Potential Mechanisms for NNS-Induced Metabolic Deviances: Satiety Hormone Secretion and Alterations in the Gut Microbiota

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ABSTRACT Rising rates of obesity and type 2 diabetes have prompted the usage and recommendation of nonnutritive sweeteners (NNS) as harmless sugar substitutes in attempts to decrease caloric intake. Contrary to the common belief that NNS remain physiologically inert post-consumption, evidence highlights their ability to alter metabolic processes via interactions in the gastrointestinal tract. An extensive review was conducted on the potential NNS-induced metabolic deviances by way of two non-mutually exclusive mechanisms. One possible mechanism involves their ability (or inability) to induce the secretion of GLP-1, a hormone produced in the gut that promotes satiety and accelerates glucose-dependent insulin secretion by interacting with sweet-taste receptors in the small intestine. Though NNS (sucralose, Ace K, and Rebaudioside A) show a high rate of GLP-1 secretion during *in vitro* studies, there are many discrepancies in results from human *in vivo* studies. A second mechanism proposes that NNS alter the composition of the gut microbiota, a vast community of microorganisms responsible for digesting food, releasing metabolites, and synthesizing vitamins. Differing forms of dysbiosis, alterations in bacterial composition, are observed, including an increased ratio of Firmicutes to Bacteroidetes and an increase in *Lactobacilli* spp. in exclusive studies, upon NNS exposure. Few experiments assessing NNS impact on the gut microbiota have been conducted with human subjects. Further investigations, specific to human subjects, should be explored in order to assess the true extent to which NNS impact incretin secretion and alterations in the gut microbiota.

INTRODUCTION

In recent decades, the usage and implementation of high-sugar content food products have accelerated the presence of metabolic syndromes like obesity and diabetes (Low et al., 2016). If current trends in diet and lifestyle continue to accelerate, an estimated 38% of the world's

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population will be overweight and another 20% will be obese by the year 2030 (Smith et al., 2016). Abnormally high consumption rates of energy-dense foods with large contents of sugars and saturated fats not only serve as a leading cause for obesity but also contribute largely to type 2 diabetes, hypertension, and cardiovascular disease (Laffitte et al., 2014). In attempt to diminish the overbearing and unhealthful trends in diet, substitutes for sugars, nonnutritive sweeteners (NNS), have been developed. NNS can deliver a high potency content of sweetness in a miniscule concentration with negligent caloric intake by binding with strong affinity to one or more sites on the sweet taste receptor in the oral cavity (Burke et al., 2015). Until recent consideration, NNS were recommended for people who were approaching obesity and/or onset diabetes to aid in health improvement by providing a sweet taste without affecting caloric intake and glycemic responses; however, on accounts of multiple studies, NNS consumption over a prolonged period of time may promote glucose intolerance, obesity and its associative commodities (Nettleton et al., 2016). Currently, the FDA has recognized six NNS for use in the United States: acesulfame potassium, aspartame, neotame, saccharin, sucralose, and Rebaudioside A. The presence of these sweeteners has dominated many components of our diet and personal care products including "diet" or "zero-calorie" drinks, chewing gum, toothpaste, and many other foods and drinks for enhancement of flavor (Sylvetsky et al., 2017). The discovery of sweet taste receptors in the gut has prompted the possibility for NNS to interact with them in a similar fashion to which they bind to the taste receptors in the oral cavity (Laffitte et al., 2014; Margolskee et al., 2007; Pepino et al., 2011).

Contrary to the common belief that NNS are physiologically inert compounds, there is overwhelming evidence that highlights their ability to alter metabolic processes that control homeostasis post-consumption via interactions in the gastrointestinal tract. This review focuses on the impact of NNS on the secretion of satiety hormone glucagon-like peptide-1 (GLP-1) and NNS-induced alterations in the gut microbiota. In terms of evolution, the highly innate sensation and perception of taste has provided vertebrates with an expansive pallet capable of detecting beneficial and toxic nutrients, thus enabling them to successfully navigate their surroundings. Most notably, the innate preference to sweet taste evolved to detect the presence of valuable carbohydrates, and the interactions between sweeteners and sweet taste receptors (in the oral and extra oral cavities) predict caloric content and evoke physiological responses that prepare the gastrointestinal tract for digestion according to the intensity and quantity (Pepino et al., 2011). The gastrointestinal tract is home to an elaborate network of enteroendocrine cells that release hormones to signal digestive, homeostatic, and behavioral responses by responding to the enteric nervous system and effectively communicating to target organs. One of these processes involves the secretion of a satiety hormone, GLP-1 which serves a vital function by amplifying glucose-dependent insulin secretion (Baggio et al., 2007; Jang et al., 2007; Takai et al., 2015).

A large part of the human metabolism is dependent on the gut microbiota for processing food that would otherwise be indigestible and synthesizing valuable vitamins, such as vitamin B12 (D'Argenio et al., 2015). Additionally, the gut microbiota has been identified as a key player in the intricate mechanisms of nutrient metabolism in the gut and in the neuronal communication and regulation of the hypothalamic-pituitary-adrenal axis (Sudo, 2014). Though the recently proposed mechanisms of such interactions are not definitive, their presence and predicted impact makes the microbiome a likely contributor to metabolic syndrome. Metabolic syndrome is characterized by a combination of three or more of the following symptoms: abdominal obesity, high triglycerides, low high-density lipoprotein-cholesterol (HDL-C) and heightened blood pressure and blood sugar (Sudo, 2014). Those affected by metabolic syndrome are subject to a significant increase risk to cardiovascular disease, diabetes mellitus, non-alcoholic fatty liver disease, polycystic ovarian syndrome, dementia, and cancer. A predicted contributor and common characteristic of metabolic syndrome is chronic inflammation which can be attributed by various environmental and genetic factors (Melvin et al., 2016). One such

environmental factor includes alterations in the gut microbiota. Changes in gut bacterial composition have been linked to inflammatory side-effects that promote insulin resistance, fat storage and weight gain in the host which makes it a potential contributor to the development of metabolic syndrome, obesity and/or type 2 diabetes (Daly et al., 2014; Palmnäs et al., 2014).

Being that both metabolic components, gut hormone secretion and the gut microbiota, are localized in the gastrointestinal mucosa and enteric nervous system, they are likely to have intersecting impacts when affected by NNS. Specifically, recent studies have indicated that the gut bacterial composition can affect the development and regulation of the hypothalamic-pituitary-adrenal axis and behavior via mechanisms of gut hormone secretion (Sudo, 2014). Additionally, host-derived satiety hormones were found to increase local bacterial proliferative capacity as well as pathogenicity (Sudo, 2014). The interactive communication between the microbiome and enteroendocrine cell hormone secretion reveals their non-mutually exclusive relationship and has further implications when assessing their response to NNS. Though the mechanistic connection between the two are not solidified, both aspects serve as important targets individually when assessing the metabolic impacts of NNS. In exploring the impact of NNS on satiety hormone

secretion and the gut microbiota, several mechanisms will be proposed that enable these discrepancies and serve as prospective points of interest for future research on metabolic health. The directionality of this review is provided by a study outline (see Figure 1).

METHODS

PubMed database searches were carried out using keywords and phrases relevant to this review: non-nutritive sweeteners, gut hormones, GLP-1, GIP, gut microbiota, body-weight, insulin, homeostasis, and glucagon. Searches were filtered by "Best Match," and their publishing dates were considered in order to include the most current information.

RESULTS
POTENTIAL MECHANISMS FOR NNS-INDUCED METABOLIC DEVIANCES

1.a. Gut Function: The Enteroendocrine System

The gastrointestinal tract consists of a series of hollow organs that enable the breakdown, metabolism, and distribution of nutrients ingested from the environment. Specific to the gut, the enteric nervous and enteroendocrine systems work together to initiate digestion and effectively communicate proper metabolic signals (via gut-derived peptides) to the brain to commence
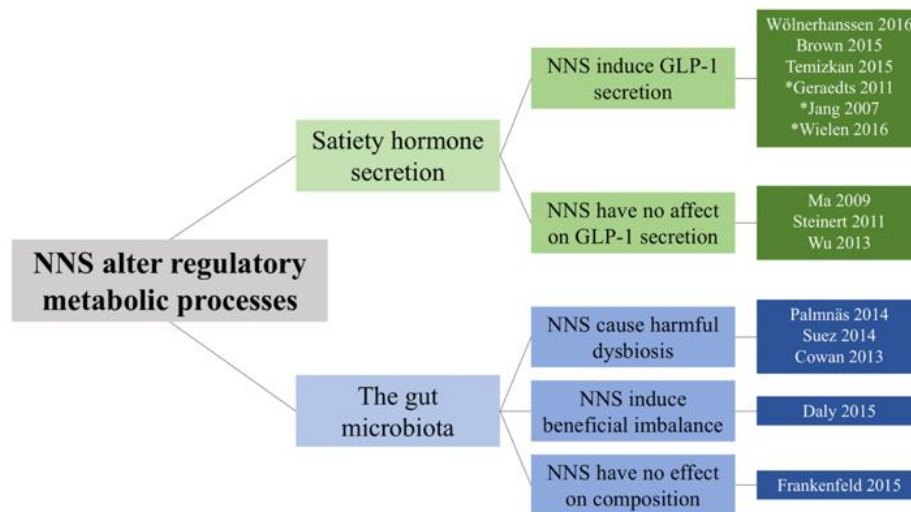

Figure 1. Study Overview. Outlined above, the direction of the review began with a general concern for NNS and their impact on metabolic process then succinctly moved toward their impact on satiety hormone secretion and the gut microbiota. Studies marked with "*" were conducted *in vitro*.

chemical and behavioral responses. Sensory neurons in the gastrointestinal tract partake in homeostasis, danger detection, and protective responses by communicating with surrounding intestinal cells (Melvin et al., 2016). The gastrointestinal tract produces a vast amount of peptide hormones that initiate specific responses. Specialized cells in the gastrointestinal tract, enteroendocrine cells, are responsible for the endocrine action of the gut (Melvin et al., 2016). During periods of fasting, ghrelin is released by specific enteroendocrine cells found in the gastric fondus. In contrast, leptin, produced by adipose cells, inhibits hunger and regulates long-term energy storage (Melvin et al., 2016). Additionally, a high presence of lipid initiates peptide YY (PYY) secretion in enteroendocreine cells in the ileum and results in a reduction in caloric intake (30%) (Melven et al., 2016). Also termed as "incretins," gut hormones, GLP-1 and gastric inhibitory polypeptide (GIP), are directly involved in the transmission of sweet-taste signals from carbohydrates by accelerating glucose-dependent insulin secretion in pancreatic ß-cells (Henquinn et al., 2012; van der Wielen et al., 2016). GLP-1, the more influential of the two, is secreted by intestinal L cells located in the gastrointestinal mucosa and acts locally within the intestinal wall to initiate enteroenteric reflexes which slow the rate of gastric emptying (Nadkarni et al., 2014). Additionally, GLP-1 regulates glucose homeostasis by inhibiting the release of glucagon, therefore decreasing the breakdown of glycogen and lowering blood glucose levels (van der Wielen et al., 2016). For these reasons, GLP-1 has been actively researched as a possible target for the treatment and prevention of obesity and type 2 diabetes. The relationship and communication between intestinal L cells and pancreatic β-cells via incretins to secrete insulin has been dubbed the *incretin effect*. It was discovered that oral glucose administration promoted substantially greater amounts of insulin secretion compared to an intravenous injection of glucose, indicating the role of incretins in amplifying insulin secretion (Delgado-Aros et al., 2002; Swithers et al., 2013). Incretins are estimated to account for 50-70% of the total insulin production post-meal consumption (Steinert et al., 2011). Considering the direct correlation with enhanced insulin

secretion, inhibition of glucagon release, and promoted feelings of satiety, GLP-1 is a vital messenger examined.

1.b. The T1R2/T1R3 Taste Receptor Expressed In Extraoral Tissues

NNS have the unique ability to elicit a sweet-taste response that ranges between 100-800 times sweeter than an equivalent amount of glucose, thus allowing their implementation for food/drink product to be miniscule. Due to their unique chemical structure, the predicted mechanism for the heightened response is an increase in bonding affinity for the sweet taste receptor. Sweet taste perception is initiated in the oral cavity by the binding of a sweet-tasting molecule to a sweet taste receptor, a G-protein coupled receptor with two 7-transmembrane subunits, taste receptor type 1 member 2 and taste receptor type 1 member 3 (T1R2/T1R3) (Burke et al., 2015). The heterodimeric receptor provides input on the caloric contents of ingested food by initializing cellular and neural responses when in contact with natural sugars or non-nutritive sweeteners (Laffitte et al., 2014). Within the last 5 years, the T1R2/T1R3 sweet taste receptor and its associated taste signal transduction molecules have been discovered in tissues beyond the mouth, including the gastrointestinal tract, pancreas, adipose tissues, and brain (Kyriazis et al., 2012; Laffitte et al., 2014; Nakagawa et al., 2009; Oya et al., 2011; Takai et al., 2015). The presence of such receptors in tissues beyond the oral cavity suggests an additional physiological purpose than simply sweet-taste detection; Specific to the intestinal tract, the T1R2/T1R3 receptors, integrated within the membrane of enteroendocrine cells, trigger physiological responses that facilitate metabolic processes and stimulate glucose absorption (Margolskee et al., 2007; Sclafani et al., 2007; Shirazi-Beechey et al., 2011). Glucose absorption in the gut is essential for the host as nutrients from food must be transferred from the gut into the bloodstream for delivery to target organs. Significant evidence supports that glucose uptake via sodium-dependent glucose transporter isoform 1 (SGLT1) in enteroendocrine cells is regulated, to a degree, by the activation of the T1R2/T1R3 receptors and their related components of G-

protein-linked signaling pathways (i.e. α-gutducin, phospholipase C β type 2, and transient receptor potential channel M5 [TRPM5]) (Jang et al., 2007; Margolskee et al., 2007; Merigo et al., 2011; Rozengurt et al., 2006). Margolskee et al., and others have demonstrated that knockout mice lacking T1R3 or α-gustducin were not able to increase SGLT1 mRNA and protein expression, whereas mice with the components significantly elevated expression of SGLT1 upon consumption of sugars or artificial sweeteners, indicating that the heterodimeric sweet taste receptor had an influence on glucose uptake in mice.

The main function of enteroendocrine cells is to produce and secrete gastrointestinal hormones, incretins, which act as local messengers and aid in digestive processes (Baggio et al., 2007). The two incretins, GLP-1 and gastric inhibitory polypeptide (GIP), also relay information to sensory neurons in close proximity via enhancing secretion neurotransmitters (Nadkarni et al., 2014). Incretins, hormones that facilitate the rapid digestion of ingested nutrients, are released within minutes of meal consumption by enteroendocrine cells in the small and large intestine (Baggio et al., 2007; Delgado-Aros et al., 2002; Nadkarni et al., 2014). Similar to glucose uptake via SGLT1, GLP-1 secretion is initiated, at least partially, by the activation of the T1R2/T1R3 receptors in the small intestine (Takai et al., 2015).

1.c. The Initiation of T1R2/T1R3 Receptor, GLP-1 Secretion, and Glucose-Dependent Insulin Release

There is functional evidence linking the T1R2/T1R3 taste receptor and the release of incretins in enteroendocrine cells; however, a definite mechanism has not been developed. Having discovered the same components involved in the perception of taste in type II taste cells in the tongue, one proposed mechanism mirrors this same pathway. As seen in Figure 2, the secretion of incretins (GLP-1 & GIP) is stimulated by the signal transduction pathway initiated at the T1R2/T1R3 receptor. In type II taste cells in the oral cavity, stimulation of the T1R2/T1R3 receptor causes the dissociation of the heterotrimeric guanine-nucleotide-binding G

protein and its α, β, and γ subunits (α-gustducin, Gβ3, and Gγ13). This dissociation leads to an increase in phospholipase C-β2 (PLC-β2) activity which causes the inositol 1,4,5-triphosphate (IP$_3$) receptor to release Ca^{2+} from intracellular stores and the opening of transient potential ion channel, transient receptor potential cation channel subfamily M member 5 (TRPM5). As a result, the membrane is depolarized, and an action potential is generated, causing a release of adenosine triphosphate (ATP) which acts as a transmitter for further gustatory afferents.

Though the mechanism has not been directly proven, eneteroendocrine cells are thought to follow a similar pathway involving the T1R2/T1R3 receptor and the heterotrimeric G protein (α-gustducin, Gβ3, and Gγ13) and the resulting depolarization of the cell membrane (Behrens et al., 2011; Gheni et al., 2014; Wu et al., 2013). Instead of continuing the transmission of gustatory sensory information from the tongue to the brain, the action potential generated in the enteroendocrine cells facilitates the release of hormone-containing vesicles. Further, GLP-1 and GIP, are able to effectively aid in post-digestive responses. In a study conducted by Jang et al. (2007), α-gustducin or T1R3 knockout mice did not show an increased secretion of GLP-1 to direct glucose ingestion. Additionally, GLP-1 secretion in L cells was significantly lower in mice exposed to a T1R2/T1R3 receptor inhibitor, lactisole. Together, their results indicate that taste receptor components in enteroendocrine cells are directly involved in the secretion of GLP-1 in mice.

The two dominant signals that drive insulin secretion in pancreatic β cells are generated ATP via glucose metabolism and the influx of Ca^{2+} ions. ATP generated by glucose metabolism closes the ATP sensitive $K^+(K_{ATP})$ channels, depolarizing the cell membrane. Voltage-dependent Ca^{2+} channels open, allowing the influx of Ca^{2+} and exocytosis of insulin granules (Côté et al., 2014; Gheni et al., 2014;). Incretins, GLP-1 and GIP, act as paracrine signaling molecules by interacting with G-protein coupled receptors (GPCRs) to accelerate insulin secretion
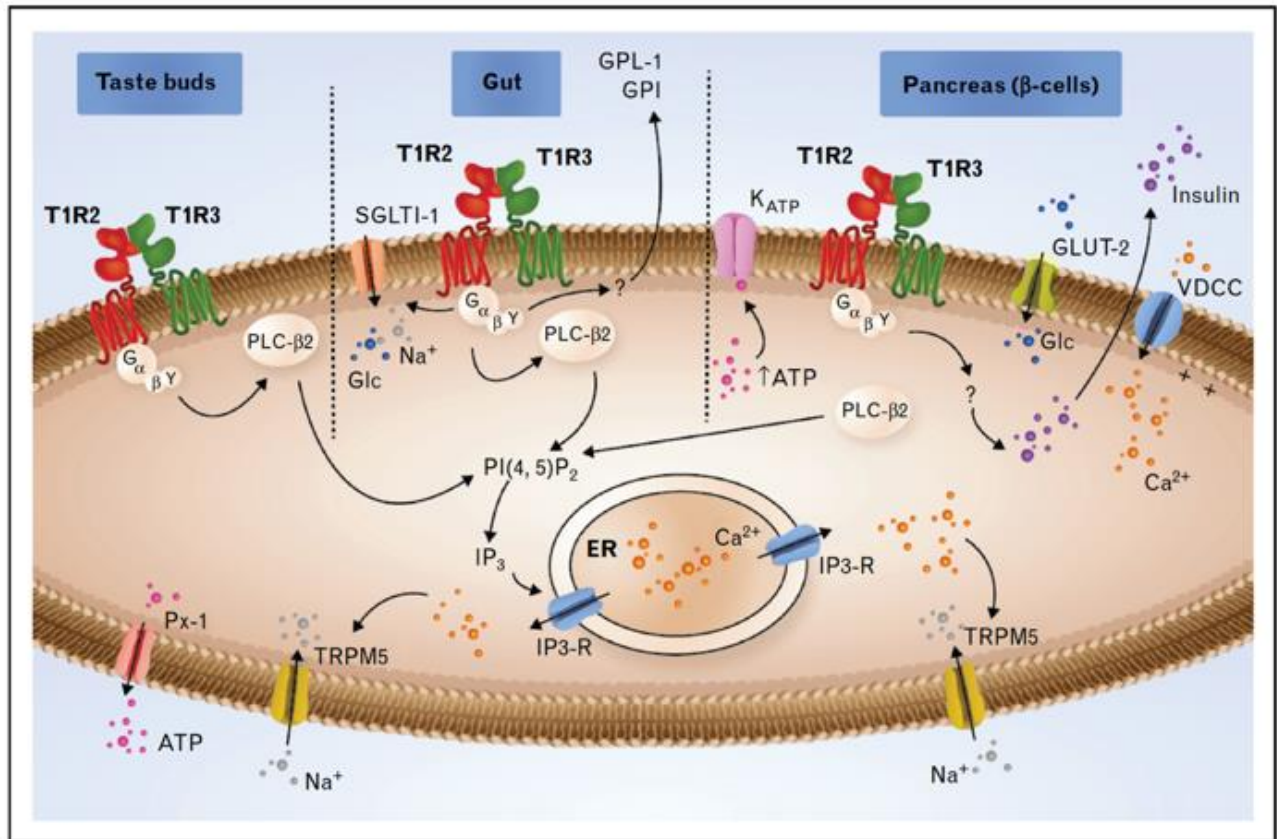
Figure 2. GLP-1 Secretion via T1R2/T1R3 Receptor. The intracellular mechanism outlined in the above figure provides a possible relationship between the intracellular cascade observed in type II taste cells and enteroendocrine cells in the gut. The mechanism in the taste buds is as follows: T1R2/T1R3 receptor stimulation; dissociation of the heterotrimeric guanine-nucleotide-binding G protein and α, β, and γ subunits (α-gustducin, Gβ3, and Gγ13); increase in phospholipase C-β2 (PLC-β2); inositol 1,4,5-triphosphate (IP$_3$) receptor releases Ca^{2+} from intracellular stores; opening of transient receptor potential cation channel subfamily M member 5 (TRPM5); membrane depolarized; action potential built; release of ATP. Enteroendocrine cells are predicted to follow a similar pathway via stimulation of the T1R2/T1R3 receptor where the action potential from Ca^{2+} built up acts as the driving force for the secretion of incretins, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1(GLP-1). Upon stimulation of the T1R2/T1R3 receptor via sweet-tasting molecule, incretins are released, and the expression of sodium-glucose transporter-2 (GLUT-2) increases. This figure (Lafitte et al., 2014) has been reproduced with permission from Wolters Kluwer Health, Inc.

in a glucose-dependent manner via cyclic AMP (cAMP) signaling; however, this phenomenon is not presented in Figure 2. Proposed by multiple sources, a possible mechanism for the amplifying effects of incretins on insulin secretion links glutamate, a product of the malate-aspartate (MA) shuttle, as the key factor for combination (Côté et al., 2014; Gheni et al., 2014). Upon stimulation of a specific G protein in the membrane of a pancreatic β cell via an incretin molecule, cAMP/protein kinase A signaling allows insulin granules to uptake glutamate produced from the malate-aspartate shuttle, thereby increasing the secretion of insulin.

1.d. Satiety Hormone Secretion via T1R2/T1R3 Receptor: NNS Glucose Structural Analogy and Macronutrient Pairing

Nonnutritive sweeteners have been predicted to elicit a response, similar to glucose, which potentiates levels of GLP-1; however, many studies dealing specifically with human subjects saw no response to predicted incretin levels, indicating a deficiency in the release of incretins via enteroendocrine cells. Incretins' ability to potentiate insulin secretion, promote satiety, and prolong gastric emptying make them a critical component to glucose regulation and

consumption patterns. Several studies, summarized in Table 1, assessed the implications of various NNS (sucralose, acesulfame potassium, aspartame, and Rebaudioside A) and polyol sweeteners (erythritol and exylitol) on GLP-1 secretion.

While focusing on studies dealing with human subjects, the experiments done in vivo were discordant in their findings. Wölnerhanssen et al. (2016) found that xylitol and erythritol, polyol sweeteners, caused a significant increase in GLP-1 levels in both lean (5) and obese (5) subjects compared to a control, an intragastric infusion of just water. Methods that delivered treatments via intragastric infusion involved a catheter tube that allowed for direct administration to the duodenum, bypassing the digestive processes that occur prior. Additionally, administering a "glucose load" consisted of consuming 75 g of glucose after NNS exposure. Polyols are not technically nonnutritive because they are partially metabolized, but over 90% of erythritol is absorbed then excreted by the kidney, and xylitol is readily fermented by bacteria in the gut. Hence, their actual caloric intake is much lower than that of glucose. With no prior intake or glucose administration, insulin levels remained low, indicating the glucose-dependent nature of insulin secretion. It is worthy to note that this study used extremely high amounts of sweetener in their infusions (50g of xylitol and 75g of erythritol), which are unrealistic to typical consumption amounts as these usually appear in lower concentrations. The structural similarity between polyols and glucose was a proposed cause for the increased levels of GLP-1. Two other studies that followed a similar intragastric infusion method in human subjects; Ma et al. (2009) and Steinert et al. (2011) observed no difference in GLP-1 levels upon sucralose, aspartame, and acesulfame potassium administration. Steinert et al., suggested that the secretion of GLP-1 depended more than just the detection sweetness or that it was reliant on a structural analogy to glucose. Rather than using infusions, many *in vivo* studies assessed NNS impact on GLP-1 by method of a dissolved drink, either commercial diet sodas or custom concoctions. This method more accurately represents the physiological conditions in which NNS would exist as the quantities of administered NNS were lower and the digestive path incorporated all components of the gastrointestinal tract. In doing this, they all also administered a 75g oral glucose tolerance test (OGTT) 10-15min after exposure to NNS to test responses to GLP-1, insulin, and blood glucose levels. Temizkan et al. (2015) administered sucralose to normal and diagnosed type 2 diabetic

Legend:
- ■ Not measured (black)
- ■ No change (gray)
- ■ Statistical increase (green)
- ■ Statistical decrease (red)

IN VIVO

Study	NNS	Participants	Sex	Method	Glucose Load	Blood glucose	GLP-1	Insulin
Wölnerhanssen 2016	Xylitol & Erythritol	10 Humans	Male & Female	Infusion	No	No change	Statistical increase	No change
Ma 2009	Sucralose	7 Humans	Male & Female	Infusion	No	No change	No change	No change
Steinert 2011	Aspartame, Ace K & Sucralose	12 Humans	Male & Female	Infusion	No	No change	No change	No change
Wu 2013	Sucralose & Ace K	10 Humans	Male	Drink	Yes	No change	No change	No change
Brown 2015	Sucralose & Ace K (*Diet Soda*)	22 Humans	Male & Female	Drink	Yes	No change	Statistical increase	No change
Temizkan 2015	Sucralose	16 Humans	Male & Female	Drink	Yes	Statistical decrease	Statistical increase	No change

IN VITRO

Study	NNS	Participants	Sex	Cell Type	Blood glucose	GLP-1	Insulin
Geraedts 2011	Sucralose & Ace K	10 Humans	Male & Female	Duodenal tissue	Not measured	Statistical increase	Not measured
Jang 2007	Sucralose	Human (post mortem)	Anonymous	Duodenal cell line	Not measured	Statistical increase	Not measured
Wielen 2016	Rebaudioside A	Mice (C57BL/6J)	Male	Duodenal cell line	Not measured	Statistical increase	Not measured

Table 1. Summarized Results of NNS Impact on GLP-1 Secretion.

subjects and found that it enhanced GLP-1 levels in only healthy subjects. Additionally, they saw lower levels of blood glucose in the normal patients after NNS introduction compared to the control (water). This study highlights the metabolic deficiencies in diabetic patients and proposes NNS as possible targets for prevention of this onset disease. Brown et al. (2009) saw a greater increase in GLP-1 levels after drinking 240mL diet soda (sweetened with sucralose and Ace K) compared to the control (carbonated water). They concluded that NNS has an enhancing effect on the GLP-1 release by synergizing with glucose. With aims to clarify the real impact of NNS on GLP-1 release, a study conducted by Wu et al., conducted similar conditions; however, instead of using a commercial diet soda, they used pure sucralose and Ace K dissolved in water with no added substances found in the diet soda used by Brown et al. (caramel color, gum acacia, natural flavors, citric acid, potassium benzoate, phosphoric acid, and potassium citrate). Their results yielded no difference in GLP-1 secretion when exposed to NNS versus the control (water). These two studies, Brown et al. and Wu et al. (2009), present the possibility that the added components found in the diet soda that Brown et al. used could have contributed, in combination with NNS, to the enhancing effect of GLP-1 more so than the NNS alone.

Approximately half of the *in vivo* studies observed an increase in GLP-1 levels, whereas the other half saw no increase. The majority of the results are not in complete agreement; however, there is moderate evidence suggesting that NNS can an impact GLP-1 secretion within *in vivo* experimentation. The experiments conducted in controlled cell lines (*in vitro*) were more congruent in their findings. Geraedts et al. (2012) took eight healthy human mucosal tissue samples from the duodenum via gastroduodenoscopy and found that sucralose and Ace K alone could induce GLP-1 secretion, but their effects were enhanced when pea protein was added in combination to the cell lines. In agreement with Brown et al., they deduced that NNS sucralose and Ace K may synergize with a macronutrient to enhance satiety hormone secretion. Jang et al. demonstrated a positive correlation between NNS

sucralose and GLP-1 secretion in post mortem human duodenum cell lines. When lactisole, a known sweet receptor inhibitor was introduced, the secretion of GLP-1 was diminished, supporting the hypothesis that involves the T1R2/T1R3 sweet taste receptor and incretin release. In derived mouse cell lines, van der Wielen et al. (2016) also observed a clear increase in GLP-1 secretion (4.3 fold in the ileum) when directly exposed to Rebaudioside A. More so than the *in vivo* experiments, the *in vitro* studies provide evidence for a direct correlation between the stimulation of the T1R2/T1R3 receptor and GLP-1 secretion. Remarkably, they proved that NNS can induce this pathway; however, *in vitro* experiments are restricting in their results due to the controlled setting and limiting factors.

NNS DISRUPT THE GUT MICROBIOTA COMPOSITION

2.a. The Importance of the Gut Microbiota on Metabolism

The human gut microbiota, consisting of mostly bacteria (>90%), archaea, viruses, and unicellular organisms, is linked to physiological and digestive capacities that humans have yet to evolve independently. Specific to the intestinal tract, the gut microbiota aids in detoxification, vitamin synthesis, immunity, and digestion by secreting enzymes for substances otherwise non-digestible (D'Argenio et al., 2015). Alterations in the gut microbiome have been linked to abnormal metabolic deficiencies as well as obesity, diabetes, and chronic inflammation (Philippaert et al., 2017). These alterations can be stimulated by internal and external factors including diet, age, hormonal cycles, therapies, and illness. It is important to note that the composition of microflora within host species develops from distinct selective pressures and inherited genetic factors; therefore, the composition will differ significantly from person to person and even more so from humans to other mammals (mice, swine, and rats) (Daly et al., 2014). Disruptions in the gut microbiota are widely recognized as an operative contributor that lead to the development of obesity and insulin resistance (Nettleton et al., 2016). Consisting of 400-1000 adherent and non-adherent bacterial species, the majority of the bacteria in the microbiome (>90%) belong to one

of the two major phyla, the Firmicutes and the Bacteroidetes (Nettleton et al., 2016; Philippaert et al., 2017). A convincing, but not definite, characteristic of obesity and type II diabetes is associated with a high ratio of Firmicutes to Bacteroidetes (Drasar et al., 1972; Nettleton et al., 2016; Palmnäs et al., 2014). The ratio between them is thought to influence metabolic processes and susceptibility to onset metabolic syndrome (Nettleton et al., 2016), but due to the specificity and variability of microbiomes, this ratio has been questionable as plenty of healthy patients showed high ratios of Firmicutes to Bacteroidetes and vice versa (Frankenfeld et al., 2015). In accordance with these recent findings, the impact of NNS on the gut microbiota has gained serious attention.

2.b. NNS-Induced Disruptions in the Gut Microbiota via Membrane-Spanning Receptors and Production of SCFA

Literature research on NNS and the gut microbiota yielded very few studies that failed to disprove their null hypothesis, but the majority of the experiments conducted were carried out with non-human mammals as their subjects. The core studies are summarized in Table 2 and are referenced throughout the text, ranging from results that showed NNS to be harmful (impaired metabolic responses), beneficial, or non-affecting to the function of metabolic responses. Additionally, a supplementary figure, detailing the taxonomic classification of bacteria involved in the following studies, was developed to display

the relationship between bacterial species and their predicted metabolic impacts (see Figure 3).

Palmnäs et al. (2014) assessed the effect of low-dose aspartame on the microbiome composition and glycemic responses on male Sprague-Dawley rats. They found that aspartame-consuming rats consumed fewer calories and gained less weight than the control diet (water); however, the aspartame group displayed elevated fasting glucose levels and impaired insulin-stimulated glucose disposal. Analysis of their microbiome composition revealed an increase in the Firmicutes to Bacteroidetes ratio, specifically, overall increases in the abundance of Enterobacteriaceae, *Roseburia*, and *Clostridium leptum*. In addition, they observed increased amounts of the short chain fatty acid (SCFA) propionate, a highly gluconeogenic substrate, in the aspartame-consuming group. The rising abundance of SCFA propionate was most likely a result of the increase in *Clostridium* spp. as it produces this metabolite during fermentation (Puertollano et al., 2014). This study shows the possibility of NNS interacting with bacteria in the gut to induce production of a bacterial end product, SCFA propionate, which is known to impair insulin function and elevate glucose levels (Palmnäs et al., 2014; Ximenes et al., 2007). Suez et al. also observed a NNS-induced glucose intolerance and attributed it to alterations in the gut microbiota. Exposure to NNS saccharin in mice lead to increasing levels of Enterobacteriaceae, Bacteroides, and some
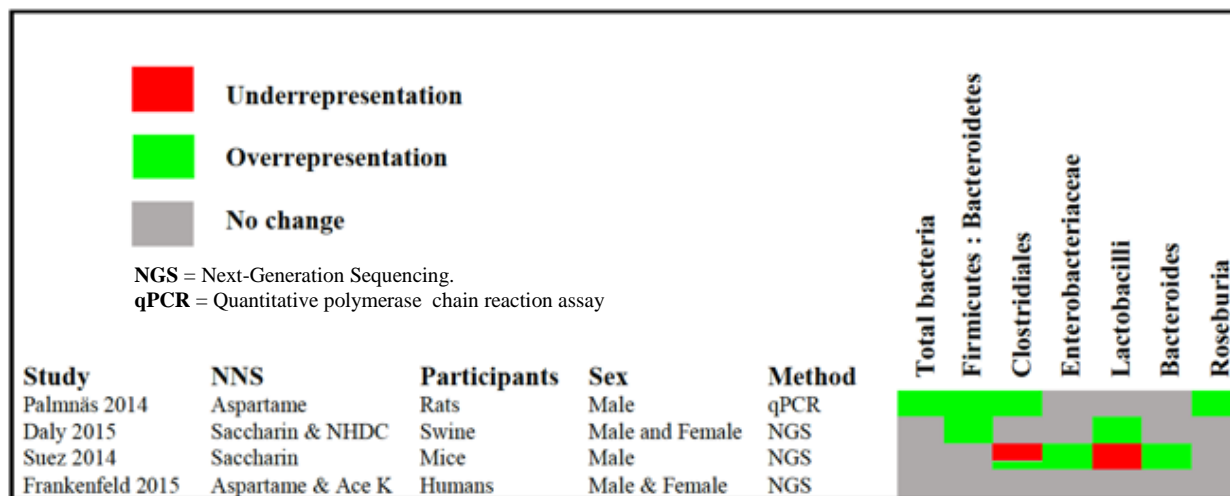

Study	NNS	Participants	Sex	Method	Total bacteria	Firmicutes : Bacteroidetes	Clostridiales	Enterobacteriaceae	Lactobacilli	Bacteroides	Roseburia
Palmnäs 2014	Aspartame	Rats	Male	qPCR		Over		Over			Over
Daly 2015	Saccharin & NHDC	Swine	Male and Female	NGS					Over		
Suez 2014	Saccharin	Mice	Male	NGS			Under	Over	Under	Over	
Frankenfeld 2015	Aspartame & Ace K	Humans	Male & Female	NGS							

Legend:
- Red = Underrepresentation
- Green = Overrepresentation
- Grey = No change

NGS = Next-Generation Sequencing.
qPCR = Quantitative polymerase chain reaction assay

Table 2. Summarized Results of NNS Impact on the Gut Microbiota.

Clostridiales, and decreasing amounts of *Lactobacilli spp.* and other Clostridiales. These bacterial compositional characteristics were accompanied by increased glycemic responses. After introducing antibiotic A (ciprofloxacin and metronidazole) and antibiotic B (vancomycin), the glycemic responses returned to normal levels that were comparable to the control group (water), suggesting that the changes in bacteria abundance were directly responsible for the impaired glycemic response. Failing to do an analysis of the microbiome post-antibiotic treatment, this study did not determine what differentiated between the two microbiome compositions. In an additional phase, they introduced saccharin treated bacterial colonies from affected mice and 7 humans to germ-free mice and saw similar trends in their rising blood glucose levels, verifying the importance of the gut microbiota composition on glucose homeostasis. Possible mechanisms for the alterations in bacteria and the resulting glycemic responses were not identified in this study. Frankenfeld et al. (2015), one of the few studies dealing with human subjects, assessed fecal bacterial composition upon exposure to aspartame and Ace K. They found no significant alterations in composition makeup (See Table 2).

In contrast to the previous studies, Daly et al. (2014) demonstrated a scenario in which

commercial NNS SUCRAM™ (saccharin/ NHDC) was beneficial to swine hosts by increasing the amounts of anti-inflammatory and immune-protecting *Lactobacillus*. Consisting of two experimental groups (standard wheat diet plus lactose or SUCRAM™) and a control group (standard wheat diet), they found a significant enhancement of caecal *Lactobacillus* populations in the lactose and SUCRAM™-exposed swine groups. *Lactobacillus* grow anaerobically by fermenting sugars to produce lactic acid, a metabolic product that was found in noticeably larger quantities in their experimental groups. In order to maximize their fermentation capacities, *Lactobacillus* have evolved multiple sugar transport and metabolic systems, which are initiated at their receptors by substrates (Daly et al., 2014). In the conclusion of their study, they proposed that certain members of the genus *Lactobacillus* have specific membrane-spanning receptor proteins that interact with one or both of the components of SUCRAM™, saccharin and/or neohesperidin dihydrochalcone (NHDC), to induce rapid growth in a similar way that lactose would. In a follow up study, Daly et al. narrowed the exact *Lactobacilli* species (*Lactobacillus* 4228) responsible for the highest rates of growth and determined the cause of the proliferation to be a result of a reduced lag-phase in cell division (Daly et al., 2016). *In vitro* analysis revealed that NHDC (one component of SUCRAM™) was
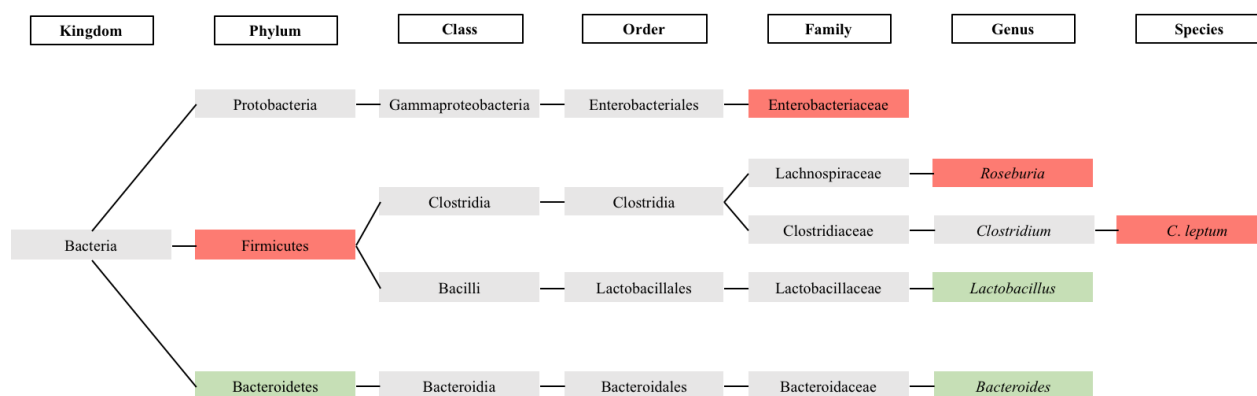

Figure 3. Taxonomic Classification of Notable Bacteria Impacted by NNS. Groups outlined in green are predicted to have a positive impact on metabolic and glycemic responses when abundance is increased. In contrast, those outlined in red are predicted to have negative impacts on metabolic processes when abundance is increased. An increased ratio of phyla Firmicutes : Bacteroidetes is associated with various metabolic deficiencies, such as type II diabetes and obesity, and can be visualized in the figure.

held most responsible for the accelerated growth patterns, confirming NHDC as the substrate for a specific membrane-spanning receptor protein. NNS NHDC is not approved for use in the United States; however, it is generally recognized as safe by the FDA.

CONCLUSIONS

NNS have established a strong presence in the common diet and continue to surround us in areas beyond perceived ingestion such as personal care products like toothpaste (Sylvetsky et al., 2017). As they are regularly recommended to individuals either prone to or experiencing metabolic deficiencies due to their non-caloric reputation, their impact post-consumption should be further examined due to the overwhelming evidence that supports the notion that NNS are likely metabolically active. While comparing studies that used different NNS and concentrations, species of subjects, and administrative processes (i.e. *in vitro* vs. *in vivo*), it is essential to consider their experimental differences as a contributor to their disagreement in results. The evidence surrounding the NNS-induced secretion of GLP-1 are consistent in most *in vitro* studies conducted with both human and other mammalian cell lines, supporting the hypothesis that NNS (sucralose & Ace K) interact with the T1R2/T1R3 taste receptor to aid in GLP-1 secretion (Geraedts et al., 2012; Jang et al., 2007; van der Wielen et al., 2016). Additionally, xylitol and erytrhitol, two sweeteners that are not technically nonnutritive, potentiated GLP-1 levels at high concentrations *in vivo* possibly due to their structural similarity to glucose (Wolnerhanssen et al., 2016). Contrary to this, the majority of studies done *in vivo* on humans determined that NNS (sucralose and Ace K) could not potentiate GLP-1 secretion independently (Ma et al., 2009; Steinert et al., 2011; Temizkan et al., 2015; Wu et al., 2013), and those that saw increased GLP-1 levels deduced that a synergistic effect between NNS and a macronutrient caused the enhanced incretin secretion (Brown et al., 2009). This intensified release of GLP-1 via NNS and macronutrients in combination was supported in Geraedts et al. where they observed significantly higher amounts of GLP-1 secretion when pea protein

was added to cell lines affected by sucralose and Ace K (Geraedts et al., 2012). As NNS are commonly consumed in combination with a meal in hopes of reducing sugar content, the pairing of NNS and macronutrient will likely induce secretion of GLP-1 and elicit an uptake response similar to that of glucose. In contrast, an inability for NNS to trigger post-ingestive responses, such as the secretion of GLP-1, can influence downstream digestion in a negative manner where conditioned response would be disrupted and secretion levels of insulin would be reduced; therefore, further experimentation is necessary to determine their definite effect on the secretion of satiety hormone, GLP-1.

Dysbiosis in the human gut microbiota is now considered a legitimate cause and characteristic of people either approaching or exemplifying obesity (David et al., 2014; Turnbaugh et al., 2006). The majority of studies assessing the effect of NNS on the gut microbiome composition observed some degree of dysbiosis. One damaging result of the dysbiosis after aspartame consumption in rats, observed by Palmnäs et al. (2014), increased the production of SCFA propionate, a gluconeogenic substrate, via *Clostridium* spp. Propionate impairs insulin function and elevates glucose levels, creating a detrimental environment for someone attempting to avoid obesity and type 2 diabetes. In converse, Daly et al. (2016) observed a positive form of dysbiosis by the increase in beneficial *Lactobacillus* spp. in saccharin and NHDC induced swine. The specific cause of increase in Lactobacilli population was attributed to a reduced lag-phase induced by NHDC, a NNS component of commercial SUCRAM™.

Altogether, the evidence presented in this review highlights the conflicting results regarding NNS and their integration into diet; NNS and their impact on hormone secretion and the gut microbiome require additional research to succinctly link their relationships. In combatting obesity and diabetes, these two non-mutually exclusive components of our metabolism serve as excellent target points for treatments and prevention. GLP-1 agonists are currently implemented as a new treatment method for type 2 diabetes due to their insulin-enhancing and

satiety characteristics, and probiotic/antibiotic treatments on patients have shown potential to change human bacterial composition in ways that are beneficial for regulating intestinal permeability, inflammation, and glycemic responses (Cani et al., 2008; Yadav et al., 2013).

Further investigations, specific to human subjects, should be explored in order to assess the true extent to which NNS impact incretin secretion and alteration in the gut microbiota as well as the inter-communication between these two components.

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