The Association between Non-Nutritive Sweetener Intake and Metabolic Syndrome in Adults

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Abstract

Non-nutritive sweeteners (NNS) have been used to replace added sugars in foods/beverages. Research related to NNS consumption and metabolic syndrome (MetS) is of great importance as NNS are often used by individuals who are looking to improve their health. The objectives of this investigation were to determine whether an association between NNS consumption (total and individual types) and MetS exists, and if any of the five risk factors for MetS were more significantly impacted by NNS consumption. Four NNS were included in this study: saccharin, sucralose, aspartame, and acesulfame potassium. Adult participants (n = 125) from Southwest Virginia were recruited for a cross-sectional investigation. Demographics, three 24-hour dietary recalls, and values for MetS (blood pressure, waist circumference, and glucose, triglyceride, and HDL levels) were collected. Statistical analyses included descriptives and multiple linear regression models. Of the 125 participants, 63 were classified as NNS consumers and 18 met the criteria for MetS. There was a significant positive relationship between MetS and total NNS consumption ($p=0.007$) and MetS and aspartame ($p=0.012$). When looking at individual MetS risk factors, waist circumference, triglyceride and glucose values were significantly positively associated with NNS consumption ($p\leq0.001$) and aspartame, sucralose, and saccharin (all $p\leq0.027$). Some limitations to current NNS research were addressed, such as, examining associations between individual NNS types and not using diet soda as a proxy for NNS consumption. More research is needed to address the bias of self-reported data and the lack of randomized controlled trials to inferentially test the impact of NNS consumption.

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General Audience Abstract

Non-nutritive sweeteners (NNS) have been used to replace added sugars in foods/beverages because they provide little to no calories. Research related to NNS consumption and metabolic syndrome (MetS) is of great importance as NNS are often used by individuals who are looking to improve their health by reducing added sugar intake. MetS consists of five risk factors: high blood pressure, large waist circumference, high fasting blood glucose values, high triglyceride values, and low high-density lipoprotein. The goals of this study were to determine if there was a relationship between NNS consumption (total and individual types) and MetS, and if any of the five risk factors for MetS were more heavily affected by NNS consumption. Four NNS were included in this study: saccharin, sucralose, aspartame, and acesulfame potassium. For this cross-sectional study, participants (n=125) from Southwest Virginia were recruited. Three 24-hour dietary recalls and values for risk factors of MetS were collected. Of the 125 participants, 63 were classified as NNS consumers and 18 met the criteria for MetS. Based on the data collected, there was a significant positive association between MetS and total NNS consumption and aspartame consumption. When looking at individual MetS risk factors, waist circumference, triglyceride and glucose values were significantly positively associated with total NNS consumption and aspartame, sucralose, and saccharin consumption. Some limitations to current NNS research were addressed, such as, examining relationships between individual NNS types and not using diet soda as the only source for NNS consumption. More research is needed to address the bias of self-reported data and the lack of randomized controlled trials to better test the impact of NNS consumption.

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List of Abbreviations

ADA: American Diabetes Association

ADI: Acceptable Daily Intake

AHA: American Heart Association

BMI: Body Mass Index

FDA: Food and Drug Administration

FFQ: Food-Frequency Questionnaire

GLP-1: Glucagon-Like Peptide 1

GRAS: Generally Recognized As Safe

GTT: Glucose Tolerance Test

HDL: High-Density Lipoprotein

HR: Hazard Ratio

MetS: Metabolic Syndrome

NDSR: Nutrition Data System for Research

NNS: Non-Nutritive Sweetener(s)

OGTT: Oral Glucose Tolerance Test

US: United States

Chapter 1: Review of Literature

Non-Nutritive Sweeteners

Non-nutritive sweeteners (NNS), or artificial sweeteners, are regulated as food additives in the United States (US) and can be used in place of sugar in foods and beverages. Foods and beverages made with these sweeteners are often labeled as "sugar-free" or "diet". NNS are added in minute amounts because they are much sweeter than traditional table sugar, providing little to no calories.[1] Currently, there are six NNS approved by the Food and Drug Administration (FDA). These include saccharin, aspartame, acesulfame potassium, sucralose, neotame, and advantame.[2] According to the Academy of Nutrition and Dietetics, NNS can be consumed safely when incorporated within a proper eating plan. The Academy also states that replacing added sugar with NNS can be a good way to moderate carbohydrate intake.[3] The FDA also recognizes a few NNS as "generally recognized as safe" (GRAS). The GRAS label signifies that researchers have no reason to believe that the product will cause harm when eaten for its intended purpose and within its recommended quantity.[1] These NNS include highly purified steviol glycosides and luo han guo fruit extracts.[2]

Saccharin

Saccharin, the oldest NNS, is 200-700 times sweeter than sugar. This NNS is heavily researched because of its carcinogenic potential.[4] In the 1970s, a study linked saccharin intake to an increased risk of bladder cancer in rats.[5] Further mechanistic studies have shown these results are only applicable in rats.[5] On the market, saccharin is labeled as Sweet'N Low, Sugar Twin, and Necta Sweet. The Acceptable Daily Intake (ADI) for saccharin is 15 mg/kg of body weight for children and adults.[4] This NNS is most commonly used in soft drinks, baked goods, jams, canned fruit, candy, salad dressing, dessert topping, and chewing gum.[4]

Aspartame

Aspartame was not approved by the FDA until 1981. On the market, aspartame is labeled as Equal, NutraSweet, and Natra Taste. Aspartame provides 4 kcals/g, however it is 200 times sweeter than sugar, so only minute amounts are used. The ADI for aspartame is 50 mg/kg of body weight for adults and children.[4] Examples of products that contain aspartame include chewing gum, diet soda, dry drink mixtures, yogurt and pudding, and instant tea/coffee.[4] All products containing aspartame must be labeled for individuals with phenylketonuria due to their inability to process phenylalanine, which is found in aspartame.[6] Aspartame is also a heavily researched NNS and is the most controversial of the NNS that are used in the US food industry[4] due to its history of being a potential carcinogen.[7] For example, one study suggested that the increase in brain tumors in the 1980s was linked to aspartame consumption, but researchers later discovered that the trend began in the 1970s, before aspartame was being used.[7]

Acesulfame Potassium

Despite acesulfame potassium having inadequate and poor-quality toxicity tests in relation to being a potential carcinogen, it was still approved by the FDA in 1998.[4,8] Reasons why these studies were inadequate include improper randomization, improper dosage levels in the rats and mice, and the amount of time the rats and mice were studied.[8] Acesulfame potassium is used in a wide variety of products such as sugar-free baked goods, chewing gum, gelatin desserts, and diet sodas. On the market, acesulfame potassium is labeled as Sunette, Sweet One, and Swiss Sweet. This NNS is 200 times sweeter than sugar. The ADI for acesulfame potassium is 15 mg/kg of body weight.[4] When used in food products, acesulfame potassium is typically paired with other NNS, especially sucralose, to provide the right flavor profile.[4]

Sucralose

Sucralose was approved by the FDA in 1999 to be used as a general purpose sweetener.[2] In the market, it is labeled as Splenda, which is a mixture of sucralose and maltodextrin, and is 600 times sweeter than sugar. Sucralose is the most similar to sugar in terms of mouthfeel. It has been promoted as a great sugar alternative for individuals with diabetes as it claims it does not affect carbohydrate metabolism.[4] Sucralose is a widely used NNS, found in 2,500 products.[2] The ADI for sucralose is 5 mg/kg of body weight.[2]

Neotame

Neotame was approved as a general use sweetener by the FDA, for everything but meat and poultry, in 2002. It is 7,000 to 13,000 times sweeter than sugar, so very little of it is required when replacing sugar in food products. Although it is FDA approved, it is rarely used in food processing.[3] The ADI for neotame is 0.3 mg/kg of body weight.[2] Neotame is a heat stable NNS, meaning it remains sweet in high temperatures, so can be used in baked goods.[2]

Advantame

Advantame is approved by the FDA as a general use sweetener and is 20,000 times sweeter than sugar. It was approved by the FDA for use in everything, except meat and poultry, in 2014. It is a heat stable sweetener, which makes it acceptable for use in baked goods.[2] The ADI for advantame is 32.8 mg/kg of body weight.[2]

Steviol Glycosides and Luo Han Guo Fruit Extract

Steviol glycosides and luo han guo fruit extract are examples of natural NNS. Steviol glycoside is a natural component of the leaves of the Stevia rebaudiana plant.[2] It is pulled from the leaves of the stevia plant and highly purified (95% minimum purity)[2] before it is used in baked goods, diet sodas, cereals, energy bars, and as a tabletop sweetener.[3] Extracts of this NNS,

including rebaudioside A, stevioside, and rebaudioside D are roughly 200 to 400 times sweeter than sugar.[2] The ADI for stevia is 4 mg/kg of body weight.[3] Extracts of the stevia plant are also mixed with erythritol sugar alcohol to make Truvia. The extract used to make Truvia is rebiana.[9] Luo han guo, also known as monk fruit extract, is 150 to 300 times sweeter than sugar. To date, no ADI has been determined for luo han guo fruit extract. This NNS is typically used as a tabletop sweetener, a food ingredient, and a component of other sweetener blends.[3]

Potential Implications of Non-Nutritive Sweetener Intake

Obesity and other chronic diseases are on the rise in the US and evidence suggests they may be tied to diets high in added sugar.[10] NNS producers are utilizing this information to advertise their products to the American public, but consumers do not have access to adequate information on NNS to determine if they are beneficial for them or not.[4] Research shows that consumption of low-calorie sweeteners and NNS are increasing rapidly. In a study done by Sylvetsky et al., researchers discovered that the percentage of individuals consuming low and no calorie sweeteners nearly doubled in children, from 9% in 1999-2000 to 15% in 2007-2008. During the same time period, the percentage of adults consuming low and no calorie sweetener increased from 27% to 32%.[11] With consumption on the rise, it is important to provide the general population with concrete information on the impact of NNS on health outcomes. Current research has not kept up with this rapid increase resulting in insufficient clinical and epidemiological data available to make a conclusion regarding the impact of NNS intake.[4] For example, some long-term, randomized-control trials have found NNS to be beneficial for weight loss, whereas other research that is more observational in nature shows a negative effect or no effect at all.[12] Also, studies have shown an association between NNS and type 2 diabetes, however, there is not enough evidence to make a causal link.[13]

In 2012, the American Heart Association (AHA) and the American Diabetes Association (ADA) sought to review the present literature on NNS to determine if there was adequate data to provide proper guidance for NNS use.[14] They concluded that there was not sufficient evidence or data to advertise that replacing caloric sweeteners with NNS was beneficial.[14] Although scientists have studied the impact of NNS on a wide variety of health conditions, the evidence is contradictory and sparse.[15]

Metabolic Effects of Non-Nutritive Sweeteners

Many wonder why and how NNS can lead to obesity, increased risk of type 2 diabetes, and increased risk of metabolic syndrome if they provide little to no calories. There are three main hypothesized mechanisms used to explain the metabolic effects of NNS. First, NNS may interfere with learned responses that help to control glucose and energy homeostasis. Second, NNS may interfere with gut microbiota and induce glucose intolerance. Third, NNS may interact with sweet-taste receptors that are expressed throughout the digestive tract that play a role in glucose absorption and trigger insulin secretion.[16]

For the first mechanism, many researchers believe that long-term exposure to NNS ingestion weakens cephalic responses triggered by sweet taste in rats. It is important to note that these responses were only seen when NNS was given orally. This mechanism has not been tested in humans yet, but many believe it is applicable to humans.[16] For the second mechanism, some NNS (saccharin, sucralose, aspartame, and stevia) have been shown to have bacteriostatic effects. This means that they are resistant to fermentation by bacteria. Researchers believe this effect goes past the microbial of the mouth and into the microbial of the gut where it can affect host metabolic phenotype and disease risk.[16] Lastly, for the third mechanism, a lot of the research and support for this mechanism came after the discovery of taste-receptors in non-taste tissues.

Researchers believe the sweet-taste signaling pathways in the gut play an important role in regulating glucagon-like peptide 1 (GLP-1) secretion and regulating glucose absorption from the intestinal lumen into enterocytes.[16]

To further investigate the possible metabolic effects of NNS, Palkowska-Gozdzik et al. studied the influence of NNS on certain parameters of thyroid axis activity.[17] The thyroid axis, also known as the hypothalamic-pituitary-thyroid axis, function can be affected by the quantity and quality of one's diet.[17] Male rats (n=105) were divided into three groups and fed isocaloric diets, ad lib, for three weeks. Two of the three groups had the same sweetness intensity, equal to 10 grams of sucrose per 100 grams of food, with sucrose and sucralose, and the third group consumed an unsweetened diet. At the end of the three week period, the rats were euthanized after a 16-hour fast and 30, 60, 120, 180 minutes after a meal. Seven rats per group were euthanized at the various time points. For rats in the sucralose group, their intake did not exceed the ADI. The study concluded that sucralose intake appeared to reduce thyroid axis activity by decreasing thyroid peroxidase activity, thyroid stimulating hormone, and plasma total thyroid concentrations. It also increased free triiodothyronine and thyroxine indexes. The findings show that sucralose may be physiologically active and can cause disturbances in thyroid axis activity.[17] In another study by Gul et al., researchers hypothesized that aspartame consumption may contribute to the development of metabolic syndrome (MetS) because of phenylalanine's inhibition of endogenous intestinal alkaline phosphatase.[18] Intestinal alkaline phosphatase is a gut enzyme shown to prevent the development of MetS in mice. The study concluded that chronic aspartame consumption in mice may lead to increased weight gain, glucose intolerance, and increased tumor necrosis factor-alpha levels. Researchers also found that aspartame reduced the activity of intestinal alkaline phosphatase in vitro and in vivo in mice models.[18]

To date, there is no definitive evidence that NNS causes metabolic disorders in humans. More research needs to be done in this area to better understand the mechanisms behind how NNS drives metabolic effects.[16-18]

Weight Status

Individuals may consider switching to NNS for weight loss management because of the minimal calories they provide. Peters et al. studied the effects of water and NNS beverage intake during a 12-week weight loss program.[19] Participants in both groups participated in a behavioral weight loss treatment program. In this prospective randomized trial, researchers concluded that individuals in the NNS group lost significantly more weight than those in the water group. In the water group, 43% of participants lost > 5% of body weight, whereas 64% of participants in the NNS group lost > 5% of body weight.[19] However, researchers were unable to decide on the specific mechanism between the NNS and water group that led to greater weight loss.[19] Limitations in this study included no blinding for researchers or participants, the study design inhibited researchers from drawing conclusions, the water group had a drastic lifestyle change they had to adhere to, and the study did not control for diet. Similarly, Tate et al. looked at the effects of NNS on weight loss over a 6-month period in an intent-to-treat analysis.[20] Caloric beverages were either replaced with water, NNS beverages, or a weight loss technique of the participant's choice. Individuals in the water or NNS beverage group were twice as likely to experience a 5% weight loss compared to those individuals who chose their own weight loss technique. However, there was not a significant difference between the water and NNS groups.[20] Limitations of this study included self-reporting bias and lack of long-term follow-up.[20]

Madjd et al. found conflicting results to the studies mentioned above when comparing water to NNS beverages for weight loss in a single-blind, randomized clinical trial.[21] In this

study, 89 overweight and obese women who usually consumed NNS beverages were either asked to consume water after lunch or continue drinking NNS beverages 5 times/week after lunch. The other two days of the week, they were asked to consume water after lunch. The groups were asked to consume their designated beverages for a total of 24 weeks while participating in a weight loss program. Over the 24 week period, both groups experienced significant weight loss. There was also a significant change in body mass index (BMI) in both groups, but there was a larger change in the water group compared to the NNS beverage group. The final conclusion of these findings was that drinking water as opposed to NNS beverages after the main meal (lunch) may lead to greater weight loss in overweight and obese female adults.[21] The greater weight loss could be attributed to the greater reduction in calories or the reduction in absolute carbohydrate intake in the water group compared to the NNS beverage group.[21] In a similar study by Madjd et al., researchers examined whether replacing NNS beverages with water after lunch promoted greater weight loss.[22] In this single-blind, randomized control trial, 81 type 2 diabetic women were recruited to participate. Participants were asked to either consume water after lunch, or continue consuming NNS beverages 5 times/week after lunch over a 24 week period. On the other days of the week, participants in the NNS beverages group were asked to consume water. Like the first study[21], the second study[22] had significant weight loss in both groups. There was also a significant difference in weight loss between the water group (-6.40±2.42 kg) and the NNS beverages group (-5.25±1.60 kg) ($p = 0.006$). The change in BMI was also greater in the water group (-2.49±0.92 kg/m^2) compared to the NNS beverages group (-2.06±0.62 kg/m^2).[22] Limitations of these two studies include not having a sample that was representative of the general public, no recording of fluid intake, their brevity for a weight loss study, the studies relied on subjective reporting, and energy expenditure was not recorded by participants.[21,22]

In a randomized trial performed by Ebbeling et al., researchers examined whether or not a change in beverage consumption reduced weight and BMI in adolescents.[23] The study took place over two years. The first year was the intervention period and the second year was the follow-up period. The emphasis of the study was to replace sugar-sweetened beverages with non-caloric beverages. During the first year, participants in the experimental group received home delivery of water and diet beverages every two weeks, along with motivational phone calls and check-ins. At the end of year one, there was a significant difference between the control and experimental group (decreased to almost no consumption) in terms of caloric beverage intake ($p< 0.001$). Also at the end of year one, the difference in BMI between the two groups was significant ($p=0.045$). At the end of the two years, there was no significant difference in weight or BMI between the experimental and control groups.[23] Limitations in this study included a small sample size, the fact that it is unclear whether or not water or diet sodas were more important, and self-reporting bias.[23] In a long-term, observational weight loss study by Fowler et al., researchers found that there was a positive dose-response relationship between NNS beverage consumption and change in BMI.[24] Participants were split into NNS consumers and non-consumers and assessed at baseline and again 7-8 years later. At follow-up, non-consumers had a mean change in BMI of 1.01 and individuals consuming 22 or more NNS beverages per week had a mean change in BMI of 1.78. That equates to a 78% greater change in BMI for the individuals consuming 22 or more NNS beverages per week compared to non-consumers.[24] Limitations of this study included not specifying the type of NNS consumed, using beverages containing NNS as a proxy for NNS consumption, not controlling for NNS consumption in the non-consumer group, and neglecting to include less-costly NNS beverages in the questionnaire which could have resulted in an underestimation.[24] Chia et al. also wanted to examine the effects of long-term NNS use on

weight gain, specifically, abdominal obesity.[25] Participants and data for this investigation came from the Baltimore Longitudinal Study of Aging, an on-going continuous enrollment cohort study. Men and women (n=1,454) with at least one visit that included anthropometric measurements and complete dietary record data beginning in 1984 were included in the study. Dietary intake was recorded using a 7-day dietary record and NNS consumption was collected when individuals indicated a food or drink containing NNS in his/her dietary record. The NNS included on the record were aspartame, saccharin, acesulfame potassium, and sucralose. When compared to individuals that did not consume NNS, those that did had on average 0.80 kg/m^2 higher BMI, 2.6 cm larger waist circumference, a 36.7% higher prevalence of abdominal obesity, and were at greater risk for type 2 diabetes. Lastly, individuals who indicated consuming NNS at baseline that were normal weight had a significantly greater incidence of obesity at follow-up compared to participants who did not report NNS use at baseline. It is important to note that consumers and non-consumers had similar diet quality. This study is unique in that it looked at beverage and food consumption of NNS over an extended period of time. Limitations of this study include the fact that the cohort study is a highly motivated and health conscious group, the age of the study population (average age was 60), NNS use was either recorded as "yes" or "no" so estimates of actual intake may be conservative, and, although it took place over a long period of time, it was still an observational study so no definitive conclusions could be made.[25]

Rats have been used to study the potential relationship between NNS consumption and weight. Studies have shown that rats given yogurt sweetened with NNS, as opposed to glucose, gained significantly more weight.[26-28] In one experiment, rats were either given glucose or saccharin,[27] and in another experiment, the rats were either given glucose, saccharin, or acesulfame potassium.[26] There were no significant differences between weight gain in rats that

consumed acesulfame potassium and those that consumed saccharin.[26] In another study, by Swithers et al., overweight rats that consumed NNS gained weight as opposed to losing weight.[28] The increase in weight could have been caused by the overconsumption of the maintenance chow that was given along with the saccharin-sweetened yogurt.[26] The main limitation of the three studies listed above is whether or not the study findings can be translated to humans.

Based on the research summarized, there is no concrete answer to whether or not NNS is beneficial for weight loss purposes. The main limitations seen across all studies included using diet soda as a proxy for NNS consumption and the conflicting results found between studies. As a general note, diet sodas are used in many NNS studies as a proxy as the majority of NNS are heat sensitive and cannot be used in food products.

Cancer

The role of NNS on development of cancer has been a heavily debated topic since the 1970s.[29] NNS have been associated with cancer since early studies linked them to bladder cancer in laboratory animals.[5] Some studies have found an association between saccharin and bladder cancer in rodents when treated with extremely high doses.[30] In one study by Soffritti et al., aspartame was significantly associated with an increase in lymphomas and leukemia in female rats when exposed to equivalent levels of human consumption.[31] There have also been studies that found the risk for development of bladder cancer in relation to saccharin consumption in humans.[30,32] Weihrauch et al. concluded that heavy NNS use lead to an increased risk of 1.3 for development of bladder cancer in humans.[30] Although there have been studies that have found a positive association with cancer and saccharin intake, the majority of studies investigating the carcinogenic effects of saccharin do not support the association and have concluded that these effects are species specific.[33] Although there was an association between NNS and bladder

cancer in some studies, Walter et al. and Kessler found no association between NNS and bladder cancer in humans.[34,35] Both studies looked specifically at saccharin, cyclamate, or a combination of the two.[34,35] The association of other cancers, like cancer of the stomach, pancreas, and endometrium, with artificial sweetener consumption have also been studied, and currently, no association of risk between these types of cancer and NNS use exists.[29]

Other studies have examined an array of different cancer types. Lim et al., completed a 5-year prospective cohort study looking at beverages containing aspartame and found no relationship between beverage consumption and the development of haematopoietic (blood cellular components) and brain cancers.[36] Schernhammer et al. found the opposite. Their study looked at whether the consumption of aspartame and sugar-containing sodas were associated with hematopoetic cancers in both men and women in a long-term epidemiologic study.[37] Diet soda consumption was assessed by twelve fluid ounce servings for diet cola with caffeine, diet cola without caffeine, and other diet sodas. In men, risk of non-Hodgkin lymphomas was significantly increased in individuals who consumed > 1 serving per day compared to non-consumers. This association showed a linear trend with greater consumption of diet sodas. In women, there was no association found. For multiple myeloma, risk of development increased linearly with increased consumption of diet sodas in men. Once again, no association was found in women. Lastly, greater risk of leukemia was seen in both men and women who consumed higher levels of diet sodas, but the sex specific results were not significant.[37] Gallus et al., completed case-control studies looking at various NNS, specifically saccharin, on the development of multiple cancers including cancers of the oral cavity and pharynx, colon, breast, etc. and found a lack of association between development of neoplasms, abnormal growths, and consumption of saccharin, aspartame, and other NNS.[38]

A review in the literature showed that most instances of cancer related to NNS consumption were found in laboratory animals, although there were some instances found in humans[37]. It is important to highlight the fact that the human studies were observational whereas the animal studies were interventions. This could mean that the carcinogenic effects of NNS may be species specific[33] or these results could be the result of the study type. The studies summarized above have a similar strength in that they specify the type of NNS studied. A gap in the literature is that only a few NNS have been examined in relation to their association with cancer. More research needs to be done in this area of research to determine if NNS have a carcinogenic effect in laboratory animals and humans.

Metabolic Syndrome and Non-Nutritive Sweeteners

According to the AHA, about 34% of adults in America have MetS.[39] The rates of individuals with MetS are increasing in the US and researchers are trying to determine why.[40] MetS is the name given to a group of five risk factors that increase an individual's risk for heart disease and other health related problems. In order to be diagnosed with MetS, an individual must have three or more of these risk factors.[39] To determine whether or not NNS have a role in MetS, researchers must look at the five risk factors: large waist circumference (≥35 inches for women and ≥40 inches for men), high blood pressure (≥130/85 mmHg), high triglyceride levels (≥150 mg/dL), low high-density lipoprotein (HDL) cholesterol (<50 mg/dL for women and <40 mg/dL for men), and high fasting blood glucose levels (≥100 mg/dL).[39]

Nettleson et al. found that individuals who consumed ≥ 1 serving of diet soda daily had a 36% greater chance of developing MetS compared to non-consumers.[41] Serving size was reported as small, medium, or large and weighted as intake frequency multiplied by 0.5, 1.0, and 1.5 respectively. The MetS risk factors most often seen in participants were high waist

circumference and high fasting blood glucose. No other risk factors of MetS were associated with diet soda consumption.[41] The observational data collected from this study allowed researchers to form an association between daily diet soda consumption and a greater risk of developing MetS.[41] Limitations in this study included using diet soda as a proxy for NNS consumption, participant reporting bias on the quantity and the types of NNS consumed, and the inability to calculate amount of NNS consumed via food products.[41]

Dhingra et al. used data from the Framingham Heart Study to determine if soft drink consumption, regular or diet, played a role in the development of MetS in middle-aged adults who were initially free of MetS in this cross-sectional analysis.[42] Offspring of the members of the original cohort who attended two consecutive visits between 1998 and 2001 were selected for this investigation. Researchers found that 41% of participants who recorded consuming 1 to 6 diet sodas per week developed MetS. Of the 819 individuals who consumed $\geq$ 1 diet soda per day, 40% of them developed MetS. Also, individuals who consumed $\geq$ 1 soft drink per day, diet or regular, had a 25% to 32% higher risk of incidence for each trait of MetS, except for high blood pressure.[42] Limitations of this study included the inability to control for diet and other lifestyle factors, using diet soda as a proxy for NNS consumption, and that all participants in this study were white Americans.[42]

Crichton et al. examined and compared two studies to determine if there was an association between diet soda consumption and MetS.[43] The first study, done in the US, was a community-based, longitudinal study consisting of 803 middle-aged participants. The second study, done in the United Kingdom, was a cross-sectional study consisting of 1,323 participants aged 18 to 69. The total number of participants in the comparison study was 2,126. In study one, dietary intake of the participants was evaluated using the food frequency questionnaire (FFQ)

portion of the Nutrition and Health Questionnaire and diet soft drink consumption was determined by glasses/cans of "diet" and "regular" carbonated drinks consumed daily. The specific quantity consumed was not asked. In the second study, a validated, semi-quantified FFQ was used. Diet and regular soft drinks were included in this FFQ. For beverage intake, participants chose between "never or rarely", "1-3 times/month", "1-2 times/week", "3-5 times/week", and "every day". Participants were also asked to indicate the total quantity in milliliters (mL). Twenty-four percent of participants in study one consumed diet soft drinks compared to 11% in study two. In both studies, diet soft drink consumption was positively associated with waist circumference ($p<0.05$). In study two, diet soft drink consumption was positively associated with systolic blood pressure and fasting plasma glucose levels ($p<0.05$). In **Table 1,** depicted below, the percent of participants from each study and the prevalence of MetS is shown.

Table 1: Comparison of Metabolic Syndrome (MetS) Incidence between Study Samples[43]

Diet Soft Drink Consumption (Servings/day)	Study 1: % (n) of population (n= 803)	Study 1: % (n) with MetS within each group	Study 2: % (n) of population (n= 1,323)	Study 2: % (n) with MetS within each group
None	73.5 (590)	42.4 (250)	76.3 (1,009)	25.8 (260)
1 per day	16.9 (136)	49.3 (67)	21.7 (287)	25.8 (74)
2 or more per day	9.6 (77)	46.8 (36)	2.0 (27)	44.4 (12)

In both studies, a positive association between diet soft drink consumption and development of MetS was observed. Limitations of this comparative study include the use of two different study designs, drink and diet consumption were all self-reported, different FFQ's were used for each study, and different measurement scales for amounts consumed were used.[43]

A lot is still unknown about what contributes to MetS, especially when it comes to diet. Lutsey et al. examined participant's total diet, focusing on specific categories like diet soda consumption. In this prospective cohort study, participants from the Atherosclerosis Risk in Communities study were followed for nine years.[44] Individuals who already had MetS and cardiovascular disease were excluded. Over the nine year period, 40% of participants developed MetS. Researchers found that diet soda consumption was strongly associated with increased risk of MetS across the two models ($p \leq 0.001$). In model one, researchers controlled for age, sex, race, education, center, and total calories. Model two included model one, as well as smoking status, pack-years, and physical activity. Model three included models one and two, as well as intakes of dairy, meat, fruits and vegetables, whole grains, and refined grains.[44] Limitations of this study included the FFQ used was not specifically developed to assess NNS consumption and participant reporting bias on types of NNS consumed.[44]

Although Gardener et al. did not specifically focus on diet soda consumption and MetS, their results supported a relationship between the two.[45] In this population-based cohort study, researchers collected data for blood pressure, blood glucose, low- and high-density lipoprotein cholesterol, triglycerides, waist circumference, and BMI. The data collected for little to no diet soda consumption, daily diet soda consumption and daily regular soda consumption is shown in **Table 2**. Frequent diet soft drink consumption was associated with hypertension, elevated blood glucose, lower HDL cholesterol, elevated triglycerides, increased waist circumference, and BMI, as compared to individuals with no diet soda consumption. All values lead to an increased risk of MetS. There was not an increased risk with regular soft drink or low diet soft drink consumption.[45] Limitations in this study included random misclassification of NNS type and recall bias with the FFQ used, type of regular and diet soft drinks consumed, using regular and

diet soda as a proxy for NNS consumption, and an inability to form a dose-response relationship due to the lack of participants that consumed diet soft drinks daily.[45]

Table 2: Metabolic Syndrome (MetS) Risk Factor Values[1]

	Blood Pressure (Mean)	Blood Sugar (Mean)	LDL Cholesterol (Mean)	HDL Cholesterol (Mean)	Triglycerides (Mean)	Waist Circumference (Mean)	BMI (Mean)
< 1 Diet Soda/Month (n=1,948)	144/83	101	129	48	131	36	28
Daily Diet Soda Consumption (n=163)	146/84	118*	128	45*	146*	39*	31*
Daily Regular Soda Consumption (n=338)	143/83	103	128	45	131	37	28

[1]Adapted from Gardener et al.[45]
*$p \leq 0.05$ between categories of diet soft drink consumption

Similarly, the CARDIA (Coronary Artery Risk Development in Young Adults) study did not focus on MetS, but examined the effects of diet soda consumption.[46] Participants were surveyed over a span of twenty years and were placed into clusters based on their dietary patterns, Western versus Prudent. Using hazard ratios, Duffey et al. found that consumers of diet beverages were at an increased risk for high waist circumference, high fasting glucose, low HDL cholesterol, and high triglycerides, compared to the other three groups (Prudent non-consumers, Prudent consumers, Western non-consumers), which consequently put them at a higher risk for developing MetS.[46] Overall, this study found that overall dietary pattern and diet beverage consumption were both important when it comes to different metabolic outcomes.[46] Limitations of this study included using self-reported dietary data, underreporting of energy intake, and the variation in types of beverages consumed by NNS non-consumers. Some NNS non-consumers

reported drinking beverages that had negative associations with risk factors of MetS (i.e., regular soft drinks) whereas other reported healthy alternatives (i.e., water).[46]

Overall, the studies mentioned above support the association between NNS intake and the development of MetS, but it is important to note that most were observational studies. Therefore, researchers are unable to make a conclusion on causation. These studies all have a major limitation in common, in that they did not look at the impact of individual types of NNS, just at NNS as a whole. Further, some studies only looked at diet soda as a proxy of NNS intake. Another limiting factor that was consistent between studies was the presence of recall bias due to self-reported data. Lastly, researchers acknowledged that there is still a lot that is unknown about MetS.[47] This puts limitations on the research done because if researchers do not fully understand the disease, then they cannot fully understand the potential causes.

Waist Circumference

Individuals with a high waist circumference typically store most of their fat around their waistline, which puts them at a higher risk for heart disease and type 2 diabetes. This risk increases significantly when women have a waist circumference higher than 35 inches, and men have a waist circumference higher than 40 inches.[48] In a study by Duffey et al., described in the previous section, individuals in both diet groups that were consumers had a higher waist circumference compared to individuals who were non-consumers.[46] In another study by Odegaard et al., researchers looked at whether or not sugar-sweetened and diet beverages had a relation to visceral adipose tissue.[49] Non-Hispanic, white participants (n=791) between the ages of 18 and 70 were included in this study. Dietary intake was assessed using the Willett semi-quantitative FFQ. Individuals who consumed ≥ 1 diet beverage per day had a waist circumference of 96.02 cm compared to 94.28 cm for individuals who consumed no diet

beverages. The study concludes that more research needs to be done in this area of research for an association to be formed.[49] In another study by Fowler et al., researchers examined whether diet soda consumption caused a change in long-term waist circumference.[50] This prospective cohort study included 749 Mexican-American and European-American individuals 65 and older. These participants were recruited from the San Antonio Heart Study cohort. The average follow-up period for individuals attending at least one follow-up was 9.4 years. To measure diet soda consumption, participants were asked, "How many bottles or cans of soft drinks do you drink per week?". Individuals who had a mean diet soda consumption of 0.05/d or more were considered users, and those with consumption of less than 0.05/d were non-users. Participants were then further broken down into non-users, occasional users (>0 but <1/day) and daily users (>1/day). Consumers of diet soda typically had a higher waist circumference than non-consumers at baseline, but this was not significant ($p = 0.06$). Increases in waist circumference were seen in all diet soda consumption categories. The mean change in waist circumference for users was almost three times that of non-users. When looking at the occasional users and daily users, a dose-response relationship emerged between diet soda consumption and waist circumference. Lastly, the change in waist circumference between users and non-users was significantly different in men, but was not statistically significant in women.[50] Limitations in this study include generalizability of results to a younger population and uncalculated confounding variables such as family history.[50]

Contradictory to the studies presented above, Tate et al., also described in the "weight status" section, found that waist circumference decreased significantly in the diet beverage group after the six month period. A reduction in waist circumference was also seen in the water and control groups.[20]

Blood Pressure

To be a metabolic risk factor, an individual's blood pressure needs to be 130/85 mmHg or higher.[51] NNS consumption has been linked to an increased risk of hypertension in adults, specifically women.[52] In fact, in a cross-sectional study, Cohen et al. discovered that NNS consumption had approximately the same negative effect on blood pressure as sugar-sweetened beverages.[53] Researchers hypothesized that the negative effect in both NNS and sugar-sweetened beverages could be due to similarities between the two drinks, like carbonation or if the drink was cola or not.[53] Limitations in this study included misclassification of beverage type by participants, variations in serving sizes, self-reports of hypertension, and that the findings could be due to residual confounding variables.[53] In a population-based cohort study, researchers found that diet soft drink consumption was linked to hypertension.[45] Diet and regular soft drink consumption was assessed at baseline (none, light, or daily) using an FFQ and participants completed a follow-up roughly ten years later.[45] Limitations of this study included using diet and regular soft drinks as a proxy for NNS and sugar-sweetened beverage consumption respectively, unavailable information on the type of regular and diet soft drinks consumed, participant reporting bias on types of NNS consumed, and the failure to reassess soft drink consumption at follow-up.[45]

Other studies evaluating the effects of NNS on blood pressure have demonstrated contradictory results. In one study that looked at metabolic syndrome, risk of high blood pressure was not associated with NNS consumption.[42] In a prospective analysis study using participants from the PREMIER study, Chen et al. also concluded that there was no association between NNS and blood pressure, but researchers did notice a significant decrease in blood pressure when

participants decreased their use of sugar-sweetened beverages.[54] A limitation of this study included a lack of diversity in participants.[54]

More research needs to be done to determine the link between NNS consumption and risk of hypertension. The studies reviewed are split. Limitations of these studies included lack of specificity in the types of NNS studied and the use of diet beverages as a proxy for NNS consumption.

Triglyceride Levels

Triglycerides are a type of fat found in the blood, and are used for energy in the body. In order for triglyceride levels to be a metabolic risk factor, individuals must have a level of 150 mg/dL or higher.[51] Raben et al. compared a sucrose-rich diet and an artificially sweetened diet in a ten week parallel intervention study with two groups.[55] The groups were randomized to receive supplemental foods and drinks that contained either sucrose or artificial sweeteners. Participants consumed the supplements as part of their daily food intake. The study comprised of 41 participants, with a sub-group of 23 participants receiving additional measurements of fasting and postprandial metabolic profiles. All participants had to be between the ages of 20 and 50 and overweight. Fasting blood draws were taken at zero weeks and again at ten weeks and a postprandial blood draw was taken at ten weeks. At week ten, fasting triglyceride levels were significantly higher in the sucrose group compared to the NNS group. For the postprandial triglycerides, levels were much higher in the sucrose group compared to the NNS group ($p<0.05$). This difference became insignificant after including change in body weight and fasting concentrations.[55] In a study by Colagiuri et al., researchers compared the effects of adding sucrose and aspartame to the typical diets of individuals with noninsulin-dependent diabetes.[56] This double-blind, cross-over study looked at nine subjects. The subjects were randomly

assigned to one of the two groups. Forty-five grams of sucrose was added to the subject's diet of one group, whereas 162 mg of aspartame was added to the subject's diet in the other. The subjects remained in their selected groups for six weeks, and then transferred groups for an additional 6 weeks. Triglycerides were tested via fasting serum lipids. Triglyceride levels were not significantly different at the end of the supplement periods compared with pretreatment levels for both groups.[56] A limitation of both of these studies includes their women to men ratio.[56] In the study by Duffey et al., researchers found that NNS consumers had higher triglyceride levels compared to non-consumers. In this study, there were a total of 3,602 participants with high triglycerides. Western consumers had a hazard ratio (HR) of 1 and Western non-consumers had a HR of 0.93. Prudent consumers had a HR of 0.80 and Prudent non-consumers had a HR of 0.72.[46]

Toigo et al. examined sixteen adult female Wistar rats and their offspring. The rats were divided in four groups; control (receiving water), sucrose (45g/L), saccharin (1.35 g/L) and aspartame (2 g/L).[57] Rats had free access to standard rat chow and their different solutions. The solutions were administered as the only drinking water for thirty days. The animals continued to receive treatment until they gave birth. At 112 days of age, the offspring were killed to perform biochemical evaluations. Compared to the control group, male offspring whose mothers consumed sucrose or aspartame had increased triglycerides. This study is one of the first to look at the impact of NNS during gestation.[57]

<u>High-Density Lipoprotein (HDL) Cholesterol</u>

HDL cholesterol, also known as "good" cholesterol, helps remove cholesterol from your arteries. For HDL levels to be a metabolic risk factor, levels need to be less than 50 mg/dL for women and less than 40 mg/dL for men. HDL cholesterol can also be considered a metabolic

risk factor if an individual is on medicine to treat low HDL cholesterol.[51] The apoA-I gene gives instructions for making a protein called apolipoprotein. This protein is a component of HDL cholesterol. This protein attaches to cell membranes and promotes the movement of cholesterol and phospholipids from inside the cell to its outer surface. ApoA-I also triggers cholesterol esterification. This reaction converts cholesterol to a form that can integrate into HDL and be transported through the bloodstream.[58] In a study by Kim et al., researchers isolated lipoproteins from blood samples given by healthy males who had fasted sixteen hours before a blood draw. HDL_3 was incubated with aspartame, acesulfame potassium, saccharin, and fructose for 48 hours at 37 degrees Celsius in the presence of 5% CO_2. After incubation, each HDL_3 sample was characterized by electrophoresis and fluorescence spectroscopic analysis to confirm glycation. In the presence of 25 millimolar of aspartame, the apoA-I band, a protein in HDL cholesterol, was slightly fragmented. In the presence of 100 millimolar, the apoA-I band was severely fragmented. Data also showed that the antioxidant activity of HDL_3 was severely impaired by the artificial sweetener treatment. This paper suggests that accumulation of artificial sweetener can speed up the modification of HDL/apoA-I and incidence of atherosclerosis.[59] In another study by Jang et al., apoA-I was purified from human plasma.[60] Three millimolar of aspartame, acesulfame potassium, and saccharin were used to treat the protein for 168 hours. The results from this study suggest that long-term consumption of NNS may cause modification of the protein and protein cleavage. The modification in the protein causes it to lose its antioxidant and phospholipid binding abilities. The proteins treated with saccharin appeared to have the most dramatic results.[60]

Fasting Blood Glucose: Overall NNS Consumption

Fasting blood glucose levels must be 100 mg/dL or higher to be considered a metabolic risk factor. A value between 100-125 mg/dL is considered pre-diabetes and a value greater than 126 mg/dL is considered diabetes.[51] The increase in chronic disease prevalence, like diabetes, has resulted in an increase in the production and consumption of NNS.[4] NNS are thought to be a good alternative for individuals with diabetes, because they have little to no calories and help to reduce sugar intake.[4] Many studies have examined the effects of added sugar consumption on glucose control in individuals with and without diabetes, but far less have examined the impact of NNS consumption on glucose levels.[4] The research that is available is inconsistent and can be difficult to interpret for the general population. As stated previously, NNS are considered healthy alternatives for individuals with diabetes because of their minimal calories and their assistance in helping individuals decrease sugar consumption.[4] The ADA has advertised NNS as a good alternative to "curb your craving" for sugar.[61] The amount of research available as well as the inconsistency of the research makes it difficult for someone to come to a definitive conclusion on the benefits/detriments of NNS consumption and their impact on glucose levels. A major gap in the literature is that a large portion of studies examine NNS consumption as a whole as opposed to looking at individual NNS consumption.

Nettleson et al., looked at data from the Multi-Ethnic Study of Atherosclerosis study in this observational study.[41] Researchers found that daily consumers of diet soda had a 67% increase in risk of developing type 2 diabetes over non-consumers. Researchers also found that diet soda consumption was linked with high fasting glucose values, in that 30% of participants that consumed ≥ 1 serving of diet soda a day developed high fasting glucose values. Researchers controlled for age, sex, race/ethnicity, examination site, energy intake, socio-economic status,

and lifestyle confounders. The study concluded that diet soda consumption may lead to impaired glucose control in adults.[41] Limitations in this study included using diet soda as a proxy for NNS consumption, misclassification of type of NNS, and the inability to calculate amount of NNS consumed via food products.[41] *Beverage Intake, Diabetes, and Glucose Control of Adults in America,* a cross-sectional study, by Mackenzie et al., studied the effects of multiple beverage choices on HbA1c levels in individuals with and without diabetes.[62] HbA1c measures glycated hemoglobin. Glycated hemoglobin is created when hemoglobin combines with glucose. This measure allows clinicians to see average blood glucose levels over an extended period of time.[63] Participants with diabetes that consumed diet sodas had higher mean HbA1c values as compared to those that did not consume diet soda. These values can be seen in **Table 3**.[62] Limitations of this study included the inability to make firm conclusions on causality, self-reporting bias, the FFQ was not designed to measure NNS intake, diet was not controlled for, and diet beverages were used as proxy for NNS consumption.[62]

Table 3: Mean HbA1c Values by Diet Soda Consumption Frequency[62]

	0 NNS drinks per month	1-29 NNS drinks per month	30-59 NNS drinks per month	≥ 60 NNS drinks per month	p (trend)
Diabetes	7.3	7.7	8.0	8.2	0.0005
No Diabetes	5.2	5.2	5.2	5.2	0.33

Suez et al. also found NNS intake to have a negative impact on glucose values.[64] In this observational study, researchers determined that non-diabetic individuals became increasingly glucose intolerant from consumption of NNS. Long-term NNS consumption was determined by a validated FFQ and glucose values were collected via a Glucose Tolerance Test (GTT).[64] A limitation in this study included recall bias.

In the study by Koning et al., men from the Health Professionals follow-up study participated in a prospective cohort study to determine if NNS consumption contributed to type 2 diabetes.[65] After adjusting for age, individuals who consumed NNS beverages had an increased risk of type 2 diabetes. However, when adjusted for other factors, such as smoking status, physical activity, alcohol intake, BMI, etc., the relationship between NNS and development of type 2 diabetes became insignificant. This study concluded that the relationship between NNS intake and diabetes risk may be related to current health conditions of the individual as opposed to NNS consumption.[65] Limitations of this study included a lack of generalizability in that all participants were white men, and residual and unmeasured confounders.[65]

Individuals may switch from regular sodas to diet sodas in order to lose weight and potentially help with diabetes.[66] Whether or not this switch is actually beneficial is still unknown. A major limitation in this area of research is that the studies described above all examined the associations between glucose levels and NNS intake as a whole. In the following sections, available research completed on specific types of NNS and their relation to glucose levels will be summarized.

Fasting Blood Glucose: Individual NNS Consumption

Rats were given yogurt sweetened with either 20% glucose or 0.3% saccharin. In the rats that consumed the saccharin mixture, blood glucose levels were significantly higher fifteen minutes after consumption compared to the rats given the glucose mixture.[67] Oral glucose tolerance tests (OGTT) were also significantly different between the two groups. For the OGTT, the rats were given a glucose solution and blood glucose values were tested. Researchers found that blood glucose values were significantly higher eight, twelve, and forty-eight minutes following the glucose solution in the rats consuming the saccharin mixture as opposed to the

glucose mixture.[67] However, another study done in rats found that drinking saccharin had no effect on blood glucose values.[68] This study only provided the rats with saccharin once every two days,[68] whereas the previous study provided the rats with the either glucose or saccharin daily for three consecutive days.[67] Limitations of these studies included whether or not the results could be replicated in human subjects and the variation of how much saccharin was given to the rats in the two studies. Also, it is important to note that in the first study, researchers measured blood glucose in response to drinking glucose[67], and in the second study, researchers measured blood glucose in response to drinking saccharin[68], meaning the two studies cannot be compared.

In humans, in a study by Althausen et al., twenty-one diabetes-free individuals and fourteen individuals with diabetes were asked to consume 200 cc of water with 0.15 g of saccharin.[69] Blood glucose was measured five, fifteen, and thirty minutes after consumption. Researchers found no hyperglycemic effect for the diabetes-free group and the diabetic group.[69] Furthermore, in another study, participants experienced hypoglycemia after orally consuming 80 mL of water with 0.5 g of saccharin.[70] The hypoglycemia ended roughly thirty minutes after consumption.[70] Another study by Jorgensen also resulted in a fall of blood glucose values when a saccharin mixture was given orally.[71] An experimental study by Suez et al. contradicts the previous studies.[64] Researchers followed seven healthy individuals, who did not normally consume NNS, over a week-long period. On days 2-7, participants consumed the ADI for saccharin. At the end of the seven day period, four of the seven participants had developed significantly worse glycemic responses.[64] The participant's glycemic responses were tested via a GTT. This means, when given glucose, the participant's responses did not respond as well to the glucose as they had before consuming saccharin.[64] Based on the majority of the research

summarized, saccharin does not appear to be associated with increased blood glucose values in humans.

In an intervention study by Brown et al., researchers found that consumption of diet soda containing acesulfame potassium and sucralose may cause an increase in GLP-1 secretion by synergizing with glucose.[72] Wu et al. re-examined the study conducted by Brown et al. and controlled for more variables.[73] As opposed to diet soda consumption, Wu et al. gave acesulfame potassium and sucralose orally in the same amounts that would have been consumed via diet soda. In the latter study, the opposite was found in that no effect on blood glucose concentrations were demonstrated.[73] A major limitation regarding the association between acesulfame potassium and glucose levels is the lack of investigations looking solely at acesulfame potassium intake, i.e., it is often combined with other NNS.

Seven healthy adults were given various mixtures of sucrose and saline, saline, or sucralose and saline.[74] The two treatment groups receiving the sucralose-saline mixture had no change in blood glucose levels after ingestion. The only group that experienced a change in glucose values was the sucrose-saline group.[74] A 3-month, randomized, double-blind study tested the effects of sucralose on glucose levels in individuals with type 2 diabetes.[75] Researchers concluded that sucralose had no effect on fasting plasma glucose levels.[75] Mezitis et al. examined the effects of a single high-dose of sucralose on patients with diabetes after an overnight fast and concluded that sucralose consumption does not negatively affect short-term blood glucose control in individuals with diabetes.[76] From the research summarized, it does not appear that sucralose has an effect on blood glucose values.

In a study conducted on forty-three adult, non-insulin dependent diabetics, 1.8 g of aspartame were administered via an oral capsule daily over a 90-day period. Participants were

told to maintain their current diets and to take the pills three times daily. Compared to the control group, which was given a placebo, aspartame had no effect on the individual's ability to control their diabetes and had no significant effect on fasting blood glucose.[77] In a study by Colagiuri et al., researchers also looked at the effect aspartame had on non-insulin dependent diabetics. Over a 6-week period, participants were either given a predetermined amount of sucrose or aspartame. The data showed no significant difference between fasting blood glucose values for the sucrose and aspartame groups.[56] Rodin looked at the effect of aspartame on overweight and normal weight individuals in his within-subjects design study, a type of experimental study. Similar to the previous two studies, the aspartame load did not have an effect on fasting plasma glucose values.[78] All studies summarized above do not support a link between aspartame consumption and glucose values.

In a study by Raskovic et al., researchers pretreated mice with two different commercial sources of stevia.[79] Group one was pretreated for four days with 200 mg/kg of Stevia, group two was pretreated for four days with 20 mg/kg of Clear Steviosides Liquid, and group three, the control group, was pretreated for four days with physiological solution. To test the changes in blood glucose values, the mice underwent an OGTT. Four days after the pretreatments were completed, researchers found that blood glucose levels were significantly lower in groups one and two compared to the control.[79] In another study conducted on rats, researchers looked at the effects of stevia rebaudiana in rats with diabetes.[80] When given 0.5 mg/kg of stevioside, researchers observed a lowering in blood glucose values.[80] In a study conducted with human subjects, twenty-two healthy individuals were given stevia rebaudiana extract following OGTT.[81] Each participant was given 13-65 g doses of the extract. The control group was given 13-250 mg of arabinose, a sugar found in plant gums, on the same schedule. Researchers found that the

group given the stevia extract had significantly lower blood glucose values post OGTT compared to the control group.[81]

The three investigations summarized all came to the same conclusion: stevia, has a hypoglycemic effect on blood glucose values.[79-81] More research needs to be done, especially on human subjects, in order to come to a definitive conclusion on the effects of stevia on blood glucose values.

Gaps in the Literature Related to Non-Nutritive Sweetener Intake and Metabolic Syndrome

Research on the association between NNS and MetS is limited. Rates of MetS are rising in the US, therefore, it is important for researchers to determine the reasons why.[40] A majority of the studies reviewed look at NNS as a whole as opposed to individually when assessing its association with MetS. This limitation will be addressed in the current investigation by not only looking at NNS as a whole, but also looking at four individual NNS and their association with MetS. A majority of studies also use diet soda as a proxy for NNS consumption. This has the potential to underestimate NNS consumers. This limitation will be addressed in the current investigation by calculating the amount of NNS consumed from foods and beverages into milligrams (mg). Another limitation in this area of research is that not all risk factors for MetS have been assessed individually. In this investigation, we will be examining the association between NNS and MetS, as well as NNS and each of the five risk factors. Lastly, many foods and beverages that contain NNS contain more than one, resulting in NNS consumers ingesting multiple types of NNS. This makes it difficult to determine if one individual NNS has a greater impact on health status than another. In this investigation, Nutrition Data System for Research (NDSR) 2015 will be used to calculate the amounts and types of NNS consumed. It is important

to look at multiple combinations of NNS, as people typically do not consume just one type of NNS. As stated previously, rates of MetS have increased in the US. From 2003-2004 to 2011-2012, the percentage of individuals with MetS went from 32.9% to 34.7%.[40] Thus, it is important to increase the amount of research being done in this area so we can better understand the impact that these sweeteners, as a whole and individually, have on our health.

Not only are there limitations when looking at NNS and MetS, there are also many limitations with NNS research in general. It is difficult to accurately measure the amount of NNS that is actually being ingested due to the limited amount of information available on food and drink labels.[11] A majority of the studies that have been done in this area of research have been observational studies. A limiting factor for observational studies is that researchers are unable to formulate a definitive conclusion on causality, especially as many studies do not control for diet, so there is no way of determining potential confounding variables.[62] Another common attribute of studies done in this area of research is the use of diet soda as a proxy for total NNS consumption. Also, many of these studies examined NNS intake as a whole instead of specific types of NNS. Lastly, many investigations use self-reported dietary intake data. Limitations that stem from this include misclassification of NNS type and self-reported bias.

Conclusion

Consumption of NNS by children and adults is increasing in the US.[11] Among adults, prevalence of MetS is also increasing.[40] There are studies that have examined the association between NNS consumption and MetS, but few have studied the association between individual NNS and MetS and the association between NNS consumption and each of the five risk factors of MetS. Among the studies summarized, there does seem to be an association between NNS consumption and MetS. A major limitation to these studies is that they are mostly observational.

There have also been studies that have shown an association between NNS consumption and waist circumference, HDL levels, and fasting blood glucose levels. Studies that examine the impact of individual NNS on each of the five risk factors are scarce. To better understand this association, analyses need to be run to determine if NNS consumers are at increased risk for MetS.

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Chapter 2: Association between Non-Nutritive Sweetener Intake and Metabolic Syndrome

Introduction

Non-nutritive sweeteners (NNS) are regulated as food additives in the United States (US). Foods and beverages made with NNS are typically labeled as "sugar-free" or "diet" as they provide little to no calories.[1] There are six NNS that are approved by the Food and Drug Administration (FDA); saccharin, aspartame, acesulfame postassium, sucralose, neotame, and advantame.[2] The FDA also recognizes two NNS as "generally recognized as safe" (GRAS)[1]; high purified steviol glycosides and luo han guo fruit extract.[2] The GRAS label signifies that researchers have no reason to believe that the product will cause harm when eaten for its intended purpose and within its recommended quantity.[1] For the purpose of this investigation we looked specifically at saccharin, aspartame, acesulfame potassium, and sucralose.

With obesity and other chronic diseases on the rise in the US[3], NNS producers are advertising their products to the American public[4] by using evidence that suggests these trends are due to diets high in added sugar.[3] The issue is that NNS consumers do not have adequate access to information on NNS to determine if they are beneficial or detrimental.[4] In a study by Sylvetsky et al., researchers found that the percentage of children who consumed NNS nearly doubled, from 9% in 1999-2000 to 15% in 2007-2008. During the same time period, the percentage of adults who consumed NNS increased from 27% to 32%.[5]

As stated previously, NNS consumption is on the rise[5], but so are rates of metabolic syndrome (MetS). According to the American Heart Association (AHA), there was a 2% increase in individuals with MetS from 2003-2004 to 2011-2012.[6] MetS is the name given to a group of five risk factors; large waist circumference (≥35 inches for women and ≥40 inches for men), high blood pressure (≥130/85 mmHg), high triglyceride levels (≥150 mg/dL), low high-

density lipoprotein (HDL) cholesterol (<50 mg/dL for women and <40 mg/dL for men), and high fasting blood glucose levels (≥100 mg/dL).[6] Each risk factor can increase an individual's risk for heart disease and other health related problems, but in order to be diagnosed with MetS, an individual must meet the criteria for three or more of these risk factors. [6]

The impact NNS consumption has on health can be hard to decipher. In 2012, the AHA and the American Diabetes Association (ADA) did a review of the current literature on NNS and concluded that there was not sufficient evidence or data to advertise that replacing caloric sweeteners (i.e., sugar) with NNS was beneficial.[7] NNS provide little to no calories. Therefore, it can be difficult to see the potential link between NNS consumption and the possible increase an individual's risk for things like obesity, type 2 diabetes, and MetS. Pepino et al. summarizes three main mechanisms that explain the proposed metabolic effects of NNS, 1) NNS may interfere with learned responses that help to control glucose and energy homeostasis, 2) NNS may interfere with gut microbiota and induce glucose intolerance, and 3) NNS may interact with sweet-taste receptors that are expressed throughout the digestive tract that play a role in glucose absorption and trigger insulin secretion.[8] Other studies have also shown that NNS may interfere with thyroid axis activity[9] and can lead to an increased risk in development of MetS.[10] More research needs to be done with NNS and its potential metabolic effects to determine if they can cause metabolic disorders in humans.[8-10] Therefore, the purpose of this investigation is to determine if there is an association between NNS consumption and MetS.

When looking at studies that focused on MetS as a whole, researchers have found positive associations between NNS consumption and MetS.[11-17] Unfortunately these studies have several major limitations. These limitations include looking at NNS consumption as a whole as opposed to individual types of NNS, using diet soda as a proxy for NNS intake, presence of

recall bias, and, in some, the inability to control for diet.[11-17] There are also studies that look at the association between NNS consumption and each risk factor of MetS. Based on the literature, the risk factors associated with total NNS consumption are waist circumference[17-19] and fasting blood glucose values.[11,20,21] Researchers acknowledge that there is still a lot that is unknown about MetS[22] and the impact of NNS consumption[7], therefore more research needs to be done in order to determine whether or not there is an association between these two variables.

Aims

The present investigation has one main objective and two secondary objectives. The main objective of this investigation is to determine if there is an association between NNS consumption and MetS. The secondary objectives are to determine if individual NNS have an association with MetS and to determine if each of the five individual risk factors of MetS has a greater association with NNS consumption. The four individual NNS examined are saccharin, acesulfame potassium, sucralose, and aspartame. Based on the current research, it is hypothesized that there is a positive association between daily total NNS consumption (not specified) and MetS.[11-17]

Waist Circumference

Based on the current research, there is a positive association between daily NNS consumption (not specified) and waist circumference.[17-19]

Blood Pressure

Based on the current research, it is unknown whether or not there is an association between daily NNS consumption (not specified) and blood pressure, specifically hypertension.[12,15,23,24]

Triglyceride Levels

Based on the current research, there is not an association between daily NNS consumption and high triglyceride levels in humans.[17,25-27]

High-Density Lipoprotein (HDL) Cholesterol

Based on the current research, there is a negative association between daily NNS consumption and HDL cholesterol levels.[28,29]

Fasting Blood Glucose

Based on the current research, there is a positive association between daily NNS consumption (not specified) and fasting blood glucose levels[11,20,21,30], but there is no association between daily NSS consumption (specified) and fasting blood glucose levels.[21,26,30-45]

Methods

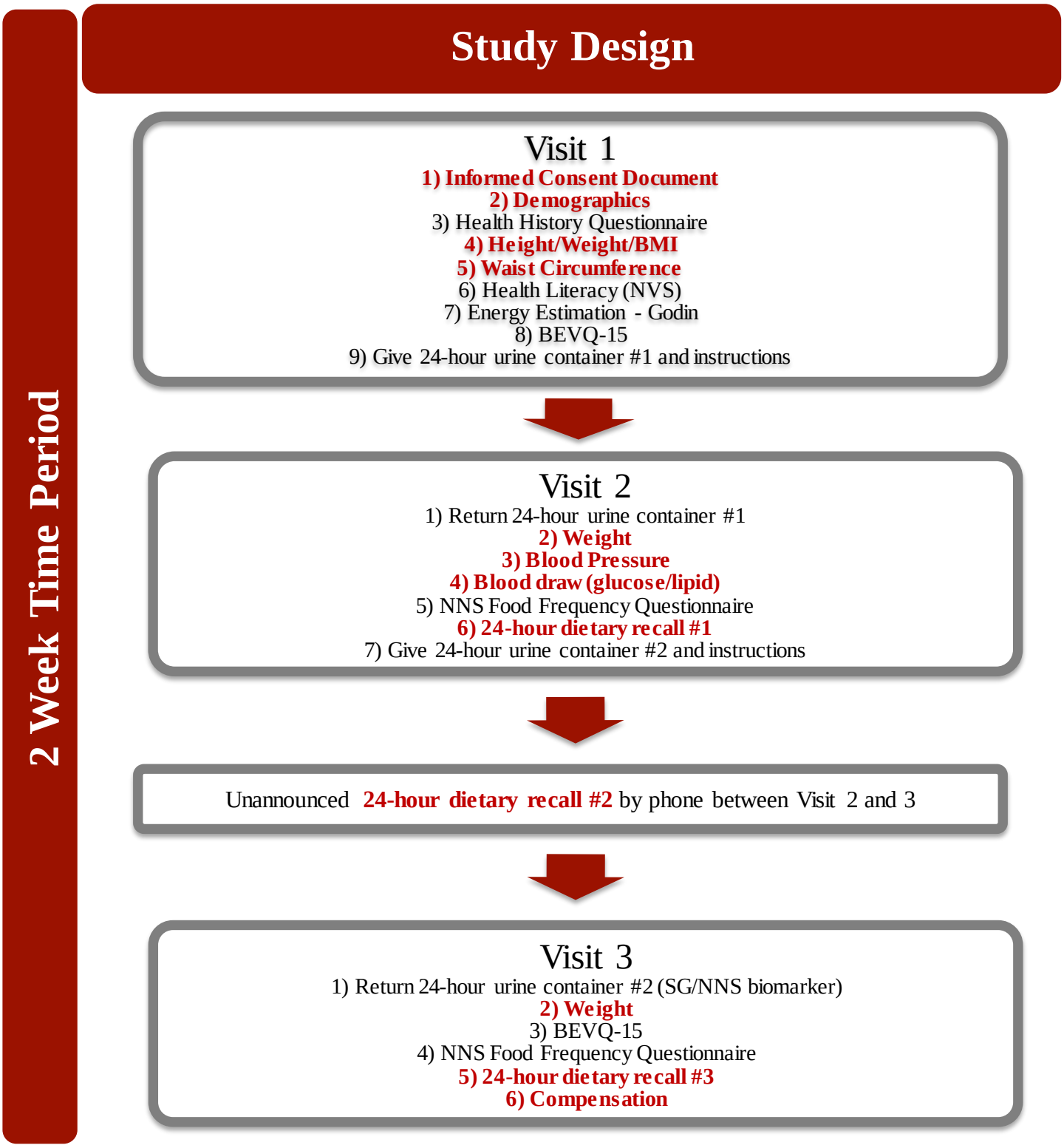
Study Design

This investigation is a cross-sectional study designed to examine associations between NNS consumption and MetS. Participants (n=125) completed a total of three visits over a two week period with an unannounced phone call between visits two and three to collect a 24-hour dietary recall. For participants to be eligible for the study, they had to be 18 years or older. The overall design of the study is shown in **Figure 1**; items in red are measures relevant to this investigation.

During visit one, participants completed the informed consent, demographic information, and height, weight, and waist circumference were measured. During visit two, participants had their weight taken. They also completed a 24-hour dietary recall, and underwent a fasting blood draw for glucose, triglyceride, and HDL cholesterol measurements. During visit three, participants completed a 24-hour dietary recall and had their weight taken for the third time.

Once a participant had successfully completed all three visits, they received compensation of $75.00 and a results packet with information regarding their dietary intake, anthropometrics, and MetS risk factor values.

Figure 1: Flow Chart of Study Design



Materials and Methods

Three 24-hour dietary recalls were collected over the course of two weeks. Two captured weekdays and one captured a weekend day. One of the three 24-hour dietary recalls was unannounced.[46] All three recalls were done by the same data collector to avoid any inconsistencies in collection. Nutrition Data System for Research (NDSR) 2015, nutrient analysis software, was used to analyze the three 24-hour dietary recalls collected from participants. NDSR breaks down the specific amounts of NNS consumed in milligrams (mg). These NNS include saccharin, acesulfame potassium, sucralose, and aspartame. A participant was considered a consumer if they consumed the equivalent of NNS in 1 fluid ounce of diet soda, from either foods or beverages. This equates to 3 mg of acesulfame potassium, 17 mg of aspartame, 12 mg of saccharin, and 6 mg of sucralose.[47]

Anthropometrics were also taken during the two week period. A waist circumference tape measure was used to measure waist circumference (Gulick II tension measuring tape; model 67019). Two measurements were taken, and if measurements were more than a half centimeter apart from one another, both measurements were taken again. Height was measured via a wall mounted, digital stadiometer and was recorded in centimeters. Weight was measured at each visit, using a digital scale (Tanita, TBF-310GS). Clothing was accounted for by inputting 1.6 kilograms (kg) on the scale. Weight was recorded to the nearest 0.1 kg. Blood pressure was measured with an automated oscillometric device (OMRON; model: HEM-907XL). The machine took two measurements and the average of the two measurements was recorded. A fasting venipuncture blood draw was taken to collect fasting blood glucose, triglyceride, and HDL cholesterol values from participants.[46]

Statistics

Descriptive statistics (frequencies, mean±standard deviation) were used to characterize participants by demographics. Participants were labeled as NNS consumers or non-consumers. ANOVAs and χ^2 were used to assess demographic differences between NNS consumers and non-consumers. The main aim of this investigation was to determine whether there was an association between NNS consumption and MetS. Multiple linear regression models were used to determine if there was an association between NNS consumption, as a whole, and MetS. A second aim of this investigation was to determine if any of the five risk factors for MetS are more heavily impacted by NNS consumption. A multiple linear regression model was used to determine if there was an association between NNS consumption, as a whole and individually, and each risk factor of MetS. Lastly, a third aim of this investigation was to determine if individual NNS types had a greater association with MetS. Multiple linear regression models were used to determine if there was an association between individual NNS consumption and MetS. Appropriate demographics were controlled for including age and sex.

Results

Demographic Characteristics

Of the 125 participants, 54 were male and 71 were female and the mean age of these participants was approximately 37 years old and the mean average body mass index (BMI) was 25.9 kg/m^2 (slightly overweight). A majority of participants were white (76%) and had completed post-college work (43%). A total of 12 participants did not report their household income (n=113). There were a total of 18 cases of MetS (i.e., met three of the five criteria) **(Table 1)**.

Table 1: Participant Demographic Characteristics

Characteristics	Total Sample (n = 125)
Age (years), M (SD)	36.7 (16.5)
Sex, n (%)	
Male	54 (43)
Female	71 (57)
Race/Ethnicity, n (%)	
White, not of Hispanic origin	95 (76)
Asian or Pacific Islander	17 (14)
Black, not of Hispanic origin	7 (6)
Hispanic	3 (2)
More than one	3 (2)
Education, n (%)	
High school	6 (5)
Some college	20 (16)
College graduate	45 (36)
Post-college work	54 (43)
Household Income, n (%)	
< $15,000	19 (15)
$15,000 – $29,999	31 (25)
$30,000 – $49,999	12 (9.5)
$50,000 – $99,999	29 (23)
>$100,000	22 (18)
Not Reported	12 (9.5)
Metabolic Syndrome, n (%)	
With 3 of 5 factors present	18 (14)
With less than 3 factors present	107 (86)
BMI (kg/m^2), M (SD)	25.9 (5.7)

Characteristics of Non-Nutritive Sweetener Consumers versus Non-consumers and Consumption Type

Among the 125 participants, 63 were classified as NNS consumers and 62 were classified as non-consumers. The mean age of consumers was slightly higher at 39 years old as compared to non-consumers at approximately 36 years old. Characteristically, BMI was the only significant value between consumers and non-consumers, with NNS consumers having a mean BMI that was 3 kg/m^2 higher ($p=0.002$). Consumers were mostly white, college graduates, with 48% having a household income greater than $50,000. Non-consumers had the same trend in

characteristics with the exception of household income, with only 34% having an income greater than $50,000. Of the 18 total cases of MetS, 12 were consumers and 6 were non-consumers **(Table 2)**.

Table 2: Characteristics of NNS Consumers and NNS Non-consumers

Characteristics	NNS Consumers (n = 63)	NNS Non-Consumers (n = 62)	Significance Between Groups
Age (years), M (SD)	38.8 (16.9)	34.5 (16.0)	0.153
Sex, n (%)			0.519
Male	29 (46)	25 (40)	
Female	34 (54)	37 (60)	
Race/Ethnicity, n (%)			0.238
White, not of Hispanic origin	52 (83)	43 (69)	
Asian or Pacific Islander	8 (13)	9 (15)	
Black, not of Hispanic origin	2 (3)	5 (8)	
Hispanic	1 (1)	2 (3)	
More than one	0 (0)	3 (5)	
Education, n (%)			0.170
High school	4 (6)	2 (3)	
Some college	14 (22)	6 (10)	
College graduate	19 (30)	26 (42)	
Post-college work	26 (41)	28 (45)	
Household Income, n (%)			0.419
< $15,000	9 (14)	10 (16)	
$15,000 – $29,999	12 (19)	19 (31)	
$30,000 – $49,999	5 (8)	7 (11)	
$50,000 – $99,999	16 (26)	13 (21)	
>$100,000	14 (22)	8 (13)	
Not Reported	7 (11)	5 (8)	
Metabolic Syndrome			0.136
With 3 of 5 factors present	12 (19)	6 (10)	
With less than 3 factors present	51 (81)	56 (90)	
BMI (kg/m^2), M (SD)	27.5 (6.5)	24.3 (4.4)	**0.002**

To get an idea of what type of NNS participants were consuming, specific mg values for each type of NNS were used to determine if individuals were classified as a consumer based on the specific type of NNS. As mentioned previously, a participant was considered a consumer if they consumed the equivalent of NNS in 1 fluid ounce of diet soda, either from foods or

beverages. This equates to 3 mg of acesulfame potassium, 17 mg of aspartame, 12 mg of saccharin, and 6 mg of sucralose. The values for NNS can be seen in the table below (**Table 3**).

Table 3: Classification of NNS Consumers by Type of NNS (n=63)

	NNS Consumers n	NNS Non-Consumers n
Aspartame	33	92
Sucralose	26	99
Saccharin	6	119
Acesulfame Potassium	42	83

Characteristics of Participants With and Without Metabolic Syndrome (MetS)

The difference in age and BMI between participants with and without MetS was statistically significant ($p \leq 0.001$). The mean BMI for participants with MetS was approximately 8 kg/m^2 higher than those without MetS and the mean age for participants with MetS was 54 versus 34 years old for those without MetS **(Table 3)**.

Table 3: Characteristics of Participants With and Without Metabolic Syndrome (MetS)

Characteristics	Participants with MetS (n= 18)	Participants without MetS (n = 107)	Significance Between Groups
Age (years), M (SD)	53.9 (12.7)	33.7 (15.3)	**≤ 0.001**
Sex, n (%)			0.908
Male	8 (44)	46 (43)	
Female	10 (56)	61 (57)	
Race/Ethnicity, n (%)			0.404
White, not of Hispanic origin	16 (89)	79 (74)	
Asian or Pacific Islander	1 (5.5)	16 (15)	
Black, not of Hispanic origin	0 (0)	7 (6)	
Hispanic	1 (5.5)	2 (2)	
More than one	0 (0)	3 (3)	
Education, n (%)			0.688
High school	1 (6)	5 (5)	
Some college	2 (11)	18 (17)	
College graduate	5 (28)	40 (37)	
Post-college work	10 (56)	44 (41)	
Household Income			0.062
< $15,000	2 (11)	17 (16)	
$15,000 – $29,999	1 (6)	30 (28)	
$30,000 – $49,999	2 (11)	10 (9.5)	
$50,000 – $99,999	4 (22)	25 (23.5)	
>$100,000	7 (39)	15 (14)	
Not Reported	2 (11)	10 (9)	
BMI (kg/m^2), M (SD)	33.3 (6.6)	24.7 (4.5)	**≤ 0.001**

Prevalence of Individual Risk Factors for Metabolic Syndrome (MetS)

The highest percentage of NNS consumers and non-consumers had 0 risk factors (35% and 53% respectively). It is important to note that 21% of NNS consumers and 16% of non-consumers were on the borderline of being diagnosed for MetS, with the presence of two risk factors (**Table 4 & Figure 2).**

Table 4: Prevalence of Individual Risk Factors for Metabolic Syndrome

Number of Risk Factors	Total Sample (n = 125) n (%)	NNS Consumers (n = 63) n (%)	NNS Non-Consumers (n = 62) n (%)
0 Risk Factors	55 (44)	22 (35)	33 (53)
1 Risk Factor	29 (23)	16 (25)	13 (21)
2 Risk Factors	23 (18)	13 (21)	10 (16)
3 Risk Factors	11 (9)	7 (11)	4 (6)
4 Risk Factors	4 (3)	3 (5)	1 (2)
5 Risk Factors	3 (2.5)	2 (3)	1 (2)

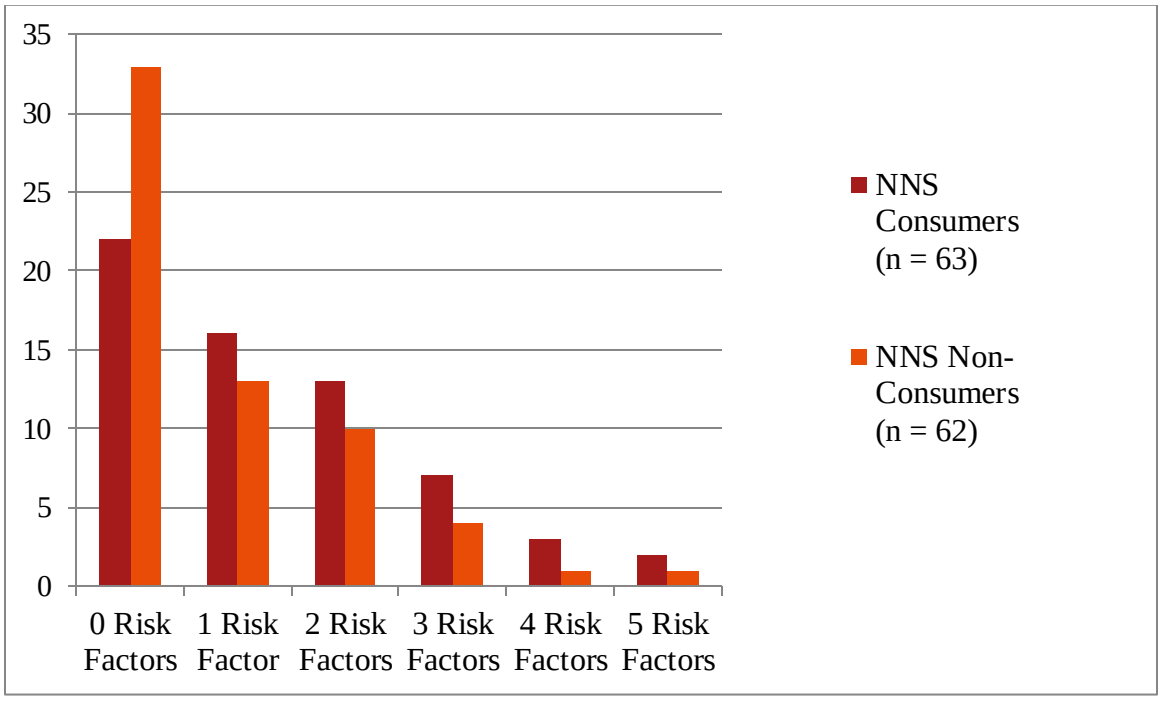


Figure 2: Prevalence of Number of Risk Factors for Metabolic Syndrome

Linear Regression Models

Multiple linear regression models help to describe how one "response" variable relates to multiple "explanatory" variables. Each regression model was run using a continuum for MetS

risk factors (0-5 risk factors present) as the "response" variable. R^2 shows the amount of variation that is caused by the "explanatory" variables and β states that for one standard deviation in the "explanatory" variable, the "response" variable will increase by β. To address aim one and three, MetS on a continuum was the "response" variable and the various NNS were the "explanatory" variables, significant values were found in model one for total NNS consumption (p=0.007) and model two for aspartame consumption (p=0.012) **(Table 5).**

Table 5: Linear Regression Models for Metabolic Syndrome and Total NNS and Individual NNS Consumption Calculated Separately*

	R^2	β (standardized)	Significance
Model 1			
Total NNS Reported	0.058	0.241	**0.007**
Model 2			
Aspartame	0.050	0.225	**0.012**
Model 3			
Saccharin	0.010	0.102	0.258
Model 4			
Sucralose	0.021	0.144	0.109
Model 5			
Acesulfame Potassium	0.010	0.101	0.264

*Response/Independent Variable was Continuum of MetS Risk Factors

To further explore aim three, a multiple linear regression model looking at MetS on a continuum as the "response" variable that included all four individual NNS as the "explanatory" variables was also run. When aspartame, saccharin, sucralose, and acesulfame potassium were included in the same model, aspartame consumption remained statistically significant (p=0.043). The β value and statistical significance both decreased **(Table 6)**.

Table 6: Linear Regression Model for Metabolic Syndrome and NNS Consumption*

	R^2	β (standardized)	Significance
Model 1			
4 Individual NNS	0.063		0.098
Aspartame		0.197	**0.043**
Saccharin		0.073	0.417
Sucralose		0.096	0.361
Acesulfame Potassium		-0.028	0.795

*Response/Independent Variable was Continuum of MetS Risk Factors

To address the potential for confounding variables, multiple linear regression models were run to determine if total NNS consumption and aspartame consumption remained significant with the inclusion of age and sex. When age was included in the models, both total NNS (p=0.078) and aspartame (p=0.129) consumption lost their statistical significance. In the models that included age (models 1, 3, and 4), the R^2 value remained consistent (**Table 7**).

Table 7: Linear Regression Models for Metabolic Syndrome and Age, Sex, Total NNS Consumption and Aspartame Consumption*

	R^2	β (standardized)	Significance
Model 1			
Age	0.232	0.481	**≤ 0.001**
Model 2			
Sex	≤ 0.001	-0.012	0.894
Model 3	0.251		**≤ 0.001**
Age		0.450	**≤ 0.001**
Total NNS Reported		0.143	0.078
Model 4	0.246		**≤ 0.001**
Age		0.454	**≤ 0.001**
Aspartame		0.123	0.129

*Response/Independent Variable was Continuum of MetS Risk Factors

To address aim two, multiple linear regression models were run to examine the five individual risk factors of MetS. A total of five models were run for each risk factor as the "response" variable. Model 1 looked at total NNS reported and models 2 through 5 looked at the individual (aspartame, saccharin, sucralose, and acesulfame potassium) as the "explanatory" variables. Total NNS consumption was positively associated with three of the five risk factors: blood glucose values, triglyceride values, and waist circumference ($p \leq 0.001$). For blood glucose values, aspartame ($p \leq 0.001$) and sucralose consumption ($p=0.027$) were both significant. For triglyceride values, only aspartame was significant ($p=0.001$). For waist circumference, aspartame ($p=0.002$), saccharin ($p=0.015$), and sucralose ($p=0.003$) were all significant. The β values for these models were also higher compared to the models run for MetS. None of the regression models run for HDL cholesterol values or systolic/diastolic blood pressure were statistically significant **(Table 8).**

Table 8: Linear Regression Models for the Five Characteristics of Metabolic Syndrome and Total NNS and Individual NNS Consumption Calculated Separately*

	R^2	β (standardized)	Significance
Blood Glucose Values			
Model 1			
Total NNS Reported	0.138	0.371	**≤ 0.001**
Model 2			
Aspartame	0.131	0.361	**≤ 0.001**
Model 3			
Saccharin	0.015	0.121	0.185
Model 4			
Sucralose	0.041	0.202	**0.027**
Model 5			
Acesulfame Potassium	0.007	0.085	0.353
	R^2	β (standardized)	Significance
Triglyceride Values			
Model 1			
Total NNS Reported	0.092	0.316	**≤ 0.001**
Model 2			
Aspartame	0.093	0.304	**0.001**
Model 3			
Saccharin	0.022	0.147	0.102
Model 4			
Sucralose	0.012	0.112	0.217
Model 5			
Acesulfame Potassium	0.020	0.141	0.118
	R^2	β (standardized)	Significance
Waist Circumference			
Model 1			
Total NNS Reported	0.110	0.332	**≤ 0.001**
Model 2			
Aspartame	0.075	0.274	**0.002**

Model 3			
Saccharin	0.047	0.218	**0.015**
Model 4			
Sucralose	0.071	0.266	**0.003**
Model 5			
Acesulfame Potassium	0.030	0.174	0.053

	R^2	β (standardized)	Significance
High-Density Lipoprotein Values			
Model 1			
Total NNS Reported	0.001	-0.024	0.791
Model 2			
Aspartame	0.001	-0.026	0.776
Model 3			
Saccharin	0.002	0.044	0.625
Model 4			
Sucralose	≤ 0.001	0.010	0.916
Model 5			
Acesulfame Potassium	0.006	-0.078	0.392

	R^2	β (standardized)	Significance
Systolic Blood Pressure			
Model 1			
Total NNS Reported	≤ 0.001	0.010	0.912
Model 2			
Aspartame	≤ 0.001	-0.002	0.986
Model 3			
Saccharin	0.001	0.032	0.724
Model 4			
Sucralose	0.005	0.070	0.440
Model 5			
Acesulfame Potassium	0.001	-0.037	0.685

	R^2	β (standardized)	Significance
Diastolic Blood Pressure			
Model 1			
Total NNS Reported	0.002	0.048	0.599
Model 2			
Aspartame	0.003	0.051	0.574
Model 3			
Saccharin	≤ 0.001	0.011	0.900
Model 4			
Sucralose	0.001	0.034	0.703
Model 5			
Acesulfame Potassium	≤ 0.001	-0.017	0.855

*Response/Independent Variables included each MetS Risk Factor Individually

Discussion

The purpose of this study was to determine if there was an association between NNS consumption and MetS. When looking at the demographic characteristics, NNS consumers followed the trend seen in the current literature. NNS consumers tended to be older, female, and white, and have a higher BMI as compared to non-consumers.[7] By including beverage and food consumption in our calculations for NNS intake, 63 participants were NNS consumers and 62 were non-consumers. If we were to follow the trend of the current literature and use diet soda as a proxy, we would only have 28 NNS consumers and 97 NNS non-consumers. This highlights the fact that using diet soda as a proxy can significantly underestimate the amount of individuals classified as NNS consumers. In the beginning, NNS were typically only seen in diet beverages because they were not heat stable. With the discovery of heat stable NNS, they are now used in a variety of food products. This evolution in the food industry makes diet soda an inappropriate proxy for NNS consumption and is something that needs to be addressed in NNS research.

Based on the data collected from multiple linear regression models, several associations were found. When looking at MetS on a continuum, there was a positive association with total NNS consumption and aspartame consumption. The association found between MetS and total NNS consumption follows the trend of the current literature.[11-17] For aspartame consumption and MetS, there is not enough evidence in the current literature to support or disprove this association. When age was added to the model for total NNS consumption and aspartame consumption, both values were not statistically significant, indicating age was a stronger predictor. These values could raise the question of reverse causality (i.e., as people age they consume more NNS or are more susceptible to abnormal MetS risk factors). It could also mean that older individuals have just been consuming NNS longer, and therefore, have experienced the possible metabolic derangements of NNS consumption.

When looking at each individual risk factor of MetS, three risk factors had an association with NNS consumption. Blood glucose values were associated with total NNS, aspartame, and sucralose consumption. Based on the current literature, a positive relationship between blood glucose and aspartame is not supported.[26,31,42] While this association was found in this investigation, it does not correspond with what has been found in previous research. In terms of total NNS consumption, there is not enough evidence in the current literature to support or disprove this association. Triglyceride values were positively associated with total NNS and aspartame consumption. Based on the current literature, there are mixed reviews for the association between triglyceride values and total NNS consumption. One study reviewed supported this association[17], while the other did not.[25] The same situation occurred for the association between triglyceride values and aspartame consumption. One study reviewed supported this association,[26] whereas the other did not.[27] It is important to note that the study by

Toigo et al., that supported this positive association, was done on rats, so the results may not translate to humans.[27] Lastly, waist circumference was positively associated with total NNS, aspartame, saccharin, and sucralose consumption. Based on the current reviewed literature, two studies support this positive association between waist circumference and total NNS consumption[17,19], one study states more research needs to be done[18], and one does not support this positive association.[48] In the study by Tate et al., researchers found that NNS users actually lost weight and had a smaller waist circumference at the end of the study period. Also, the study by Tate et al. was a randomized control trial and not observational.[48] It is important to note that the studies summarized did not use multiple linear regression models and were active weight loss studies, therefore the current literature and the data collected are not directly comparable.

In regards to the hypotheses, the evidence is equivocal as compared to previous research. The hypotheses for waist circumference and NNS consumption, which stated that there is a positive association between NNS consumption and waist circumference, and fasting blood glucose values and total NNS consumption, which stated that there is a positive association between daily NNS consumption and fasting blood glucose values, were supported by the data. The hypotheses for blood pressure, which stated that a conclusion could not be made on whether or not there was an association, triglycerides, which stated that there is not an association between daily NNS consumption and high triglyceride levels, HDL cholesterol, which stated that there is a negative association between daily NNS consumption and HDL cholesterol, and individual NNS consumption and fasting blood glucose, which stated that there is no association between daily NNS consumption and fasting blood glucose values, were not supported. No associations were found for blood pressure and HDL cholesterol. However, associations were

found between the other three risk factors (triglyceride values, waist circumference, and fasting blood glucose) and total NNS, aspartame, saccharin, and sucralose consumption.

Strengths of this study include the retention rate of participants, using food and beverage intake to accurately calculate whether a participant was a NNS consumer versus a non-consumer, the use of state-of-the-art nutritional analysis software (NDSR) to specify the types and exact amounts (mg) of NNS being consumed by participants, looking at four individual NNS and their relation to MetS, and looking at each individual risk factor of MetS and its relation to NNS consumption. Limitations of this study include the possibility of reporting bias from the 24-hour dietary recalls, our limited power with number of participants (n=125), not controlling for diet, not controlling for confounding variables such as weight history, the inability to determine causation due to a cross-sectional study design, and the limited number of participants with MetS (n=18).

Conclusion

The rates of NNS consumption and MetS are increasing.[5,6] Research is attributing the rise in MetS and other chronic diseases to diets high in added sugar[3], giving fuel to NNS producers to support their products.[4] Unfortunately, there is not enough available evidence for the general public to form an opinion on whether NNS consumption is beneficial or harmful.[4] This investigation was unique, as it not only looked at the association between NNS consumption and MetS, it also examined individual types of NNS and identified potential associations with MetS as a whole and individual MetS risk factors. Also, it used food and beverage consumption to calculate amount of NNS consumed. The present data suggests that there could be a positive association between NNS consumption and MetS; however, causality cannot be assumed due to

the observational data collected. For example, the data shown could be due to reverse causality with confounding variables that were not accounted for, such as diet and weight history.

This investigation did address some of the gaps in the current literature, such as, looking at individual types of NNS and looking at NNS consumption from all foods and beverages and not just diet soda. The majority of current literature uses diet soda as a proxy for NNS consumption. As seen by this study, this can significantly underestimate the amount of individuals classified as NNS consumers. It did not address other limitations, such as diet, sample size, and limitations presented in completing an observational study. More research needs to be completed to determine the exact relationship between NNS consumption and MetS to provide registered dietitian nutritionists, other health care providers, and the general public with adequate information regarding the impact of NNS consumption on health.

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Chapter 3: Future Direction and Conclusion

Non-nutritive sweeteners (NNS), or artificial sweeteners, are regulated as food additives in the United States (US) and can be used in place of added sugars in foods and beverages. Foods and beverages made with these sweeteners are often labeled as "sugar-free" or "diet". NNS are added in minute amounts as they are much sweeter than traditional table sugar, providing little to no calories.[1] Currently, there are six NNS approved by the Food and Drug Administration (FDA). These include saccharin, aspartame, acesulfame potassium, sucralose, neotame, and advantame.[2] The FDA also recognizes a few NNS as "generally recognized as safe" (GRAS). The GRAS label signifies that researchers have no reason to believe that the product will cause harm when eaten for its intended purpose and within its recommended quantity.[1] These NNS include highly purified steviol glycosides and luo han guo fruit extracts.[2] The percent of children and adults that consume NNS in the US has increased significantly.[3] NNS producers advertise NNS as a good sugar alternative, but consumers do not have sufficient access to information regarding NNS and whether or not consumption will benefit them.[4] Along with the increase in NNS consumption, rates of metabolic syndrome (MetS) have also increased. From 2003-2004 to 2011-2012, the percentage of individuals with MetS increased from 32.9% to 34.7%.[5]

The present investigation had one main objective and two secondary objectives. The main objective of this investigation was to determine if there was an association between NNS consumption and MetS. The secondary objectives were to determine if individual NNS had an association with MetS and to determine if each of the five individual risk factors of MetS had a greater association with NNS consumption. Using multiple linear regression models, positive associations were found between total NNS consumption and MetS, aspartame consumption and

MetS, and an association with blood glucose values, waist circumference, and triglyceride values and total/individual NNS consumption.

There are limitations to current NNS research, the first of them being that a majority of the research looks at total NNS as opposed to individual types of NNS. Also, studies looking at NNS consumption typically use diet soda as a proxy for NNS intake. Using diet soda as a proxy can underestimate the amount of NNS an individual is consuming because it underestimates the amount of individuals that are NNS consumers, it leaves out the consumption from foods, candies, and gums; furthermore, it limits investigations regarding the impact of individual types of NNS. Lastly, it is difficult to measure NNS intake. This is due to multiple reasons, such as, having a mixture of NNS in foods, the lack of information on food and beverage labels, and the use of self-reported dietary intake data. As NNS all have different chemical makeups, it is imperative to examine the impact of individual types, as differences in metabolic impacts can be great. These limitations make it difficult to accurately assess the potential impact NNS may have on health.

More research is required to determine if there is a definite relationship between NNS consumption and MetS. It would be advantageous for future studies to not use diet soda as a proxy for NNS consumption and control for diet, age, and weight history. A large and diverse study population with a greater number of individuals with MetS would also be beneficial in determining an association between the two variables. Lastly, it would be advantageous if researchers could determine if participants have always been NNS consumers or have transitioned recently. This can help determine if individuals switched to NNS after discovering they had MetS or another negative health diagnosis. For example, with the current investigation,

it is unclear whether or not individuals with MetS switched to NNS and their health improved or they have always consumed NNS and their health has declined.

Future directions for NNS research includes continuing observational studies to determine intake patterns, randomized controlled trials to determine causation, a refined method to determining NNS intake other than self-reporting diet (i.e., development of a dietary biomarker), and potential policy changes to improve NNS labeling of foods and beverages. These future directions would improve research by allowing researchers to fully understand intake patterns, determine if there is a link between NNS consumption and health status, and allow collection of more concrete NNS intake data with limited potential for bias. With these advancements, the public will be better informed regarding the health impact of NNS and researchers and clinicians will be able to develop specific recommendations based on an individual's need.

In conclusion, this investigation contributes to the current literature by examining the association between NNS consumption and MetS. It also expanded on the current literature by looking at individual NNS and by looking at each of the five risk factors of MetS and the association each one has to NNS consumption (total and individual), and by using foods and beverages to calculate NNS consumption in mg. However, more research needs to be conducted to inferentially test the impact of non-nutritive sweeteners on metabolic syndrome.

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