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# Artificial sweeteners and perceived obesity and diabetes

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**ARTIFICIAL SWEETENERS AND PERCEIVED OBESITY AND DIABETES**

by

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B.S., University of Connecticut, 2014

Submitted in partial fulfillment of the  
requirements for the degree of  
Master of Science

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# **ARTIFICIAL SWEETENERS AND PERCEIVED OBESITY AND DIABETES**

**YEACHAN KIM**

## **ABSTRACT**

Artificial sweeteners have been increasingly incorporated into our diets. Contrary to what is believed to alleviate the obesity and diabetes epidemic seen today, artificial sweeteners have shown to induce the very problem it was meant to repress. Studies found that the consumption of artificial sweeteners ultimately lead to an increased risk of weight gain and diabetes. Exposure has shown to induce problems ranging from dysbiosis, inflammation, overconsumption, metabolic derangements, and much more that highly suggests the counterintuitive effects of artificial sweeteners.

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## LIST OF ABBREVIATIONS

BMI .....	Body Mass Index
FDA .....	Food and Drug Administration
GMP .....	Good manufacturing practices
GRAS .....	Generally recognized as safe
LCS .....	Low-calorie Sweetener
REG .....	Food additives with regulation imposed
NAS .....	Non-caloric Artificial Sweetener
NNS .....	Non-nutritive sweetener
NUTRS .....	Nutritive sweetener

## INTRODUCTION

Saccharin was the very first artificial sweetener to be discovered by Professors Fahlberg and Remsen (Echhardt et al, 1980). The use of artificial sweeteners has become widely popularized as a sugar substitute since the turn of the 20<sup>th</sup> century (Arnold, Krewski & Munro, 1983). As of this date, the Food and Drug Administration (FDA) has approved six different artificial sweeteners for the human use of consumption.

Sweetener	FDA Status	Acceptable Daily Intake (ADI)	Sweetness Relative to Sucrose
Acesulfame Potassium	NNS, REG	15 mg/kg (~ 30 cans of diet soda)	200X
Aspartame	NUTRS, REG, GMP	50 mg/kg (~ 18 cans of diet soda)	160–220X
Neotame	NNS, REG, GMP	2 mg/kg	7,000–13,000X
Saccharin	NNS, REG/ITEM	5 mg/kg	300X
Stevia	GRAS	5 mg/kg	300X
Sucralose	NNS, REG, GMP	5 mg/kg (~ 6 cans of diet soda)	600X

**Table 1. Six artificial sweeteners approved by FDA.** FDA approved sweeteners with an acceptable daily intake (ADI), which is the amount of sweetener deemed safe for daily consumption (Sylvetsky, Rother & Brown, 2011).

However, there have been multiple controversies surrounding the use of artificial sweeteners as part of a regular diet. In a series of studies conducted in 1977, researchers found a positive correlation between intake of saccharin and the rate of bladder cancer formation in rats (Hicks & Chowanec, 1977). These findings led to the temporary ban of saccharin use by the FDA, but was overturned due to the lack of evidence and association

of cancer formation in the human bladder (Kessler & Clark 1978). In addition, a prospective cohort study showed that daily intake of artificially sweetened soft drinks was linked to increased risk of stroke and dementia (Pase et al, 2017). Moreover, several other conditions such as cardiovascular disease and metabolic disorders have been listed as potential risks when consuming artificial sweeteners (Dhingra et al, 2007). But amongst all these potential associated risks, obesity and diabetes have been one of the more prevalent risks linked to artificial sweeteners.

Generally, artificial sweeteners are considered to be healthy alternatives to sugar, contributing zero calories while providing the same sweet taste that conventional sugar gives (FDA, 2015). Over the past 3 decades, the consumption of artificial sweeteners increased significantly as well:

Year	Foods containing added NS			Foods containing NNS		
	Intake per capita <i>g</i>	Percentage of population %	Intake per consumer <i>g</i>	Intake per capita <i>g</i>	Percentage of population %	Intake per consumer <i>g</i>
<b>Beverages</b>						
1965	190	41.1	455	10	2.5	368
1977	242	49.5	491	22	4.8	417
1989–1991	302	50.5	581	71	10.1	546
1999–2000	599	67.6	881	109	9.1	736
2001–2002	568	66.2	857	108	9.4	711
2003–2004	585	66.6	872	129	10.8	752
<b>Foods</b>						
1965	396	94.2	398	1	0.8	60
1977	352	95.4	357	1	3.8	23
1989–1991	376	94.3	383	7	3.2	204
1999–2000	381	90.0	388	19	4.9	305
2001–2002	357	89.9	363	15	5.2	232
2003–2004	375	90.3	381	17	5.8	233
<b>Total</b>						
1965	586	94.3	589	11	3.3	304
1977	594	95.8	599	23	8.0	258
1989–1991	677	95.5	683	78	12.7	493
1999–2000	979	91.6	987	128	12.9	658
2001–2002	924	91.2	931	123	13.5	619
2003–2004	960	91.5	963	146	15.1	663

**Table 2. Trend in intake of food and beverage containing NNS.** There is an increasing trend in the consumption of non-nutritive sweetener (NNS) through food and beverage among Americans aged  $\geq 2$  years old (Mattes & Popkin, 2009).

Increased trend in the consumption of artificial sweetener is observed among all age groups, socioeconomic backgrounds, and race (Sylvetsky et al, 2012). Specifically, the most dramatic increase in consumption rate in the United States could be seen among females, non-Hispanic black children, and Hispanic adults (Sylvetsky et al, 2012). As show in table 2 above, 15.1% of total US population would consume artificial sweetened food or beverage on a given day, compared to 2.5% in 1965 (Mattes & Popkin, 2009).

Although artificial sweeteners are deemed safe and great alternative for diabetic and obese patients, there has been many contradictory findings across the research field. Consumption of artificial sweeteners have shown to have damaging effects through inducing glucose intolerance (specifically linked to type 2 diabetes) by altering the gut microbiota (Suez et al, 2014). In a similar fashion, consumption of artificial sweeteners has shown to promote energy intake behaviors, showing an indirect link to obesity and diabetes as well (Mattes & Popkin 2009). However, in the midst of these findings linking artificial sweeteners to obesity and diabetes, several studies found that the correlation was inconclusive due to lack of evidence and limitations on studies (Gardner et al, 2012; Pepino 2015; Canty & Chan 1991).

Despite all these misconceptions and contradictory results, there are significant evidence that links artificial sweeteners to obesity and diabetes. Unlike what was expected of the artificial sweeteners to provide weight loss and a solution to obesity, the effects are often counterintuitive. This thesis will look at several specific studies and mechanisms showing that there is a strong correlation that links artificial sweeteners to obesity and diabetes.

## **Specific Aims**

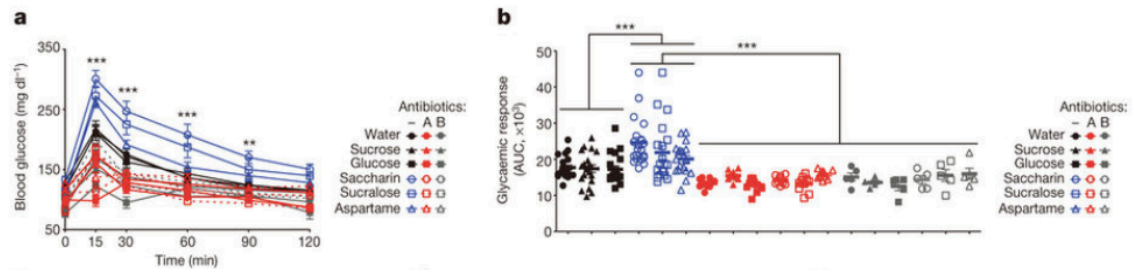
Specific Aim of this thesis is to identify and review previously published studies and mechanisms that show positive correlation of artificial sweetener consumption to obesity and diabetes:

1. Alterations to the gut microbiota leading to weight gain and diabetic conditions
2. Metabolic changes that occur through consumption of artificial sweeteners
3. Associated behavioral change leading to energy consumption or imbalance

## **Effects of Artificial Sweeteners on Gut Microbiota**

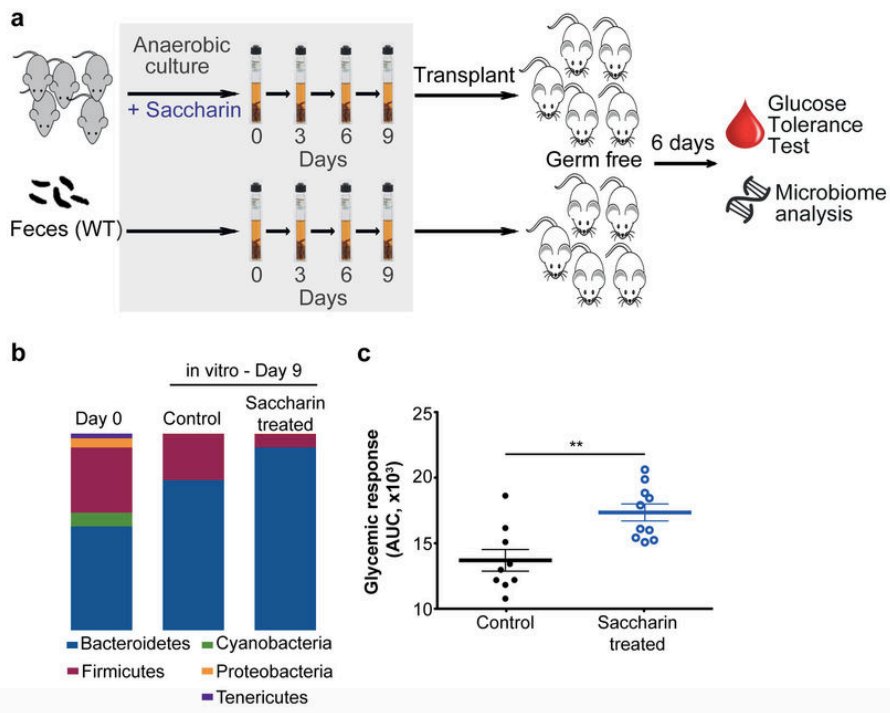
As artificial sweeteners (i.e. saccharin) are passed through the digestive system, it is undigested and not metabolized (Byard & Goldberg, 1973). However, these artificial sweeteners are left directly to interact with the gut microbiota (John, Wood & Hawkins, 2000). Gut microbiota has a profound effect on human health and alterations to these cells can lead to metabolic and immune disorders in both animals and humans alike (Boulangé et al, 2016).

A recent study in 2014 has shown that intake of artificial sweeteners does indeed alter gut microbiota (Suez et al, 2014). The first series of studies were done on 10-week-old C57Bl/6 mice (Suez et al, 2014). To see the effects of artificial sweeteners on these mice, three groups were created (Suez et al, 2014). Two of the three groups were control groups with one mice group drinking only water and the other control group drinking water enhanced with either glucose or sucrose (Suez et al, 2014). The experimental group received water with a selection of saccharin, sucralose, and aspartame (Suez et al, 2014). The experimental group showed the greatest glucose intolerance while control groups showed similar glucose tolerance curves as seen in figure 1 below (Suez et al, 2014).



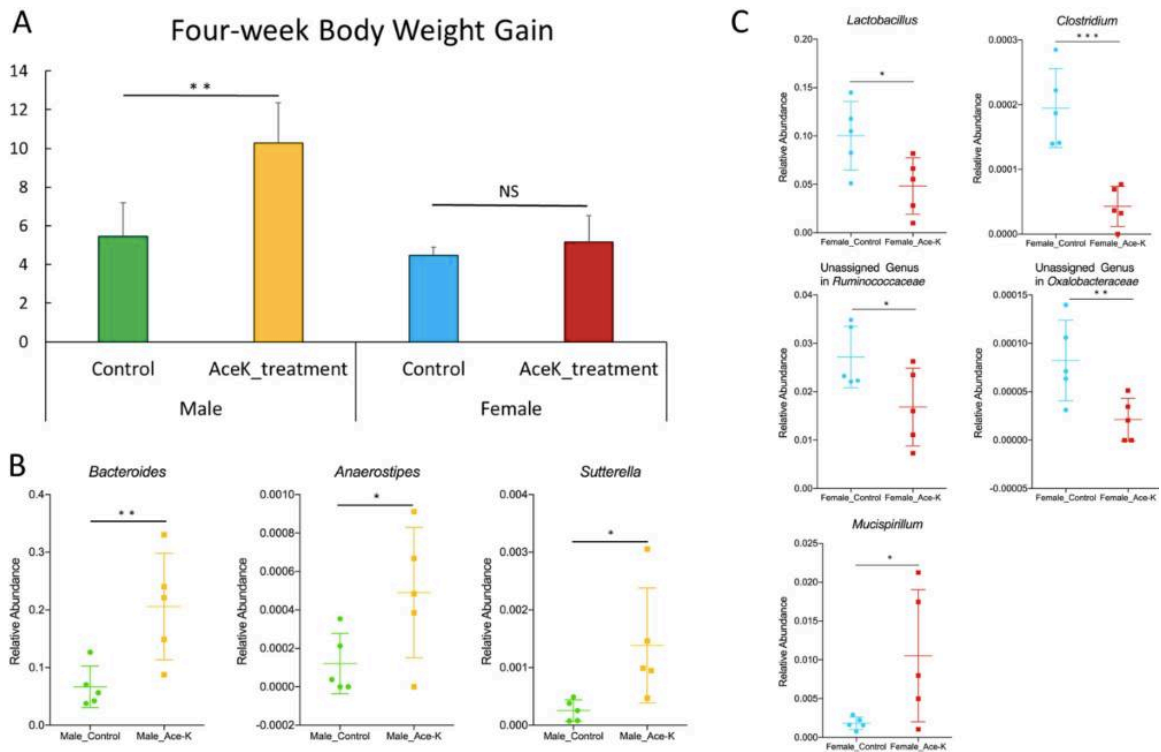
**Figure 1. Oral Glucose Tolerance Test (Week 11).** Experimental group drinking water mixed with artificial sweetener showed the greatest glucose intolerance (Suez et al, 2014).

In addition to the mice study, Suez and colleagues collected and analyzed the fecal compositions of the mouse groups (Suez et al, 2014). Mice drinking water treated with artificial sweetener showed distinct microbiota composition when compared to the control groups (Suez et al, 2014). These fecal microbiota samples were also cultured in an anaerobic environment in presence of saccharin or the respective control group media (Suez et al, 2014). The saccharin treated microbiota was then transferred to germ-free mice, which resulted in markedly higher glucose intolerance when compared to germ-free mice treated with the control group fecal samples (Figure 2a) (Suez et al, 2014). The composition of the bacteria also differed significantly. The stool culture of the saccharin treated mice showed an increase in the *Bacteroidetes* and also the reduction in the *Firmicutes* (Figure 2b) (Suez et al, 2014). Studies show that the misbalance of these two phyla is associated with obesity (Turnbaugh et al, 2006). In sum, the results of oral administration of artificial sweeteners and *in vitro* experimentations using fecal samples showcases that artificial sweeteners alters the gut microbiota, leading to dysbiosis and glucose intolerance in that mammal (Suez et al, 2014).



**Figure 2. Saccharin’s effect on gut microbiota.** Figure 2a shows the timeline of *in vitro* experiment using fecal sample of the control group and the experimental group. Figure 2b shows the stool sample composition. Figure 3c shows the glycemic response of the treated mice (Suez et al, 2014).

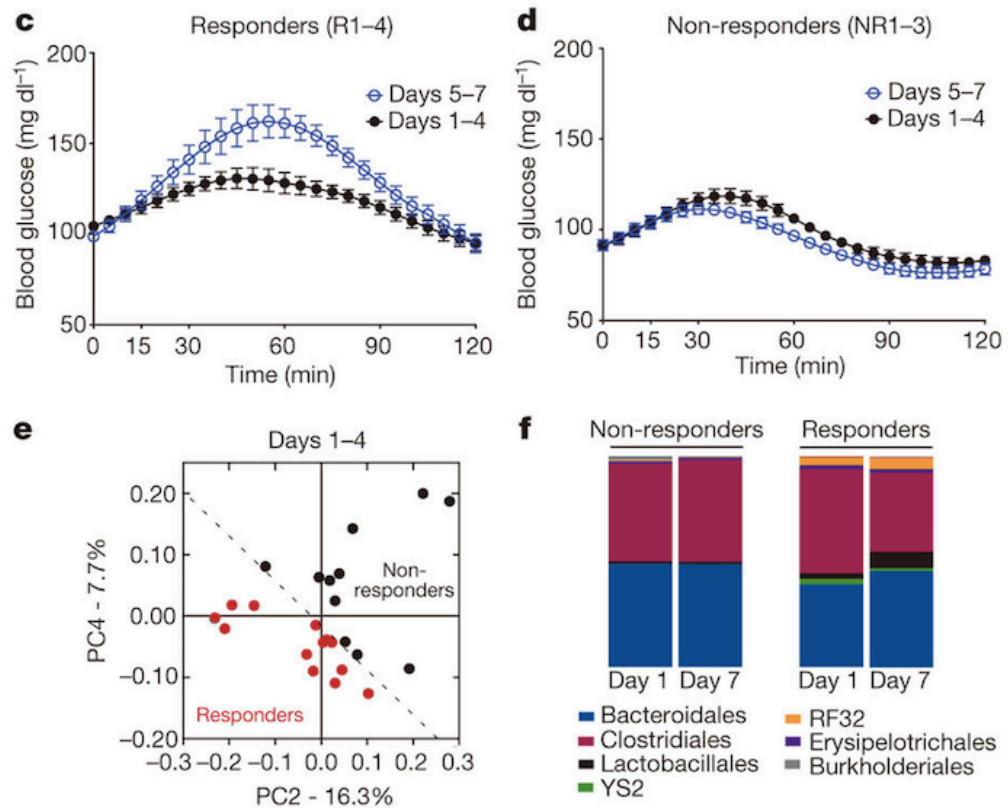
A similar experiment took part in 2017, where 10 male and 10 female mice were given either water (control) or acesulfame-k (ace-k) enriched water (Bian et al, 2017) for the duration of 4 weeks. It was shown that ace-k enriched water produced dysbiosis in the gut and resulted in weight gain (Bian et al, 2017). However, only the male mice resulted in weight gain while the female mice showed very minimal changes, suggesting a gender specific reaction to the artificial sweetener (Figure 3) (Bian et al, 2017).



**Figure 3. Gender specific response of mice to artificial sweeteners.** Ace-k treatment in male mice showed significant weight change while female mice showed minimal changes. Treatments also led to upregulation of multiple genes associated with pro-inflammatory mediators, suggesting liver and tissue inflammation in response to artificial sweeteners (Bian et al, 2017).

After the conclusion of studies with mice, which may yield unique results, follow up studies were done to test artificial sweeteners in human subjects (Suez et al, 2014). Seven volunteers consumed the acceptable daily intake of saccharin (5mg/kg of body weight) from days 2-7 (Suez et al, 2014). 4 of the 7 individuals developed glucose intolerance in this short period of time, and they were termed Non-caloric Artificial Sweetener (NAS) Responders (Suez et al, 2014). The remaining 3 individuals did not

show statistically significant changes in their glucose tolerance, and they were termed NAS non-responders (Suez et al, 2014).



**Figure 4. Effects of artificial sweeteners on human participants.** Figure 3c shows the NAS responders’ development of glucose intolerance. Figure 3d shows minimal changes for NAS non-responders. Figure 3ef shows clear changes in microbiome composition of NAS responders while minimal change was observed in NAS non-responders (Suez et al, 2014).

The differing results seen by the NAS responders and NAS non-responders show that humans have a personalized response to artificial sweeteners, mainly due to the

contrast in the composition of each microbiota (Suez et al, 2014). Bian and colleagues has shown that there exist multiple factors to explain such a phenomenon such as gender-specific responses of the gut microbiome to artificial sweeteners (Bian et al, 2017). Collectively, these findings suggest that the increase in consumption of artificial sweeteners directly correlates to the increase seen in obesity and diabetes.

### **Observed Adverse Effects of Artificial Sweeteners**

As many would speculate, foods that are high in sugars and sweeteners have been linked with an increase in the incidence of various adverse health effects. Studies have shown that consumption of sweet drinks and sweetened substances often led to higher incidents of type 2 diabetes (O'Connor et al, 2015). However, multiple studies have implicated non-caloric sweeteners to actually induce weight gain while others have identified specific factors that may be secondary effects ultimately caused by the sweeteners. Due to the highly accepted reality of sucrose and other normal sugars being so closely linked to issues such as weight gain and diabetes, the fact that the artificial sweeteners may also indirectly lead to the same underlying issues that people were trying to avoid is quite alarming.

### **Weight Changes Following Consuming Artificial Sweeteners**

The immediate benefits of artificial sweeteners seem to be that it intuitively causes weight loss due to the absence of caloric values in the substance consumed. Miller and Perez found a rather positive and favorable outcome in terms of weight loss when

low-calorie sweeteners replaced normal sweeteners (Miller & Perez, 2014). In overweight individuals, similar results were observed when given a trial period of 10 weeks of sucrose or artificial sweetener (Raben et al, 2002). Individuals who consumed the artificial sweetener were less likely to manifest adverse effects such as high blood pressure and fat mass (Raben et al, 2002). However, there have still been many contradictory studies and outcomes that has suggested that weight loss is not always the predicted outcome (Stellman & Garfinkel, 1988; Andrejic et al, 2013)

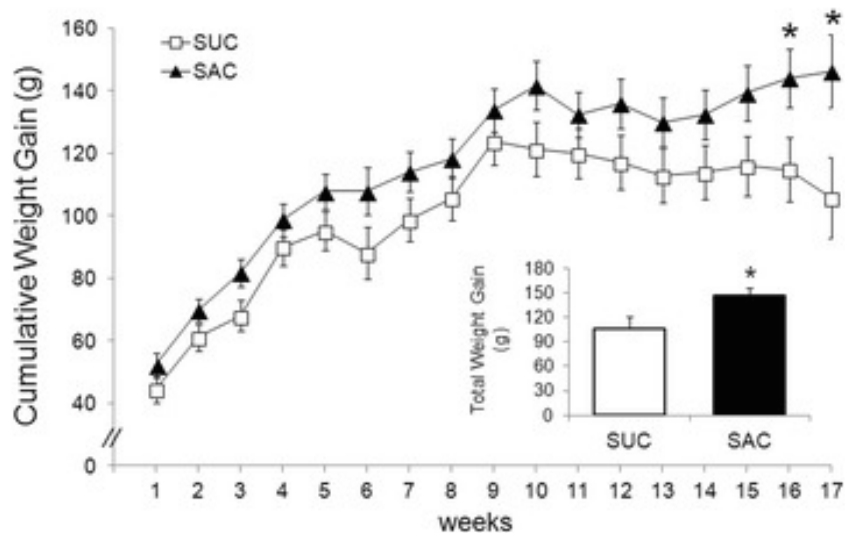
In terms of the obesity, the results were many times counter-intuitive to what was expected. It was reasonable to assume that by using artificial sweeteners, there would be a lower BMI due to the absence of calories (Gardner et al, 2012). However, the results were rather surprising. In a study conducted by Fowler and colleagues, the relationship between the consumption of artificial sweeteners and weight gain were measured in a population of Mexican Americans and non-Hispanic white individuals (Fowler et al, 2008). Exercise frequency and dietary intake of food was recorded daily (Fowler et al, 2008). Overall, instead of a decrease in BMI, there was actually an increase in the BMI of the individuals following documented use of artificial sweeteners (Fowler et al, 2008). In mice, similar results have been measured with a greater increase of weight following consumption of a solution of artificial sweeteners (Polyak et al, 2010). Polyak and colleagues noted that despite the weight gain in groups of mice that were given artificial sweeteners relative to control groups that did not have the artificial sweetener, the weight increased even without increasing the amount of food intake (Polyak et al, 2010).

AS beverage type	$\Delta$ BMI with no use of specified beverage	<i>n</i>	$\Delta$ BMI with any use of specified beverage (kg/m <sup>2</sup> )	<i>n</i>	Difference <sup>b</sup> (95% CI)	<i>P</i>
Diet soft drinks	1.10 ± 0.06	2,301	1.52 ± 0.09	1,070	0.42 (0.21, 0.63)	<0.0001
AS tea	1.14 ± 0.06	2,527	1.54 ± 0.10	844	0.40 (0.18, 0.63)	<0.0001
AS coffee	1.19 ± 0.05	2,894	1.52 ± 0.13	477	0.32 (0.05, 0.60)	<0.0001

AS, artificial sweetener;  $\Delta$ BMI, change in BMI; CI, confidence interval.  
<sup>a</sup>BMI, adjusted for gender and ethnicity; baseline age, education, socioeconomic index, BMI, exercise frequency, and smoking status; and interim change in exercise level and smoking cessation. <sup>b</sup> $\Delta$ BMI in users minus  $\Delta$ BMI in nonusers.

**Table 3. Consumption of artificial sweeteners and BMI.** Participants who consumed more artificial sweeteners had higher BMI measures. (Fowler et al, 2008)

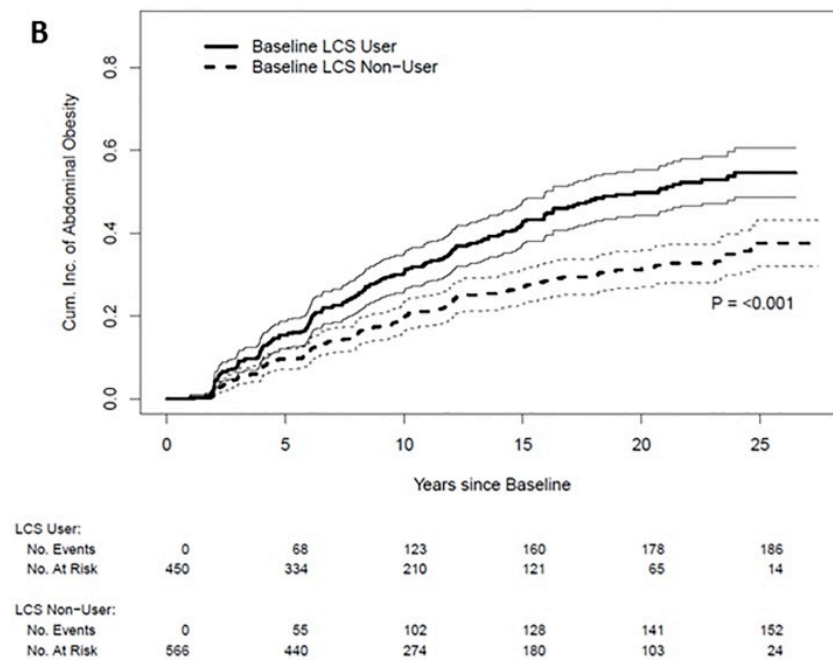
Swithers, Sample and Davidson found that the predispositions also mattered in determining whether or not weight gain was observed (Swithers, Sample and Davidson, 2013). Rats that were fed a diet that was maintained by a lower fat content did not gain significantly more weight when comparing between a supplement with saccharin fed group and a supplement with glucose fed group (Swithers, Sample and Davidson, 2013). However, a secondary group of rats that were fed a “Westernized” diet that was higher in fat content did have a difference in the amount of fat that was gained between the sucrose supplement group and the saccharin fed group (Swithers, Sample and Davidson, 2013). The female rats that were fed the Westernized diet initially and given the saccharine supplement had a significant change in weight and body fat content with a greater increase compared to the glucose supplement group (Swithers, Sample, and Davidson, 2013).



**Figure 5. The effect of either sucrose or saccharine solutions on the cumulative weight gain.** Rats who consumed saccharine had a higher weight gain compared to the sucrose group (Pinto et al, 2017).

Lavery and colleagues found, in British children, that boys tended to consume more artificial sweeteners than girls at the age of approximately 11 years (Lavery et al, 2015). Participants recorded the types of food consumed and the amount exercise that was taken on a given day (Lavery et al, 2015). Variety of factors were controlled, such as dietary behaviors, physical activities, sex, ethnic group, and socioeconomic backgrounds (Lavery et al, 2015). Following an increased consumption of the artificially sweetened substances, increase in fat content or adiposity was observed throughout children age 7 to 11 (Lavery et al, 2015). Multiple studies have shown the tendencies of artificial sweeteners to be connected with a higher measured waist measurement. In a recent study by Fowler, Williams and Hazuda, the waist circumference of people who

had a history of consumption of diet sodas were shown to be significantly larger than the individuals who did not drink the diet sodas (Fowler, Williams & Hazuda, 2015). Chia and colleagues showed the same results of a larger waist and increased incidence of abdominal obesity in a study of individuals with chronic use of artificial sweeteners (Chia et al, 2016). Figure below shows LCS users, solid lines, with increased abdominal obesity when compared to non LCS users represented by the dotted line (Chia et al, 2016).



**Figure 6. Years since the baseline and the cumulative increase in the abdominal obesity.** There is an increasing trend for abdominal obesity when consuming artificial sweeteners in comparison with those that did not chronically consume it. Chronic low-calorie sweetener users had a higher baseline of abdominal obesity relative to individuals who did not have an extensive history of chronic use. (Chia et al, 2016)

## **Diabetes and Metabolic Issues**

In one report by de Koning and colleagues, a prospective study was conducted on a cohort of men over a course of 20 years (de Koning et al, 2011). The type of beverage consumption and the frequency was tracked using questionnaires and the cases of type 2 diabetes that arose were tracked and documented (de Koning et al, 2011). Compared to the group that consumed regular sweetened beverages such as juices, the group that engaged in consumption of beverages with an artificial sweetener had a significantly lower incidence of diabetes (de Koning et al, 2011). For women who consumed a higher frequency of sugary beverages, the incident of type 2 diabetes was also observed in a study conducted by Schulze and colleagues (Schulze et al, 2004). However, this relationship has had also very conflicting results in others studies (Fagherazzi et al, 2013).

Multiple clinical studies have shown that when participants consumed artificial sweeteners, there was not much difference in outcome for diabetes with another group that consumed normal sweeteners. Both sweeteners and non-caloric sweeteners seemingly could lead to increased cases of diabetes. In a French study conducted by Fagherazzi and colleagues, a population of French women were chosen to be tracked for their consumption of either fruit juice, beverages that were artificially sweetened, or sugar sweetened drinks over a 14 year period (Fagherazzi et al, 2013). Both women who consumed sugar sweetened and artificially sweetened beverages had an increase in the cases of type 2 diabetes that was documented (Fagherazzi et al, 2013). Huang and colleagues also found that in a population of postmenopausal women, there was a higher incidence of type-2 diabetes when participants consumed artificial sweeteners or sugar

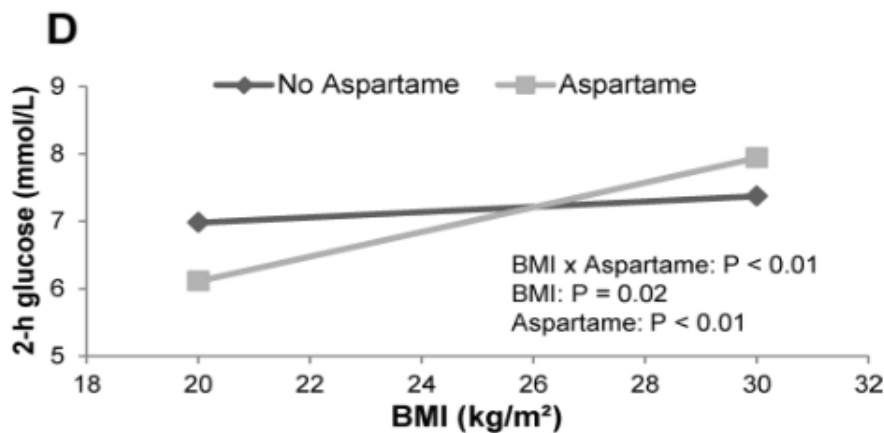
sweeteners (Huang et al, 2017). This general trend towards diabetes was only combatted when the artificially substituted and regularly substituted drinks were replaced with regular water (Huang et al, 2017).

Kuk and Brown found that obese patients were found to be more susceptible to issues with diabetes following consumption of artificial sweeteners (Kuk and Brown, 2016). Individuals who were obese had a higher likelihood of demonstrating glucose intolerance, which likely led to these results (Kuk and Brown, 2016). It was also documented by Nettleton and colleagues that the consumption of diet sodas was also associated with a higher likelihood of developing either type 2 diabetes or metabolic syndrome (Nettleton et al, 2009). Although metabolic issues changed according to other factors such as the adiposity of subjects, Nettleton and colleagues found that type-2 diabetes was independent of these factors and mainly seemed to be influenced by the diet sodas (Nettleton et al, 2009).

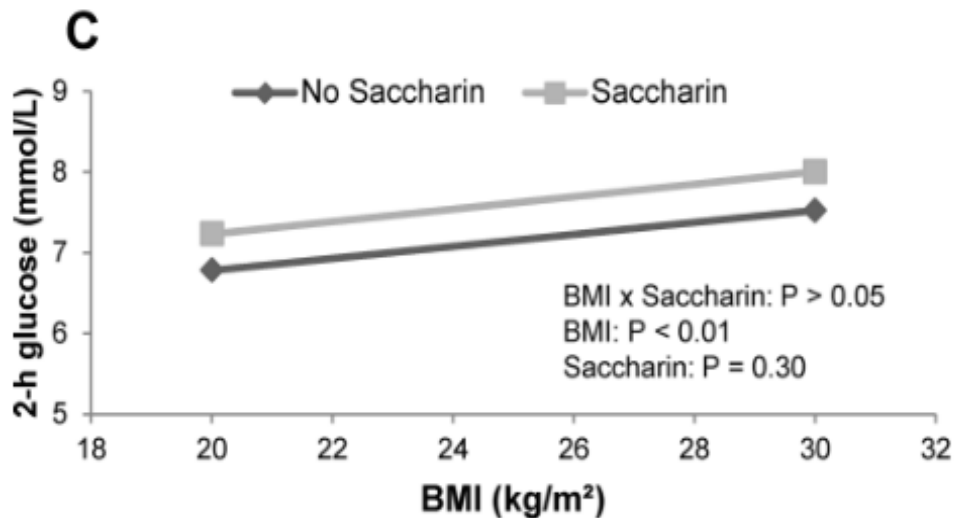
	Rare or never	> rare/never but <1 serving/week	≥1 serving/week to <1 serving/day	≥1 serving/day	<i>P</i> <sub>trend</sub> *
Metabolic syndrome					
<i>n</i>	2,288	367	722	501	
Cases	478	95	169	129	
HR (95% CI)	1.00†	1.34 (1.07–1.67)	1.20 (1.00–1.43)	1.31 (1.07–1.60)	0.003
	1.00‡	1.42 (1.14–1.78)	1.28 (1.06–1.53)	1.36 (1.11–1.66)	<0.001
	1.00§	1.31 (1.05–1.64)	1.13 (0.94–1.37)	1.18 (0.96–1.44)	0.06
	1.00	1.30 (1.04–1.62)	1.15 (0.95–1.38)	1.17 (0.96–1.44)	0.06
Type 2 diabetes					
<i>n</i>	2,961	455	914	681	
Cases	221	33	84	75	
HR (95% CI)	1.00†	1.06 (0.73–1.52)	1.39 (1.07–1.80)	1.63 (1.24–2.13)	<0.001
	1.00‡	1.10 (0.76–1.59)	1.46 (1.12–1.89)	1.67 (1.27–2.20)	<0.001
	1.00§	1.00 (0.69–1.45)	1.23 (0.94–1.60)	1.40 (1.06–1.84)	0.01
	1.00	0.98 (0.68–1.42)	1.25 (0.96–1.62)	1.38 (1.04–1.82)	0.01

*n* = 5,011. \**P*<sub>trend</sub> with categorical variable modeled continuously. †Model 1 adjusted for study site, age, sex, race/ethnicity, and energy intake. ‡Model 2 adjusted for the variables in model 1 above plus education, physical activity, smoking status, pack-years, and weekly or more supplement use. §Adjusted for the variables in model 2 above + waist circumference (centimeters). ||Adjusted for the variables in model 2 above + waist circumference (centimeters) and BMI (weight in kilograms divided by the square of height in meters).

**Table 4. The incidents of Type 2 diabetes and metabolic syndrome with consumption of diet sodas.** Participants who drank more diet soda had higher incidents of developing type 2 diabetes. Factors controlled were age, sex, race/ethnicity, socioeconomic status, and energy intake (Nettleton et al, 2009).



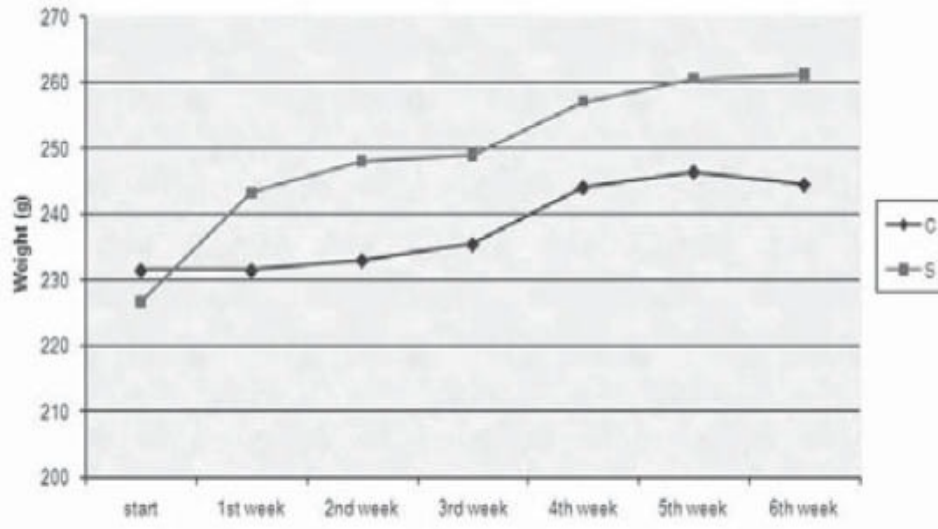
**Figure 7. Obesity and glucose intolerance.** Participants who received the aspartame non-caloric sweetener had higher glucose intolerance when they had a higher BMI. (Kuk and Brown, 2016)



**Figure 8. Body Mass Index and glucose intolerance following the consumption of the non-caloric sweetener saccharin.** There was a parallel increase of the intolerance between both the control and the experimental groups. Both groups increased intolerance with increasing BMI. 2856 adults tested (Kuk and Brown, 2016).

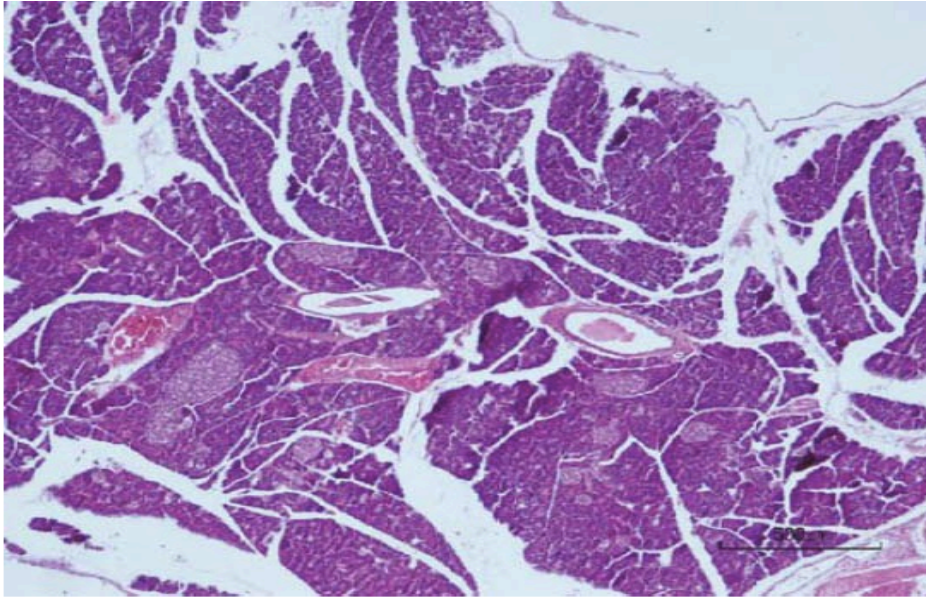
From histological studies of rodent models, there has been more insight into the reasons for the difference biological outcomes between the artificial sweetener groups and the group without an artificial sweetener. According to a study conducted by Andrejic and colleagues, the regions of the pancreas were different between the saccharin group and the non-saccharine group (Andrejic et al, 2013). The densities of the exocrine acini and the islets of Langerhans, both critical to the function of the metabolism, were greater in the saccharine group (Andrejic et al, 2013). Furthermore, other markers such as the enzyme aspartate transaminase (AST) were greater for the saccharine group (Andrejic et al, 2013). In addition, the levels of glucose were much higher in the saccharine treated

group relative to the control group that was not exposed to the artificial sweetener (Andrejic et al, 2013).

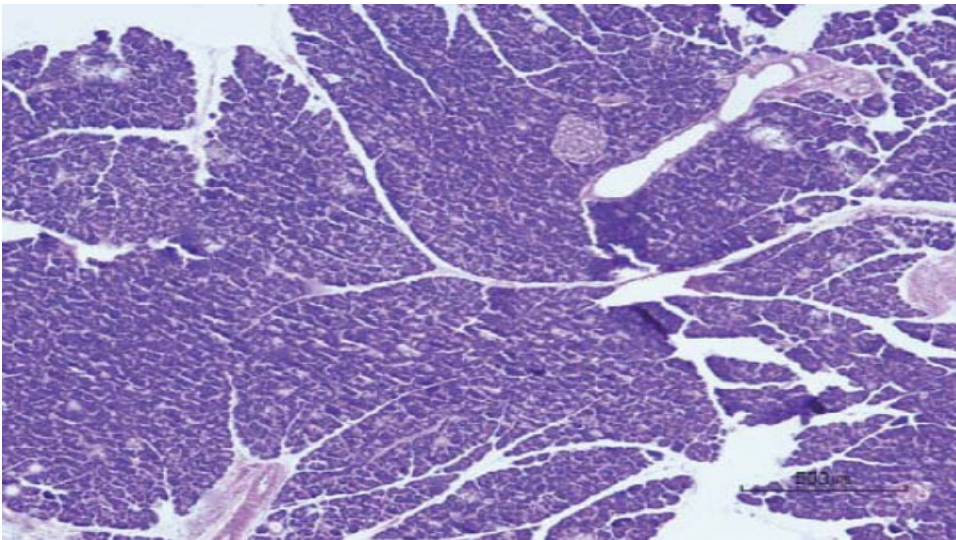


Weight gain in control (C) and saccharin treated (S) groups during a period of 6 weeks.

**Figure 9. Weight of saccharine exposed vs non-saccharine exposed group over a 6 week duration.** The saccharine group had a higher weight compared to the control group (Andrejic et al, 2013).



**Figure 10. Pancreatic images of saccharine exposed rodents.** The pancreas of a male rodent following continued use of saccharine as a sweetener. (Andrejic et al, 2013).



**Figure 11. Image of the pancreas for a control group rodent that did not consumed the artificial sweetener saccharine.** (Andrejic et al, 2013).

## **Non-Superficial Biological Consequences**

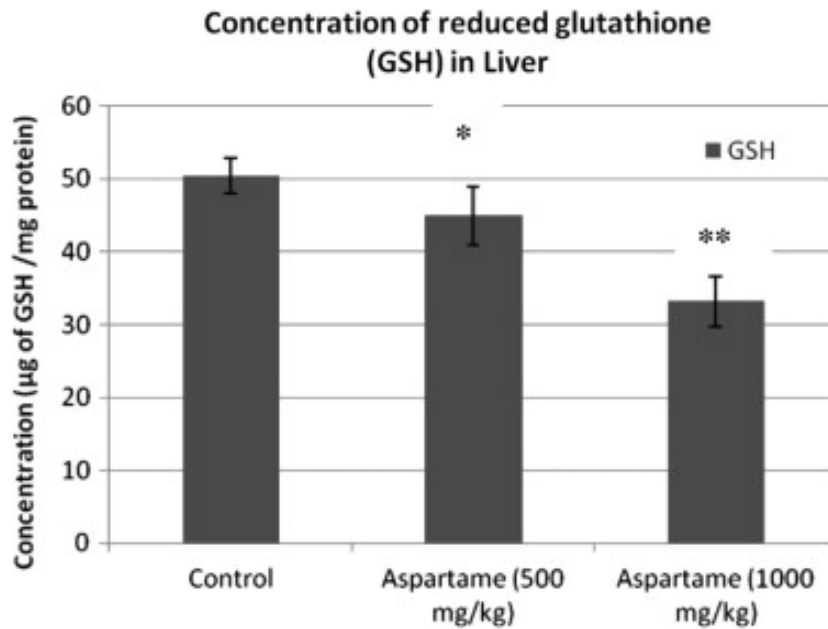
From multiple studies, the biological implications following consumption of artificial sweeteners have been rather alarming. Jang, Jeoung and Cho studied the consequential nature of artificial sweeteners on a modified version of apolipoprotein A-I and the causative effects (Jang, Jeoung, & Cho, 2011). According to the findings of the study, effects of sugar fructose and artificial sweeteners were measured by assessing the high-density lipoproteins (HDL) along with apolipoprotein A-I (Jang, Jeoung, & Cho, 2011). Due to the protective effects of both the HDL and the apolipoprotein A-I, any negative change would lead to adverse effects on both atherosclerosis progression along with early senescence (Jang, Jeoung, and Cho, 2011). In patients diagnosed with human immunodeficiency virus (HIV), the adverse health consequences were observed in patients who ate artificial sweeteners (Hall et al, 2017). Negative consequences included plaque build-up in blood vessels and the increased risk for cardiovascular disease (CVD) (Hall et al, 2017).

In addition to the cardiovascular effects, there has been some study into the effects of consuming artificial sweeteners on more on genotoxic effects. Bandyopadhyay, Goshal, and Mukherjee tested mice bone marrow cells following administration of three artificial sweeteners (Bandyopadhyay, Goshal, and Mukherjee, 2008). The artificial sweeteners acesulfame K, saccharin, and aspartame were given orally to an experimental group of 8-10 week old Swiss albino mice (Bandyopadhyay, Goshal, and Mukherjee, 2008). For aspartame, mice were divided into five groups and given 0, 7, 14, 28, and 35 mg/kg through gastric intubation (Bandyopadhyay, Goshal, and Mukherjee, 2008).

Acesulfame K experimental group was divided into 0, 150, 300, and 600 mg/kg and saccharin experimental group was divided into 0, 50, 100, and 200 mg/kg (Bandyopadhyaya, Goshal, and Mukherjee, 2008). After a period of administration of the artificial sweeteners, the mice were sacrificed and their cells were processed and tested via a comet assay to identify any genetic changes in the bone marrow cells (Bandyopadhyaya, Goshal, and Mukherjee, 2008). DNA breaks were observed in the regions of the bone marrow cells (Bandyopadhyaya, Goshal, and Mukherjee, 2008). Furthermore, there was difference in the effect between the three sweeteners, aspartame causing the least damage to the DNA out of the three sweeteners (Bandyopadhyaya, Goshal, and Mukherjee, 2008).

Amin and AlMuzafar found that saccharin also has the potential to cause other biological damage in the renal tissue of rats (Amin and AlMuzafar, 2015). Rats were given the saccharin orally at two different dose regimens (Amin and AlMuzafar, 2015). For those rats given saccharin, there was significant decrease in cholesterol, LDL, and serum triglycerides (Amin and AlMuzafar, 2015). On the other hand, liver biomarkers increased in serum ALT, ALP, AST, albumin, total protein, and urea levels (Amin and AlMuzafar, 2015). Abhilash also found a similar effect of increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are all important in metabolism of converting and breaking down food (Abhilash et al, 2011). The levels of glutathione (GSH), an antioxidant, has also been shown to be significantly decreased following the consumption of aspartate (Abhilash et al, 2011). Moreover, saccharin

seemingly caused higher hepatic MDA level and a decrease in catalase and SOD activities, which are the signs of oxidative stress (Amin and AlMuzafar, 2015).



**Figure 12. Aspartame and glutathione concentration in liver of rodent models.**

Rodents who received more aspartame had a significant decrease in the amount of glutathione. (Abhilash et al, 2011).

	Control	Low Saccharin	High Saccharin	Low Methyl-salicylate	High Methyl-salicylate
GSH (nmol/100 mg)	72.9 ± 1.41 <sup>a</sup>	68.13 ± 0.87 <sup>a</sup>	58.12 ± 0.48 <sup>b</sup>	73.10 ± 1.44 <sup>a</sup>	72.24 ± 1.24 <sup>a</sup>
MDA (nmol/g/h)	4.82 ± 0.41 <sup>a</sup>	5.03 ± 0.19 <sup>a</sup>	7.64 ± 0.62 <sup>b</sup>	5.08 ± 0.23 <sup>a</sup>	4.91 ± 0.25 <sup>a</sup>
SOD (u/g)	70.68 ± 1.50 <sup>a</sup>	73.58 ± 0.98 <sup>a</sup>	42.33 ± 0.86 <sup>b</sup>	70.27 ± 0.82 <sup>a</sup>	73.22 ± 1.02 <sup>a</sup>
Catalase (kx10 <sup>2</sup> )	47.03 ± 0.41 <sup>a</sup>	48.85 ± 0.84 <sup>a</sup>	29.65 ± 2.62 <sup>b</sup>	44.56 ± 0.43 <sup>a</sup>	46.2 ± 0.65 <sup>a</sup>

Data expressed as MEAN ± SE. Means with the same superscript letters (s) <sup>a</sup>, <sup>b</sup>, are not significantly different. Means having

**Table 5. Saccharin treated rats had higher levels of MDA.** High concentrations of saccharin also caused a decrease in the levels of catalase. Furthermore, the SOD was decreased in the high saccharine group relative to all the other groups. (Amin and AlMuzafar, 2015)

According to Adaramoye and Akanni, aspartame may also have some serious cellular consequences (Adaramoye and Akanni, 2016). 20 adult Wistar rats ranging from 185 to 193 grams were assigned to four different groups, a control receiving distilled water and three experimental groups receiving aspartame doses of 15, 35, and 70 mg/kg body weight (Adaramoye and Akanni, 2016). For the two highest levels of the aspartame groups, there was a significant change in the weight of the brain and the liver with an increase in the size and weight (Adarmoye and Akanni, 2016). In addition, there was a decrease in the levels of antioxidants creating the potential for more adverse effects because of the lack of the buffering effect of antioxidants against harmful substances (Adarmoye and Akanni, 2016). In the liver of rodent models, reductions in the number of antioxidants following the consumption of aspartame were also noted (Abhilash et al, 2011). Furthermore, there was a reduction in the levels of multiple enzymes that

included catalase and glutathione peroxidase (Adarmoye and Akanni, 2016). Aspartame was also shown to cause a decrease in the total cholesterol and low-density lipoproteins (Adarmoye and Akanni, 2016). Although the results are still limited to small number of parameters, this result has the potential to lead to significant changes in the long run.

Treatment	Body weight, g		Weight of organs, g			Relative weight of organs		
	Initial	Final	Liver	Kidney	Brain	Liver	Kidney	Brain
Control	194.00±3.54	237.50±2.32	4.14±0.24	1.10±0.10	0.57±0.03	1.74±0.35	0.46±0.03	0.24±0.02
ASP 1	193.33±2.70	233.70±4.11	4.69±0.35	0.97±0.07	0.59±0.06	2.01±0.28	0.42±0.02	0.25±0.03
ASP 2	190.83±3.03	241.20±3.05	5.62±0.5 <sup>a</sup>	1.01±0.08	0.68±0.02 <sup>a</sup>	2.33±0.20 <sup>a</sup>	0.42±0.03	0.28±0.04
ASP 3	192.09±2.31	247.40±3.10	5.80±0.3 <sup>a</sup>	1.06±0.10	0.71±0.03 <sup>a</sup>	2.34±0.31 <sup>a</sup>	0.43±0.04	0.29±0.05

Values are means±SD of five animals per group; <sup>a</sup>significantly different from control (p<0.05); ASP 1, aspartame at a dose of 15 mg/kg; ASP 2, aspartame at a dose of 35 mg/kg; ASP 3, aspartame at a dose of 70 mg/kg.

**Table 6. Body Weight, Relative weight of organs, and Weight of organs after Aspartame consumption.** (Adarmoye and Akanni, 2016).

The implications for the influence of artificial sweeteners on cancer have also been quite alarming. Ashok and Sheeladevi tested the toxic effects of aspartame via a mechanism that signals the release of methanol (Ashok and Sheeladevi, 2014). From the increase of methanol, it was proposed that there would be an increase in the amount of oxidative stress for the brain (Ashok and Sheeladevi, 2014). Furthermore, the expression of pro-apoptotic Bax and anti-apoptotic Bcl-2 along with caspase-3 could also cause an adverse change (Ashok and Sheeladevi, 2014). For the aspartame treated rats, there was increase in the pro-apoptotic Bax increase while the anti-apoptotic Bcl-2 was lower (Ashok and Sheeladevi, 2014). These results suggest that artificial sweeteners may have a role in

cell death that can be detrimental to the body and the normal processes of clearing harmful substances or growths.

### **Behavioral vs. Biological Underlying Reasons for Adverse Effects**

There have been continuous questions as to how and why people gain weight when they are consuming a non-caloric substance that would seemingly not contribute much to a gain in weight. However, a few studies have shown very eye-opening results as to the potential mechanisms or reasons behind this odd trend. Furthermore, the trends have forced the need for identifying whether the fundamental driving force is more along the lines of behavioral mechanisms or actual physiological manifestations or possibly even both.

### **Behavioral Explanations and Questions:**

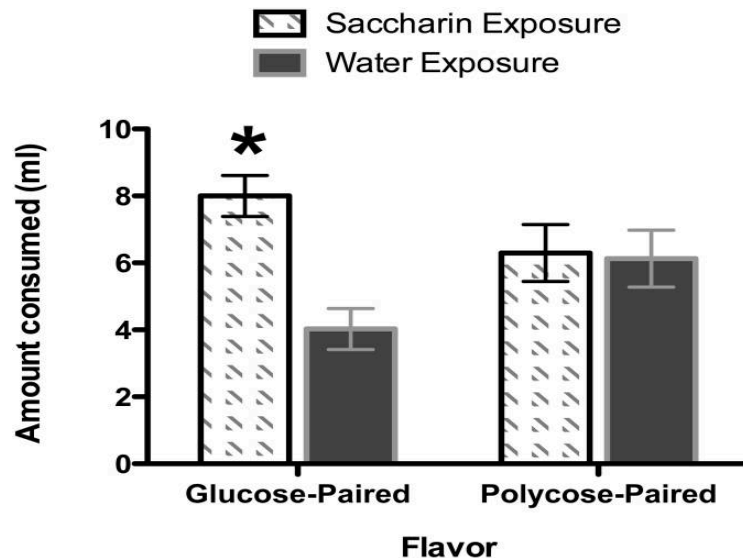
Pinto and colleagues found that saccharin also seemed to influence how much energy rat subjects exerted (Pinto et al, 2017). In their study, Pinto and colleagues measured the energy expenditure of rats following either sucrose and saccharin (Pinto et al, 2017). The saccharin group had more weight gain compared to the sucrose diet group and was shown to exert less energy when measured (Pinto et al, 2017). From the data, Pinto and colleagues concluded that the reasonable explanation was that a longer exposure of sucrose stimulated a greater energy expenditure (Pinto et al, 2017). On the other hand, the usage of saccharin may lead to the rats at rest to stop any further energy usage (Pinto et al, 2017).

Stellman and Garfinkel showed a surprising observation on the aftermath following the use of an artificial sweetener (Stellman & Garfinkel, 1988). According to the prospective study that was conducted, both male and female participants who had a history of consuming artificial sweeteners had a greater weight gain irrespective of the initial BMIs that were documented than the group that did not consume artificial sweeteners (Stellman & Garfinkel, 1988). Furthermore, the type of food ingested, such as fatty and non-fatty foods, did not influence the weight gain (Stellman & Garfinkel, 1988). Rather, even though the artificial sweetener group consumed more of what most people would consider to be health foods such as chicken and fish instead of sweets and butter, they were more likely to gain more weight (Stellman & Garfinkel, 1988).

Various questions still remain on why the weight gain actually occurs following consumption of artificial sweeteners. According to studies with rodents, the relationship between consumption of sweets and the amount of calories that are consumed following initial consumption of an artificial and regular sweetener is differential (Davidson et al, 2011). Davidson and colleagues initially exposed two groups to either a normal sweetener or the artificial sweetener saccharine (Davidson et al, 2011). Following the initial exposure, the rodents were given nutritive solutions that had a sweet component or a normative component, all being a normative nutritive value, and were taught the difference between the sweetened and unsweetened substances (Davidson et al, 2011). When first exposed to artificial sweetener, the rodents were less likely to have an association between sweeter substances and the metabolic consequences that normally arise from the consumption (Davidson et al, 2011). The saccharin group was found to eat

more substances with glucose following the initial exposure due to wanting to compensate for the lack of sweetness (Davidson et al, 2011). Although not a human model, it would seem plausible that this association could also explain partially the trends that arise in humans following consumption of artificial sweeteners.

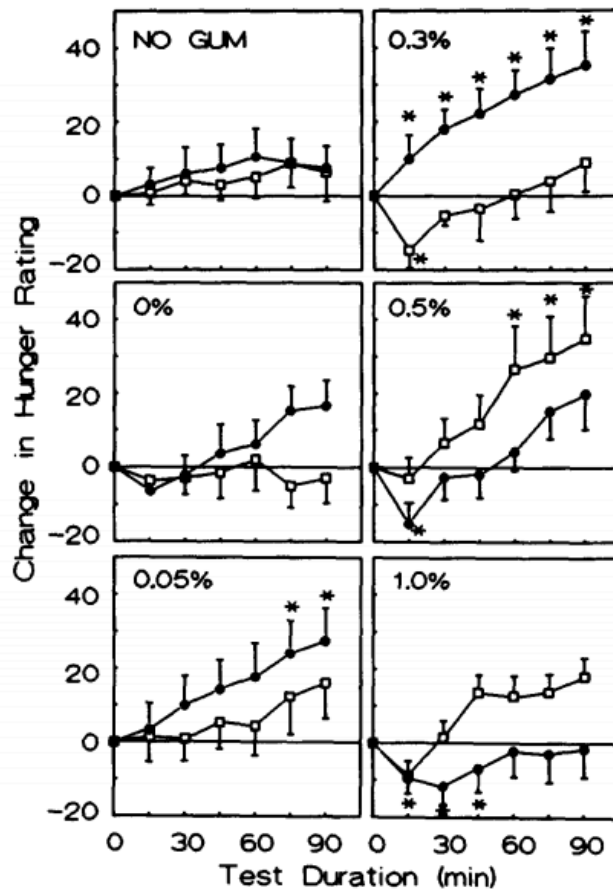
The predispositions may also be a key factor in determining whether or not the outcomes of consuming an artificial sweetener leads to weight loss or gain. In a secondary meta-analysis on a data set from a trial study in children, Katan and colleagues showed that there may be also differential outcomes in weight loss or gain depending on the initial BMI of participants prior to exposure of either sweeteners or artificial sweeteners (Katan et al, 2016). Children who had a higher BMI at the beginning of the trial were shown to not have the same level of compensation in terms of caloric consumption when compared to those who were below the median BMI level (Katan et al, 2016).



**Figure 13. The consumption of either glucose-paired or polycose-paired solution following the initial conditions of early saccharin or water exposure. (Davidson et al, 2006)**

Multiple studies have confirmed that artificial sweeteners lead to hunger and increased appetite (Mattes & Popkin, 2009; Gardner et al, 2012). Even the simplest experiment using oral stimulation by aspartame has shown to increase hunger (Tordoff & Alleva, 1990). Study by Tordoff and Alleva took 20 participants into two groups, 10 male and 10 female (Tordoff & Alleva, 1990). In each group, 5 were given no gum as control while the remaining 5 were given gum containing 0%, 0.05%, 0.3%, 0.5%, or 1.0% aspartame (Tordoff & Alleva, 1990). Collectively, gums with higher concentration of aspartame resulted in hunger but 0.3% and 0.5% showed higher results than 1.0% (Tordoff & Alleva, 1990). This points out that many factors play into stimulating hunger such as taste, osmolarity, and liquidity of the sweetener’s substance (Tordoff & Alleva,

1990). Tordoff and Alleva concludes that sweetness influences appetite, which is also confirmed in other studies that shows saccharin increasing food intake and food preference in rat models (Tordoff & Alleva, 1990; Tordoff & Friedman, 1989).



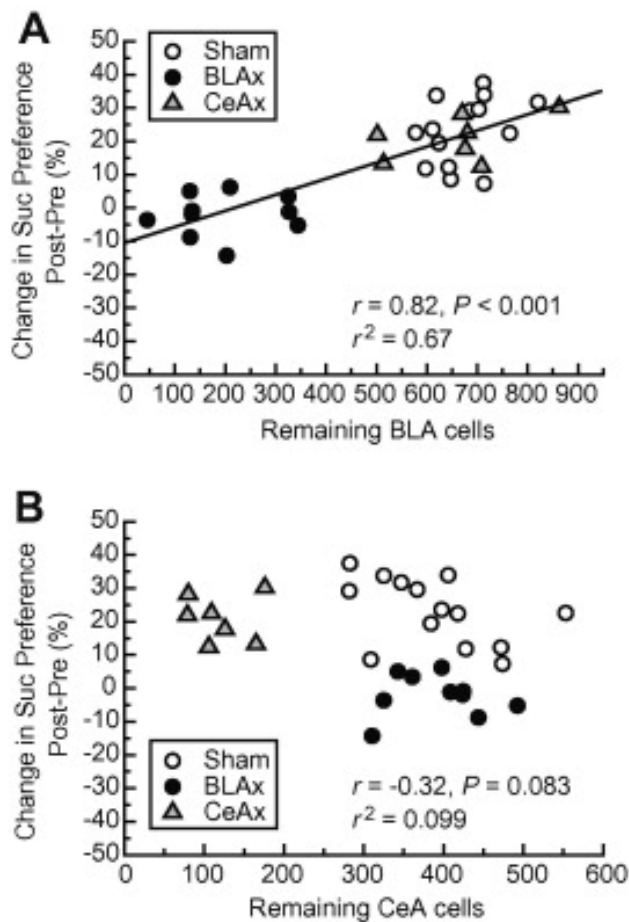
**Figure 14. Oral stimulation of aspartame and hunger ratings.** Solid dot represents female subjects while clear dots represent male subjects. Differences in sexes can be seen with higher sweetness rating given by male subjects. 0.3% and 0.5% resulting in greater hunger ratings than 1.0% shows that concentration of aspartame and hunger is not linear (Tordoff & Alleva, 1990).

## **Physiological Perspective Behind Choosing Sweeteners or No Sweeteners**

Although the behavioral elements have been widely accepted as a plausible explanation on explaining the adverse effects of consuming the artificial sweeteners, the biological elements and the evidence that has been produced are also equally important to evaluate an accurate depiction of everything. Furthermore, the underlying biological reasons for how a difference or preference for a caloric substance over a non-caloric sweetener in rat studies are equally necessary to identify in order to gain a more thorough understanding how the biological and behavioral changes arise.

Yasoshima and colleagues studied the different regions of the amygdala to determine which regions were primarily the areas that had more of a sugar preference (Yasoshima et al, 2015). In their study, they examined the central and the basolateral regions of the amygdala via “sham lesions” in these two areas (Yasoshima et al, 2015). When the mice with sham lesions were given training periods of either the real sucrose sugar or the artificial sweetener saccharine while being subjected to forced food deprivation, all mice showed a learned preference for the caloric solution of sucrose following the starvation (Yasoshima et al, 2015). However, when an excitotoxic lesion was placed in both the basolateral and the central regions, the results indicated a difference between the two groups (Yasoshima et al, 2015). Mice who were administered microlesions followed by an excitotoxin injection in the basolateral regions were not able to choose the more concentrated solution of the sucrose as the sham lesion group had done (Yasoshima et al, 2015). In contrast, the microlesions and the toxin injections in the central area of the amygdala did not yield any changes and the mice again chose the more

concentrated sucrose solution (Yasoshima et al, 2015). From these findings, it could be extrapolated that the basolateral region played a larger role in this mechanism for realizing the nutritive value in sucrose when compared to the artificial sweetener and when sucrose was compared to itself (Yasoshima et al, 2015).



**Figure 15. The change in the sweetener preferences.** There was a positive correlation for preference in sucrose concentration for more concentrated basolateral cell regions compared to the CeA cells that seemingly did not have a positive correlation. (Yasoshima et al, 2015)

Sclafani, Zukerman, and Ackroff showed that it might not be all about the difference in calories that motivates a preference for caloric content but may be the actual glucose content in the substances offered (Sclafani, Zukerman, and Ackroff, 2015). According to their research, rats were initially more drawn to the non-caloric sweetener sucralose (Sclafani, Zukerman and Ackroff, 2015). However, in the long run, the rats showed an affinity for the glucose and sucrose solutions (Sclafani, Zukerman, and Ackroff, 2015).

### **Metabolic Changes and Proposed Mechanisms**

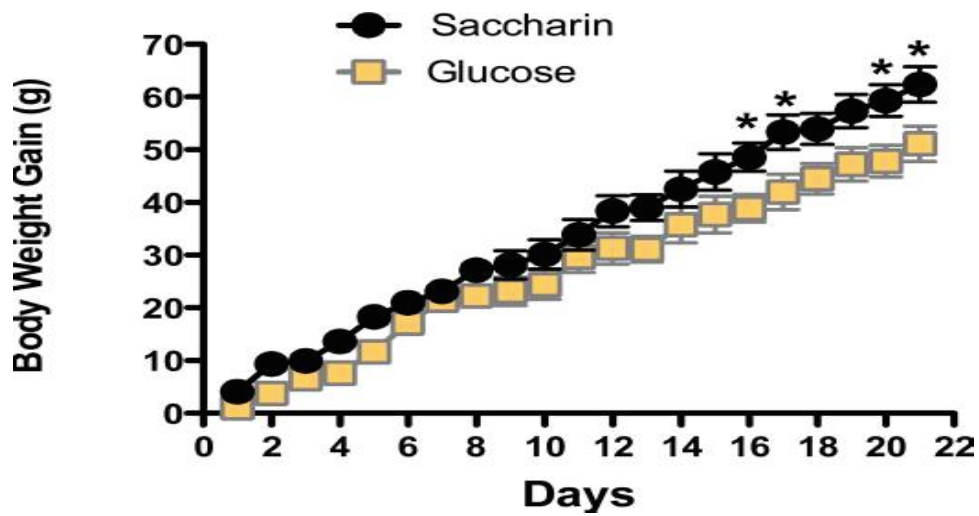
From a study by Malaisse and colleagues, three types of artificial sweeteners induced a greater secretion of insulin (Malaisse et al, 1998). However, not all sweeteners induced a greater secretion of insulin (Malaisse et al, 1998). Participants that were given the aspartate sweetener did not have greater secretion compared to when the artificial sweeteners such as sodium cyclamate, sodium saccharine or stevioside (Malaisse et al, 1998). Simon and colleagues investigated the mechanisms of how adipogenesis and the process of lipolysis may be influenced by artificial sweeteners (Simon et al, 2013). The results of these studies yielded data that was suggestive of an influence on metabolic processes that may not be dependent on the actual sweet receptors (Simon et al, 2013). According to Simon and colleagues, the T1R2 and the T1R3 G-coupled taste receptors were expressed during the process of adipogenesis (Simon et al, 2013). When the rat models were treated with saccharine, the levels of adipogenesis were higher (Simon et al, 2013). Furthermore, lipolysis, or the process of breaking down fats, was also lower once

the saccharin was introduced (Simon et al, 2013). However, the influence of the artificial sweeteners was seemingly independent of the changes in metabolism (Simon et al, 2013).

Furthermore, there are suggested actual physical mechanisms that could explain the differential metabolic outcomes that arise due to the prior exposure with either artificial or regular sweetener. Mitsutomi and colleagues found that, although the hyperglycemia seemed to have been decreased following consumption of a non-nutritive sweetener in rat subjects, there was some changes in the metabolism that could not be ignored (Mitsutomi et al, 2014). In particular, the metabolic changes were primarily seen in the rats that been induced into obesity through diet (Mitsutomi et al, 2014). Metabolic markers such as the UCP-1 were changed in the diet-induced obese (DIO) rodents suggesting a change in their metabolism following saccharine consumption (Mitsutomi et al, 2014).

According to Swithers and colleagues, the actual homeostasis of glucose could be differential relative to whether or not rodent subjects had been prior exposed to the saccharine sweetener (Swithers et al, 2012). Interestingly, there was a difference in the administration of glucose and the outcomes of the glucose homeostasis between the rats with prior exposure to saccharine or the group without the exposure (Swithers et al, 2012). In particular, when rats were given glucose orally, the rats that had been exposed to saccharine showed a higher level of glucose in the blood (Swithers et al, 2012). This effect was not witnessed when rats were given glucose directly injected into their stomachs suggesting a difference in the processing (Swithers et al, 2012). Moreover, the levels of the GLP-1 were much lower in the saccharine group compared to the non-

saccharine group of rats (Swithers et al, 2012). Ultimately, Swithers and colleagues suggested that the predictive association between the sweetened flavors and the amount of calories may not be established (Swithers et al, 2012).



**Figure 16. Saccharin exposure and glucose exposure and consequential weight gain among subjects.** The saccharin exposed group had higher weight gain compared to the glucose exposed group. (Swithers et al, 2012).

## **Discussion**

There has been growing support for the cautious evaluation of artificial sweeteners due to their perceived negative affect on areas such as weight gain and diabetes (Kuk and Brown, 2016). However, there are still published studies that have not been able to document any changes following consumption. Furthermore, numerous studies are able to still maintain the greater adversity consequential to regular sugars rather than artificial sweeteners. When compared to diet drinks, sugar-sweetened beverages have been shown to still lead to pre-diabetes and other health issues (Ma et al, 2016). Furthermore, when measuring for difference in fatty liver disease between artificial sweeteners and non-artificial sweeteners, participants who had consumed the traditional non-artificial sweeteners had more incidents of fatty liver disease (Ma et al, 2015).

Studies have also shown artificial sweeteners to disrupt gut microbiota, leading to glucose intolerance and liver inflammation that ultimately increases the risk of obesity, diabetes, and cardio-metabolic problems (Suez et al, 2014; Jensen et al, 2015; Bian et al, 2017). Rodent models have confirmed that artificial sweetener leads to dysbiosis and glucose intolerance, however, humans have shown to have selective response to artificial sweeteners (Suez et al, 2014; Bian et al, 2017).

Although most evidence has suggested that there are, indeed, observable patterns of change following the ingestion of artificial sweeteners, it is still a debatable topic. Some studies have shown that there is a tendency to consume more calories following consumption of an artificial sweetener in order to compensate for a possible underlying

understanding and desire to compensate for the lack of calories from the artificial sweeteners (Davidson et al, 2011). Although this process does seemingly make sense, there are also contradictory measures that have shown that there may be no difference between consuming an artificial sweetener and a normal sweetener. In recent studies, individuals with similar BMI and dietary habits who were given either stevia, aspartame or sucrose were shown to have no difference in the appetite or feeding patterns (Anton et al, 2010). Furthermore, there was no difference in the satiety following the consumption of aspartame, stevia or sucrose foods (Anton et al, 2010).

Saccharine and aspartame have been associated with potential differential effects on internal mechanisms and potential adverse consequences on the cellular and molecular levels for metabolic processes. Artificial sweeteners were documented to be linked with an increase of insulin secretion, which was harmful for the overall sugar balance within the body (Malaisse et al, 2018). Additionally, glucose homeostasis was observed to be offset following the consumption of saccharine leading to assumptions that the presence of artificial sweeteners can have indirect and direct influences on internal body mechanisms that would lead to the conspicuous changes in the body systems (Swithers et al, 2012). Although the natural body mechanisms seem to have a direct impact following the exposure to artificial sweeteners, the effects have yet to be seen on other drug mechanisms (Jo et al, 2017). In mice models, the drug metabolism of bupropion was measured by the 5 cytochrome P450 activity, which yielded no significant changes following consuming the saccharine (Jo et al, 2017).

## **Conclusion**

There is still a great misconception about artificial sweeteners. The absence of calories may lead to think that artificial sweeteners can be a great substitute for sugary substances and a great alternative for overweight and diabetic patients. However, studies have found that artificial sweeteners result in weight gain and increase the risk of diabetes, which is contrary to what was believed. Both short and long-term studies associate the consumption of artificial sweeteners to have consequential effects leading conditions such as dysbiosis, overconsumption, changes in metabolic outcomes, and much more. However, many of these studies derive from rodent models. Further studies must include human subjects to confirm the findings that suggest a correlation between artificial sweeteners and increased risk of obesity and diabetes. Nonetheless, there is overwhelming data that suggest that artificial sweeteners bring potential risk to our overall health. Continued monitoring of artificial sweetener consumption should be highly enforced.

## REFERENCES

- Abhilash M., Paul M.V.S., Varghese M.V., Nair R.H. (2012) Effect of long term intake of aspartame on antioxidant defense status in liver. *Food Chemical Toxicology*. 49:1203–1207.
- Adaramoye OA, Akanni OO (2016). Effects of long-term administration of biochemical indices, lipid profile and redox status of cellular system of male rats. *Journal of Basic and Clinical Physiology and Pharmacology*. 27(1): 29-37.
- Amin, K. A., & AlMuzafar, H. M. (2015). Alterations in lipid profile, oxidative stress and hepatic function in rat fed with saccharin and methyl-salicylates. *International Journal of Clinical and Experimental Medicine*, 8(4), 6133–6144.
- Andrejić, B. M., Mijatović, V. M., Samojlik, I. N., Horvat, O. J., Čalasan, J. D., & Đolai, M. A. (2013). The influence of chronic intake of saccharin on rat hepatic and pancreatic function and morphology: gender differences. *Bosnian Journal of Basic Medical Sciences*, 13(2), 94–99.
- Anton, S. D., Martin, C. K., Han, H., Coulon, S., Cefalu, W. T., Geiselman, P., & Williamson, D. A. (2010). Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. *Appetite*, 55(1), 37–43.
- Arnold DL, Krewski D, Munro IC. (1983). Saccharin: a toxicological and historical perspective. *Toxicology*. 27(3-4):179–256.

- Ashok I., Sheeladevi R. (2014). Biochemical responses and mitochondrial mediated activation of apoptosis on long-term effect of aspartame in rat brain. *Redox Biology*. 2:820–831.
- Bandyopadhyay A, Ghoshal S, Mukherjee A. (2008). Genotoxicity Testing of Low-Calorie Sweeteners: Aspartame, Acesulfame-K, and Saccharin. *Drug Chemical Toxicology*. 31(4):447–57.
- Bian, X., Chi, L., Gao, B., Tu, P., Ru, H., & Lu, K. (2017). The artificial sweetener acesulfame potassium affects the gut microbiome and body weight gain in CD-1 mice. *Public Library of Science ONE*. 12(6), e0178426.
- Boulangé, C. L., Neves, A. L., Chilloux, J., Nicholson, J. K., & Dumas, M.-E. (2016). Impact of the gut microbiota on inflammation, obesity, and metabolic disease. *Genome Medicine*. 8, 42.
- Byard JL, Goldberg L. (1973). The metabolism of saccharin in laboratory animals. *Food and Cosmetics Toxicology*. 11:391–402.
- Canty DJ, Chan MM. (1991). Effects of consumption of caloric vs noncaloric sweet drinks on indices of hunger and food consumption in normal adults. *The American Journal of Clinical Nutrition*. 53(5):1159–64.
- Chia, C. W., Shardell, M., Tanaka, T., Liu, D. D., Gravenstein, K. S., Simonsick, E. M., Ferrucci, L. (2016). Chronic Low-Calorie Sweetener Use and Risk of Abdominal Obesity among Older Adults: A Cohort Study. *Public Library of Science ONE*. 11(11), e0167241.

- Davidson, T. L., Martin, A. A., Clark, K., & Swithers, S. E. (2011). Intake of High-intensity Sweeteners alters the Ability of Sweet Taste to Signal Caloric Consequences: Implications for the Learned Control of Energy and Body Weight Regulation. *Quarterly Journal of Experimental Psychology*, *64*(7), 1430– 1441.
- De Koning, L., Malik, V. S., Rimm, E. B., Willett, W. C., & Hu, F. B. (2011). Sugar-sweetened and artificially sweetened beverage consumption and risk of type 2 diabetes in men. *The American Journal of Clinical Nutrition*, *93*(6), 1321–1327.
- Dhingra R., Sullivan L., Jacques P.F., Wang T.J., Fox C.S., Meigs J.B. (2007) Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. *Circulation*. 116:480-488.
- Echhardt K., King M.T., Gocke E., & Wild D. (1980). Mutagenicity study of Remsen-Fahlberg saccharin and contaminants. *Toxicology Letter*. 7:51–60
- Fowler, S.P, Williams K, Resendez RG, Hunt KJ, Hazuda HP, Stern MP. (2008). Fueling the obesity epidemic? Artificially sweetened beverage use and long-term weight gain. *Obesity Journal*. 16:1894–1900.
- Fagherazzi G, Vilier A, Saes Sartorelli D, Lajous M, Balkau B, Clavel-Chapelon F. (2013). Consumption of artificially and sugar-sweetened beverages and incident type 2 diabetes in the Etude Epidémiologique auprès des Femmes de la Mutuelle Générale de l'Education Nationale-European Prospective Investigation into Cancer and Nutrition cohort. *The American Journal of Clinical Nutrition*. 97(3):517–523.

- Fowler, S. P., Williams, K., & Hazuda, H. P. (2015). Diet soda intake is associated with long-term increases in waist circumference in a bi-ethnic cohort of older adults: The San Antonio Longitudinal Study of Aging. *Journal of the American Geriatrics Society*, 63(4), 708–715.
- Gardner C., Wylie-Rosett J., Gidding S. S., Steffen L. M., Johnson R. K., Reader D., et al. (2012). Nonnutritive sweeteners: current use and health perspectives: a scientific statement from the American heart association and the American diabetes association. *Diabetes Care*. 35, 1798–1808. 10.2337/dc12-9002
- Hall, L. N., Sanchez, L. R., Hubbard, J., Lee, H., Looby, S. E., Srinivasa, S., Fitch, K. V. (2017). Aspartame Intake Relates to Coronary Plaque Burden and Inflammatory Indices in Human Immunodeficiency Virus. *Open Forum Infectious Diseases*. 4(2), ofx083.
- Hicks RM, Chowanec J. (1977) The importance of synergy between weak carcinogens in the induction of bladder cancer in experimental animals and humans. *Cancer Research*. 37(8 Pt 2):2943–2949.
- Huang, M, Quduss, A, Stinson, L., Shikany, J.M., Howard, B.V., Kutob, R.M., Lu, B., Manson, J.E., Eaton, C.B. (2017). Artificially sweetened beverages, sugar-sweetened beverages, plain water, and incident diabetes mellitus in postmenopausal women: the prospective Women's Health Initiative observational study. *The American Journal of Clinical Nutrition*. Aug;106(2):614-622.

- Jang W, Jeoung NH, Cho KH. (2011). Modified apolipoprotein (apo) A-I by artificial sweetener causes severe premature cellular senescence and atherosclerosis with impairment of functional and structural properties of apoA-I in lipid-free and lipid-bound state. *Molecules and Cells*. 31:461–70.
- Jensen, A. B., Ajslev, T. A., Brunak, S., & Sørensen, T. I. A. (2015). Long-term risk of cardiovascular and cerebrovascular disease after removal of the colonic microbiota by colectomy: a cohort study based on the Danish National Patient Register from 1996 to 2014. *British Medical Journal Open*. 5(12), e008702.
- Jo, J. H., Kim, S., Jeon, T. W., Jeong, T. C., & Lee, S. (2017). Investigation of the Regulatory Effects of Saccharin on Cytochrome P450s in Male ICR Mice. *Toxicological Research*. 33(1), 25–30.
- John B.A., Wood S.G., Hawkins D.R. (2000). The pharmacokinetics and metabolism of sucralose in the mouse. *Food and Chemical Toxicology*. 38(Suppl. S2):107–110.
- Katan, M. B., de Ruyter, J. C., Kuijper, L. D. J., Chow, C. C., Hall, K. D., & Olthof, M. R. (2016). Impact of Masked Replacement of Sugar-Sweetened with Sugar-Free Beverages on Body Weight Increases with Initial BMI: Secondary Analysis of Data from an 18 Month Double–Blind Trial in Children. *Public Library of Science ONE*. 11(7), e0159771.
- Kessler II, Clark JP. (1978) Saccharin, cyclamate, and human bladder cancer. No evidence of an association. *The Journal of American Medical Association*. 240(4):349–355.

- Kuk JL, Brown RE. (2016). Aspartame intake is associated with greater glucose intolerance in individuals with obesity. *Applied Physiology, Nutrition, and Metabolism* 41:795–8.
- Lavery, A. A., Magee, L., Monteiro, C. A., Saxena, S., & Millett, C. (2015). Sugar and artificially sweetened beverage consumption and adiposity changes: National longitudinal study. *The International Journal of Behavioral Nutrition and Physical Activity*. 12, 137.
- Malaisse WJ, Vanonderbergen A, Louchami K, Jijakli H, Malaisse-Lagae F. (1998). Effects of artificial sweeteners on insulin release and cationic fluxes in rat pancreatic islets. *Cellular Signalling*. 10(10):727–33.
- Ma J, Jacques PF, Meigs JB, Fox CS, Rogers GT, Smith CE, Hruby A, Saltzman E, McKeown NM. (2016). Sugar-sweetened beverage but not diet soda consumption is positively associated with progression of insulin resistance and prediabetes. *Journal of Nutrition*. 146:2544–2550.
- Ma, J., Fox, C.S., Jacques, P.F., Speliotes, E.K., Hoffmann, U., Smith, C.E., McKeown, N.M. (2015). Sugar-sweetened beverage, diet soda, and fatty liver disease in the Framingham Heart Study cohorts. *Journal of Hepatology*, 63(2), 462–469.
- Mattes, R. D., & Popkin, B. M. (2009). Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. *The American Journal of Clinical Nutrition*, 89(1), 1–14

- Miller, P. E., & Perez, V. (2014). Low-calorie sweeteners and body weight and composition: a meta-analysis of randomized controlled trials and prospective cohort studies. *The American Journal of Clinical Nutrition*, *100*(3), 765–777.
- Mitsutomi K., Masaki T., Shimasaki T., Gotoh K., Chiba S., Kakuma T., Shibata H. (2014). Effects of a nonnutritive sweetener on body adiposity and energy metabolism in mice with diet-induced obesity. *Metabolism*. *63*: 69–78.
- Nettleton, J. A., Lutsey, P. L., Wang, Y., Lima, J. A., Michos, E. D., & Jacobs, D. R. (2009). Diet Soda Intake and Risk of Incident Metabolic Syndrome and Type 2 Diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care*. *32*(4), 688–694.
- O'Connor, L., Imamura, F., Lentjes, M. A. H., Khaw, K.-T., Wareham, N. J., & Forouhi, N. G. (2015). Prospective associations and population impact of sweet beverage intake and type 2 diabetes, and effects of substitutions with alternative beverages. *Diabetologia*. *58*(7), 1474–1483.
- Pase MP, Himali JJ, Beiser AS, Aparicio HJ, Satizabal CL, Vasan RS, Seshadri S, Jacques PF. (2017) Sugar- and artificially sweetened beverages and the risks of incident stroke and dementia: a prospective cohort study. *Stroke* *48*:1139–46.
- Pepino MY. (2015). Metabolic effects of non-nutritive sweeteners. *Physiological Behavior*. *152*(Part B):450–455.

- Pinto, DE, Foletto, KC, Nunes, RB, Lago, PD, Bertoluci, MC. (2017). Long-term intake of saccharin decreases post-absorptive energy expenditure at rest and is associated to greater weight gain relative to sucrose in wistar rats. *Nutrition Metabolism* (London). Feb 20; 14:18.
- Polyak E, Gombos K, Hajnal B, Bonyár-Müller K, Szabó S, Gubicskó-Kisbenedek A, et al. (2010). Effects of artificial sweeteners on body weight, food and drink intake. *Acta Physiologica Hungarica*. 97(4):401–7.
- Raben A., Vasilaras T. H., Møller A. C., Astrup A. (2002). Sucrose compared with artificial sweeteners: different effects on *ad libitum* food intake and body weight after 10 wk of supplementation in overweight subjects. *American Journal of Clinical Nutrition*. 76, 721–729.
- Schulze MB, Manson JE, Ludwig DS, et al (2004). Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. *The Journal of American Medical Association*. 292: 927–934.
- Sclafani, A., Zukerman, S., & Ackroff, K. (2015). Postoral Glucose Sensing, Not Caloric Content, Determines Sugar Reward in C57BL/6J Mice. *Chemical Senses*. 40(4), 245–258.
- Simon BR, Parlee SD, Learman BS et al. (2013). Artificial sweeteners stimulate adipogenesis and suppress lipolysis independently of sweet taste receptors. *Journal of Biological Chemistry*. 288:32475–89.
- Stellman SD, Garfinkel L. (1988). Patterns of artificial sweetener use and weight change in an American Cancer Society prospective study. *Appetite*. 11(Suppl 1):85–91.

- Suez J., Korem T., Zeevi D., Zilberman-Schapira G., Thaiss C.A., Maza O., Israeli D., Zmora N., Gilad S., Weinberger A., et al. (2014) Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature*. 514:181–186.
- Swithers, S. E., Laboy, A. F., Clark, K., Cooper, S., & Davidson, T. L. (2012). Experience with the high-intensity sweetener saccharin impairs glucose homeostasis and GLP-1 release in rats. *Behavioral Brain Research*, 233(1), 1–14.
- Swithers, S. E., Sample, C. H., & Davidson, T. L. (2013). Adverse effects of high-intensity sweeteners on energy intake and weight control in male and obesity-prone female rats. *Behavioral Neuroscience*, 127(2), 262–274.
- Sylvetsky A, Rother KI, Brown R. (2011) Artificial sweetener use among children: epidemiology, recommendations, metabolic outcomes, and future directions *Pediatric Clinics of North America*. 58(6):1467–80, xi.
- Sylvetsky A, Welsh JA, Brown RJ, Vos MB. (2012) Low-calorie sweetener consumption is increasing in the United States. *American Journal of Clinical Nutrition*. 96:640–646.
- Turnbaugh P.J., Ley R.E., Mahowald M.A., Magrini V., Mardis E.R., Gordon J.I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444, 1027–1031.
- Tordoff MG, Alleva AM (1990) Oral stimulation with aspartame increases hunger. *Physiology and Behavior*. 47: 555–559.

Tordoff MG, Friedman MI. (1989) Drinking saccharin increases food intake and preference: In Comparison with other drinks. *Appetite*. 12:1–10.

United States Food and Drug Administration. Additional Information about High-Intensity Sweeteners Permitted for use in Food in the United States. (2015) Retrieved 2017, from <https://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm397725.htm>

Yasoshima Y, Yoshizawa H, Shimura T, Miyamoto T (2015). The basolateral nucleus of the amygdala mediates caloric sugar preference over a non-caloric sweetener in mice. *Neuroscience*. 291:203–215.

**CURRICULUM VITAE**

