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## The Impact of Single Nucleotide Polymorphisms on Cortisol Receptor Activity in Populations with Obesity

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The Impact of Single Nucleotide Polymorphisms on Cortisol Receptor Activity  
in Populations with Obesity

By

Cassidy Michalicka

\* \* \* \* \*

Submitted in partial fulfillment  
of the requirements for  
Honors in the Department of Neuroscience

UNION COLLEGE

June 2022

## **Abstract**

Cassidy Michalicka

The Effect of Cortisol Hypersensitivity Polymorphisms on Cortisol Activity in Obesity

Department of Neuroscience, June 2022.

Advisor : Brian Cohen

Cortisol is a crucial part of the endocrine system; it has the capacity to affect nearly every organ and tissue in the human body. When functioning correctly, cortisol is known to regulate the body's stress response, control metabolism, suppress inflammation, regulate blood pressure, regulate blood sugar, regulate our body's circadian rhythm, and much more. When the concentration of cortisol in the blood is elevated for an excessive period, the body responds with symptoms such as hyperglycemia, hypertension, weight gain, and moon face. Commonly this is known as Cushing's Syndrome (CS), and interestingly, we have seen a phenotypic resemblance when contrasted alongside Metabolic Syndrome, a subtype of obesity. Several specific nucleotide polymorphisms (SNPs) have been found along the glucocorticoid (GR) and mineralocorticoid (MR) receptors that are expected to present in a higher concentration amongst populations with obesity. Our current research is investigating the relationship between the allele frequency of four SNPs (rs7901695, rs12772424, rs3753519, and rs1051052), and obesity. Through a collaboration with Ellis Hospital Bariatric Care Center, we obtained the patients' body mass index (BMI), blood glucose levels, serum triglycerides levels, HDL and LDL cholesterol levels, and systolic and diastolic blood pressures to evaluate. Amongst the SNPs tested, there was a significant difference between different genotypes of the rs3753519 SNP in original weight, diastolic pressure, extra body weight, and extra body weight percentage. This

data supports the hypothesis that SNPs involved in the activity of cortisol can alter metabolic profiles and could be contributing to the development of diseases such as Metabolic Syndrome and CS. Furthermore, this could aid the deeper understanding of how these SNPs influence weight gain and could provide better predictions and outlooks on the success of weight loss significantly improving the overall quality of life for individuals with obesity.

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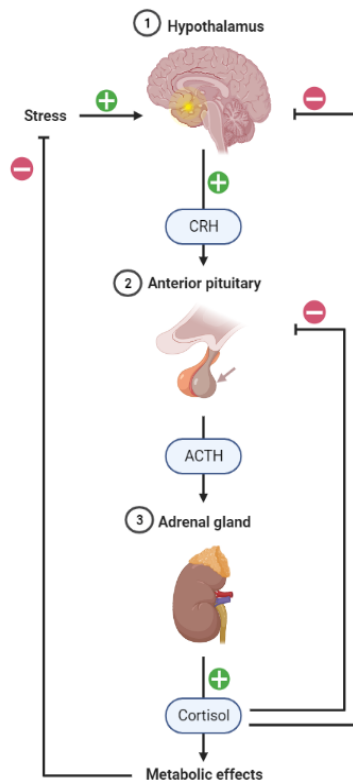
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## **Introduction**

Obesity is the pandemic of the 21st century.<sup>1</sup> Obesity occurs when energy intake is greater than energy expenditure resulting in excess adipose tissue. We categorize obesity within individuals with a body mass index score of 30 or greater. In the United States, the epidemic of obesity associated with a high prevalence of comorbidities is present and growing. These comorbidities are commonly portrayed by several diseases such as hypertension, dyslipidemia, insulin resistance, type 2 diabetes, cardiovascular disease, stroke, sleep apnea, and osteoarthritis.<sup>2</sup> According to the National Health and Nutrition Examination Survey (NHANES) of 2013, more than 1 in 3 adults were characterized as having obesity<sup>3</sup>. Today, this number has nearly doubled to 2 in 3 adults, resulting in the average risk for diseases associated with obesity in the population increasing significantly. Obesity can result from an array of environmental and genetic factors such as overeating, chronic sleep loss, insufficient physical activity, various medications, and pollutants and stress.<sup>2</sup>

When our bodies are exposed to stress our initial response is immediately mediated by the sympathetic nervous system, here epinephrine and norepinephrine are secreted.<sup>4</sup> Their role is to prime our bodies to deal with the stress nearly instantly; within a short time period they commonly increase heart rate, increase blood pressure, expand air passages in lungs, enlarge pupils, redistribute blood to muscles, and maximize blood glucose levels.<sup>4</sup> Shortly after, our body will recognize the elevated norepinephrine levels and trigger a more long term and sustainable response via the hypothalamic pituitary adrenal axis (HPA axis).<sup>4</sup> When the hypothalamus recognizes the elevated norepinephrine, it secretes corticotropin releasing hormone (CRH) into the bloodstream where it will travel to the anterior pituitary gland. The

anterior pituitary will then release adrenocorticotrophic hormone (ACTH) in response to CRH, where it will travel to the cortex of the adrenal gland through the bloodstream. Once ACTH reaches the surfaces of the adrenal gland, it will bind and trigger a series of intracellular events which result in the secretion of cortisol.<sup>5</sup> Cortisol is regulated through a negative feedback loop where the adrenal gland will recognize the excess levels of cortisol and release an inhibiting hormone through the blood that will travel to the hypothalamus and stop the production of CRH; the original messenger through this pathway. This pathway is primarily responsible for returning our bodies to homeostasis, while fundamentally controlled by the central nervous system (hypothalamus), havoc can arise when our bodies lose the ability to carry out this process efficiently and correctly.<sup>5</sup>



**Fig 1.** The Hypothalamic Pituitary Axis<sup>6</sup>

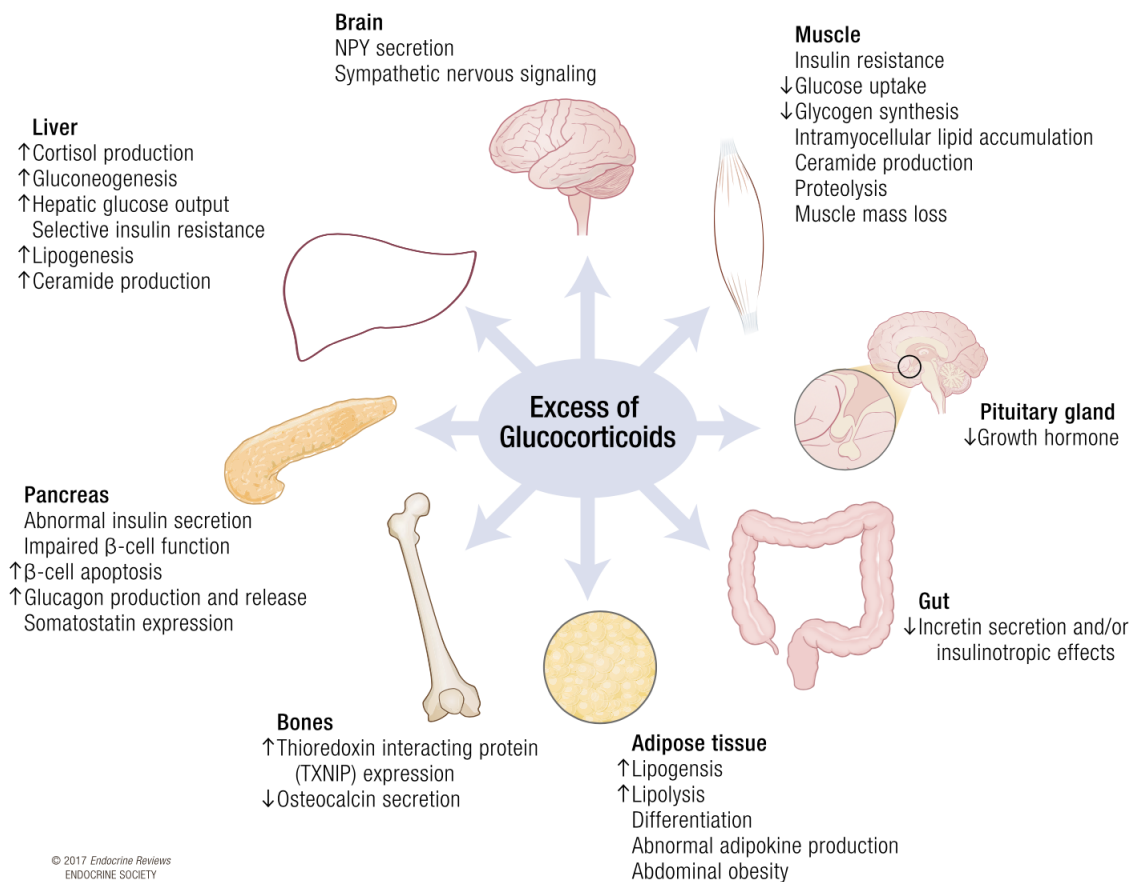
Cortisol is a steroid hormone produced by the adrenal gland in response to stress.<sup>7</sup>

Cortisol has the power to increase blood sugar, suppress the immune system, stimulate fat and carbohydrate metabolism, and initially decrease any tired, sluggish, or sore feelings throughout our bodies.<sup>4</sup> Cortisol receptors are present in nearly every tissue within the body. This gives cortisol the power to affect the nervous, immune, cardiovascular, respiratory, reproductive, musculoskeletal, and integumentary organ systems.<sup>7</sup> Cortisol is synthesized from cholesterol and is the most essential glucocorticoid in the body.

Glucocorticoids are steroid hormones synthesized from the cortex of the adrenal glands, they have a multi-dimensional role involved in production and maintenance of glucose, protein, and fat in the body.<sup>7</sup> The best predictor for the comorbidities associated with obesity is not correlated to the total body adipose mass, but rather, the quantity of visceral fat.<sup>2</sup> Visceral fat is the fat known to wrap around the organs inside the abdomen, commonly associated with diseases which correlate with obesity. One supported explanation of visceral obesity is exposure to excessive levels of glucocorticoids.<sup>2</sup> Excess levels of glucocorticoids affect our bodies in numerous ways in addition to the increase in adipose tissue which ultimately results in visceral obesity. As illustrated in Figure 2, within muscle tissue, the effect of excess glucocorticoids has the ability to decrease glucose uptake and therefore decrease muscle mass, this can be problematic as with a higher percentage of muscle mass our metabolic rate increases and consequently, with a decrease in muscle mass, metabolic rate will decrease which will contribute to additional abdominal obesity. More relevantly, we see insulin resistance, insulin plays a central role in the regulation of blood sugar levels, it determines how the body stores glucose and fat.<sup>8</sup> When the body is resistant to insulin, the body struggles to maintain blood

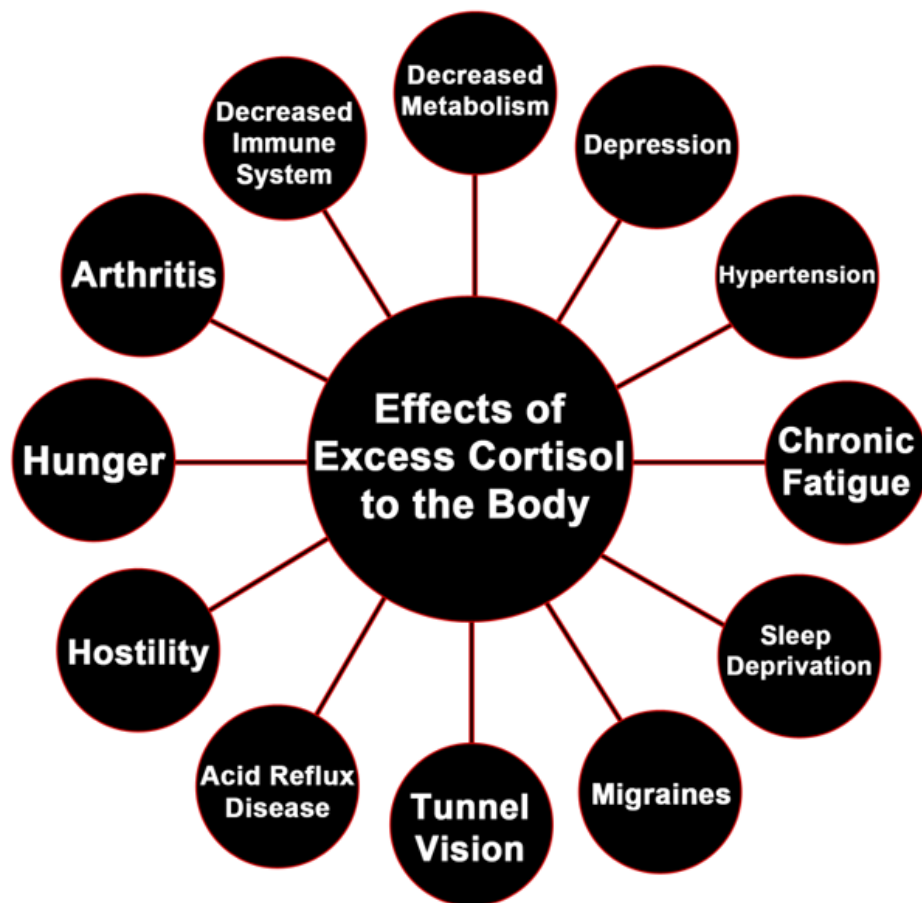


sugar at the correct level which can directly translate to type 2 diabetes and also obesity. From the liver, Figure 2 supports the notion that excess levels of glucocorticoids results in excess cortisol production in the liver; this individually triggers excess gluconeogenesis; where glucose is synthesized abnormally from non-carbohydrate substrates.<sup>9</sup> As previously mentioned, excess glucose levels like we saw with insulin resistance can inadvertently lead to type 2 diabetes and obesity. From a neural perspective, excess levels of glucocorticoids trigger the sympathetic nervous signaling pathway that stimulates our focus and alertness for tasks. In addition, excess glucocorticoids also triggers neuropeptide Y (NPY) secretion in the brain, NPY is known to directly stimulate food intake (the feeling of hunger), decrease energy expenditure, increase energy stored as adiposity, and indirectly stimulate obesity.<sup>10</sup>



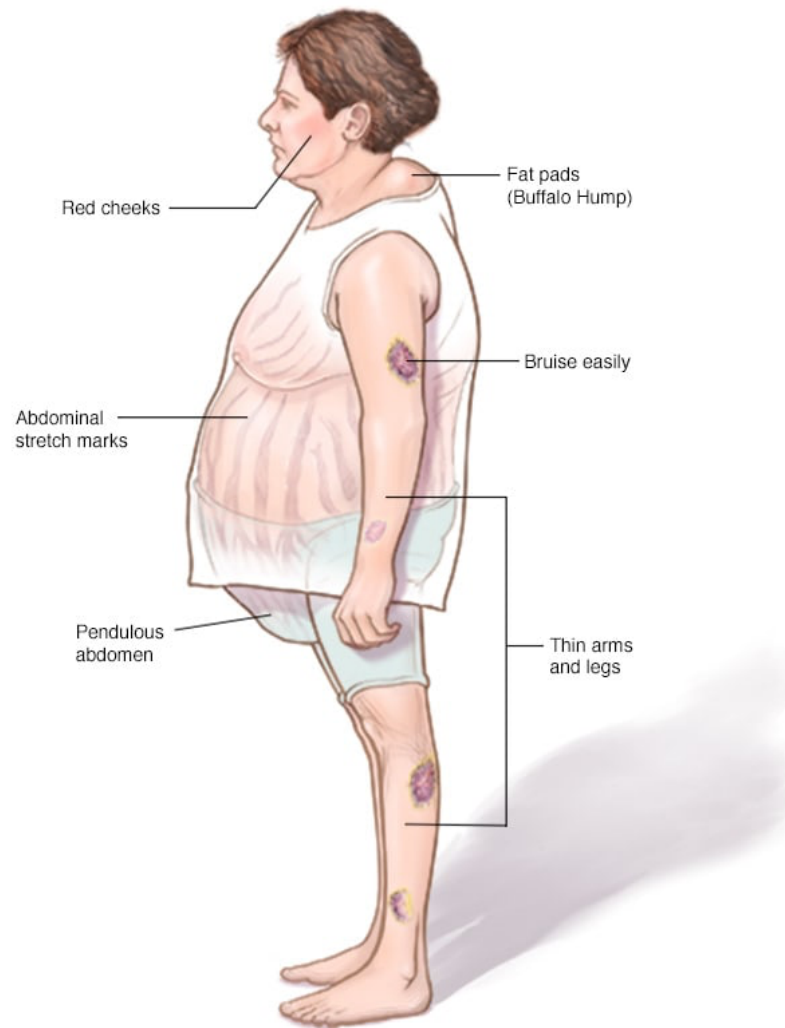
**Fig 2.** Excess Glucocorticoids Effect on the Body<sup>11</sup>

Although cortisol is essential for maintaining normal bodily function, there are negative aspects concerning the versatility of cortisol. Demonstrated in the figure above we see the immediate effects of excess levels of glucocorticoids and how this can affect the human body. However, more specifically, when cortisol is excessively secreted and elevated levels are circulating through the body we see a link between decreased immune system, decreased metabolism, depression, hypertension, chronic fatigue, sleep deprivation, an increase in hostility and hunger, as well as arthritis.<sup>12</sup> Moreover, from the beginning it was elaborated that poor sleep quality and stress can cause obesity through the HPA axis pathway, however it is evident cortisol is the real villain.



**Fig 3.** Excess Cortisol on the Body<sup>12</sup>

There are actually a few disorders that are directly related to cortisol; relevant to my research is Cushing's Syndrome. Also referred to as hypercortisolism, it stems from long term exposure to excess cortisol on the blood stream.<sup>13</sup> Cushing's is a subtype of obesity, and is characterized specifically by abdominal obesity and visceral fat which we know is from those excess glucocorticoids.<sup>2</sup> The symptoms of Cushing's syndrome are dependent on how elevated the levels of cortisol are, common symptoms include weight gain (most specific to the face and abdomen areas), fatty deposits between shoulder blades, diabetes, hypertension, muscle weakness, and osteoporosis.<sup>14</sup>



**Fig 4. Symptoms of Cushing's Syndrome<sup>14</sup>**

Interestingly, some patients record cortisol levels in the normal range, however, the subjects report an abundant number of symptoms that typically correlate with elevated cortisol levels.<sup>15</sup> It is possible that mutations along the glucocorticoid receptor gene have modified the sensitivity to cortisol and may be responsible for the adverse effects on a variety of subjects.<sup>15</sup> Meaning, the receptor could be hypersensitive to cortisol and therefore activates excessively despite cortisol levels residing in the normal range. In one study performed, patients developed the phenotype typically identified with Cushing's syndrome despite having normal or even low cortisol levels.<sup>15</sup>

Therefore, not everyone who suffers from obesity, symptoms that correspond with Cushing's Syndrome, or elevated cortisol levels has Cushing's Syndrome. Furthermore, the question evolves into how do we treat obesity? The most common first steps through a weight loss process is emphasizing a nutrition plan catered to your specific weight loss goals alongside a regular physical activity.<sup>16</sup> While these alone are not the solution for everyone, weight loss management programs that promote a sense of community and camaraderie to help propel an individual's motivation and sense of responsibility can be helpful. However, it is important to find a program that will support and guide the individual to reach their goals in a safe and healthy manner. Prescription medication prescribed by a licensed physician can also be an option with treatments for obesity, these commonly can be linked to managing cortisol levels, stress, or other hormonal issues that may be negatively impacting an individual's ability to maintain a healthy BMI.<sup>16</sup> Behavioral therapy is also an option that may aid individuals who specifically struggle to identify or control their underlying eating habits, their mindset through the weight loss process, and any other mental blocks that may impede the process.<sup>17</sup> Lastly, for

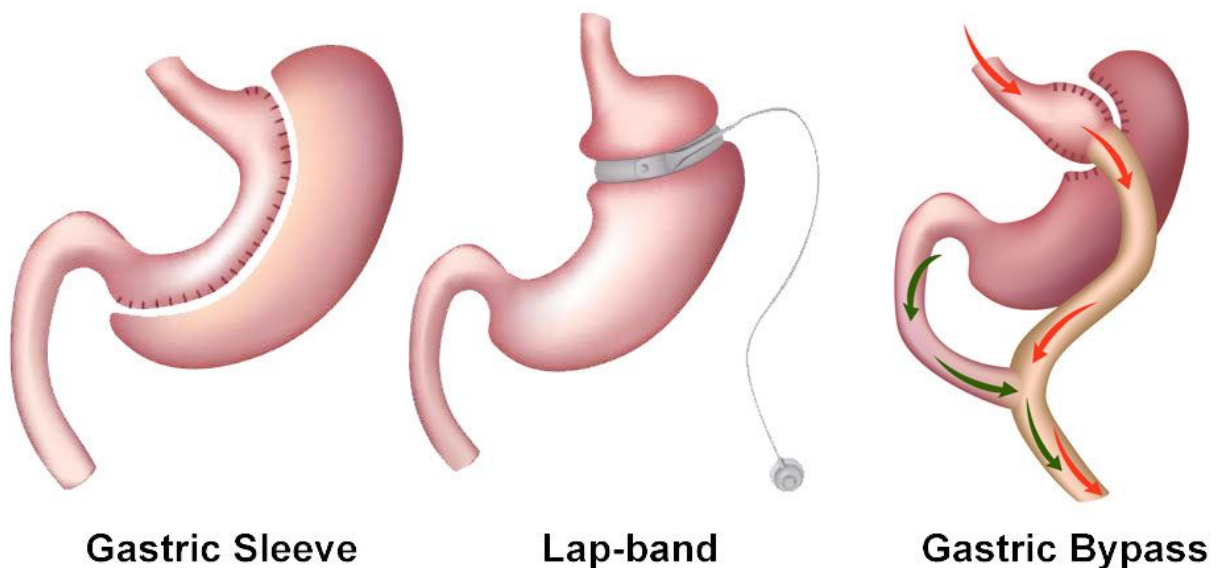
a specific group who qualify, bariatric surgery can be an invasive yet rewarding option to boost the weight loss journey of an individual.

Bariatric surgery is the most effective single therapy, aiding complications concerning glucose homeostasis, body weight loss, plasma triglyceride levels, type 2 diabetes, and HDL cholesterol.<sup>1</sup> To qualify for bariatric surgery, you must have a BMI of 40 or higher, or a BMI of 35-40 alongside one of the following: diabetes, high blood pressure, high cholesterol, fatty liver disease, or sleep apnea. They also could qualify with a BMI of 30-35 alongside uncontrollable diabetes. There are three main procedures when it comes to bariatric surgery, the Roux-en-Y gastric bypass, as well as the sleeve gastrectomy, and gastric banding.<sup>18</sup>

The gastric sleeve is the most common surgery option and the only irreversible option, where 80% of the stomach is removed so that patients feel less hungry and become full quicker.<sup>18</sup> While there is a risk the irreversibility could cause issues long term, it can induce gut hormonal changes that can prevent and help manage type 2 diabetes.<sup>19</sup> The lap band is where a rubber band is placed on the superior portion of the stomach ultimately reducing volume and appetite, this procedure is reversible as well as adjustable.<sup>18</sup> With the option to expand the portion of the available stomach this often can be difficult for individuals to stay on track with their weight loss journey. The Roux-en-Y procedure is when the inferior portion of the esophagus is rerouted to the duodenum of the small intestine. This allows food and nutrients to enter the small intestine directly bypassing up to 95% of the stomach, this results in optimal weight loss due to the patient feeling full much faster than other similar methods.<sup>18</sup> Roux-en-Y is also the most effective procedure for patients with severe reflux disease, people with high BMIs, and those with diabetes.<sup>18</sup> Roux-en-Y also has shown to reduce the occurrence of type 2

diabetes in most patients.<sup>19</sup> More specifically, massive weight loss has shown to decrease insulin resistance in adipose tissue, lower the rate at which cortisol is secreted and reduce the production of glucocorticoids.<sup>20</sup> This infers specific types of the Roux-en-Y could be favored to maximize success rates of treatment in those who have been diagnosed with Cushing's Syndrome by targeting obesity at its foundation.

## Revision Bariatric Surgery Procedures



**Fig 5.** Three Most Common Types Of Bariatric Surgery<sup>22</sup>

At this point, not everyone who has these procedures is equally successful, which leads us to our hypothesis. Single nucleotide polymorphisms (SNPs) associated with cortisol hypersensitivity are negatively associated with the success of bariatric surgery. We inferred this because genetic factors cannot be completely overcome by the process of a physical surgery. Therefore, despite the initial success of the surgery, if an individual has unaddressed excess cortisol or cortisol hypersensitivity this success will not be long term. If what we hypothesize is

supported then the presence of cortisol hypersensitive SNPs located on the glucocorticoid receptors found in higher concentrations in patients who have obesity could imply that there is a potential risk that bariatric surgery will not be a successful treatment alone.

In order to evaluate the prevalence of potential variations in the DNA sequence or hypersensitive SNPs in the obese population, we analyzed four SNPs in particular. Two are located on the same locus, rs7901695 and rs12772424, each located on the TCF7L2 gene. TCF7L2 protein is a key transcriptional factor regulating gene expression, it is linked to type 2 diabetes, metabolic disorders, and homeostasis.<sup>22</sup> Variants of this gene confer potentially the greatest risk for the development of type 2 diabetes and impaired glucose intolerance, alongside psychiatric conditions, cancer, and small bowel diseases.<sup>22</sup> We hope to investigate whether our two SNPs will underlie a possible pathway for hypersensitivity to cortisol.

Another SNP we will be investigating is rs3753519, commonly referred to as HSD11B1. We chose this SNP because while we know excess cortisol can lead to central obesity, we learned a particular variance in the gene HSD11B1 has been associated with obesity and insulin resistance.<sup>23</sup> Many factors can influence insulin resistance including environmental and genetic, however cortisol levels have a huge impact on the ability to accept insulin.<sup>23</sup> As mentioned above, insulin resistance can ultimately lead to chronically elevated glucose levels, type 2 diabetes, and visceral obesity.

The last SNP we chose is rs1051052 found along the gene serpinA1. From literature we learned that specifically rs1051052 was associated with an elevated risk of obesity, diabetes, cardiomyopathy, and kidney disease.<sup>24</sup>

These could expose the vital roles cortisol and hypersensitive glucocorticoid receptors play in the success of an individual's weight loss management program. If supported, this would aid in guidance of which specific treatments of weight loss may correspond with their physical goals but also their genetic composition.

## **Methods:**

### *DNA Collection:*

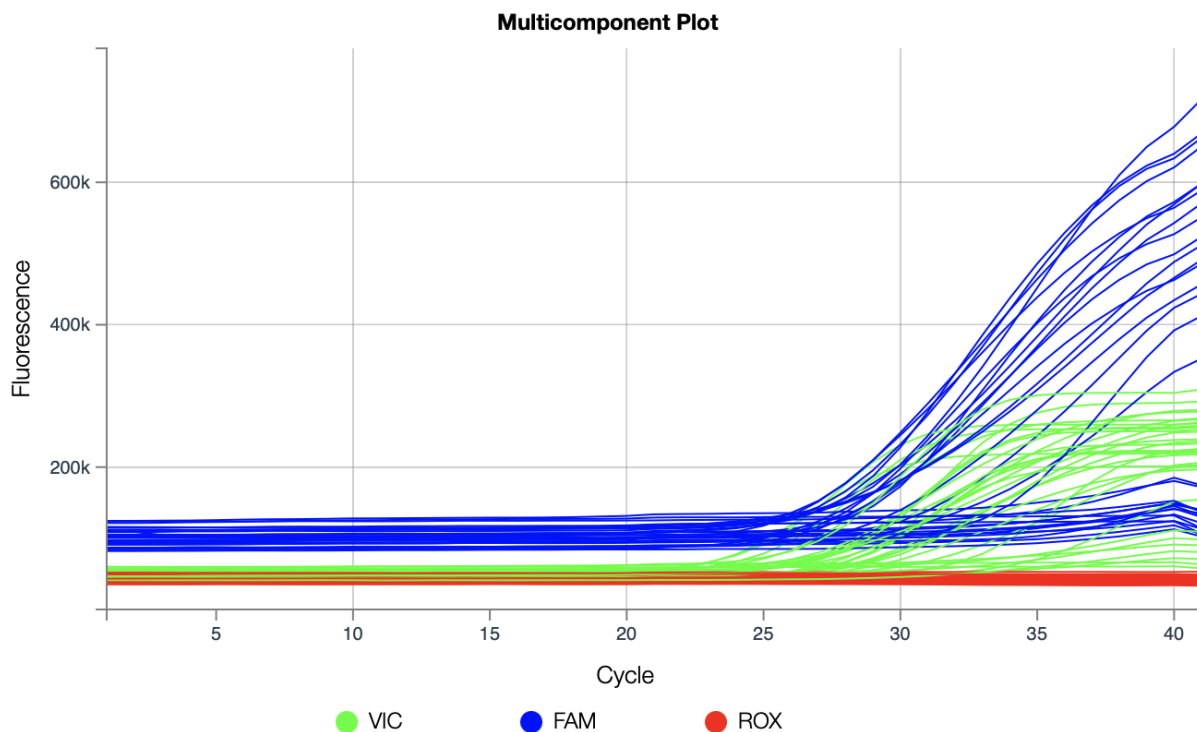
To test our hypotheses, previously collected DNA samples from our lab's collaboration with the bariatric program at Ellis Medicine Bariatric Care Center were analyzed through qPCR analysis. Past members of the lab obtained the DNA extractions by performing buccal swabs where patients cheeks were swabbed for 15-30 seconds. They stored the tips of the swabs in 1.5 µL tubes to preserve for future analysis. To extract the DNA, they added 600 uL of 50 mM NaOH to the tubes and vortexed for 10 minutes. The tubes were then heated at 95°C for 10 minutes. Following heating, 120 uL 1 M Tris (pH 8.0) was added to the tubes and the tips of the swabs were discarded. Extracted DNA was then stored at 4°C to be used in subsequent qPCR reactions.

### *Quantitative Polymerase Chain Reaction:*

qPCR or quantitative polymerase chain reaction, measures DNA amplification in real time with fluorescence measurements.<sup>25</sup> To run the reaction, a DNA template is combined with DNA polymerase, free deoxynucleotides (dNTPs), and specifically designed primers in order to amplify the target DNA sequences of interest for SNPs : rs7901695 , rs12772424, rs3753519, rs1051052. First, the amplified DNA is fluorescently labeled, the amount of fluorescence



released during amplification is directly proportional to the amount of amplified DNA.<sup>26</sup> To achieve the amplification measurements in real time, qPCR relies on a fluorometer to monitor the amount of fluorescence released through the entire reaction.<sup>26</sup> In our lab, the primers are designed to anneal to SNPs in the template and differentiate between genotypes, this way both primer sets can be added to the same DNA sample and the real time amplification differentiates between sequences being amplified.<sup>25</sup> Our results for qPCR in real time are displayed on an amplification plot with the measuring cycle values depicted on the x axis and fluorescence on the y axis. Threshold for the fluorescence is provided, and a positive result for the primer set is obtained once amplification exceeds the threshold.<sup>27</sup>



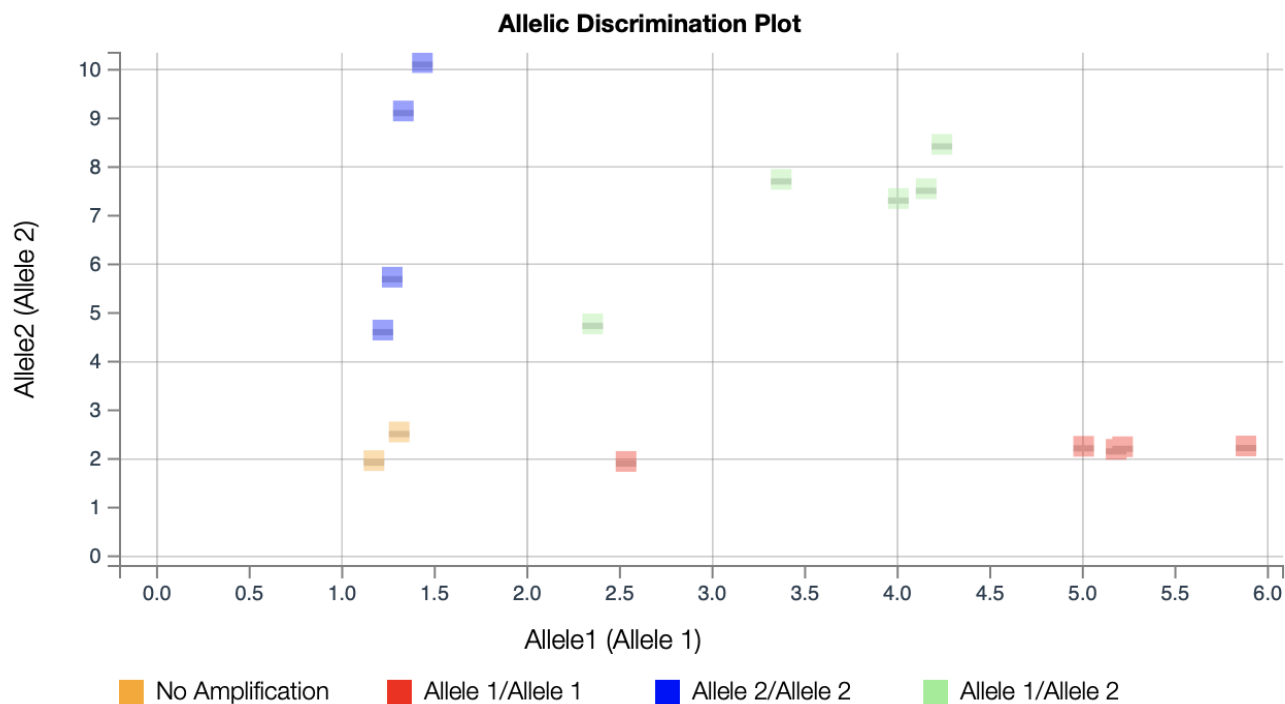
**Fig 6.** Example of qPCR amplification plot featuring SNP rs12772424 where cycle values are measured alongside fluorescence. A positive result for the primer set is obtained once the amplification meets the threshold value (~150k).

### *Data Analysis and Statistical Testing:*

JMP was used to compare genotypic frequencies of our bariatric population across all SNPs investigated. JMP allowed us to analyze the relationship between the genotype and the original weight, BMI, weight loss, ideal body weight, and excess body weight. JMP was also used to analyze the relationship between genotype and the success of bariatric surgery as measured by a percent of excess body weight lost. All relationships were identified using a significance criterion of  $p < 0.05$ .

### **Results:**

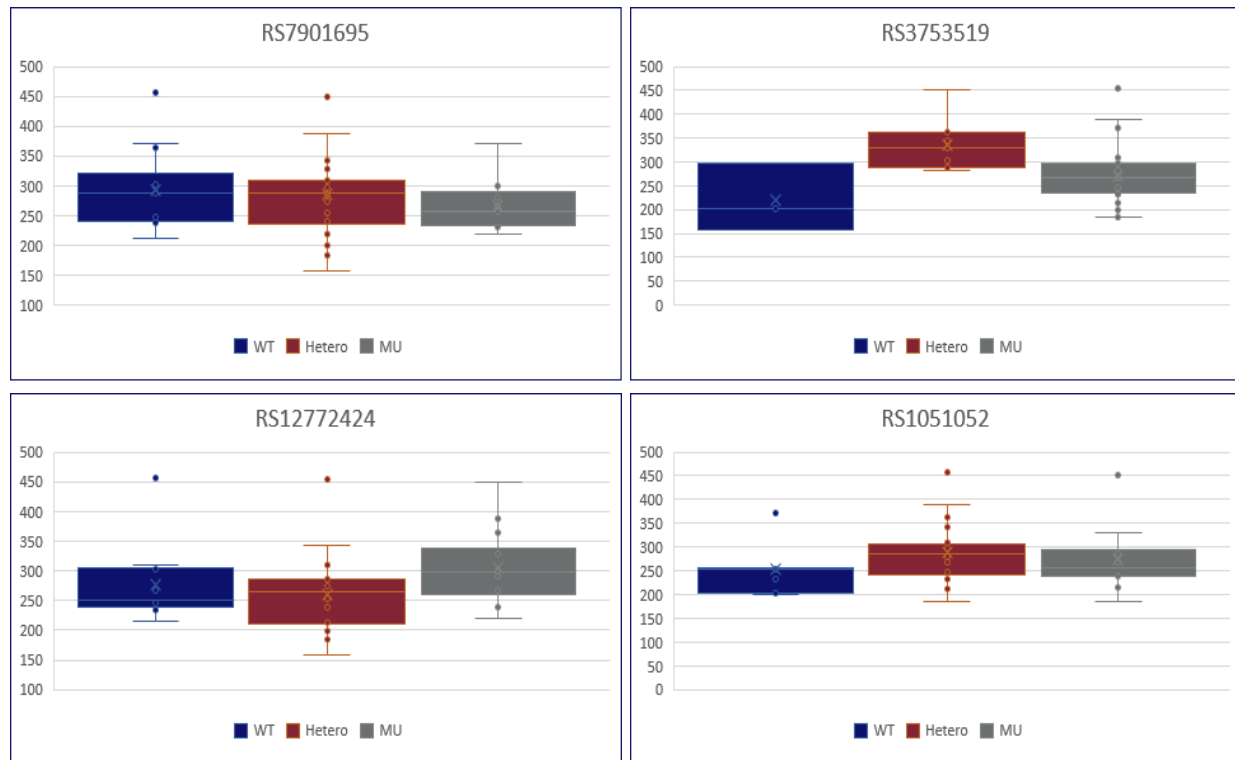
Polymorphisms for rs7901695, rs12772424, rs3753519, and rs1051052 were run through quantitative polymerase chain reaction. Figure 7 depicts a representative run for rs12772424 for 16 patients, each of their allelic frequencies are analyzed and categorized to determine their genotypes. A homozygous wild type genotype (WT) is represented by a blue square on the allelic discrimination plot. A homozygous mutant (MU) genotype is represented by a red square and a heterozygous genotype resembles a green square respectively. For the occasions where an orange square is present, these resemble the situations where no amplification was recorded. Through our experimentation with qPCR, these could represent our negative control, a faulty sample of DNA was used, or simply experimental error.



**Fig 7.** Patient samples analyzed through qPCR to analyze genotypes in rs12772424.

Genotypic data was extracted from qPCR allelic discrimination plots as described within the experimental approach. From this, allelic frequencies of the bariatric population were contrasted against the four SNPs investigated. The first subcategory that was analyzed within the bariatric population was their original weight prior to bariatric surgery. This information is insightful when deciphering further trends, such as excess body weight and excess body weight lost. It would be logical and expected to see a direct relationship between a greater original weight and a greater excess body weight and hopefully excess body weight lost. Within figure 8, specifically rs3753519, the heterozygous individuals had a significantly higher original weight than the mutant and wild type genotypes within that SNP. No other SNPs were found to have any other significant differences, however, within rs12772424 the mutant genotype was trending higher than both wild type and heterozygous genotypes. This could be attributed to a

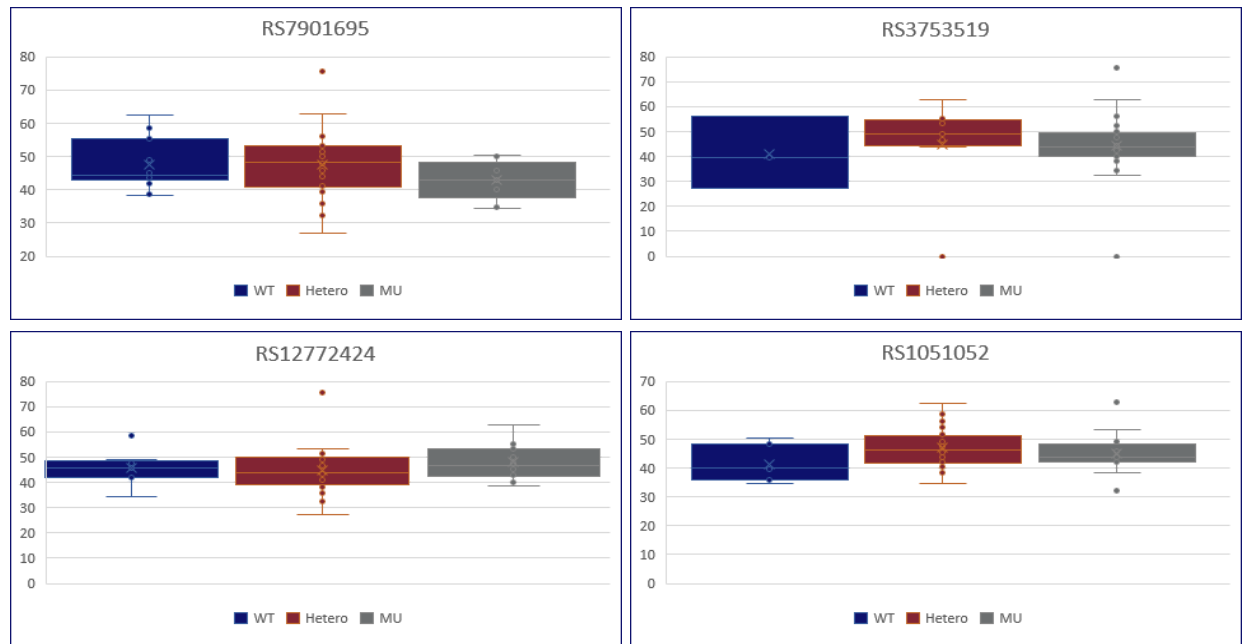
smaller sample size and it is predicted that in the future if more patients were included in the study, a significant finding may occur.



**Fig 8.** Original body weight allelic frequencies of the bariatric population contrasted against all SNPs investigated. The blue represents the WT genotypic population, the red represents the heterozygous genotypic population, and the grey represents the mutant genotype population for each SNP respectively.

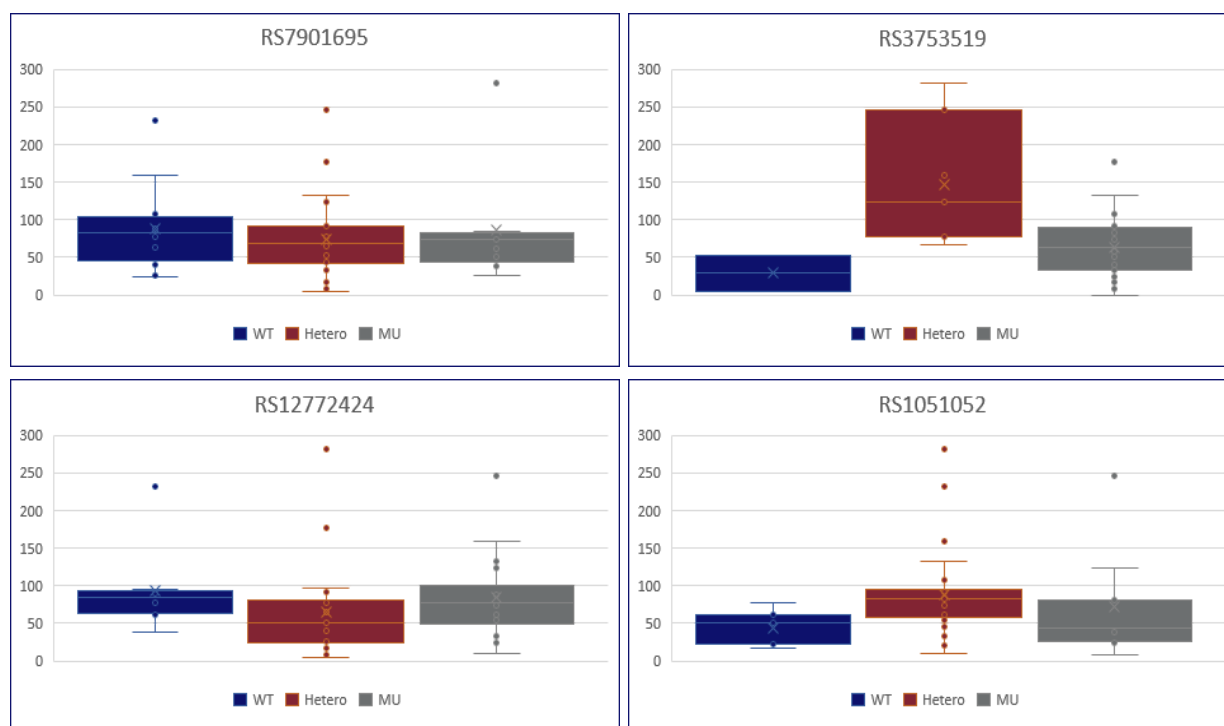
The next subcategory chosen to be analyzed within the bariatric population was their starting BMI prior to bariatric surgery. The reason this statistic was chosen in addition to original weight is because the original weight values were very dimensional once all experiments were completed and all data was collected. It was a great indication of whether patterns emerged abruptly or they were trending from the beginning of their weight loss surgery. Meaning, do these SNPs impact a patient's ability to lose excess body weight, or their risk of weight regain? However, BMI is crucial as obesity and the threshold for who qualifies for bariatric surgery procedures is measured and characterized through BMI. In figure 9, no early trends and no

significant correlations could be made since all calculated p-values were greater than 0.05.



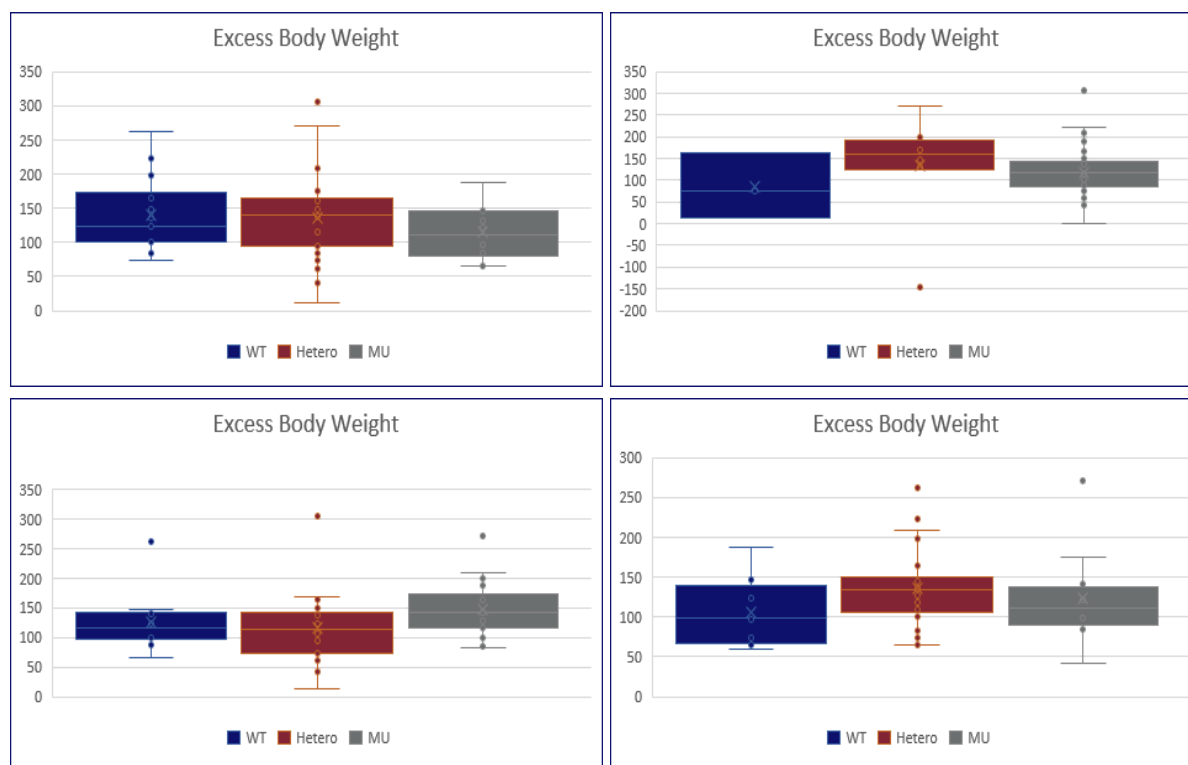
**Fig 9.** Allelic frequencies of the bariatric population categorized by BMI while contrasted against all SNPs investigated. The blue represents the WT genotypic population, the red represents the heterozygous genotypic population, and the grey represents the mutant genotype population for each SNP respectively.

Weight loss across the bariatric population was then analyzed amongst all SNPs. This subcategory is not directly relevant to our research as it is not clear what portion of the weight was excess body weight. Our research focuses on the holistic approach of the weight loss story of the bariatric population. To gain access to this information, we calculated the arbitrary ideal body weight of each individual through the height and body weight metric. In figure 10 of rs3753519, individuals of the heterozygous genotype had a significantly greater weight loss than mutant and wild type genotypes of that SNP. While not statistically relevant, the efforts of these individuals to better themselves and their lives is.



**Fig 10.** Allelic frequencies of the bariatric population categorized by weight loss while contrasted against all SNPs investigated. The blue represents the WT genotypic population, the red represents the heterozygous genotypic population, and the grey represents the mutant genotype population for each SNP respectively.

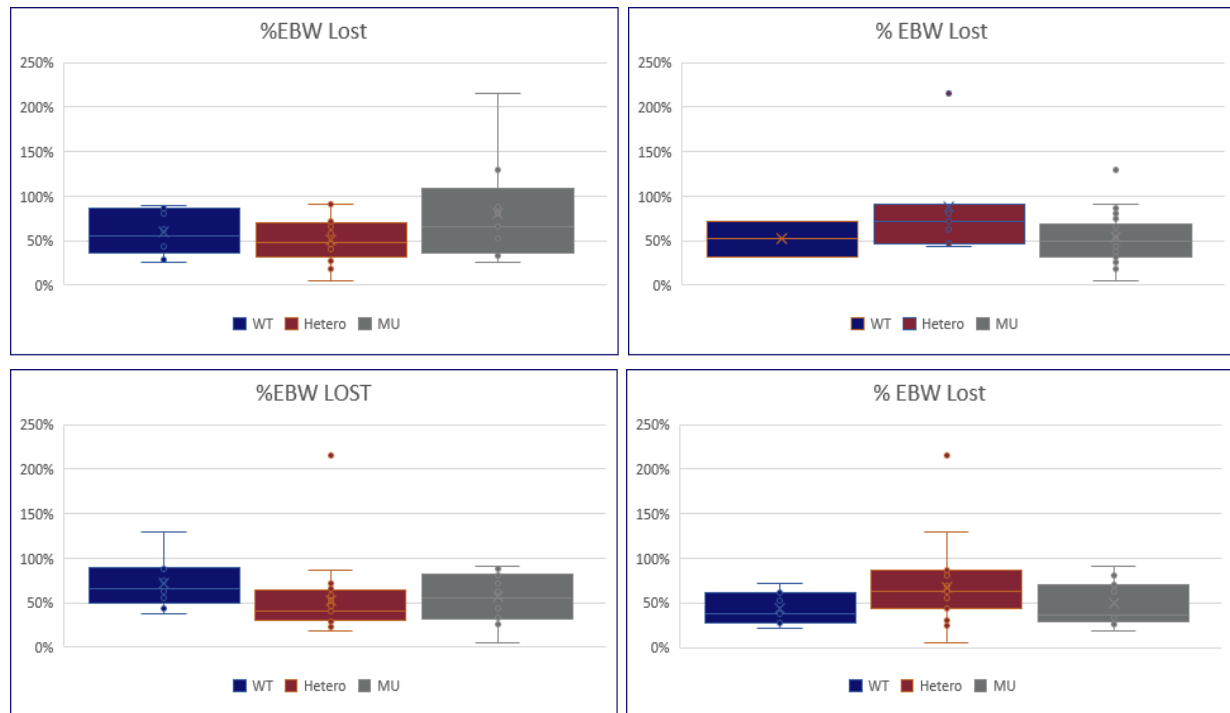
The portion of original weight considered excess body weight was calculated through the subtraction of the ideal body weight from original body weight. Ideal body weight was arbitrarily calculated through the height and BMI metric. As expected, in figure 11 of rs3753519, the heterozygous genotype had a significantly higher portion of body weight that was considered excess body weight. It is also important to note the trend within rs12772424 of the mutant genotype is trending to be significantly higher than both heterozygous and wildtype genotypes. While the p value is still greater than 0.05 and cannot be considered statistically significant, with the addition of more individuals in the study it would be expected to see another significant finding.



**Fig 11.** Allelic frequencies of the bariatric population categorized by excess body weight while contrasted against all SNPs investigated. The blue represents the WT genotypic population, the red represents the heterozygous genotypic population, and the grey represents the mutant genotype population for each SNP respectively.

The last sub category investigated is percent excess body weight lost (%EBW Lost), this data was derived from what portion of their bodies were excess body weight, which was calculated from the ideal body weight metric subtracted from original weight, which stimulated this research by categorizing the weight of these individuals as obese from the BMI standard. As seen in figure 12, the percent of excess body weight lost by patients tested for each SNP was compared to the prevalence of genotypes identified for each SNP. As seen in previous data, the heterozygous individuals of the rs3753519 genotype lost a significantly greater percent of their excess body weight than mutant or wild type genotypes of that SNP. This trend is consistent with original weight, weight loss, and excess body weight, meaning, individuals with greater starting weights would have the opportunity and logically should lose more weight than

individuals who have a lower starting weight. Interestingly, in rs12772424 the wild type genotype is trending towards being statistically significant, this trend is opposite of what was demonstrated in previous data. However, with the addition of more individuals in the study it would be expected to see another significant finding.



**Fig 12.** Allelic frequencies of the bariatric population categorized by %EBW Lost while contrasted against all SNPs investigated. The blue represents the WT genotypic population, the red represents the heterozygous genotypic population, and the grey represents the mutant genotype population for each SNP respectively.

## **Discussion:**

The goal of my senior thesis research project was to investigate the impact of single nucleotide polymorphisms on cortisol receptor activity in populations with obesity. Our goal was to determine how the prevalence of these SNPs may predispose individuals to having chronic obesity and how they can be treated efficiently to maximize their weight loss goals,



maximize success rates of bariatric surgery, improve their physical as well as mental health, and maintain these results to live a better quality of life. I hypothesized that single nucleotide polymorphisms associated with cortisol hypersensitivity are negatively associated with the success of bariatric surgery. We inferred this because genetic factors cannot be completely overcome by the process of a physical surgery. Meaning, despite the initial success of the surgery, if an individual has unaddressed excess cortisol or cortisol hypersensitivity this success will not be long term. Therefore, the presence of cortisol hypersensitive SNPs located on the glucocorticoid receptors found in higher concentrations in patients who have obesity could imply that there is a potential risk that bariatric surgery will not be a successful treatment alone.

After careful analysis of each SNP and their role in original weight, BMI, excess body weight, and excess body weight loss, we unfortunately did not find significance within three of our SNPs, rs7901695, rs12772424, and rs1051052. With rs7901695 and rs12772424, literature suggested a link from their corresponding gene towards the development of type 2 diabetes, metabolic disorders, and defective homeostasis.<sup>22</sup> This could be attributed to their loci, TCF7L2, a key protein transcriptional factor known to regulate gene expression. Concerning rs1051052, this SNP was known to be associated with an elevated risk of obesity, diabetes, cardiomyopathy, and kidney disease.<sup>24</sup> Support from these SNPs could have exemplified the vital roles cortisol and hypersensitive glucocorticoid receptors play in the success of an individual's weight loss management program.

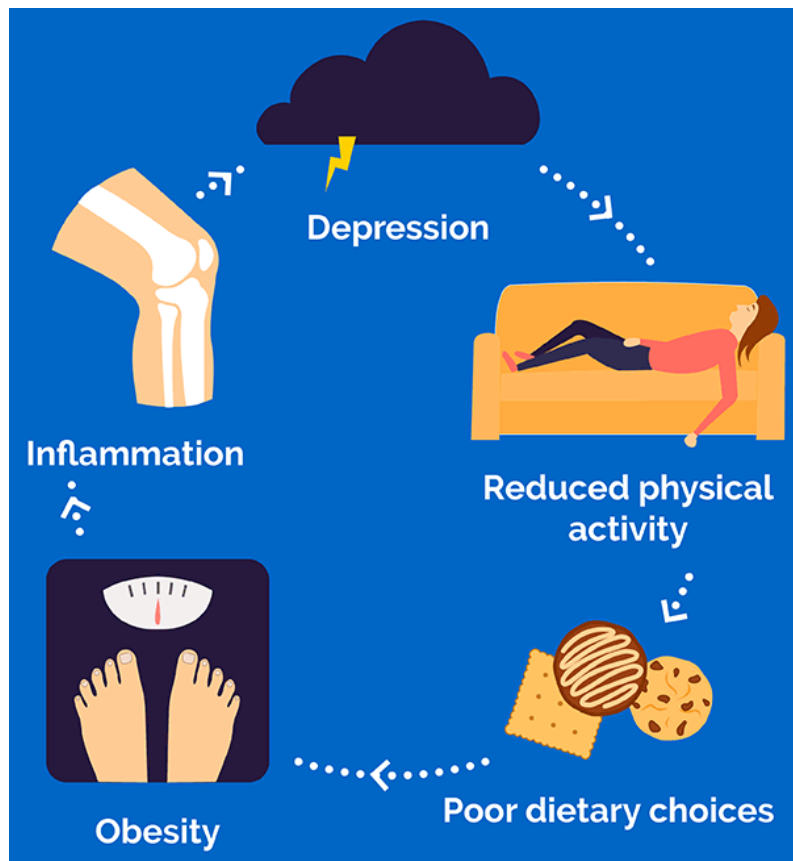
We did not anticipate to not obtain any significance through these SNPs, however, we must attribute some possible experimental error alongside our smaller sample size as we only have roughly 50 DNA samples of sufficient data for each SNP. If we were to continue this

research, I believe it would be worthwhile to gather more DNA buccal samples from Ellis Bariatric Center to ensure the samples are fresh and not degraded over time.

Fortunately, we found significance with one of our SNPs, rs3753519. This means a SNP in the HSD11B1 gene is associated with a difference in excess body weight loss, this suggests the variation of this gene may affect the outcome of bariatric surgery. We did anticipate this SNP to show significance through analysis, as through literature we learned a particular variance in the gene had been associated with obesity and insulin resistance.<sup>23</sup> As mentioned above, insulin resistance can ultimately lead to chronically elevated glucose levels, type 2 diabetes, and visceral obesity. What we did not expect was that it was the heterozygous genotype of the population that was significantly different compared to the wild type and mutant genotypes. This was unusual because we predicted if the gene had any association with obesity we would expect the mutant genotype to be the most significant as there would be two copies of the affected gene.

Since our goal was to expose the vital roles cortisol and hypersensitive glucocorticoid receptors play in the success of an individual's weight loss management program, through the success of rs3753519 of the HSD11B1 receptor, this can aid the guidance of which specific treatments of weight loss may correspond with physical alongside genetic composition of those who suffer from obesity. To best understand what methods of treatment would best serve each individual through their weight loss journey, it is important to acknowledge why bariatric surgery alone may fail. While the immediate weight loss for these individuals is a remarkable accomplishment, too often, this transformation is not long term and can lead them to falling into a negative cycle we commonly refer to as depress-obesity.

Obesity is a risk factor for depression, especially in women, and consequently, depression is a risk factor for obesity most commonly seen in males.<sup>28</sup> The link and comorbidities accustomed to both diseases, specifically evident within bariatric programs can accrue from several reasons, including failure of weight maintenance, poor mental health pre-surgery, lack of support from medical providers, and lack of acceptance from society.



**Fig 13.** Viscous cycle referred to as depress-obesity.<sup>29</sup>

From our results, we can deduce cortisol had a great impact on the depression-obesity negative cycle as detailed in figure 13. This is because when individuals have chronically elevated cortisol in their bloodstream or their glucocorticoid receptors are highly sensitive to cortisol, the body reacts as if levels are increasingly elevated despite being in normal range. Despite bariatric surgery removing a large portion of the excess body weight that excess cortisol

added to their bodies, genotypically they are still identical. The elevated cortisol response is still present within each of their bodies as bariatric surgery alone cannot reduce or impact their cortisol levels or response. This means the risk of phenotypic regression, or failing to maintain their weight loss is very high for these individuals and will be until the issue concerning cortisol and the body's reaction is treated accordingly.

From this we can emphasize the importance of a holistic approach to treating obesity at the root. For the individuals who qualify for bariatric surgery, these results exemplify the importance of DNA tests and pre-screening of each SNPs and their respective genotypes are present in the body. This would allow us to know who is pre-dispositioned not only to developing obesity, but also at risk for failure of weight maintenance after bariatric surgery. This information would give providers a head start on the treatment plan, specifically stating the importance of not only following an appropriate diet plan for each individual, but also encouraging regular physical activity, ensuring their state of mental health is in high spirits and if it falters, they have the resources to adjust and heal accordingly. Most importantly, the villain of this story, cortisol must be addressed.

Currently, there are several medications available for treating elevated cortisol response from the body. These include but are not limited to stopping the production at the zona fasciculata layer of the adrenal gland or blocking the glucocorticoid receptors from receiving the hormone itself. This is exceptionally important as these medications could result in aiding the comorbidities associated with obesity and or elevated cortisol levels like type 2 diabetes, hypertension, dyslipidemia, and insulin resistance, but also depression, anxiety, poor sleep quality, and inflammation.

And this speaks volumes as to why continuing this research is so crucial. To continue identifying SNPs that are associated with obesity, so we know how to best treat individuals in the future who are pre-dispositioned to developing obesity and who ultimately will continue to suffer until projects like these are able to accumulate enough to make a real difference. Despite how small of a difference this project makes in the grand scheme of medicine, this is what medicine and research is about, answering questions and to hopefully improve the quality of life of others.

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