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Development of a Mouse Model of Obesity via High Sucrose Consumption

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Abstract

The purpose of this project was to generate a mouse model of obesity, glucose intolerance and insulin resistance using high sucrose consumption for undergraduate research of metabolism, obesity and diabetes. The goal was to achieve diet induced obesity in these animals within a single semester (approximately 4 months), so that organs could be harvested and studied in the following semester. A commonly used mouse strain that is sensitive to diet induced obesity, the C57BL/6, was placed on a diet that replaced their water intake with a 30% sucrose solution. These animals gained significant weight on the sucrose solution and were glucose intolerant by the end of 12 weeks as demonstrated by a glucose tolerance test. However, an insulin tolerance test was performed and results from this test indicated that, while fasting blood glucose is elevated, insulin resistance was not confirmed at the end of 12 weeks. While insulin sensitivity may need to be investigated in another manner, the results of this study indicate that a mouse model of obesity and glucose intolerance can be generated within a single semester, which makes this a useful model for undergraduate research of metabolism, obesity and potentially Type 2 Diabetes.

Keywords: Obesity, Glucose Intolerance, Insulin Resistance

1. Introduction

Obesity and Type 2 Diabetes (T2D) are becoming overwhelming health concerns in the United States and other developed nations.[1,2] Both are associated with a high caloric intake and an increased intake of refined/simple sugars.[3,4] Research indicates that 25% of Americans regularly consume more than 200 Kcal per day in sugar-sweetened beverages (SSB) such as soda and juice.[5] Retrospective studies have found that those who consume SSB regularly will gain significantly more weight over a 20 year period than those who do not.[6]

The purpose of this project was to develop a model of SSB over consumption in female mice that will lead to obesity, glucose intolerance and insulin resistance in order to evaluate its metabolic effects in various tissues throughout the body. There are several laboratory strains of mice that have been intentionally developed to be sensitive to obesity. One such strain, the C57BL/6 mouse, has been extensively characterized and is commonly used to study diet induced obesity.[7–11] This mouse strain has been shown to exhibit increased weight gain and impairment in glucoregulation when placed on a high fat diet, as well as increased fasting glucose, body mass, pancreatic mas, pancreatic fat, and increased fasting glucose.[12,13] Other studies on high fat diets have shown diet-induced glucose intolerance, as well as greater mass and fat gains and insulin resistance.[14,15] Studies on obesity development have been successful in mice given a high fat diet, but the when comparing high fat to high sugar diets, high sugar diets have not been as successful. During a 55 week study high sugar diets did not develop obese mice.[9] However, other studies have been able to produce weight gain in C57BL/6 mice if the sucrose is in solution and provided in place of water.[16,17] Therefore, although this model is not novel, it was used in order to mimic the effect of SSB consumption described above and to

determine if it was possible to develop an obese, glucose intolerant and insulin resistant mouse within the limited time period of a single semester ideal for an undergraduate research project.

These mice were obtained from a science lab supplier and placed on a 30% concentration of sucrose solution for 12 weeks. It was hypothesized that consumption of the 30% sucrose solution in place of water over the period of 12 weeks would lead to significant weight gain, glucose intolerance and insulin resistance in the C57BL/6 mice. While this does not mimic the typical American consumption of SSB, the primary goal was to develop a model of obesity within the constraints of a single semester for undergraduate research. Since this research project was performed by undergraduate students, the goal was to create a cost efficient experiment that complemented the schedules and availability of the students. Though other researchers have done similar studies to generate comparative results, this study focuses on the data obtained in a 12 week time period.[15,18,19]

2. Methodology

2.1. Subjects

For this study, female C57BL/6 mice were obtained from a science supplier (Simonsen Laboratories, Gilroy, CA) at approximately 6 weeks of age. Mice were acclimated for 2 weeks in individual cages and maintained on a 12 hour light/ dark cycle with ad libitum access to food and water. They were then weight matched and distributed into a control group (CTR, n=5) that received standard diet and water or a sucrose supplemented group (SUG, n = 5).The SUG group was placed on a 10% sugar solution and increased to 30% sucrose at regular intervals over a 4 week period, increasing the percentage 5% each week.. They were then maintained on 30% sucrose in place of pure water for 12 weeks. Animals were weighed and food and liquid intake were measured weekly. At the end of the study the animals were euthanized and dissected.

2.2. Glucose Tolerance Test

After approximately 12 weeks, the mice were fasted for 16 hours then underwent a glucose tolerance test (GTT). Briefly, baseline fasting blood glucose was measured from tail vein blood using a handheld glucometer. After baseline, mice received an intraperitoneal injection of dextrose based upon their body weight (2.5 mg/Kg). Blood glucose was measured again at 10, 20, 30 and 60 minutes post injection. The GTT is the most widely used measure for assessing glucose homeostasis in rodents and comparison of the glucose area under the curve (AUC) is a commonly used methodology to determine a reasonable summary of systemic glucose load and clearance after an infusion of a glucose bolus.[20]

2.3 Insulin Tolerance Test

Insulin tolerance tests (ITTs) were performed to measure the effect of SSB intake on insulin sensitivity. At the end of 12 weeks, an ITT was performed on the mice. Baseline glucose was measured from the tail vein blood using a handheld glucometer. After baseline, mice received an intraperitoneal injection of insulin based upon their body weight (1 Unit/ Kg body weight). Blood glucose was measured again at 15 minutes post injection. The ITT's K index was calculated to determine insulin resistance. This is a commonly used methodology to rapidly determine a subjects level of insulin resistance without the employment of a hyperglycemic-euglycemic (HE) clamp.[21] The HE clamp is the gold standard in diabetes research, yet it is quite invasive and expensive.[22]

2.4 Analysis And Statistics

All results were analyzed using SPSS (IBM). To determine if statistical differences between the CTR and SUG groups existed, t-tests were performed.

3. Results

3.1 Mouse Body Weight Change

After 12 weeks of 30% sucrose consumption, the C57BL/6 SUG mice were 46.9 % heavier than the CTR (Figure 1). A t-test analysis demonstrated that the difference was significant by week 11 and continued through week 12 ($p = 0.02$). While 30% sucrose consumption led to significant weight gain for the SUG mice, the individualized mice experienced different levels of weight increase. Some animals appear to be much more sensitive to the SSB, possibly because of a difference in activity level between individual animals, which was anecdotally observed, but not measured.

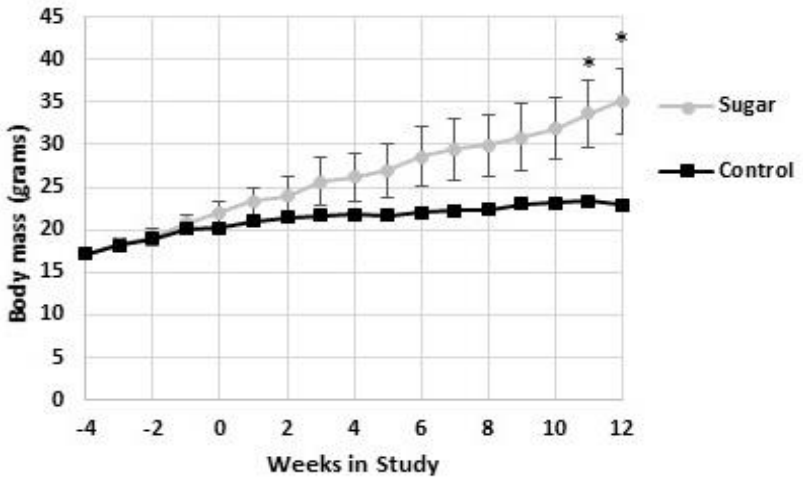

Figure 1. Comparison of Average Mouse Body Mass in CTR Versus SUG Animals

Week 0 corresponds to the beginning of the first week of the 30% sucrose solution. Error bars represent the standard error of the mean (SEM). Error bars are not visible on control values. * indicates significance as analyzed by a t-test ($p < 0.05$)

3.2 Caloric Intake

In order to determine their total caloric intake, liquid and food intake were measured weekly. Liquid intake more than doubled once the 30% sucrose solution was provided to the SUG mice. Food consumption in the SUG mice dropped by nearly three-quarters when they were administered the 30% sucrose solution. However, the total caloric intake of the SUG mice remained about the same and was never statistically greater than the CTR mice (Figure 2).

The 30% sucrose solution is sufficient to produce significant weight gain in a 12 week period in the C57BL/6 mice. Animals in the SUG group compensated for their increased caloric intake via the SSB by reducing food intake, which normalized caloric intake to CTR levels. A little more than two-thirds (72.4%) of the 30% SUG calories came from the sucrose solution rather than from food (Table 1).

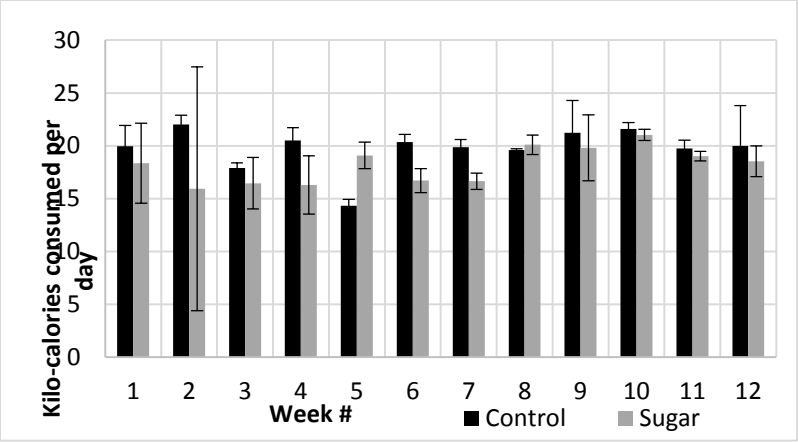

Figure 2. Comparison of Caloric Intake Each Week of the Study

Error bars represent the standard error of the mean (SEM). Results not significant.

Table 1. Food Intake Data

Comparison of average body mass, weight gain over a 12 week period and daily food, liquid and caloric intake. Standard error is in parenthesis. Asterisk (*) indicates a significant difference between the CTR and SUG group.

	CTR	**SUG**
Body mass (grams)	22.93 (0.38)	35.09 (3.99) *
Weight Gain (%)	9.36 (0.45)	48.62 (4.03) *
Food intake (grams/day)	5.33 (1.82)	1.36 (1.10) *
Liquid intake (mLs/day)	5.97 (0.19)	14.08 (1.35) *
Caloric intake (Kcal/day)	2.85 (0.14)	2.65 (0.19)

3.3 Glucose Tolerance Test

The glucose tolerance test (GTT) is designed to measure disturbances in glucose metabolism.[23–25] After the injection of dextrose, blood glucose levels are measured to determine how quickly it is cleared from the blood. This is a commonly used measure to determine how efficient systemic glucose is cleared.[26] The 30% SUG group had significantly impaired glucose tolerance (Figure 3). Analysis demonstrated that area under the curve (AUC) more than doubled in the SUG mice (20,367 ± 2093 SUG vs. 8922 ± 677 in CTR; $p = 0.004$), indicating that the SUG animals were much slower at clearing systemic glucose.

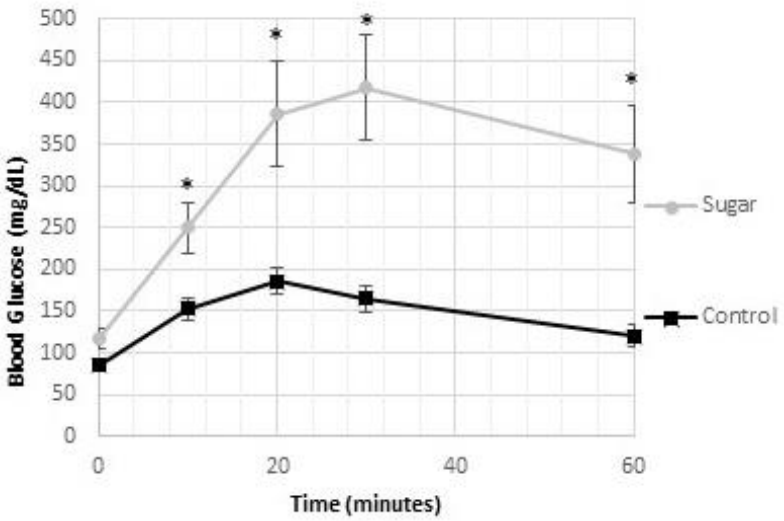

Figure 3. Glucose Tolerance Test

The SUG animals were significantly glucose intolerant as demonstrated by the GTT at the end of 12 weeks. Their fasting glucose level was elevated and they cleared glucose much more slowly. Error bars represent the standard error of the mean (SEM). * indicates significance as analyzed by a t-test ($p < 0.05$)

3.4 Insulin Tolerance Test

The insulin tolerance test (ITT) is designed to measure metabolic response to insulin.[25] Glucose levels are measured before and 15 minutes after injection in order to determine the sensitivity of insulin affected tissues. In some tissues, insulin enables translocation of glucose transporters to the cell membrane, thus enabling glucose removal from circulation.[27,28] The ITT is a measure of how efficiently this occurs. Due to the high variability of the data there is insufficient evidence to demonstrate that the SUG group is resistant to insulin (Table 2).

Table 2. Insulin Tolerance Test

Comparison of individual animal results from insulin tolerance test. While baseline fasting glucose started out higher in the SUG animals, it was not significantly different at 15 minutes. The percent change in blood glucose, though highly variable, was also not affected.

Group	Mouse	Baseline	15 Minute	% Change
Control	1	75	64	-14.67
	2	67	91	35.82
	3	78	42	-46.15
	4	82	95	15.85
	5	85	55	-35.29
	Mean	77.4	69.4	-8.89
Sugar	1	168	169	0.60
	2	122	87	-28.69
	3	117	122	4.27
	4	120	71	-40.83
	5	97	102	5.15
	Mean	124.8	110.2	-11.90
t-test	**p-value**	**0.004**	**0.073**	**0.872**

4. Discussion

4.1 Weight Gain

The mouse strain C57BL/6 was chosen because it was known to be sensitive to diet which induced obesity. The consumption of the high concentration of sucrose in the C57BL/6 led to a drastic effect on weight gain in 12 weeks. This indicates that the model of 30% sucrose is sufficient to cause significant weight gain in the 12 week period allotted and, thus, will be used for future studies to examine the effects of SSB on metabolic factors.

4.2 Caloric Intake

Despite high sucrose content of the 30% SSB, caloric intake did not vary significantly between the SUG and CTR groups. In fact, while the SUG mice drank more, they significantly reduced their food intake to compensate for the calories that they drank. Yet, this reduction was not sufficient to prevent body weight gain, indicating that calories from refined, simple sugars lead to accumulation of body weight and body fat when compared to the more healthy CTR diet of complex carbohydrates and protein.

4.3 Glucose Tolerance Test

The GTT performed on the mice demonstrated that the SUG started with higher blood sugar and were not able to clear the glucose from their blood as efficiently as the CTR group after the injection. This indicates that the long term sucrose over-consumption can lead to impaired glucose tolerance. Since the SUG mice overconsumed sucrose in the form of SSBs, they likely had consistently elevated blood sugar levels (hyperglycemia) that will result in glucotoxicity. Glucotoxicity has been demonstrated to damage pancreatic β cells, which secrete insulin, and insulin targeted tissues, such as the liver, which absorb glucose from circulation.[25,26,29–34] Ultimately, these effects may result in insulin resistance and Type 2 Diabetes in the SUG mice.

4.4 Insulin Tolerance Test

An insulin tolerance test (ITT) was performed on SUG mice after 12 weeks; however, results indicate that they are not significantly insulin resistant at this time. There was significant variability in the SUG group, which likely is one of the main factors that contributed to the lack of evidence to support our hypothesis. Yet, it should be noted that these results do not conclusively indicate that the SUG animals are still insulin sensitive. On the contrary, the elevated fasting blood glucose indicates that basal, fasting insulin levels may not be sufficient to keep the SUG blood glucose at CTR levels. This indicates that they may be insulin resistant, but an assay of fasting insulin levels and insulin during a GTT would provide more conclusive results. In addition, according to the theory of glucotoxicity, long term elevation in blood glucose in these animals will likely lead to insulin resistance and Type 2 Diabetes.[29,35,36] The small sample size could also be insufficient to demonstrate the difference that was expected.

4.5 Mouse Model For Undergraduate Research

It was necessary to consider a variety of factors when attempting to develop a model of diet induced obesity for undergraduate research. A major factor influencing study design was time. Many mouse strains that are sensitive to diet induced obesity are available for reasonable pricing and the scientific literature on these models in extensive. Since the researchers will be investigating the molecular mechanisms involved in obesity, the C57BL/6 is well characterized and the entire genome sequence is freely available through the National Center for Biotechnology Information (National Institute of Health, USA) for use in future molecular biology studies.

Twelve weeks was sufficient time to develop significant obesity and glucose intolerance in this model. However, the ITT did not indicate that they were insulin resistant at the end of 12 weeks. In the future, researchers will be analyzing fasting insulin levels in these animals and comparing them to fasting glucose levels to determine insulin resistance through a homeostatic model assessment test.[37] Only once these tests have been performed will the researchers be able to determine if 12 weeks is sufficient time to induce insulin resistance in the SUG mice. A larger sample size would also contribute to more significant results.

Using this model, undergraduates will be investigating molecular pathways involved in glucose homeostasis and adiposity in tissues throughout the body. Specifically, students will be investigating the pancreas, liver, fat and skeletal muscle and determining effects on glucose transport, metabolism, insulin signaling and adipose development using techniques such as polymerase chain reaction (PCR) and Western blotting. By developing this model, students will gain a deeper understanding of how whole body physiology corresponds to tissue specific molecular biology.

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