Non-nutritive sweeteners: evidence on its association with metabolic diseases and potential effects on glucose metabolism and appetite

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Running title: Non-nutritive sweeteners and metabolic diseases

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Abstract

There is an ongoing debate concerning non-nutritive sweeteners (NNS), their usage and its effects on metabolism. The association between NNS consumption, development of metabolic diseases, and changes in appetite regulating hormones is not clear. The aim of this article is to present an overview of NNS and to examine the scientific evidence regarding their effects on glucose metabolism and appetite regulating hormones. Some observational studies suggest an association between NNS consumption and development of metabolic diseases; however, adiposity is a confounder frequently found in these studies. The results of the available clinical trials are heterogeneous and not comparable because of major differences between them. Future controlled studies evaluating specific NNS, with an appropriate sample size, including a uniform study group, with a sufficient exposure time, and considering adjustment for confounder variables such as anthropometric characteristics, previous consumption of NNS, and coexistence of significant metabolic comorbidities, are needed.

Key words: non-nutritive sweeteners, diabetes, metabolic syndrome, obesity, appetite hormones
Introduction

By definition nonnutritive sweeteners (NNS), otherwise known as very low-calorie sweeteners, artificial sweeteners, non-caloric sweeteners, or intense sweeteners, are substances with a higher intensity of sweetness per gram than caloric sweeteners such as sucrose, corn syrup, and fruit juice concentrates, used in small quantities providing no or few calories (1).

At present, there are eight NNS approved to be used as sweeteners in foods and other products, which include sucralose, aspartame, saccharin, acesulfame-K, neotame, advantame, steviol glycosides, and Luo Han Guo fruit extracts. All of these NNS are generally recognized as safe (GRAS) for humans by institutions like the US Food Drug Administration (FDA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the European Food Safety Authority (EFSA). The last two NNS mentioned are obtained from natural sources; this is why nowadays it is inappropriate to name all the NNS, artificial sweeteners, as in the past (2,3).

Although these compounds have very different chemical structures, they all have in common the ability to powerfully activate some of the multiple potential ligand binding sites of the heterodimeric T1R1 + T1R3 sweet-taste receptor in humans (4).

NNS consumption is suggested as a strategy in the nutritional therapy to reduce sugar and energy intake in persons with obesity, diabetes, and other metabolic diseases. Its intake has increased in the latter years, and nowadays there is a widespread availability of products containing NNS. In addition, some recent
studies have described potential metabolic effects of the NNS. Therefore, the objective of this article is to review and analyze the information regarding the association between NNS consumption and the development of metabolic diseases and to discuss the possible effects of these substances on glucose metabolism and appetite hormones.

**Overview of non-nutritive sweeteners**

**Saccharin**

Saccharin was the first approved and used NNS in 1879. It has been used as a non-caloric sweetener in foods and beverages for more than 100 years (5). It is 200 to 700 times sweeter than table sugar (sucrose), and it does not contain any calories. It is not metabolized in the body, and it is heat stable (2). It is known that saccharin is eliminated from the body without changes, primarily through urine (6).

Concerns about saccharin use increased after a study in 1960 showed that saccharin at high levels may cause bladder cancer in laboratory rats, which led to mandate additional studies and a warning label on saccharin-containing products until such warning could be shown to be unnecessary. Since then, more than 30 human studies have demonstrated that the results found in rats were not relevant for humans, and that saccharin is safe for human consumption. Products containing saccharin are no longer required to have the warning label (5,7).

**Aspartame**

Since aspartame is composed of amino acids, it contains a minimum amount of calories (4 kcal per gram), however it is about 200 times sweeter than table sugar,
therefore it is consumed in minimal quantities providing insignificant amounts of energy (8).

Aspartame is unique among low-calorie sweeteners due to the fact that it is completely broken down into its constituent amino acids, aspartic acid and phenylalanine, and a small amount of ethanol. These components are found in greater amounts in common foods, such as meat, milk, fruits, and vegetables, and are metabolized in the body in the same way whether they come from aspartame or from foods (5).

In 1981, the FDA approved aspartame to be used under specific conditions: as a table sweetener, in chewing gum, cold breakfast cereals, and dry bases for some foods (i.e., beverages, instant coffee and tea, jellies, puddings, fillings, dairy products, and toppings) (8). In 1996, the FDA approved aspartame as a general-purpose sweetener. It is not heat stable and loses its sweetness when heated, so typically it is not contained in baked goods (2,7). Labels of aspartame-containing foods and beverages must include a statement that informs that the product contains phenylalanine (7,8). Nevertheless, long-term studies in both children and adults have shown that aspartame does not alter fasting plasma phenylalanine levels (6).

Acesulfame potassium (Ace-K)

Ace-K is a combination of an organic acid and potassium and is named on the food labels as acesulfame K, acesulfame potassium, or Ace-K (7). It is about 200 times sweeter than sugar and it is often combined with other sweeteners (2,5).
In 1988, acesulfame K was approved by the FDA in non-caloric beverages. Later, in 2003 its general use was approved (5). Ace-K is 100% absorbed but not metabolized, and it is excreted within 24 hours. This substance is only 20% potassium, so even a 12-ounce diet soda with Ace-K, would only add 12 mg of potassium to the dietary intake (6).

Ace-K is heat stable, meaning that it stays sweet even when used at high temperatures during baking, making it suitable as a sugar substitute in baked goods (2).

Sucralose

Sucralose is a disaccharide in which three chlorine molecules replace three hydroxyl groups on the sucrose molecule (7). It is about 600 times sweeter than sugar (2). It has been shown to have little or no absorption and it is not metabolized in either human or animal models (6). The limited metabolism of sucralose occurs directly in tissues, as opposed to the gut lumen (9). In 1998, the FDA approved sucralose for use in 15 food categories, and in 1999 to be used as a general-purpose sweetener. Sucralose can be found in a variety of foods including baked goods, beverages, chewing gums, jellies, and frozen dairy desserts. It is heat stable, making it suitable as a sugar substitute in baked goods (10).

Neotame

Neotame is a derivative of the dipeptide phenylalanine and aspartic acid (7). It is approximately 7,000 to 13,000 times sweeter than table sugar. This substance is rapidly metabolized and completely eliminated from the body, reducing the
availability of phenylalanine (11). In 2002, the FDA approved neotame to be used as a general purpose sweetener and as a flavor enhancer in foods, except in meat and poultry. It is heat stable making it suitable as a sugar substitute in baked goods (2).

Stevia

Stevia is the generic term used for food ingredients derived from the herb *Stevia rebaudiana*, a plant native from South America, discovered by Bertoni. Steviol glycosides are a more precise term for a group of intensely sweet compounds extracted and purified from the leaves of this plant (12). Steviol glycosides are NNS and they are 200 to 400 times sweeter than table sugar. In contrast to the other NNS, stevia has an unusual metabolic route in the body. It is absorbed as steviol after bacterial degradation in the colon, glucuronidated by the liver and transported back to the intestine where it is metabolized and excreted (6). The use of purified stevia extracts as sweeteners is currently permitted in a wide number of countries. The FDA has categorized steviol glycosides of high-purity (95% minimum purity) as GRAS. The different types of steviol glycosides include rebaudioside A, also known as Reb A; stevioside, rebaudioside D, and steviol glycoside mixture preparations with rebaudioside A and/or stevioside as the predominant components. In contrast, the use of the stevia leaf and crude stevia extracts is not considered GRAS, and their use as sweeteners is not permitted (12,13).
Advantame

Advantame is the most recently NNS approved by the FDA and the JECFA to be used as a general purpose sweetener and flavor enhancer. It is structurally related to aspartame, but sweeter (approximately 20,000 times sweeter than sucrose). It is stable to low-pH and high-temperature, which indicates that, it can be used as a sugar substitute in many products including baked goods (2,14).

Due to its high sweetness, advantame is used in very small quantities, does not provide significant amounts of calories, and can be consumed by people with phenylketonuria (14,15).

Luo Han Guo

The Siraitia grosvenorii Swingle fruit extract (SGFE), usually known as Luo Han Guo or monk fruit is an extract with sweet taste containing different non-nutritive mogrosides (mostly mogroside V) obtained from a plant native to Southern China and recently received the GRAS status by the FDA (2,15).

This sweetener has gained popularity and well acceptance at the same time with stevia because both are NNS derived from natural sources, but the Luo Han Guo has not been commercialized globally yet (16). It is about 100 to 250 times sweeter than sucrose and its acceptable daily intake (ADI) has not been specified yet (2).

In table 1 the characteristics of NNS are summarized (1,2).
**Metabolic effects of NNS: mechanisms**

Recently, some investigations have evaluated the effects of NNS on glucose metabolism and appetite regulating hormones, postulating diverse mechanisms by which NNS may have an influence on glucose, insulin and other peptides concentrations.

Taste receptors, located in the taste buds cells, trigger the secretion of diverse hormones implicated in the satiety sensation that works as a negative feedback mechanism after food ingestion. These include cholecystokinin, neuropeptide Y, peptide YY, glucagon, glucagon like peptide (GLP) type 1 and 2, ghrelin, oxytocin, galanin, and leptin, among others. It has been observed that taste buds and Langerhans islets have similar phenotypic, structural, and functional characteristics involved in the processing and regulation of nutrients ingestion. In addition, many of the hormones secreted by taste buds in the tongue are expressed by intestinal cells, where they have a key role in food intake and digestion (17).

*In vitro* studies have shown that sucralose increases GLP-1 and GLP-2 secretion in mice intestinal L cells through interaction with taste receptors. Similarly, sucralose, Ace-K, and saccharin stimulate insulin secretion activating taste receptors in mice pancreatic beta cells. However, these findings have not been replicated in human studies (18).

Sugar sensing in the intestinal tract alters nutrient absorption and hormone secretion (19). Margolskee et al. (20) showed in an animal model that enteroendocrine cells express T1R2 and T1R3. In their study, glucose and NNS in
the intestinal lumen stimulated taste receptors and increased GLP-1 and glucose-dependent insulinotropic peptide (GIP) secretion, promoting expression of SGLT1 and glucose absorption. In addition, Mace et al. (21) found that sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. They showed that NNS stimulate intestinal T1R2 and T1R3 increasing GLUT2 expression and glucose transport on the luminal intestinal cells. However, these effects have not been demonstrated in humans and the increase on passive and active intestinal glucose transport cannot be considered a conclusive effect of NNS.

Lastly, another postulated mechanism for the metabolic effects of NNS is the alteration of the intestinal microbiome. Data from studies in animal models and from a small study in human subjects suggest that NNS affect gut microbiota. Schiffman et al. showed that gut microbiota in rats was altered after 12 weeks of exposure to sucralose. The numbers of total anaerobes, bifidobacteria, lactobacilli, Bacteroides, clostridia, and total aerobic bacteria were significantly decreased without an effect on enterobacteria (22).

Suez et al. (23), in a study performed in rodents showed that after an eleven-week exposure to high doses of saccharin, sucralose, and aspartame, glucose concentrations significantly augmented. After a four-week broad-spectrum antibiotic treatment, the glucose variation was abolished. Subsequently, a feces transplant from saccharin-exposed humans to non-exposed rodents was performed. After the transplant, glucose concentrations showed an increase. The microbiome exhibited a significant imbalance with an increase in the Bacteroides
genus and Clostridiales order suggesting that saccharin modify intestinal microbiome in detriment of glucose tolerance.

Palrnäš and cols. showed in rats that after 8 weeks of low-dose aspartame consumption, glucose levels increased and insulin-stimulated glucose disposal was impaired. This was associated with an increase in gut total bacteria, Enterobacteriaceae, Clostridium leptum and Roseburia ssp. In addition, in rats fed with a high fat diet, aspartame attenuated the increase in the firmicutes:bacteroidetes ratio (24).

The role of the gut microbiota in health and disease has been highlighted in recent years. Although the gut microbiota is generally constant over life in adults; it is known that it can be altered importantly by many factors like the use of antibiotics, anti-inflammatory drugs, laxatives, antacids, and chemotherapy. To a lesser degree, gut microbiota can be modified by diet, stress, and consumption of chlorinated water (25). Furthermore, the impact of diet and lifestyle on gut microbiota are less well defined, and it could take months to see considerable changes in the microbiome induced by diet (26, 27).

The evidence regarding the relationship between NNS intake and changes in gut microbiota are contradictory. Specific changes in gut microbiota have been associated with diseases like obesity, metabolic syndrome, inflammatory bowel disease, irritable bowel syndrome, diabetes, neurodevelopmental disorders, autoimmune diseases, and allergies (28). It is well described microbiota dysbiosis occurring in obesity characterized by an increment of Firmicutes and a decrement of Bacteroidetes (26). The changes observed in the gut microbiota induced by NNS
until now do not allow to establish a certain modification. However, this is a new research area in the study of the potential NNS metabolic effects, and it is necessary to continue exploring the relationship between NNS, metabolic alterations and changes in gut microbiota.

Figure 1 summarizes the postulated mechanisms of NNS metabolic effects.

**Security aspects of NNS**

The safety of NNS is demonstrated according to a series of studies that include: 1) preliminary *in vitro* tests with or without metabolic activation demonstrating security based on the following tests: Amos test, micronucleus test, and cell cultures, 2) studies of biological absorption, kinetics, excretion, metabolic, and biochemical pathways, 3) *in vivo* acute toxicity, 4) *in vivo* chronic toxicity, 5) reproductive effects in males and females during gestation, 6) reproductive effects in second and third generations, 7) special studies, 8) security limits in population at high risk of extremely high consumption of NNS, 9) determination of the maximal tolerated dose, meaning the amount that is considered safe and that does not cause alterations when it is consumed daily during all lifetime, and 10) determination of the ADI, which is 100 or more times inferior to the maximal tolerated dose (29).

Before a NNS is approved for human consumption, in a first stage, studies need to probe that it does not produce cellular mutations, does not cause cellular neoplastic changes or nuclear atypia, does not change membranes composition, and does not modify oxygen consumption and utilization. In a second stage, constant intestinal absorption is verified, and that its depuration do not alter renal...
and/or hepatic function, that it does not cause in vitro adverse effects, does not modify energetic and plastic metabolic pathways, and does not produce hydric, electrolyte, acid-base or osmotic changes. In a third stage, it is demonstrated that consumption in healthy volunteers does not cause secondary effects, tissue damage, neoplastic changes or short, middle or long term metabolic modifications. Similarly, it is verified that it does not induce fetal malformations or fertility problems in at least three generations in laboratory animals. Finally, the maximal amount that a human can tolerate without any local or systemic manifestation is determined. Once this maximal amount is known, the ADI is established to be at least 100 or more times inferior to this quantity (29).

**Scientific evidence: observational and clinical trials in humans**

In recent years evidence about the NNS, provided from clinical trials and observational studies, has questioned the absence of metabolic effects of these substances in the body.

More than 10 observational prospective studies have evaluated the association between the consumption of artificially sweetened beverages (ASB) and the development of metabolic diseases like diabetes, metabolic syndrome or obesity. The majority of these studies concluded that there is a significant association between the consumption of ASB and the risk to develop these metabolic diseases; however, most of these associations were attenuated after adjustment for variables including age, smoking, physical activity, alcohol consumption, family history of diseases, diet quality, and energy intake. Interestingly, when an additional adjustment for variables related to adiposity such as body mass index
(BMI) and waist circumference was performed, these associations did not remain statistically significant and the relationship could not be confirmed.

Among these observational studies, the Nurses’ Health Study (NHS) I and II followed 74,749 and 91,249 women over 8 and 24 years, respectively (30, 31). In the NHS I the association between the consumption of artificially sweetened carbonated beverages and the incidence of type 2 diabetes (T2D) was significant for both caffeinated and caffeine-free beverages, with a 1.59 relative risk (RR) (95% CI 1.47-1.71; P<0.001) and 1.76 (95% CI: 1.63-1.89; P<0.001), respectively. After adjustment for several variables including medical history, lifestyle factors, other concomitant diseases, total energy intake, and BMI using Cox proportional hazards regression models, the association remained significant for caffeine-free beverages but not for caffeinated beverages (RR: 1.09; 95% CI: 1.00-1.18; P=0.02) and (RR: 1.01; 95% CI: 0.93-1.10; P=0.99), respectively (30).

The NHS II did not found a relationship between the consumption of one or more diet soft drinks per day and the development of diabetes (RR: 1.21; 95% CI: 0.97-1.50; P=0.12), this association did not change after an additional adjustment for caloric intake (31).

The Health Professionals Follow-Up Study (HPFS) followed 40,389 men over 20 years identifying 2,680 incident cases of T2D during the study. Consumption of ASB was associated with the development of T2D (HR: 1.91; 95% CI: 1.72-2.11; P<0.01), however, this association was not maintained after multivariable adjustment including total energy intake and BMI (HR: 1.09; 95% CI: 0.98-1.21; P=0.13) (32).
The European Prospective Investigation into Cancer and Nutrition (EPIC) Study is a large cohort that included 8 European countries and followed 340,234 adults over 16 years. A significant association was found between the consumption of one or more servings per day of ASB and the incidence of T2D (HR: 1.84; 95% CI: 1.52-2.23; P<0.001). After multivariable adjustment including BMI, the association did not remain statistically significant (HR: 1.13; 95% CI: 0.85-1.52; P=0.24) (33).

Greenwood and cols. performed a meta-analysis to evaluate the association between sugar sweetened and artificially sweetened soft drinks and the incidence of T2D and found that both types of beverages increased the risk to develop this disease with a RR of 1.20 (95% CI: 1.12-1.29; P<0.001) and 1.13 (95% CI: 1.02-1.25; P=0.02), respectively (34).

Another recent meta-analysis found that sugar sweetened beverages, ASB, and fruit juices had a positive association with incident T2D. After multivariable adjustment considering adiposity and calibration for information and publication bias, the relationship between ASB and the development of T2D lost significance (RR: 1.22; 95% CI: 0.98-1.52; P=0.07). However, it was concluded that both ASB and fruit juices ingestion are not recommendable as a prevention strategy to develop T2D (35).

All the observational studies evaluating the relationship between ASB consumption and the incidence of metabolic syndrome have reported significant associations even after a multivariable adjustment (36–38). Nevertheless, none of these studies performed adjustment for variables related to adiposity, except for The Multi-Ethnic Study of Atherosclerosis (MESA), in which the association (HR: 1.36; 95% CI:
1.11-1.66; P<0.001) lost statistical significance (HR: 1.17; 95% CI: 0.96-1.44; P=0.06) after the adjustment for BMI and waist circumference (39).

The relationship between the consumption of beverages containing NNS and obesity development must be explored. To date only one observational study has been identified assessing this association, and a definite conclusion cannot be established (40).

The evidence from the observational prospective studies indicate that there could be a relation between the ASB intake and chronic metabolic diseases development. The possible explanation for the attenuation or loss of these associations after the adjustment for adiposity is that people that are prone to gain weight tend to consume this kind of beverages as a strategy to lose weight or reduce their energy intake. In this situation, there could be other lifestyle or genetic factors that may impact in the development of these diseases beyond the ingestion of ASB. In addition, subjects with obesity or overweight are at risk to develop metabolic diseases. Another limitation of these studies is that they only measured the consumption of ASB, and these are not the unique source of NNS, therefore the “non-consumers” could also be exposed to NNS in other products. In table 2 we show the summary of observational prospective studies evaluating the association between the ASB consumption and T2D or metabolic syndrome development, adjusted by adiposity related variables. The studies that did not found a significant association in the crude risk were not included because no further adjustment was needed.
In a systematic review we found that there are 28 clinical trials evaluating the effect of different NNS on variables related to glucose metabolism and appetite (41).

Some clinical trials have reported lower glucose and insulin concentrations after the consumption of diverse NNS; however, in most cases these results are comparing NNS to caloric sweeteners (glucose or sucrose) which are expected to have an impact on glucose and insulin concentrations (42–44). There are three studies comparing water or placebo to NNS consumption, and found lower glucose levels (43, 45, 46). Two studies have observed greater insulinogenic index after the consumption of stevia or aspartame (44, 45). Horwitz and cols. found a higher mean insulin area under the curve (AUC) after aspartame consumption compared to saccharin or an unsweetened beverage (47).

Pepino (48) and Suez (23) found in their clinical trials deleterious effects of NNS on glucose metabolism. Pepino and cols. showed higher glucose and insulin levels after sucralose consumption compared to water. Also, they demonstrated a decrement in insulin sensitivity and in the insulin clearance rate. However, it is difficult to extrapolate the results of this study because they evaluated an acute exposition to only one NNS (sucralose), and the experiments were made in morbidly obese subjects, most of them women.

Suez and cols. performed a small study in humans after different experiments in rodents, observing that saccharin had a strong impact on glucose levels. During 7 days, 7 subjects (with characteristics not specified) consumed 100% of the ADI of saccharin and four of the participants developed a significant increase in glucose concentrations. A microbiota transplantation to mice was performed from the
saccharin-responders subjects and 7 days after the transplant, the high glucose response was also observed in rodents.

The results of this study have been questioned for many reasons: the reduced sample size, the absence of a control group, the insufficient description about the procedures, the inclusion criteria, and the characteristics of the participants. Also, it is important to mention that the effects of the NNS on glucose concentrations have been evaluated and these results have not been previously shown.

While it can be possible that the ingestion of NNS have an impact on glucose tolerance and other metabolic aspects, we consider that the current evidence is not conclusive and there is a need to reevaluate the safety of these substances with well-designed controlled clinical trials.

It is not possible to perform statistical analysis of the effects observed in the different clinical trials because there is high heterogeneity in their methodologies, included population, outcome variables, and results presentation.

In relation to appetite, studies have used visual analogue scales to measure subjective ratings of variables related to appetite including hunger, fullness, satiety, and desire to eat, among others (44, 49–54). Another used strategy is to measure _ad libitum_ energy intake from a buffet after the ingestion of NNS (44, 52, 55, 56). None of these studies could confirm any effect of NNS on appetite except for one recently published trial that found significantly higher _ad libitum_ intake (P=0.01) after the ingestion of beverages containing aspartame, monk fruit and stevia compared to sucrose (57). In this randomized crossover study, Tey et al. observed
that the energy “saved” with the consumption of NNS was compensated at subsequent meals with no differences in total daily energy intake between the caloric sweetener and the non-nutritive sweeteners studied (57).

Some appetite regulating hormones including GLP-1, GIP, PYY, glucagon, ghrelin, and cholecystokinin have been measured in clinical trials in order to investigate if NNS have an impact on their concentrations. In accordance to some in vitro studies, sucralose increased GLP-1 concentration in two trials performed on healthy subjects and in subjects with type 1 diabetes (46, 58). Hall and cols. found lower GLP-1 concentrations after the consumption of aspartame (49); however, Sylvetsky et al. found that diet sodas containing NNS slightly increased GLP-1 responses to glucose in normal weight, overweight, and obese individuals (59). Additional effects in other appetite related peptides have not been found.

It has been described that the ingestion of NNS may dissociate sweetness from energy, disturbing the balance between taste response, appetite, and food preferences, being this problem more important in children (60). If the preference for sweet taste is encouraged by consuming NNS it could promote the consumption of other sweetened and energy dense foods, increasing energy intake and weight gain. In addition, studies in rodents support that long-term exposure to NNS debilitates cephalic responses activated by sweet taste (4). NNS have been associated with a variety of central and peripheral metabolic consequences, including stimulation of oral and extra-oral sweet taste receptors, alterations in gut microbiota, impaired ability of sweet taste to predict energy availability, although the mechanisms are still poorly understood (61).
Conclusions

The consumption of NNS can be recommended in specific conditions, for example in people with obesity and diabetes in whom the glycemic and weight control are fundamental. The American Diabetes Association (ADA) and the American Heart Association (AHA) suggest that NNS can be useful in a structured diet to replace sources of added sugars; thereby, promoting both energy and carbohydrate intake reduction (1). The consumption of foods and beverages containing NNS should not encourage a compensatory increase of energy intake from other sources or affect diet quality. For this reason, it is necessary that the consumption of NNS is tied to a healthy lifestyle according to the recommendations of the health professionals and also to ingest less than the established ADI for each NNS by the FDA and the JECFA.

It is recommendable to avoid both non-nutritive and caloric sweeteners to elude the preference for sweet taste and the consumption of sweet foods that in most cases provide significant amounts of additives and energy from other nutrients like fats. Also, in several circumstances, the consumption of ASB is accompanied with fast food or other types of non-healthy foods.

We consider that well-founded conclusions regarding the effect of NNS on metabolism and appetite hormones cannot be proven and there is a need for additional controlled studies evaluating each NNS, with a proper sample size, including a uniform study group, with a sufficient exposure time, and adjusting for confounder variables such as anthropometric characteristics, previous consumption of NNS, and coexistence of significant metabolic comorbidities.
References


43. Härtel B, Graubaum H, Schneider B, Bier A. The influence of sweetener
solutions on the secretion of insulin and blood glucose level.


57. Tey SL, Salleh NB, Henry J, Forde CG. Effects of aspartame-, monk fruit-,
stevia- and sucrose-sweetened beverages on postprandial glucose, insulin and energy intake. Int J Obes. 2017; [Epub ahead of print].


Figure 1. Potential mechanisms involved in non-nutritive sweeteners metabolic effects.

Small intestine. In animal models NNS thought stimulation of T1R2 and T1R3 receptors increase secretion of GLP-1 and GIP, these in turn augment the expression of SGLT-1 increasing active glucose transport. Another postulated mechanism is that NNS induce expression of GLUT-2 increasing passive glucose transport. In pancreas
Colon. Animal studies and a small study in humans demonstrated changes in the gut microbiota induced by NNS associated with effects in glucose metabolism (see text for details).

Pancreas. In vitro studies have postulated that some NNS thorough interaction with taste receptors (T1R2 and T1R3) stimulate insulin release.
Table 1. Characteristics of the non-nutritive sweeteners

<table>
<thead>
<tr>
<th>Non-Nutritive Sweetener</th>
<th>ADI FDA (mg/kg body weight)</th>
<th>ADI JECFA (mg/kg body weight)</th>
<th>Year FDA Approved</th>
<th>Times sweeter than sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saccharin</td>
<td>15</td>
<td>5</td>
<td>1958</td>
<td>200-700</td>
</tr>
<tr>
<td>Aspartame</td>
<td>50</td>
<td>40</td>
<td>1981</td>
<td>200</td>
</tr>
<tr>
<td>Acesulfame-K</td>
<td>15</td>
<td>15</td>
<td>1988</td>
<td>200</td>
</tr>
<tr>
<td>Sucralose</td>
<td>5</td>
<td>15</td>
<td>1999</td>
<td>600</td>
</tr>
<tr>
<td>Neotame</td>
<td>0.3</td>
<td>2</td>
<td>2002</td>
<td>7,000-13,000</td>
</tr>
<tr>
<td>Stevia</td>
<td>4</td>
<td>4</td>
<td>2008</td>
<td>200-400</td>
</tr>
<tr>
<td>Luo Han Guo</td>
<td>-</td>
<td>-</td>
<td>2010</td>
<td>100-250</td>
</tr>
<tr>
<td>Advantame</td>
<td>32.8</td>
<td>5</td>
<td>2014</td>
<td>20,000</td>
</tr>
</tbody>
</table>


*ADI is a measure of the amount of a specific substance in food or drinking water than can be ingested over a lifetime without an appreciable health risk. It is usually expressed in milligrams of sweetener per kilogram of body weight per day (mg/kg body weight). The amount is usually set at 1/100 of the maximum level at which no adverse effects was observed in animal experiments (1).
Table 2. Observational prospective studies that adjusted by adiposity the association between the consumption of artificially sweetened beverages and the risk to develop metabolic diseases.

<table>
<thead>
<tr>
<th>Study</th>
<th>Disease</th>
<th>Follow-up (years)</th>
<th>n</th>
<th>Crude risk</th>
<th>Risk adjusted by adiposity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPIC (33)</td>
<td>T2D</td>
<td>16</td>
<td>340,234</td>
<td>1.93 (1.47-2.54)</td>
<td>1.13 (0.85-1.52)</td>
</tr>
<tr>
<td>EPIC-France (62)</td>
<td>T2D</td>
<td>14</td>
<td>66,118</td>
<td>3.50 (2.49-4.93)</td>
<td>1.68 (1.19-2.39)</td>
</tr>
<tr>
<td>EPIC-Norfolk (63)</td>
<td>T2D</td>
<td>10.8</td>
<td>24,653</td>
<td>1.70 (1.35-2.14)</td>
<td>1.17 (0.93-1.48)</td>
</tr>
<tr>
<td>HPFS (30)</td>
<td>T2D</td>
<td>22</td>
<td>39,059</td>
<td>1.87 (1.65-2.12)</td>
<td>1.06 (0.93-1.22)</td>
</tr>
<tr>
<td>HPFS-2 (32)</td>
<td>T2D</td>
<td>20</td>
<td>40,389</td>
<td>1.91 (1.72-2.11)</td>
<td>1.09 (0.98-1.21)</td>
</tr>
<tr>
<td>JEFS (64)</td>
<td>T2D</td>
<td>7</td>
<td>2,037</td>
<td>1.99 (1.33-2.98)</td>
<td>1.70 (1.13-2.55)</td>
</tr>
<tr>
<td>MESA (39)</td>
<td>MS</td>
<td>7</td>
<td>5,011</td>
<td>1.31 (1.07-1.60)</td>
<td>1.17 (0.96-1.44)</td>
</tr>
<tr>
<td>MESA (39)</td>
<td>T2D</td>
<td>7</td>
<td>5,011</td>
<td>1.63 (1.24-2.13)</td>
<td>1.38 (1.04-1.82)</td>
</tr>
<tr>
<td>NHS I (30)</td>
<td>T2D</td>
<td>24</td>
<td>74,749</td>
<td>1.59 (1.47-1.71)</td>
<td>1.01 (0.93-1.10)</td>
</tr>
</tbody>
</table>

EPIC: the European Prospective Investigation into Cancer and Nutrition study, HPFS: the Health Professionals Follow-up Study, JEFS: Japan employee factory study, MESA: the Multi-Ethnic Study of Atherosclerosis, NHS: the Nurses’ Health Study, T2D: type 2 diabetes, MS: metabolic syndrome, n: number of subjects followed in the study. The associations showed in the table are between the highest amount of artificially sweetened beverages consumption and the incidence of type 2 diabetes or metabolic syndrome in the study. Associations represented as relative risks, hazard ratios or odds ratios with their respective 95% confidence intervals. Adiposity adjustment include a multivariable adjustment plus adjustment by body mass index and/or waist circumference.