A review of the effects of Aspartame and Saccharin on human health, with special emphasis on the gut microbiome

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Abstract

The use of artificial sweeteners, such as aspartame and saccharin, are becoming more prevalent due to its popularity for low calorie diets and sugar alternatives, especially to those with diabetes. Artificial sweeteners have been found to cause adverse health effects ranging from headaches to cancer (Whitehouse, 2008). However, much of the research that has been done on the harmful effects of aspartame and saccharin is centered on their possible carcinogenic effects. Little to no research has been conducted on the effects of aspartame and saccharin on the gut microbiome. The gut microbiome has been linked to the progression of multiple disorders based on the alterations in its composition. Studies have also found a bidirectional interaction between the gut microbiome and the brain (Ma, 2019). As many side effects of artificial sweeteners have been reported in relation to the nervous system, we will explore if these artificial sweeteners are affecting the health of the gut microbiome. The aim of this study is to discuss the effects of aspartame and saccharin on human health, with emphasis on the gut microbiome.

Introduction

The current research on the effects of aspartame and saccharin on the gut microbiota is limited, but literature suggests a link between the health of the gut microbiome and the nervous system. Many side effects of artificial sweeteners have been reported in relation to the nervous and immune systems, and this study will explore if these artificial sweeteners are affecting the health of the gut microbiome. The aim of this review is to discuss the effects of aspartame and saccharin on human health, with emphasis on the gut microbiome.

Artificial sweeteners have become part of a large controversy in relation to food, health and nutrition. They are derived from chemical synthesis of organic compounds that have little to no nutritional value, and are used to substitute the taste of natural sweeteners that do have some nutritional value. Artificial sweeteners are commonly used as a sugar substitute in diets and by obese and/or diabetic patients for a lower calorie effect while controlling blood glucose levels (Saad, 2014). Due to the growing "diabesity" epidemic, the popularity and use of artificial sweeteners is becoming more prevalent but research on the adverse effects of such sweeteners on human health, particularly on the gut microbiome, is limited.

Artificial Sweeteners

Non-nutritive sweeteners (NNS), such as aspartame and saccharin, have the effect of tasting sweeter than sucrose (table sugar) with no nutritional or caloric value. Aspartame, found in "Equal" and "Nutrasweet", is the most used artificial sweetener and has been found to be 200 times sweeter than sucrose (Saad, 2014). It is often found in products such as "diet" or "low calorie" beverages, desserts, and chewing gum. Consuming higher than the recommended amounts of aspartame has been directly linked to an increase of phenylalanine in the brain, promoting seizures for those who are susceptible to them (Maher, 1987). Saccharin, found in

"Sweet n' Low," is the first artificial sweetener discovered, and was found to be 300 times sweeter than sucrose. After multiple studies were performed on rats, saccharin was believed to cause bladder cancer and hepatotoxicity (Saad, 2014). Both artificial sweeteners have been found to cause both acute and chronic effects, ranging from headaches to cancer (Whitehouse, 2008). However, much of the research that has been done on the harmful effects of aspartame and saccharin is centered on their putative carcinogenic effects. Little to no research has been conducted on the effects they have on the gut microbiome.

Overview of the Effects of Aspartame

Multiple studies tested the effects of aspartame consumption administered at both safe and high dosages on different cells and organs. The high dosages included anything greater than 45 mg/kg, while the safe dosage was based on the optimum dosage for humans anything below or equal to 40 mg/kg. The effects tend to result because of a change in the balance of oxidants and antioxidants leading to oxidative stress. Numerous studies have been reviewed and analyzed to determine and summarize the overall effects of aspartame on human health (Table 1).

Both safe and high doses of aspartame have led increased production of free radicals, causing an alteration in the oxidant and antioxidant balance, inducing oxidative stress in blood cells. Erythrocyte membranes are damaged by oxidative stress, this damage causes an impairment to their flow through microcirculation and oxygen delivery to tissues inducing erythrocyte aging and inflammation (Barodka, 2014 & Mohanty, 2014). Exposure to reactive oxygen species (ROS), a free radical, induces the loss of T lymphocyte signaling molecules, leading to decreased T-cell proliferation, and apoptosis (Cemerski, 2003). After incubating, human erythrocyte membranes in high and safe doses of aspartame, metabolites caused an inhibition in acetylcholinesterase (AChE) activity. A decrease in AChE activity could potentially

lead to neurological symptoms (Tsakiris, 2006). As free radicals overwhelm the antioxidant defense systems, oxidative stress results in blood cells. This leads to alterations in neutrophil function and humoral immunity in aspartame fed wistar albino rats (Arbind, 2014).

More than any other tissue, neuronal cells are vulnerable to oxidative stress. The high concentrations of polyunsaturated fatty acids makes these cells more susceptible to lipid peroxidation. As the free radicals attach to the fatty acids neuronal cell function is interfered with (Mariani, 2005). Neurobehavioral changes in aspartame fed rats were found to be caused by neuronal oxidative damage. This damage led to alterations in neurobehavioral, including emotional and anxiety behaviors. ROS was also linked to neuronal apoptosis, indicating that chronic aspartame consumption leads to oxidative damage in discrete brain regions (Ashok, 2015). A neutrotropic effect was observed when male mice were fed a high dose of aspartame (1000 mg/kg) (Villareal, 2016). Oxidative stress causes an overexpression of proinflammatory cytokines leading to damaged neurons and a disruption in the blood-brain barrier (Gu, 2012).

Immune cells are especially sensitive to changes in the oxidant/antioxidant balance. These cells use ROS to carry out cell functions including: cellular proteins, nucleic acids, and the cell membrane (Knight, 200). Immune cells contain high levels of polyunsaturated fatty acids in their plasma membranes, making them very sensitive to oxidative stress (Victor, 2003). Elevated levels of lipid peroxidation (LPO), evidence of oxidative stress, was present in immune organs of aspartame fed rats. As aspartame metabolizes methanol is released causing these elevated levels of LPO (Choudhary, 2014). Methanol intoxication caused a reduction in organ weight and immune cells count, which was potentially caused by an increase in shrunken and dead cells. The reduction in organ weight led to oxidative damage (Skrzydlewska, 1997). Oxidative stress led to

variation in serum cytokine levels and alterations in cellular and humoral immunity (Choudhary, 2015).

Oxidative stress, caused by aspartame consumption, leads to complications in multiple organs including: the liver, kidney, and heart. Antioxidant levels were affected by both high and safe doses of aspartame, leading to hepatocellular injury by overproduction of ROS (Muriel, 2009). Free radicals, including ROS, activate Kupffer cells, immune cells in the liver, leading to the release of proinflammatory cytokines, and inflammation causing the modulation of hepatocyte metabolism (Wheeler, 2003). Oxidative stress was present in the kidneys after aspartame consumption potentially leading to the progression of kidney fibrosis and chronic kidney diseases (Kim 2009). Oxidative stress in cardiac tissue impaired cardiac function and loss of vagal tone, such results were found present in aspartame fed rats. A loss of vagal tone makes humans significantly susceptible to cardiovascular disease (Choudhary, 2016).

Overview of the Effects of Saccharin

Frequent consumption of saccharin has been linked to type 2 diabetes, metabolic syndrome, weight gain, and cardiovascular disease (Swithers, 2013). Multiple studies have been reviewed and analyzed to determine and summarize the overall effects of saccharin on human health (Table 2).

Liver damage was observed in saccharin fed rats. Both low (10 mg/kg) and high (500 mg/kg) doses of induced hypocholesterolemia, while hypertriglyceridemia was only induced by a high dose of saccharin. The decreased serum cholesterol levels in combination with the increased alkaline phosphatase (ALP) levels indicate liver damage. One of the signs of hepatotoxicity, seen in both high and low dose fed rats, is elevated aminotransferase levels caused by the release of free radicals. Hepatic function is significantly altered by the elevated serum ALT and ALP,

caused by consumption of saccharin, especially in high doses (Amin, 2016). Oxidative stress was also induced by saccharin, by lowering catalase activity and total antioxidant concentration, leading to inflammation of liver cells (Abdallah, 2002). Saccharin consumption resulted in a significant decrease in protein profile, this decrease in protein and albumin indicates liver dysfunction (Helal, 2019). Histological changes were viewed in liver sections of aspartame and saccharin fed rats. These changes include congestion of the central vein and hepatic sinusoids, a disorganized hepatic parenchyma, and hyperplasia of the bile duct (Andrejić, 2013).

Similarly saccharin also induced toxic effects on the kidney by elevating serum creatinine and urea in both high and low doses of saccharine. These elevations lead to a reduction in glomerular filtration and kidney dysfunction (Turley, 2003; Helal, 2019). The higher and more frequent the dose of saccharin the higher the risk of oxidative stress causing damage to cells, and eventually leading to carcinomas (Amin, 2015). Rats that were fed high concentrations of saccharin developed urinary bladder hyperplasia, and those that were diabetic experienced bladder cancer development (Howe, 1977; and Taylor,1980).

The Gut Microbiome

The gut microbiome refers to the microbe population that is living in the human intestines. It contains thousands of different bacterial species and more than 3 million genes. Approximately two-thirds of each person's microbiota is unique to them and about one third of it is common among others. The main functions of the gut microbiome include food digestion, production of vitamins B and K, and helping the immune system by performing a barrier effect. The gut microbiota begins developing at birth, becoming stable and similar to that of adults by 3 years of age. Our microbiotas are evolving and changing throughout our lives due to our dietary

habits and various environmental factors (Bäckhed, 2012 and Ma, 2019). The microorganisms that are found in the gut microbiome work to metabolize the substrate inputs from the host into metabolites that affect the host by entering the bloodstream (Kumar, 2016).

The Human Microbiome Project Consortium is a gene sequence-based study that identified Bacteroidetes as a dominant bacterial phylum in a healthy human gut. There are four main bacterial phyla located in the gut microbiome: Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria. Bacteroidetes are gram-negative bacteria that are generally found to be beneficial when found in the gut, but when found outside of the gut could be considered pathogenic. They have been found to have the highest resistance rates of all anaerobic pathogens and have the most antibiotic resistance mechanisms. They are first introduced into the gut flora during vaginal birth, supporting that the uniqueness of the gut microbiome develops as early as birth (Wexler, 2007). One of the most abundant species of Bacteroidetes is *Bacteroides vulgatus.* A healthy gut flora would also consist of a greater Bacteroidetes to Firmicutes ratio. Firmicutes are mostly gram-positive bacteria and play a role in nutrient and energy resorption in the gut (Ley, 2016). The Microbiome Project found that Bacteroidetes and the phylum Firmicutes are directly correlated with obesity. Studies suggest that an increase in Firmicutes is correlated with an increase in weight and obesity, while a decrease in Bacteroidetes is related to an increase in weight and obesity (Kumar, 2016).

The phylum Proteobacteria is found to be the most unstable phylum present in the gut microbiome, responding sensitively to environmental factors. Bacteria of this phylum stain gram-negative and are mostly pathogens. A great increase and unstable level of Proteobacteria could represent a state of disease (Shin, 2015). The most abundant Proteobacteria found in the gut microbiome is *Escherichia coli* (*E. coli*) which functions to assist in normal digestive

processes in the intestines and ferments lactose using lactase. Pathogenic strains promote the development of chronic diseases of the intestines, such as Crohn's disease (Delmas, 2015).

Actinobacteria are gram-positive bacteria with a number of important functions such as decomposing organic substances. *Bifidobacterium*, a genus of the phylum Actinobacteria, is commonly used as part of a treatment for intestinal disorders and common colds (Ranjani, 2016). It is commonly used as a probiotic to prevent the death of the beneficial bacteria during antibiotic treatment (Araya-Kojima,1995).

Gut-Brain Axis

The gut microbiome has been found to contribute to illnesses in the intestines and other organs by alterations in the gut microbes. Studies have found a bidirectional interaction between the gut microbiome and the brain. The key route for communication between the central nervous system (CNS) and gut microbiota is the vagus nerve (VN). VN bidirectionally interacts between the gut microbiome and brain to maintain homeostasis in the cerebrum and intestine. Perturbations of the VN may cause CNS dysfunctions or gastrointestinal pathologies, such as neurodegenerative diseases or inflammatory bowel syndrome (Ma, 2019). The gut-brain axis includes the interaction through immunological signaling between the gut microbiota metabolites with cellular components of the CNS; this leads to the progression of various CNS disorders.

Although the direct connection between the gut microbiome and the CNS is not fully understood, studies suggest a connection between the blood-brain barrier (BBB) and the gut microbiome (Logsdon, 2018). A study on germ-free mice shows that the absence of gut microorganisms makes the BBB more permeable to macromolecules (Sharon, 2016). After the consumption and breakdown of aspartame, phenylalanine levels increase and cross the BBB causing changes in the production of neurotransmitters. When phenylalanine crosses the BBB

and reaches the brain it becomes neurotoxic and causes the deterioration of brain neurons, this neurotoxic effect is linked to memory loss (Humphries, 2008). The consumption of aspartame has been linked to causing oxidative stress in the brain, liver, and kidney.

The local and long distance effects of the gut microbiome attributes greatly to the overall health of the body. The gut microbiome contains approximately 70% of the immune system cells (Szefel, 2015). There is a special relationship between the gut microbiome and the immune system, where it enables the immune system to recognize and defend itself against bacteria to prevent bacterial invasions and infections (Gopalakrishnan, 2018). Aspartame consumption leads to an increase in corticosterone levels, indicating it as a chemical stressor. It also caused an increase in lipid peroxidation and nitric oxide indicating free radical production. In combination, the corticosterone increase and free radical production suppress the immune system and alter cytokine secretion (Choudhary, 2014).

Monocyte derived dendritic cells (moDC) mediate T-helper cells 1 and 17 after stimulation by *Escherichia coli Schaedler* or *Morganella morganii*, however *Bacillus Subtilis* limits this effect. Mediated by moDCs, *E. coli Schaedler* induced full maturation in moDCs causing strong inflammatory and microbiota-induced effector helper T responses. On the other hand, *B. subtilis* induces inflammation in the absence of interleukin-12 and - 23; it also causes a decrease in the effector Th1/th17 immune response (Bene, 2017).

Diseases & the Gut Microbiome

Several diseases have also been directly correlated to the gut microbiome including Type 2 diabetes mellitus, where the altered microbiota bacteria are Proteobacteria, *Bifidobacterium*, and *Lactobacillus*. Studies have also found that *Bacteroides vulgatus* is suggested in the development of Crohn's disease and colon cancer (Wexler, 2007). Analysis of gut microbiota of

patients with Crohn's disease shows the ratio of Proteobacteria is increased, which the diversity and total amount of firmicutes is decreased (Bene, 2017). *E. Coli* strains, that are usually non-pathogenic, have also been found to contribute to the development of chronic intestinal diseases, such as Crohn's disease and colorectal cancer (Delmas, 2015). Fecal and mucosal microbial populations of Parkinson's disease patients display different bacterial abundances than people without the disease, with a decrease in *Prevotellaceae* (*Bacteroidetes*) and an increase in *Lactobacillaceae* (*Firmicutes*). Microbial populations in feces could also be used to distinguish between specific forms of PD, in the tremor-dominant form of PD levels of *Enterobacteriaceae* (*Proteobacteria*) were lower in abundance than in the postural and gait instability forms (Sharon, 2016).

In an attempt to develop more information on the pathogenesis of Alzheimer's disease (AD), researchers decided to analyze gut microbiota composition hoping to find a link similar to the gut microbiome effects on amyloid deposition that was seen in rodents. Results showed that AD patients had a decreased microbial diversity and were compositionally different than healthy individuals, who were matched by gender and age. Bacterial abundance differed including increased Bacteroide and decreased Firmicutes and Bifidobacterium (Vogt, 2017). While there is no direct cure for AD multiple nutritionists suggest potential patients to take part of the MIND diet, with hopes to lessen the severity or delay the progression of symptoms. This diet emphasizes the importance of eating whole grains, nuts, greens, vegetables, poultry, and avoiding foods such as red meats, anything fried, and most importantly sweeteners.

Artificial Sweeteners & the Gut Microbiome

Few studies have been conducted to demonstrate the effects of NNS on the gut microbiome. NNS have been found to interfere with the gut microbiome composition by having

short-chain fatty acids (SCFAs) act as ligands for the G protein-coupled receptors in the gastrointestinal tract resulting in NNS permeability and altering gut microbiota composition. These alterations result in insulin resistance and an increased risk of metabolic syndrome (Laiuchonal, 2019). Insulin resistance, type 2 diabetes mellitus, and cardiovascular diseases have all been associated with high sugar diets, and are in the realm of metabolic syndrome (Seetharaman, 2016).

NNS have been linked to metabolic syndrome through three potential mechanisms: an interaction between NSS and sweet taste receptors, NNS interfering with usual sweetness response, and NNS interfering with gut microbiota composition (Pepino, 2015). These mechanisms are illustrated in figure 1 (adapted from; Liauchonak, 2019).

The perception of sweet tastes begins at the type 2 taste receptor cells that are clustered in G protein-coupled receptors (GCPRs). The two classes of GCPRs are taste 1 receptor (T1R) and taste 2 receptors (T2R), the receptors responsible for perceiving sweetened by humans are subtypes of the TR1 family. NNS contribute to metabolic syndrome and insulin resistance through an interaction with the T1R taste receptors that are associated with G protein α-gustducin, resulting in increased intracellular cAMP levels and neurotransmitter release (Liauchonak, 2019).

The association between NNS and insulin and hormone secretion impacts learned behavior and response to sweetness. Consumption of NNS has been associated with weight gain, by interfering with the physiological responses caused by the separation of sweetness from calories. By weakening the caloric compensation, excess energy intake leads to increased weight gain (Booth, 1972). An interference with learned responses that contribute to energy homeostasis was observed in a study, after glucose and saccharin were given orally but not directly into the

stomach glucagon-like peptide-1 (GLP-1) was released (Swithers, 2012). The disruption is shown when GLP-1 was suppressed when saccharin was given orally. The lowered GLP-1 levels decreased glucose utilization in muscle, liver, and adipose tissue; inhibiting glucagon release. This interference leads to elevated blood glucose levels, and possible weight gain (Liauchonak, 2019).

The gut microbiota composition is altered by NNS through the SCFAs acting as ligands for GPCRs in the gastrointestinal tracts (GI), regulating permeability and gut composition. The increased ratio of Firmicutes to Bacteroidetes has been linked to the consumption of NNS and metabolic syndrome, and this ratio is directly correlated with glucose tolerance (Liauchonak, 2019). This ratio is thought to be of significant relevance in human gut microbiota composition. During the first year of life, infant's microbiotas consist of high levels of bifidobacterium. As infants grow and consume diverse diets an increase in the firmicute/bacteroidetes ratio occurs, this is one of the main alterations that differentiates between infants and adults (Mariat, 2009). Studies have shown that obese and nonobese subjects have different gut microbiota compositions. The ratio of firmicutes/bacteroidetes was higher among the obese and overweight subjects (Kasai, 2015).

Changes in the gut microbiota have been linked to metabolic endotoxemia, inflammation in the gut promoting insulin resistance, gut permeability, and obesity. These changes include a decrease in *bifidobacteria* in combination with an increase in *enterobacteria* (Cani, 2007). The release of inflammatory cytokines is caused by the release of lipopolysaccharides (LPS) into the gut. When LPS is absorbed it binds to CD14 proteins, nucleotide oligomerization, and Toll-like receptors of macrophages and dendritic cells (Tanti, 2013). The excess release of inflammatory

cytokines also causes insulin desensitization, complications in glucose transport, oxidative stress, and inflammation in adipose tissue (Cani, 2007).

Alterations in microbiota composition has been associated with the development of type 2 diabetes mellitus (Liauchonak, 2019). While researching the different effects of NNS on metabolism, researchers added doses of saccharin to mice and rats' diets. To determine the effects of saccharin on glucose intolerance of the microbiome, a fecal transplant was taken from experimental mice and introduced into germ-free mice. Mice exposed to saccharin associated microbiomes experienced significant changes to their own microbiomes, ultimately leading to glucose intolerance. Microbial composition began showing an increase in *Bacteroides* and *Clostridiales*, while there was a decrease in *Lactobacilli*. These alterations have been linked with type 2 diabetes in humans (Suez, 2015). The effects of the NNS, sucralose and saccharin, were examined on the growth of the intestinal *E. coli.* Results showed that both sucralose and saccharin reduced the size of bacterial colonies, and inhibited *E. coli* strain growth (Wang, 2018). However, no such studies were done using aspartame.

To further determine the relationship between the consumption of NNS and blood glucose levels, a study was conducted on seven healthy volunteers that did not regularly consume NNS in their daily diet. After a week, four out of the seven individuals developed poorer glycemic responses, while the other three had no improved glucose tolerance. By consuming large amounts of NNS, glucose intolerance increases in both mice and humans. This suggests that these effects are related to the composition and function of the microbiota (Suez, 2014). Pepino's proposed mechanism of NNS interfering with gut microbiota composition supports these findings on the potential alteration in microbiota composition (Pepino, 2015)

For many years the consumption of aspartame was debated and observed. The consumption of minor concentrations of aspartame was found to modify bacterial communities. The changes in the gut bacteria interfere with the ordinary physiological responses that control homeostasis (Choudhary, 2017). Each molecule of aspartame releases a molecule of methanol which then metabolizes into formaldehyde, a carcinogen (Pretorius, 2012). A fecal analysis of gut bacteria was done to test the impact of low-doses of aspartame on male rats. Results showed that aspartame increased total bacteria, including Firmicutes, *Enterobacteriaceae*, and *Clostridium leptum*. Increases in Proteobacteria such as *Enterobacteriaceae* have been linked to inflammation and insulin resistance. These results show that even low-doses of aspartame have multiple effects on gut bacteria (Palmnas, 2014). When mice were fed aspartame, plasma phenylalanine levels increased, and also caused an increase in the frequency of seizures.

Saccharin induced alterations in the gut microbiome of mice, by increasing the abundance of proinflammatory bacterial genes and decreasing anti inflammatory metabolites. An increase in *Corynebacterium parvum* induces chronic inflammation by overproducing nitric oxide leading to hepatic necrosis (Chamulitrat, 1995). Saccharin induced gut microbiome perturbations, by inducing nitric-oxide synthase and tumor necrosis factor alpha in the liver, leading to elevated inflammation in mouse liver (Bain, 2017). Nitric oxide is commonly related to inflammation in liver diseases including tumors and liver fibrosis. Tumor necrosis factor alpha is a key cytokine in inflammation and could induce damage to cells, such as liver damage leading to inflammation (La Mura, 2014; Wu, 2010).

Discussion

Within the human body there are trillions of different microorganisms that play a role in maintaining homeostasis. It is well known that the foods consumed throughout our lifetime are

what control the bacteria present in the gut. By enhancing the growth of certain bacterias the healthy to harmful bacterial ratio may be changed, potentially leading to disorders and health issues. Artificial sweeteners are one of the many foods that have created a controversy based on their potential alterations to the gut microbiome, and other parts of the body. This review summarizes the known effects of artificial sweetener, aspartame and saccharin, consumption on human health , with special emphasis on the gut microbiome.

Aspartame was found to cause multiple issues in the body ranging from the brain to the gut microbiome. As it's metabolized aspartame releases methanol leading to oxidative stress. The release of ROS leads to the damage of multiple cells, including neuronal apoptosis. Both saccharin and aspartame had similar effects on the liver, by inducing inflammation and hepatotoxicity. Oxidative stress, induced by aspartame and saccharin consumption, caused alterations in normal serum levels that alter both kidney and liver function. It is inferred that consumption of both high or safe doses of aspartame and saccharin have an effect on the microorganisms present in the human gut. Constant consumption will cause a more noticeable effect, the studies mentioned in this review show that the long term consumption potentially increases the risk of developing disorders such as diabetes, Alzheimer's disease, and cancers; as shown in tables 1 and 2.

These studies suggest a direct relationship between the gut microbiome and the body's immune system. With about 70% of the body's immune cells located in the gut microbiome, maintaining a healthy microbiota is even more of a priority. Consumption of aspartame leads to methanol release causing reduction in immune cells. Furthermore, alterations in the microbiome may be caused by the consumption of artificial sweeteners. Several studies suggest that the consumption of aspartame or saccharin could lead to immunological problems, caused by the

alterations in the gut microbiota. These effects on the gut bacteria may lead to the development of different disorders, including ones associated with the brain.

Three mechanisms have been proposed to describe the connection between NNS consumption and its effects on the body: an interaction between NSS and sweet taste receptors, NNS interfering with usual sweetness response, and NNS interfering with gut microbiota composition. These mechanisms link NNS consumption to shifts in the gut microbiota affecting insulin signaling and the development of metabolic syndrome.

In conclusion, it is suggested that further testing and research must be conducted to better understand the effects of artificial sweeteners, such as aspartame and saccharin, on the gut microbiome. Further testing may include inoculating dominant gut bacteria in different concentrations of aspartame and saccharin to view and analyze their inhibitory or enhancing effects. An extensive review on the effects of saccharin, similar to that of Choudhary (2017) on aspartame, would help provide better insight on its effects on human health. Deciphering the effects of artificial sweeteners' on the gut microbiome could provide great insight on how the consumption of certain foods contribute to the pathophysiology of diseases by altering gut microbes.

Acknowledgments

Thank you to all my family and friends for always supporting me. Special thanks to Dr.Mortellaro and Dr.Callahan for being amazing advisors and their assistance with this review paper.

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Table 1				
Reference	**Cells/tissue**	**Model**	**Dosage of Aspartame**	**Result**
Ashok & Sheeladevi (2015)	Brain	Wistar albino male rats	40 mg/kg	Increase in neuronal oxidative damage leading to neuronal cell apoptosis in the brain
Kim et al. (2011)		Zebra fish (10 weeks old)	3 mM	Impaired learning and memory, increased brain inflammation
Villareal et al. (2016)		Male mice	1000 mg/kg	Apoptosis was observed in the hippocampus
Ashok & Sheeladevi (2014)		Wistar albino male rats	75 mg/kg	Changes in locomotor activity and anxiety levels. Imbalances in cell membrane homeostasis led to oxidative stress in multiple brain regions.
Simintzi et al. (2007)		Wistar rats	150-200 mg/kg	Decrease in AChE activity in frontal cortex and hippocampus
Tskiris et al. (2006)	Blood	Human erythrocyte membrane	150 -200 mg/kg	Significant decrease in AChE activity
Choudhary et al. (2015)	Immune System	Wistar albino male rats	40 mg/kg	Homeostasis of immune organs was altered. Variations in serum cytokine levels, and alteration of cellular and humoral immunity
Abhilash et al. (2011)	Liver	Wistar albino male rats	500 and 1000 mg/kg	Alterations in liver antioxidant status
Choudhary et al (2014)		Wistar albino male rats	40 mg/kg	Induced oxidative stress, alterations in serum protein and bilirubin levels.
Kim et al.		Zebrafish	3mM	Infiltrated inflammatory cells

(2011)				in liver
Ashok et al. (2014)	Kidney	Wistar albino male rats	75 mg/kg	Histological changes in renal cortex
Choudhary et al (2014)		Wistar albino male rats	40 mg/kg	Induced oxidative stress, alterations in creatinine, urea, and uric acid levels
Martins & Azoubel (2007)		Wistar female rats	14 mg/kg heated to 40 °C	Fetal kidney morphological alterations in renal structures during organogenesis
Choudhary & Sundareswaran (2016)	Heart	Wistar albino male rats	40 mg/kg	Oxidative stress, reduced heart rate, imparied cardiac function
Palmnas et al. (2014)	Gut Microbes	Male Albino rats	5-7 mg/kg	Alterations in gut microbiota led to elevated fasting glucose levels and impaired insulin tolerance
Suez et al. (2014)		Mice	4% aspartame	Alterations in gut microbiota caused high glucose
Sections adapted from: Choudhary, A. K., & Pretorius, E. (2017). Revisiting the safety of aspartame. *Nutr. Rev. 75*(9):718-730.				

Table 2				
Reference	**Cells/tissue**	**Model**	**Dosage of Saccharin**	**Result**
Kim et al. (2011)	Brain	Zebra fish	3mM	Infiltrated cells
Amin et al. (2016)	Liver	Rat	10 and 500 mg/kg	Hypercholesterolemia and high alkaline phosphatase levels (these indicate liver damage)
Helal et al. (2019)		Male Albino Rat	5 mg/kg	Significant decrease in protein profile indicating liver damage. Increase in aminotransferase is related to breakdown of liver parenchyma. Functional changes caused by hepatocellular impairment and release of intracellular enzymes into the blood
Andrejić et al. (2013		Albino Rat	0.0005% saccharin	Histological changes
Amin et al. (2016)	Kidney	Rat	10 and 500 mg/kg	Elevated creatinine and urea
Helal et al. (2019) Turley et al. (2003)		Male Albino Rat	5 mg/kg	Elevated creatinine and urea leading to renal dysfunction and reduction in glomerular filtration
Bain et al. (2017)	Gut Microbiota	Mice	0.3 mg/ml	Alterations in gut microbiota linked to inflammation and liver damage

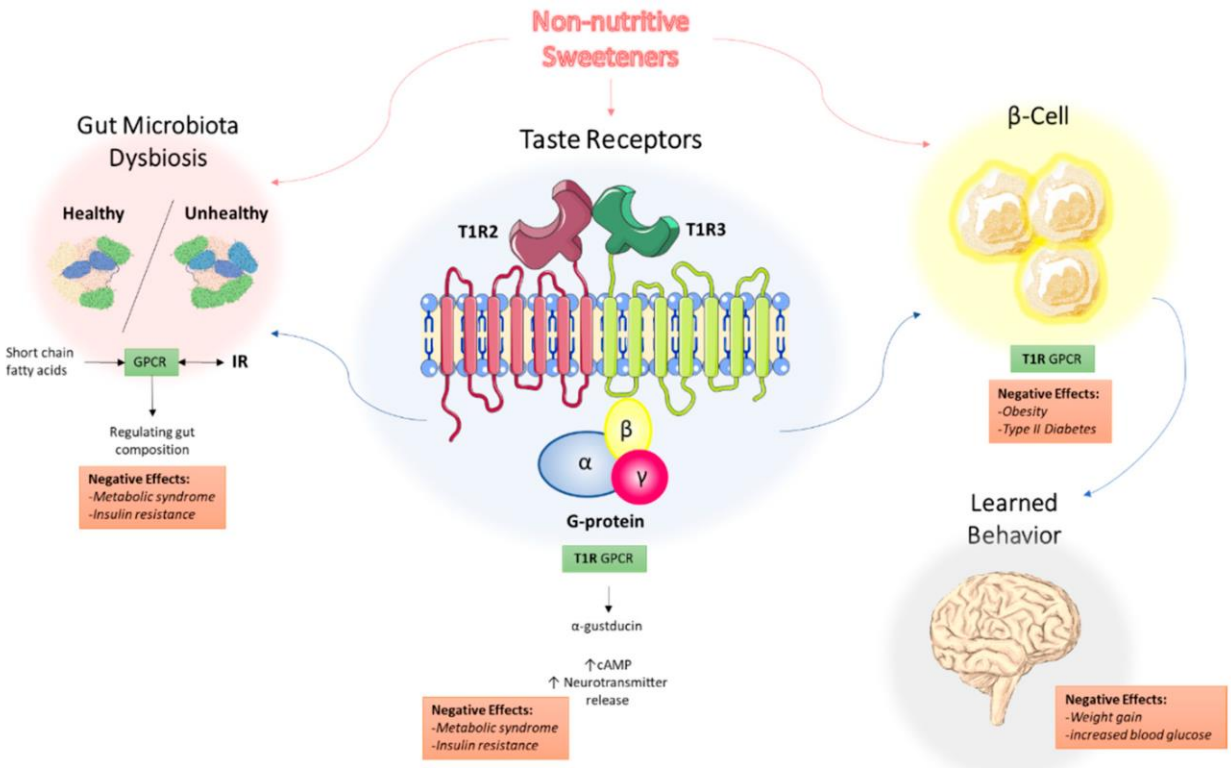

Figure 1: Mechanisms linking Non-nutritive Sweeteners to metabolic syndrome
Abbreviations: Non-nutritive sweeteners (NNS), G protein-coupled receptor (GPCR), short-chain fatty acid (SCFA).
Adapted from: Liauchonak, 2019