraines, counseling to avoid aspartame and other formaldehyde-releasing products was associated with resolved symptoms<sup>107</sup>. An expert work group under the Academy of Nutrition and Dietetics has responded to these concerns using the evidence analysis process to investigate the relationship between aspartame, methanol, and formaldehyde<sup>109</sup>.

Aspartame forms two amino acids, phenylalanine and aspartic acid, as well as an alcohol, methanol. Commonly consumed food items like non-fat milk and tomato juice provide four to thirteen times more aspartic acid, phenylalanine, and/or methanol than an aspartame-containing beverage<sup>109</sup>. After degradation of methanol, formaldehyde is formed and immediately utilized by the body; if the body does not need it, formaldehyde is converted to formic acid, which is excreted or degraded into carbon dioxide and water<sup>109</sup>. Consumers should be informed that formaldehyde is produced by the human body daily, in amounts thousands of times higher than any aspartame-containing beverage and this substance is actually needed as a metabolite in many bodily processes<sup>109</sup>. The Academy of Nutrition and Dietetics also responded to concerns regarding the safety of aspartame consumption in pregnant or lactating women. Studies have overlooked the fact that aspartame does not enter the bloodstream as aspartame,



meaning direct contact with the fetus and/or breast milk is impossible<sup>109</sup>. This means studies using direct injection of aspartame into the body or into a dish of cells have no safety implications during pregnancy or lactation<sup>109</sup>. Furthermore, the amounts typically consumed are considered safe for this population<sup>109</sup>.

### **Limitations to Non-nutritive Sweetener Research**

Common limitations to NNS research include differing metabolic pathways between humans and animals<sup>84,110</sup>, small sample sizes and short study duration of randomized controlled trials<sup>19,52</sup> and potential for many confounders in observational studies<sup>35,36,67,69,70,101</sup>.

A majority of the research investigating the relationship between NNS use and cancer and physiological and intestinal changes is conducted in animal models, which is problematic due to the differing metabolic pathways between animals and humans<sup>84,110</sup>. Additionally, perceived sweetness levels vary widely among species<sup>110</sup>, making it difficult to understand compensatory behavior and intestinal changes in humans. These limitations emphasize the need for future studies to focus on the effects of NNS on the human metabolic pathway<sup>84</sup>.

Most samples in randomized controlled trials are small<sup>45,46,51,83</sup> and predominantly consist of women participants<sup>45,47</sup>, which limits the generalizability of study findings<sup>19</sup>. Short study durations ( $\leq 6$  months)<sup>19</sup> in randomized controlled trials cannot capture the complete scope of dietary patterns<sup>52</sup> or the accrual of long-term outcomes of NNS use<sup>49</sup> (Table 2).

While observational studies offer longer study durations than randomized controlled trials, the potential for many confounders exists. As evidenced in previous

sections, many observational studies have reported adverse health outcomes with NNS consumption. However, individuals may consume more NNS foods and beverages in an effort to lose weight<sup>37</sup> or cope with preexisting health conditions like high triglycerides, impaired fasting glucose, or high blood pressure<sup>111</sup>. This consumer decision explains the attenuation of significant weight- and disease-related health outcomes in observational studies after multivariate adjustments<sup>13,37,52,53,70,71</sup>. In other words, preexisting weight or medical conditions may make it appear that NNS is associated with poor health status, but poor health status may actually predetermine NNS consumption; this is called reverse causality<sup>13,111</sup>. As such, it is important to consider the factors that contribute to reverse causality, referred to as confounding variables, which include but are not limited to baseline health and weight status, dietary (total calorie, fat, carbohydrate, sugar intake) and lifestyle (physical activity level) habits, and/or BMI, in associations between cardiometabolic health risk and NNS intake, especially in observational studies<sup>13,53,68,71,101</sup>. Even though extensive adjustment analyses<sup>35,36,67,69,70,101</sup> are conducted to control for these factors, residual confounding cannot be ruled out in any observational study.

The current body of literature on NNS needs longer-term randomized controlled trials to determine causality<sup>19,67,68</sup>, especially mechanistic studies that address how NNS affects metabolic function<sup>53</sup>. These study designs would better control for reverse causality and confounding factors between NNS intake and various health complications like diabetes and CVD<sup>13,68</sup>. Observational studies will also continue to play a role in assessing long-term health outcomes of NNS use, however a variety of limitations need to be addressed, including inadequate dietary methodology<sup>19</sup>.

Inadequate dietary methodology is a major obstacle, especially for observational studies, in understanding the effects of NNS on health<sup>19</sup>. To develop sound assessment tools that accurately measure overall NNS intake, it is important to become familiar with the current tools being utilized as well as their systemic issues, which will be reviewed in the next section.

**Table 2. Randomized Controlled Trials Assessing Cardiometabolic Risk in Humans**

Study Population	Study Purpose/Intervention	Dietary Assessment Tool	Variables Controlled	Limitations <sup>a</sup>	Outcomes
n=50 males n=268 females 42±11 yr old Overwt/obese Participants were SSB consumers (≥200kcal/day) <sup>49</sup>	To compare wt loss results of replacing SSB with water or NNS (aspartame, sucralose, ace-K) beverages over 24 week. Participants provided with ≥2 servings/day of water or NNS beverage, or made non-beverage diet changes (control).	2 unannounced 24-hour recalls used to assess dietary intake at baseline, 12 week, & 24 week. NDS-R (2000) used to analyze diet.	Excluded participants with recent wt loss, cancer, history of heart issues, or if pregnant or on certain meds. No differences in baseline characteristics (age, sex, race, education, BMI) or energy intake between groups. Participants blinded to other treatments.	Potentially underpowered to detect group differences (because provided instructions for better health). Limited generalizability: mostly female, overwt/obese. Short duration limited investigation of long-term NNS benefits.	NNS beverage & water consumers more likely to achieve a significant 5% wt loss than control. NNS beverage consumers lost wt faster & reduced daily energy intake more consistently than water consumers. All groups improved systolic BP.
n=34 males n=176 females 42.2±0.9 yr old Overwt/obese Participants were SSB consumers (≥200kcal/day) <sup>50</sup>	To compare energy intake & diet habits between water & NNS (aspartame, sucralose, ace-K) beverage consumers over 24 week. Participants substituted ≥2 servings/day of SSB with water or NNS beverage.	2 unannounced 24-hour recalls used to assess dietary intake at baseline, 3 mo, & 6 mo. NDS-R (2000) used to analyze diet.	Excluded participants with recent wt loss, cancer, history of heart problems, or if pregnant or on certain meds. No differences in baseline characteristics (age, sex, race, education, BMI) or energy intake between groups. Participants blinded to other treatments.	Potentially underpowered to detect group differences (because provided instructions for better health). Limited generalizability: mostly female, overwt/obese. Short duration limited investigation of long-term NNS benefits.	NNS beverage & water consumers significantly ↓ energy intake. No between group differences.
n=6 males n=35 females 35.2±2.1 yr old Healthy overwt <sup>45</sup>	To assess effects of daily addition of NNS (aspartame, ace-K, cyclamate, saccharin) or sucrose beverages & foods on ad libitum energy intake & wt over 10 week. Groups received similar amounts & types of foods & drinks containing NNS or sucrose.	7 day weighed dietary records used to assess dietary intake at week 1, 5, & 10. Participants told to consume a minimum amount of NNS or sucrose products. Nutrition analysis program not reported.	Excluded participants with recent wt loss, cancer, history of heart issues, or if pregnant or on certain meds. No differences in baseline characteristics (age, sex, race, education, BMI) or energy intake between groups. Participants blinded to other treatment.	Small sample. Limited generalizability: mostly females, overwt. Participants became aware of sweetener received. Controlled eating behavior ↓ in sucrose consumers & ↑ in NNS consumers. Short duration limited investigation of long-term NNS benefits.	NNS consumers significantly ↓ sucrose intake, energy intake, wt (-1.0 kg), fat mass (-0.3 kg), & BP (-3.1/-1.2 mmHg). Significantly ↑ protein & fat intake through NNS products. Sucrose consumers significantly ↑ energy intake (+380 kcal/day), wt (+1.6 kg), fat mass (+1.3 kg), BP (+3.6/+4.1 mmHg), and carbohydrate & sucrose intake through sucrose products.

Study Population	Study Purpose/Intervention	Dietary Assessment Tool	Variables Controlled	Limitations <sup>a</sup>	Outcomes
n=9 females n=21 males 25.6±1.8 yr old Normal wt Participants willing to keep dietary record <sup>46</sup>	To investigate effect of NNS (aspartame) beverage intake on energy intake & wt over 3 week. Participants underwent 3 total conditions for 3 week each: 4 bottles NNS beverages/day, 4 bottles SSB/day, & no soda.	Daily dietary records used to assess dietary intake for 9 week. <i>NUTRITIONIST-3</i> used to analyze diet.	Excluded participants with diabetic predisposition or if dieting or pregnant. Monitored eating restraint, med use, and food preferences. Controlled for temporal issues (weather, weekends).	Small sample. Limited generalizability: mostly males. Potential for inaccurate diet records (tracked for 9 week). Short duration limited investigation of long-term NNS benefits.	NNS beverage intake period resulted in significant ↓ in energy intake (-530 kcal/day) & wt (-0.47±0.55 kg) in males. SSB intake significantly ↑ energy intake (+0.97±0.23 kg) in females & wt (+0.52±0.23 kg).
n=6 males 39.8 yr old. Normal wt Participants regular consumers of sucrose Participants willing to be inpatients on metabolic unit <sup>83</sup>	To assess energy compensation & wt when sucrose replaced with NNS (aspartame) over 3.5 week. Participants underwent 3, 6 day diet periods: 1-normal diet (sucrose), 2-NNS substitution, 3- normal diet. NNS sources: beverages, desserts, condiments, & sweetener packets.	Food weighed before & after each meal & snack to assess dietary intake over 3.5 week. USDA's Handbook Number 8 used to analyze diet.	Excluded participants with history of overweight/obese, if not in good health, dieting, or if sharing food with visitors. Participants blinded to treatment.	Limited generalizability: all men & conducted in metabolic research ward (free-living people aware of NNS intake). Small sample. Short duration limited investigation of long-term NNS benefits.	NNS period resulted in significant ↓ in energy intake (-622 kcal/day) & wt (-0.8 kg). Sucrose period resulted in significant ↑ in energy intake (+622 kcal/day) & wt (+0.8 kg).
n=163 females 42.8±9.8 yr old Healthy obese Participants willing to use or avoid NNS products <sup>47</sup>	To examine effect of NNS (aspartame) on wt loss & maintenance in wt control program: 19 week active wt loss, 1 yr maintenance, & 2 yr follow-up. Participants either used or avoided NNS foods & beverages.	3 day food records used to assess dietary intake at week 1, 19, 71, & 175. Accompanied by 7 day form containing list of NNS foods. Food records sent to Nutritional Coding Center to analyze diet.	Excluded participants if pregnant or dieting. No differences in baseline characteristics (age, wt, NNS intake, energy intake, PA level, education, hunger, & eating control) between groups. Trained to look for NNS in products. Participants blinded to treatment.	Limited generalizability: highly motivated participants, educated, middle to upper-class, obese, white women. Limited intervention compliance over time (reported ↑ of NNS intake in NNS non-consumer group).	Both NNS consumers & non-consumers lost 10 kg in active wt loss period. NNS consumers regained significantly less wt during maintenance & follow-up periods (+2.6 kg, +4.6 kg, respectively) than NNS non-consumers (+5.4 kg, +9.4 kg, respectively).
n=47 males. n=56 females. 13-18 yr old. Participants regular SSB consumers (≥1 serving/day) <sup>51</sup>	To investigate effect of replacing SSB with NNS (aspartame, sucralose) beverages or water on wt over 25 week. Participants received home deliveries of beverages each week (4 servings/day). Control group continued normal beverage patterns.	2 unannounced 24-hour recalls used to assess dietary intake at baseline & week 25. NDS-R (2003) used to analyze diet.	Excluded participants if dieting, smoking, on certain meds or with major illness or eating disorder. No differences in gender & BMI between groups. Adjusted for baseline characteristics (age, gender, race, BMI, & socioeconomic status) & daily energy intake, PA level, & TV time.	Limited generalizability: young participants. Small sample. Short duration limited ability to see long-term NNS benefits. Did not stage pubertal status (effect modifier).	NNS & water consumers with higher wt status had significant BMI change compared to SSB consumers with higher wt status (-0.63±0.23, +0.12±0.26, respectively).

Study Population	Study Purpose/Intervention	Dietary Assessment Tool	Variables Controlled	Limitations <sup>a</sup>	Outcomes
n=41 males n=45 females 57.6 yr old Normal wt, overweight, obese Participants diabetic & nondiabetic <sup>102</sup>	To evaluate effects of NNS (stevia) on blood glucose & BP on 3 groups of participants: type I diabetics, type II diabetics, & nondiabetics for 12 week. Participants received either 3-250 mg NNS capsules/day or placebo.	Did not assess diet.	Excluded participants with certain diseases, very low levels or very high BP or HbA1C, if pregnant or on certain meds. No differences in baseline characteristics (clinical & biochemical) between groups. Double-blinded.	Potentially underpowered to detect significant difference in diabetic's blood glucose levels. Limited generalizability: NNS delivered in capsule form. Short duration limited investigation of long-term NNS benefits.	NNS consumers & non-consumers did not have significant changes in BP, glucose levels, or HbA1C values from baseline to week 12.
n=88 males n=48, females 57.6±1.0 yr old Obese Participants diabetic <sup>103</sup>	To examine effect of high daily dose of NNS (sucralose) on glycemic control over 13 week. Participants received either 667 mg NNS capsule/day or placebo.	Did not assess diet.	Excluded participants with unstable or new diabetes. No differences in baseline characteristics (age, sex, race, BMI, type of diabetes meds, HbA1C, fasting glucose, & C-peptide) between groups. Double-blinded.	Limited generalizability: obese, diabetic participants using insulin or hypoglycemic meds, & NNS delivered in capsule form.	NNS consumers did not significantly differ from non-consumers in terms of HbA1C values, fasting glucose, or C-peptide from baseline to week 13. No between-group differences.
n=62 males n=60 females 60.3±1.1 yr old Obese Participants diabetic <sup>104</sup>	To assess effect of high daily dose of NNS (stevia) on diabetes over 16 week. Participants received either 1000 mg NNS capsule/day or placebo.	3 day food records used to assess dietary stability at baseline & week 16. Food Processor Nutrition Analysis & Fitness Software (version 8.60 ESHA Research) used to analyze diet.	Excluded participants with unstable hypertension, certain diseases, recent heart issue, or if using certain meds or pregnant. No differences in baseline characteristics (fasting glucose, insulin, C-peptide, wt, BP, lipids, diet) between groups. Participants kept stable diet. Double-blinded.	Limited generalizability: obese, diabetic participants & NNS delivered in capsule form.	NNS consumers did not significantly differ from non-consumers in terms of HbA1C values, fasting glucose, insulin levels, C-peptide, BP, body wt, or fasting lipids from baseline to week 16. No between-group differences.

<sup>a</sup> Self-reported dietary intake subject to bias (misreport, recall, response, misclassification biases).

Ace-K = acesulfame potassium

BMI = body mass index (kg/m<sup>2</sup>)

BP = blood pressure

HbA1C = Hemoglobin A1C

NNS = non-nutritive sweeteners

PA = physical activity

SSB = sugar-sweetened beverage

USDA = United States Department of Agriculture

Wt = weight

**Table 3. Observational Studies Assessing Cardiometabolic Risk in Humans**

Study Population	Study Purpose/ Intervention	Dietary Assessment Tool	Differences between NNS Consumers & Non- Consumers	Variables Controlled	Limitations <sup>a</sup>	Outcomes
n=5,158 males and females n=3,301 Mexican n=1857 white 25-64 yr old <sup>37</sup>	Assessed relationship between NNS beverage intake & long-term wt gain over 8 yr. Measured height, & wt at baseline & at 1 follow-up.	Baseline 24-hour recall used with cohort I. Study questionnaire used to assess NNS beverage & sugar substitute intake in cohort II. Participants put into beverage groups based on intake frequency. Nutrition analysis program not reported.	NNS consumers had greater age, education, socioeconomic status, & PA level. More likely to be dieting, female, overweight/obese. Less likely to be Hispanic or smokers. Lower intake of total energy, carbohydrate, & sucrose kcal, alcohol, SSB, & milk. Higher intake of protein & total & saturated fat.	Exclusion criteria not reported. Adjusted for age, BMI (baseline), changes in PA level, education, gender, & socioeconomic status.	Limited generalizability: mostly Mexican. Follow-up NNS use administered with cohort II only. Cannot specify between NNS type. Underestimation of NNS intake possible (analyzed NNS cola, coffee, tea only).	Significant positive dose-response relationship between baseline NNS intake & incidence of overweight/obese & BMI change. Risk of overweight/obese significantly higher in baseline normal weight & overweight participants consuming >21 NNS beverages/week than non-consumers. BMI change significantly higher for NNS consumers; insignificant for diabetics or if PA levels changed.
n=749 males and females Mexican & White participants ≥65 yr old <sup>35</sup>	Assessed relationship between NNS cola intake & waist circumference change over 12 yr. Measured waist circumference, height, & wt at baseline & 4 follow-ups.	Study questionnaire used to assess NNS cola intake at baseline & follow-ups. Participants put into beverage intake groups based on intake frequency. Nutrition analysis program not reported.	NNS consumers had higher education level & baseline prevalence of overweight/obese. More likely to be white & less likely to be low-income. NNS consumers not different from non-consumers in terms of age or sex. NNS non-consumers highest SSB consumers.	Exclusion criteria not reported. Adjusted for age, BMI (baseline), education, ethnicity, diabetes, neighborhood, PA level, & waist circumference (baseline).	Limited generalizability: older adults. Complete diet data unavailable so results unadjusted for energy intake. Underestimation of NNS intake possible (analyzed NNS cola only).	Significant positive dose-response relationship between high NNS cola intake & ↑ in waist circumference. Mean interval waist circumference change for NNS consumers 3 times higher than non-consumers (2.11 cm vs. 0.77 cm, respectively).
n=6,814 males and females. White, Black, Hispanic, & Chinese participants 45-84 yr old <sup>53</sup>	Examined relationship between NNS cola intake & risk of MetS & diabetes over 5 yr. Assessed diabetes & MetS & components at 3 follow-ups.	Study FFQ used to assess NNS cola & SSB (cola) intake at baseline. Participants put into beverage intake groups based on intake frequency. Nutrition analysis program not reported.	NNS consumers more likely to be white. SSB consumers more likely to be black or Hispanic.	Excluded participants with unreliable dietary data. Adjusted for age, BMI (baseline), education, energy intake, ethnicity, PA level, socioeconomic status, sex, waist circumference (baseline), & diet habits specific to diabetes, & MetS.	Limited generalizability: older adults. Mediation by adiposity & fasting glucose change & by diabetes. Misreporting (portions reported as small, medium, or large) likely. FFQ paired NNS cola with mineral water. Underestimation of NNS intake possible (analyzed NNS cola only).	Significantly greater risk of diabetes & high waist circumference associated with daily NNS cola intake. Daily intake of NNS cola associated with 36% higher risk for incident MetS (insignificant after adjusting for adiposity) & 67% greater risk for diabetes (significant) compared to non-consumers.

<b>Study Population</b>	<b>Study Purpose/ Intervention</b>	<b>Dietary Assessment Tool</b>	<b>Differences between NNS Consumers &amp; Non-Consumers</b>	<b>Variables Controlled</b>	<b>Limitations<sup>a</sup></b>	<b>Outcomes</b>
n=74 males n=90 females. White children participants Grades 3-6 <sup>52</sup>	Evaluated relationship between beverage intake & BMI in children over 2 yr. Collected total energy intake, height, & wt at baseline & yr 2.	24-hour recall used to assess NNS cola, SSB (cola), milk, juice & energy intake at baseline & yr 2. Nutritionist IV (ESHA Research, Salem, Oregon) used for beverage & energy analysis.	NNS consumers more likely to be female, overweight/weight gaining, & less likely to drink milk.	Exclusion criteria not reported. Adjustment factors not reported.	Small sample size. Limited generalizability: rural, children, white, & girls. Data collected only on school days. 2 24-hour recalls occurred in fall & spring, limiting accurate comparisons. Underestimation of NNS intake possible (analyzed NNS cola only).	NNS cola intake significantly higher in overweight & weight gaining participants than normal weight participants. Intake of 1 NNS cola/day ↑ BMI Z-score by 0.156 but only explained 3.5% of variance in BMI Z-score (rest explained by baseline BMI). NNS cola only beverage linked to yr 2 BMI Z-score.
n=2,569 males n=3,470 females White participants Mean age=52.9 yr old <sup>67</sup>	Assessed relationship between NNS cola & SSB (cola) intake & MetS incidence over 4 yr. Compared effects of SSB to NNS colas. ≥1 follow-ups.	Self-administered Willett FFQ used to assess NNS cola & SSB (cola) intake at follow-ups. Participants put into beverage groups based on intake frequency. Nutrition analysis program not reported.	NNS & SSB consumers were not compared (beverages combined in defining intake groups).	Excluded participants with CVD, MetS, or missing diet or health info. Adjusted for age, diet habits, PA level, sex, & total energy intake. Also adjusted for baseline covariates (blood sugar, BP, HDL, triglyceride, & waist circumference).	Limited generalizability: all white. Underestimation of NNS intake possible (analyzed NNS cola only).	Intake of ≥1 cola/day (NNS or SSB, similar risk) associated with higher risk for MetS, obesity, ↑ waist circumference, impaired fasting glucose, high BP, high triglyceride, & low HDL. New diagnoses of MetS reported by 22.6% of participants consuming ≥1 cola/day at 4 yr follow-up.
n=4,197 males n=5,317 females White & black participants 45-64 yr old <sup>68</sup>	Assessed relationship between diet & MetS incidence over 9 yr. Categorized diets: Prudent (healthy) & Western (unhealthy). 3 follow-ups.	Modified Willett FFQ used to assess diet pattern & NNS cola & SSB (cola & fruit drinks) intake at baseline & yr 6. Nutrition analysis program not reported.	NNS consumers more likely to be women.	Excluded participants with MetS, CVD or unreliable diet info. Adjusted for age, anthropometrics (baseline), education, race, PA level, total energy intake, & diet habits.	Limited generalizability: middle-aged adults. Relationship with NNS cola intake unexpected. Underestimation of NNS intake possible (analyzed NNS cola only).	NNS cola intake significantly associated with ↑ risk of MetS (34% greater risk for highest NNS category than lowest). Consuming Western diet significantly adversely associated with incident MetS.
n=14,900 males and females White, black, Mexican participants 18-75 yr old <sup>101</sup>	Used cross-sectional NHANES data to assess relationship between cola intake & glucose control in diabetics & nondiabetics.	NHANES III FFQ used to assess beverage intake at time 1. 1 day recall used to measure diet habits. Nutrition analysis program not reported.	NNS consumers more likely to have diabetes.	Exclusion criteria not reported. Adjusted for age, BMI, education, ethnicity, diet habits, poverty, PA level, sex, & yr since diabetes diagnosis. Generalizable	Portion size unspecified. Survey outdated (NNS products change). Underestimation of NNS intake possible (analyzed NNS cola only).	Diabetics drank 3x amount of NNS cola as nondiabetics. Diabetics drinking ≥1 NNS cola/day had significantly higher HbA1C level (0.7 units) than diabetics who were not NNS cola consumers.



Study Population	Study Purpose/ Intervention	Dietary Assessment Tool	Differences between NNS Consumers & Non- Consumers	Variables Controlled	Limitations <sup>a</sup>	Outcomes
n=4,161 males and females White & black participants 18-30 yr old <sup>36</sup>	Examined relationship between diet type, NNS cola intake, & cardio-metabolic risk over 20 yr. Evaluated interaction between diet (Western & Prudent) & NNS cola intake. 6 follow-ups.	Interviewer- administered Coronary Artery Risk Development in Young Adults Diet History questionnaire & quantitative diet history tool used to assess diet at baseline. NDS-R used to analyze diet.	NNS consumers more likely to have Prudent diet, be female, white, older, college-educated, & have baseline overweight status. NNS consumers with Prudent diet: more PA than non-consumers with Prudent diet. NNS consumers with Western diet: higher BMI, waist circumference, HDL levels, & baseline obese status than NNS non- consumers with Western diet. NNS non-consumers had higher total energy intake in both diets.	Excluded participants at baseline with high BP, high fasting glucose, high triglyceride, high waist circumference, low HDL, & MetS. Adjusted for age, education, ethnicity, PA level, sex, wt (baseline), & total energy intake.	Limited generalizability: young adults. Did not exclude subjects with 2 baseline criteria for MetS. NNS non- consumer group included those who drank SSB & water. Underestimation of NNS intake possible (analyzed NNS cola <sup>70</sup> only).	NNS non-consumers with Prudent diet had lowest risk of MetS, high waist circumference, & high triglyceride compared to NNS consumers with Western diet. ↓ risk of MetS for Prudent diet maintained even after stratification for NNS intake.
n=43,371 males 40-75 yr old n=84,085 females 30-55 yr old White participants <sup>70</sup>	Assessed relationship between cola intake & stroke risk over 22-28 yr. Participants reported medical history & lifestyle at baseline & every 2 yr.	Willett FFQ used to assess NNS cola & SSB (cola) intake at baseline (only men) & every 4 yr (women & men). 5- 6 follow-up FFQ sent. Nutrition analysis program not reported.	NNS consumers had higher BMI & rates of chronic disease.	Excluded participants with unreliable diet info, cancer history or diagnosis, diabetes, angina, MI, stroke, & CVD. Adjusted for BMI, dietary & non- dietary CVD risk factors, PA level, & total energy intake.	Limited generalizability: white adults. Associations between NNS & stroke risk not prevalent in previous findings. No clear biologic mechanism. Underestimation of NNS intake possible (analyzed NNS cola only).	Significant stroke risk posed by intake of ≥1 serving/day of NNS or SSB cola. Slight attenuation of associations when adjusted for hypertension, diabetes, BMI, total energy intake, & baseline wt change.
n=40,389 males White participants 40-75 yr old <sup>13</sup>	Evaluated relationship between SSB & NNS beverage (colas & non-colas) intake and incident diabetes over 20 yr. Participants reported medical history & lifestyle at baseline & every 2 yr.	Willett FFQ used to assess NNS beverage & SSB intake at baseline every 4 yrs. 5 follow-up FFQ sent. Nutrition analysis program not reported.	NNS consumers had better overall diet quality (lower intake of red/processed meats, carbohydrate, & total energy; higher intake of protein & total fat). NNS consumers had higher BMI & PA levels & more likely to have diabetic predisposition & high triglyceride & BP levels.	Excluded participants with early incidences of diabetes. Adjusted for age, BMI, BP, family history, dieting, total energy intake, wt change (pre- enrollment) & triglyceride (baseline).	Limited generalizability: white men. Underestimation of NNS intake possible (analyzed NNS beverages only).	Significant association between NNS beverage intake & diabetes in age- adjusted analysis but not in multivariate-adjustment. Association between NNS beverage intake & diabetes mostly explained by health status, baseline wt change, dieting, & BMI. Significant association between high SSB intake & diabetes risk.

Study Population	Study Purpose/ Intervention	Dietary Assessment Tool	Differences between NNS Consumers & Non- Consumers	Variables Controlled	Limitations <sup>a</sup>	Outcomes
n=2,564 males and females Hispanic, white, & black participants Mean age 69±10 yr old <sup>69</sup>	Assessed relationship between cola intake & vascular events (stroke, MI, vascular death) over 10 yr.	Interviewer- administered Modified Block FFQ used to assess NNS cola & SSB (cola) intake & diet at baseline. Participants put into beverage groups based on intake frequency. Nutrition analysis program not reported.	NNS consumers more likely to be white & have high BP, high triglyceride, high BMI, hypertension, increased waist circumference, lower HDL, & chronic diseases. SSB consumers more likely to be male, black, with higher carbohydrate intake, higher BP, higher cholesterol, & lower prevalence of diabetes.	Excluded if participant had history of stroke or heart attack or unreliable diet info. Adjusted for age, blood glucose, BMI, BP, cholesterol, CVD, diabetes, diet, education, race, PA level, sex, triglyceride, & waist circumference. Sensitivity analysis excluded obese participants with history of diabetes or MetS (limited power).	Limited generalizability: mostly older Hispanics & high BMI. Cannot specify brands. Only collected diet & cola habits at baseline (patterns change). Limited power (NNS intake low in sample). Underestimation of NNS intake possible (analyzed NNS cola only).	Daily NNS cola consumers had significantly higher risk of vascular events (remained after adjusting for MetS, diabetes, CVD, hypertension, & high cholesterol). Light SSB & NNS cola intake (1 serving/month) not associated with ↑ risk of vascular event.
n=66,118 females French participants Mean age 52.6±6.6 yr old <sup>71</sup>	Assessed relationships between SSB, NNS beverage, & 100% fruit juice intake & diabetes risk over 14 yr. Subjects updated health info every 2-3 yr.	Self-administered French Diet History Questionnaire used to assess food & SSB, NNS beverage & 100% fruit juice intake at baseline. Nutrition analysis program not reported.	NNS consumers had more variation in NNS intake than non-consumers in SSB intake (standard deviation 568±129.5 ml/week vs 328±69.4 ml/week). NNS consumers more likely to be obese women & have prediabetes or diabetes.	Excluded participants with diabetes or unreliable diet info. Adjusted for BMI, cholesterol, diet, diabetes history, education, hypertension, & PA level. Excluded early diabetic cases in models to identify reverse causation between intake of NNS & diabetes risk (similar associations).	Limited generalizability: middle-aged, overwt, French females. Cannot specify sweetener type. Beverage intake not updated during study (changes). Limited power when stratified by BMI. Underestimation of NNS intake possible (analyzed NNS cola and fruit beverages only).	Strong positive relationship between diabetes risk & SSB & NNS beverage intake across all intake groups. Highest consumers of SSB & NNS beverage at higher risk of diabetes. BMI & adiposity slightly attenuated magnitude for relationship between high SSB & NNS beverage intake & diabetes but significance remained.

<sup>a</sup> Observational data cannot determine causality due to potential for reverse causality & residual confounding. Self-reported dietary intake subject to bias (misreport, recall, response, misclassification biases).

FFQ = food frequency questionnaire

BMI = body mass index (kg/m<sup>2</sup>)

BP = blood pressure

CVD = cardiovascular disease

HbA1C = Hemoglobin A1C

HDL = high density lipoprotein cholesterol

MetS = metabolic syndrome

NDS-R = Nutrition Data System for Research

NHANES = National Health and Nutrition Examination Survey

NNS = non-nutritive sweeteners

PA = physical activity

SSB = sugar-sweetened beverage

Wt = weight

## Difficulties in Measuring Non-nutritive Sweetener Intake

The research community needs to address the uncertainties in measuring NNS intake to better understand the associations between NNS intake and certain disease states and conditions. Self-reported dietary intake assessment methods, which include dietary intake records or diaries, 24-hour dietary recalls, and food frequency questionnaires (FFQ) are often used for diet and health research studies (Table 2 and Table 3). A dietary intake record is an approach that requires the respondent to record the amount of all foods and beverages consumed over a consecutive three to four day period<sup>112</sup>. In a 24-hour dietary recall, respondents report all foods and beverages consumed during the past 24 hours or previous day in an interview which takes place face-to-face or via telephone. A FFQ requires respondents to report typical frequency and quantity of listed food and/or beverage items. FFQ can be developed to estimate habitual intake of particular food groups or items of interest, such as NNS<sup>112</sup>. Unlike food intake records and 24-hour recalls which only provide data on recent intake, FFQ can provide a representative summary of an individual's food intake over a longer period of time (i.e. habitual intake)<sup>112</sup>.

Misreporting is a common limitation of all self-reported dietary methods. Misreporting occurs when respondents inaccurately estimate portion sizes during report of previously consumed foods<sup>113</sup>. Low literacy can affect a respondent's ability to accurately report portion sizes due to literacy and language barriers and/or low motivation levels, decreasing the reliability and quality of the response. Often times, large portion sizes are underestimated while small portion sizes are overestimated<sup>112,113</sup>. For low literate populations, interviewer-administered diet tools are preferable over self-

administration, as interviewers can provide additional assistance and use portion size models to elicit a more accurate estimation of diet habits<sup>112</sup>. Similarly, it is common for participants to experience recall or information bias, which occurs when they are unaware of or unknowingly consume foods that contain NNS or they simply do not remember eating a NNS-containing food<sup>19,36-38</sup>.

In early randomized controlled trials, food intake records were often used to assess NNS intake<sup>46,47</sup>. However more recent randomized controlled trials utilize 24-hour dietary recalls<sup>49-51</sup> as well as food frequency questionnaires<sup>52</sup>. Observational studies typically use food frequency questionnaires<sup>13,35-37,53,67-71,101</sup> either alone or combined with 24-hour dietary recalls. The literature shows a shift toward food frequency questionnaires to assess NNS intake, as they are inexpensive in terms of cost and time and can be self-administered and optically scanned, which facilitates analysis of typical dietary intake in large studies<sup>112</sup>. Common food frequency questionnaire include modified versions of the Willett Food Frequency Questionnaire<sup>13,67,68,70</sup>, the Block Questionnaire from the National Cancer Institute<sup>69</sup>, and the NHANES III Food Frequency Questionnaire<sup>101</sup>. Some studies developed their own questionnaires to contain information specifically pertaining to the study<sup>35-37,53,71</sup>. However, a variety of limitations exist with the NNS dietary assessment tools, specifically food frequency questionnaires, used up to this point. These limitations go beyond the inherent issues of self-reported dietary assessment tools.

Limitations pertaining specifically to current food frequency questionnaires include the lack of type or brand name description of NNS beverages<sup>35,37,69,71,101</sup>, the pairing of NNS products with non-NNS products (like unsweetened mineral water)<sup>53</sup> which can lead to food and beverage misclassification, and the use of subjective serving

sizes (small, medium, or large beverages)<sup>53,101</sup>. Often times, participants are asked very general questions about their NNS use; questions that only probe about the frequency and/or quantity of sugar-free beverage consumption, not the specific beverage type or brand<sup>35,37,69,71,101</sup>. This nondescript approach limits the accuracy of intake data because the NNS type (acesulfame-potassium, aspartame, saccharin, sucralose) is not captured. Another issue is that the majority of the assessment tools are only intended to measure NNS intake in beverages<sup>35-37,53,69</sup>, including the modified versions of the Willett food frequency questionnaire<sup>13,67,68,70</sup>, the Block Questionnaire from the National Cancer Institute<sup>69</sup>, and the NHANES III food frequency questionnaire<sup>101</sup>. Even more, many of these tools collect diet intake data on NNS colas (diet sodas) only<sup>35,36,52,53,67,68,101</sup>, while not accounting for juice drinks, mixes, teas, or other non-carbonated NNS beverages. The focus on NNS beverages, particularly colas, can lead to underestimation of NNS intake, which could affect conclusions. Clearly, this approach to measuring NNS intake based mostly on beverages, specifically colas, limits the adequacy of the defining terms or the defining intake level that constitutes a NNS consumer in studies. Current observational studies label participants as NNS consumers or NNS non-consumers based solely on the frequency and quantity of their cola intake, without considering the contribution of NNS from food items<sup>35,36,52,53,67,68,101</sup>. While the health effects of replacing SSB with NNS beverages is important, it is imperative to assess the effects of NNS-containing food products on health outcomes as well<sup>19</sup>.

Another difficulty in measuring NNS intake pertains to the databases used for dietary intake analysis. Analyzing 24-hour recalls and developing food frequency questionnaires requires access to a reliable nutrition database. Research quality U.S.

databases include the University of Minnesota Nutrition Coordinating Center's (NCC) Food and Nutrient Database, the USDA National Nutrient Database for Standard Reference (USDA SR), and the USDA Food and Nutrient Database for Dietary References (USDA FNDDS)<sup>114</sup>. The NCC is more comprehensive than the others, providing access to the highest number of food items, brand name products, and nutrients/food components (18,200, 8,000, 165, respectively) compared to the USDA SR (8,463, 1,300, 148, respectively) and the USDA FNDDS (7,253, 2,000, 64, respectively)<sup>114</sup>. In terms of completeness of nutrient values (i.e. specific macro- and micro-nutrient content of foods in database) in these databases, the NCC provides 90-100% completeness, the USDA SR provides 0-100%, and the USDA FNDDS provides 100%<sup>114</sup>. While commercial databases also exist and generally contain more products than research databases, they are limited to the nutrients included on the nutrition facts panel<sup>114</sup> which is not sufficient for NNS intake studies, as NNS is not quantified on nutrition facts panels. Comparing these databases highlights the importance of using reliable dietary information to investigate health effects.

With a high consumer demand for low-calorie and sugar-free products, databases will need to enhance the effort to keep up with the ever-changing NNS market<sup>19</sup>. This is critical because new products often contain varying combinations and concentrations of different types of NNS<sup>40</sup>. Thus, a reliable nutrition database needs to keep up with the marketplace and continually update its products for research purposes. An updated nutrition database containing current NNS food products will help to guide accurate development of dietary intake assessment tools<sup>19</sup>. In the past, nutrition databases were not providing accurate NNS composition values<sup>40</sup>. The lack of accurate data in nutrition

databases was caused by food processors and manufacturers not making specific information about the amount of NNS in foods and beverages widely accessible<sup>40</sup>, which was permitted because NNS is classified as a food additive<sup>38</sup>. This left both consumers and researchers struggling to accurately report NNS consumption patterns<sup>40</sup>. For example, Piernas et al. had to approximate NNS consumption because older versions of Nutrition Data System for Research (specifically version 4.03\_31, 2000) [NDS-R]), a software program developed by the NCC, only listed estimates of NNS in typical products<sup>50,115</sup>; in fact until 2005, NDS-R did not contain acesulfame-potassium or sucralose<sup>116</sup>. Previous NNS estimation in products complicates analysis of the current body of literature on NNS, as it is difficult to determine the accuracy of older assessment tools. However, recent versions of NDS-R report none to very low estimate percentages for NNS in products (acesulfame-potassium and sucralose percent estimated = 0% [2006-2014], aspartame and saccharin = 2% [2006] and 1% [2014])<sup>117,118</sup>. Additionally, NDS-R releases a new version of their database every year, with the goal to update and expand specific nutrients and keep pace with the dynamic marketplace<sup>119</sup>. A systematic process, driven by relevance to science, is employed in the adding of new and reformulated products to NDS-R<sup>119</sup>. This process also takes special consideration of categories that are more subject to change, such as ready-to-eat cereals, and of products that experienced a marketplace shift, such as margarines after the reformulation to reduce trans fatty acids<sup>119</sup>. Updates also occur when more accurate analytical methods are used to provide nutrient values for food items in the database. Furthermore, updates in foods and nutrients in USDA SR and USDA FNDDS are also incorporated into NCC's NDS-R<sup>119</sup>. Although NDS-R currently has nutrient information for only 36 NNS brand name

products and despite the lack of information regarding monk fruit extract and stevia, as previously mentioned, the database contains over 18,000 products<sup>114</sup> and plans to update their NNS category in 2015<sup>120</sup>. These efforts indicate the ability of NDS-R to keep pace with the marketplace.

To revise and develop effective assessment tools, these systemic issues will need to be addressed using participant training in portion sizes and NNS-containing products, psychosocial questions to estimate level of underreporting<sup>112</sup>, and descriptive questionnaires containing both beverage and food items developed using updated versions of nutrition databases. These updates will facilitate the research community in accurately measuring NNS intake, which will enable the thorough investigation of potential NNS-related health outcomes<sup>19</sup>.

## **Conclusion**

NNS has been suggested as a method to facilitate reductions in energy intake, weight, and cardiometabolic risk. However potential health outcomes, including cancer, weight loss, weight gain, physiological and intestinal changes, cardiovascular disease, diabetes, and migraines, have been reported in both observational studies and randomized controlled trials. This uncertainty makes it increasingly important to address the limitations of current NNS literature. Difficulties in measuring NNS intake stem from differing metabolic pathways between animal and human models, small sample sizes and short study durations of randomized controlled trials, numerous potential confounders in observational studies, and inadequate dietary methodology. Currently, the common dietary assessment methods include 24-hour recalls and food frequency questionnaires. The accuracy of previously used food frequency questionnaires is limited by the lack of



type or brand name description of NNS beverages, use of subjective serving sizes, and the emphasis on beverage, specifically cola, NNS products. Additionally, there is a need for dietary assessment tools to utilize updated databases that keep pace with the dynamic marketplace. To understand the relationship between NNS and various disease states, it is important to address these limitations. This process starts with the characterization of current NNS intake patterns, which will facilitate the development of more comprehensive and representative dietary assessment tools.

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## **Chapter 2: Characterization of Non-nutritive Sweetener Patterns**

### **Abstract**

Controversy surrounds the use of artificial sweeteners (non-nutritive sweeteners [NNS]) as an effective weight-loss and maintenance strategy. Minimal data assessing NNS intake is available, and no literature exists for rural, health-disparate areas, where obesity and related comorbidities are prevalent. The objective of this investigation was to characterize the NNS consumption patterns of a rural adult population. A cross-sectional sample of Southwest Virginian adults completed three 24-hour dietary recalls. NNS intake was characterized by exploring differences in demographic characteristics, anthropometrics, biochemical markers, dietary quality, and dietary intake between NNS consumers and NNS non-consumers, as well as by evaluating the overall frequency, type, and sources of NNS consumption. NNS consumption was extracted from the component/ingredient level of dietary intake using nutrition analysis software (NDS-R). Among participants (n=301) (aged  $41.8 \pm 13.4$  years; mean BMI= $33.0 \pm 9.1$ ), 33% (n=100) reported consuming NNS. Although NNS consumers had better overall Healthy Eating Index 2010 (HEI-2010) scores for dietary quality and healthier dietary factors than NNS non-consumers, they were found to have a higher body mass index (BMI) status and lower Healthy Eating Index 2010 (HEI-2010) scores for intake of sodium and refined grains. There were no differences in biochemical markers between NNS consumers and NNS non-consumers. Mean daily NNS intake for NNS consumers was  $869 \pm 3,553$  mg, with sucralose being the largest contributor by weight, followed by aspartame, acesulfame potassium, and saccharin. NNS added at the table, diet tea, and diet soda were the top contributors to absolute NNS intake (38%, 34%, 27%). Diet soda, juice drinks, and table NNS were the most frequently consumed NNS dietary sources (39%, 17%, and 16% respectively). NNS consumption for this sample was similar to the

national average intake (2007-2008 NHANES and 2012 Food and Health Survey) (33% vs 28-30%). Implementing weight loss/control strategies that utilize NNS as a replacement for sugar-sweetened items may be a plausible intervention within this rural population. These findings could inform the development of a dietary intake assessment tool that accurately measures NNS intake, which would facilitate the inferential testing of associations of NNS consumption on weight status and related co-morbidities.

## Introduction

Replacing added sugars in the diet with non-nutritive sweeteners (NNS) to reduce appetite, body weight, and/or cardiometabolic risk factors is a controversial topic<sup>1</sup>. About 30% of U.S. adults consume NNS on any given day<sup>2,3</sup>. However, potential associations between cardiometabolic health outcomes and NNS intake have been reported in randomized controlled trials and observational studies, including weight loss<sup>4-10</sup>, weight gain<sup>11-14</sup>, physiological and intestinal changes<sup>15-27</sup>, cardiovascular disease<sup>12,14,28-32</sup>, and diabetes<sup>14,33,34</sup>. Still, a variety of issues limit determination of causality in these studies. Limitations include small sample sizes and short study duration of randomized controlled trials<sup>1,13</sup>, potential for many confounders in observational studies<sup>12,28,30-32,35</sup>, and inadequate dietary intake assessment tools<sup>1</sup> that focus mainly on NNS beverage intake, specifically diet sodas<sup>36-38,40,70,71,101</sup>. Thus, NNS intake is often underestimated in observational studies<sup>36-38,40,70,71,101</sup>. Additionally, these tools do not specify type or beverage brand, as general questions about NNS intake are used<sup>35,37,39,41,101</sup>; this approach limits determination of type of NNS (acesulfame-potassium, aspartame, saccharin, and sucralose) consumed in products<sup>1,3</sup>. To gain a better understanding of the potential health outcomes associated with NNS consumption, it is necessary to understand these NNS intake patterns, as well as the characteristics of NNS consumers.

An extensive literature review identified studies that assessed NNS intake patterns, along with demographic, dietary, lifestyle, and biochemical characteristics of NNS consumers. Consumers of NNS were typically older<sup>11,30</sup>, Caucasian<sup>12,14,30,31</sup> women<sup>11,13,30,34</sup> with higher weight statuses<sup>11,12,30-34</sup>, and more chronic disease risks<sup>13,11,30,32,33</sup>. NNS consumers also tended to have higher levels of education<sup>11,12,30</sup> and socioeconomic status<sup>11,12</sup>, and were more likely to engage in dieting behavior<sup>11,33</sup> than NNS non-consumers. Usually their diets were lower in

overall calories, as well as lower in calories from carbohydrates, sugar and sugar-sweetened beverages, alcohol, and milk<sup>11,30,33</sup>. These findings were also corroborated by Sylvetsky et al., in the first study to assess national consumption trends of NNS foods and beverages using National Health and Nutrition Examination Survey (NHANES) data<sup>3</sup>. These national trends showed that 28% of the U.S. population (33% of females and 25% of males) consumed NNS during 2007-2008<sup>3</sup>. Of these consumers (both male and female), 38% were  $\geq 55$  years old (29% 39-54 and 15% 18-34 years old), 36% were Caucasian (22% Black and 25% Hispanic), 36% were obese (30% overweight and 22% normal), and 38% were in the highest income tertile (28% middle and 22% low)<sup>3</sup>. Similarly, the *Food and Health Survey: Consumer Attitudes toward Food Safety, Nutrition & Health*<sup>2</sup>, commissioned by the International Food Information Council Foundation, reported that among a nationally representative sample of 1,057 U.S. adults (18-80 years old), 30% of consumers reported consumption of NNS in 2012<sup>2</sup>. However, characterization of NNS intake in rural populations has yet to be explored. NNS intake patterns in rural populations are especially pertinent, as this group is at a higher risk for obesity and a variety of chronic health conditions<sup>36-39</sup>. Furthermore, since rural-residing adults have higher prevalence and levels of sugar-sweetened beverage consumption than urban-residing adults, NNS intake could serve as a method to facilitate weight loss and/or maintenance in rural populations<sup>40</sup>. The first step in determining the applicability of this strategy is characterizing NNS intake patterns in a rural population to determine who uses NNS and how is NNS being used.

In 2010, the most prevalent NNS in products was sucralose (2,500), followed by acesulfame-potassium (1,103), and aspartame (974)<sup>41</sup>. While the most prevalent NNS in products has been assessed, the most commonly consumed *type* of NNS, (i.e., acesulfame-potassium, aspartame, saccharin, and sucralose) has not been investigated<sup>1,3</sup>. This is important because each

sweetener is composed of different compounds and is metabolized differently, which could impact health outcomes. Acesulfame-potassium is composed of organic acid and potassium and is excreted mostly unchanged in the urine<sup>42</sup>, while aspartame, a methyl ester of aspartic acid and phenylalanine dipeptide<sup>43</sup>, is metabolized in the intestines to aspartic acid, methanol, and phenylalanine<sup>43</sup>, and then absorbed into the general circulation<sup>43</sup>. Similar to acesulfame-potassium, saccharin is not metabolized and is excreted unchanged<sup>44</sup>. Sucralose, which is sucrose with three chlorine molecules instead of three hydroxyl groups<sup>45,46</sup>, is minimally absorbed and mostly excreted unchanged in the feces or urine<sup>47</sup>.

### **Aims and Hypotheses**

**Aim 1:** To explore differences between NNS consumers and NNS non-consumers in:

- a) baseline demographic characteristics (gender, age, race, education status, socioeconomic status, and health literacy),
- b) anthropometrics (weight and BMI),
- c) biochemical markers (blood pressure, blood glucose, cholesterol [total, high density lipoprotein, low density lipoprotein], and triglyceride concentrations),
- d) dietary quality (Health Eating Index scores-2010), and
- e) dietary intake (total energy, total beverages, sugar-sweetened beverages, water, carbohydrates, added sugar, protein, fat, alcohol, sodium, and energy density).

**Hypothesis 1:** Based on previously reviewed observational studies, it is hypothesized that compared to NNS non-consumers, NNS consumers will:

- a) be older<sup>11,30</sup>, Caucasian<sup>12,14,30,31</sup> women<sup>11,13,30,34</sup> with higher levels of education<sup>11,12,30</sup> and socioeconomic status<sup>11,12</sup>. Based on higher education and income levels, it is also hypothesized that NNS consumers will have higher health literacy.

b) have higher weight and BMI statuses<sup>11,12,30-34</sup>,  
c) have biochemical values associated with risk for chronic disease<sup>28,30,31,33,35</sup>,  
d) and e) have healthier dietary quality and dietary factors than NNS non-consumers because many observational studies reported that NNS consumers' diets were typically lower in total energy, sugar-sweetened beverages, alcohol, carbohydrates, and added sugars<sup>11,30,33</sup>.

**Aim 2:** To characterize non-nutritive sweetener intake (frequency, type, and sources of sweetener) in a sample of rural Southwest Virginian adults from the Talking Health study.

**Hypothesis 2:** Although 30% of U.S. adults consume NNS<sup>1,3</sup>, it is hypothesized that less of this rural population will consume NNS, as rural adults have been shown to be high sugar-sweetened beverage consumers<sup>40</sup>. Based on production trends<sup>41</sup>, it is hypothesized that the most commonly consumed types of NNS in order from highest to lowest will be sucralose, acesulfame-potassium, and aspartame. Based on national consumption trends, it is hypothesized that the most prevalent source of NNS is diet soda, followed by reduced-calorie beverages (light fruit juices and lemonades)<sup>2,3</sup>.

### **Study Design and Subjects**

This characterization will utilize baseline, cross-sectional dietary data, collected from 2014, from a large, community-based, randomized-controlled behavioral trial, Talking Health. The primary purpose of Talking Health was to evaluate the effectiveness of a six-month intervention aimed at decreasing the consumption of sugar-sweetened beverages versus a matched contact comparison group aimed at increasing levels of physical activity<sup>48</sup>.

Eligible participants were English-speaking adults ( $\geq 18$  years old) consuming  $\geq 200$  sugar-sweetened beverage kcal per day, with access to a telephone and no physical activity

limitations<sup>48</sup>. Sugar-sweetened beverage consumption was determined with the Beverage Intake Questionnaire (BEVQ-15), which is a quantitative food-frequency tool that evaluates the frequency and volume of consumption of different beverages over the previous month<sup>49</sup>.

This study targeted adults residing in an eight county rural region in southwest Virginia, with a goal to recruit participants of low socioeconomic and literacy levels. This region's average rurality status is 6.1 (SD±2.5) out of 9 on the Rural-Urban Continuum Codes (where 1=urban and 9=very rural)<sup>50</sup>. Residents of this region are mostly Caucasian (95%), with a high school education or less (58%), and 18% live below the federal poverty line, according to the U.S. Census Bureau<sup>51</sup>.

Trained Extension Program Assistants and/or community members assisted with recruitment of participants, however recruitment methods were tailored to each county. Organizations serving low resource populations, like Head Start and the Department of Social Services, were also targeted. Other recruitment methods included flyers in community sites, newspaper postings, and recruitment postcards sent to addresses provided by a preexisting Cooperative Extension database and/or a mailing company that identified low socioeconomic communities<sup>48</sup>.

Eligible participants were randomized to one of two study arms: the SIPsmartER (sugar-sweetened beverage reduction) intervention group or the MoveMore (targeting physical activity behaviors) comparison group. The goal of the SIPsmartER intervention was to decrease sugar-sweetened beverage consumption to the recommended level of  $\leq 8$  fluid ounces per day; conversely, the goal of the MoveMore intervention was to increase physical activity to the recommended duration of 150 minutes of moderate-intensity and/or muscle strengthening activities per week. Over six months, both groups' intervention structures consisted of three



small group classes, one live teach-back call, and eleven interactive voice response telephone calls; additional intervention components included individual action plans and behavior charts to track behavior and interactive voice response telephone calls<sup>48</sup>.

## **Methods**

Baseline data collection included anthropometrics (height, weight, BMI), blood pressure, glucose, cholesterol (total, high density lipoprotein [HDL], and low density lipoprotein [LDL]), and triglyceride concentrations. Additionally, measures of dietary intake (24-hour dietary recalls) and demographic characteristics (age, gender, ethnicity/race, income, education status, health literacy) were also collected<sup>48</sup>.

Height and weight were assessed in light clothing without shoes using a research-grade stadiometer and a calibrated digital Tanita scale (Model: 310GS). An automated oscillometric device OMRON (Model: HEM-907XL) was used to measure blood pressure<sup>52</sup> before any blood sampling, following the American Heart Association guidelines. Two measurements, one minute apart, with appropriate cuff size, were taken while the participant was in an upright sitting position. The average of the measurements was determined. Routine finger sticks with a One Touch Fine Point Lancet (Lifescan, Johnson & Johnson Company) were used to measure fasting blood glucose, cholesterol (total, LDL, HDL), and triglyceride levels. Collection of blood samples occurred with a capillary tube and the CardioChek PA system, which adhered to the accuracy guidelines of the National Cholesterol Education Program of the National Institutes of Health<sup>53</sup>.

To assess dietary intake, trained researchers, supervised by a PhD-level Registered Dietitian, conducted three non-consecutive 24-hour dietary recalls within a two-week period, capturing two weekdays and one weekend day<sup>54</sup>. Interviewer-administered methods were used to

collect dietary recalls, with one completed at baseline data collection in-person and the following two completed via unannounced telephone calls. Nutritional analysis software (Nutrition Data System for Research [NDS-R] 2011, University of Minnesota) was used to analyze these 24-hour recalls<sup>48</sup>. Through dietary recalls, dietary quality was calculated using the Healthy Eating Index-2010 (HEI-2010)<sup>55</sup>, which assesses a person's diet and their adherence to the 2010 Dietary Guidelines for Americans<sup>56</sup>. The HEI-2010 consists of twelve dietary factors (nine adequacy and three moderation categories) including total fruit, whole fruit, total vegetables, dark-green vegetables and beans, whole grains, dairy, total protein foods, seafood and plant proteins, fatty acids, refined grains, sodium and empty calories (solid fat, alcohol, and added sugars)<sup>55</sup>. Higher HEI-2010 scores (on a scale ranging from 1-100) indicate greater conformity to the 2010 Dietary Guidelines for Americans<sup>56</sup>. HEI-2010 scores can be further categorized into good (>80), needs improvement (51-80), or poor (<51)<sup>55</sup>. Health literacy status was assessed with the Newest Vital Sign; possible scores range from 0-6, with 0-3 indicating a low health literacy status and 4-6 indicating a high health literacy status<sup>57</sup>.

### Data Analysis

To characterize the frequency, type, and sources of NNS intake for this sample, baseline 24-hour dietary recalls were analyzed. NNS content in participants' diets was calculated by NDS-R<sup>58</sup>; NDS-R extracted average mg of NNS in NNS-containing food and beverage items from the component/ingredient level of participants' dietary intake<sup>58</sup>.

From here, categorization of NNS consumers and non-consumers was determined based on the total mg of NNS from both foods and beverages in a participant's diet. In many previous observational studies, classification of a NNS consumer was determined solely from a participant's beverage, specifically cola, intake<sup>12-14,28-30,35</sup>. We do not believe this method

adequately defines the intake level to classify someone as a NNS consumer, as foods also contribute to NNS intake. Thus, we used a novel method where a participant was considered a NNS consumer if they consumed the *equivalent* of NNS in 1 fl oz of diet soda, from either foods or beverages. This intake level corresponds to 3 mg acesulfame potassium, 17 mg aspartame, 12 mg saccharin, or 6 mg sucralose. For example, if a participant only consumed 5 mg of sucralose from all foods and beverages in one day, then he would be considered a NNS non-consumer. Since these participants were already sugar-sweetened beverage consumers (by study design), consumption of at least the *equivalent* of 1 fl oz of diet soda was considered intentional intake.

The most and least prevalent NNS types (acesulfame-potassium, aspartame, saccharin, and sucralose) were identified, along with major dietary sources of NNS. Additionally, quantification of consumers' average daily NNS intake by mg content was determined.

### Statistics

Using SPSS (version 21.0 for Windows, 2012; IBM), statistical analyses on descriptive statistics (means  $\pm$  standard deviations and frequencies) were performed to look at baseline demographic characteristics, anthropometrics, biochemical markers, dietary quality, and dietary intake for the entire sample, as well as for NNS consumers and non-consumers. These analyses were also performed to determine NNS frequency, type, and food source. Independent t-tests (continuous variables) and  $\chi^2$  tests (categorical variables) were used to compare demographic characteristics, anthropometrics, biochemical markers, dietary quality, and dietary intake between NNS consumers and non-consumers. The  $\alpha$  level was set *a priori* at  $P \leq 0.05$ .

## Results

### Demographic Characteristics

Participants from Talking Health (n=301) were primarily female (81% of sample), Caucasian (93%), with a mean age of  $41.8 \pm 13.4$  years and a mean BMI of  $33.0 \pm 9.1$  kg/m<sup>2</sup> (57% were obese [BMI $\geq$ 30 kg/m<sup>2</sup>]). Of the sample, 68% had greater than a high school degree. Mean annual income was  $\$23,173 \pm 17,145$ , but most participants earned less than  $\$14,999$  (43%). One-third of the sample was found to be NNS consumers (**Table 1**).

**Table 1. Participant Demographic Characteristics (n=301)**

Characteristic	n (%)
Gender	
Male	56 (19)
Female	245 (81)
Mean age $\pm$ SD	41.8 $\pm$ 13.4
Race/Ethnicity	
White	280 (93)
African American	13 (4)
Other	8 (3)
BMI (kg/m <sup>2</sup> )	
Mean BMI $\pm$ SD	33.0 $\pm$ 9.1
Underweight ( $\leq$ 18.4)	6 (2)
Normal weight (18.5-24.9)	59 (19.5)
Overweight (25-29.9)	65 (21.5)
Obese ( $\geq$ 30)	171 (57)
Education Level	
<High school	30 (10)
High school graduate	66 (22)
Some college	109 (36)
College graduate	73 (24)
Graduate school	23 (8)
Household Income (\$)	
Mean Income (\$) $\pm$ SD	23,173 $\pm$ 17,145
$\leq$ \$14,999	129 (43)
\$15,000-34,999	96 (32)
\$35,000-54,999	39 (13)
$>$ \$55,000	37 (12)
Mean NVS Score <sup>a</sup> $\pm$ SD	4.0 $\pm$ 1.9
NNS Consumer	100 (33)

<sup>a</sup>NVS, Newest Vital Sign (0-3 = low health literacy, 4-6 = high health literacy)  
NNS = non-nutritive sweetener.

Comparison of NNS consumers (n=100) and NNS non-consumers (n=201) revealed no significant differences between gender, mean age, race, education level, or household income (**Table 2**). However, the mean income for NNS consumers was slightly higher than NNS non-

consumers (mean difference = \$2,624 ± 2,096). Additionally, health literacy scores were slightly higher for NNS consumers than NNS non-consumers (mean difference = 0.20 ± 0.24).

**Table 2. Demographic Characteristics of NNS Consumers (n=100) Versus NNS Non-Consumers (n=201)**

Characteristic	NNS Consumers n (%)	NNS Non-Consumers n (%)	Significance
Gender			
Male	16 (16)	40 (20)	X <sup>2</sup> = 0.67 p = 0.41
Female	84 (84)	161 (80)	
Mean age ± SD (years)	42.8 ± 13.5	41.4 ± 13.3	t = -0.87 p = 0.39
Race/Ethnicity			
White	92 (92)	188 (93.5)	X <sup>2</sup> = 0.97 p = 0.81
African American	5 (5)	8 (4)	
Other	3 (3)	5 (2.5)	
Weight (kg)	94.41 ± 28.63	88.48 ± 23.41	t = -1.92 p = 0.06
Mean BMI ± SD (kg/m <sup>2</sup> )	34.7 ± 10.6	32.1 ± 8.2	t = -2.33 p = 0.02
BMI Category			
Underweight (≤18.4)	2 (2)	4 (2)	X <sup>2</sup> = 0.09 p = 0.99
Normal weight (18.5-24.9)	19 (19)	40 (20)	
Overweight (25-29.9)	21 (21)	44 (22)	
Obese (≥30)	58 (58)	113 (56)	
Education Level			
High school graduate or less	33 (33)	63 (31)	X <sup>2</sup> = 0.08 p = 0.77
Some college or more	67 (67)	138 (69)	
Household Income (\$)	24,925 ± 18,022	22,301 ± 16,668	t = -1.25 p = 0.21
Mean Income ± SD (\$)			
≤14,999	40 (40)	89 (44)	x <sup>2</sup> = 1.83 p = 0.61
15,000-34,999	30 (30)	66 (33)	
35,000-54,999	15 (15)	24 (12)	
>55,000	15 (15)	22 (11)	
Mean NVS Health Literacy Score ± SD	4.1 ± 1.9	3.9 ± 2.0	t = -0.84 p = 0.40

NNS = non-nutritive sweetener

### Anthropometrics

NNS consumers had higher weight (mean difference = 5.93 kg ± 3.30) and BMI (mean difference = 2.58 kg/m<sup>2</sup> ± 1.20) statuses than NNS non-consumers. However, only BMI difference was statistically significant for NNS consumers compared to NNS non-consumers.

### Biochemical Markers

No significant differences were found between biochemical markers for NNS consumers and NNS non-consumers (**Table 3**). NNS consumers had slightly higher systolic and diastolic blood pressure and blood glucose levels than NNS non-consumers. However, they had lower LDL, total cholesterol, and triglyceride levels, and higher HDL levels than NNS non-consumers.

**Table 3. Biochemical Variables of NNS Consumers (n=100) Versus NNS Non-Consumers (n=201)**

Biochemical Variables	NNS Consumers Mean ± SD	NNS Non-Consumers Mean ± SD	Mean Difference ± Std. Error	Significance
Systolic Blood Pressure (mmHg)	122 ± 15	122 ± 16	-0.6 ± 2	t = 0.72 p = 0.75
Diastolic Blood Pressure (mmHg)	80 ± 11	79 ± 11	-1 ± 1	t = 0.49 p = 0.61
Blood Glucose (mg/dL)	80 ± 26	78 ± 24	-2 ± 3	t = 0.60 p = 0.55
Low Density Lipoprotein (mg/dL)	94 ± 32	99 ± 32	5 ± 4	t = 1.22 p = 0.23
High Density Lipoprotein (mg/dL)	48 ± 16	45 ± 15	-3 ± 2	t = -1.59 p = 0.11
Total Cholesterol (mg/dL)	163 ± 39	167 ± 37	4 ± 5	t = 0.96 p = 0.34
Triglyceride (mg/dL)	122 ± 73	130 ± 78	7 ± 9	t = 0.80 p = 0.43

NNS = non-nutritive sweetener

### Dietary Quality

Dietary quality was assessed via HEI-2010 scores (**Table 4**), where maximum scores for each category are noted. Overall, NNS consumers had significantly higher dietary quality than

NNS non-consumers ( $46.7 \pm 11.9$  vs.  $42.4 \pm 12.6$ ). Contributing to this higher overall score were significantly higher scores for total fruit, dark-green vegetables and beans, dairy, and empty calories in NNS consumers versus NNS non-consumers. Furthermore, NNS consumers had slightly higher, yet non-significant, scores for whole fruit, total vegetable, whole grain, total protein foods, and seafood and plant proteins. These scores were indicative of better adherence to the 2010 Dietary Guidelines for these groups. However, NNS consumers had significantly lower scores for refined grains and sodium and a slightly lower score for fatty acids, indicating less adherence in these groups.



**Table 4. Dietary Quality of NNS Consumers (n=100) Versus NNS Non-Consumers (n=201)**

<b>HEI-2010 Dietary Components (maximum score)</b>	<b>NNS Consumers Mean <math>\pm</math> SD</b>	<b>NNS Non- Consumers Mean <math>\pm</math> SD</b>	<b>Mean Difference <math>\pm</math> Std. Error</b>	<b>Significance</b>
Total Fruit (5)	1.5 $\pm$ 1.6	1.0 $\pm$ 1.5	-0.4 $\pm$ 0.2	t = -2.29 p = 0.02
Whole Fruit (5)	1.6 $\pm$ 1.9	1.1 $\pm$ 1.8	-0.5 $\pm$ 0.2	t = -2.11 p = 0.04
Total Vegetables (5)	2.8 $\pm$ 1.4	2.5 $\pm$ 1.5	-0.3 $\pm$ 0.2	t = -1.60 p = 0.11
Dark-green Vegetables and Beans (5)	1.5 $\pm$ 1.9	1.0 $\pm$ 1.6	-0.5 $\pm$ 0.2	t = -2.36 p = 0.02
Whole Grains (10)	2.6 $\pm$ 3.3	2.5 $\pm$ 3.2	-0.1 $\pm$ 0.4	t = -0.14 p = 0.89
Dairy (10)	5.4 $\pm$ 3.0	4.3 $\pm$ 2.8	-1.1 $\pm$ 0.4	t = -3.18 p < 0.001
Total Protein Foods (5)	4.4 $\pm$ 1.0	4.2 $\pm$ 1.2	-0.1 $\pm$ 0.1	t = -0.10 p = 0.32
Seafood and Plants Proteins (5)	1.9 $\pm$ 2.1	1.7 $\pm$ 2.1	-0.2 $\pm$ 0.3	t = -0.86 p = 0.39
Fatty Acids (10)	4.0 $\pm$ 3.4	4.1 $\pm$ 3.3	0.1 $\pm$ 0.4	t = 0.13 p = 0.90
Refined Grains (10)	5.5 $\pm$ 3.3	6.6 $\pm$ 3.1	1.1 $\pm$ 0.4	t = 2.84 p = 0.01
Sodium (10)	3.1 $\pm$ 3.0	4.5 $\pm$ 3.1	1.4 $\pm$ 0.4	t = 3.67 p < 0.001
Empty Calories (20)	12.4 $\pm$ 4.8	8.9 $\pm$ 5.6	-3.6 $\pm$ 0.7	t = -5.49 p < 0.001
HEI Total Score (100)	46.7 $\pm$ 11.9	42.4 $\pm$ 12.6	-4.3 $\pm$ 1.5	t = -2.84 p = 0.005

NNS = non-nutritive sweetener

### Dietary Factors

Dietary intake was assessed via 3-24 hour dietary recalls (**Table 5**). Healthier dietary factors for NNS consumers versus NNS non-consumers included significantly lower values for total daily energy, total beverage (kcal), sugar-sweetened beverage (kcal and fl oz), total sugar (g), added sugar (% total kcal and g), alcohol (% total kcal), and energy density (kcal/g); NNS consumers also had a significantly higher intake of protein (% total kcal). Less healthy dietary

factors for NNS consumers were found as well, however none of these factors were statistically significant. NNS consumers had slightly higher intake of fat (% total kcal) and saturated fat (% total kcal), along with slightly less intake of water (fl oz).

**Table 5. Dietary Factors of NNS Consumers (n=100) Versus NNS Non-Consumers (n=201)**

<b>Dietary Variables</b>	<b>NNS Consumers Mean ± SD</b>	<b>NNS Non-Consumers Mean ± SD</b>	<b>Mean Difference ± Std. Error</b>	<b>Significance</b>
Total Energy (kcal)	1719 ± 671	1955 ± 990	235 ± 110	t = 2.14 p = 0.03
Total Beverage (kcal)	297 ± 217	476 ± 393	179 ± 42	t = 4.25 p < 0.001
Total Beverage (fl oz)	63.4 ± 29	66.6 ± 36.9	3.3 ± 4.2	t = 0.78 p = 0.44
Sugar-sweetened beverage (kcal)	238 ± 215	384 ± 361	146 ± 39	t = 3.72 p < 0.001
Sugar-sweetened beverage (fl oz)	21.8 ± 20.3	32.7 ± 30.0	11.0 ± 3.3	t = 3.30 p < 0.001
Water (fl oz)	21.9 ± 21.2	24.6 ± 27.4	2.7 ± 3.1	t = 0.88 p = 0.38
Carbohydrate (% total kcal)	49.2 ± 8.8	51.4 ± 10.1	2.2 ± 1.2	t = 1.81 p = 0.07
Total Sugar (g)	97.3 ± 55.1	137.3 ± 92.9	40.0 ± 10.1	t = 4.00 p < 0.001
Added Sugar (% total kcal)	16.7 ± 8.5	23.5 ± 12.4	6.8 ± 1.4	t = 4.92 p < 0.001
Added Sugar (g)	74.1 ± 52.1	114.8 ± 87.6	40.7 ± 9.5	t = 4.28 p < 0.001
Protein (% total kcal)	15.7 ± 3.7	14.5 ± 4.5	-1.3 ± 0.5	t = -2.48 p = 0.01
Fat (% total kcal)	34.8 ± 7.2	33.1 ± 7.4	-1.7 ± 0.9	t = -1.92 p = 0.06
Saturated Fat (% total kcal)	12.2 ± 3.3	11.4 ± 3.2	-0.7 ± 0.4	t = -1.85 p = 0.07
Alcohol (% total kcal)	0.2 ± 0.8	1.1 ± 4.2	0.9 ± 0.4	t = 2.05 p = 0.04
Sodium (mg)	3010 ± 1280	3089 ± 1477	79 ± 173	t = 0.46 p = 0.65
Energy Density (kcal/g)	0.7 ± 0.3	0.8 ± 0.3	0.1 ± 0.0	t = 2.15 p = 0.03

NNS = non-nutritive sweetener

### Intake Frequency and Total Daily Intake of Non-nutritive Sweeteners Overall and by Type

Among participants (n=301), 33% (n=100) reported consuming NNS (Table 1). Mean daily intake of any type of NNS was  $869 \pm 3,553$  mg (Table 6). This value is equivalent to about 3.5 cans of diet soda. Sucralose contributed the most to mean daily NNS consumption, with 2.5 times more milligrams consumed than aspartame, the second biggest contributor to mean daily NNS intake. Acesulfame potassium contributed similarly to aspartame, while saccharin contributed very minimally.

**Table 6. Total NNS Intake Categorized by Type of NNS Among NNS Consumers (n=100) Versus NNS Non-Consumers (n=201)**

	<b>NNS Consumers Mean Daily Intake (mean <math>\pm</math> SD)</b>	<b>NNS Non- Consumers Mean Daily Intake (mean <math>\pm</math> SD)</b>
Total NNS intake (mg)	$869 \pm 3,553$	$1.4 \pm 2.7$
By artificial-sweetener type		
Sucralose (mg)	$1,034 \pm 2,788$	$0.03 \pm 0.4$
Aspartame (mg)	$414 \pm 1,815$	$0.3 \pm 1.7$
Acesulfame Potassium (mg)	$367 \pm 2,257$	$0.05 \pm 0.3$
Saccharin (mg)	$52 \pm 52$	$0 \pm 0$

NNS = non-nutritive sweetener

### Dietary Sources of Non-nutritive Sweeteners

Tabletop NNS (i.e., NNS from packets or NNS added to foods after preparation) made up 37% of total NNS intake, followed by diet tea (33%), and diet soda (27%) (Table 7). Among 100 NNS consumers, there were 144 *instances* of NNS intake; diet soda was the most frequently consumed dietary source of NNS and was consumed 39% of the time, followed by juice and flavored drinks (17%), and tabletop NNS (16%) (Table 8 and Figure 1).

**Table 7. Dietary Sources of NNS Intake Ranked by Amount of NNS Among NNS Consumers (n=100)**

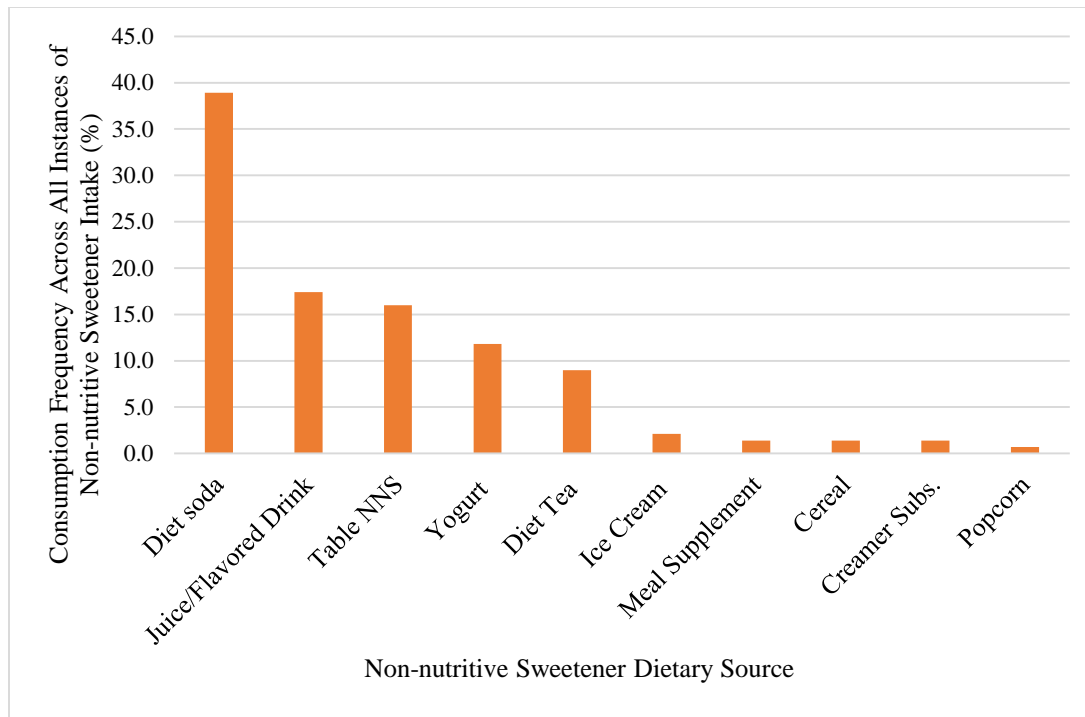
Dietary source	Sucralose (%)	Aspartame (%)	Acesulfame Potassium (%)	Saccharin (%)	Total NNS (%)
Tabletop Sweetener	68.2	0.7	0.0	95.9	36.6
Diet Tea	0.0	60.5	92.0	0.0	33.7
Diet soda	30.1	34.9	4.1	4.1	27.0
Juice and Flavored Drinks	1.0	1.3	2.4	0.0	1.3
Yogurt	0.5	1.4	0.2	0.0	0.7
Meal Replacement Supplements	0.1	0.6	1.2	0.0	0.4
Ice Cream	0.0	0.0	0.0	0.0	0.1
Cereal	0.0	0.3	0.0	0.0	0.1
Coffee Cream Substitutes	0.0	0.0	0.1	0.0	0.0
Popcorn	0.0	0.0	0.0	0.0	0.0

NNS = non-nutritive sweetener

**Table 8. Dietary Sources of NNS Shown as Number of Total Instances (n=144) and Percentage of Total Instances Among NNS Consumers (n=100)**

NNS Dietary Source	n (%)
Diet Soda	56 (39)
Juice and Flavored Drink	25 (17)
Table NNS	23 (16)
Yogurt	17 (12)
Diet Tea	13 (9)
Ice Cream	3 (2)
Meal Replacement Supplement	2 (1)
Cereal	2 (1)
Coffee Creamer Substitute	2 (1)
Popcorn	1 (1)

NNS = non-nutritive sweetener



**Figure 1. Dietary Sources of NNS Shown as Percentage of Total Instances (n=144) Among NNS Consumers (n=100)**

## Discussion

These findings demonstrated that demographically, NNS consumers and NNS non-consumers were similar, with the exception of BMI. However, in terms of dietary intake, NNS consumers tended to have better overall dietary habits than NNS non-consumers. NNS consumers had a significantly higher BMI than NNS non-consumers, which is consistent with findings from observational studies<sup>11,12,30-34</sup>. For example, Fowler et al. saw a significant positive dose-response relationship between baseline NNS intake and incidence of overweight/obesity and BMI change<sup>11</sup>. Other studies reported a significant relationship between high NNS intake and increased waist circumference, with NNS consumers having significantly higher gains in waist circumference than NNS non-consumers<sup>12,14</sup>. It is also possible that NNS consumers in this sample switched from drinking predominantly sugar-sweetened beverages to drinking some NNS beverages in an effort to cope with weight gain<sup>11</sup> or other health conditions<sup>59</sup>, which has been

well documented in NNS-related literature<sup>11,33,59</sup>. Despite previous findings of NNS consumers having biochemical markers associated with chronic disease risk<sup>28,30,31,33,35</sup>, this sample had no significant differences in these markers between NNS consumers and NNS non-consumers. These findings are consistent with results of randomized controlled trials that saw no differences in blood pressure or blood glucose levels between NNS consumers and NNS non-consumers<sup>60-62</sup>. Another explanation for the lack of difference in biochemical markers is that NNS consumers, who have been known to use NNS in an effort to cope with pre-existing conditions like diabetes, may have been using medications to control these markers when their blood pressure and blood values were tested.

In terms of dietary quality, NNS consumers had a significantly better HEI-2010 total score, as well as significantly better scores for total fruit, dark green vegetables and beans, dairy, and empty calories. These results indicate NNS consumers' diets in this sample generally adhered to the 2010 Dietary Guidelines for Americans more so than NNS non-consumers. Furthermore, NNS consumers had lower total energy intake and density, and consumed less total beverages, sugar-sweetened beverages, total sugar, added sugar, and alcohol than NNS non-consumers. These lower total energy intake and density results are supported by the finding that NNS consumers have been able to reduce their energy intake, on average, by 10%, which corresponds to ~222 kcal per day<sup>7</sup>. Furthermore, in an observational study by de Koning et al., NNS consumers had a better overall dietary quality than NNS non-consumers, consuming less red and processed meat and carbohydrate, and more protein, while also maintaining a lower total energy intake level<sup>33</sup>. Another observational study reported that NNS consumers were more likely to follow a healthier diet containing high amounts of fruit, whole grains, and milk<sup>30</sup>. However, HEI-2010 scores for refined grains and sodium were lower for NNS consumers. These

values indicate that NNS consumers might be compensating for missed calories with foods that are highly processed and contain large amounts of sodium and refined grains. There is some speculation that individuals might justify consumption of a higher calorie food after consuming NNS<sup>63</sup>, which could explain the lower HEI-2010 for refined grains and sodium. Another potential explanation for the higher intake of refined grains is the possibility that NNS created an enhanced preference for sweet taste due to the high sweetness levels<sup>18</sup>.

To characterize intake patterns in a rural population, frequency, type, and sources of NNS intake were investigated. Despite this region's rural status<sup>50</sup>, 33% of this sample consumed NNS (Table 1). This rate is fairly consistent with national NNS consumption rates<sup>2,3</sup>, which report NNS consumption ranging from 28-30%. However, it was expected that this sample's NNS consumption would be lower due to reports of higher sugar-sweetened beverage intake among rural populations<sup>40</sup>. The high consumption rate found in this investigation could reflect a shift in beverage patterns among rural populations to cope with high obesity and chronic health disease risk<sup>36-39</sup>. Furthermore, when asked about NNS beverages, participants from rural southwest Virginia reported in a qualitative study (n=54) that NNS beverages had mostly positive attributes, including taste and health outcomes, acknowledging that NNS beverages contained less calories and sugar<sup>64</sup>. Participants also explained that their doctor's recommendations, along with a diagnosis of diabetes, would influence their consumption of NNS beverages<sup>64</sup>. These qualitative findings support the idea that participants in this rural region are aware of the health benefits of NNS and may have started to consume NNS in response to a weight or health problem.

Sucralose was the most prevalent NNS type in this sample's diet, followed by aspartame, acesulfame potassium, and saccharin. This list corresponds well to NNS prevalence in food and

beverage products, as sucralose is the most common, followed by very similar levels for acesulfame potassium and aspartame<sup>41</sup>. The most frequently consumed sources of NNS were diet soda, juice and flavored drinks, and tabletop NNS, which correlated with national consumption trends that reported the that diet soda was the most prevalent source of NNS, followed by reduced-calorie beverages (light fruit juices and lemonades)<sup>2,3</sup>.

The purpose of this investigation was to determine differences between NNS consumers and non-consumers in terms of demographic characteristics, anthropometrics, biochemical markers, dietary quality, and dietary factors and to characterize intake patterns in a rural population, by assessing frequency, type, and sources of NNS intake. This investigation was not without limitations. Data from another study, Talking Health, was utilized. Talking Health aimed to evaluate the effectiveness of a six-month intervention to decrease sugar-sweetened beverage consumption versus a matched contact comparison group to increase physical activity levels. Thus, by design these participants were high sugar-sweetened beverage consumers, which could affect their NNS intake, as well as the generalizability of the study. However, rural populations are shown to consume high levels of sugar-sweetened beverages<sup>40</sup> and therefore, it is expected to see sugar-sweetened beverage consumption in rural NNS consumers. Furthermore, since characterization of NNS consumption was not the goal of this study, the specificity of 24-hour dietary recalls in regards to NNS foods consumed could be affected; it is common for participants to experience recall or information bias, which occurs when they are unaware of or unknowingly consume foods that contain NNS or they simply do not remember eating a NNS-containing food<sup>1,3,11,30</sup>. However, because Talking Health's investigation pertained to beverage consumption, it is likely that special attention was given to all beverages consumed. With that being said, misreporting is a common limitation of self-reported dietary intake assessment



methods<sup>65</sup>, including 24-hour dietary recalls and food frequency questionnaires (BEVQ-15). This sample is also at risk for low health literacy levels, which can affect a respondent's ability to accurately report portion sizes<sup>66</sup>. Nevertheless, the study was supervised by a doctorate-level Registered Dietitian and interviewer-administered methods (including USDA's automated multiple pass method) were used to assess dietary intake, which helps combat misreporting in low literature populations<sup>66</sup>. Additionally, an older version of NDS-R (2011) was used to analyze this sample's NNS intake. Although this database contains nutrition content information for over 18,000 products, NDS-R only has information for 36 NNS brand name products<sup>67,68</sup>. Thus, NNS intake may be underestimated in this sample due to the inability to analyze all dietary sources of NNS. Furthermore, no significant differences in demographics were found between NNS-consumers and NNS non-consumers, which is not supported in the existing literature. However, this sample was predominantly Caucasian females of low income status and moderate education level, so it may be underpowered to detect significant differences between groups for demographics. Lastly, there may be limited generalizability of these results due to the rurality of the sample.

This investigation had many strengths as well. It adequately explored the existing literature on weight and cardiometabolic outcomes associated with NNS, reviewing more than 20 randomized controlled trials and observational studies. Multiple studies were also reviewed pertaining to cancer and physiological and intestinal changes. Furthermore, this investigation is the first to characterize NNS intake (consumer characteristics and frequency, type, and source) in a large (n=301) rural population. NNS intake patterns in rural populations are particularly relevant because this group is more at risk for obesity and a variety of chronic health conditions<sup>36-39</sup>. NNS could serve as a potential method to reduce or maintain weight in rural

populations, especially since rural-residing adults have higher prevalence and levels of sugar-sweetened beverage consumption than urban-residing adults<sup>40</sup>. Also, this investigation is unique in that it explores the most commonly consumed types of NNS in both beverages and foods<sup>1,3</sup>. This differentiation between NNS type is important as each type of NNS is composed of different compounds and is metabolized differently, which could affect health outcomes. Additionally, by assessing NNS intake through both beverages and foods, the current investigation adds to the pre-existing NNS literature that has a heavy emphasis on NNS beverages, specifically diet sodas.

## **Conclusion**

The characterization of NNS intake will facilitate the understanding of potential health outcomes associated with NNS consumption. This current research investigated differences in demographic characteristics, anthropometrics, biochemical markers, dietary quality, and dietary factors between NNS consumers and NNS non-consumers in a rural sample. NNS consumers did not differ from NNS non-consumers in terms of demographic characteristics or biochemical markers. However, NNS consumers had a higher BMI status and lower HEI-2010 scores for intake of sodium and refined grains. Nevertheless, NNS consumers had better overall HEI-2010 scores of dietary quality. This sample's NNS intake was similar to the national consumption patterns and production trends, with 33% of the population consuming NNS, sucralose as the most prevalent type of NNS, and diet soda as the most frequently consumed source of NNS. The characteristics of these NNS consumers and the common intake patterns can be used to develop a dietary intake assessment tool that accurately measures NNS intake, which can facilitate a better understanding of the associations between health outcomes and intake of NNS.

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### **Chapter 3: Future Directions and Conclusion**

Non-nutritive sweeteners (NNS) have been suggested as a method to facilitate reductions in energy intake, weight, and cardiometabolic risk. However, various potential health outcomes, such as cancer, weight loss, weight gain, physiological and intestinal changes, cardiovascular disease, diabetes, and migraines, have been associated with NNS intake in both observational studies and randomized controlled trials. This uncertainty makes it increasingly important to address the limitations of current NNS research. The current literature review and findings demonstrate the challenges in reaching conclusions about NNS and weight. In this review, there are many short-term randomized controlled trials that observe associations between NNS intake and weight loss, perhaps due to their inability to observe long-term weight changes<sup>1</sup>; there are also many long-term observational studies in this review that showed associations between NNS intake and weight gain, which could be the result of inaccurate assessment of NNS intake and the potential for many confounding variables<sup>1</sup>. The results of this study also illustrate the controversy of NNS as a weight loss/maintenance strategy, with NNS consumers having significantly higher body mass index (BMI) while at the same time, having significantly better diet quality (Healthy Eating Index-2010 scores) than NNS non-consumers. NNS consumers also had lower intakes of total daily energy, total beverages, sugar-sweetened beverages, total sugar, added sugar, and alcohol.

Due to this uncertainty, there has been a call for well-designed randomized controlled trials and observational studies<sup>1</sup>. Randomized controlled trials will help in determining causation and long-term effects of NNS on weight and cardiometabolic risk<sup>1</sup>; it is suggested that for weight loss studies, duration should be at least one year to adequately assess causation<sup>1</sup>. Observational studies are useful for identifying associations between NNS intake and various outcomes and

also facilitate understanding of long-term changes in weight and cardiometabolic risk<sup>1</sup>. These two study designs will continue to be useful in guiding the recommendations for use of NNS and its role in weight and cardiometabolic risk management. Even more, mechanistic studies are needed to explore the proposed underlying mechanisms for weight gain, specifically pertaining to NNS-driven physiological and intestinal changes<sup>1</sup>.

The current review of limitations and characterization of NNS consumers and intake patterns in a rural sample will assist the effort to develop accurate self-reported dietary intake assessment methods. Currently, the common dietary assessment methods include 24-hour recalls and food frequency questionnaires. The accuracy of previously used food frequency questionnaires is limited by the lack of type or brand name description of NNS beverages, use of subjective serving sizes, and the emphasis on beverages, specifically diet sodas. These dietary assessment tools also need to utilize updated databases that keep pace with the dynamic marketplace.

Another aspect of accurately assessing NNS intake is finding a way to overcome the bias of self-reported dietary intake methods. As mentioned previously, a major limitation of self-reported assessment methods is misreporting. This limitation indicates a need for methodological advances, specifically pertaining to tools that measure dietary intake of NNS. Dietary intake biomarkers are an objective methodological advancement that would combat the subjectivity and biases inherent to self-reported dietary intake methods<sup>2,3</sup>. Additionally, biomarkers can provide validation to self-reported methods, like 24-hour recalls and questionnaires that assess NNS intake<sup>4,5</sup>. Biomarkers can reflect dietary intake over the past few hours or days (short-term), weeks or months (medium-term), and even months or years, depending on the type of biomarker that is collected (blood, hair, urine, tissue)<sup>5</sup>. Biomarkers also address a very pertinent problem for

NNS research by providing intake information that food databases may not be able to provide<sup>5</sup>. Not only can biomarkers provide more accurate measures of dietary intake, but they can also aid in predicting nutritional-disease risk<sup>5</sup>. However, dietary biomarkers are still subject to limitations including cost and invasiveness<sup>6</sup>, so it is important moving forward to identify a minimally invasive and low cost dietary marker<sup>7</sup> to measure NNS intake. From here, the biomarker's validity, reliability, and ability to detect change should be determined to ensure that certain factors (genetic, lifestyle, and dietary) do not affect the biomarker's intended purpose<sup>8</sup>.

In summary, future directions for NNS research include well-designed randomized controlled trials to determine causality and observational studies to assess long-term associations and outcomes. The accuracy of subjective self-reported dietary intake assessment methods that will likely be used in these studies can be improved by including type or brand name description of NNS beverages, using objective serving sizes, collecting intake information on all beverages (not just diet sodas) and food products, and utilizing updated food databases. These methods can potentially be further improved by utilizing dietary biomarkers to objectively validate NNS intake. These advancements in NNS research will help to understand NNS use and health outcomes, which can be translated to epidemiological studies, national recommendations, and clinical settings.

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