POTENTIAL METABOLIC EFFECT OF SUCRALOSE FOLLOWING AN ORAL GLUCOSE LOAD IN SUBJECTS WITH OBESITY AND NORMAL-WEIGHT SUBJECTS

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Food Science and Human Nutrition
with a concentration in Human Nutrition
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2018

Urbana, Illinois

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ABSTRACT

Objective: Whether sucralose, the most commonly used non-nutritive sweetener (NNS), affects glucose metabolism in people is unclear. It has been reported that, when consumed acutely before an oral glucose tolerance test (OGTT), sucralose enhances insulinemic responses and decreases insulin sensitivity in subjects with obesity who are not regular consumers of NNS. However, studies in normal-weight adults, none of which control for use of NNS, found sucralose does not affect insulin responses to the ingestion of glucose or other carbohydrates. The objectives of the current study are to determine if those effects of sucralose can be replicated in subjects with obesity, are generalizable to normal-weight subjects when controlling for history of NNS use, and are caused merely by the sweet taste of sucralose (i.e., sham-feeding). In addition, with the aim of identifying potential mechanisms by which sucralose may decrease postprandial insulin sensitivity, we here investigated whole-body glucose kinetics by using a dual-tracer approach. Finally, we tested the hypothesis that, due to the compromised intestinal permeability associated with obesity, sucralose consumption is associated with higher plasma sucralose concentrations in people with obesity.
Research Design and Methods: Ten normal-weight subjects (BMI: 22.8 ± 0.9 kg/m^2) and nine subjects with obesity (BMI: 37.7 ± 6.1 kg/m^2), all non-regular users of NNS, non-diabetic, and without significant insulin resistance (based on a homeostatic model assessment of insulin resistance score < 3), underwent a 5-hour modified OGTT in which ingested glucose was labeled with one tracer while a second glucose tracer was infused intravenously at a constant rate on three separate testing days. Each testing

day differed only by which pre-load solution (water ingestion (control condition), sucralose ingestion (experimental condition), or sucralose sham-fed (taste condition)) was administered 10 minutes prior to the OGTT in a randomized crossover design. Blood samples were taken throughout observation to determine plasma glucose, insulin, C-peptide, glucose-dependent insulinotropic polypeptide (GIP), glucose tracer-to-tracee ratio, and sucralose concentrations.

Results: In subjects with obesity, compared to the control condition, sucralose sham-feeding reduced insulin and C-peptide concentrations within the first hour of the OGTT (both p-values ≤ 0.05), whereas sucralose ingestion increased plasma C-peptide incremental area under the curve (iAUC) by 22 ± 10% ($p < 0.05$) and tended to increase plasma insulin iAUC by 16 ± 9% ($p = 0.09$) and plasma glucose iAUC by 34 ± 12% ($p = 0.06$). No condition affected GIP. There were no differences among conditions in the degree of suppression of endogenous glucose production (EGP) after the glucose load, but both sucralose conditions affected the pattern of oral glucose rate of appearance (Ra) and glucose rate of disappearance (Rd). Compared to the water condition, sucralose affected the pattern of change with time including i) a greater decline (50-60 min) and subsequently a reduced decline (120-140 min) in oral glucose Ra ($p = 0.03$), and ii) a reduced glucose Rd (50-80 min) and subsequently an increased glucose Rd (140-160 min) after the glucose load ($p < 0.05$). While the reduction in glucose Rd during the taste condition and the increase in Rd 140-160 min after the glucose load could be explained by prevailing plasma insulin concentrations, the reduction in glucose Rd 50-80 min after the glucose load on the experimental condition cannot. During this time period, plasma insulin concentrations after sucralose ingestion were similar or

higher than after water ingestion, which is in agreement with previous results of a decreased insulin sensitivity in subjects with obesity when sucralose ingestion preceded an OGTT. In normal-weight subjects, there were no significant differences among conditions in plasma glucose, C-peptide, GIP, or plasma glucose or hormones iAUCs, but both sucralose conditions tended to equally lower mean plasma insulin concentrations following the OGTT (p = 0.09) and suppressed EGP to a greater degree (p = 0.09) compared to water. There were no significant differences between groups for peak sucralose concentrations, the time to reach peak sucralose concentration, and plasma sucralose iAUCs (all p-values > 0.19).

Conclusions: These data demonstrate that sucralose differentially affects hormonal responses to an oral glucose load in subjects with obesity and normal weight subjects. Sucralose ingestion (not merely its taste) increases insulin secretion to an oral glucose load and transiently reduces glucose Rd in people with obesity, but not in normal-weight subjects. These findings support the hypothesis that sucralose may have adverse effects on glucose metabolism in people with obesity, which is the group that most frequently consumes NNS to facilitate weight management. These data also underscore a physiological role for sweetness perception in glucose homeostasis, which supports the notion that sweetness, regardless of an associated caloric contribution, should be consumed in moderation.

ACKNOWLEDGEMENTS

I would like to thank all of those from the university who have helped in some way during my time as a graduate student. Dr. Yanina Pepino, my advisor, who accepted me into her lab and provided me the opportunity to work on this project. Dr. Ruopeng An, who supported my idea for a review paper on non-nutritive sweeteners and helped in every step of the way towards achieving my first first-author publication. Dr. Alexander Lipka, who has helped me become much more skilled at statistics and offered me a teaching assistantship for his statistics course. My committee members, Dr. Megan Dailey and Dr. John Erdman, for their suggestions regarding my research and guidance in choosing classes.

Thank you to all of my lab mates: Raul, Katie, Belen, and Ramiro, for helping me whenever I needed it, providing valuable feedback, and creating an enjoyable environment to work in. Thank you to Maxwell Holle, who showed me what it takes to be a successful graduate student and worked alongside me the entire way towards publishing our review paper together. Thank you to Adam Kriska, who shared his experiences in graduate school with me and urged me to make decisions with my own self-interest in mind.

Thank you to all of my friends back home, whose friendship has never dwindled despite the distance. It means so much to me to pick up right where we left off every time I see any of you.

Thank you to my family, especially my parents and grandmother, who have supported and encouraged me throughout this process. I would not be here today if not for you all.

And finally, thank you to my girlfriend Layla Alazawy, who is my partner throughout life and who has been with me every step of the way. Your support means more to me than you could ever know, and your presence alone makes difficult times easy.

Dedicated to Anna Dudziak

TABLE OF CONTENTS

ABBREVIATIONS

NNS – non-nutritive sweetener(s)

BMI – body mass index

AUC – area under the curve

iAUC – incremental area under the curve

GLP-1 – glucagon-like peptide 1

GIP – glucose-dependent insulinotropic peptide

SGLT1 - sodium-dependent glucose co-transporter isoform 1

ADME - absorption, distribution, metabolism, and excretion

OGTT – oral glucose tolerance test

ADI – acceptable daily intake

FDA – United States Food and Drug Administration

PYY – peptide tyrosine tyrosine

3-OMG – 3-O-methyl glucose

TIM – tagatose/isomalt mixture

Ace-K – acesulfame potassium

HOMA-IR – homeostatic model assessment for insulin resistance

Total Ra – total rate of glucose appearance

Oral Ra – oral rate of glucose appearance

EGP – rate of endogenous glucose production

Rd – rate of glucose disappearance

CHAPTER 1: INTRODUCTION

A hypercaloric diet potentiated by excess sugar consumption is known to increase risk of obesity, cardiovascular disease, and type 2 diabetes (Rippe and Angelopoulos, 2016a, Rippe and Angelopoulos, 2016b). Therefore, limiting sugar consumption, specifically added sugar, is recommended by governmental and health organizations around the world (Hess et al., 2012). However, humans have an innate attraction towards sweet taste (Steiner, 1974), which makes avoiding sweet-tasting foods difficult. Non-nutritive sweeteners (NNS), which are sugar substitutes, provide intense sweet taste without the addition of sugar or calories. Therefore, NNS use is growing in popularity, with about 25% of children and 41% of adults reportedly consuming them (Sylvetsky et al., 2017).

Despite their negligible caloric contribution, data from animal models and human cell lines suggest that NNS are not metabolically inert. The sweet taste receptor, T1R2+T1R3, is a heterodimer that detects sugars and NNS and is expressed in many tissues beyond the oral cavity (Laffitte et al., 2014). In the intestine, activation of these sweet taste receptors by sugars results in the secretion of the incretins glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) from enteroendocrine L-cells and K-cells, respectively (Jang et al., 2007, Kojima and Nakagawa, 2011). Activation of these receptors also increases active glucose absorption through upregulation of sodium-dependent glucose co-transporter isoform 1 (SGLT1) (Margolskee et al., 2007, Moran et al., 2010, Stearns et al., 2010) and passive glucose absorption through increased translocation of GLUT2 to the intestinal lumen

(Mace et al., 2007). In the pancreas, activation of sweet taste receptors expressed in beta-cells mediates insulin secretion (Kojima et al., 2015, Kyriazis et al., 2012, Malaisse et al., 1998, Nakagawa et al., 2009).

Additionally, it has been reported that sucralose, one of the most widely used NNS, stimulates the secretion of incretins in cell lines (Jang et al., 2007, Margolskee et al., 2007), indicating that sucralose is capable of interacting with the sweet taste receptors located beyond the oral cavity. The above findings have raised the question of whether sucralose consumption may affect glucose absorption and alter the glycemic response to a caloric load in humans. Numerous studies have been conducted investigating the post-ingestive effects of sucralose in human and animal models. Presented hereafter will be a comprehensive review of all of the current literature related to the absorption, distribution, metabolism, and excretion (ADME) of sucralose and its effect on fasting and postprandial glycemic control in both chronic and acute studies. Included in this review is a study conducted by Pepino and collaborators (Pepino et al., 2013), which was the first to examine the effects of sucralose consumed prior to a glucose load strictly in people with obesity who were not regular consumers of NNS (review in detail later). They found that the acute ingestion of sucralose prior to an oral glucose tolerance test (OGTT) enhanced insulinemic responses. However, in contrast with such findings in people with obesity, data from most studies conducted in healthy, normal-weight people show that sucralose does not affect glycemic or insulinemic responses to the ingestion of a carbohydrate load (Brown et al., 2011, Wu et al., 2013, Wu et al., 2011). The reason(s) for the discrepancy between the results from these studies and Pepino and collaborators data is not clear, but we hypothesize it is

related to sucralose having differential effects in subjects with obesity and normal-weight subjects.

The primary goals of our present study were to evaluate the acute effects of sucralose ingestion, versus merely perceiving the sweet taste of sucralose in the mouth, on hormonal responses to an OGTT and whole-body glucose metabolism in people with obesity and in normal-weight people. We hypothesized that the ingestion of sucralose before a glucose load, but not the mere perception of its sweet taste, would cause an increase in glucose-stimulated insulin secretion, a reduced suppression in endogenous glucose production, and a reduced glucose rate of disappearance in subjects with obesity, but would have no effects in normal-weight subjects. Accordingly, we assessed effects of acute sucralose ingestion versus water ingestion or sucralose sham-fed (i.e., tasted without being swallowed) on plasma glucose, glucose kinetics, and hormonal responses to a glucose load. In addition, we hypothesized that people with obesity were more vulnerable to the metabolic effects of sucralose than their normal-weight peers because, due to the compromised intestinal permeability associated with obesity (Gummesson et al., 2011), sucralose consumption would result in higher peak sucralose concentrations in plasma in people with obesity.

For our study, we selected sucralose because 1) it is one of the eight (sucralose, saccharin, aspartame, acesulfame-K, neotame, advantame, stevia, and luo han guo fruit extract) NNS currently approved for use by the US FDA; 2) of the eight NNS, it is the most commonly used and found in the most food products (Yang 2010); and 3) it is the NNS used in Pepino and collaborators' previous study (Pepino, Tiemann et al. 2013), where it was demonstrated that sucralose alters glycemic and hormonal

responses to an OGTT in subjects with obesity. In the following chapter, I present a detailed review on sucralose, including its chemistry, sensory properties, pharmacokinetic profile, and metabolic effects both in pre-clinical and clinical studies.

CHAPTER 2: LITERATURE REVIEW OF SUCRALOSE

2.1 *Chemistry, Sensory Properties, and Nutritional Value of Sucralose*

Sucralose is artificially derived from the disaccharide sucrose by substituting three hydroxyl groups with chlorine. These subtle changes keep sucralose structurally similar to sucrose, and maintain its high water solubility (Jenner and Smithson, 1989). However, the substitution with chlorine causes the conformation of the sucralose molecule to be oriented in such a way that glycosidic enzymes are unable to digest sucralose into monosaccharides, thus becoming unable to undergo metabolism (Magnuson et al., 2016). As will be later discussed, most sucralose leaves the body intact and unchanged (Roberts et al., 2000).

Sucralose stimulates the human sweet taste receptor by binding to its Venus Fly Trap domain located on the N-terminal domain (Liu et al., 2011, Masuda et al., 2012). In determining the binding location of sucralose to the sweet taste receptor, Masuda *et al.* were unable to identify the binding location of sucrose because of the faint cellular responses compared to sucralose and other artificial sweeteners (Masuda et al., 2012). This supports sensory data indicating that the chlorine substitutions make sucralose about 400-700 times sweeter than sucrose on a weight for weight basis (Wiet and Beyts, 1992). This means that the addition of very small amounts, relative to sucrose, are necessary to achieve similar intensities of sweetness.

The combination of the potent sweet taste along with the inability to provide a caloric value make sucralose an appealing replacement for sugar. Sucralose is the most commonly used NNS today, an ingredient in over 1,500 food products (Yang, 2010),

and is most commonly known for sweetening Splenda. Because of its widespread use, investigations into this sweetener are important to identify its efficacy as a substitute for sugar.

2.2 *Sucralose Acceptable Daily Intake and Estimating Average Intake*

The acceptable daily intake (ADI) is an estimate of the amount of a substance, on a per body weight basis, that can be ingested daily over a lifetime without appreciable risk. The FDA and Joint FAO/WHO Expert Committee on Food Additives have set the ADI for sucralose at 5 mg/kg of body weight (FDA, 2018) and 15 mg/kg of body weight (JECFA, 1991), respectively. These values are important to reference when determining how high of a dose is considered safe when given in a study. However, it is also crucial to know if consumers of sucralose are within these ranges, and if they are, where the low, average, and high users fall within this range. When conducting a study, administering amounts that are typically consumed can give results that better reflect an outcome representative of the population. Hereafter, all references to the ADI will be to the amount set by the FDA.

An overview of 11 studies, which estimated NNS intake in various countries and age groups mainly using food diaries and 24-hour dietary recalls, has compiled the results and estimated that the average user only consumes 3% to 9% of the ADI (0.15-0.45 mg/kg) and high users consume 18% to 45% of the ADI (0.9-2.25 mg/kg) (Renwick, 2006). The highest reported consumption in these 11 studies was 2.25 mg/kg/day, which is less than half of the ADI (FSANZ, 2004).

Several more studies have been conducted to estimate the average intake since the work of Renwick *2006*. In Belgium, 24-hour recalls were completed by 3083

participants over 15 years of age, which determined that the highest users (95th percentile) consumed 1.53 mg/kg/day (30% of the ADI) (Huvaere et al., 2012). Another study in Belgium, which used a more conservative approach to estimating daily sucralose intake, estimated that the average user consumed 0.8 mg/kg/day (16% of the ADI) and high users consumed 3.1 mg/kg/day (62% of the ADI) (Van Loco et al., 2015). In Korea, 24-hour recalls were completed by 8081 participants aged 1-65 years old (Ha et al., 2013). Two estimates were made based on the assumption of randomly choosing foods with and without sweeteners, or deliberately choosing foods sweetened with sucralose. The randomly chosen estimate came out to 1.2 mg/kg/day (24% of the ADI) and 3.4 mg/kg/day (68% of the ADI) for average and high users, respectively. The deliberately chosen estimate came out to 6.5 mg/kg/day (130% of the ADI) and 17.7 mg/kg/day (354% of the ADI) for the average and high users, respectively. The deliberately chosen estimate is much greater because it is a worst-case scenario, and most of the estimate comes from the assumption that soju, an alcoholic beverage in Korea sweetened with sucralose, is consumed frequently.

2.3 *Absorption, Distribution, Metabolism, Excretion (ADME) of Sucralose*

2.3.1 *Animal Models*

ADME studies of sucralose have been conducted in mice (John et al., 2000a), rats (Sims et al., 2000), dogs (Wood et al., 2000), and rabbits (John et al., 2000b), which all show that most of the sucralose is recovered unchanged, and that a majority of it is excreted in the feces, with a smaller percentage being absorbed and excreted through the urine.

2.3.1.1 *Mice*

Sixteen mice were divided into 3 treatment groups receiving 100 mg/kg (4 male, 4 female), 1500 mg/kg (2 male, 2 female), and 3000 mg/kg (2 male, 2 female) of sucralose during a single oral gavage administration (John et al., 2000a). A fourth group also received a 20 mg/kg IV dose of isotopically labelled ^{14}C-sucralose into their tail vein. Urine and feces samples were taken for the next 5 days to record the amount of sucralose excreted. The group receiving the dose in their tail vein excreted 80% of the sucralose in their urine, 22% in their feces, and 2% was found in their cage debris, for a total recovery of 104%. The group receiving 100 mg/kg by oral gavage excreted 23% of the dose in the urine, 70% in the feces, and 3% was found in the cage debris, for a total recovery of 96%. Comparing the amount of sucralose excreted in the urine, when the dose was orally administered, to that of an IV dose, the researchers estimated that about 29% of the dose was absorbed in the oral gavage group. The groups receiving 1500 and 3000 mg/kg in a single oral gavage had similar results to each other, with 15 and 16% excreted in the urine, 74 and 72% excreted in the feces, and 3 and 5% found in the cages, for a total recovery of 92 and 94%, respectively. It is estimated that these groups absorbed around 20% of the dose each. Nearly all of the sucralose recovered was unchanged, with about 2% of other metabolites found, but there was so little that they could not be identified.

2.3.1.2 *Rats*

Three acute studies have been conducted in regards to oral gavage administration of sucralose in rats (Sims et al., 2000). Twelve adult rats were divided into 2 treatment groups receiving 100 mg/kg (3 male, 3 female) and 1000 mg/kg (3

male, 3 female) of sucralose and isotopically labelled [^{36}Cl]sucralose through oral gavage. Additionally, four adult rats with their bile ducts cannulated were divided into 2 groups receiving 50 mg/kg (1 male, 1 female) and 100 mg/kg (1 male, 1 female) of sucralose and isotopically labelled [^{36}Cl]sucralose through oral gavage. In the third acute study, six rats aged 3 months received 10 mg/kg of sucralose and isotopically labelled ^{14}C-sucralose through oral gavage. The researchers found that the group receiving 100 mg/kg excreted 8.5% of the dose in urine and 88% in the feces, for a total recovery of 96.5%; the group receiving 1000 mg/kg excreted 5% of the dose in urine and 90% in the feces, for a total recovery of 95%; and the group receiving 10 mg/kg excreted 5% of the dose in urine and 93% in the feces, for a total recovery of 98%. The groups that had their bile ducts cannulated excreted 6% and 1.5% of the doses through the bile at 50 mg/kg and 100 mg/kg, respectively. The researchers declared that these amounts are similar to those found in urine, thus the urinary excretion underestimates the total amount of sucralose absorbed by the intestinal tract in rats. About 91% of the recovered sucralose in the urine was unchanged, with two more polar conjugates found, but unidentified. As nearly 100% of the sucralose found in the feces was unchanged, the two conjugates made up less than 1% of the total recovered dose.

Thirty four adult rats were divided into two groups receiving either a control diet (8 male, 8 female) or the same diet containing 3% sucralose (9 male, 9 female) for 26, 52, or 85 weeks (Sims et al., 2000). At 26, 52, and 85 weeks, rats from both groups were given an acute dose of 100 mg/kg ^{14}C-sucralose through oral gavage. The group receiving the control diet excreted 7.7% of the dose in the urine and 83.5% in the feces, for a total recovery of 91.2% over all 3 of the time points measured. The group receiving

the diet with sucralose excreted 6.6% in the urine and 83.9% in the feces, for a total recovery of 83.9% over all 3 of the time points measured. The researchers determined that there were no differences in percentages of the dose eliminated in the urine and feces between the groups receiving the two diets. The recovered sucralose was primarily unchanged, with about 10% of similar conjugates found in the urine in the acute studies mentioned above. Again, because the feces was nearly 100% unchanged sucralose, the conjugates made up less than 1% of the total dose administered.

2.3.1.3 *Dogs*

Two male and two female beagle dogs were administered 10 mg/kg sucralose containing ^{14}C-sucralose through a single oral gavage (Wood et al., 2000). After 5 days, 27.6% of the dose was excreted in the urine, 68.4% in the feces, and 1.6% in the cage, for a total recovery of 97.6%. Comparing the urinary excretion of a sucralose dose given by oral gavage to that of an IV dose, which is considered to 100% absorption, the researchers determined that about 35% of the oral gavage dose was absorbed in the gastrointestinal tract. After the 5 day period, small amounts of radioactivity was still detectable in the plasma, indicating that it may take more than 5 days for all of orally administered sucralose to be excreted. A more polar, glucuronic acid conjugate of sucralose was found in the urine, which accounted for about 2-8% of the total oral dose.

2.3.1.4 *Rabbits*

Three pregnant and three non-pregnant rabbits received single oral gavage doses of 10 mg/kg of sucralose that included ^{14}C-sucralose (John et al., 2000b). After 5 days, the pregnant group excreted 21.5% of the dose in urine, 65.2% in the feces, and 0.4% in the cage, for a total recovery of 87%. The non-pregnant group had similar

results, with 22.3% excreted in the urine, 54.7% in the feces, and 3.4% in the cage, for a total recovery of 80.5%. The sucralose recovered was primarily unchanged in both groups, with unidentifiable small amounts of more polar conjugates present.

2.3.1.5 *Summary of Animal Models*

Overall, animal models including mice, rats, dogs, and rabbits demonstrate that most orally administered sucralose is not absorbed into plasma and is excreted in the feces, and nearly all of the recovered sucralose is unchanged. No differences were found between naïve and chronic consumers, and the amount of sucralose in the dose does not significantly alter the percentages of total or route of excretion.

2.3.2 *Human Models*

A similar study design to those presented above in animal models was used to determine the pharmacokinetics of sucralose in man at two separate doses (Roberts et al., 2000). Eight healthy males aged 30-48 received a solution of 1 mg/kg of sucralose that contained ^{14}C-sucralose, and two of these subjects were chosen to receive 10 mg/kg sucralose in a similar follow-up study. After 5 days, the participants receiving the dose of 1 mg/kg excreted 14.5% in the urine and 78.3% in the feces, for a total recovery of 92.8%; the participants receiving the 10 mg/kg dose excreted 11.2% in the urine and 85.5% in the feces, for a total recovery of 96.7%. The sucralose found was primarily unchanged, with two more polar glucuronide conjugates found as 2.6% and 1.6-1.9% of the total dose in the 1 mg/kg and 10 mg/kg groups, respectively. Following the 1 mg/kg dose, the average peak concentration was 262 ng equivalents per mL and occurred about 2 hours after consumption. After 12 hours the concentration dropped to 36 ng equivalents per mL, and very slowly declined for the remainder of the study. This human

model demonstrates that, similar to animal models, most sucralose is unabsorbed and excreted through the feces, while the amount that is absorbed passes through the body primarily unchanged.

As I am aware of only one published ADME study in humans, which included only 8 subjects (Roberts et al., 2000), more research should be included in this area to confirm the findings presented here. Furthermore, each of these eight subjects were healthy males, which excludes a large portion of the population, including females and people with metabolic dysregulation, such as obesity or type 2 diabetes.

2.3.2.1 *Metabolic Responses to Sucralose: Human Clinical Trials*

Studies investigating the metabolic responses of sucralose typically measure fasting or postprandial parameters related to plasma glucose control, such as the insulinemic and incretin responses, C-peptide, HbA1C, and gastrointestinal peptides. These studies can be separated into either acute or chronic exposure to sucralose, as well as the population studied (healthy subjects or subjects with diabetes).

2.3.2.1.1 *Chronic Consumption*

2.3.2.1.1.1 *Healthy Subjects*

Two studies investigating repeated exposure to sucralose were conducted by *Baird et al. 2000* (Baird et al., 2000). In one of these studies, eight subjects (mean weight: 70kg) received ascending doses of sucralose (up to 10 mg/kg) in a solution every other day for 10 days, followed by daily doses of 2 mg/kg (3 days) and 5 mg/kg (4 days). The results showed that sucralose consumption did not affect blood concentrations of glucose or insulin measured after an overnight fast. Additionally, a 50g oral sucrose tolerance test was performed a week after the final sucralose dose,

which showed a normal postprandial insulin response. Blood sucralose measurements were taken following the 10 mg/kg dose, which showed a peak concentration around 1 hour, which decreased afterwards until it was barely detectable after 24 hours. In the second study from this group, 108 participants were divided into two groups receiving either a sucralose (47 males, mean weight: 79 ± 11 kg; 30 females, mean weight: 60 ± 7 kg) or fructose (50 g/day) (17 males, mean weight: 73 ± 9 kg; 14 females, mean weight: 65 ± 7 kg) solution for 12 weeks. The group receiving sucralose had doses of 125 mg/day for weeks 1-3, 250 mg/day for weeks 4-7, and 500 mg/day for weeks 8-12. These doses of sucralose ranged between 4.8 to 8.0 mg/kg/day for males and 6.4 to 10.1 mg/kg/day for females, nearly all above the FDA ADI for sucralose. The researchers did not find any changes to fasting blood insulin or glucose between groups or within groups from the beginning to end of the study. One week after the last dose of sucralose, glucose and insulin responses to a 50 g sucrose load were similar between groups. There was also no accumulation of sucralose in the blood during prolonged daily exposure.

In another study investigating the effects of high doses of sucralose consumption per day, 48 males received either 1000 mg/day of sucralose or a cellulose placebo, both administered as capsules for 12 weeks (Binns, 2003). This amount of sucralose averaged 13.22 mg/kg/day across participants, well above the 5 mg/kg FDA ADI. Results from weekly blood samples showed that there was no affect throughout the study on fasting glucose, serum insulin, serum C-peptide, HbA1c, insulin secretion, or insulin sensitivity.

2.3.2.1.1.2 *Subjects with Type 2 Diabetes*

One hundred and twenty eight subjects with type II diabetes were investigated in a double-blind, placebo-controlled, randomized trial with daily dosing of sucralose for 13 weeks (Grotz et al., 2003). Subjects were separated into groups receiving sucralose (667 mg/day) (n = 67) or cellulose (n = 69) administered in capsules. This amount of sucralose was approximately 7.5 mg/kg/day, 50% more than the FDA ADI. Following the 13 weeks, all subjects received the cellulose capsules for 4 weeks. Blood samples were taken every two weeks to measure fasting plasma glucose, serum C-peptide, and HbA1c. The results showed no differences between any of the parameters measured throughout the 13-week trial. At the end of the 4-week follow-up with cellulose capsules, fasting plasma glucose was significantly lower in the sucralose group compared to baseline and to the cellulose group.

2.3.2.1.1.3 *Summary of Chronic Consumption*

Together, the results from these four studies show that consumption of sucralose up to 13 weeks, at concentrations in various ranges both within and above the FDA ADI, does not have any measurable alterations to biochemical measurements, specifically in regards to maintaining glucose homeostasis, in healthy subjects or subjects with diabetes. However, this data is in contradiction with findings from epidemiological studies, which indicate that chronic NNS consumption is associated with weight gain, type 2 diabetes, and metabolic syndrome (reviewed by Swithers, 2013).

It is important to note that many of the doses used in the aforementioned studies were much higher than the estimated daily intake values from the previous section, which, in fact, may be a limitation as opposed to a strength. It has been shown that at

high concentrations, some NNS, such as saccharine and acesulfame potassium (Ace-K), become antagonists to the sweet taste receptor and are perceived as more bitter than sweet (Galindo-Cuspinera et al., 2006). The suppression of sweet taste perception, along with bypassing the oral cavity by using capsules, does not provide study designs capable of determining the role that matching sweet taste with caloric value has on metabolic regulation. *Swithers and Davidson* have demonstrated that rats exposed to sweet tastes without a caloric value (consuming NNS) consumed more calories, expended less calories, and increased their adiposity and body weight (Swithers and Davidson, 2008). The reasoning behind this may be that animals predict the caloric value of food based on the accompanying taste perception, in this case sweetness. When this predictive relationship is no longer valid, it may diminish physiological responses responsible for regulating energy homeostasis.

Furthermore, in regards to the interaction between sucralose and the sweet taste receptor, using capsules as a route of administration for sucralose consumption does not mimic consumer use. It is not clear where the capsules dissolve and release sucralose, so it is unknown which post-oral sweet taste receptors may have also been bypassed. It is important for future studies investigating sucralose chronic consumption to make sure that the sucralose administered is perceived as sweet by the mouth and is not given in a capsule.

2.3.2.1.2 *Acute Consumption*

2.3.2.1.2.1 *Healthy Subjects*

2.3.2.1.2.1.1 *Intragastric Infusion of Sucralose Alone: Bypassing the Oral Cavity*

The studies included here include participants who acutely received sucralose alone through intragastric infusions, therefore eliminating the potential for cephalic phase responses related to the sweet taste of sucralose in the mouth.

Ma et al. administered four treatments of saline, 50 g sucrose, 80 mg sucralose, and 800 mg sucralose to seven normal-weight subjects (BMI: 21.6 ± 1.2 kg/m^2) on separate testing days in a randomized, single-blinded crossover study (Ma et al., 2009). Blood samples were taken at the time of infusion and up to 4 hours following to measure blood glucose, plasma insulin, GLP-1 and GIP. The results demonstrate that neither concentration of sucralose had effects different from the water treatment, whereas blood glucose, plasma insulin, GLP-1 and GIP all increased following the sucrose treatment.

In the other study utilizing administration through intragastric infusion, 6 males and 6 females, all normal-weight (BMI: 23.0 kg/m^2), received 6 different treatments (water, 169 mg aspartame, 220 mg Ace-K, 62 mg sucralose, 25 g fructose, and 50 g glucose) to investigate both NNS and caloric sweeteners (Steinert et al., 2011). Blood samples were taken at infusion and up to 2 hours afterwards to measure blood glucose, insulin, and glucagon and plasma GLP-1, peptide tyrosine tyrosine (PYY), and ghrelin. None of the NNS elicited responses different from water in regards to any of the parameters measured.

2.3.2.1.2.1.2 *Sucralose Consumption Alone*

Studies included here will administered sucralose without the addition of substantial calories to fasted normal-weight subjects. Seven females and one male participated in a randomized, single-blinded, crossover design (Ford et al., 2011). Subjects consumed or were sham-fed 41.5 mg of sucralose on two separate testing occasions. Neither condition elicited altered responses in plasma glucose, insulin, GLP-1, or PYY, nor measurements of appetite compared to a water control.

Six men and ten women, all without obesity, participated in a randomized, crossover, double-blind study receiving three different strawberry jams on separate testing days (Ibero-Baraibar et al., 2014). The jams were 60 g, one sweetened with naturally occurring sugars (25 g) and two with sucralose (15 and 17 mg). The results showed that jams containing sucralose did not raise blood glucose or insulin concentrations above baseline, while the sugar containing jams increased both.

Additionally, we have systematically reviewed and conducted a meta-analysis on the effects of all NNS consumption without calories, including sucralose, on measurements of fasting blood glucose (Nichol et al., 2018). The overall results indicated that sucralose consumption did not alter fasting glycemic concentrations at any measured time point. Overall, the data strongly suggest that acute exposure to sucralose without a substantial caloric load does not affect parameters related to glycemic control in fasted, non-obese, healthy subjects.

2.3.2.1.2.1.3 *Intraduodenal Infusion of Sucralose with a Caloric Load: Bypassing the Oral Cavity*

To the best of my knowledge, only a single study has utilized intraduodenal infusion as a vehicle of administration for sucralose and the preceding caloric load (Ma et al., 2010). Eight males and two females, all normal-weight (BMI: 23.4 ± 0.8 kg/m^2), received a total of 960 mg sucralose, or a saline control, by continuous fusion over 150 minutes. 30 minutes into the infusion, glucose and 3-O-methyl glucose (3-OMG) were infused at a rate of 1 calorie/min for the next 2 hours. Blood samples were taken for the entirety of the 150-minute infusion to measure blood glucose, GLP-1, and 3-OMG. For the 30 minutes prior to the caloric load, sucralose did not affect baseline measurements of blood glucose or GLP-1. Following the caloric load, the sucralose and saline treatments had similar increases to blood glucose, GLP-1, and 3-OMG concentrations.

2.3.2.1.2.1.4 *Sucralose Consumption with a Caloric Load*

The studies included here evaluated the effect that sucralose consumption has on postprandial glucose regulation following a caloric load in healthy subjects. Twenty two healthy subjects (BMI: 25.6 ± 4.6 kg/m^2) consumed either diet soda (sweetened with sucralose and Ace-K) or unflavored carbonated water ten minutes prior to an OGTT (Brown et al., 2009). The results showed that postprandial glucose and insulinemic responses were unchanged between the two treatments. However, the GLP-1 area under the curve (AUC) and peak were significantly greater after diet soda consumption compared to the carbonated water.

Eight healthy females (BMI: 22.2 ± 1.7 kg/m^2) took part in a randomized cross-over study with pre-load treatments of 6 g of Splenda (which contained ~72 mg of

sucralose and ~5.928 g of glucose and maltodextrin combined), 6 g Splenda in combination with 50 g sucrose, 50 g sucrose, or water (Brown et al., 2011). Each participant consumed the four treatments on separate testing days, with a breakfast of scrambled eggs, cheese, orange juice, and buttered toast given an hour after the treatment. Blood samples were taken at fasting, for an hour after the pre-load treatment, and for 2 hours following the breakfast consumption to measure blood glucose, insulin, glucagon, triglycerides, and ghrelin. Splenda consumption alone did not cause differences in any measured parameters compared to the water treatment at fasting (0-1 hours after pre-load) or post-prandial (0-2 hours after the breakfast meal) times. Additionally, Splenda consumption in combination with sucrose was no different from sucrose in any measured parameters after pre-load or breakfast consumption.

Ten healthy males (BMI: 25.5 ± 1.0 kg/m^2) received four different pre-load treatments as part of a randomized, single-blinded crossover study on four separate testing days (Wu et al., 2013). The treatments were water, 52 mg sucralose, 200 mg Ace-K, and 46 mg sucralose in combination with 26 mg Ace-K, which were all consumed 10 minutes prior to a 75 g OGTT. Blood samples were taken prior to the pre-load and up to four hours after the OGTT to measure blood glucose, insulin, and GLP-1. The results showed that none of the sweetener treatments were different from water in terms of any of the parameters measured.

In a randomized, single-blinded crossover trial, eight subjects (four male, four female) with an average BMI of 30.3 ± 4.5 kg/m^2 were administered 3 pre-load treatments (200 mL water, 24 mg sucralose in solution, or 72 mg aspartame in solution) 15 minutes prior to a 75 g OGTT (Temizkan et al., 2015). Blood samples were taken

prior to pre-load ingestion and up to two hours after the OGTT to measure blood glucose, insulin, C-peptide, and GLP-1. The results indicated that on the day of sucralose consumption, blood glucose concentrations peaked 15-30 minutes earlier, blood glucose AUC was lower, and the GLP-1 AUC was greater compared to water. Insulin and C-peptide concentrations and AUCs were similar between the sucralose and water conditions.

Ten healthy subjects (seven males, three females; BMI: 25.5 ± 1.5 kg/m^2) participated in a single-blind, randomized crossover trial testing the effects of four pre-load treatments on post-prandial glycemia and gastric emptying (Wu et al., 2011). The treatments consisted of 60 mg sucralose, 40 g glucose, 40 g 3-OMG, or 40 g tagatose/isomalt mixture (TIM), which were consumed 15 minutes prior to a meal containing potatoes, glucose, and egg yolk. Blood samples were taken before the pre-load and up to four hours post-meal ingestion to measure blood glucose, serum insulin, plasma GLP-1 and GIP. Sucralose and TIM did not affect any of the parameters for the 15 minutes between the pre-load and meal, while glucose increased all measurements and 3-OMG increased only plasma GLP-1 and GIP. Following the meal, blood glucose, was significantly lower on the day of sucralose and TIM consumption compared to glucose, and sucralose was significantly lower than 3-OMG. Postprandial serum insulin incremental AUC (iAUC) was greater following the glucose pre-load compared to all others. Plasma GLP-1 was significantly lower on the day of sucralose consumption compared to all other pre-loads following the meal. Postprandial plasma GIP was significantly lower for both the sucralose and TIM pre-loads compared with glucose and 3-OMG.This study did lack a water control to better evaluate the effects of sucralose on

the measured metabolic responses, but the results presented here show differences between sucralose and caloric sweeteners.

Sylvetsky et al. designed a randomized crossover study to determine the effects of sucralose consumed in water (study arm 1, n = 30, BMI: 25.8 $\pm$ 4.2 kg/m^2 and consumption of sucralose-containing sodas (study arm 2, n = 31, BMI: 26.3 $\pm$ 7.5 kg/m^2) 10 minutes prior to a 75 g OGTT (Sylvetsky et al., 2016). Blood samples in both study arms were taken prior to the pre-load consumption and up to 2 hours following the OGTT to measure blood glucose, insulin, C-peptide, GLP-1, and 3-OMG. Both study arms also had subjects come on four separate testing days to receive the treatments. In study arm 1, the 4 pre-load treatments contained water, 68 mg sucralose, 170 mg sucralose, and 250 mg sucralose. The results showed that none of the concentrations of sucralose were different from water in regards to any of the measured parameters. In study arm 2, the 4 pre-load treatments were carbonated water, diet cola (68 mg sucralose, 41 mg Ace-K, and other ingredients), diet lemon-lime soda (18 mg sucralose, 18 mg Ace-K, 57 mg aspartame, and other ingredients), and carbonated water (68 mg sucralose and 41 mg Ace-K). The results showed that the carbonated water sweetened with sucralose and Ace-K was no different from the plain carbonated water in any of the measured parameters. Each of the three treatments containing NNS increased insulin AUC by 22-25% compared to the water control, but did not reach statistical significance. Diet cola did demonstrate a higher GLP-1 AUC compared to carbonated water, which indicates that this may have been due to other ingredients found in the soda aside from the artificial sweeteners. Whether the rise in GLP-1 AUC is due to the other ingredients alone, or an interaction between certain ingredients and the NNS, was not determined.

Pepino and collaborators performed a randomized crossover study in people with obesity (BMI: 41.0 $\pm$ 1.5 kg/m^2) who were considered healthy (not diagnosed with diabetes and a homeostatic model assessment for insulin resistance (HOMA-IR) < 2.6) and were non-regular consumers of NNS (Pepino et al., 2013). Subjects (15 females and two males) ingested pre-load treatments of water or 68 mg sucralose ten minutes prior to a 75 g OGTT on two separate testing days. Blood samples were taken prior to the pre-load treatments and up to 5 hours following the OGTT to measure plasma glucose, insulin, C-peptide, glucagon, GLP-1 and GIP. They found that concentrations of glucose, insulin, and C-peptide in plasma reached higher peaks and insulin AUC was greater on the day of sucralose consumption compared to water.

2.3.2.1.2.2 *Subjects with Diabetes*

2.3.2.1.2.2.1 *Sucralose Consumption with a Caloric Load*

A follow-up study was conducted based on the findings from *Brown et al.* 2009 (Brown et al., 2012). Using a similar study design (as previously mentioned), subjects included in this study were diagnosed with type 1 (n = 9, BMI: 21.7 $\pm$ 2.4 kg/m^2) or type 2 (n = 10, 35.0 $\pm$ 6.8 kg/m^2) diabetes. The authors did not indicate whether subjects with type 1 diabetes continued their ongoing insulin treatment during the study. The results in these participants showed that subjects with type 1 diabetes had an increase in GLP-1 AUC following ingestion of diet soda compared to carbonated water that was similar to that observed in their previous study in healthy subjects. However, the consumption of the diet soda failed to modify any of the parameters measured in subjects with type 2 diabetes.

A second subject pool including people with type 2 diabetes was included in the study conducted by *Temizkan et al.* (Temizkan et al., 2015). Four males and four females (BMI: 33.7 $\pm$ 5.4 kg/m^2) participated in the study, following the aforementioned design. The results here indicated that blood glucose, insulin, C-peptide, and GLP-1 AUCs were not different between the sucralose and water treatments.

In a randomized, double-blind crossover study, subjects with type 1 diabetes (eight males, five females, BMI: 32.0 $\pm$ 1.9 kg/m^2) and type 2 diabetes (eight males, five females, BMI: 23.7 $\pm$ 0.9 kg/m^2) ingested, by capsules, both a cellulose control and 1000 mg sucralose on two separate testing occasions (Mezitis et al., 1996). Subjects with type 1 diabetes received their usual insulin or sulfonylurea dose 30 minutes before receiving their capsules. Immediately after consumption, participants received a liquid breakfast comprised of 360 calories. Blood samples were taken prior to and up to 4 hours following any consumption to measure blood glucose and serum C-peptide. The results indicated that both groups showed similar results across all parameters between the sucralose and cellulose control treatments.

2.3.2.1.2.3 *Summary of Acute Consumption*

The studies presented here indicate that, regardless of route of administration, sucralose alone does not alter any measured parameters related to fasting glucose regulation in healthy participants. A study has yet to be conducted investigating the effect of acute consumption of sucralose alone including subjects with diabetes. Diet cola consumed prior to an OGTT caused a greater post-prandial GLP-1 AUC in non-obese, healthy subjects (Brown et al., 2009, Sylvetsky et al., 2016) and non-obese, type 1 diabetics (Brown et al., 2012), but not in subjects with obesity and type 2 diabetes

(Brown et al., 2012). The increases in GLP-1 AUC were not replicated in carbonated water sweetened with the same amounts of sweetener as the diet cola (Sylvetsky et al., 2016); however, the results from *Temizkan et al.* demonstrate that post-prandial GLP-1 AUC was greater after a sucralose pre-load compared to water in non-obese, healthy subjects, but not subjects with obesity and type 2 diabetes (Temizkan et al., 2015). Furthermore, the findings from *Sylvetsky et al.* and our group (Pepino et al., 2013) suggest that a pre-load of sucralose (in diet soda or in water alone) increases post-prandial plasma insulin AUC in non-diabetic normal-weight subjects and subjects with obesity.

Overall, the data summarized here on the effects that sucralose consumption may have on post-prandial glucose regulation is discrepant. It is unclear whether differing results are due to being normal-weight vs. obese or insulin sensitive vs. resistant. Increases in post-prandial GLP-1 AUC were observed in non-obese, insulin sensitive subjects (Brown et al., 2009, Sylvetsky et al., 2016, Temizkan et al., 2015), but not insulin sensitive subjects with obesity (Pepino et al., 2013) or subjects with obesity and type 2 diabetes (Brown et al., 2012, Temizkan et al., 2015). Post-prandial plasma insulin AUC was increased in both insulin sensitive normal-weight subjects (Sylvetsky et al., 2016) and insulin sensitive subjects with obesity (Pepino et al., 2013). Nonetheless, a majority of the studies presented in this review found no effect of sucralose consumption on any parameters related to post-prandial glucose control in normal-weight subjects.

Based on the overall review of the literature, we are interested in the effects that sucralose has on post-prandial glycemic control in people with obesity who are insulin

sensitive. People with obesity are of particular interest because there are fewer studies including this population, more people with obesity use NNS compared to their normal-weight counterparts (36% vs 22%, respectively) (Sylvetsky et al., 2012), and data from animal models suggest that subjects with obesity may be most vulnerable to the effects of NNS consumption (Swithers et al., 2013). Additionally, we believe it is important to control for prior NNS use because of data from animal models suggesting chronic NNS use is associated with increases in active (Margolskee et al., 2007, Moran et al., 2010, Stearns et al., 2010) and passive glucose absorption (Mace et al., 2007). Therefore, we would expect frequent users to have greater glycemic responses to a glucose load than non-users, and would have a blunted response to a sucralose pre-load (experimental condition) due to the similarities between the experimental condition and control condition (water pre-load) in this population. Correspondingly, our current study was designed to investigate the impact of sucralose consumption, and of its sweet taste alone, on post-prandial glucose regulation in both normal-weight subjects and subjects with obesity, all without significant insulin resistance and non-regular consumers of NNS.

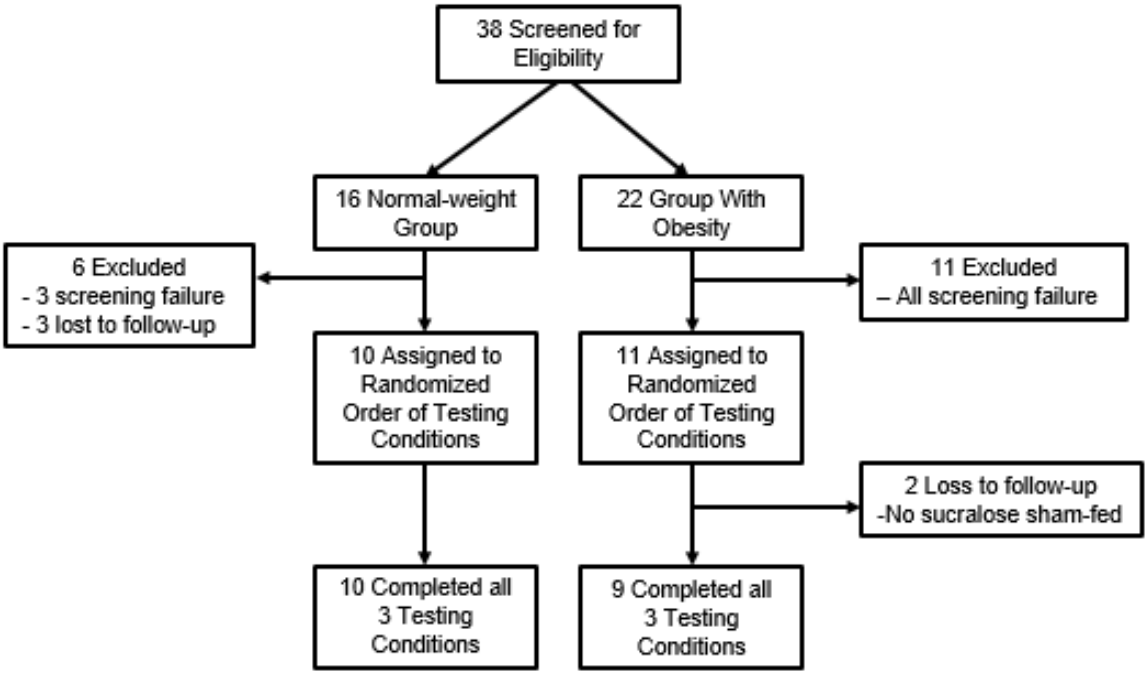
CHAPTER 3: RESEARCH DESIGN AND METHODS

3.1 *Subjects*

Thirty-eight potential participants (16 normal-weight subjects and 22 subjects with obesity) enrolled in the study and completed a screening visit that included a 2 hour OGTT as part of a comprehensive medical evaluation, and a detailed questionnaire used in previous studies that inquired about typical NNS use (Klein et al., 2006, Pepino et al., 2013). Subjects with a fasting plasma glucose concentration ≥ 7 mmol/L, a 2-hour post glucose load plasma glucose concentration ≥ 11.1 mmol/L, or significant insulin resistance based on a HOMA-IR score > 3, were excluded from participation. Also, those who were regular consumers of NNS (i.e., reported consuming more than one diet beverage per week, one spoonful of NNS per week, or an equivalent amount of NNS in foods); smoked tobacco cigarettes in the past six months; were pregnant or breastfeeding; had a history of malabsorptive syndromes, bariatric surgery, or inflammatory intestinal disease; or were taking any medication which may affect glucose metabolism were excluded. Out of 38 subjects, 14 failed screening, 3 were lost to follow-up after their screening visit, and 2 did not complete one of the study visits (Figure 1). Data are therefore presented for the 9 subjects with obesity and 10 normal-weight subjects who completed the study (Table 1). This study was approved by the institutional review board at Washington University School of Medicine in St. Louis, and all screened subjects gave informed written consent before participation.

Subjects were evaluated on three study visits separated by ~ 1 week with a crossover-randomized design. For each study visit, subjects were admitted in the

Figure 1. Subject Recruitment Flowchart



Flowchart showing the number of participants screened for eligibility, the amount excluded in each group, and the amount lost to follow up. The bottom two boxes show that we had ten normal-weight subjects and nine subjects with obesity included in our final data set.

Table 1. Subject Characteristics

	Normal-weight	Obese
Age (years)	27.0 (4.2)	29.9 (4.1)
Weight (kg)	66.0 (10.7)	103.7 (17.0)*
BMI (kg/m^2)	22.8 (0.9)	37.7 (6.1)*
Female/Male	7/3	8/1
Race		
White	7 (70%)	4 (44%)
Black	2 (20%)	4 (44%)
Other	1 (10%)	1 (11%)
Plasma concentrations		
Fasting Glucose (mmol/L)	5.0 (0.2)	5.1 (0.4)
Fasting Insulin (pmol/L)	41.4 (18.7)	84.8 (36.7)*
Fasting C-Peptide (nmol/L)	0.5 (0.1)	0.7 (0.2)*
Fasting GIP (pmol/L)	7.7 (4.1)	11.2 (5.8)

Data are mean (SD), with the exception of race data which are n (%)
* denotes a significant difference from normal-weight ($p < 0.05$)

morning to the Clinical Research Unit at Washington University School of Medicine at 0700 h. Subjects were instructed to fast overnight (12 hours) at home and to avoid physical exercise for 3 days before each study visit. After obtaining vital signs, one catheter was inserted into a forearm vein for infusion, and a second catheter was inserted into a radial artery to obtain blood samples. A primed, continuous infusion of [6,6-$^{2}H_{2}$] glucose (priming dose, 22 µmol/kg; infusion rate of 0.22 µmol/kg*min) was started and maintained until the end of the study. After 3.5 hours of tracer infusion, subjects ingested a 75 g OGTT that included 1.5 g of [U-$^{13}C_{6}$] glucose. Blood samples were obtained at 40, 30, 20, 10, 8, 6, 4, and 2 minutes before and at 0, 10, 20, 30, 40, 50, 60 minutes after ingestion of glucose. Following 1-hour post-ingestion, blood samples were taken every 20 minutes for four hours to determine glucose kinetics and plasma hormone and sucralose concentrations. In a randomized order, subjects drank 60 mL of 2 mmol/L sucralose (i.e., 48 mg sucralose) (sucralose ingestion condition) or an equivalent volume of distilled water (water condition), or tasted the same concentration of sucralose and expectorated (sucralose sham-fed condition) 10 minutes prior to glucose ingestion.

3.2 *Rationale for Dose of Sucralose Chosen*

The dose of sucralose was chosen to replicate the amount used in the previous study (Pepino et al., 2013). The logic underlying the selection of this sucralose concentration is that 2 mmol/L of sucralose effectively stimulated incretin release in human intestinal cells in vitro (Jang et al., 2007), and 48 mg closely matches the total amount of sucralose present in 8-ounces of diet soda if it was sweetened exclusively with sucralose (Sucralose, 2017).

3.3 *Rationale For Choice of Primary Metabolic Outcomes*

Pepino and collaborators have shown that the acute ingestion of sucralose increases peak plasma glucose, plasma C-peptide and insulin concentrations, and insulin AUC after an OGTT, while there was a trend for GIP concentrations to be elevated (Pepino et al., 2013). Therefore, insulin was selected because 1) it is a primary regulatory hormone for plasma glucose concentrations and 2) we are interested in determining if the results previously reported are replicable in people with obesity and generalizable to normal-weight people. C-peptide is also an important outcome because it is secreted in equimolar amounts to insulin and is not metabolized by the liver. Unlike insulin, which undergoes a substantial and variable amount of hepatic extraction during first pass metabolism (Polonsky and Rubenstein, 1984), C-peptide is removed from circulation, by renal uptake and filtration, at a more constant rate which is preserved during fasting and post-prandial conditions (Licinio-Paixao et al., 1986). Therefore, changes to C-peptide concentrations better represent changes to insulin secretion than insulin concentrations alone because changes to insulin concentrations can be largely due to the variability of hepatic extraction. GIP was selected as an outcome variable because there was a trend for sucralose to increase concentrations after an OGTT, which suggests that GIP, an incretin, may have contributed to the increase in glucose-stimulated insulin secretion. Additionally, we measured for plasma sucralose concentrations to see if different metabolic responses to sucralose between people with obesity and normal-weight people were due to different amounts of sucralose absorbed by each group.

3.4 *Biochemical Measurements*

At all time-points, plasma glucose was measured immediately after collection by using an automated glucose analyzer (YSI 2300 STAT plus; Yellow Springs Instruments, Yellow Spring, OH). Blood samples were also collected in chilled EDTA tubes containing a protease inhibitor cocktail (Millipore, Billerica, MA). These samples were placed on ice and centrifuged at 4^{o}C and the plasma was stored at -80^{o}C for subsequent analyses. Plasma insulin and C-peptide concentrations were measured at the same time-points as plasma glucose up to 60 minutes post-glucose ingestion. Following the 60-minute post-ingestion, they were measured every 40 minutes until the end of the test. Plasma insulin concentrations were determined by using a two-site immunoenzymatic assay (DxI 800; Beckman Instruments, Chaska, MN), and C-peptide by using a solid-phase two-site chemiluminescent immunometric assay (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Plasma GIP was measured at 10 and 2 minutes before and at 0, 10, 30, 50, 60, 100, 140, and 180 minutes after glucose consumption by using commercially available immunoassay kits from Millipore (Billerica, MA). The glucose tracer-to-tracee ratio in plasma was determined using gas chromatography-mass spectrometry (Hewlett-Packard MSD 5973 system with a capillary column) after derivatizing glucose with acetic anhydride.

Plasma sucralose concentrations were measured right before and at 10, 40, 70, 130, 190, and 310 minutes after sucralose consumption by using liquid chromatography-mass spectrometry (Sylvetsky et al., 2017a). Sucralose was first extracted from plasma by vortexing a 50 µL aliquot of plasma and 500 µL methanol containing D_6-sucralose internal standard for 5 minutes. The tube was then centrifuged

at 14,000 RPM (revolutions per minute) for 10 minutes. Supernatant (300 µL) was transferred to a high-performance liquid chromatography (HPLC) vial and sealed. Assays were performed with an Acquity I-Class UPLC (Waters Corp., Milford MA, USA) and an Acquity UPLC BEH C-18 column (2.1 mm x 50 mm, 1.7 µm) coupled with a Q-Exactive MS (Thermo Scientific, Waltham, MA, USA) with an HESI-II electrospray source. The relative standard deviation across all samples and replicates was < 4%.

3.5 *Calculations*

The AUCs and iAUCs were calculated using the trapezoid method (Allison et al., 1995). HOMA-IR values were calculated using the HOMA2 calculator (available from www.OCDEM.ox.ac.uk). Total glucose rate of appearance (total Ra) was calculated using Steele's equation for non-steady state conditions (Steele, 1959). Oral rate of glucose appearance (oral Ra), endogenous glucose production (EGP), and rate of glucose disappearance (Rd) were calculated as previously described (Gastaldelli et al., 2007). For glucose kinetics statistical analyses, we included data collected between 0 and 180 minutes post glucose ingestion, instead of 0 and 300 minutes, due to cumulative errors in the model beyond 180 minutes.

3.6 *Statistical Analyses*

Differences between groups for demographics, measurements of fasting outcome variables, plasma sucralose peak, time to peak, and iAUC were determined using an unpaired t-test. To determine whether plasma sucralose concentrations were higher in subjects with obesity than in normal-weight subjects, we used a repeated measure ANOVA with time as a within factor and group as a between factor. We did not have data on plasma sucralose concentrations from one subject with obesity, so

assessments conducted for this outcome variable include eight subjects with obesity and ten normal-weight subjects.

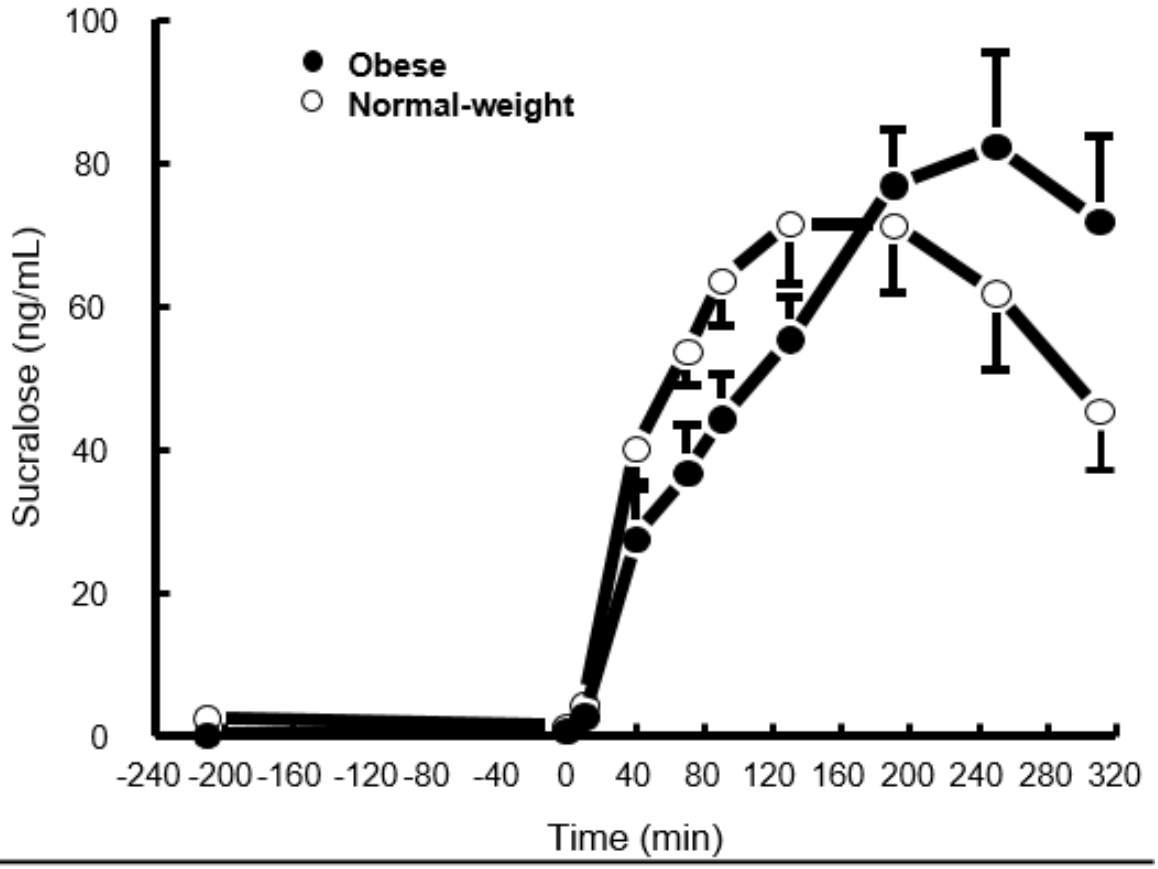
The statistical significance of the effect of tasting and ingesting sucralose (or just tasting sucralose) on plasma glucose, insulin, C-peptide, and GIP concentrations, as well as glucose kinetics (total glucose Ra, oral glucose Ra, EGP, and glucose Rd), during the OGTT in normal-weight subjects and subjects with obesity was determined by conducting separate repeated ANOVAs for each group and each outcome variable including condition (water, sucralose-ingestion, and sucralose sham-fed) and time-point as within-subject factors. For the same outcome variables, repeated measures ANOVAs were also conducted for the AUCs, iAUCs, and incremental peaks with condition (water, sucralose-ingestion, and sucralose sham-fed) as within subject factor. If the sphericity assumption of the analysis of variance was violated, the Huynd-Feldt correction was used. When ANOVAs revealed significant effects, *post-hoc* Fisher Least Significant Difference analyses were conducted. Based on data from our previous study in subjects with obesity (Pepino et al., 2013), we tested the a priori hypothesis that the iAUC of insulin was greater after sucralose ingestion than after the water control using planned comparisons in each group. Data in the tables and figures are presented as means and standard errors of the mean (SEM), unless otherwise stated. All analyses were performed with STATISTICA 13.2 (TIBCO Software Inc., Palo Alto, CA) and criterion for statistical significance was $p \leq 0.05$.

CHAPTER 4: RESULTS

4.1 *Subject Characteristics and Plasma Sucralose Concentrations*

There were no significant differences in age, sex, and race distribution between groups (Table 1). Although fasting plasma glucose concentrations were not significantly different between groups ($t_{(17)}$ = -0.69, p = 0.50), fasting plasma insulin concentrations ($t_{(17)}$ = -3.30, p = 0.004) and HOMA-IR ($t_{(17)}$ = -4.38, p < 0.001) were significantly higher in the group with obesity than in the normal weight group (Table 1). The shape of the plasma sucralose concentration versus time curve tended to be different between groups ($F_{(2,30)}$ = 3.14, p = 0.06), with higher sucralose concentrations during the last hour of the test in subjects with obesity than in normal-weight subjects (Figure 2). However, peak sucralose concentrations, the time to reach peak sucralose concentration, and plasma sucralose iAUCs were not significantly different between groups (all p-values > 0.19; Table 2).

Figure 2. Plasma Sucralose Concentrations



Mean plasma sucralose concentrations in normal-weight subjects and subjects with obesity following sucralose ingestion at time 0. Data are the mean ± SEM for eight subjects with obesity and ten normal-weight subjects.

Table 2. Hormonal Responses, Glucose Kinetics, and Plasma Sucralose

	Normal-weight			Obese		
	Water	Sucralose ingestion	Sucralose sham-fed	Water	Sucralose ingestion	Sucralose sham-fed
Metabolic responses						
Incremental peak						
Glucose (mmol/L)	5.7 ± 0.4	5.4 ± 0.5	5.5 ± 0.4	4.7 ± 0.4	5.0 ± 0.4	4.5 ± 0.4
Insulin (pmol/L)	683 ± 131	548 ± 92	590 ± 115	1030 ± 123	1141 ± 185	1041 ± 184
C-Peptide (nmol/L)	2.8 ± 0.3	2.6 ± 0.3	2.8 ± 0.3	3.4 ± 0.2	3.7 ± 0.3	3.6 ± 0.3
GIP (pmol/L)	49.5 ± 6.8	45.0 ± 6.0	48.5 ± 5.5	58.4 ± 3.7	61.7 ± 6.2	56.0 ± 4.4
iAUC						
Glucose (mmol/L)*300 min	470 ± 56	560 ± 66	491 ± 60	413 ± 63	499 ± 48[#]	455 ± 59
Insulin (pmol/L)*300 min	65356 ± 13104	60656 ± 10187	59668 ± 10639	121550 ± 20278	143412 ± 28588[#]	128246 ± 25681
C-Peptide (nmol/L)*300 min	375 ± 40	394 ± 43	375 ± 45	488 ± 53	564 ± 50[*]	541 ± 43
GIP (pmol/L)*300 min	6736 ± 1070	6238 ± 975	6425 ± 742	7538 ± 643	7566 ± 643	7221 ± 720
Glucose Kinetics						
Incremental Peak						
Total Ra (µmol/min)	2415 ± 253	2464 ± 336	2198 ± 313	2691 ± 230	2734 ± 238	2552 ± 229
Oral Ra (µmol/min)	2697 ± 194	2566 ± 226	2619 ± 272	3254 ± 253	3227 ± 266	3094 ± 244
Rd (µmol/min)	2185 ± 289	2071 ± 280	2169 ± 297	1857 ± 203	1750 ± 152	1656 ± 149
Nadir						
EGP (µmol/min)	93.1 ± 39.5	74.2 ± 50.5	67.9 ± 39.5	0	0	0
AUC						
Total Ra (µmol/min)*180 min	316178 ± 22105	307314 ± 22501	336264 ± 28450	298426 ± 7840	296648 ± 11129	284548 ± 9769
Oral Ra (µmol/min)*180 min	263720 ± 17303	260716 ± 10319	288719 ± 21131	273059 ± 9715	278233 ± 11272	265530 ± 11220
Rd (µmol/min)*180 min	317343 ± 26019	300479 ± 26382	333157 ± 31326	290588 ± 15205	286916 ± 14422	273900 ± 16371
Plasma Sucralose						
Incremental Peak (ng/ml)	-	78.6 ± 8.4	-	-	99.7 ± 13.0	-
Time to Peak (min)	-	143 ± 19	-	-	183 ± 26	-
*iAUC (ng/L*300 min)*	-	16997 ± 1975	-	-	18090 ± 1582	-
Total Oral Glucose Appearing in Circulation (g)	52.0 ± 2.6	53.5 ± 1.3	59.2 ± 3.5	52.3 ± 1.9	53.4 ± 2.1	53.5 ± 2.3

Data are mean ± SEM for nine subjects with obesity and ten normal-weight subjects.
* indicates a significant difference from water control ($p < 0.05$)
[#] indicates a trend towards a difference from water control ($p < 0.10$)

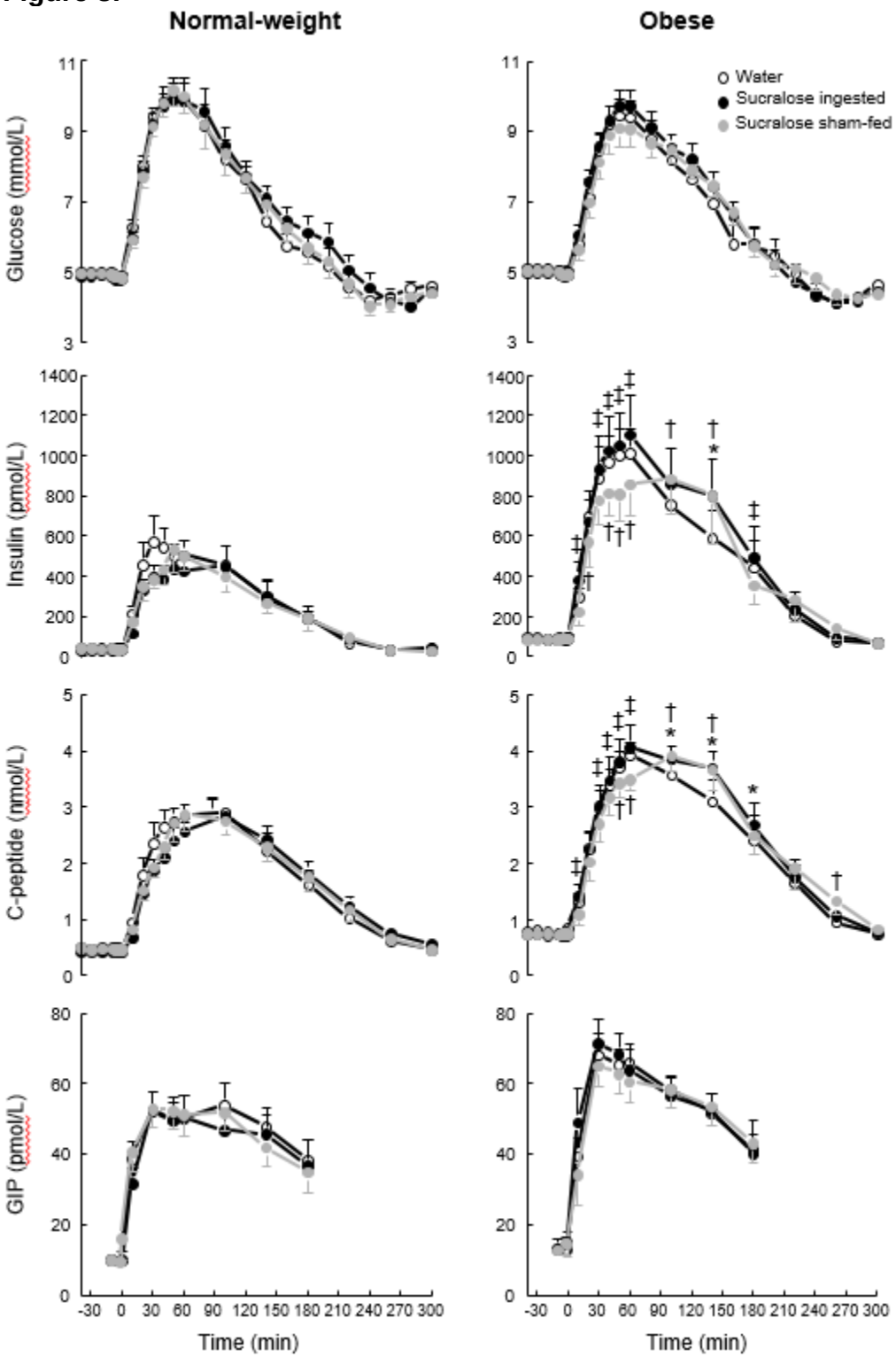
4.2 *Plasma Glucose and Hormone Concentrations Within Groups*

In the normal-weight group, neither sucralose ingestion nor sucralose sham-fed conditions were different from water in regards to mean plasma concentrations, iAUCs, and incremental peaks for glucose, C-peptide, and GIP (Table 2, Figure 3, Figure 4). However, there was a trend for the average plasma insulin concentrations over time to be lower on the sucralose ingestion and sucralose sham-fed conditions compared to water ($F_{(2,18)}$ = 2.73, p = 0.09; Figure 3).

In the group with obesity, sucralose ingestion increased the plasma glucose iAUC by 34 ± 12 % compared to water, but the differences between conditions did not reach statistical significance ($F_{(2,14)}$ = 3.66, p = 0.06; Table 2, Figure 4). There were no differences to mean or incremental peak plasma glucose concentrations between conditions (Table 2, Figure 3). Compared to the water and sucralose ingestion conditions, sucralose sham-fed decreased plasma insulin concentrations within the first hour of the OGTT in the group with obesity ($F_{(9,72)}$ = 2.01, p = 0.05; Figure 3). Similarly, sucralose-sham fed decreased C-peptide concentrations compared to the water and sucralose ingestion conditions from 50 to 60 minutes ($F_{(13,105)}$ = 2.23, p = 0.01; Figure 3). Compared to water, sucralose ingestion increased C-peptide iAUC by 22 ± 10% ($F_{(2,16)}$ = 3.85, p < 0.05; Fisher LSD p < 0.02) and insulin iAUC by 16 ± 9%, although for insulin iAUC, the difference between conditions only tended to approach statistical significance ($F_{(1,8)}$ = 3.67, p = 0.09) (Table 2, Figure 4). In the group with obesity, the same seven out of nine subjects had greater insulin and C-peptide iAUCs after sucralose consumption than after water consumption (Figure 5). There were no

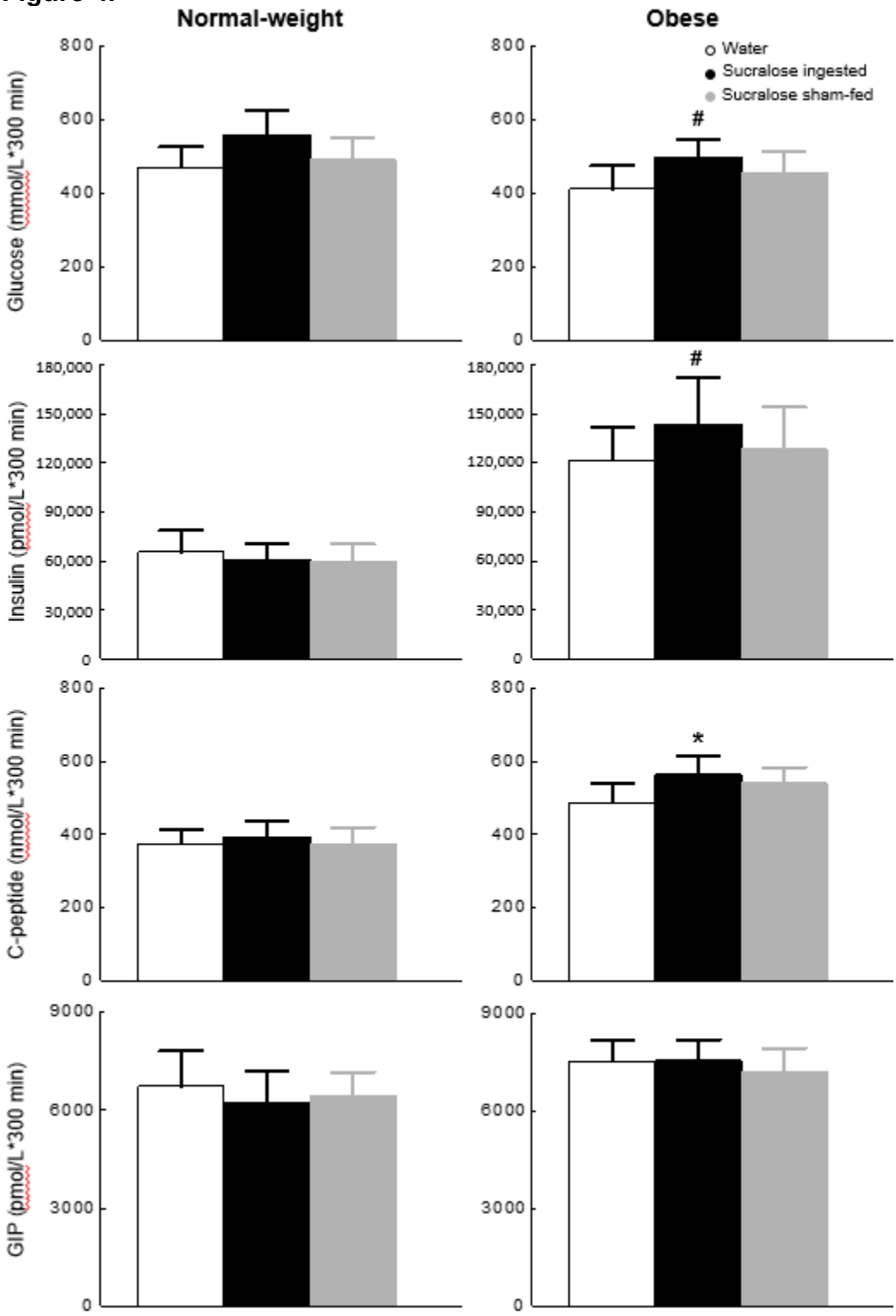
significant differences for GIP mean concentrations, iAUCs, or incremental peaks between conditions within the group with obesity (Table 2, Figure 3, Figure 4).

Figure 3.



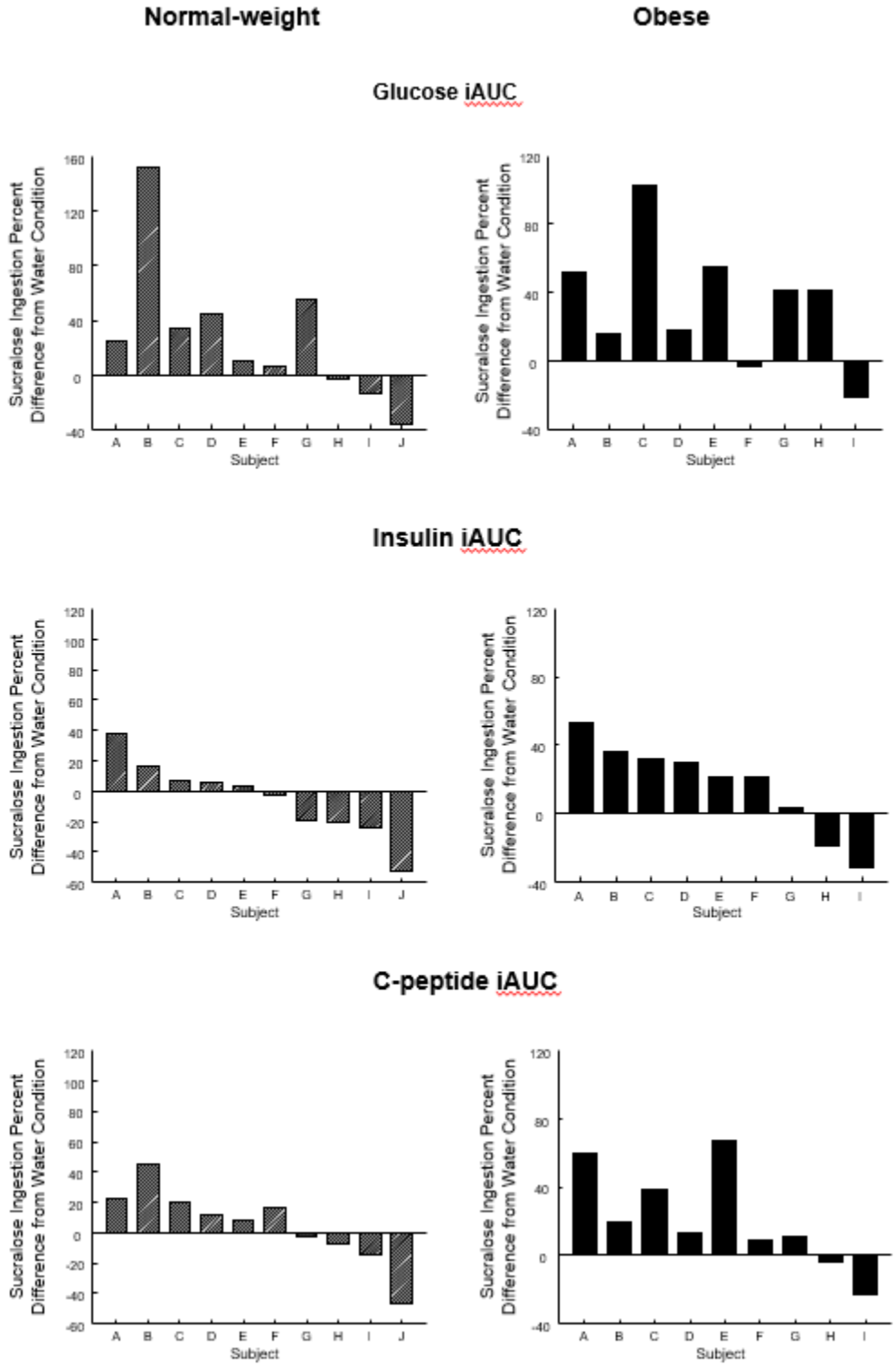
Data are mean ± SEM for plasma glucose, insulin, C-peptide, and glucagon concentrations in ten normal-weight subjects and nine subjects with obesity after consuming water or sucralose, or being sham-fed sucralose, 10 minutes prior to an OGTT (given at time 0). * indicates a significant difference between sucralose ingestion and water, † indicates a significant difference between sucralose sham-fed and water, ‡ indicates a significant difference between sucralose ingestion and sucralose sham-fed ($p \leq 0.05$).

Figure 4.



Data are the mean ± SEM of the incremental area under the curves for glucose, insulin, C-peptide, and GIP in ten normal-weight subjects and nine subjects with obesity after consuming water or sucralose, or being sham-fed sucralose, 10 minutes prior to an OGTT. * indicates a significant difference between water and sucralose ingestion conditions ($p < 0.05$). # indicates a trend towards a difference between water and sucralose ingestion conditions ($p < 0.10$).

Figure 5.



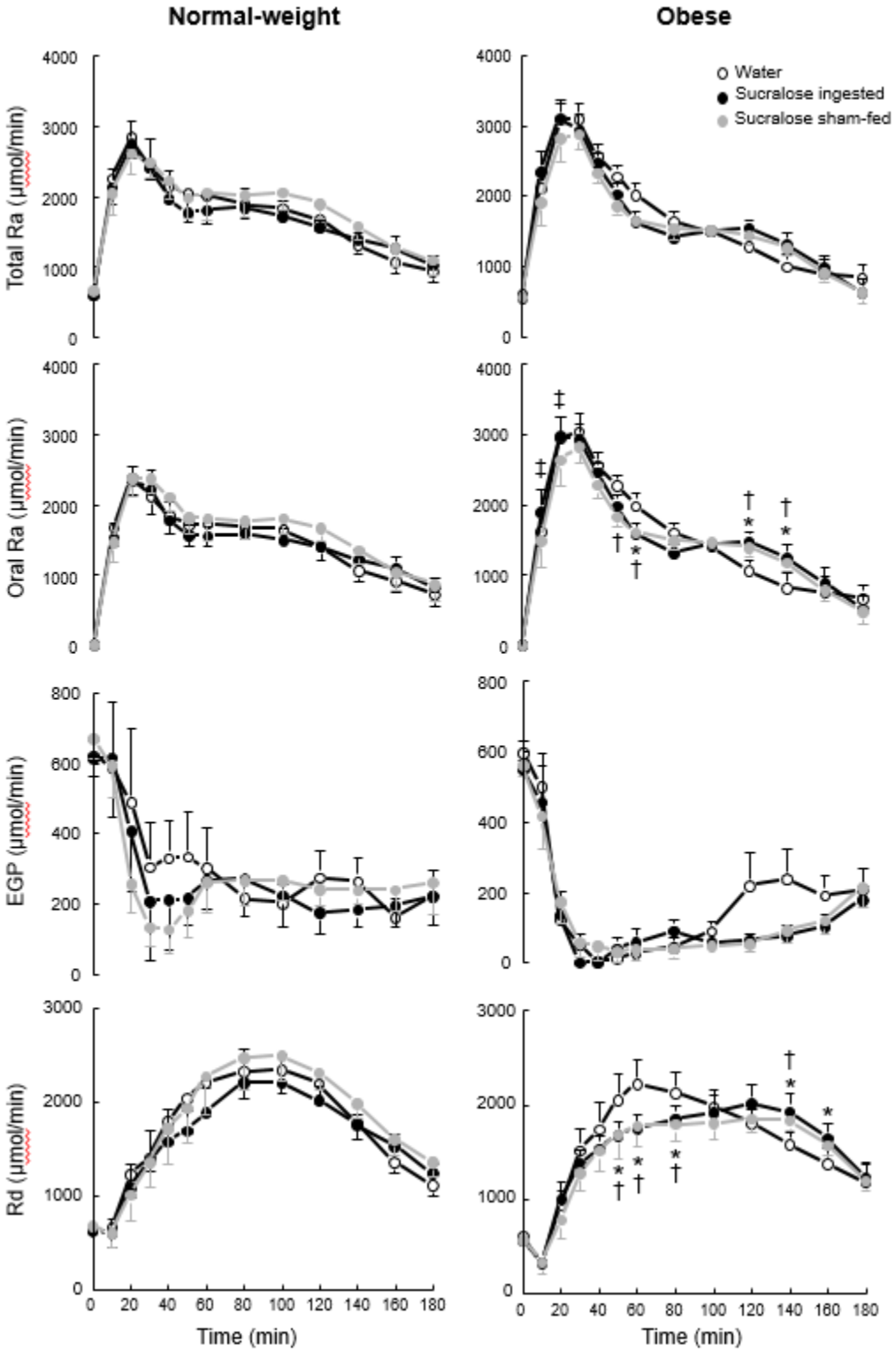
Percent changes to glucose, insulin, and C-peptide iAUCs between water and sucralose ingestion conditions in ten normal-weight subjects and nine subjects with obesity. Bars above the 0 line indicate that there was a positive change between conditions.

4.3 *Glucose Kinetics*

There were no differences to the time point curves, AUCs, or incremental peaks between conditions for total Ra, oral Ra, or Rd, nor the time point curves and nadirs for EGP, in this group (Table 2, Figure 6, Figure 7).

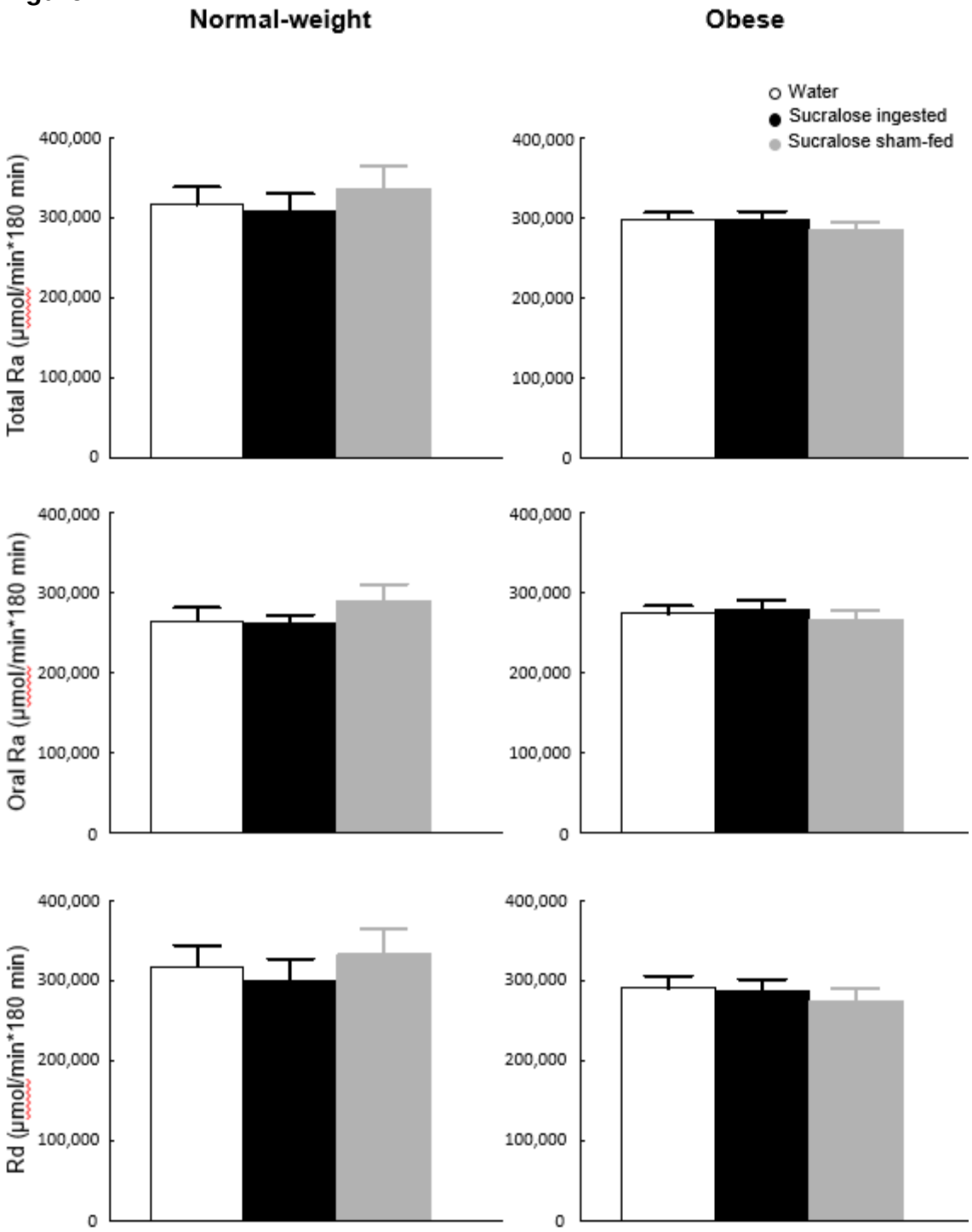
Unlike that observed in normal-weight subjects, condition did affect total and orally derived glucose rates of systemic appearance and total glucose disappearance in subjects with obesity. The average total Ra across time points 0-180 min was lower following sucralose sham-fed than water ($F_{(2,16)}$ = 3.60, p = 0.05; Fisher LSD p = 0.02; Figure 6), and there were significant condition by time interactions for oral glucose Ra ($F_{(12,95)}$ = 1.99, p = 0.03) and for glucose Rd ($F_{(6,51)}$ = 2.27, $p < 0.05$). Post-hoc analyses of these interactions revealed that, compared to water, both sucralose conditions lowered oral Ra at 60 minutes and increased oral Ra from 120-140 minutes. They also caused a lower Rd from 50-80 minutes and a higher Rd at 140 minutes, but only sucralose ingestion maintained the higher Rd until 160 minutes (Figure 6). There were no differences between conditions in regards to EGP.

Figure 6.



Data are mean ± SEM rates of total Ra, oral Ra, EGP, and Rd in ten normal-weight subjects and nine subjects with obesity after consuming water or sucralose, or being sham-fed sucralose, 10 minutes prior to an OGTT (given at time 0). * indicates a significant difference between sucralose ingestion and water, † indicates a significant difference between sucralose sham-fed and water, ‡ indicates a significant difference between sucralose ingestion and sucralose sham-fed ($p \leq 0.05$)

Figure 7.



Data are the mean ± SEM of the area under the curves for total glucose Ra, oral glucose Ra, and glucose Rd in ten normal-weight subjects and nine subjects with obesity after consuming water or sucralose, or being sham-fed sucralose, 10 minutes prior to an OGTT.

CHAPTER 5: DISCUSSION

Our results indicate that the acute ingestion of sucralose compared to water, prior to an OGTT, increased post-prandial plasma C-peptide concentrations and tended to increase post-prandial plasma insulin concentrations in people with obesity who were not regular consumers of NNS, but did not alter plasma C-peptide concentrations and tends to decrease average plasma insulin concentrations in normal-weight people. The group with obesity also experienced lower plasma insulin and C-peptide concentrations during the sucralose sham-fed condition for the first 60 minutes and from 50-60 minutes, respectively, after the OGTT. Following sucralose consumption, plasma glucose iAUC during the OGTT tended to be greater compared to the water condition only in the group with obesity. These results suggest that both the ingestion of sucralose and its taste alone alter glycemic and insulinemic responses to an oral glucose load in people with obesity.

The finding that the group with obesity experienced a trend of elevated plasma glucose iAUC compared to water on the day of sucralose ingestion, but not the day of sucralose sham-fed, indicates that these changes were due to the post-ingestive effects of sucralose. Previous research in murine models have shown that the presence of sucralose in the intestine increases the rate of glucose absorption through increased mRNA expression (Margolskee et al., 2007) and increased translocation of GLUT2 to the apical membrane (Mace et al., 2007). While our data do not indicate an overall increase in oral Ra, our data cannot be used to estimate rates of intestinal glucose absorption. Because our plasma glucose measurements were taken from systemic

circulation, glucose appearance rates are potentially impacted by a combination of several processes, including 1) the fraction of ingested glucose that is emptied from the stomach across time; 2) the rate of glucose absorption in the intestine and 3) net hepatic glucose uptake when glucose passes through the liver via the portal vein. (Moore et al., 2012). Nonetheless, because total Ra (the sum of oral Ra and EGP) is unchanged, the likely cause for the increase plasma glucose iAUC was from a decreased rate of glucose disappearance from 50-80 minutes during the sucralose ingestion condition (Figure 6). These findings agree with our hypothesis of a reduced rate of glucose disappearance in subjects with obesity, but also reject the hypothesis that this would be accompanied with a lesser suppression of EGP.

The finding that the increased sucralose-mediated insulin response was not accompanied by decreased plasma glucose during the OGTT in the group with obesity suggests that acute sucralose consumption caused insulin resistance in these subjects. Also, by matching time points on the plasma insulin and glucose Rd curves, it is clear that similar concentrations of insulin between the water and sucralose ingestion conditions do not result in similar rates of glucose clearance. Following sucralose ingestion, rate of glucose clearance was reduced from 50-80 minutes, although insulin concentrations were comparable (Figure 3, Figure 6). We propose that the ingestion of sucralose may be potentiating the “anti-incretin effect”, which is a state of relative insulin resistance occurring post-prandially.

To better understand the “anti-incretin effect”, it is important to understand the “incretin effect”. First, incretins are hormones that help decrease blood glucose levels by promoting the secretion of insulin from the pancreas. The “incretin effect” is the

increased stimulation of insulin secretion after oral compared to after intravenous administration of glucose, both resulting in similar plasma glucose concentrations. The increase in insulin secretion is due to the release of the incretins GLP-1 and GIP from enteroendocrine L-cells and K-cells, respectively, which otherwise would not be released without the presence of glucose in the gastrointestinal tract. However, despite enhanced insulin secretion caused by the incretin effect, insulin sensitivity is lower during oral than intravenous glucose administration, suggesting that oral glucose ingestion causes a state of relative insulin-resistance, the so-called “anti-incretin effect” (Salinari et al., 2017). It has been hypothesized that the “anti-incretin effect” is the state of relative insulin resistance reached following a meal to protect against post-prandial hyperinsulinemia and hypoglycemia caused by the “incretin effect”. *Salinari et al.* have provided evidence of this in their study measuring insulin sensitivity when glucose was given by OGTT or administered through an IV in both normal-weight people and people with obesity (Salinari et al., 2017). The IV glucose administration was isoglycemic to the OGTT, so subjects reached similar concentrations of plasma glucose during both treatments. Despite experiencing similar changes to glycemia, normal-weight subjects and subjects with obesity were 24.5% and 70.3% less insulin sensitive after oral consumption compared to IV administration, respectively. By utilizing the Monte Carlo simulation, they found that plasma insulin levels measured during the OGTT, coupled with insulin sensitivity values estimated during the IV administration, would result in severe hypoglycemia in subjects with obesity. Therefore, the authors interpreted that subjects with obesity are protected from hypoglycemia by becoming less insulin sensitive during the OGTT. In relation to our findings that suggest acute sucralose

consumption decreases insulin sensitivity in subjects with obesity, *Salinari et al.* demonstrated that the “anti-incretin effect” was exacerbated in their subjects with obesity compared to normal-weight subjects. If sucralose could amplify the relative state of insulin resistance of the OGTT through post-ingestive effects in the gastrointestinal tract, this may explain the greater amount of plasma insulin in the sucralose ingestion versus the sham-fed condition in the group with obesity.

Surprisingly, the mere perception of sucralose sweetness before drinking a glucose load decreased plasma insulin concentrations an hour later in the group with obesity, which was reciprocated in the glucose Rd curve. Our finding that sweetness perception before a glucose load decreases insulin response to an OGTT complements well with recent findings that demonstrate that the inhibition of sweetness perception before a glucose load increases insulin response to an OGTT (Karimian Azari et al., 2017). Karimian Azari and collaborators recently found that the addition of lactisole, a broad sweet taste receptor antagonist, to a glucose load increased plasma insulin concentrations during the OGTT (Karimian Azari et al., 2017). Because the lack of sweet taste perception in the study design of Karimian and collaborators lead to an increase in plasma insulin, we believe that the additional sweet taste of the sucralose before the OGTT in our study design caused the decline in plasma insulin in the sucralose sham-fed condition in the group with obesity. Because the C-peptide data closely matches the plasma insulin curve, we hypothesize that the decline in plasma insulin was due to a decreased rate of insulin secretion. However, further investigation into the role of sweet taste perception on post-prandial insulin secretion and clearance are required to determine the cause of the decline.

The finding of sucralose lack of effect on any measurement of GIP in both groups is consistent with other studies conducted in humans which also found no effects of sucralose on incretin responses (Brown et al., 2011, Brown et al., 2012, Ford et al., 2011, Ma et al., 2009, Ma et al., 2010, Pepino et al., 2013, Steinert et al., 2011, Sylvetsky et al., 2016, Temizkan et al., 2015, Wu et al., 2013, Wu et al., 2011), despite sucralose activating sweet taste receptors and stimulating incretin release in cell lines (Jang et al., 2007, Margolskee et al., 2007). *Saltiel et al.* conducted a study attempting to determine why results in human models are not similar to data from cell lines (Saltiel et al., 2017). The researchers used a rat model to either luminally administer or intravenously infuse the proximal small intestine with various sweeteners, including sucralose. They demonstrated that intra-luminal administration of 31.4 mmol/L of sucralose (nearly 16 times the concentration used in our study) did not elicit an incretin response. Additionally, intravenous administration of 10 mmol/L sucralose, but not 1mmol/L or 0.1 mmol/L, significantly increased GLP-1 concentrations and showed a trend for increased GIP concentrations. The researchers suggest that the reason for the lack of incretin response during the luminal administration compared to the intravenous infusion may be due to the small amount of sucralose absorbed by rats (5-8% (Sims et al., 2000)). This theory fits well with our data, as we only administered 2 mmol/L sucralose, of which ~15% (Roberts et al., 2000) is absorbed in humans. According to *Saltiel et al.*, the circulating concentration in our study (< 1 mmol/L) would not be high enough to elicit an incretin response. Despite this, we take caution extrapolating the intra-luminal administration data. The concentration used is 50 times the sweetness of a 20% glucose sweetened solution. As mentioned previously in the literature review,

some artificial sweeteners become antagonists to the sweet taste receptor at high concentrations (Galindo-Cuspinera et al., 2006). Therefore, it is unclear whether the lack of incretin response was due to an antagonistic relationship with the sweet taste receptor in the lumen or the insufficient concentration of sucralose in circulation. Further investigation is still required to determine why data from *in vivo* studies do not replicate data found *in vitro* studies.

Contrary to our hypothesis, subjects with obesity and normal weight subjects achieved a similar peak plasma sucralose concentration after sucralose ingestion. However, it may still be the case that people with obesity absorb more sucralose than normal-weight people. Concentrations in the group with obesity continued to rise 130 minutes following sucralose consumption, but began to decline at this same time point in the normal-weight group. Arguably, if groups were challenged with larger sucralose doses or with frequent consumption of sucralose, subjects with obesity may achieve higher plasma sucralose concentrations. The prolonged rise of plasma sucralose observed in subjects with obesity may be due to the increased permeability of the large intestine, which has been shown to be associated with increased visceral adiposity in women (Gummesson et al., 2011). In addition, plasma sucralose concentrations are influenced by other factors beyond intestinal absorption. A decreased rate of sucralose clearance, via excretion in the urine (Roberts et al., 2000), or diminished P-glycoprotein function in the enterocytes (Schiffman and Rother, 2013), may also explain a delayed decline in plasma concentrations in the group with obesity.

This study has some limitations. For one, our entire study population comprised people who do not normally consume NNS. The logic for this strict inclusion criteria was

based on the finding from several studies that chronic NNS ingestion upregulates the expression of SGLT1, which in turn increases the initial rate of Na+-dependent glucose uptake in three different mammalian species (mice (Margolskee et al., 2007), pigs (Moran et al., 2010) and cattle (Moran et al., 2014)), and increases the glycemic response to an oral glucose load in rodents (Suez et al., 2014, Swithers et al., 2012). Second, our group with obesity comprised people who had a HOMA-IR < 3, so our findings might not extrapolate to people with obesity who are more insulin resistant. Additional studies including chronic NNS users and people who are more insulin resistant are needed.

In conclusion, our data suggest that acute consumption of sucralose prior to an OGTT augments insulin responses in people with obesity but not in normal weight people. These findings add to the growing evidence that sucralose is not metabolically inert, and is the first study, to our knowledge, to demonstrate that the sweet taste of sucralose alone may alter metabolic outcomes. Future studies may include a sucralose ingestion condition without taste (i.e., taking a pill or intragastric/intraduodenal infusion), prior to an OGTT, to better understand the post-ingestive effects of sucralose independent of the perception of its sweet taste in people with obesity. Also, investigating sham-feeding of other sweet-taste stimuli (caloric and non-caloric) prior to an OGTT could help identify if sweet taste in itself caused the changes observed in our sucralose sham-fed condition, or if the effects were exclusive to the sweet taste of sucralose.

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