University of Cincinnati

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I, Puja Sharma, hereby submit this original work as part of the requirements for the degree of Master of Science in Biomedical Research Technology.

It is entitled:

High Sugar Consumption Results in Mammary Epithelial Hyperplasia and Adipocyte Hypertrophy in a Mouse Model of Hyperglycemia

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UNIVERSITY OF Cincinnati

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High Sugar Consumption Results in Mammary Epithelial Hyperplasia and Adipocyte Hypertrophy in a Mouse Model of Hyperglycemia

A thesis submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of

Master of Science

In the Department of Molecular and Developmental Biology of the College of Medicine

By Puja Sharma

B.S. California State University, Northridge

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Committee

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Abstract
Increasing evidence has shown the impact of external factors on increased cancer risk. The chances of being diagnosed with breast cancer increases in female patients diagnosed with diabetes and/or obesity. Lifestyle behaviors, such as diet, can impact the anatomy of the mammary gland, but the exact mechanism is not well known. In this study, we explore the anatomical changes that arise in the mammary gland in response to an unhealthy diet, specifically studying the adipocytes and epithelial cells of the mammary glands. Our hyperglycemic mouse model showed signs of diabetes and obesity and has higher risk factors for breast cancer. In addition, not only was there a reversal in obesity and diabetic phenotypes, but also in adipocyte hypertrophy. Quantification of the mammary gland lobules and proliferation showed irreversible epithelial hyperplasia. We believe that this preliminary data will lead to further studies that focuses on identifying a mechanistic link between hyperglycemia and cancer.

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Introduction

Despite being a genetic disease, accumulating evidences indicate that external risk factors, including diet, contribute to up to 70% of human cancers (42, 43). Breast cancer is one of the many cancers that show a strong correlation with lifestyle behavioral factors (e.g. diet). Many of those who have been diagnosed with breast cancer have a high likelihood of also being obese and diabetic (5, 9, 25, 26). Incidence rates of breast cancer that are attributable to lifestyle behavioral factors are increasing, suggesting a pressing need to understand the mechanistic link between these non-communicable diseases. Center for disease control and prevention (CDC) data indicates significant geographical overlap of diabetes and breast cancer cases in the US (Figure 1). Many of the states in the northeast and southeast regions of the United States show elevated cases compared to the rest of the country (11, 37). In addition, there is a correlation between the factors that contribute to the two. Both are associated with unhealthy diets, little exercise, and increased body weights (7, 25, 29). In this study, we investigated how lifestyle behavioral factors, particularly diet, influence the risk of developing breast epithelial cell hyperplasia. In order to explore this correlation, it is essential to understand how hyperglycemia can impact the structural and physiological features of the mammary gland.

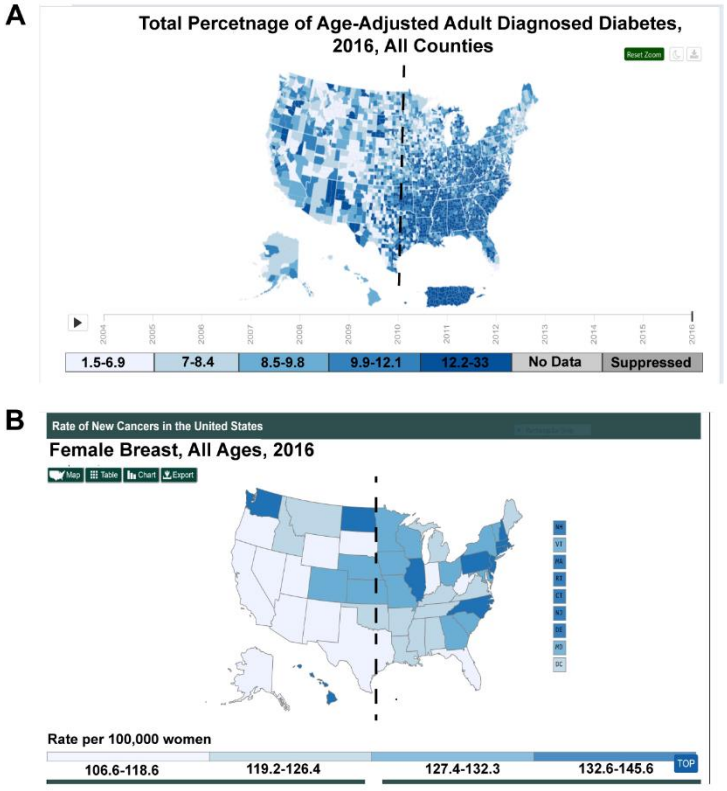

Figure 1: 2016 CDC data of (A) diabetes and (B) breast cancer cases. There is an increase in the number of cases in northeast and southeast regions compared to the rest of the country, represented by the dashed line. Darker blue colors represent higher cases.

It's well known that a common characteristic of breast cancer is increased epithelial cell proliferation. In order to understand whether hyperglycemia could be contributing to dysregulated cell proliferation and, consequently, structural changes within the mammary gland, a study was set up to detect changes in response to high sugar consumption. With this model, we hope to explore the varying proliferation changes, as well as the developmental and structural changes, that may be associated with an increased risk to breast cancer. We hypothesized that, compared to the control group, the high sucrose group will exhibit a significantly higher risk for diabetes, as well as factors associated with an increased risk to breast cancer. Thus, our aim was to test if high sugar consumption induced hyperplastic changes using a clinically relevant mouse model of hyperglycemia. With these results, further experiments can be geared toward understanding, mechanistically, how and why these changes take place.

The mouse mammary gland contains one lobe that forms a branching system, which spreads throughout the gland (Figure 2A). Like humans, this branching is composed of luminal epithelial cells and myoepithelial cells (Figure 2B). It is embedded in a stroma, which is surrounded by adipocytes (8, 13). This mouse model is appropriate for our study as it allows us to investigate the epithelial and adipocyte changes on a smaller scale.

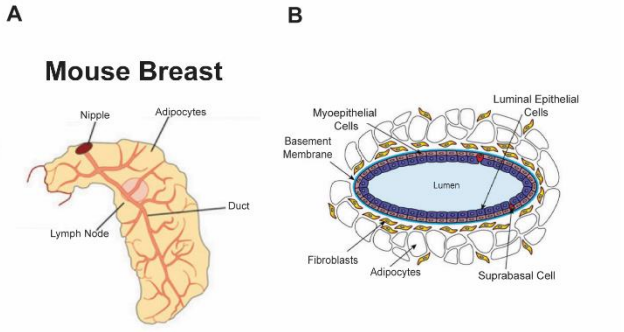

Figure 2: Anatomical representation shows that the majority of the mammary pads are composed of adipocytes and branching ducts. (A) The mouse mammary pad contains a single complex branching system that consists of a single lactiferous duct. (B) Lobule schematic showing cellular components that make up the ducts.

Materials and Methods

Animal Work:

Eight-week old in-house-bred C57BL/6 female mice were fed, *ad libitum*, either plain water or 25% sucrose water. All mice were provided with standard regular chow as the diet. Body weights were measured at biweekly intervals. Various parameters of hyperglycemia/diabetes including levels of blood glucose, HbA1C and insulin were also measured between the groups. A subset of mice that were on sucrose diet, and showed signs of full-blown diabetes, were switched back to plain water to check if the phenotype could be reversed. All mice were sacrificed at week 40, at the diestrus stage of estrus cycle, by using ketamine/xylazine as an anesthetic agent. At the time of sacrifice, blood was collected by retro-orbital bleeding. Serum was separated for insulin assay. In addition, a small amount of blood was also collected separately using heparinized capillary tubes for HbA1C measurement. Mammary glands were harvested and weights were measured. Bottom right and left mammary glands were used for preparing whole mounts and immunohistochemistry, respectively. All animal work was done in compliance of the animal and welfare regulations. Protocols are approved under IACUC Protocol Number 2018-0034.

Calorie Consumption:

The chow consumed was measured in grams and water consumed was measured in milliliters. Both were converted to calories based on the dietary information.

HbA1c:

HbA1c levels were detected using a Mouse Hemoglobin A1c Kit (Crystal Chem 80310). Duplicate samples were processed and the mean was used to calculate the HbA1c percentage.

Insulin Level Detection:

Insulin levels were detected using an Insulin ELISA kit (Alpco 80-INSHU-E01.1). Duplicate samples were processed. The mean was calculated and converted to ng/dL.

Hematoxylin and Eosin (H&E) Staining:

Deparaffinized and rehydrated breast sections were introduced to Hematoxylin, 2% Acetic Acid, Bluing Solution, and Eosin. The slides were dehydrated and coverslips were mounted using Histomount (Life Technologies). Slides were imaged using Cincinnati Children's Hospital Pathology Core's Aperio ImageScope.

Estrus cycle synchronization of mice:

Prior to sacrifice, vaginal lavage was performed using phosphate buffered saline (PBS) to determine the phase of estrus cycle of each mouse. Cells were mounted on the cover slip and allowed to dry followed by staining with crystal violet dye. After 1-2 minutes, cells were washed gently with running water and observed under microscope to confirm the phase of the estrous cycle. Because the rate of mammary gland proliferation varies between different stages of estrous cycle, to maintain consistency, we sacrificed all mice at diestrous cycle.

Whole Mount:

Whole mounts were fixed using Carnoy's Solution, and stained with Carmine overnight and coverslips were mounted using Histomount (Life Technologies). Due to the large area of the mammary glands, partial images of slides were taken then merged using Adobe Photoshop.

Sholl Analysis:

Sholl analysis was used to measure the branching density. It was performed on merged whole mount mammary gland images using Fiji Image J. Based on the contrast, the green channel images were used. Their background was removed manually and confirmed by overlaying the images. The length of each breast was measured in centimeters and was set as the scale to measure mammary epithelial area and lymph node area. Total branching area was determined by subtracting the mammary epithelial area by the lymph node area. Sholl Analysis output provided a linear graph and either a log-log or semi-log graph of the number of intersections versus the radii. Branching density was calculated by dividing the sum of intersections by the total branching area with units N/cm^2.

Adipocyte Quantification:
Adipocyte number and area was quantified using the Adiposoft Plugin on ImageJ. The minimum and maximum values were set based on the adipocyte diameters. Five representative images were taken per Hematoxylin and Eosin slide. The scale was set to represent the area in micrometer based on the pixel-to-micron conversion of 8x images.

Lobule Quantification:
H&E stained slides were used to measure the areas and number of lobules. Area measurements were taken using Image J. The scale was set to represent the area in micrometer based on the 20x pixel-to-micron conversion.

Immunohistochemistry:
Deparaffinized and rehydrated mammary gland sections were antigen retrieved using citrate buffer followed by peroxide quenching and blocking with 10% goat serum (GS).Sections were then incubated with Ki-67 (CST 12202S, 1:400 dilution in 2% GS) primary antibody and anti-rabbit (Vector Laboratories BA-1000, 1:200 dilution in GS) secondary antibody. Sections were treated with Avidin-Biotin Complex followed by 3,3′-Diaminobenzidine (DAB Substrate Kit, Peroxidase; Vector Laboratories SK4100). The sections were counterstained and dehydrated. Coverslips were mounted using Histomount (Life Technologies). Slides were imaged using a Leica DM2500 microscope.

Statistical Analysis:
Four female mice were used per treatment group. Data was graphed using GraphPad Prism Version 8.4.2. One-way ANOVA was performed and significance was determined using p-value calculations from Tukey's multiple comparisons test.

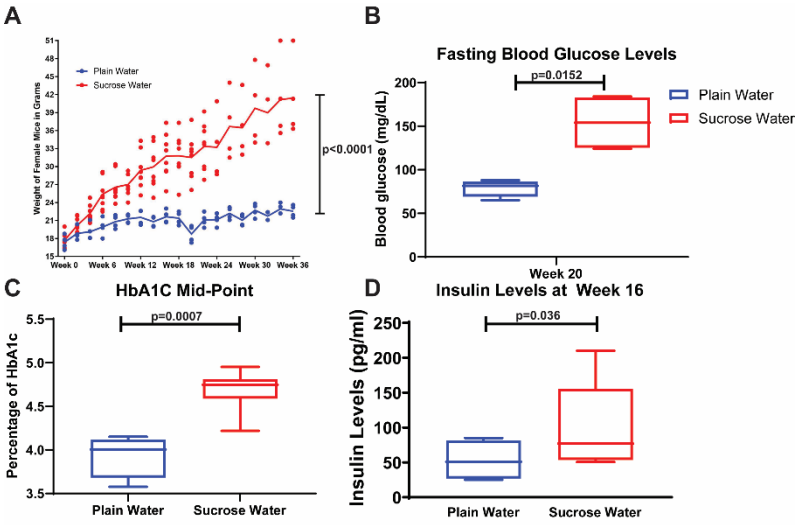

Figure 3: Weight gain, insulin levels, and HbA1c levels increase in response to an increased sugar intake. (A) Mice on a high sucrose diet gain more body weight than mice on a plain water diet; plain water n=4, sucrose water n=8. (B) Blood glucose increased in the sucrose water-fed mice; plain water: n=4, sucrose water: n=8. (C) HbA1c Levels confirm that there is an increase in blood glucose in sucrose water-fed mice compared to the control. (D) Insulin levels are increased in sucrose water-fed mice compared to the control group, confirming hyperinsulinemia.

Results

High sugar consumption led to obese and diabetic phenotypes.

The sucrose water-fed group gained significantly more weight than the control group (Figure 3A), suggesting that sucrose consumption as a single component in diet directly leads to obesity in mice. We also found that these mice also exhibited all necessary hallmarks of diabetes, including significantly high blood glucose, HbA1C and insulin levels compared to the control group (Figure 3B, 3C, and 3D). HbA1c percentage, a marker to check the average blood glucose levels for a period of 8-10 weeks(3, 17, 19)were found to be significantly higher in sucrose water-fed mice when compared to its control counterparts. Overall, our results indicate that high sucrose consumption leads to diabetes and obesity in mice.

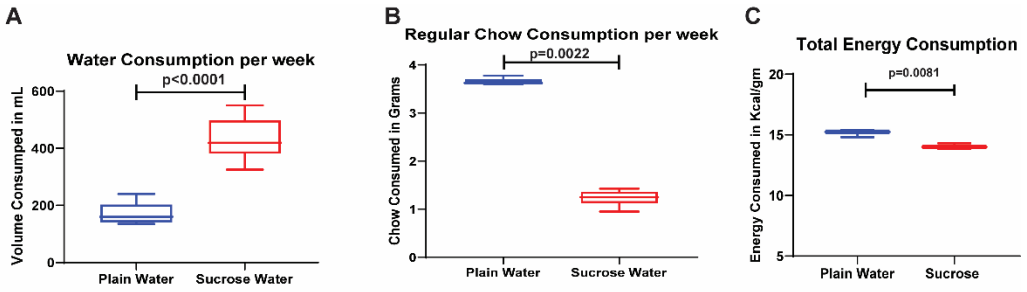

Figure 4: High sucrose water is consumed more than plain water, while chow intake is adjusted to match calorie intake. (A) On average, mice drank more sucrose water than plain water over a span of 12 weeks; plain water: n=4, sucrose warer: n=8. (B) In compensation to drinking more water, sucrose-fed mice reduced their chow intake; plain water: n=4, sucrose warer: n=8 (C) Overall calorie intake of both groups remained relatively similar.

Quality of Calorie matters: Sucrose water-fed mice compensates for total calorie intake by reducing solid food intake but still becomes obese and diabetic.

We measured the levels of sucrose and plain water consumption for a period of several weeks and found that mice that were given sucrose water consumed nearly twice as much volume compared to mice that were provided with plain water (Figure 4A). Unexpectedly, sugar water-fed mice compensated the calorie consumption by significantly decreasing the intake of the solid food (regular chow) (Figure 4B). When the total calorie intake was calculated, it was seen that both groups consumed a similar amount of calories, indicating that diabetes and obesity in sucrose water-fed mice are due to increased sugar intake, rather than increased calorie intake (Figure 4C). Thus, we conclude that consumption of excess liquid sucrose (resembling excess consumption of sugary drinks in humans) as a single dietary component causes obesity and diabetes in mice with comparable total calorie consumption.

Obesity and diabetes are reversible in mice.

At week 22, when all eight sucrose water-fed mice showed significant increase in body weight, half the animals (four mice) were switched to plain water to test whether the obese and diabetic phenotypes are reversible. Switching to plain water rapidly and significantly reduced body weight of these four mice (Figure 5A). In addition, the levels of fasting blood glucose and insulin were also decreased (Figure 5B and 5D). However, we observed significant variability in the HbA1C levels in the reversal group within the duration of our study (Figure 5C). This could possibly be due to the fact has HbA1c has a long half-life period.(20). We expect that continued feeding plain water for a longer duration would reduce HbA1C levels in the reversal group.

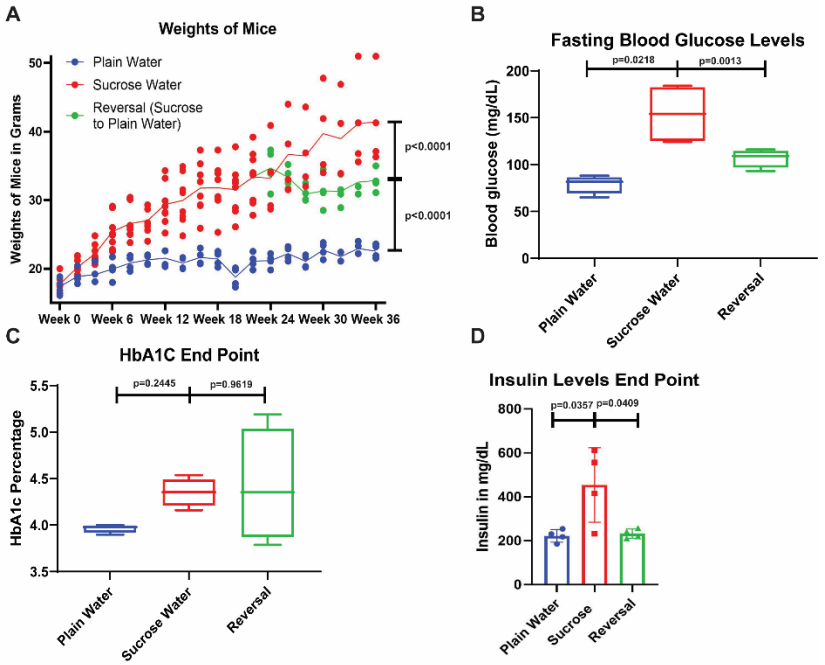

Figure 5: Reversal of high sucrose to plain water was able to decrease body weights, fasting blood glucose, and insulin levels, though the HbA1c levels did not decrease. (A) When sucrose water-fed mice were reversed back to plain water, the body weights decreased; n=4. (B) Fasting blood glucose levels also show a decrease when sucrose was reversed; n=4. (C) HbA1c levels at the time of sacrifice show that the sucrose-fed mice had higher HbA1c levels, but this did not significantly decrease in the reversal group. (D) At the time of sacrifice, insulin levels of the reversal mice were significantly decreased compared to the sucrose water-fed mice; n=4.

High Sugar consumption altered breast adiposity

Weights of mammary glands of sucrose water-fed mice were significantly higher when compared to that of control mice suggesting that higher sugar consumption resulted in heavier breasts – a phenotype that was significantly reversed in the reversal group. (Figure 6A). In addition there were significant increases in the size of the breasts of sucrose water-fed mice as compared to plain water-fed group (Figure 6B). Again, this phenotype was largely reversed in the reversal group. Increased weights and sizes of breasts indicate that there could be significant changes in the structure and anatomy of breasts. To examine these changes, we performed H&E staining on the breast sections. H&E staining showed a distinct difference in the appearance of the adipocytes (Figure 7A). We observed that the number of adipocytes within a 1.3mm x 0.85mm area of the sucrose water-fed mice were significantly lower than that in the same area of plain water-fed mice (Figure 7B), which is indicative of increased size of adipocytes (Figure 7C) due to higher fat accumulation. Switching to plain water partially

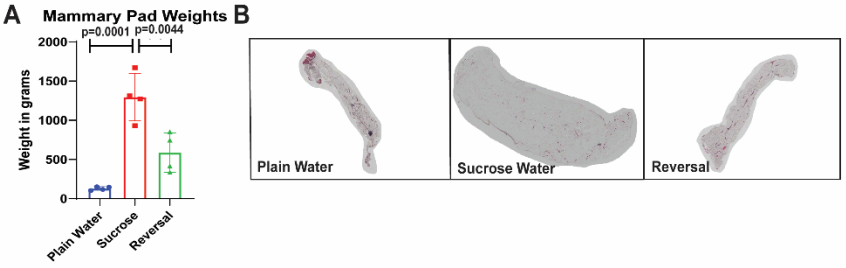

Figure 6: Mammary pads weight and size change in response to sugar intake. (A) Mammary pad weights show an increase in sucrose-fed mice compared to the control, the weight was reduced when sucrose was reversed; **, ***p=0.0001, n=4. (B) H&E stained whole mammary pads of the control, sucrose-fed, and reversal mice show the overall mammary pad size difference.

reversed adipocyte structure, adipocyte number and size (Figure 7A-C) within the time-frame of our reversal study. We expect this phenotype to be completely reversible following continued exposure of this animals to plain water. These together show that in a given area plain water-fed mice contain many smaller adipocytes, while the sucrose-fed mice contained larger and fewer adipocytes. Because much of the mouse mammary gland is composed of adipocytes, it can be inferred that the differences seen in the mammary gland weights could mainly be due to the changes seen in adiposity. In addition, hyperinsulinemia, and altered cytokine profile may likely contribute to breast adipocyte hypertrophy. Studies are underway to determine the molecular changes in these adipocytes.

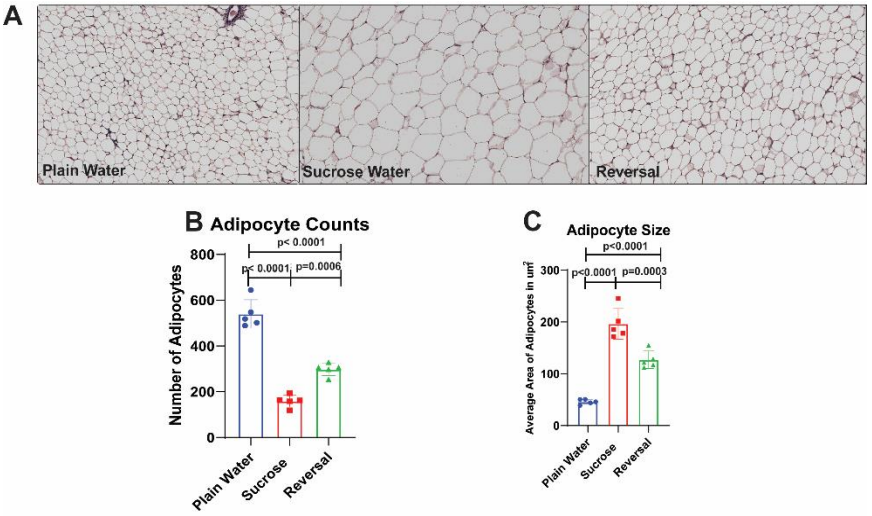

Figure 7: Hemoxylin and Eosin staining show an increase in adipocyte size, indicating the changes in mammary pad weight is a result in the lipid content of the breast. (A) Adipocytes in a 1.3mm x 0.85mm area of control mice, sucrose-fed mice, and reversal diet mice show a change in size. (B) Total number of adipocytes in a 1.3mm x 0.85mm area show that the control group have significantly more adipocytes compared to the sucrose-fed group, the number of adipocytes in this area increased in the reversal group, data is obtained from five representative images per mouse; n=4 (C) Average areas of counted adipocytes show that the size within the control group were significantly smaller than those of the sucrose-fed group, the size returned to a smaller size in the reversal group, data is obtained from five representative images per mouse; n=4.

High sucrose consumption increased the number of lobules in the breasts.

In addition to the adipocytes, the H&E images were used to analyze the mammary gland lobules (Figure 8A). The lobules are important in understanding the structure and development within the mammary gland as they are the main source of epithelial cells. Upon quantification of lobules, we observed that breasts of the sucrose water-fed group contained significantly higher number of lobules (Figure 8B). Contrary to water-fed mice, the size of individual lobules also greatly varied in sucrose-fed mice with many markedly large lobules. The distribution of lobule areas are shown in Figure 8C. Surprisingly, despite near-complete reversal of breast adiposity, lobule number and size was not reversed in the reversal group. This key piece of data indicates that at least within the time frame of our experimental setup, switching hyperglycemic mice back to plain water was adequate to reverse adipocyte hypertrophy but insufficient to reverse the increases in the number and size of lobules, leading to a high epithelial content both in sucrose-fed and reversal group. Further analysis is underway to understand the molecular changes in these epithelial cells.

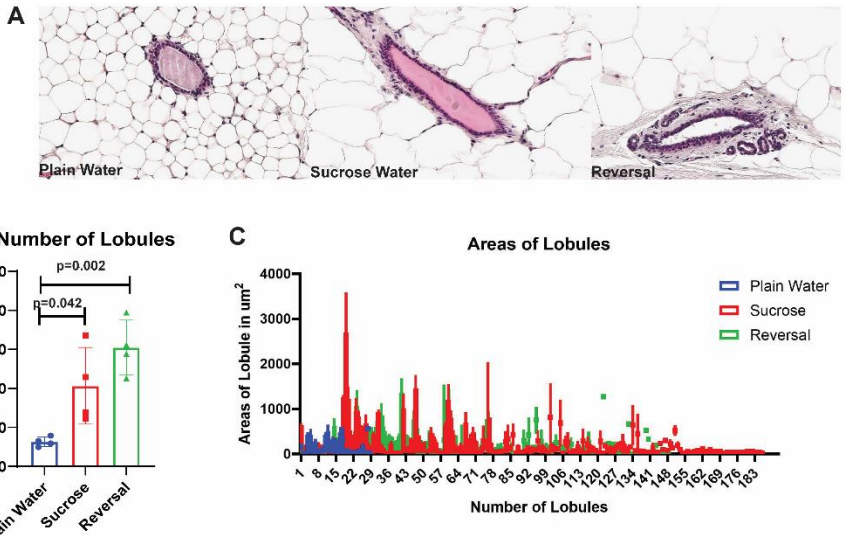

Figure 8: When mammary pad lobules were quantified, it was seen that there was an increase in sucrose-fed mice, but there was not a decrease in the reversal group. (A) H&E stained lobules of control mice, sucrose-fed mice, and reversal mice show the structural differences between the three groups. (B) Total number of lobules show a significant increase in the sucrose-fed mice and the reversal mice; n=4 (C) Areas of lobules show there are many more lobules with larger areas in the sucrose-fed mice and the reversal mice compared to the control, indicating that the reversal treatment is not effecting the lobule structure.

Branching Density is increased in high sucrose water-fed mice

In order to explore the anatomical changes in the mammary gland caused by high sucrose diet, branching density was quantified via Sholl analysis of breast whole mounts. We observed that breasts of sucrose water-fed mice were not only larger but also showed significantly more branching than those of the plain water-fed mice (Figure 9A and 9B). The size and branching was partially reversed in the reversal group of mice. When quantified using Sholl analysis, we found a significant increase in the branching density of the sucrose water-fed mice compared to the control mice that

was partially reversed in the reversal group of mice (Figure 9C). It has been shown that increased branching density is associated with an increased risk to breast cancer (6, 12, 15, 36, 39). This indicates that higher sucrose intake induces branch formation predisposing breast tissue to oncogenic changes.

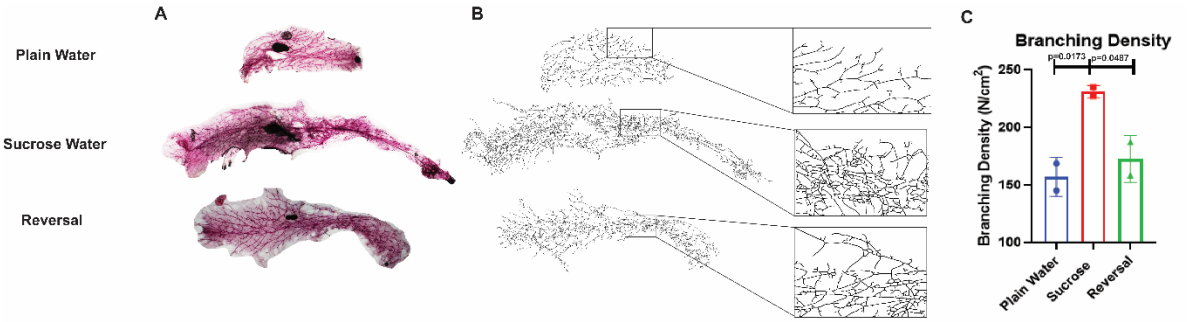

Figure 9: Sholl Analysis results show branching density increases in response to increased sugar intake. (A) The control, sucrose water-fed, and reversal groups show differences in their mammary pad size. (B) Upon removal of the background it is seen there is a difference in their branching. (C)When this was quantified, it was seen that there was an increase in the branching density in the sucrose-fed group, which was decreased in the reversal group; n=2.

High sucrose consumption caused irreversible changes in the proliferation index of the breast epithelia.

Because we observed changes in the breast epithelial content, we then went on to measure the proliferation index of luminal epithelial cells. Immunohistochemical staining for ki-67, a well-known marker of proliferation, showed a significant increase in the number of ki-67 positive cells in the breast epithelia of sucrose water-fed mice (Figure 10A and B). These results support our findings in Figure 8 that showed higher expression of lobules in the breasts of sucrose water-fed mice when compared to plain water-fed mice. When comparing the number of proliferating lobules, the sucrose water-fed and reversal groups had significantly more proliferating lobules than the control group (Figure 10C). Besides increase in total number of lobules with proliferating cells and total number of proliferating cells in the entire breast, the number of proliferating cells per lobule was also increased in sucrose-water-fed mice that was not reversed in the reversal group (Figure 10D). This confirms that increased sugar intake increases breast epithelial hyperplasia. This change seems durable since switching mice back to plain water failed to reverse this phenotype.

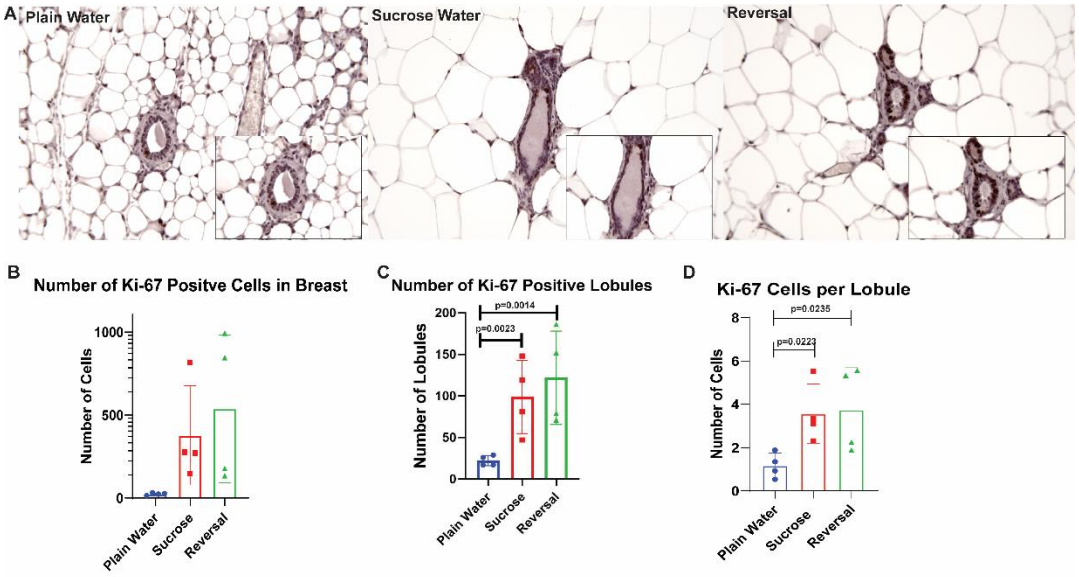

Figure 10: Quantification of cell proliferation within mammary gland lobules show an increase when mice are fed high sucrose water, this does not decrease when reversed. (A) Ki-67 stained lobules of control mice, sucrose-fed mice, and reversal mice show the difference in cell proliferation. (B) The total number of Ki-67 cells was increased in both sucrose-fed mice and reversal mice compared to the control mice. (C) The number of Ki-67 positive lobules followed a similar pattern, where sucrose-fed and reversal mice have significantly more proliferating lobules than the control mice; n=4. (D) The average number of Ki-67 positive cells per lobules showed that the average proliferation rate between sucrose and reversal groups were very similar and were both significantly higher than the control group; n=4.

Conclusion and Future Directions

The goal of this study was to identify changes in the mammary glands of mice fed with plain water versus 25% sucrose water and relate this to the risk of breast cancer. It was hypothesized that high sucrose consumption would cause a significant increase in factors associated with increased breast cancer risk in comparison to the control group. While administering the sucrose, we were able to confirm that the mice showed increase in body weight, blood glucose, HbA1C, and insulin levels. All of these followed the hypothesized pattern and confirmed that sucrose in drinking water induced obesity and diabetes in mice.

We were able to show that consumption of unhealthy diet increased the risk for diabetes and obesity. The finding that mice were able to sense a greater calorie intake through sucrose feeding and adjust total calorie intake by reducing the amount of solid food intake was surprising. The finding is in contrast to what is known in humans where an increase in consumption of sugary drinks co-occurs with an increase in the consumption of solid food, increasing total calorie intake (10, 40). We do not fully understand the reason for this difference. Whether satiety is differently regulated in rodents and mice and/or the abundance of novelty in food type tempts humans to continue eating after satiety has been reached are intriguing questions that needs to be examined.

To determine the anatomical and proliferative changes in the breast, we performed several quantitative studies. Increase in breast size was largely due to breast adipocyte hypertrophy. It's been shown that increased mammary adiposity is associated with an increase in insulin and insulin growth factor-1 signaling (4, 27, 32). Because hyperinsulinemia has been shown to be associated with increased estrogen, it can be argued that hyperinsulinemia may increase risk for breast cancer in the sucrose-water fed mice (30). Diabetes causes systemic inflammation (18, 23, 35). Therefore, in addition to the role of insulin, or IGF1, a surge in estrogen may be caused due to inflammation-induced upregulation of Nf-kB (24, 32, 33, 44). Further studies are needed to determine molecular links between hyperglycemia and breast adiposity.

The lobules were also analyzed, and it was found that the there was an increase in lobule number and area with an increased sugar intake, but this was not decreased when the sugar intake was reversed. This indicates that the effect a high sugar diet has on the mammary epithelial cells may be irreversible. This was confirmed further when epithelial cell proliferation was measured. The cause of this irreversibility is currently being explored. This may be a result of excess glucose available within the body, as shown by the end point HbA1c levels. As indicated, this can be confirmed by introducing a plain water diet for a longer period of time. On the other hand, chronic exposure to sugary drinks may induce durable changes in breast epithelia. We are performing comprehensive cell cycle analysis including using additional markers such as phopsho-Histone H3 and EdU to understand the effect of chronic hyperglycemia on breast epithelial hyperplasia.

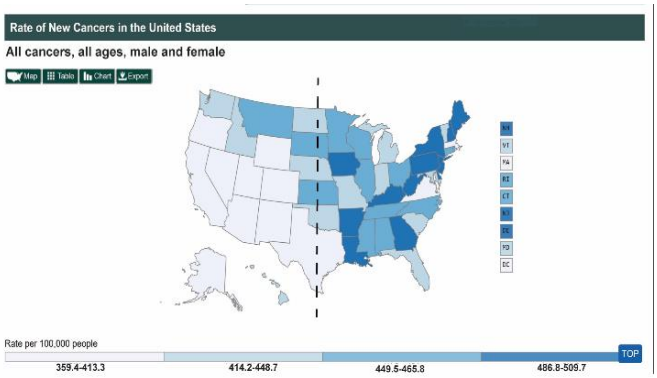

Figure 11: The CDC data of total cancer cases in the US in 2016 shows that the most cases are in the northeast and southeast regions, shown to the right of the black dashed line.

Because a murine model was used, certain limitations and considerations were taken into account when analyzing the mammary glands. The first being that mice contain five pairs of mammary glands that each have a single branching complex, while humans have one pair of mammary glands with approximately fifteen lobes. Also, mouse mammary glands contain significantly more adipocytes than human mammary glands. Because mice have a simpler mammary gland structure than humans, these analyses would be more complex in the human breast. Lastly, the estrus cycle of the mice were taken into consideration. Not only were the estrus cycles matched at the time of dissection, it was also compared to the analogous human phase, which is the luteal phase. During this phase, proliferation is reduced as there are lower levels of estrogen impacting the breast (2, 13, 28, 34).

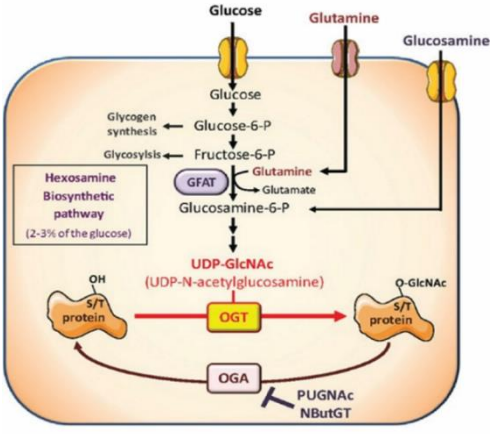

Figure 12: In our model, it is hypothesized that the production of O-GlcNAc via the Hexosamine Biosynthesis Pathway is contributing to the increased cancer risk in our mouse model.

The results of this study are part of a larger project that focuses on more than just breast cancer risk. The CDC data of breast cancer cases is a representative of total cancer cases within the United States (Figure 11) (37). The data shows that states in the northeast and southeast regions not only have increased breast cancer cases, but also increased cases of cancer overall. This includes cancers within the prostate, lung, uterus, bladder, and many others organs. In addition, many of these cancers are included in the top ten highest cancer death rates of 2016. As indicated, an increase in breast cancer risk is associated with poor eating habits,

lack of exercise, and weight gain. This is not just seen in breast cancer, these factors have been shown to affect many other organs within the body and contribute to multiple forms of cancer risks.
How this is directly associated with the increased risk of cancer is not well known, but we are hypothesizing that it is associated with glycosylation. Glycosylation is a post-translational modification where a carbohydrate covalently bonds to a protein, lipid, nucleic acids, or organic compound (22). It has been shown that glycosylated proteins are increased in a variety of cancers (22, 38). When excess sucrose is taken in via diet, it gets converted to glucose so it can be used up by the body. Glucose can then be converted and enter multiple pathways, one of which is the hexosamine biosynthesis pathway (Figure 12). In a healthy cell, only two to three percent of glucose molecules follow the hexosamine pathway. When a cell is in a stressful environment, including a cancerous environment, there is an elevated flux into this pathway (21). The end product is known as UDP-linked β-N-acetylglucosamine (UDP-GlcNAc). When this molecule interacts with a naked protein, it forms a bond via a serine or a threonine and forms O-GlcNAc. This process is known as O-GlcNAcylation.

O-GlcNAc is known to be elevated within many cancers. (14, 16, 21). Future directions of this project focus on understanding the role of O-GlcNAc in cancer and how it is expressed under different conditions. We hypothesize that in our model, both in vivo and in vitro, the consumption of excess sugars is associated with both increased markers of cancer and increased O-GlcNAcylation. By targeting different enzymes within this pathway, we would like to test how lifestyle behaviors can contribute to an increased cancer risk.

Statement of Work

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