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# The Effect of Moderate Consumption of Non-Nutritive Sweeteners on Glucose Tolerance and Body Composition in Rats

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# THE EFFECT OF MODERATE CONSUMPTION OF NON-NUTRITIVE SWEETENERS ON GLUCOSE TOLERANCE AND BODY COMPOSITION IN RATS

By

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ABSTRACT

The Effect of Moderate Consumption Of Non-Nutritive Sweeteners On Glucose Tolerance And Body Composition In Rats

by

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**Introduction**: A comorbidity often seen with obesity is the development of impaired glucose tolerance. Abnormalities in the ability to metabolize glucose can lead to increased risk of developing pre-diabetes and if continued, diabetes mellitus type 2. To combat the effects of excess energy intake on obesity and glucose intolerance, low-energy and non-nutritive sweeteners have been introduced as a replacement for traditional sucrose and fructose sweeteners that contribute more energy density. Limited research has been done concerning the effects of moderate consumption of nonnutritive sweeteners on blood glucose tolerance and body composition.

**Purpose**: The purpose of this study was to determine the effect of moderate consumption of NNS (aspartame and sucrose) on glucose tolerance and body composition in an animal model.

**Methods**: Sprague-Dawley rats (N=30) were housed in pairs in a 12 hr light/dark cycle. Animals were randomized into one of three groups where they were each fed a standard chow diet, with the inclusion of a treatment. Treatments include the addition of aspartame (8.5 mg/kg/day) or sucralose (2.6 mg/kg/day) to water, or a control of unflavored water. All animals were given food *ad libitum* for 6 weeks prior to testing and

sacrifice. The three treatment groups were as follows (n=10) aspartame (ASP), (n=10) sucralose (SUC), and (n=10) a control of water (CON). Assessments of lean mass and fat mass were determined from weighing of epididymal fat pads and the use of dual energy x-ray absorptiometry with small animal software prior to sacrifice at the completion of the 6 week treatment. After overnight fasting, an oral glucose tolerance test was administered. Blood glucose concentrations were measured with a tail prick sample, using a Bayer blood glucose monitor. Rats were then given an oral glucose load via oral gavage of 2 mg/kg, with samples then being taken every 15, 30, 60, and 120 minutes of load with blood glucose being examined immediately. Insulin was collected from a tail bleed, with samples being stored at -70° C for later analysis. Insulin assessment was completed with the use of a radioimmunoassay for insulin sensitive rats.

**Results:** Following the 6 week intervention treatment of water with aspartame (ASP), water with sucralose (SUC), or control (CON), no significant differences were seen in the results of oral glucose tolerance testing. ASP (10,150 ± 595 mg/dL/120 min, $p$=0.282) and SUC (9,147 ± 231 mg/dL/120 min, $p$=0.870) areas under the glucose concentration curve (AUC) were not significantly different from the CON group AUC (9147.85 ± 465 mg/dL/120 min). The areas under the insulin concentration curve were not significantly different between the NNS groups and the control (ASP $p$= 0.120, SUC $p$=0.456). Changes in body mass from the beginning of treatment to final were not significantly different between each of the NNS groups and the control group (ASP $p$= 0.787, SUC $p$=0.587). Epididymal fat pad mass was significantly higher in the ASP group compared with the control group (5.50 ± 0.34 g, $p$=0.042).

**Conclusion:** No significant effect was seen from the moderate consumption of aspartame or sucralose on glucose tolerance. No significant differences were seen in weight or overall body fat. However, while percent body fat was unaffected, aspartame consumption at low doses may alter body fat distribution. These results may be of importance in preventing increased abdominal obesity.

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# CHAPTER 1

# INTRODUCTION

Obesity has become a significant health concern as it continues to be an underlying factor for many preventable disease states. Currently affecting more than one-third of Americans, obesity has been linked to life altering illnesses such as heart disease, certain types of cancer, musculoskeletal disorders, and diabetes mellitus type 2.[1] A comorbidity often seen with obesity is the development of impaired glucose tolerance. Abnormalities in the ability to metabolize glucose can lead to increased risk of developing pre-diabetes and if continued, type 2 diabetes mellitus (T2DM).[2] Those who are even considered to be overweight, but not obese, are three times more likely to develop T2DM.[3] Type 2 diabetes mellitus is responsible for 90-95% of all diabetes cases in the United States.[4]

To combat the effects of excess energy intake on obesity and glucose intolerance, low-energy and non-nutritive sweeteners (NNS) have been introduced as a replacement for traditional sucrose and fructose sweeteners, which contribute more energy. NNS have been seen as beneficial due to their lack of glucose and energy density for those attempting to regulate blood glucose and reduce excess adiposity. This provides an alternative for those who consume traditionally sweetened beverages, which are correlated with increased weight gain,[5] insulin resistance through inflammation and b-cell dysfunction, increased visceral adiposity,[6] cardiovascular disease,[7] and more recently are linked to rheumatoid arthritis in women.[8] Previous findings have shown the use of NNS to be correlated with weight loss and are recommended for those with glucose intolerance.[9]

Acesulfame potassium, aspartame, saccharin, and sucralose are the most common low-energy sweeteners to be made available in the United States.[10] These artificial sweeteners are sweeter than traditional sucrose. Aspartame has the effect of being 180 times sweeter, while sucralose is 600 times sweeter. This allows consumers to take in such low doses that they are deemed low-energy or nonnutritive. While found in a variety of foods, NHANES 2007-2008 reported diet beverages were the primary source of intake of NNS in the American diet.[11] Other sources can include yogurts, gum, desserts, as well as other food products. While consumption is found in all ages and ethnicities, adult females were the most likely to consume NNS beverages on a regular basis.

The increase in obesity and prevalence in T2DM may be correlated with an increased reliance on NNS.[11] Current research, which has mostly examined the physiological effects of consumption at the approved upper limits, has produced contrasting results. A significant effect from NNS consumption to increased BMI or adiposity, as well as impaired glucose tolerance, has been reported in most,[12-16] but not all, studies.[17] One study has reported on the moderate effects of aspartame consumption on blood glucose changes and body composition.[9] Since many Americans rely on moderate consumption of NNS,[18] a further examination of the relationship between moderate NNS consumption and body composition and blood glucose may be warranted. Therefore, the purpose of this study is to determine the effect of moderate consumption of NNS (aspartame and sucrose) on glucose tolerance and body composition in an animal model.

**Purpose:** The purpose of this study was to determine the effect of moderate consumption of NNS (aspartame and sucralose) on glucose tolerance and body composition in an animal model.

**Research Hypothesis**

1) Is there a causal relationship between nonnutritive sweeteners and body composition?

    Ho: Non-nutritive sweeteners will have no effect on body composition.

    H1: Non-nutritive sweeteners will have an effect on body composition.

2) Is there a causal relationship between nonnutritive sweeteners and glucose intolerance?

    Ho: Non-nutritive sweeteners will have no effect on glucose tolerance.

    H1: Non-nutritive sweeteners will have an effect on glucose tolerance.

**Definition of Terms**

**ASB**: Artificially sweetened beverages. Diet beverages that use non-nutritive sweeteners such as aspartame, sucralose, saccharin, stevia rebaudiana extract, and acesulfame potassium. These are used to replace traditional sweeteners in beverages such as high-fructose corn syrup or sucrose.

**NNS**: Non-nutritive sweetener. High intensity sweeteners used in place of traditional sweeteners such as sucrose or fructose which do provide energy. Non-nutritive sweeteners are used in low doses relative to traditional sweeteners, leaving them with a minimal energy contribution.

**OGTT**: Oral glucose tolerance test examines the effect of a glucose load on the body to determine glucose tolerance concerns such as pre-diabetes, diabetes, or gestational diabetes. Measurements of serum insulin and blood glucose are tested every 15, 30, 60, 90, and 120 minutes from the time of glucose load consumption.

**CPIR**: Cephalic phase insulin response is the release of insulin following digestion of a meal. It has been seen in both humans and rats.[19]

# CHAPTER 2

# REVIEW OF LITERATURE

## Non-Nutritive Sweeteners

NNS are a common ingredient in many types of foods, as well as used as an added sweetener for many attempting to reduce the added energy intake from traditional sweeteners. Two popular sources of NNS are aspartame and sucralose. Both are found in the most common sources of NNS for adults and children, which are diet beverages, followed by packaged artificial sweetener pouches, which are used as a topical additive for foods or beverages.[11]

Aspartame is enzymatically broken down from its molecular formula $C_{14}H_{18}N_2O_5$ by esterases and peptidases. It is then metabolized as phenylalanine, aspartic acid, and methanol.[20] Phenylalanine is an essential amino acid and precursor to melanin, dopamine, norepinephrine, and thyroxine. Aspartic acid is a nonessential amino acid commonly found in sugar cane that can be used as a neurotransmitter.[21] Both phenylalanine and aspartic acid are commonly found in natural food sources such as meats, grains, and dairy products. As with their natural counterparts, the metabolized products of aspartame are broken down in the body and do not accumulate. Methanol is metabolized at a slower rate than other alcohols, such as ethanol, but is readily digested.[22] Methanol is initially converted to formaldehyde in the liver. It then is converted to formic acid, and is excreted through urine. The amount of formaldehyde derived from aspartame is minute, as the body naturally contains amounts significantly greater than what is found in a serving of this NNS.[18] Long term consumption of aspartame is considered safe up to 50 mg/kg per day, although most adults consume 3

mg/kg per day. When long term users of aspartame at the dose of 75mg/kg per day were compared to a control group, no significant differences in standard serum values, blood formate, or 24-hour urinary excretion were seen.[23]

Sucralose $C_{12}H_{19}Cl_3O_8$ is a chlorinated NNS derived from sucrose $C_{12}H_{22}O_{11}$. Three hydroxyl groups in sucrose are replaced with three chlorine atoms to form sucralose.[24] Unlike aspartame, which is broken down through digestion, sucralose remains unchanged and is excreted intact.[18] Sucralose remains stable when exposed to higher temperatures. It then provides an appropriate use for baked goods and expands its use outside of diet beverages. Sucralose's added range of thermal flexibility allows it to be used in 15 food and beverage categories. Sucralose has fewer concerns for health implications than other NNS, resulting in its use in 80 countries. Sucralose has an acceptable daily intake of 5 mg/kg per day, but the average adult consumes 1.6 mg/kg per day.

## Obesity

Obesity has become a significant health concern as it continues to be an underlying factor for many preventable disease states. Overweight is defined by a body mass index (BMI) over 25, while obesity is indicated with a BMI over 30. The increased body mass is related to excessive adipose tissue which can present health risks.[25] The rising prevalence of overweight and obesity leads to increased medical costs. As of 2003, there were an estimated 75 billion dollars spent on obesity related costs, with half being financed through Medicare and Medicaid.[26] As of 2008 this estimate grew

drastically to 147 billion dollars having been spent on obesity related medical care, proving it to not only be a health issue, but an economic concern as well.[1]

Increased adiposity leads to the development of overweight and obesity. These conditions resulting from increased weight lead to stress on the body and further health implications in many body systems.[4] Hypertension is three times greater in those who are obese than those of a normal weight class. Elevated levels of LDL cholesterol and reduced HDL from increased weight lead to greater risk for coronary artery disease. These effects on the heart increase the risk of angina, sudden cardiac death, and myocardial infarction in obese individuals. Weight gain affects other systems, such as respiratory issues with the development of sleep apnea. Reproductive complications also occur from the development of excess weight, including reduced testosterone, increased estrogen, increased risk of polycyclic ovary syndrome, increased gestational diabetes, and increased birth defects in those who are pregnant.

While NNS were originally intended to decrease energy consumption and provide a solution for weight control, current research has reported a significant effect between increased BMI and higher NNS consumption.[12-14] Increases in the incidence of obesity and NNS consumption may be coincidental or causal. Yet further examination of this relationship has been researched without a definitive conclusion.

## NNS's Effect on Energy Intake

It has been reported that NNS reduces energy consumption, yet increases in adiposity and BMI are still correlated to the use of NNS. Long term prospective research by Stellman and Garfinkel (1988) found the use of NNS users had an increased BMI.[14]

Of the 78,692 subjects studied, BMI in the lowest quartile of NNS users was increased by 12.9%, while the BMI in the highest quartile increased by 29.8%. An examination of the diet patterns of NNS users found that they consumed significantly more vegetables and lean proteins, favoring less energy dense foods than those who did not use NNS. This suggested that BMI increases in NNS users were not likely to be from poor diet choices or that the consumption of NNS lead to increased energy consumption.

Fowler et al. (2008) studied the effects of NNS intake in 3,682 participants over a 9 year period, and found that BMI increased by 47% in NNS users.[12] The percent of energy from protein, total fat, and saturated fat was higher in artificially sweetened beverage users (ASB). However, Fowler found total energy, energy from carbohydrates and sucrose, as well as alcohol consumption, were significantly lower in the NNS consumers than in subjects who abstained from NNS,[12] which contrasts findings of Stellman and Garfinkel (1988).[14] This suggests that the use of NNS lead to reduced energy intake when used as a substitute to energy dense sweeteners.

When energy intake was controlled in Winstar rats, consumption of NNS flavored yogurt led to a 28% greater weight gain in those that consumed saccharin, and a 20% increase in weight in the rats that consumed aspartame.[13] However other research in animal models found aspartame based NNS did not lead to increased intake in food or increases in weight in mice.[15]

The effect of NNS on reducing energy intake has been demonstrated, but showed contrasting results on BMI. The effect of moderate consumption of NNS on body composition in rats found that rats who consumed aspartame had lower body fat percentages, which was attributed to decreased energy intake.[9] This could be related to

the lower amounts of NNS used in this study, since other research used on greater quantities in the diet for treatment groups and found that body fat was increased.[16, 17] However, a meta-analysis of 1,951 adults and children found consumption of low energy sweeteners, such as diet NNS beverages, to decrease body weight by 0.80 kg.[27] While this loss appears to be minimal, a significant overall reduction in BMI, adiposity, and waist circumference was found.

## Digestive Signaling from NNS

An alternative hypothesis on the relationship between increased BMI and NNS is that NNS do not provide satiety in the way traditional sweeteners do, which may lead to the increased consumption of food.[28] The cephalic phase of digestion begins with the activation of hormonal secretions necessary for the digestion and absorption of nutrients. The cephalic phase can be triggered by sensations such as sight, smell, and the detection of sweet flavored foods.[29] Taste sensations are detected by G protein-coupled receptors (GLP-1), while the specific T1R2 and T1R3 receptors are responsible for signaling sweetness by coupling with GLP-1.[30] These taste receptors have been found to be stimulated by NNS, such as saccharin and acesulfame potassium.[31] However, the effect of sucralose is inconclusive as it has been shown to activate GLP-1 through T1R2 and T1R3 in vitro,[32] yet when examined in healthy human subjects, there were no increases in GLP-1 indicating sucralose did not stimulate sweetness receptors.[30] For those NNS which have shown an effect, the signaling of sweetness would then elicit activation of the cephalic phase, which has been found to possibly increase hunger signals leading to increased energy consumption.[33]

Part of the cephalic phase is the activation of the insulin response (CPIR), a neural stimulation from the vagus nerve that elects a cascade of hormones necessary for activation of digestion, particularly the stimulation of insulin secretion from beta cells in the pancreas.[19] Initial insights on CPIR suggested that it could be activated by energy stimulation. However, more recent research has found a stimulation of CPIR from the sensation of sweetness, even when it is derived from non-energy dense sources. It has been found that the NNS saccharin has elicited a response similar to glucose, resulting in stimulation of the CPIR.[34] However, no stimulation of the CPIR was reported when aspartame was ingested.[29,35] If NNS are causing a stimulation in CPIR, ingestion of NNS with a carbohydrate load could result in greater glucose absorption, which places consumers at greater risk for glucose intolerance, and possibly obesity.[36]

**Glucose Intolerance**

A comorbidity often seen with obesity is the development of impaired glucose tolerance. Abnormalities in the ability to metabolize glucose can lead to increased risk of developing pre-diabetes and if continued, T2DM.[37] Those who are even considered to be overweight, but not obese, are three times more likely to develop T2DM.[3] T2DM is responsible for 90-95% of all diabetes cases in the United States.[4] Increased abdominal adiposity correlates to increased plasma free fatty acids which lead to the secretion of insulin from the pancreas. Excessive stimulation can increase insulin resistance in the peripheral tissues, reduce the uptake of glucose, and increase hepatic glucose production. T2DM isn't initially correlated with the inability to produce insulin, but the recipient cells become defective, forming a resistance to insulin. This leads to increased

stress on the pancreas to increase insulin production, resulting in damage to the beta cells. Fasting hyperglycemia occurs from glucose circulating as decreased sensitivity to insulin or a decreased insulin response prevents glucose uptake.[37]

T2DM can result from multiple risk factors contributing to metabolic syndrome.[4] Metabolic syndrome is characterized by displaying at least three of the following: abdominal obesity (≥ 40in waist circumference in men or ≥ 35in waist circumference in women), insulin resistance (100mg/dL fasting plasma glucose), dyslipidemia from high triglyceride (≤ 150mg/dL) or cholesterol values (<40 mg/dL in men and <50 mg/dL in women), or hypertension (≥130/85 mmHg). Weight gain of 11-18 pounds can double the risk of developing T2DM. Excessive visceral adipose tissue contributing to a greater waist circumference increases risk of developing glucose intolerance further.

Prediabetes is characterized by a fasting blood glucose level of 100 - 125 mg/dL.[1] Those with pre-diabetes alone have an 8.5% greater risk of developing cardiovascular disease over a ten year period. This is twice the risk that would be seen in a normoglycemic individual.[2] As of 2011, the development of diabetes had occurred in 20.9 million Americans, having tripled in occurrence since 1980.[38] Rates of death due to diabetes related complications are expected to double between 2005 and 2030.[39]

NNS have been marketed to assist those who are sensitive to glucose due to metabolic derangements contributing to hyperglycemia. The use of NNS as a replacement for traditional sweeteners was thought to be beneficial in that it would provide a lower rise in blood glucose, as they do not contain larger amounts of carbohydrate as traditional sweeteners do. The effects of NNS on blood glucose and insulin release, which can affect metabolic conditions, were not heavily investigated until

recently. Research has led to findings showing that the use of NNS may contribute to glucose intolerance, providing the opposite effect of what many hyperglycemic patients depend on it for.[9,16,40,41]

Daily consumers of NNS containing diet sodas were assessed for diabetic risk in a study of 6,814 participants between the ages of 45-84. Those who consumed at least one serving of diet soda per day had a 67% higher risk of developing type 2 diabetes when compared to non-consumers.[40] Larger waist circumferences were also found in users of NNS, which has been found to be a risk factor for diabetes.[42]

When sucralose was administered to 17 obese subjects prior to a 5 hour glucose tolerance test, significant increases were seen, indicating glucose intolerance.[43] A single serving of sucralose similar to dosing found in a 12 oz can of diet soda was ingested in the treatment group prior to testing, while the control subjects consumed water. The results of the OGTT showed a significantly increased peak insulin response ($p$=0.02), increased insulin area under the curve ($p$=0.03), and increased peak plasma glucose concentrations ($p$=0.03).

Due to the significant affect that dietary intake and physical activity can have on blood glucose and insulin, animal models have been the primary form of research on this topic. Human studies leave a greater margin for error in the validity of results, as controlling diet and limiting physical activity are not easily obeyed. In order to ensure confounding variables have not swayed results, rat and mice studies have been conducted where diet and activity can be easily controlled.

To examine the effects of NNS on blood glucose changes with a controlled diet, Palmnäs et al. (2014) used male Sprague-Dawley rats as a model.[9] The rats were put

on either a standard chow or a high fat diet, with the control of water or a moderate dose (5-7 mg/kg per day) of aspartame infused water as a treatment. The aspartame consumption correlated to increased fasting hyperglycemia as well as an impaired insulin tolerance, regardless of diet. The authors suggested that the aspartame impaired insulin mediated suppression of hepatic glucose output. Mitsutomi et al. (2013) had examined the relationship of NNS intake to insulin and glucose responses, by adding aspartame to the water of mice.[15] After 8 weeks of consuming either a control of water, water with sucrose, or water with aspartame, glucose tolerance was assessed with an oral glucose tolerance test (OGTT). Insulin, glucose, and triglyceride levels were significantly increased in the NNS group compared to controls, suggesting that NNS intake impaired glucose tolerance.

Suez et al. confirmed these findings by adding saccharin, sucralose, and aspartame to the drinking water of mice.[16] After 11 weeks of consumption, the NNS groups had significantly increased glucose intolerance. To determine if the NNS were the causative factor for glucose intolerance, the authors examined NNS consumption among 381 non-diabetic individuals. NNS consumption was correlated with markers/risk factors for hyperglycemia, including increased waist-to-hip ratio and glycosylated hemoglobin.

The source of changes in glucose tolerance from NNS is an emerging field of research. In rat models, the consumption of NNS was seen to alter glucose absorption on the membrane of enterocytes.[41] The addition of sucralose or acesulfame potassium doubled the rate of post prandial glucose absorption 5 to 20 minutes after consumption. This was the result of the NNS increasing the presence of GLUT2 transporters in the

intestine to assist with glucose uptake. Glucose absorption into the intestinal epithelial cell is dependent on sodium co-transport (SGLT transport protein) and GLUT2 transporters, which facilitate glucose uptake. If NNS and carbohydrate sources are consumed together, their collaborative effect could increase glucose uptake into the blood, causing hyperglycemia. Long term elevation of blood glucose could then damage pancreatic islet cells from over production of insulin leading to glucose intolerance.

Other research using rat models has focused on changes in body composition and glucose metabolism related to the NNS induced changes in the microbiota in the gut.[9,16] Suez et al. investigated the relationship of NNS on glucose intolerance by performing a fecal transplant with the microbiota from NNS treated rats, into rats who had not consumed NNS. Following transplantation, the recipient mice exhibited glucose intolerance, leading to the suggestion that NNS consumption's effects on metabolism of glucose are related to changes in the gut microbiota.

Contrasting results on glucose tolerance have also been seen. In ten healthy subjects, differences in blood glucose and plasma GLP-1 were assessed, after receiving an infusion of sucralose or saline. No significant differences were found between treatments, in contrast to previously reported studies.[32] Further research is needed to determine the definitive effects of NNS on glucose tolerance and weight gain. Currently there is one study that looks at the effects of moderate consumption of aspartame on these variables,[9] while other research has focused on higher intakes that may be above what the average adult consumer uses.[16]

# CHAPTER 3

# METHODOLOGY

Sprague-Dawley male rats (Taconic Biosciences, Rensselaer, NY) were housed in pairs (N=30) in a 12 hr light/dark cycle in UNLV's Laboratory Animal Care Facilities. Animals were randomly assigned to one of three groups where they were each fed a standard chow diet, with the inclusion of a treatment. Treatments include the addition of commercially available aspartame (Merisant, Chicago, IL) or sucralose (Heartland Food Products Group, Carmel, IN). Treatment dosing for aspartame (8.47 mg/kg/day) and sucralose (2.6 mg/kg/day) was formulated based on each groups mean weight, which was measured weekly. The three treatment groups were as follows (n=10) aspartame (ASP), sucralose (SUC), and a control of water (CON). Animals had no significant differences in weight between the ASP (147.2 ± 2.2 g), SUC (152.9 ± 2.6 g), and CON (152.2 ± 1.4 g) at the start of the study ($p$=0.13). All animals were given food *ad libitum* for 6 weeks prior to testing and sacrifice.

Subjects were weighed once per week to monitor growth and formulate weekly treatment dose. Following overnight fasting, an oral glucose tolerance test was administered at the completion of the 6-week study. A fasting blood glucose and serum insulin sample was taken prior to administering a glucose load. Rats were then administered an oral glucose load via oral gavage of 2 g/kg dose of 50% weight/volume dextrose solution, with samples then being taken every 15, 30, 60, and 120 minutes of load. Blood glucose was examined immediately through use of a Bayer glucose monitor and test strips. Blood samples (300 µl) for insulin were collected into microcapillary tubes via tail bleeding. Insulin samples were centrifuged to isolate plasma and stored at

-70° C for later analysis. Insulin assessment was completed with the use of a radioimmunoassay for insulin sensitive rats (Millipore Corp, Billerica, MA).

Assessments of lean mass and fat mass were determined from the use of dual energy x-ray absorptiometry (DEXA) with small animal software (Lunar Prodigy, General Electric) immediately following sacrifice at the completion of the 6 week treatment period. Standard values for animals were used for compliance with the small animal software. Standard DEXA values included height of 10 inches, and weight of 0.3 lbs. Removal of the epididymal fat pads was conducted following the DEXA scan, and were immediately weighed.

**Statistical Analysis**

Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) 22.0 software (Armonk, New York). One-way analysis of variance (ANOVA) and post hoc Tukey's tests was used to identify the differences in the dependent measures between groups. Significance was set to $p<0.05$. Area under the glucose and insulin response curves (AUC) were calculated via the trapezoid method. Data are reported by analyzing the mean ± SEM.

# CHAPTER 4

# RESULTS

## Glucose Tolerance

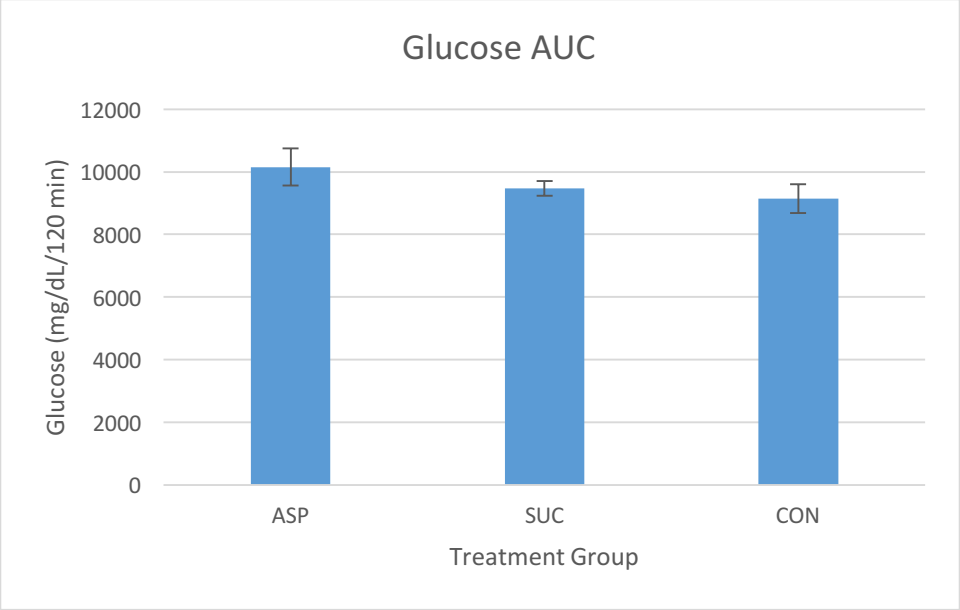
Following the 6 week intervention treatment of water with aspartame (ASP), water with sucralose (SUC), or control (CON), no significant differences were seen in the results of oral glucose tolerance testing. ASP ($p$=0.282) and SUC ($p$=0.870) areas under the glucose concentration curve (AUC) were not significantly different from the CON group AUC (Table 1, Figure 1). Individual time points were analyzed for significance. No significant differences were found at 0 min (ASP $p$=0.897, SUC $p$=0.435), 15 min (ASP $p$=0.930, SUC $p$= 0.952), 30 min (ASP $p$=0.319, SUC $p$=0.472), 60 min (ASP $p$=0.550, SUC $p$=0.999), or 120 min (ASP $p$=0.161, SUC $p$= 0.849) between the NNS groups and the control group (Table 1, Figure 2).

**Table 1: OGTT Serum Glucose Comparison**

| Treatment | 0 min (mg/dL) | 15 min (mg/dL) | 30 min (mg/dL) | 60 min (mg/dL) | 120 min (mg/dL) | AUC (mg/dL/120min) |
|---|---|---|---|---|---|---|
| **ASP (n=10)** | 52.8 ± 2.8 | 84.3 ± 5.2 | 94.6 ±6.2 | 91.1 ± 6.3 | 75.4 ± 8.0 | 10,150.5 ± 595.0 |
| **SUC (n=10)** | 58.9 ± 2.1 | 79.2 ± 3.7 | 92.5 ± 3.7 | 84.5 ± 3.1 | 65.3 ± 2.3 | 9,472.5 ± 231.0 |
| **CON (n=10)** | 54.4 ± 2.7 | 81.5 ± 6.9 | 83.9 ± 5.2 | 84.2 ± 3.6 | 61.2 ± 3.8 | 9147.9 ± 465.7 |

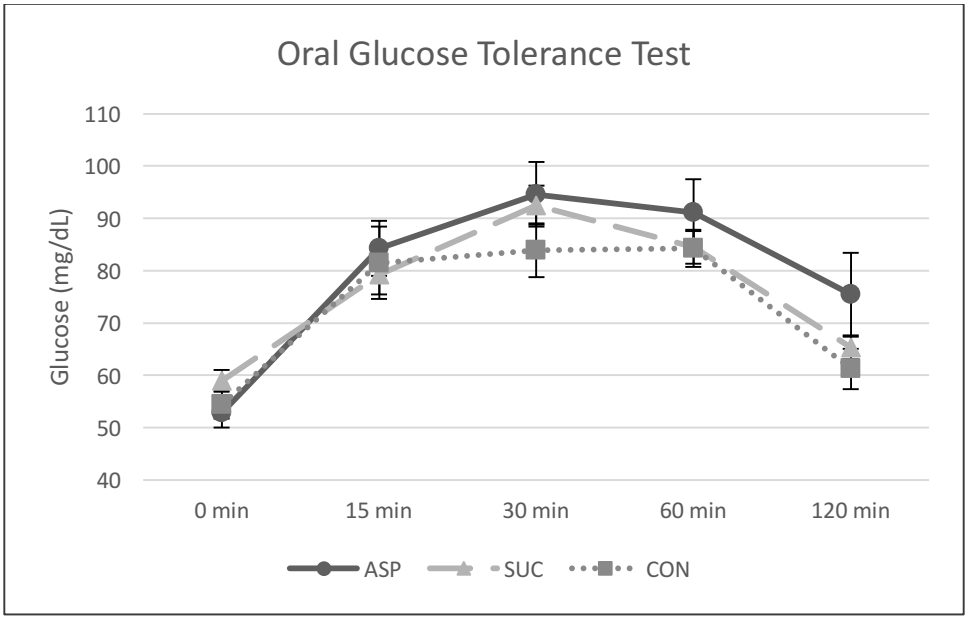
**Values are mean ± standard error; ($p$>0.05)**

## Figure 1: OGTT Serum Glucose Area Under The Curve




**Area under the glucose concentration curve for aspartame (ASP), Sucralose (SUC), and control (CON).**

## Figure 2: OGTT Serum Glucose




**Glucose response for aspartame (ASP), Sucralose (SUC), and control (CON) from OGTT.**

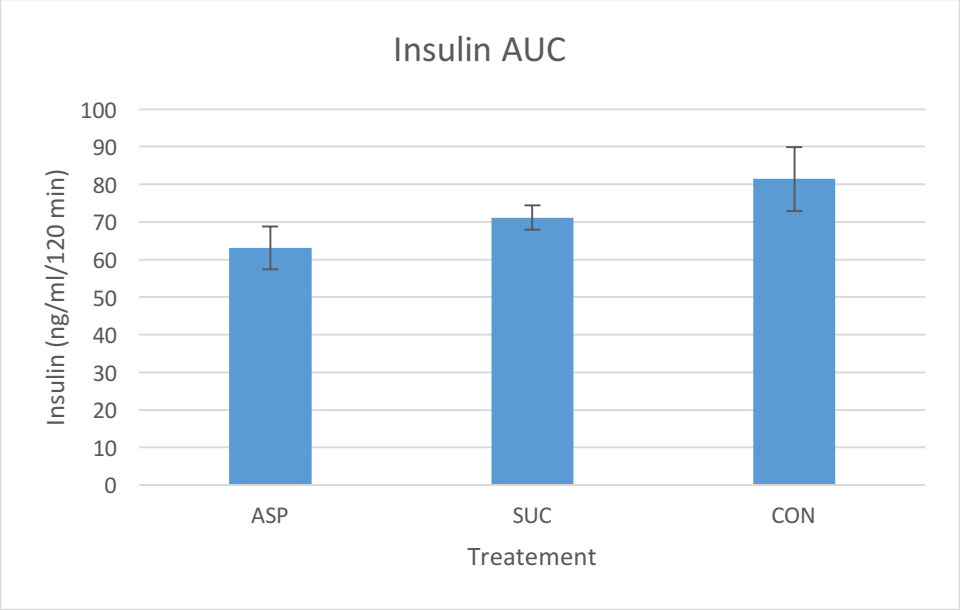
A viable sample for plasma insulin at each time point was collected on animals from the ASP group (n=6), SUC group (n=7), and CON group (n=6). The areas under the insulin concentration curve were not significantly different between the NNS groups (ASP $p$= 0.120, SUC $p$=0.456) and the control (Table 2, Figure 3). No significant differences were seen at 0 min (ASP $p$=0.980, SUC $p$=0.350), 15 min (ASP $p$=0.159, SUC $p$= 0.601), 30 min (ASP $p$=0.667, SUC $p$=0.411), 60 min (ASP $p$=0.677, SUC $p$=0.806), or 120 min (ASP $p$=0.756, SUC $p$= 0.282) between either NNS group and the control group (Table 2, Figure 4).

**Table 2: OGTT Plasma Insulin Comparison**

| **Treatment** | **0 min** (mg/dL) | **15 min** (mg/dL) | **30 min** (mg/dL) | **60 min** (mg/dL) | **120 min** (mg/dL) | **AUC** (mg/dL/120min) |
|---|---|---|---|---|---|---|
| **ASP (n=6)** | 0.63 ± 0.19 | 0.60 ± 0.07 | 0.51 ± 0.08 | 0.52 ± 0.09 | 0.49 ± 0.09 | 63.1 ± 5.7 |
| **SUC (n=7)** | 0.49 ± 0.08 | 0.77 ± 0.11 | 0.75 ± 0.06 | 0.55 ± 0.05 | 0.48 ± 0.06 | 71.2 ± 3.2 |
| **CON (n=6)** | 0.66 ± 0.12 | 0.93 ± 0.15 | 0.61 ± 0.09 | 0.63 ± 0.12 | 0.72 ± 0.18 | 81.4 ± 8.6 |

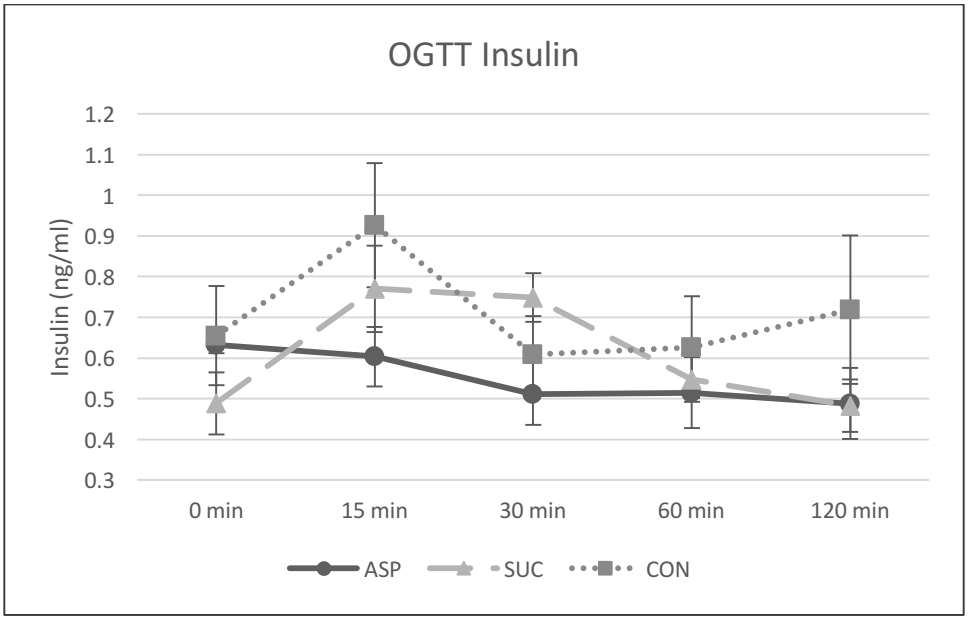
**Values are mean ± standard error; ($p$>0.05)**

## Figure 3: OGTT Plasma Insulin Area Under The Curve




**Area under the glucose concentration curve for aspartame (ASP), Sucralose (SUC), and control (CON).**

## Figure 4: OGTT Plasma Insulin

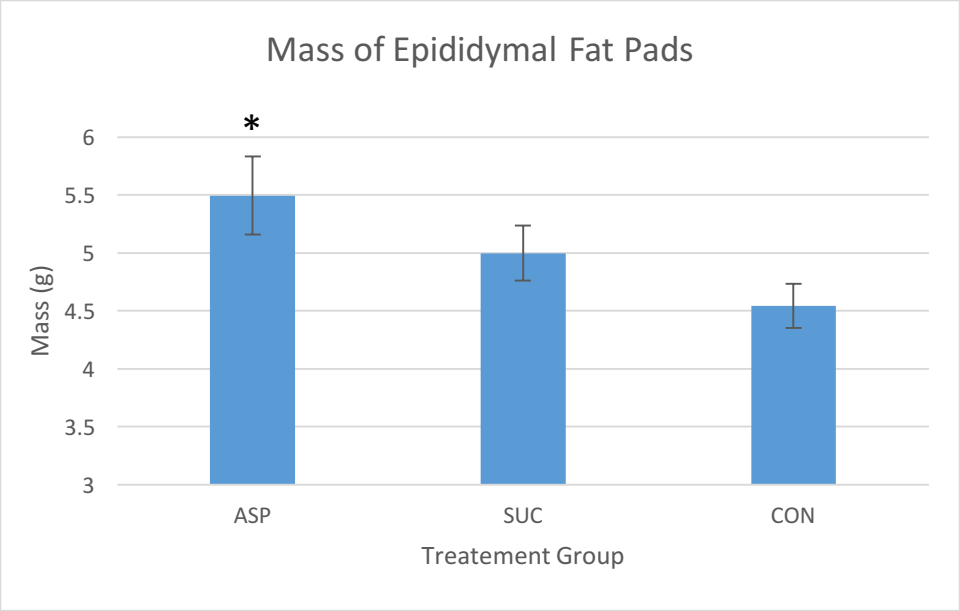



**Insulin response for aspartame (ASP), Sucralose (SUC), and control (CON) from OGTT.**

## Body Composition

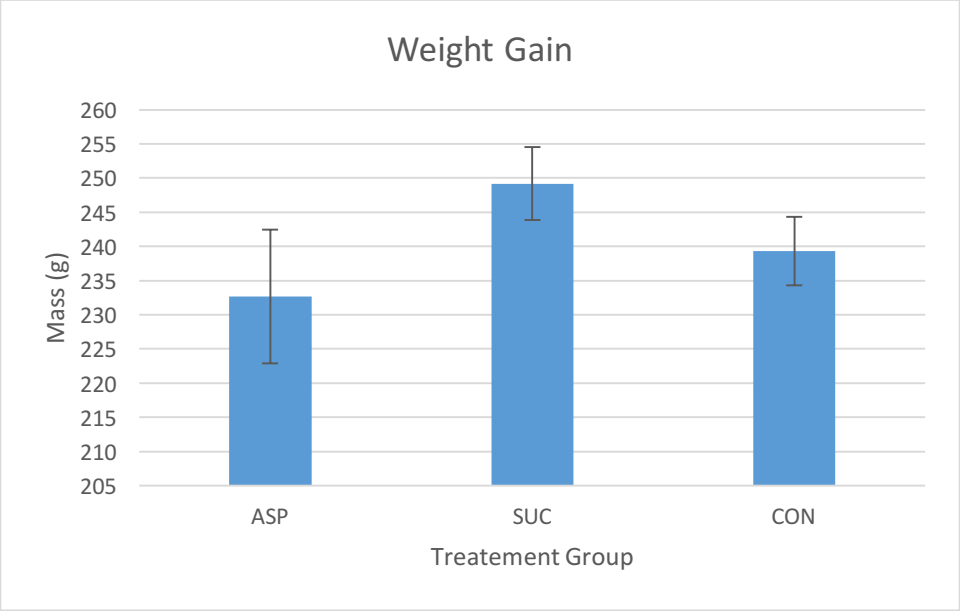
Epididymal fat pad mass was significantly higher in the ASP group compared with the control group (5.50 ± 0.34 g, $p$=0.042) (Table 3, Figure 5). However, changes in body mass from the beginning of treatment to the completion were not significantly different between each of the NNS groups (ASP $p$= 0.787, SUC $p$=0.587) and the control group (Table 3, Figure 6). Percent body fat was measured by DEXA (Table 3, Figure 7). While the ASP group (19.30 ± 0.70%) had a higher mean body fat percentage than both SUC (17.7 ± 0.67%) and CON group (17.79 ± 0.91%), results were not significantly different (ASP group, $p$= 0.273; SUC group, $p$=0.994; vs. CON).

**Figure 5: Mass of Epididymal Fat Pads**



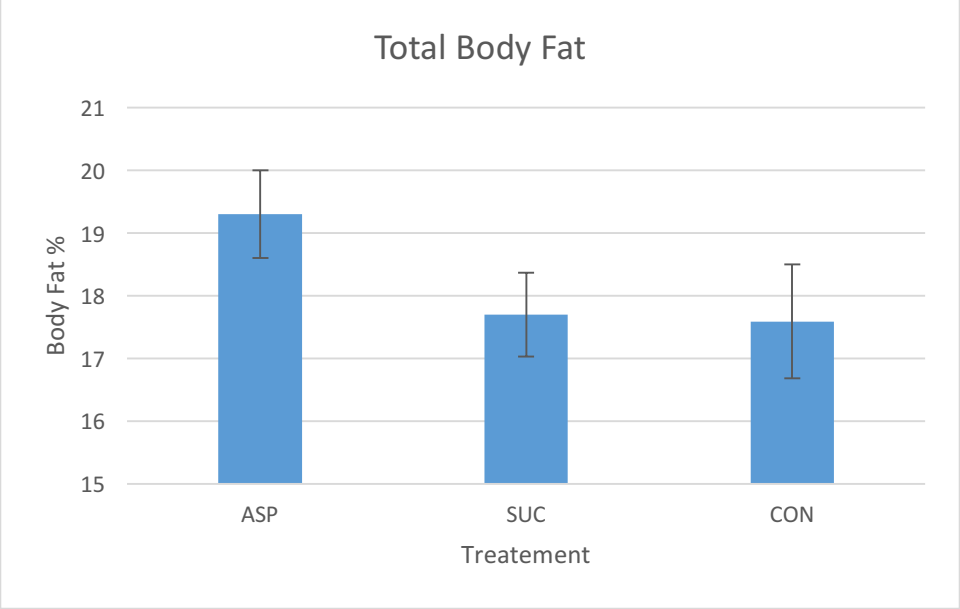

**Epididymal fat pad weight for aspartame (ASP), Sucralose (SUC), and control (CON). * Significantly different than control ($p \leq 0.05$)**

## Figure 6: Body Mass Change




**Body mass changes for aspartame (ASP), Sucralose (SUC), and control (CON).**

## Figure 7: Total Body Fat Percent




**Total body fat percent for aspartame (ASP), Sucralose (SUC), and control (CON).**

## Table 3: Body Composition Assessments Comparison

| | ASP (n=10) | SUC (n=10) | CON (n=10) |
|---|---|---|---|
| **Body Fat (%)** | 19.3 ± 0.70 | 17.7 ± 0.67 | 17.59 ± 2.88 |
| **Weight Gain (g)** | 232.7 ± 9.79 | 249.2 ± 5.29 | 239.30 ± 5.00 |
| **Weight of Epididymal Fat Pads (g)** | * 5.5 ± 0.34 | 5.0 ± 0.24 | 4.55 ± 0.19 |

**Values are mean ± standard error; ($p$>0.05)**

# CHAPTER 5

# DISCUSSION AND IMPLICATIONS

Artificial sweeteners (NNS) are a common ingredient in many types of foods to reduce the added energy intake from traditional sweeteners. Two of the most popular NNS are aspartame and sucralose, which are found in diet beverages and packaged artificial sweetener packets.[11] While NNS were originally intended to decrease energy consumption and provide a solution for weight control, a connection between increased BMI and higher NNS consumption has been reported.[12-14]

Previous research on the effects of NNS on glucose tolerance and body composition changes in humans consist primarily of observational studies, with few intervention studies.[9,13,15,16] When energy intake was controlled in rats, consumption of NNS flavored yogurt led to a 28% greater weight gain in those that consumed saccharin, and a 20% increase in weight in the rats that consumed aspartame.[13] However other research in animal models found aspartame based NNS did not lead to increased intake in food or increased weight in mice.[15]

The effects of NNS on blood glucose changes have been studied using rats as a model.[9] Fasting hyperglycemia as well as an impaired insulin tolerance was correlated to aspartame consumption in rats given a moderate dose (5-7 mg/kg per day) of aspartame infused water as a treatment, regardless of diet, suggesting that aspartame impaired insulin mediated suppression of hepatic glucose output. Similarly, insulin, glucose, and triglyceride levels were significantly increased in the NNS treated mice compared to control animals, suggesting that NNS intake impaired glucose tolerance.[15,16] Research has led to findings showing that the use of NNS may

contribute to glucose intolerance; the opposite effect of what many hyperglycemic patients are expecting.[9,16,40,42] Further, several studies that included an intervention treatment of aspartame used doses significantly exceeding the average adult intake of 3mg/kg/day.[13,15,16,18] The body of research examining glucose tolerance or body composition changes from the NNS sucralose is much smaller. Sucralose has been seen to increase post prandial glucose consumption by raising the amount of glucose transporters.[41] However, intervention strategies assessing moderate doses of sucralose consumption have not been seen. Therefore, the purpose of this study was to determine the effect of moderate consumption of non-nutritive sweeteners on glucose tolerance and body composition in an animal model.

In this study, no significant differences were found in either NNS group when compared to the control for glucose or insulin from oral glucose tolerance testing. While the ASP group did have higher trending OGTT values for serum blood glucose, significance was not found. This result varies from previous research which found a relationship between moderate levels of aspartame consumption and impaired glucose tolerance.[9] Palmnäs et al., used a similar dose (5-7 mg/kg/day) of aspartame to that used in this study (8.5 mg/kg/day). However, significantly higher values in fasting glucose were seen in the aspartame vs control group in the previous study. No significant differences in the plasma insulin response to the oral glucose load were found in our study, which deviates from the previously published research on aspartame at low doses.[9] The primary variation in methods between this study and that of Palmnas et al., was in the the duration of treatment (8 weeks of NNS treatment in the previous study vs. 6 weeks in the present study).

Previous research on sucralose and impaired glucose tolerance has been limited. Research in humans found a significant effect from the consumption of sucralose, however this effect was not replicated in the current findings.[43] Disparities may be seen from the different subject models or methods. In the human research, sucralose was administered once immediately prior to a OGTT, whereas this study focused on low doses over a 6 week period, and animals were fasted prior to oral glucose tolerance testing.

Other research examining possible relationships between NNS and glucose tolerance focused on higher treatment doses of aspartame, concluding a potential effect on reducing glucose tolerance.[15,16] Increases in area under the glucose concentration curve were seen in NNS treated groups compared to the control. This follows trends seen in previous studies.[15,16] However, differences seen in AUC from the moderate dose of NNS administered were not significant. Differences in the significance found in this study and results previously published may be due to the higher doses of NNS administered in previous research. The aspartame dose in this study was a 0.02% solution, which is significantly lower than the 4-5% solution used in similar studies that found significantly impaired glucose tolerance.[15,16] Increasing the administered dose beyond what is typically consumed in humans makes it difficult to determine if the effects seen with these higher doses will be replicated in a normal human population. Based on the findings of this study, moderate consumption of the artificial sweeteners aspartame and sucralose does not have a significant effect on glucose tolerance in a rat model.

The epididymal fat pads were found to be significantly higher for the ASP group ($p$=0.042) compared to the CON group, suggesting that ASP may lead to redistribution of body fat stores. This confirms findings by Mitsutomi et al., which found the epididymal fat

pads to have greater mass following aspartame treatment.[15] Epidemiological studies performed on humans found consumption of NNS in the form of ≥1 serving of diet soda can increase waist circumference significantly, supporting that a change in body composition may occur from NNS intake.[40] Researchers have suggested increases in epididymal fat pads may relate to increased visceral adiposity in humans, indicating a higher risk for atherosclerosis and metabolic conditions.[44] However, more research should be done to establish this specific connection. Weight changes from the start to the completion of the study were not significantly different between groups. Body fat percent was not significantly different between the ASP or SUC group when compared to the control. While the current findings do indicate increases in total body fat compared to the controls, results were not significant. While significance was not found, this is on trend with the findings seen in Palmnas et al., which had a significantly higher body fat percent for the low dose aspartame group. Both studies observed a lower final weight in the aspartame treated groups, however weight changes were not significant in either study. The NNS groups did exhibit higher total body fat than the control, but results were not significant, contrasting previous research conducted with higher doses of NNS.[16] Evidence presented in this study suggests a possible change in body fat distribution from the consumption of the NNS aspartame at low doses.

## Future Research

Future research regarding the relationship between NNS consumption and glucose tolerance and body composition in human models would be of benefit. A study design ensuring the inclusion of a treatment rather than observational analysis in humans

could further confirm findings seen at low doses of NNS treatment. This can be difficult as other factors such as diet and physical activity would require monitoring. Results seen in the study regarding glucose tolerance and body fat percent were not significant with the low dose treatment. As this contrasts findings at larger doses,[13,15,16] a more in depth dose response relationship would be warranted to determine the threshold of significance from NNS consumption on glucose tolerance and body composition.

## Conclusion

In conclusion, this study showed no effect from the consumption of aspartame or sucralose on glucose tolerance. No significant differences were seen in weight or overall body fat. However, while percent body fat was unaffected, aspartame consumption at low doses may alter body fat distribution. These results may be of importance in preventing increased abdominal obesity. Increased abdominal fat stores are a marker for metabolic syndrome and can be a risk factor for glucose intolerance.[4]

# APPENDIX I: ANOVA AND DESCRIPTIVE STATISTICS

**Descriptives**

Glucose AUC

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Asp | 10 | 10150.5000 | 1881.43296 | 594.96134 | 8804.6039 | 11496.3961 | 7980.00 | 14025.00 |
| Suc | 10 | 9472.5000 | 730.59051 | 231.03301 | 8949.8670 | 9995.1330 | 8025.00 | 10530.00 |
| Con | 10 | 9147.8500 | 1469.36647 | 464.65448 | 8096.7285 | 10198.9715 | 6757.50 | 10995.00 |
| Total | 30 | 9590.2833 | 1454.22028 | 265.50308 | 9047.2686 | 10133.2981 | 6757.50 | 14025.00 |

**ANOVA**

Glucose AUC

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 5234628.817 | 2 | 2617314.408 | 1.260 | .300 |
| Within Groups | 56093313.025 | 27 | 2077530.112 | | |
| Total | 61327941.842 | 29 | | | |

**Multiple Comparisons**

Dependent Variable: Glucose AUC

Tukey HSD

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Asp | Suc | 678.00000 | 644.59757 | .551 | -920.2266 | 2276.2266 |
| | Con | 1002.65000 | 644.59757 | .282 | -595.5766 | 2600.8766 |
| Suc | Asp | -678.00000 | 644.59757 | .551 | -2276.2266 | 920.2266 |
| | Con | 324.65000 | 644.59757 | .870 | -1273.5766 | 1922.8766 |
| Con | Asp | -1002.65000 | 644.59757 | .282 | -2600.8766 | 595.5766 |
| | Suc | -324.65000 | 644.59757 | .870 | -1922.8766 | 1273.5766 |

**Descriptives**

Glucose 0 Min

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Asp | 10 | 52.8000 | 8.96660 | 2.83549 | 46.3857 | 59.2143 | 36.00 | 66.00 |
| Suc | 10 | 58.9000 | 6.53962 | 2.06801 | 54.2218 | 63.5782 | 48.00 | 68.00 |
| Con | 10 | 54.4000 | 8.44854 | 2.67166 | 48.3563 | 60.4437 | 40.00 | 68.00 |
| Total | 30 | 55.3667 | 8.20212 | 1.49749 | 52.3039 | 58.4294 | 36.00 | 68.00 |

**ANOVA**

Glucose 0 MIN

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 200.067 | 2 | 100.033 | 1.543 | .232 |
| Within Groups | 1750.900 | 27 | 64.848 | | |
| Total | 1950.967 | 29 | | | |

**Multiple Comparisons**

Dependent Variable: Glucose 0 MIN

Tukey HSD

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Asp | Suc | -6.10000 | 3.60134 | .226 | -15.0292 | 2.8292 |
| | Con | -1.60000 | 3.60134 | .897 | -10.5292 | 7.3292 |
| Suc | Asp | 6.10000 | 3.60134 | .226 | -2.8292 | 15.0292 |
| | Con | 4.50000 | 3.60134 | .435 | -4.4292 | 13.4292 |
| Con | Asp | 1.60000 | 3.60134 | .897 | -7.3292 | 10.5292 |
| | Suc | -4.50000 | 3.60134 | .435 | -13.4292 | 4.4292 |

**Descriptives**

Glucose 15 min

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Asp | 10 | 84.3000 | 16.62027 | 5.25579 | 72.4106 | 96.1894 | 64.00 | 115.00 |
| Suc | 10 | 79.2000 | 11.82089 | 3.73809 | 70.7438 | 87.6562 | 63.00 | 99.00 |
| Con | 10 | 81.5000 | 21.93551 | 6.93662 | 65.8083 | 97.1917 | 43.00 | 110.00 |
| Total | 30 | 81.6667 | 16.82021 | 3.07094 | 75.3859 | 87.9474 | 43.00 | 115.00 |

**ANOVA**

Glucose 15 min

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 130.467 | 2 | 65.233 | .218 | .805 |
| Within Groups | 8074.200 | 27 | 299.044 | | |
| Total | 8204.667 | 29 | | | |

**Multiple Comparisons**

Dependent Variable: Glucose 15 min

Tukey HSD

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Asp | Suc | 5.10000 | 7.73362 | .789 | -14.0749 | 24.2749 |
| | Con | 2.80000 | 7.73362 | .930 | -16.3749 | 21.9749 |
| Suc | Asp | -5.10000 | 7.73362 | .789 | -24.2749 | 14.0749 |
| | Con | -2.30000 | 7.73362 | .952 | -21.4749 | 16.8749 |
| Con | Asp | -2.80000 | 7.73362 | .930 | -21.9749 | 16.3749 |
| | Suc | 2.30000 | 7.73362 | .952 | -16.8749 | 21.4749 |

**Descriptives**

Glucose 30 min

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Asp | 10 | 94.6000 | 19.68756 | 6.22575 | 80.5164 | 108.6836 | 65.00 | 131.00 |
| Suc | 10 | 92.5000 | 11.72130 | 3.70660 | 84.1151 | 100.8849 | 73.00 | 113.00 |
| Con | 10 | 83.9000 | 16.29894 | 5.15418 | 72.2404 | 95.5596 | 62.00 | 107.00 |
| Total | 30 | 90.3333 | 16.35666 | 2.98630 | 84.2257 | 96.4410 | 62.00 | 131.00 |

**ANOVA**

Glucose 30 min

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 642.867 | 2 | 321.433 | 1.220 | .311 |
| Within Groups | 7115.800 | 27 | 263.548 | | |
| Total | 7758.667 | 29 | | | |

**Multiple Comparisons**

Dependent Variable: Glucose 30 min

Tukey HSD

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Asp | Suc | 2.10000 | 7.26014 | .955 | -15.9009 | 20.1009 |
| | Con | 10.70000 | 7.26014 | .319 | -7.3009 | 28.7009 |
| Suc | Asp | -2.10000 | 7.26014 | .955 | -20.1009 | 15.9009 |
| | Con | 8.60000 | 7.26014 | .472 | -9.4009 | 26.6009 |
| Con | Asp | -10.70000 | 7.26014 | .319 | -28.7009 | 7.3009 |
| | Suc | -8.60000 | 7.26014 | .472 | -26.6009 | 9.4009 |

**Descriptives**

Glucose 60 min

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Asp | 10 | 91.1000 | 20.01916 | 6.33061 | 76.7792 | 105.4208 | 71.00 | 135.00 |
| Suc | 10 | 84.5000 | 9.86858 | 3.12072 | 77.4404 | 91.5596 | 70.00 | 100.00 |
| Con | 10 | 84.3000 | 11.26499 | 3.56230 | 76.2415 | 92.3585 | 68.00 | 100.00 |
| Total | 30 | 86.6333 | 14.29368 | 2.60966 | 81.2960 | 91.9707 | 68.00 | 135.00 |

**ANOVA**

Glucose 60 min

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 299.467 | 2 | 149.733 | .719 | .496 |
| Within Groups | 5625.500 | 27 | 208.352 | | |
| Total | 5924.967 | 29 | | | |

**Multiple Comparisons**

Dependent Variable: Glucose 60 min

Tukey HSD

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Asp | Suc | 6.60000 | 6.45526 | .569 | -9.4053 | 22.6053 |
| | Con | 6.80000 | 6.45526 | .550 | -9.2053 | 22.8053 |
| Suc | Asp | -6.60000 | 6.45526 | .569 | -22.6053 | 9.4053 |
| | Con | .20000 | 6.45526 | .999 | -15.8053 | 16.2053 |
| Con | Asp | -6.80000 | 6.45526 | .550 | -22.8053 | 9.2053 |
| | Suc | -.20000 | 6.45526 | .999 | -16.2053 | 15.8053 |

**Descriptives**

Glucose 120 min

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Asp | 10 | 75.4000 | 25.44362 | 8.04598 | 57.1987 | 93.6013 | 58.00 | 146.00 |
| Suc | 10 | 65.3000 | 7.13442 | 2.25610 | 60.1963 | 70.4037 | 54.00 | 79.00 |
| Con | 10 | 61.2000 | 12.15456 | 3.84361 | 52.5052 | 69.8948 | 40.00 | 76.00 |
| Total | 30 | 67.3000 | 17.30288 | 3.15906 | 60.8390 | 73.7610 | 40.00 | 146.00 |

**ANOVA**

Glucose 120 min

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1068.200 | 2 | 534.100 | 1.894 | .170 |
| Within Groups | 7614.100 | 27 | 282.004 | | |
| Total | 8682.300 | 29 | | | |

**Multiple Comparisons**

Dependent Variable: Glucose 120 min

Tukey HSD

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Asp | Suc | 10.10000 | 7.51004 | .383 | -8.5205 | 28.7205 |
| | Con | 14.20000 | 7.51004 | .161 | -4.4205 | 32.8205 |
| Suc | Asp | -10.10000 | 7.51004 | .383 | -28.7205 | 8.5205 |
| | Con | 4.10000 | 7.51004 | .849 | -14.5205 | 22.7205 |
| Con | Asp | -14.20000 | 7.51004 | .161 | -32.8205 | 4.4205 |
| | Suc | -4.10000 | 7.51004 | .849 | -22.7205 | 14.5205 |

**Descriptives**

Insulin AUC

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| ASP | 6 | 63.1242 | 13.98617 | 5.70983 | 48.4466 | 77.8017 | 41.32 | 80.55 |
| SUC | 7 | 71.1643 | 8.54774 | 3.23074 | 63.2589 | 79.0696 | 55.95 | 77.70 |
| CON | 6 | 81.4017 | 20.94478 | 8.55067 | 59.4215 | 103.3819 | 54.60 | 113.33 |
| Total | 19 | 71.8582 | 16.01632 | 3.67439 | 64.1385 | 79.5778 | 41.32 | 113.33 |

**ANOVA**

Insulin AUC

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1007.537 | 2 | 503.769 | 2.233 | .140 |
| Within Groups | 3609.866 | 16 | 225.617 | | |
| Total | 4617.403 | 18 | | | |

**Multiple Comparisons**

Dependent Variable: Insulin AUC

Tukey HSD

| (I) Group | (J) Group | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| ASP | SUC | -8.04012 | 8.35666 | .610 | -29.6031 | 13.5228 |
| | CON | -18.27750 | 8.67211 | .120 | -40.6544 | 4.0994 |
| SUC | ASP | 8.04012 | 8.35666 | .610 | -13.5228 | 29.6031 |
| | CON | -10.23738 | 8.35666 | .456 | -31.8003 | 11.3256 |
| CON | ASP | 18.27750 | 8.67211 | .120 | -4.0994 | 40.6544 |
| | SUC | 10.23738 | 8.35666 | .456 | -11.3256 | 31.8003 |

**Descriptives**

Insulin 0 min

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| ASP | 6 | .6317 | .04750 | .01939 | .5818 | .6815 | .58 | .70 |
| SUC | 7 | .4886 | .20301 | .07673 | .3008 | .6763 | .22 | .69 |
| CON | 6 | .6550 | .29710 | .12129 | .3432 | .9668 | .27 | .99 |
| Total | 19 | .5863 | .21180 | .04859 | .4842 | .6884 | .22 | .99 |

**ANOVA**

Insulin 0 min

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .108 | 2 | .054 | 1.229 | .319 |
| Within Groups | .700 | 16 | .044 | | |
| Total | .807 | 18 | | | |

**Multiple Comparisons**

Dependent Variable: Insulin 0 min

Tukey HSD

| (I) Group | (J) Group | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| ASP | SUC | .14310 | .11636 | .454 | -.1572 | .4433 |
| | CON | -.02333 | .12075 | .980 | -.3349 | .2883 |
| SUC | ASP | -.14310 | .11636 | .454 | -.4433 | .1572 |
| | CON | -.16643 | .11636 | .350 | -.4667 | .1338 |
| CON | ASP | .02333 | .12075 | .980 | -.2883 | .3349 |
| | SUC | .16643 | .11636 | .350 | -.1338 | .4667 |

**Descriptives**

Insulin 15 min

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| ASP | 6 | .6033 | .17862 | .07292 | .4159 | .7908 | .42 | .87 |
| SUC | 7 | .7700 | .27899 | .10545 | .5120 | 1.0280 | .45 | 1.16 |
| CON | 6 | .9267 | .37431 | .15281 | .5339 | 1.3195 | .35 | 1.38 |
| Total | 19 | .7668 | .30192 | .06927 | .6213 | .9124 | .35 | 1.38 |

**ANOVA**

Insulin 15 min

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .314 | 2 | .157 | 1.891 | .183 |
| Within Groups | 1.327 | 16 | .083 | | |
| Total | 1.641 | 18 | | | |

**Multiple Comparisons**

Dependent Variable: Insulin 15 min

Tukey HSD

| (I) Group | (J) Group | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| ASP | SUC | -.16667 | .16023 | .563 | -.5801 | .2468 |
| | CON | -.32333 | .16627 | .159 | -.7524 | .1057 |
| SUC | ASP | .16667 | .16023 | .563 | -.2468 | .5801 |
| | CON | -.15667 | .16023 | .601 | -.5701 | .2568 |
| CON | ASP | .32333 | .16627 | .159 | -.1057 | .7524 |
| | SUC | .15667 | .16023 | .601 | -.2568 | .5701 |

**Descriptives**

Insulin 30 min

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| ASP | 6 | .5117 | .18809 | .07679 | .3143 | .7091 | .29 | .79 |
| SUC | 7 | .7486 | .15731 | .05946 | .6031 | .8941 | .49 | .93 |
| CON | 6 | .6083 | .23173 | .09460 | .3652 | .8515 | .36 | 1.05 |
| Total | 19 | .6295 | .20805 | .04773 | .5292 | .7297 | .29 | 1.05 |

**ANOVA**

Insulin 30 min

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .185 | 2 | .093 | 2.495 | .114 |
| Within Groups | .594 | 16 | .037 | | |
| Total | .779 | 18 | | | |

**Multiple Comparisons**

Dependent Variable: Insulin 3 0min

Tukey HSD

| (I) Group | (J) Group | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| ASP | SUC | -.23690 | .10718 | .100 | -.5135 | .0397 |
| | CON | -.09667 | .11123 | .667 | -.3837 | .1903 |
| SUC | ASP | .23690 | .10718 | .100 | -.0397 | .5135 |
| | CON | .14024 | .10718 | .411 | -.1363 | .4168 |
| CON | ASP | .09667 | .11123 | .667 | -.1903 | .3837 |
| | SUC | -.14024 | .10718 | .411 | -.4168 | .1363 |

**Descriptives**

Insulin 60 min

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| ASP | 6 | .5150 | .21436 | .08751 | .2900 | .7400 | .27 | .81 |
| SUC | 7 | .5471 | .14430 | .05454 | .4137 | .6806 | .32 | .78 |
| CON | 6 | .6267 | .30592 | .12489 | .3056 | .9477 | .32 | 1.15 |
| Total | 19 | .5621 | .21890 | .05022 | .4566 | .6676 | .27 | 1.15 |

**ANOVA**

Insulin 60 min

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .040 | 2 | .020 | .388 | .685 |
| Within Groups | .823 | 16 | .051 | | |
| Total | .863 | 18 | | | |

**Multiple Comparisons**

Dependent Variable: Insulin 60 min

Tukey HSD

| (I) Group | (J) Group | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| ASP | SUC | -.03214 | .12615 | .965 | -.3577 | .2934 |
| | CON | -.11167 | .13091 | .677 | -.4495 | .2261 |
| SUC | ASP | .03214 | .12615 | .965 | -.2934 | .3577 |
| | CON | -.07952 | .12615 | .806 | -.4050 | .2460 |
| CON | ASP | .11167 | .13091 | .677 | -.2261 | .4495 |
| | SUC | .07952 | .12615 | .806 | -.2460 | .4050 |

**Descriptives**

Insulin 120 min

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| ASP | 6 | .4883 | .21442 | .08754 | .2633 | .7134 | .21 | .82 |
| SUC | 7 | .4829 | .17260 | .06524 | .3232 | .6425 | .18 | .69 |
| CON | 6 | .7183 | .44678 | .18240 | .2495 | 1.1872 | .14 | 1.50 |
| Total | 19 | .5589 | .30089 | .06903 | .4139 | .7040 | .14 | 1.50 |

**ANOVA**

Insulin 120 min

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .223 | 2 | .111 | 1.267 | .308 |
| Within Groups | 1.407 | 16 | .088 | | |
| Total | 1.630 | 18 | | | |

**Multiple Comparisons**

Dependent Variable: Insulin 120 min

Tukey HSD

| (I) Group | (J) Group | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| ASP | SUC | .00548 | .16496 | .999 | -.4202 | .4311 |
| | CON | -.23000 | .17119 | .393 | -.6717 | .2117 |
| SUC | ASP | -.00548 | .16496 | .999 | -.4311 | .4202 |
| | CON | -.23548 | .16496 | .351 | -.6611 | .1902 |
| CON | ASP | .23000 | .17119 | .393 | -.2117 | .6717 |
| | SUC | .23548 | .16496 | .351 | -.1902 | .6611 |

**Descriptives**

Weight Change

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Asp | 10 | 232.7000 | 30.94457 | 9.78553 | 210.5636 | 254.8364 | 174.00 | 290.00 |
| Suc | 10 | 249.2000 | 16.73851 | 5.29318 | 237.2260 | 261.1740 | 215.00 | 278.00 |
| Con | 10 | 239.3000 | 15.79768 | 4.99566 | 227.9990 | 250.6010 | 224.00 | 269.00 |
| Total | 30 | 240.4000 | 22.56424 | 4.11965 | 231.9744 | 248.8256 | 174.00 | 290.00 |

**ANOVA**

Weight Change

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1379.400 | 2 | 689.700 | 1.391 | .266 |
| Within Groups | 13385.800 | 27 | 495.770 | | |
| Total | 14765.200 | 29 | | | |

**Multiple Comparisons**

Dependent Variable: Weight Change

Tukey HSD

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Asp | Suc | -16.50000 | 9.95761 | .240 | -41.1891 | 8.1891 |
| | Con | -6.60000 | 9.95761 | .787 | -31.2891 | 18.0891 |
| Suc | Asp | 16.50000 | 9.95761 | .240 | -8.1891 | 41.1891 |
| | Con | 9.90000 | 9.95761 | .587 | -14.7891 | 34.5891 |
| Con | Asp | 6.60000 | 9.95761 | .787 | -18.0891 | 31.2891 |
| | Suc | -9.90000 | 9.95761 | .587 | -34.5891 | 14.7891 |

**Descriptives**

Body Fat %

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Asp | 10 | 19.3000 | 2.21811 | .70143 | 17.7133 | 20.8867 | 16.50 | 23.90 |
| Suc | 10 | 17.7000 | 2.10660 | .66617 | 16.1930 | 19.2070 | 13.80 | 21.40 |
| Con | 10 | 17.5900 | 2.88230 | .91146 | 15.5281 | 19.6519 | 13.30 | 23.20 |
| Total | 30 | 18.1967 | 2.47268 | .45145 | 17.2734 | 19.1200 | 13.30 | 23.90 |

**ANOVA**

Body Fat %

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 18.321 | 2 | 9.160 | 1.556 | .229 |
| Within Groups | 158.989 | 27 | 5.888 | | |
| Total | 177.310 | 29 | | | |

**Multiple Comparisons**

Dependent Variable: Body Fat %

Tukey HSD

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Asp | Suc | 1.60000 | 1.08522 | .319 | -1.0907 | 4.2907 |
| | Con | 1.71000 | 1.08522 | .273 | -.9807 | 4.4007 |
| Suc | Asp | -1.60000 | 1.08522 | .319 | -4.2907 | 1.0907 |
| | Con | .11000 | 1.08522 | .994 | -2.5807 | 2.8007 |
| Con | Asp | -1.71000 | 1.08522 | .273 | -4.4007 | .9807 |
| | Suc | -.11000 | 1.08522 | .994 | -2.8007 | 2.5807 |

**Descriptives**

Weight of Epididymal Fat Pads

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| ASP | 10 | 5.4960 | 1.06671 | .33732 | 4.7329 | 6.2591 | 4.00 | 7.40 |
| SUC | 10 | 4.9970 | .75208 | .23783 | 4.4590 | 5.5350 | 3.76 | 6.33 |
| CON | 10 | 4.5450 | .60526 | .19140 | 4.1120 | 4.9780 | 3.68 | 5.39 |
| Total | 30 | 5.0127 | .89354 | .16314 | 4.6790 | 5.3463 | 3.68 | 7.40 |

**ANOVA**

Weight of Epididymal Fat Pads

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 4.526 | 2 | 2.263 | 3.280 | .053 |
| Within Groups | 18.629 | 27 | .690 | | |
| Total | 23.154 | 29 | | | |

**Multiple Comparisons**

Dependent Variable: Weight of Epididymal Fat Pads

Tukey HSD

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| ASP | SUC | .49900 | .37147 | .384 | -.4220 | 1.4200 |
| | CON | .95100* | .37147 | .042 | .0300 | 1.8720 |
| SUC | ASP | -.49900 | .37147 | .384 | -1.4200 | .4220 |
| | CON | .45200 | .37147 | .454 | -.4690 | 1.3730 |
| CON | ASP | -.95100* | .37147 | .042 | -1.8720 | -.0300 |
| | SUC | -.45200 | .37147 | .454 | -1.3730 | .4690 |

*. The mean difference is significant at the 0.05 level.

APPENDIX II: RAW DATA

OGTT SERUM GLUCOSE VALUES

| **Subject** | **0 min** (mg/dL) | **15 min** (mg/dL) | **30 min** (mg/dL) | **60 min** (mg/dL) | **120 min** (mg/dL) |
|---|---|---|---|---|---|
| ASP - 1 | 46 | 74 | 94 | 135 | 146 |
| ASP - 2 | 49 | 82 | 65 | 84 | 66 |
| ASP - 3 | 63 | 99 | 131 | 109 | 76 |
| ASP - 4 | 36 | 65 | 78 | 72 | 58 |
| ASP - 5 | 57 | 77 | 101 | 95 | 69 |
| ASP - 6 | 54 | 99 | 104 | 104 | 72 |
| ASP - 7 | 55 | 93 | 113 | 83 | 66 |
| ASP - 8 | 45 | 75 | 78 | 79 | 62 |
| ASP - 9 | 57 | 64 | 80 | 71 | 64 |
| ASP - 10 | 66 | 115 | 102 | 79 | 75 |
| SUC - 11 | 58 | 63 | 87 | 94 | 66 |
| SUC - 12 | 51 | 99 | 113 | 90 | 61 |
| SUC - 13 | 60 | 64 | 92 | 93 | 69 |
| SUC - 14 | 53 | 81 | 91 | 100 | 79 |
| SUC - 15 | 48 | 81 | 93 | 83 | 63 |
| SUC - 16 | 61 | 71 | 80 | 84 | 63 |
| SUC - 17 | 67 | 71 | 73 | 71 | 54 |
| SUC - 18 | 62 | 83 | 93 | 70 | 66 |
| SUC - 19 | 68 | 91 | 108 | 78 | 73 |
| SUC - 20 | 61 | 88 | 95 | 82 | 59 |
| CON - 21 | 49 | 96 | 98 | 100 | 62 |
| CON - 22 | 40 | 65 | 68 | 72 | 59 |
| CON - 23 | 55 | 43 | 64 | 68 | 40 |
| CON - 24 | 44 | 57 | 62 | 75 | 44 |
| CON - 25 | 55 | 72 | 72 | 81 | 54 |
| CON - 26 | 60 | 89 | 89 | 92 | 69 |
| CON - 27 | 60 | 103 | 86 | 76 | 76 |
| CON - 28 | 68 | 80 | 101 | 96 | 66 |
| CON - 29 | 61 | 110 | 107 | 94 | 75 |
| CON - 30 | 52 | 100 | 92 | 89 | 67 |

OGTT SERUM INSULIN VALUES

| **Subject Number** | **0 min** (ng/ml) | **15 min** (ng/ml) | **30 min** (ng/ml) | **60 min** (ng/ml) | **120 min** (ng/ml) |
|---|---|---|---|---|---|
| ASP - 1 | 0.68 | 0.42 | 0.36 | 0.81 | 0.82 |
| ASP - 5 | 0.7 | 0.77 | 0.67 | 0.4 | 0.44 |
| ASP - 6 | 0.61 | 0.53 | 0.79 | 0.74 | 0.41 |
| ASP - 7 | 0.6 | 0.46 | 0.29 | 0.38 | 0.21 |
| ASP - 8 | 0.62 | 0.57 | 0.49 | 0.49 | 0.4 |
| ASP - 10 | 0.58 | 0.87 | 0.47 | 0.27 | 0.65 |
| SUC - 13 | 0.69 | 0.57 | 0.49 | 0.65 | 0.67 |
| SUC - 14 | 0.64 | 0.8 | 0.62 | 0.78 | 0.39 |
| SUC - 15 | 0.26 | 1.16 | 0.8 | 0.48 | 0.47 |
| SUC - 17 | 0.62 | 0.45 | 0.74 | 0.5 | 0.18 |
| SUC - 18 | 0.35 | 1.09 | 0.93 | 0.52 | 0.48 |
| SUC - 19 | 0.22 | 0.81 | 0.74 | 0.58 | 0.69 |
| SUC - 20 | 0.64 | 0.51 | 0.92 | 0.32 | 0.5 |
| CON - 22 | 0.84 | 1.03 | 1.05 | 1.15 | 0.54 |
| CON - 23 | 0.32 | 0.95 | 0.52 | 0.4 | 1.5 |
| CON - 26 | 0.27 | 0.65 | 0.36 | 0.54 | 0.62 |
| CON - 27 | 0.67 | 1.2 | 0.56 | 0.54 | 0.82 |
| CON - 29 | 0.99 | 1.38 | 0.58 | 0.81 | 0.14 |
| CON - 30 | 0.84 | 0.35 | 0.58 | 0.32 | 0.69 |

WEIGHT

| Subject | Week 1 (g) | Week 2 (g) | Week 3 (g) | Week 4 (g) | Week 5 (g) | Week 6 (g) |
|---|---|---|---|---|---|---|
| ASP - 1 | 137 | 208 | 264 | 321 | 362 | 356 |
| ASP - 2 | 144 | 202 | 262 | 325 | 354 | 368 |
| ASP - 3 | 157 | 203 | 312 | 400 | 442 | 447 |
| ASP - 4 | 135 | 202 | 269 | 325 | 350 | 363 |
| ASP - 5 | 151 | 218 | 294 | 367 | 390 | 417 |
| ASP - 6 | 150 | 217 | 286 | 348 | 405 | 398 |
| ASP - 7 | 150 | 225 | 297 | 354 | 385 | 367 |
| ASP - 8 | 154 | 217 | 276 | 329 | 353 | 328 |
| ASP - 9 | 149 | 217 | 288 | 337 | 365 | 378 |
| ASP - 10 | 145 | 208 | 277 | 334 | 370 | 377 |
| SUC - 11 | 153 | 234 | 302 | 371 | 404 | 415 |
| SUC - 12 | 161 | 238 | 301 | 368 | 406 | 415 |
| SUC - 13 | 150 | 222 | 285 | 347 | 383 | 393 |
| SUC - 14 | 157 | 230 | 298 | 356 | 385 | 396 |
| SUC - 15 | 158 | 222 | 309 | 389 | 426 | 436 |
| SUC - 16 | 153 | 224 | 299 | 361 | 400 | 409 |
| SUC - 17 | 156 | 234 | 303 | 371 | 410 | 414 |
| SUC - 18 | 162 | 226 | 296 | 357 | 399 | 407 |
| SUC - 19 | 136 | 203 | 277 | 336 | 372 | 378 |
| SUC - 20 | 143 | 211 | 270 | 319 | 348 | 358 |
| CON - 21 | 155 | 226 | 287 | 337 | 372 | 381 |
| CON - 22 | 150 | 212 | 270 | 332 | 361 | 374 |
| CON - 23 | 154 | 228 | 298 | 368 | 405 | 418 |
| CON - 24 | 151 | 221 | 275 | 328 | 365 | 377 |
| CON - 25 | 158 | 224 | 289 | 354 | 388 | 388 |
| CON - 26 | 142 | 207 | 265 | 336 | 369 | 378 |
| CON - 27 | 152 | 222 | 290 | 345 | 377 | 389 |
| CON - 28 | 151 | 215 | 282 | 347 | 385 | 397 |
| CON - 29 | 153 | 225 | 296 | 367 | 404 | 422 |
| CON - 30 | 156 | 217 | 281 | 342 | 380 | 391 |

BODY COMPOSITION

| **Subject** | **Body Fat** (%) | **Epididymal Pads** (g) |
|---|---|---|
| ASP - 1 | 17.1 | 4 |
| ASP - 2 | 16.5 | 4.8 |
| ASP - 3 | 20.8 | 7.4 |
| ASP - 4 | 18.4 | 5.02 |
| ASP - 5 | 17.9 | 7.07 |
| ASP - 6 | 18.3 | 5.93 |
| ASP - 7 | 21.4 | 5.69 |
| ASP - 8 | 19.3 | 5.25 |
| ASP - 9 | 19.4 | 4.59 |
| ASP - 10 | 23.9 | 5.21 |
| SUC - 11 | 21.4 | 5.33 |
| SUC - 12 | 13.8 | 5.72 |
| SUC - 13 | 18.2 | 4.39 |
| SUC - 14 | 16.8 | 4.67 |
| SUC - 15 | 17.4 | 5.5 |
| SUC - 16 | 17.6 | 5.15 |
| SUC - 17 | 18 | 4.65 |
| SUC - 18 | 15.4 | 4.47 |
| SUC - 19 | 19.2 | 6.33 |
| SUC - 20 | 19.2 | 3.76 |
| CON - 21 | 18.2 | 5.26 |
| CON - 22 | 13.3 | 3.83 |
| CON - 23 | 15.3 | 4.45 |
| CON - 24 | 18.4 | 3.68 |
| CON - 25 | 16.9 | 5.39 |
| CON - 26 | 16.2 | 4.52 |
| CON - 27 | 18.5 | 4.11 |
| CON - 28 | 23.2 | 5.26 |
| CON - 29 | 15.2 | 4.26 |
| CON - 30 | 20.7 | 4.69 |

# APPENDIX III: PROTOCOL FOR ANIMAL CARE AND USE

UNLV IACUC

## Approved

| | |
|---|---|
| DATE: | July 8, 2015 |
| TO: | John Young |
| FROM: | UNLV IACUC |
| PROJECT TITLE: | [759773-3] The Effect of Nonnutritive Sweeteners on Glucose Tolerance and Body Composition in Rats |
| REFERENCE #: | |
| SUBMISSION TYPE: | Revision |
| ACTION: | APPROVED |
| DECISION DATE: | July 8, 2015 |
| EXPIRATION DATE: | July 7, 2018 |

Thank you for your submission of Revision materials for this research project. The UNLV IACUC has APPROVED your submission. All research must be conducted in accordance with this approved submission.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation. Please use the appropriate amendment form for this procedure.

Please report all NON-COMPLIANCE issues regarding this project to this committee.

**Annual Review**

This project requires annual review by this office. Please use the appropriate annual review form for this procedure.

**Renewals**

All projects expire three years after the approval date. You must submit and obtain approval for a new project prior to July 7, 2018 to avoid any lapse in approval.

If you have any questions, please contact Kevin Bergeron at (702) 895-5453 or kevin.bergeron@unlv.edu. Please include your project title and reference number in all correspondence with this committee.

Office of Research Integrity - IACUC & IBC
4505 Maryland Parkway . Box 454022 . Las Vegas, Nevada 89154-4022
(702) 895-5453

UNLV **Institutional Animal Care and Use Committee**

## PROTOCOL FOR ANIMAL CARE AND USE

1. PROJECT OR EXERCISE TITLE: The Effect of Nonnutritive Sweeteners on Glucose Tolerance and Body Composition in Rats

2. PRINCIPAL INVESTIGATOR/COURSE DIRECTOR:
NAME and TITLE: Dr. John Young
DEPARTMENT: Kinesiology and Nutrtion Sciences
OFFICE PHONE: 702-895-4626 EMERGENCY PHONE: 702-682-7792
LAB CONTACT (TECHNICIAN) and PHONE NUMBER:
LOCATION (BUILDING/LAB#):
E-MAIL:john.young@unlv.edu FAX: 5-1500
Does the PI have direct contact with the animals? ☒ Yes ☐ No

3. LIST RESEARCHERS AND STAFF INVOLVED WITH ANY PROTOCOL PROCEDURES:

| Name | Responsibility | Office Phone | Emergency Phone |
|---|---|---|---|
| Ashley Tovar | Student | | 702-683-2594 |
| Dr. Laura Kruskall | Co-PI | 702-895-4985 | 702-274-0370 |
| Dr. James Navalta | Data Analysis | 702-895-2344 | |

*Hit tab to add more rows to any of the tables in this form. Use the down arrow to move out of the table to the next field.*

4. TRAINING FOR ALL PERSONNEL WORKING WITH ANIMALS ON THIS PROTOCOL:

| Name | Role in this protocol (PI, co-PI, student, etc.) | Experience working with species | Training Received |
|---|---|---|---|
| Jack Young | PI | 38 Years | 1991 UNLV |
| Laura Kruskall | Co-PI | 13 years | 2001 UNLV |
| Ashley Tovar | Student | 1 year | 2015 UNLV |

5. PROTOCOL STATUS: ☒ New/Renewal ☐ Modification

START DATE: 8/2015 ANTICIPATED COMPLETION DATE (3 yr max): 11/2015

6. FUNDING SOURCE: ☒ DEPARTMENT ☒ OTHER INTRAMURAL SOURCE
☐ EXTRAMURAL SOURCE SPECIFY SOURCE:
**IF FUNDED**, GRANT NUMBER:

7. CATEGORY OF ETHICAL CONCERN APPLICABLE TO THIS PROTOCOL (see Appendix A): C

8. JUSTIFICATION: THIS SECTION IS REQUIRED FOR ANY PROJECT IN WHICH THERE IS A POTENTIAL FOR PAIN, DISTRESS, OR DISCOMFORT THAT **CANNOT BE ALLEVIATED**. EXAMPLES OF SUCH PROJECTS INCLUDE INDUCED DISEASE STATES WHICH CAUSE SEVERE SYMPTOMS OR DEATH, SURGICAL STUDIES RESULTING IN SEVERE POST-OPERATIVE DISCOMFORT OR LOSS OF FUNCTION, USE OF SEVERE AND UNAVOIDABLE NOXIOUS STIMULI, AND SO FORTH.

a. Will there be unrelieved pain or stress (defined as lasting for more than a moment, i.e. longer duration or more painful than a needlestick)? ☒ No ☐ Yes

b. If yes, provide justification and an indication of the number of animals, per year, that are going to experience unrelieved pain or stress must be provided.

c. Is death an end-point? ☒ No ☐ Yes

d. If yes, explain why some earlier end-point data cannot be used instead (provide objective supporting data).

9. WILL THE PROJECT OR EXERCISE INVOLVE EXPOSURE OF ANIMALS OR ANIMAL HANDLERS TO:

| | | | |
|---|---|---|---|
| BIOHAZARDS? | ☒ NO | ☐ YES | IF YES, SPECIFY: |
| RADIOISOTOPES? | ☒ NO | ☐ YES | IF YES, SPECIFY: |
| CARCINOGENS? | ☒ NO | ☐ YES | IF YES, SPECIFY: |
| TOXIC CHEMICALS? | ☒ NO | ☐ YES | IF YES, SPECIFY: |

IF ANY ITEM ANSWERED YES, PLEASE COMPLETE AND **ATTACH SUPPLEMENT FORM 1.** An approved Institutional Biosafety Committee protocol may be required.

10. ITEMS WHICH APPLY TO THIS PROJECT:

| | |
|---|---|
| ☒ Blood and/or tissue collection | ☐ Survival surgery |
| ☐ Antibody production and collection | ☐ Non-survival surgery |
| ☐ Behavioral studies | ☐ Aseptic surgery |
| ☐ Prolonged physical restraint | ☐ Multiple surgeries on the same animal |
| ☐ Food or water deprivation | ☐ Alleviated pain |
| ☐ Environmental extremes | ☐ Unalleviated pain |
| ☐ Electrical stimuli | ☒ Anesthetics used |
| ☐ Induction of trauma | ☐ Single housing |
| ☐ Work to be done off campus | ☐ Immobilizing agents without anesthesia |
| ☐ Field studies | |

11. IS SURGERY INVOLVED IN THE PROPOSED PROJECT?
☒ No ☐ Yes IF YES, **ATTACH SUPPLEMENT 2**

12. ARE STRESSFUL OR PAINFUL PROCEDURES, OTHER THAN SURGERY, PART OF THIS PROJECT?
☒ No ☐ Yes IF YES, **ATTACH SUPPLEMENT FORM 3**

13. ARE PROLONGED (MORE THAN A FEW HOURS) PHYSICAL RESTRAINT PROCEDURES PART OF THIS PROJECT?
☒ No ☐ Yes IF YES, **ATTACH SUPPLEMENT FORM 4**

14. WILL GENETICALLY ENGINEERED OR OTHER MUTANT ANIMALS BE PRODUCED OR MAINTAINED THAT MAY BE EXPECTED TO DEVELOP CLINICAL SIGNS AS A RESULT OF THE MUTATION?
☒ No ☐ Yes IF YES, **ATTACH SUPPLEMENT FORM 5. AN APPROVED INSTITUTIONAL BIOSAFETY COMMITTEE PROTOCOL IS ALSO REQUIRED.**

15. WILL TISSUES, CELLS OR OTHER PRODUCTS DERIVED FROM ANIMALS BE SHARED OR A TISSUE BANK ESTABLISHED FOR FUTURE SHARING WITH OTHER INVESTIGATORS DURING OR AFTER THE PROJECT?
☒ No ☐ Yes

16. ARE BEHAVIORAL STUDIES INVOLVED IN THIS PROJECT? ☐ Yes ☒ No
Do any of the following procedures or conditions apply?

| | | |
|---|---|---|
| Food Reward | ☐ | |
| Electrical Shock | ☐ | Strength and duration: |
| Food Deprivation | ☐ | Kcal/day provided: |
| Water Deprivation | ☐ | ml/kg provided: |

If a conditioning protocol applies, please explain the purpose.

Criteria for monitoring the condition of the animals during food and water deprivation:

17. ARE THERE ANY EXCEPTIONS TO THE GUIDE (*Guide for the Care and Use of Laboratory Animals, National Research Council*)? ☐ Yes ☒ No

If yes, describe the exceptions in detail including the scientific justification for the exception. The most common exceptions include; single housing, lighting, food/water variations, cage changes, temperature, ventilation, or euthanasia method that differs from the guide.

| |
|---|
| |

18. ANIMAL CENSUS AND HOUSING - Use one line/species/pain category. Repeat table rows as needed.

| Common Species Name/Scientific Name | Strain/Stock Breed | Sex | Starting Age/Weight | Total # Requested | Pain Category |
|---|---|---|---|---|---|
| Rat/ Rattus norvegicus | Wistar/Sprague-Dawley | Male | 140-160 g (10 weeks of age)<br><br>Puberty is reached at 50 days ±10 days. 10 weeks ensures no changes in weight related to puberty rather than treatment. | 30 | N/A |
| | | | | | |

During the course of the study, list the source of the animals and the expected average daily census:

| Average Daily Census: 24-30 | Source of the animals: Approved vendor |
|---|---|
| | |
| | |

Will these animals be bred? If so explain, please include numbers of animals bred and anticipated number of offspring.

| |
|---|
| No. |

19. LOCATION OF ANIMAL HOUSING AND USE AREAS:

ANIMAL HOUSING FACILITY: UNLV Lab Animal Care Facility
WILL ANIMALS BE REMOVED FROM THE HOUSING FACILITY FOR USE ELSEWHERE?
☐ No ☒ Yes IF YES, SPECIFY LOCATIONS:

| Procedures | Building or Site | Room |
|---|---|---|
| Non surgical procedures or conditions | LACF | |
| Nonsurvival surgery | N/A | |
| Survival surgery | N/A | |
| Postsurgical/Postanesthesia/Postprocedural Recovery | N/A | |

Maximum number of hours at one time the animals will be kept in the laboratory/surgery:
☒ 0-12 hrs ☐ 12-24 hrs ☐ over 24 hrs *(Animals kept over 24hours require an establishment of an IACUC approved satellite facility.)*

20. ARE THERE ANY SPECIAL REQUIREMENTS FOR ANIMAL HOUSING, DIETS, RESTRAINT, OR PROCEDURES FOR DISPOSAL? ☒ Yes ☐ No

IF NO, ANIMALS WILL BE MAINTAINED ACCORDING TO THE STANDARD OPERATING PROCEDURES. IF YES, PROVIDE (AN) EXPLANATION(S) FOR EACH PARAMETER INVOLVED (E.G., LIGHT:DARK CYCLES, HUMIDITY, AMBIENT TEMPERATURE, CAGING BEDDING, DIETS, AND SO FORTH):

| |
|---|
| 12 hr light/dark cycle, standard chow diet provided by UNLV LACF, non-nutritive sweetener added to drinking water in 2 treatment groups: group 1 aspartame(n=10) and group 2 sucralose(n=10) |

21. INSTRUCTIONS FOR DISPOSITION OF SICK OR INJURED ANIMALS

Call Investigator ☐ Veterinarian (or Designee) to Treat ☐ Euthanize ☒

22. INSTRUCTIONS FOR DISPOSITION OF DEAD ANIMALS

Call Investigator ☐ Veterinarian (or Designee) to Necropsy ☐ Bag for Disposal ☒

23. IF WILD OR EXOTIC SPECIES ARE TO BE USED:

a. ARE SPECIAL PERMITS REQUIRED? ☐ Yes ☒ No
IF YES, ATTACH A COPY OF APPLICABLE PERMITS OR PERMIT APPLICATIONS

b. WILL ANIMALS BE OBSERVED, MANIPULATED, OR TRAPPED IN THE WILD? ☐ Yes ☒ No
IF YES, AND THEY HAVE NOT ALREADY BEEN ADDRESSED, DESCRIBE THE FIELD PROCEDURE(S) INCLUDING THE GEOGRAPHIC LOCATION(S) INVOLVED:

24. ADMINISTRATION OF ANESTHETICS AND ANALGESICS.

| Species | Drug | Route | Site/location | Volume | Dosage | Frequency |
|---|---|---|---|---|---|---|
| Rat | dexmedetomidine | Injection | Intraperitoneal | | 0.25-0.3 mg/kg - per DVM | 1 time |
| Rat | antesedan | injection | intraperotineal | | 0.5-1.0 mg/kg - per DVM | 1 time |

25. ADMINISTRATION OF EXOGENOUS SUBSTANCES
Complete this section for all drugs, infectious agents, carcinogens, toxins, experimental compounds, etc.

| Species | Drug/Substances | Route | Site/Location | Volume | Dosage | Frequency |
|---|---|---|---|---|---|---|
| Rat | aspartame | Drinking water | cages | 80-110 ml/kg/day | 5-7 ml/kg/day | Ad libitum |
| Rat | sucralose | Drinking water | cages | 80-110 ml/kg/day | 1-3 ml/kg/day | Ad libitum |

**All substances must be pharmaceutical grade**. If non-pharmaceutical grade substances will be used, please list the substance and explain why a pharmaceutical grade cannot be used:

Other Comments:

Aspartame dosage: Palmnäs, M. S. A., Cowan, T. E., Bomhof, M. R., Su, J., Reimer, R. A., Vogel, H. J., Shearer, J. (2014). Low-Dose Aspartame Consumption Differentially Affects Gut Microbiota-Host Metabolic Interactions in the Diet-Induced Obese Rat. *PLoS ONE*, *9*(10), e109841. doi:10.1371/journal.pone.0109841

Sucralose dosage: Abou-Donia, M. B., El-Masry, E. M., Abdel-Rahman, A. A., McLendon, R. E., and Schiffman, S. S. 2008. Splenda alters gut microflora and increases intestinal Pglycoprotein and cytochrome P-450 in male rats. *J. Toxicol. Environ. Health A* 71: 1415–1429.

26. ANTEMORTUM FLUID/TISSUE COLLECTION

| Species | Type of Fluid or Tissue | Volume or Mass | Frequency of Collection | Method or Route of Collection | Anesthesia or Sedation Used? |
|---|---|---|---|---|---|
| Rat | Blood samples | 300 µl | 0, 15, 30, 60, 120 minutes after glucose load | Tail bleeding | Yes (dexmedetomidine) (antesedan) |

27. EUTHANASIA

| Species | Method and Secondary Method (if applicable) | Drug (if applicable) | Dose of Drug mg/kg (if applicable) | Route |
|---|---|---|---|---|
| Rat | $CO_2$ | | | inhalation |
| Rat | Bilateral pneumothorax Note: carcasses must be intact for DEXA scans, so opening chest cavity and severing heart will be done after the DEXA scan is completed | | | |

28. LAY SUMMARY

This summary may be forwarded to the Office of Public Affairs. They may use it to describe your research to the media. The lay summary should be brief and readily understandable to the general public. Provide a summary of the project that explains the need for the research, what the project goals are, and how the use of animals will help reach the stated goals:

A comorbidity often seen with obesity is the development of impaired glucose tolerance. Abnormalities in the ability to metabolize glucose can lead to increased risk of developing pre-diabetes and if continued, diabetes mellitus type 2. To combat the effects of excess caloric intake on obesity and glucose intolerance, low-calorie and non-nutritive sweeteners have been introduced as a replacement for traditional sucrose and fructose sweeteners that contribute more calories. Non-nutritive sweeteners have been seen as beneficial due to their lack of glucose and caloric density for those attempting to regulate blood glucose and reduce excess adiposity. Limited research has been done concerning the effects of moderate consumption of non-nutritive sweeteners on blood glucose tolerance and body composition. Therefore, the purpose of this study is to determine the effect of moderate consumption of non-nutritive sweeteners (aspartame, and sucrose) on glucose tolerance and body composition in an animal model. Animal models provide an appropriate form of treatment as animal studies reduce environmental factors that may affect results. Diet can also be closely controlled in an animal model. METHODS: 10 week old Simonsen albino rats will be housed in pairs at UNLV's LACF. Animals will be randomized into one of three groups where they will each be fed a standard chow diet, with the inclusion of a treatment. Treatment groups include the addition of 1) aspartame (8.5 mg/kg/day) or 2) sucralose (2.6 mg/kg/day) to water, or 3) a control of unflavored water. All animals will be given a standard chow diet for 6 weeks prior to testing and sacrifice. The result will be three treatment groups as follows (n=10) aspartame, (n=10) sucralose, and (n=10) a control of untreated water. After 8 hours of overnight fasting at the completion of the treatment period, an oral glucose tolerance test will be administered. Animals will have fasting blood glucose concentrations measured with a tail prick sample, using a standard blood glucose monitor. Rats will then be given an oral glucose load via oral gavage of 2 mg/kg, with samples then being taken at 15, 30, 60, 90, and 120 minutes after dosing. Blood glucose will be measured immediately, and 300 µl samples will be collected into micro capillary tubes. Sample tubes will be centrifuged; plasma will be removed and stored for insulin assay. Following euthanasia, assessments of lean body mass and fat mass will be determined from weighing and the use of dual energy x-ray absorptiometry with small animal software.

29. EXPERIMENTAL SUMMARY

ALL 3 QUESTIONS MUST BE ANSWERED.

a. What are the objectives or underlying hypotheses of the experiment?

Obesity has become a significant health concern as it continues to be an underlying factor for many preventable disease states. Currently affecting more than one-third of Americans, obesity has been linked to life altering illnesses such as heart disease, certain types of cancer, musculoskeletal disorders, and diabetes mellitus type 2.[1] A comorbidity often seen with obesity is the development of impaired glucose tolerance. Abnormalities in the ability to metabolize glucose can lead to increased risk of developing pre-diabetes and if continued, type 2 diabetes mellitus (T2DM).[2] Those who are even considered to be overweight, but not obese, are three times more likely to develop type 2 diabetes.[3] Type 2 diabetes mellitus is responsible for 90-95% of all diabetes cases in the United States.[4]

To combat the effects of excess caloric intake on obesity and glucose intolerance, low-calorie and nonnutritive sweeteners (NNS) have been introduced as a replacement for traditional sucrose and fructose sweeteners, which contribute more calories. NNS have been seen as beneficial due to their lack of glucose and caloric density for those attempting to regulate blood glucose and reduce excess adiposity. The trending increase in obesity and prevalence in T2DM may be correlated with an increased reliance on NNS.[5] Current research, which has mostly examined the physiological effects of consumption at the approved upper limits, has produced contrasting results. A significant correlation with NNS consumption and increased BMI or adiposity, as well as impaired glucose tolerance, has been reported in most,[6-10] but not all, studies.[11] Since many Americans rely on moderate consumption of NNS,[12] a further examination between the correlation of moderate NNS consumption and body composition and blood glucose may be warranted.

The purpose of this study is to determine the effect of moderate consumption of NNS (aspartame, and sucralose) on glucose tolerance and body composition in an animal model. There is continuing controversy over the role of NNS consumption on the risk and development of obesity, type 2 diabetes, and related disorders. Population-based studies on the associations between diet soft drink and metabolic disease have produced conflicting results, due in large part to the difficulty of controlling confounding variables in human populations. Thus, we propose to examine the impact of chronic, low-dose NNS consumption in an animal model where confounding variables can be strictly controlled. Despite the interest in this area, little actual research on the metabolic effects of NNS on glucose tolerance and body fatness has been published. After an exhaustive PubMed search we have identified only two articles specifically on OGTT and aspartame and none on OGTT and sucralose. Similarly, we identified nine articles specifically examining body fat and aspartame and two examining body fat and sucralose.

References

1. Center for Disease Control. (2014, September 9). Obesity and Overweight for Professionals: Data and Statistics: Adult Obesity - DNPAO - CDC. Retrieved from http://www.cdc.gov/obesity/data/adult.html
2. Karve, A., & Hayward, R. A. (2010). Prevalence, Diagnosis, and Treatment of Impaired Fasting Glucose and Impaired Glucose Tolerance in Nondiabetic U.S. Adults. *Diabetes Care*, *33*(11), 2355–2359. doi:10.2337/dc09-1957
3. Field AE, Coakley EH, Must A, et al. (2001) Impact of Overweight on the Risk of Developing Common Chronic Diseases During a 10-Year Period. *Arch Intern Med.* 2001;161(13):1581-1586. doi:10.1001/archinte.161.13.1581.
4. Nahikian-Nelms, M., Sucher, K., Lacey, K., & Roth, S. (2011). *Nutrition Therapy and Pathophysiology* (2nd ed.). Belmont, CA: Wadsworth, Cengage Learning.
5. Gardner, C., Wylie-Rosett, J., Gidding, S. S., Steffen, L. M., Johnson, R. K., Reader, D., Lichtenstein, A. (2012). Nonnutritive Sweeteners: Current Use and Health Perspectives. *Diabetes Care*, *35*(8), 17889-1808. doi:10.2337/ dc12-9002
6. Fowler, S. P., Williams, K., Resendez, R. G., Hunt, K. J., Hazuda, H. P. and Stern, M. P. (2008), Fueling the Obesity Epidemic? Artificially Sweetened Beverage Use and Long-term Weight Gain. Obesity, 16: 1894–1900. doi: 10.1038/oby.2008.284
7. Feijó Fde, M., Ballard, C. R., Batista, K. C., Neves, A. M., Ribeiro, M. F., & Bertoluci, M. C. (2013). Saccharin and aspartame, compared with sucrose, induce greater weight gain in adult Wistar rats, at similar total caloric intake levels. *Appetite*, *60*(1), 203-207. doi:10.1016/j.appet.2012.10.009
8. STELLMAN, S., & GARFINKEL, L. (1988). Patterns of artificial sweetener use and weight change in an american cancer society prospective study. *Appetite*, *11*(1), 85-91. doi:10.1016/0195-6663(88)90048-7
9. Mitsutomi,, K., Masaki, T., Shimasaki, T., Gotoh, K., Chiba, S., Katuma, T., & Shibata, H. (2013). Effects of nonnutritive sweetener on body adiposity and energy metabolism in mice with diet-induced obesity. *Metabolism*, *63*(1), 69-78. doi:10.1016/j.metabol.2013.09.002
10. Suez, J., Korem, T., Zeevi, D., Zilberman-schapira, G., Thaiss, C., Maza, O., Israeli, D. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature*, *514*(7521), 181-186. doi:10.1038/nature13793
11. Ma, J., Chang, J., Checklin, H. L., Young, R. L., Jones, K. L., Horowitz, M., & Rayner, C. K. (2010). Effect of the artificial sweetener, sucralose, on small intestinal glucose absorption in healthy human subjects. *British Journal of Nutrition*, *104*(6), 803-806. doi:10.1017/S0007114510001327
12. Shwide-Slavin, C., Swift, C., & Ross, T. (2012). Nonnutritive Sweeteners: Where Are We Today? *Diabetes Spectrum*, *35*(2), 104-110. doi:10.2337/diaspect.25.2.104

b. What happens to the animals from start to finish (please give a detailed description of all procedures)?

(N=30) Sprague-Dawley rats will be obtained from approved vendor. Rats will be housed in pairs in a 12 hr light/dark cycle in UNLV's Lab Animal Care Facility (LACF). Animals will be randomized into one of three groups, where they will each be fed a standard chow diet for 6 weeks with the inclusion of a treatment. Treatments include the addition of aspartame (5-7 mg/kg/day) (Palmnäs, et al. (2014) *PLoS ONE*, *9*(10), e109841. doi:10.1371/journal.pone.0109841) or sucralose (1-3 mg/kg/day) (Abou-Donia, et al. (2008) *J. Toxicol. Environ. Health A* 71: 1415–1429) to water, or a control of unflavored water. Water intake will be logged daily. The result will be three treatment groups as follows (n=10 per group) aspartame, sucralose, and a control of water.

Following the 6-week treatment period, animals will be fasted overnight (food removed from cages) in preparation for the oral glucose tolerance test. Animals will be weighed and blood will be collected by tail clip (0.5 mm tip of tail). Blood glucose will be measured immediately with a standard blood glucose meter, and samples for insulin (300 µl) will be collected in micro capillary tubes. Animals will then be sedated with dexmedetomidine via intraperitoneal injection. Following sedation, a glucose load (2 mg/kg) will be administered via oral gavage. Antesedan will then be administered and subsequent blood samples will be taken at 15, 30, 60, and 120 minutes after dosing. Blood glucose will be measured immediately with a standard blood glucose meter. Samples for insulin (300 µl) will be collected in micro capillary tubes, centrifuged, plasma removed, and stored at -60 C until assayed. Following the glucose tolerance test, animals will be returned to cages until euthanization. After euthanization via $CO_2$ inhalation, animals will be transported to BHS 335 where body composition will be determined by dual energy x-ray absorptiometry (DEXA) with small animal software. Animal carcasses will be placed on an absorbent underpad separating the carcass from direct contact with the DEXA surface. Following DEXA scans, carcasses will be returned to LACF for disposal.

c. Why was this species or strain selected?

When examining insulin and glucose tolerance changes from a stimulus, it is important to protect the subject from outside factors that may affect the results. Diet can drastically influence the release of insulin and glucose uptake, which is difficult to control in humans. Even with attempts to educate subjects on diet parameters, it is still possible that subjects will consume outside sources of non-nutritive sweeteners. Other research examining the effects of artificial sweeteners on various dependent variables has relied on animal models, specifically rats. Previous research focusing on the relationship between non-nutritive sweeteners and glucose tolerance or body composition, has used a rat model to limit outside dietary influence on the results. [1-3]

1. Palmnäs, M. S. A., Cowan, T. E., Bomhof, M. R., Su, J., Reimer, R. A., Vogel, H. J., Shearer, J. (2014). Low-Dose Aspartame Consumption Differentially Affects Gut Microbiota-Host Metabolic Interactions in the Diet-Induced Obese Rat. *PLoS ONE*, *9*(10), e109841. doi:10.1371/journal.pone.0109841
2. Mitsutomi,, K., Masaki, T., Shimasaki, T., Gotoh, K., Chiba, S., Katuma, T., & Shibata, H. (2013). Effects of nonnutritive sweetener on body adiposity and energy metabolism in mice with diet-induced obesity. *Metabolism*, *63*(1), 69-78. doi:10.1016/j.metabol.2013.09.002
3. Suez, J., Korem, T., Zeevi, D., Zilberman-schapira, G., Thaiss, C., Maza, O., Israeli, D. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature*, *514*(7521), 181-186. doi:10.1038/nature13793

d. How many animals will be used and how did you determine that this will be a sufficient number?

i. Number of animals requested:

N=30, 10 per treatment group.

-

ii. How did you determine that this would be a sufficient number? (Choose at least one)

☒ Statistical power analysis (please describe)

Based on the relevant literature, 8-10 per group/time point is sufficient to detect a significant difference due to treatment. Using the standard deviation for plasma glucose and insulin from a previous study, the power for n=5 and for n=10 is presented below.

Well designed studies typically generate a power of at least 0.8.

**Glucose**

| | 0 min | 15 min | 30 min | 60 min | 120 min |
|---|---|---|---|---|---|
| **Mean1** | 94 | 134 | 131 | 153 | 102 |
| **SD1** | 6.9 | 15.3 | 15.4 | 6.6 | 15.1 |
| **Mean2** | 107 | 162 | 157 | 162 | 120 |
| **SD2** | 10 | 30.8 | 17.1 | 14.2 | 18 |
| **Mean diff** | -13 | -28 | -26 | -9 | -18 |
| **Pooled SD** | **8.45** | **23.05** | **16.25** | **10.4** | **16.55** |
| **Power for n=5** | 0.78 | 0.61 | 0.81 | 0.39 | 0.53 |
| **Power for n=10** | **0.96** | **0.86** | **0.97** | **0.61** | **0.78** |

**Insulin**

| | 0 min | 15 min | 30 min | 60 min | 120 min |
|---|---|---|---|---|---|
| **Mean1** | 0.54 | 1.23 | 1.15 | 0.55 | 0.72 |
| **SD1** | 0.1 | 0.4 | 0.7 | 0.5 | 0.9 |
| **Mean2** | 1.65 | 3.6 | 2.97 | 3.5 | 2.17 |
| **SD2** | 0.8 | 0.9 | 1.7 | 2.2 | 1.5 |
| **Mean diff** | -1.11 | -2.37 | -1.82 | -2.95 | -1.45 |
| **Pooled SD** | **0.45** | **0.65** | **1.2** | **1.35** | **1.2** |
| **Power for n=5** | 0.99 | 1 | 0.77 | 0.96 | 0.6 |
| **Power for n=10** | **1** | **1** | **0.96** | **1** | **0.85** |

☒ Similar published experiments (cite references)

(n=5):

Mitsutomi,, K., Masaki, T., Shimasaki, T., Gotoh, K., Chiba, S., Katuma, T., & Shibata, H. (2013). Effects of nonnutritive sweetener on body adiposity and energy metabolism in mice with diet-induced obesity. *Metabolism*, *63*(1), 69-78. doi:10.1016/j.metabol.2013.09.002

(n=10-12):

Bielohuby, M., Sisley, S., Sandoval, D., Herbach, N., Zengin, A., Fischereder, M., Menhofer, D. (2013). Impaired glucose tolerance in rats fed low-carbohydrate, high-fat diets. *American Journal of Physiology*, *305*, 1059-1070. doi:10.1152/ajpendo.00208.2013

Palmnäs, M. S. A., Cowan, T. E., Bomhof, M. R., Su, J., Reimer, R. A., Vogel, H. J., Shearer, J. (2014). Low-Dose Aspartame Consumption Differentially Affects Gut Microbiota-Host Metabolic Interactions in the Diet-Induced Obese Rat. *PLoS ONE*, *9*(10), e109841. doi:10.1371/journal.pone.0109841

☐ Special consideration for difficult to obtain, rare, or endangered species, explain for clarification

e. For 3-year renewals please describe what has been accomplished in the past 3 years and how this protocol differs from the previously approved protocol (for new protocols indicate N/A):

30. ASSURANCES (See Appendix B)

I have searched the following sources for alternatives to animal model.

| Data Source | Searched From (Yr) | Searched To (Yr) | Keywords used in the Search | Date Search Performed |
|---|---|---|---|---|
| Pubmed | 2000 | 2015 | Non-nutritive sweeteners, artificial sweeteners, aspartame, sucralose, blood glucose, insulin, oral glucose tolerance test | 4/12/2015 |
| Medline via Web of Knowledge | 2000 | 2015 | Non-nutritive sweeteners, artificial sweeteners, aspartame, sucralose, blood glucose, insulin | 4/12/2015 |
| Biomed Central | 2000 | 2015 | Artifical sweeteners, non-nutritive sweeteners, insulin, glucose, diabetes | 4/12/2015 |
| | | | | |
| | | | | |

Alternatives refer to methods or approaches which result in refinement of procedures which lessen pain and/or distress; reduction in numbers of animals required; or replacement of animals with non-whole-animal systems or replacement of one animal species with another, particularly if the substituted species is non-mammalian or invertebrate.

I have determined that the following alternatives are available. Described briefly if applicable:

The dynamic process of glucose uptake and insulin secretion is best studied in an intact, whole-animal system. Replacement of animals with humans is not practical because of the inability to control diet. Diet can drastically influence the release of insulin and glucose uptake, which is difficult to control in humans. Even with attempts to educate subjects on diet parameters, it is still possible that subjects will consume outside sources of non-nutritive sweeteners.

## Investigator Certifications

***By submitting and digitally signing this package in IRBNet, you agree to the following certifications:***

I understand that 'unnecessarily duplicative' research involving laboratory animals is not permitted by the federal animal welfare law and hereby provide assurance that the research proposed herein does not, to the best of my knowledge, unnecessarily duplicate research already reported in the public literature.

I certify that the use of all animals involved in this project will be carried out according to the provisions of the Animal Welfare Act, PHS Policy, the principals of the 'NIH Guide for the Care and Use of Laboratory Animals', and the UNLV Policy Governing The Use of Animals in Research, Teaching and Testing. I agree to notify the UNLV Institutional Animal Care and Use Committee (IACUC) of any substantive changes in the research use of the animals, including the number of animals, species used, or procedures performed.

I understand that the University of Nevada, Las Vegas and its representatives on the IACUC have the authority to suspend any part of my research, should I not be in compliance at any time with USDA, PHS/NIH, or UNLV regulations for animal care and use.

I certify that all personnel having direct animal contact, including the investigator, have or will have, prior to participating in this protocol, been trained in humane and scientifically acceptable procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in this project or are under the direct (in-lab) supervision of a trained individual; and that employees will be allowed adequate time to attend training sessions.

I assure that all named individuals on this project have read and understood the procedures outlined in this protocol as approved by the IACUC.

I certify that I will obtain approval from the IACUC before initiating any significant changes in this study.

I certify that I will notify the IACUC regarding any unexpected study results that impact the animals. Any unanticipated pain or distress, morbidity or mortality will be reported to the attending veterinarian and the IACUC.

I certify that the information provided in this application is accurate to the best of my knowledge. I also understand that should I use the project described in this application as a basis for a proposal for funding (either intramural or extramural), it is my responsibility to ensure that the description of animal use in such funding proposal is identical in principle to that contained in this protocol.

I understand that the Department of Laboratory Animal Care Services is responsible for administering and assigning animal housing space within the central animal facilities. The Manager, LACF will make space assignments for the efficient utilization of space, which may result in investigators sharing animal housing space. I further realize that I must notify the Manager, LACF of animal housing needs in order to insure the availability of suitable space before animals are procured.

**UNIVERSITY OF NEVADA LAS VEGAS**
**PROTOCOL FOR ANIMAL CARE AND USE**

**SUPPLEMENT 1**

**ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS**

1. Study Involves exposure of Animals to: ☐ Biohazards ☐ Toxic Chemicals ☐ Radioisotopes ☐ Carcinogens
   a. Identify Agent(s):

| Species | Agent | Dose/Kg Body Weight | Route | Site/Location | Frequency |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |

| Agent/Material | Hazardous For | Spread By |
|---|---|---|
| | | |
| | | |

   b. What are the potential health risks to humans or animals?

2. a. Describe experimental procedures involving these agents:

   b. Do the procedures involve animal pain, distress or discomfort?

   c. What are the durations of the studies involving the agents?

   Precautions checked below apply to this experiment:
   ☐ The researcher or his/her technicians are responsible for the feeding and care of these animals. The following items must be assumed to be contaminated with hazardous material and require special handling:
   ☐ Cage ☐ Water Bottle ☐ Animal Carcasses ☐ Bedding ☐ Ventilation System ☐ Other

   d. Describe any special animal care requirements related to the use of these agents:

3. a. Describe special containment facility requirements:
   ☐ Cages must be autoclaved before cleaning
   ☐ Label cages and remove label after decontamination
   ☐ Personal protective equipment must be removed before leaving room
   ☐ Personal protective equipment must be discarded or decontaminated at the end of the project
   ☐ Hands, arms, and face must be thoroughly washed upon leaving the room
   ☐ Decontaminate room (inform SLACS when cage and or room can be returned to general use)
   ☐ Other:

   b. Describe special precautions for animal handlers:
   The following personal protective equipment is required:
   ☐ Lab Coat/Coveralls ☐ Shoe Covers/Booties ☐ Disposable Gloves ☐ Head Cover
   ☐ NIOSH Certified Dust Mask ☐ Disinfectant Footbath ☐ Eye Protection/Face Shield
   ☐ Fitted Respirator ☐ Other:

   c. Describe special waste and animal dispersal requirements:
   ☐ Animal carcasses must be labeled and disposed of as follows:
   ☐ Incineration ☐ Biohazardous Waste Container ☐ Bag and Autoclave ☐ EHS will pick up

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☐ Other:

All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:
☐ Incineration ☐ Biohazardous Waste Container ☐ Bag and Autoclave ☐ EHS will pick up
☐ Other:

4. If radioactive materials are being used, a copy of an approved use permit must be attached to the protocol. If biohazard, please attach a copy of an approved Institutional Biosafety Committee (IBC) protocol. Contact the Director of Compliance for IBC information.

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**PROTOCOL FOR ANIMAL CARE AND USE**

**SUPPLEMENT FORM 2**

**SURGICAL PROCEDURES**

1. a. Describe pre-operative care and selection procedures (including physical examinations, lab tests, and preconditioning procedures such as withholding food and/or water:

b. List pre-operative and inter-operative medications and anesthetics:

| Species | Drug | Dosage/Kg Body Weight | Route | Site/Location | Frequency |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

Anesthesia waste gases are classified as environmental hazards. These gases must be used in a fume hood or have a gas scavenging system in place. If gas anesthetics will be used, describe the method you will use to prevent or eliminate human exposure to extraneous anesthetic vapors:

c. List all people involved in the surgical procedure (include undergraduate and graduate students; medical residents; etc.) If anyone was not listed on protocol question 4, please indicate the individual's training, experience or exposure to surgery.

d. Who will administer the anesthetic?

What is the estimated duration of anesthesia?

How will the depth of anesthesia be monitored and assessed and how often will the assessment of pain and distress be made?
Monitor for: Frequency:

- ☐ Absence of withdrawal reflex to toe or ear pinch
- ☐ Absence of blink reflex
- ☐ Pupil size and light response
- ☐ Respiratory rate, depth, and character
- ☐ Monitor heart rate and rhythm
- ☐ Monitor blood pressure or arterial pulse
- ☐ Mucous membrane color and capillary refill time
- ☐ Assessment of jaw and/or skeletal muscle tone

Other:

☐ If a paralytic is used, explain its purpose and how the level of anesthesia will be monitored in the paralyzed animal.

2. Is this a survival surgery? ☐ Yes ☐ No

a. Is this a major survival procedure? ☐ Yes ☐ No (Major surgery is any procedure that enters a body cavity.)

b. Will more than one survival procedure be performed on any one animal? ☐ Yes ☐ No

**If yes, provide scientific justification. Multiple survival surgeries on a single animal are generally prohibited. Cost savings alone is not acceptable justification. The only circumstances under which multiple survival surgeries may be justified are when they are related components of a single research project or to conserve rare and endangered species.**

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**Procedures should be spaced far enough apart to allow animals time to recover in order to minimize dehydration and weight loss.**

3. Describe the surgical procedure(s). Sterile surgical techniques MUST be used in survival surgeries involving most animals; thus, a description of the procedures to be used to ensure asepsis should be provided.

4. What methods will be used to prevent dehydration and hypothermia during surgery? *Note: Inexpensive human heating pads are not recommended during surgery unless very closely monitored. They are unsuitable for postoperative care.*

5. For non-survival study, what is the duration of the surgery, and study prior to the animal being euthanized?

6. Post-Operative Care for Survival Studies:

a. Post-Anesthesia Recovery - Describe the observations that will ensure that the animals are stable and returning to a safe level of recovery from anesthesia and indicate who will be responsible for the observations, Federal regulations require someone must be present until the animal recovers fully from anesthesia:

b. Post-Surgical Recovery - Describe the frequency of examination and observation of the animal and management procedures for potential experiment-related diseases, please indicate how these observations will be documented:

c. What criteria will be used to determine that animals are ready to return to their housing (i.e. sternal recumbancy, other etc.)?

d. What criteria will be used to evaluate post-operative pain, distress, or discomfort?
☐ Vocalization ☐ Restricted mobility ☐ Poor food or water intake ☐ Change in general appearance
☐ Change in normal respiration ☐ Signs of infection ☐ Change in behavior
Other:

e. Analgesics must be given routinely, as opposed to prn or "as needed" basis. If you intend to deviate from routine analgesia please include a justification:

f. List post-operative medications, which may be used (e.g.antibiotics or analgesics). If the use of such agents would interfere with the project objectives, explain and document with objective data:

g. Describe any special diet or other supportive care required post-op.

h. How long will the animals be kept alive after surgery?

i. Describe any long-term care required post-surgically. Also, if animals are chronically implanted or instrumented, this section should be completed.

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**SUPPLEMENT 3**

**PAIN, DISTRESS OR DISCOMFORT INVOLVEMENT**

1. Indicate any clinical conditions or abnormalities expected or that could arise as a result of the proposed study or teaching.

   Clinical Signs:

   a. Change in general appearance
   ☐ Lack of grooming ☐ Rough coat ☐ Nasal discharge ☐ Ocular discharge ☐ Abnormal posture
   ☐ Swelling ☐ Tumors ☐ Ulcerations ☐ Discoloration of fur, feces, or urine
   Specify:

   Other:

   b. Change in normal respiration
   ☐ Rapid ☐ Slow ☐ Shallow ☐ Labored ☐ Sneezing ☐ Wheezing
   Other:

   c. Change in normal appetite Specify:

   d. Change in weight
   ☐ <10% weight loss ☐ 10-15% weight loss ☐ >20% weight loss
   Other:

   e. Changes in other physical characteristics
   ☐ Hypothermia ☐ Hyperthermia ☐ Muscle atrophy ☐ Bleeding ☐ Diarrhea
   ☐ Constipation ☐ Infection ☐ Blindness ☐ Paralysis
   Other:

   f. Changes in behavior
   ☐ Hyperactivity ☐ Hypoactivity ☐ Coma ☐ Tremors ☐ Convulsions ☐ Limb paralysis
   ☐ Prostration ☐ Self induced trauma ☐ Spasticity ☐ Agitation ☐ Depression ☐ Impaired ambulation
   Other:

2. If any animal pain, distress, or discomfort is expected as part of this project, describe what criteria will be used to assess the level of severity and the need for alleviation (if permissible):

3. If animal pain, distress, or discomfort cannot be alleviated for scientific reasons, explain and document with objective data:

4. If specific pain, distress, or discomfort alleviating agents will interfere with the project's objectives, list them and document their potential interference with objective data:

5. In terms of species-specific behavioral changes and physiological signs, what criteria will trigger the decision to remove an animal from the experiment or teaching exercise, or to terminate the experiment or teaching exercise?

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**SUPPLEMENT 4**

**PROLONGED PHYSICAL RESTRAINT**

Brief physical restraint of animals for examination, collection of clinical samples, or a variety of other clinical and experimental manipulations can be accomplished manually or with devices such as restraint stocks or squeeze cages; BUT, it is important that such devices be suitable in size and design for the animals being restrained and that they be operated properly to minimize stress and avoid injury to the animals. Prolonged restraint of any animal, including the chairing of non-human primates, should be avoided unless essential to research objectives. Such procedures must not be used for investigator convenience only.

1. Explain the rationale for use of prolonged restraint:

2. Describe any devices involved, including construction materials and dimension:

3. What is the duration and frequency of device use?

4. What will be the observation intervals while the animals are in, or subjected to, the device(s)?

5. Qualified faculty or staff making the observations:

   Name: Phone:

6. Will pain or discomfort be induced in the restrained animal? ☐ Yes ☐ No
   If yes, describe and justify using objective data:

7. Will electrical stimuli or other aversive stimuli, including abnormal light or sound environments, be used to modify animal behavior or train the restrained animal? ☐ Yes ☐ No
   If yes, describe in detail:

8. Will analgesics, sedative, or tranquilizers be used to provide additional restraint? ☐ Yes ☐ No
   If yes, list:

9. Will the restrained animals be fasted (food or water) or placed on a limited diet? ☐ Yes ☐ No
   If yes, explain:

   a. How will the general well-being of the animal be determined?

   b. How often will the animals' body weights be determined?

**PROTOCOL FOR ANIMAL CARE AND USE**

**SUPPLEMENT 5**

**GENETICALLY ENGINEERED OR NATURALLY OCCURRING MUTANT ANIMALS**

**Complete one Supplement Form 5 for each mutant strain**

*Transgenic lines may exhibit unforeseen impairments and investigators should provide objective endpoints at which the animal will be euthanized. The acceptability of maintaining a strain with pathologic symptoms that appear to cause pain or distress is dependent on whether the strain provides a valid model for the parameter to be studied and whether further study of this strain is likely to significantly increase our understanding or ability to treat this condition. When a transgenic line has obvious pathology, investigators are asked to evaluate the characteristics and utility of the line as promptly as possible and to decide whether there is a likely research benefit which justifies continued breeding of this strain.*

SPECIES:

MUTANT STRAIN:

SOURCE:

1. a. Describe the physiological defect induced by the mutation:

   b. Indicate the time course after birth for development of clinical signs related to the mutation

      1=birth

      2=onset of early observable signs of genetic disease

      3=onset of moderate signs of genetic diseases, pain or distress

      4=onset of severe signs of genetic disease, pain or distress

      x=death

**Time Line**

**1 2 3 4 x**

   c. Describe the clinical signs observed at (2), (3), and (4) above. Examples include diabetic mice that exhibit elevated blood glucose levels at point (2), polyuria/polydipsia at point (3) and poor condition and behavioral signs of distress at point (4); and, SOD1 transgenic mice that exhibit a minor loss of mobility at point (2), abnormal locomotion at point (3), and paralysis at point (4).

   d. Will the animals be treated to prevent observable clinical signs and /or signs of pain or distress? ☐ Yes ☐ No
   If yes, describe treatments:

   e. At what point in the disease process will the animals be euthanatized?

2. Who will monitor the animals for clinical signs and provide treatment if needed?

**An approved Institutional Biosafety Committee protocol may be required in addition to this form. Contact the Office of Research Integrity (ext. 55453) for more information.**

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## Appendix A

**CATEGORIES OF ETHICAL CONCERN FOR USE OF VERTEBRATE ANIMALS IN TEACHING AND RESEARCH PROGRAMS**

The USDA has established categories of invasiveness for animal research which must be addressed in the protocol. The University reports to the USDA how many USDA animals are used each year in each category.

**CATEGORY B:** Maintaining animals without experimentally manipulating them in any way. Standard animal care and food, water, and shelter/enclosures are all that the animals experience. Any bleeding, tail clips or other manipulations are not allowed. Some breeding colonies, conditioning periods as well as field studies with only observation and no animal contact would qualify for Category B.

**CATEGORY C:** Experiments or teaching exercises that involve no or minimal pain or distress to vertebrate animal species. For Category C procedures no pain-relieving drugs or anesthetic drugs are utilized because animals experience, at most, no more than transient slight pain or distress. These manipulations might include minor blood sampling, injections with benign substances (NOT Freund's adjuvant), use of positive rewards for training, tail clips, ear tagging or punching, and euthanasia for tissue collection. If any of these manipulations are done under anesthesia, this would then make them Category D.

((Examples - Holding animals captive for experimental purposes; simple procedures such as observing feed selection preferences; injections of relatively harmless substances such as therapeutic levels of antibiotics, and blood collection from peripheral veins; physical examinations; food and water deprivation for short periods measured in hours; or standard methods of euthanasia which induce rapid unconsciousness, such as anesthetic overdose.)

**CATEGORY D:** Research studies in which pain or distress is relieved with appropriate anesthetics, analgesics, or tranquilizer drugs (or other means of relieving pain or distress). Surgery must have adequate anesthesia, and if the animal is recovered after surgery and anesthesia, pain control must be instituted. Major surgeries include opening any body cavity, orthopedic procedures, etc.

(Examples: Exposure of blood vessels or implantation of catheters under full anesthesia; behavioral studies on conscious animals which involve short-term stressful restraint; noxious stimuli from which escape is possible; and surgical procedures under deep anesthesia that may result in only some minor post-operative discomfort or pain.)

**CATEGORY E:** Procedures that involve unrelieved pain or distress. Category E experiments will not normally be approved at the University of Nevada, Las Vegas. The IACUC must have solid scientific justification for withholding agents to alleviate the pain or distress before these studies may be approved. Toxicological or microbial testing or infectious disease research that requires that the study continue until animals show clinical signs or death occurs are an example of Category E. Many such procedures may be expressly prohibited by Federal laws, regulation, or policies. Category E procedures will not be approved for use in teaching exercises.

(Examples - Severe burn or trauma infliction on anaesthetized animals or without the use of analgesics following awakening from anesthesia; application of noxious stimuli (as in shock for behavioral studies), killing by nonapproved methods which may involve severe distress or pain before unconsciousness is obtained.)

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## Appendix B

## Guidelines for Searches to Alternatives to Animal Use

**Introduction**

The Animal Welfare Act Regulations, Section 2.31 and USDA (Policy 11 and 12 ) require that a written narrative be provided by the Principle Investigator (PI) to determine whether or not alternatives exist to procedures which may cause pain or distress in animals used for teaching or research.

**Definition of Alternatives**

Alternatives refer to methods or approaches which result in refinement of procedures which lessen pain and/or distress; reduction in numbers of animals required; or replacement of animals with non-whole-animal systems or replacement of one animal species with another, particularly if the substituted species is non-mammalian or invertebrate.

**Animal Welfare Act Regulations**

The AWA regulations require the Institutional Animal Care and Use Committee (IACUC) to determine that "the principle investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and has provided a written narrative description of the methods and sources used to determine that alternatives were not available." The PI must provide scientific justification to the IACUC if alternatives are available but not used.

**Types of Studies Requiring an Alternatives Search**

Not all animal use protocols require an alternatives search. Only studies utilizing procedures which result in more than momentary or slight pain or distress require a search. Examples of studies that require a search include: toxicity and infectious diseases research, tumor induction or transplantation studies, survival and non-survival surgical procedures, pain research, in vivo monoclonal or polygonal antibody production procedures, fluid and/or food restriction, and prolonged restraint. If you are unsure if your study requires an alternative search, either contact one of the institutional veterinarians for advice or just go ahead and do the search. If you fail to do a required search for alternatives, approval of your protocol by the IACUC may be delayed.

**Alternatives Narrative**

The minimal written narrative should include: the databases searched or other sources consulted, the date of the search and the years covered by the search, and the key words and/or search strategy used by the Principal Investigator when considering alternatives or descriptions of other methods and sources used to determine that no alternatives were available to the painful or distressful procedure. The narrative should be such that the IACUC can readily assess whether the search topics were appropriate and whether the search was sufficiently thorough. Reduction, replacement, and refinement (the three R's) must be addressed, not just animal replacement.

**Database and Web Site Searching**

In order to perform literature searches that meet regulatory requirements, use key words that would identify alternatives to any and all study procedures that may cause more than momentary or slight pain or distress to the animals. Problems often arise in choosing keywords and search strategies that will yield the most pertinent information. Appropriate search terms or keywords include animal testing alternatives, alternatives, tissue culture, cell culture, simulation, in vitro, and model. Keywords should be selected keeping the 3R principles in mind. The 3 R's represent reduction in the number of animals used, refinement of techniques and procedures that reduce pain and distress, and replacement of animal with non-animal techniques. A list of suggested terminology for alternatives from the UC Center for Animal Alternatives website is listed below (http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm). While this list of terms is not exhaustive, you can use it as a model, combining alternatives terms with those specific to your area of research. In your database search, combine appropriate text words from this list with text words that are appropriate to the particular protocol. Although it is important to include relevant keywords, adding many irrelevant keywords that are not likely to identify alternatives with the idea that "more is better" is discouraged and will not improve the likelihood that the IACUC will approve your protocol.

alternative
analges* or sedative*
anesthe* or anaesthe*
*animal model*
animal testing alternative*
anxiolytic*
artificial*
artificial intelligence systems or AI
assay* or technique* or technic* or method*
bacteria or protozoa
behavioral enrichment
cadaveric model*
computer aided instruction or CAI
computer simulation
computer software or software
culture and (cell or tissue or organ)
digital image*
environmental enrichment
euthanasia
fish or cephalopod*
handling
housing or facility design or caging
interactive
invertebrate*
mannequin or manikin or mannikin
mathematical model*
model*
plastinat*
simulat*
single-cell* organism*
software
train* or educat* or teach*
tranquiliz*
video*
virtual and (surg* or reality)

vitro and (method or model or technique)
welfare or pain or stress or distress

**Example of a Keyword Search**

This example of a keyword search is for a study involving hibernation in ground squirrels. The keywords to be used in a database search for this project include:

hibernation; ischemia AND alternatives; ground squirrel and other models; less invasive AND catheterization.

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CURRICULUM VITAE

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Bachelor of Sciences, Nutrition 2014
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Thesis Title: The Effect of Moderate Consumption of Non-Nutritive Sweeteners on Glucose Tolerance And Body Composition In Rats

Thesis Examination Committee:

Chair, John Young, Ph.D., FACSM
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Committee Member, James Navalta, Ph.D.
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Presentations:

Koschel, T.L., Manning, J.W., Tacad, D.K., Montes, J., Tanner, E., McCune, D., Tovar, A., Taylor, J., Young, J.C., DeBeliso, M., Navalta, J.W. Moderate Altitude Acclimation has no Effect on Respiratory Exchange Ratio, or Percent of CHO and Fat Utilization During a 1-Mile Trail Run. Annual Meeting of the Southwest American College of Sports Medicine, Costa Mesa, CA, 2015.

Navalta, J.W., Manning J.W., Tacad, D.K., Montes, J., Tanner, E., McCune, D., Koschel, T.L., Tovar, A., Taylor, J., Young, J.C., DeBeliso, M. Body Mass Index has no Effect on the Post Exercise Hypotension Response Following a Trail Run. Annual Meeting of the Southwest American College of Sports Medicine, Costa Mesa, CA, 2015.

Tanner, E., Manning, J.W., Taylor, J., Montes, J., McCune, D., Koschel, T.L., Tacad, D.K., Tovar, A., Young, J.C., DeBeliso, M., Navalta, J.W. Validation of Hexoskin Biometric Shirt to Cosmed K4b$^2$ Metabolic Unit in Adults During Trail Running. Annual Meeting of the Southwest American College of Sports Medicine, Costa Mesa, CA, 2015.

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