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Review

Metabolic effects of non-nutritive sweeteners

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HIGHLIGHTS

- NNS use in humans is linked to weight gain and type 2 diabetes risk.
- NNS in rodents disrupt learned responses that help control glucose homeostasis.
- NNS in rodents alterglycemic responses to a sugar load by perturbing gut microbiota.
- NNS increase intestinal glucose transporter expression in three mammalian species.

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ABSTRACT

Until recently, the general belief was that non-nutritive sweeteners (NNSs) were healthy sugar substitutes because they provide sweet taste without calories or glycemic effects. However, data from several epidemiological studies have found that consumption of NNSs, mainly in diet sodas, is associated with increased risk to develop obesity, metabolic syndrome, and type 2 diabetes. The main purpose of this article is to review recent scientific evidence supporting potential mechanisms that explain how "metabolically inactive" NNSs, which have few, if any, calories, might promote metabolic dysregulation. Three potential mechanisms, which are not mutually exclusive, are presented: 1) NNSs interfere with learned responses that contribute to control glucose and energy homeostasis, 2) NNSs interfere with gut microbiota and induce glucose intolerance, and 3) NNSs interact with sweet-taste receptors expressed throughout the digestive system that play a role in glucose absorption and trigger insulin secretion. In addition, recent findings from our laboratory showing an association between individual taste sensitivity to detect sucralose and sucralose's acute effects on metabolic response to an oral glucose load are reported. Taken as a whole, data support the notion that NNSs have metabolic effects. More research is needed to elucidate the mechanisms by which NNSs may drive metabolic dysregulation and better understand potential effects of these commonly used food additives.

Contents

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1. Introduction

It is generally believed that non-nutritive sweeteners (NNSs) are healthy substitutes for sugars because they provide sweet taste without calories or glycemic effects [1]. Currently, six NNSs (sucralose, aspartame, saccharin, acesulfame potassium, neotame and advantame) are approved to be used as a sweetener in food, and two (steviol glycosides, and Luo han guo extract) are generally recognized as safe and permitted for use in food by the US Food and Drug Administration (FDA) [2]. Although these compounds have very different chemical structures, they all have in common the ability to very potently activate some of the multiple potential ligand binding sites of the heterodimeric T1R1 + T1R3 sweet-taste receptor in human subjects [3]. Before the FDA granted final approval of NNSs, a battery of toxicology and clinical studies in a number of species, including humans, were conducted to demonstrate that NNSs are generally safe and well-tolerated. In addition, the data from several studies, conducted in human subjects with and without diabetes, found that even extremely high doses of sucralose or aspartame (many times above the estimated maximum intake), did not affect blood glucose, C-peptide, or HbA1c concentrations (e.g., [1,4–6]). However, data from several epidemiological studies have found that consumption of NNSs, mainly in diet sodas, is not linked to better health outcomes (reviewed in [7,8]). In fact, some studies found positive associations between NNS consumption and weight gain, metabolic syndrome, and type 2 diabetes [9–14], although other studies did not (e.g., [15,16]; reviewed in [17]).

At least two hypotheses, not mutually exclusive, might explain the paradoxical association between consuming NNSs and adverse metabolic outcomes: 1) reverse causation, i.e. individuals who are likely to develop metabolic disease or are gaining weight choose to consume NNSs as a strategy to reduce sugar and caloric intake; and 2) NNSs are not physiologically inert but affect biological processes involved in regulating energy and glucose homeostasis. This article reviews recent scientific evidence supporting potential mechanisms that explain how "metabolically inactive" NNSs, which have few, if any, calories, might promote metabolic dysregulation and presents some findings from our laboratory in which we explore associations between individual taste sensitivity to detect sucralose and sucralose acute effects on metabolic response to an oral glucose load.

2. Potential mechanisms underlying the association between the use of nonnutritive sweeteners and adverse metabolic outcomes

The list of potential mechanisms described below is not collectively exhaustive, nor mutually exclusive. In fact some of these mechanisms may act synergistically.

2.1. NNSs interfere with learned responses that contribute to control glucose and energy homeostasis

Much of the evidence behind this concept derives from the seminal work by Swithers and Davidson in a rodent model ([8,18–20], reviewed in [21]). Using the Pavlovian conditioning principles as the foundational context of their research, they hypothesize that the use of NNSs weakens the ability of sweet taste to predict energy and evoke autonomic and endocrine learned responses that prepare the digestive tract for the optimal process of ingested food, such as the cephalic response [19]. In their elegant animal model, rats receive differential experience with a sweet taste that either predicts (glucose) or does not predict (saccharine, acesulfame K, or stevia) increased calories. Data from a series of experiments show that compared with rats that consume a diet always sweetened with glucose (i.e. sweet predicts calories), those consuming a diet where sweet taste does not reliably predict calories (i.e. sweetened with NNSs) are heavier, accumulate more body fat, exhibit a diminished ability to compensate for calories ingested in a pre-meal, and have a reduced thermic response to eating a novel meal [18,19,22,23]. Consistent with their hypothesis that NNSs weaken cephalic responses, compared with rats in the control group (i.e. sweet predicts calories), animals consuming a diet sweetened with NNSs responded with relative hyperglycemia when given a novel sweet-tasting test meal or a standard glucose tolerance test [24]. Importantly, this altered glucoregulatory response to a glucose load, which was associated with reduced circulating levels of the incretin hormone glucagon like peptide-1 (GLP-1), was observed when the glucose load was given orally but not when glucose was infused directly into the stomach by gavage (i.e. bypassing oral taste stimulation) [24]. That previous experience with NNSs affected glucoregulatory responses to a glucose load when glucose was tasted, but not when directly released in the stomach, further supports their hypothesis that it is disruptions in learned responses elicited by tasting sweetness, not in post-absorptive consequences of consuming sugar, that alter glucose homeostasis in this rodent model.

Early studies by Deutsch [25] also strongly support the theory that in rodents, long-term exposure to NNS ingestion weakens cephalic responses triggered by sweet taste. Following up from findings that saccharin ingestion potentiated hypoglycemic effects of exogenous administered insulin [26], Robert Deutsch tested the hypothesis that the sweet taste of saccharin elicited a conditioned hypoglycemic response that could be extinguished by giving animals long-term access to the non-caloric sweetener [25]. He showed that, consistent with the conditioning theory, saccharin ingestion alone leads to relative hypoglycemia in animals with little to no prior experience with NNSs. However, such a conditioned hypoglycemic response was extinguished after animals had long-term access to saccharin (i.e. the experience of tasting sweetness without the subsequent rise in blood sugar) [25].

The hypothesis that exposure to NNSs weakens cephalic responses to sweet food has not been tested in human subjects, and future research in this area is warranted. There are important differences between humans and rodents on the type of stimuli that elicit cephalic responses. Sweet liquids, either caloric or non-caloric, are good stimuli to elicit cephalic responses in rats [27–29] but generally do not elicit cephalic responses in human subjects [30–32]. However, given that 1) classical or Pavlovian conditioning is one of the most basic forms of learning (demonstrated even in invertebrates such as the *Aplysia*) [33], 2) cephalic responses are elicited when people taste and chew food ([34], reviewed in [35]), and 3) studies in human subjects show that cephalic responses are required for a normal postprandial glucose tolerance [36,37], there is great potential that the above theory, which posits that NNSs interfere with learned responses that contribute to control glucose and energy homeostasis, is applicable to human subjects.

2.2. NNSs interfere with gut microbiota and induce glucose intolerance

Perhaps the one unquestionable benefit of NNSs is that they help reduce dental cavities [38]. The anticavity effect of saccharin, sucralose, aspartame, and stevia is not only explained by the fact that these compounds are resistant to fermentation by oral bacteria, but also because of their demonstrated bacteriostatic effects [39–41]. Data from studies in vitro [42] and in animal models [43–45], and from a small study in human subjects [45], suggest that the effects of these NNSs are not limited to the microbial inhabitants of the mouth, but extend to those in the gut, thereby affecting the host metabolic phenotype and disease risk [46]. Pioneer work from the group of Schiffman showed that 12 weeks of exposure to Splenda (a NNS comprising 1% *w*/*w* sucralose with glucose (1% *w*/*w*) and maltodextrin (94% *w*/*w*) as fillers) significantly altered gut microbiota composition by decreasing beneficial bacteria and was associated with weight gain in rats [43]. In a recent work, Suez et al. confirmed and extend these findings by identifying a microbe-mediated mechanism by which NNSs might influence metabolism [45]. Suez et al. showed that 11 weeks of exposure to saccharin, sucralose, or aspartame

induced higher glucose excursions after a glucose load than those in control animals not exposed to NNSs, and that such metabolic phenotype, at least for saccharin (which due to being the one NNS that more strongly affected glycemic responses in mice was studied further), was mediated by alteration of gut microbiota. Their elegant animal model truly determined causation because they demonstrated that saccharine induced hyperglycemia was transferable to germ-free mice that had never been exposed to saccharin in their life but that received a fecal transplant from saccharin-fed mice, or from microbiota incubated in vitro in the presence of saccharin [45]. Further, they exposed seven young healthy volunteers who were not regular users of NNSs, to one week of the FDA's maximum acceptable daily saccharin intake and evaluated their responses to an oral glucose tolerance test daily. They found that regular saccharin exposure in most subjects (i.e. responders), but not in all of them, increased glycemic responses to a glucose load. Congruent with findings from their animal model, the transplant of stool from human subjects of the responders group induced glucose intolerance in recipient germ-free mice [45]. Noteworthy, the inclusion of a control group for the exposure to saccharin in human subjects would have strengthened the conclusion of the study. Because such a control group was not included in the design, it is unclear whether some healthy individuals exposed to 7 consecutive oral glucose tolerance tests (i.e. daily consumption of 75 g of glucose) would have developed changes in glucose metabolism in the absence of saccharin, and whether transplant of stools from such a group would have caused glucose intolerance in germ-free mice.

Consistent with the findings from Suez et al., Palmnas and collaborators showed that 8 weeks of aspartame exposure (in a dose equivalent to human subjects consuming ~2–3 diet soft drinks per day) perturbed gut microbiota and resulted in elevated fasting glucose levels and impaired insulin tolerance in rats [44]. However, the mechanism by which aspartame perturbed gut microbiota is unclear, as aspartame is metabolized before reaching the colon by intestinal esterases and peptidases into amino acids and methanol [47].

2.3. NNSs interact with sweet-taste receptors in the digestive system that play a role in glucose absorption and trigger insulin secretion

2.3.1. Taste receptors are expressed in tissues beyond the tongue

One of the most exciting discoveries in recent years in the field of the chemical senses is the finding of taste receptors in non-taste tissues [48–50]. Data obtained from studies in mouse models in vivo and in vitro and human duodenal L cells in vitro [48,49] strongly support the hypothesis that the sweet taste receptor subunit T1R3 coupled to the taste G protein alpha-gustducin, underlies at least one of the components of sugar sensing in the gut. Mice lacking alpha-gustducin or *T1r3* show a severely blunted incretin response to glucose challenge [48,51]. Incretins (GLP-1 and glucose dependent insulinotropic peptide: GIP) are gut hormones that once released into the bloodstream stimulate pancreatic beta-cells to secrete insulin, among other effects (reviewed in [52]). The so-called "incretin effect", first described in the 60's refers to the fact that an oral glucose load elicits a remarkably greater insulin response than an intravenous glucose load even when both loads are matched to cause identical blood glucose levels [53]. That taste-signaling pathways in the gut intervene in the "incretin effect" is further supported by two observations. First, lactisole, a human sweet taste receptor antagonist, completely blocks GLP-1 release in vitro [48,49], and significantly reduces GLP-1 secretion in response to intraduodenal or intagrastic glucose administration in human subjects [54,55]. Second, alpha-gustducin knockout mice have significantly disrupted glucose homeostasis both after a glucose challenge and after post-fasting feeding on chow [48].

In addition to its important function of regulating GLP-1 secretion, sweet-taste signaling pathways in the gut may play a key role in the regulation of glucose absorption from the intestinal lumen into enterocytes. Data obtained in rodents suggest that intestinal sweet taste receptors control both active glucose absorption, by modulating expression of sodium-dependent glucose transporter isoform 1 (SGLT1) [49], and passive glucose absorption, by modulating apical glucose transporter 2 (GLUT2) insertion to the intestine [50]. Unlike wild type, knockout mice lacking either alpha-gustducin or *T1r3* failed to up-regulate SGLT1 intestinal expression and glucose absorptive capacity when exposed to a high carbohydrate diet (70% sucrose) [49]. In fact, recent data suggest that sweet taste receptors may contribute to the incretin response by activating SGLT1 [56]. Consistent with findings from previous research that pharmacologically blocked SGLT1 activity [57], data from research studies using SGLT1 knockout mice determined that SGLT1 plays a critical role for intestinal glucose absorption and incretin release [56].

2.3.2. NNSs and metabolic function in cell systems and animal models

The discovery of taste receptors in the gastrointestinal tract revived old speculations about the possibility that NNSs could have post-ingestive effects [58]. Supporting this hypothesis, results from studies conducted in cell systems and animal models show that NNSs, like sugars, activate sweet taste receptors localized in enteroendocrine cells and pancreatic β-cells, which trigger the secretion of incretins [48,49] and insulin [59–62], respectively. The sucralose dose–response for incretin release from L-cells is non-linear (0.004 mM to 5 mM sucralose stimulates GLP-1 release, but 20 mM sucralose does not), which might explain, at least in part, why studies that used sucralose doses many times above the estimated maximum intake (4 to 6 times) did not detect metabolic effects of sucralose ingestion. In addition, data from studies conducted in animal models demonstrate that the interaction of NNSs with sweet taste receptors expressed in enteroendocrine cells increases both active intestinal glucose absorption and passive intestinal glucose absorption by upregulating the expression of sodium-dependent glucose transporter isoform 1 (SGLT1) [49,63,64] and increasing the translocation of glucose transporter 2 to the apical membrane of intestinal epithelia [50], and that NNS dietary supplementation increases body adiposity and causes hyperinsulinemia and insulin resistance in mice with diet-induced obesity [65].

3. NNSs and metabolic function in human subjects

Data from four studies conducted in human subjects support the potential importance of NNSs in regulating glucose homeostasis. The acute consumption of NNSs, namely, a diet soda, or a small amount of sucralose (24 mg of sucralose in 200 ml of water) immediately before an oral glucose load significantly enhanced GLP-1 secretion in healthy children and young overweight/obese adults [66–68], but not in subjects with type 2 diabetes (T2D) [67,68]. Furthermore, we have recently found that the ingestion of sucralose, the most commonly used NNSs, affects the glycemic response to an oral glucose load and increases both peak plasma glucose concentration and glucose-stimulated insulin secretion in subjects with obesity [69]. We also found that sucralose ingestion tended to increase plasma GIP concentration ($P = 0.08$), and, suggesting that acute sucralose intake could promote insulin resistance, we found that ~20% higher than normal concentrations of insulin were required to maintain same glycemia when obese subjects consumed sucralose than when they consumed water before glucose ingestion [69].

In contrast, the results from studies conducted in healthy lean adults have reported that sucralose does not affect glycemic or hormonal responses to the ingestion of glucose or other carbohydrates [70–74]. The reason(s) for the discrepancy between the results from these studies and our own data [69] is not clear, but could be related to differences in study subjects and the inclusion of subjects who were regular users of NNSs in the other studies. We specifically study subjects with obesity because 1) NNSs are often promoted to help decrease calorie intake and facilitate weight management in this population; 2) the prevalence of NNS use is higher in this population than in lean subjects (36% vs. 22% [75]); and 3) data from animal models suggest that obese subjects may

be the most affected by NNS consumption [20]. In addition, we purposely tried to study a homogeneous group of subjects by only including those who were: i) "insulin sensitive" based on homeostasis model assessment of insulin resistance (HOMA-IR) ≤ 2.6, and ii) not regular users of NNSs. Controlling for the use of NNSs when evaluating potential "acute" metabolic effects of NNSs is critical because, as described above, there is considerable evidence in support of the hypothesis that chronic NNS ingestion has biological activity. It has been shown that chronic NNS ingestion 1) upregulates the expression of SGLT1, which in turn increases the initial rate of Na^+-dependent glucose uptake in three different mammalian species (mice [49], pigs [63], and cows [76]), and 2) increases the glycemic response to an oral glucose load in rodents [24,44,45,65] and in human subjects, at least for the case of 7 days of exposure to the maximum acceptable daily intake of saccharin [45].

4. Tongue and gut endocrine cells

In addition to the recent discovery that taste receptor-like cells are present in the digestive system, it has also been shown that functional gut hormones are expressed in the tongue of rodents [77–81] and macaques [80]. For example, GLP-1 and its receptor (GLP-1R) are expressed in taste buds, and their secretion modulates sweet and savory taste sensitivity in mice [80]. Furthermore, it has been proposed that a fraction of cephalic-phase rise in GLP-1 levels in rodents is directly released from taste cells into the bloodstream [79]. A recent study in cultured human taste cells shows that stimulation with very small concentrations of a free fatty acid, triggers GLP-1 release, just like what was observed in intestinal endocrine cells [82]. These observations suggest that functional gut hormones are also expressed in the tongue of human subjects.

The similarities in the molecular mechanisms of taste signal transduction in the tongue and nutrient signal transduction in the gut suggest that the study of taste perception can provide novel insights into chemical sensing mechanisms in the gut that regulate metabolic function. For example, healthy individuals with a family history of type 2 diabetes have a significant impairment in taste detection that is specific to glucose [83]. We recently tested the hypothesis that individual differences in the perception of sucralose sweetness would correlate with the effects of sucralose on metabolic responses to a glucose load (e.g., the higher the taste sensitivity to detect sucralose, the greater the effect of sucralose on glycemic responses). To test this hypothesis, we evaluated subjects' taste sensitivity to detect sucralose and sucrose by using a two-alternative, forced-choice staircase procedure [84,85] in 16 of the 17 subjects who completed the metabolic studies in which sucralose or water was consumed immediately before a glucose load (see [69]). The two-alternative, forced-choice staircase procedure provides an accurate and reliable assessment of taste detection thresholds and is recommended as the method of choice to determine individual sensitivity to taste [86]. It is important to note that a detection threshold is the lowest concentration of taste stimuli that a subject can detect, and is below an individual's threshold for conscious perception (i.e. when performing this task, subjects detect that a taste stimulus is different than water, but do not recognize a sweet taste when sucrose or NNS detection thresholds are being measured). Therefore, taste detection thresholds are resistant to subjective response bias that could be originated by exposure to sucralose during the metabolic study. We found that, consistent with the literature, detection thresholds for sucralose were ~750 times lower than for sucrose (sucralose: 0.010 ± 0.001 mM vs. sucrose: 7.5 ± 2.2 mM; unpublished observation). Supporting our hypothesis, we found a significant correlation between individual differences in sucralose taste detection thresholds and the effects of sucralose on glycemic responses ($r = -0.51$, $n = 16$, $P = 0.04$; Fig. 1; unpublished observation) such that the higher the sensitivity to detect sucralose taste (i.e., the smaller the amount of sucralose that is detectable as "a taste different than water"), the greater was the difference in the glucose peak between the sucralose and water (control)

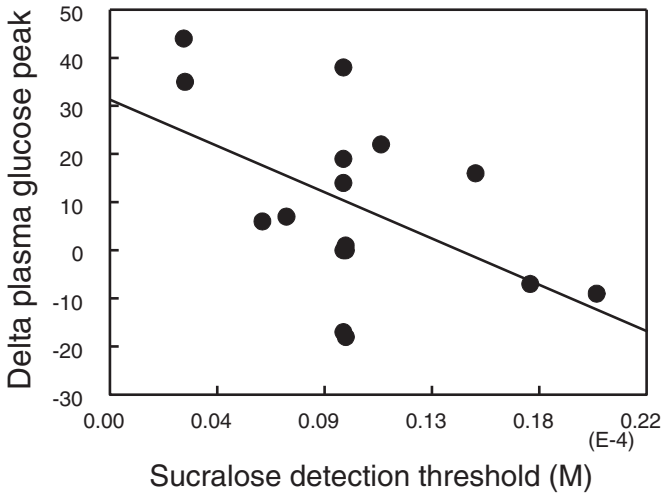

Fig. 1. Correlation between sucralose detection thresholds and sucralose effects on plasma glucose peak concentration (i.e. difference between plasma glucose peak concentrations on the day that sucralose preceded the glucose load and the day that water preceded the glucose load) in 16 obese subjects.

conditions (i.e. the bigger the effect of sucralose on glucose peak responses to a glucose load). Although the inference of molecular mechanisms of taste perception from psychophysical data has major limitations, these data are consistent with the hypothesis that individual differences in signaling pathways in taste receptor (or taste receptor-like) cells affect, at least in part, both sensing of sucralose in the mouth and sucralose acute metabolic activity in the gut. These data, although indirectly, add to the evidence of a mechanistic link between taste perception and metabolism.

5. Conclusion

Several potential mechanisms, which are not mutually exclusive, could explain the paradoxical association between the consumption of NNSs and metabolic disorders observed in epidemiological studies. First, according to Terry Davidson and Susan Swithers's theory, the dissociation of sweetness from calories could interfere with fundamental physiological responses that had evolved to control homeostasis (reviewed in [21]). Second, NNSs induce changes in the gastrointestinal environment and thus of the gut microbiota [43–45], which can trigger glucose intolerance [44,45]. Third, NNSs interact with novel sweet taste receptors discovered in non-taste tissues including the gut and the pancreas, which can influence insulin secretion [48,49,59–62]. However, to date, only the last two mechanisms have been evaluated in human subjects. The finding on the effects of NNSs on human gut microbiome is limited to potential effects of saccharin. Although provocative, and highly congruent with findings from studies in rodent models, the results from this study in humans are limited, because of its small sample size and the lack of a control group for saccharin exposure [45]. There are inconsistencies between findings from data from animal models and human subjects in regard to whether NNSs can acutely affect glycemic responses in vivo, presumably by activating sweet taste receptors in the digestive system [48,49,66–74]. The reasons for discrepancy between the results from different studies are unknown but could be related to differences in study subjects (e.g. lean vs. obese and frequent users of NNSs vs. non-users of NNSs). Importantly, most of this research in human subjects has evaluated the effects of sucralose (or sucralose in combination with acesulfame K), and therefore results from these studies should not be extrapolated to all NNSs.

Taken as a whole, despite several epidemiological studies showing an association between NNS consumption and metabolic disorders [9–14], and strong data supporting causality between NNS exposure and

metabolic disorders in animal models [18–24,43–45], there is no clear evidence that NNSs cause metabolic disorders in human subjects. However, data from at least five different mammalian species (rats, mice, pigs, cows, human) show that NNSs can be metabolically active [49,63,65,76,66–69]. Therefore, the old concept that NNSs are invariably metabolically inert no longer holds true. More research is needed to elucidate the mechanisms by which NNSs may drive metabolic effects and better understand potential effects of these commonly used food additives.

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