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Physiological effects of activity-based anorexia in female rats and an overview of eating disorders

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PHYSIOLOGICAL EFFECTS OF ACTIVITY-BASED ANOREXIA IN FEMALE RATS AND AN OVERVIEW OF EATING DISORDERS

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by

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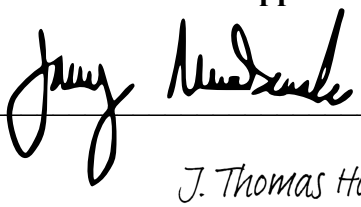

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ABSTRACT:

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PHYSIOLOGICAL EFFECTS OF ACTIVITY-BASED ANOREXIA IN FEMALE RATS AND AN OVERVIEW OF EATING DISORDERS

The aim of this thesis is to bring AN to the foreground of conversation both in a scientific and sociological framework. Nearly 40 million Americans suffer from anxiety disorders, which is characterized by the feeling of a loss of control. In some cases, another disorder called anorexia nervosa (AN) can codevelop. AN is characterized by a refusal, and inability, to maintain a healthy body weight. Some suffering from anxiety may restrict caloric intake and increase exercise to cope with stress. This results in e*xtreme* caloric deprivation. AN can be modeled in rats using an activity-based anorexia (ABA) method. In this study, we sorted 32 adolescent female rats into four main groups. One group had no running wheel and full food access (sedentary), one had a running wheel and full food access (exercise), one had no running wheel but restricted food access (chronic food restricted), and one had a running wheel and restricted food access (ABA). Over a period of two-weeks, the ABA rats reached the anorexic phenotype. From there, we will investigate the possible neural mechanisms behind anorexia by looking at two specific populations of neurons in the brainstem: glucagon-like peptide 1 and prolactin-releasing peptide. There is evidence that societal factors can trigger AN to form such as social media; however, the exact causes of AN are not well understood which makes its treatment very difficult within humans. By better understanding possible neural mechanisms that contribute to AN, we can more holistically treat patients suffering with it.

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PREFACE AND ACKNOWLEDGEMENTS:

As a fourth grader, the only concern I can vividly remember was having to memorize the capitals of all fifty states. Happening upon quicksand might also have been a concern, but that was only because I had watched *The Princess Bride* around that age. Other than those two immediate threats, I lived a pretty stress-free life. The same cannot be said for another girl in my class. Around the fourth grade she developed anxiety. This was not the acute anxiety I felt when I blanked on the capital of Delaware, this was chronic, debilitating anxiety. She would wake up with little energy and yet she came to school every day with a smile on her face. Soon, her anxiety disorder developed into an eating disorder: anorexia. By the seventh grade she was 5'8 and weighed less than 110 pounds. Her muscles atrophied and her body developed lanugo-like hair. In the ninth grade she began a five-year battle with shin splints that bother her to this day due to the low bone density caused by her period of malnutrition. The signs, the problems, the disorder were so clear to see, and yet I failed to be there when she needed me most.

I did not know of any of her struggles until she wrote me a letter. She told me of her disorders, and she asked me not to tell anyone. I did not understand what she meant by "anorexia" and so when my mom found out from her mom, I did not know why she was so serious about it. My mom reassured me that she was getting help, but I truly did not know what "anxiety" or "anorexia" meant until my sophomore year of high school in human anatomy. We learned about the effects of anxiety on the body and how eating disorders can have long term consequences. I can remember feeling my heart begin to pound as the blood rushed from my brain. Anxiety. Anorexia. Two very real and very scary realities my best friend had been facing starting in the fourth grade.

My interest in nutrition and mental health started because of my friend's experience. I wrote my high school thesis on the "epidemic" of mental health disorders with nutrition being one of the causes for the apparent increase. My interest in holistic medicine began after I saw my friend take pill after pill for years with no improvement to her anxiety. I did one quick Google search and found that caffeine should be limited in people experiencing anxiety because of its physiological effects. My friend drank coffee every morning and no doctor ever told her to stay away from caffeine. My interest in Dr. Maniscalco's research had its origins in my friend's inability to overcome anorexia. When I heard about Dr. M's research from Dr. Fricks-Gleason I could not help but walk into his office on the first day of freshman year and ask to do research with him.

What affects our lives, what shapes who we become, is not always what we may think. I would never have thought that the hardships of a twelve-year-old girl who I just happened to go to school with, and who, by chance, was my best friend since kindergarten would impact my senior thesis for both high school and college.

Bernd Heinrich wrote, "We are not just the product of our genes. We are also the product of ideas" (Life Everlasting, 261). I am a firm believer that I am who I am because of those are around me. The encouragement, support, and knowledge I have been surrounded by has molded me into the person I am today, and I do not have the words to express how much I value that. I would like to thank Dr. Maniscalco for his guidance, high expectations, and willingness to let me be a part of his research. What I have learned from him goes far beyond how to perfuse a rat. I would also like to thank my reader, Dr. Howe, for always making the time to talk to me. Thank you to the Neuroscience Department and staff at Regis University for allowing me the incredible experience of conducting research. I would not have the necessary knowledge and skills to

complete this research without your continued pursuit of excellence. Thank you to the University Research and Scholarship Committee at Regis University for their funding which helped make this research possible. Thank you to the Honors Department, specifically Dr. Narcissi and Dr. Schrier, for your continued dedication to the advancement of the program. My most challenging classes have come from this program, but they have also been my most formative. Finally, thank you to my family, without whom I would not be where I am today.

CHAPTER I: Anorexia Nervosa

Anxiety-based disorders affect about 18.1% of the United States population each year which makes them the most common mental illnesses in the country (ADAA, 2021). The prevalence of anxiety-based disorders now seems, at first glance, to be more common than in previous decades. Findings have shown, however, that there are in fact no significant increases or decreases in the prevalence of mental health disorders such as anxiety (Bandelow & Michaelis, 2015). Despite there being no increase in the overall number of people who have anxiety disorders, the number of people who seek active treatment or are more willing to talk about them has increased. It is likely that cultural, societal, and personal changes have taken away some of the negative stigma surrounding mental health, making it more acceptable to talk about from both a societal and medical standpoint. The issue, however, is that despite being the most diagnosed mental illness, anxiety-based disorders are still often underrecognized and undertreated (Bandelow & Michaelis, 2015). The other problem is that anxiety-based disorders often begin in adolescence (10-19 years old) with some types beginning as early as 7 years old. (Besedo et al., 2010; Lijster et al., 2017). Anxiety disorders are the leading cause of health-related disability in children and their development of them in adolescence has long lasting effects throughout their life (Kieling at al., 2011).

Anxiety-based disorders can take the form of five major types of disorders: Generalized Anxiety Disorder, Obsessive-Compulsive Disorder, Panic Disorder, Post-Traumatic Stress Disorder, and Social Anxiety Disorder (National Institute of Mental Health, 2021). Researchers and medical personnel do not know what the *exact* cause is for developing anxiety disorders, but like other forms of mental illness, they do not form due to personal weaknesses. This means that anxiety disorders do not form because of an “error” in their genetics or a flaw in their character.

Some known causes have roots in chemical imbalances, environmental factors, or genetic heritability (Cleveland Clinic, 2021). Anxiety disorders can make people feel out of control, irritable, fearful, stressed, worried, or confused among other symptoms in a chronic or unmanageable fashion (National Institute of Mental Health, 2021).

An issue arises when one looks at the ages of patients who are diagnosed with anxiety disorders. A majority of those with anxiety-based disorders are anywhere from 15-21 years old. This age range is generally when individuals are going through high school and college. These developmental periods often foster lifestyles that promote feelings of anxiety on an everyday basis. The pressure of performing well in classes, achieving good grades, building a social life, and even just dealing with peers can all be anxiety inducing. It is easy to see, then, how anxiety-based disorders can be misdiagnosed or not effectively explained. There are no special tests or scans that can detect an anxiety disorder which can cause doctors to undertreat patients who do not understand the extent of the difference between normal and debilitating anxiety (Cleveland Clinic, 2021). Since anxiety disorders can be undertreated, it is common for patients to have other disorders co-occur when chronic anxiety is present. Specifically, it is common for young females who suffer from anxiety in some form to develop an eating disorder (Lavender et al., 2013).

An individual with an anxiety disorder often feels as if they have little to no control in their life. This feeling of powerlessness exasperates the stressful emotions of people who have anxiety because they also commonly present with the need to have complete control in their life. Interestingly, and frighteningly, the ability to control day-to-day food intake can provide needed feelings of power and control when things feel unmanageable. Eating disorders generally manifest themselves in three distinct types: anorexia nervosa, bulimia nervosa, and binge-eating

disorder. AN has the second highest mortality rate of any mental health disorder, second only to opioid overdose, due to severe complications to health as a result of malnutrition (Arcelus, 2011). Many die from AN itself, but there are others who die from suicide because of the severe symptoms that have lifelong effects (National Institute of Mental Health, 2021). Bulimia nervosa (BN) is characterized by overeating which is then followed by behaviors that compensate for the overeating by vomiting, excessive exercise, or the use of laxatives (National Institute of Mental Health). People with BN can be underweight, normal weight, or overweight. Binge-eating disorder is the most common eating disorder in the United States and is characterized by the loss of control of eating without being followed by purging (National Institute of Mental Health, 2021).

While each eating disorder is problematic and very serious, AN produces extremely high mortality rates. The confusing part about this high mortality is that one can often tell when an individual has AN. Patients look emaciated, their muscles atrophy and in females they develop irregular or non-existent menstrual cycles. Nonetheless, more times than not, individuals who develop AN do not get help until it is too late, or the habit of decreasing food intake becomes a neurological habit (Recovery Lighthouse, 2021; Foerde et al., 2015). AN is extremely hard to recover from due to the solidification of neural connections that form the habit of not eating, which contributes to the high mortality rate as well as the high suicide rate. For this reason, AN is especially interesting to better understand because it appears to be against human nature to starve oneself.

In addition to a severe reduction of caloric intake, individuals with AN also have a tendency to excessively exercise (Caspar, 1998). These two actions coupled together lead to major weight loss when AN persists over chronic periods of time. Part of the reason why AN

develops and is maintained is because periods of food restriction can reduce anxiety (Zerwas et al., 2013), an effect that is also achieved through exercise (Caspar, 1998). There are also social implications such as societal pressures that can contribute to the persistence of AN (Bordo, 2020). All mentioned causes are relatively understood and accepted regarding why individuals develop AN in chronic ways, but there may be more basic changes in neural signaling that contribute to the reduction of stress when caloric intake is decreased, and exercise is increased. These could be alterations to the way the brain processes stressors and threatening environmental stimuli. Findings by Foerde et al. (2015) identified changes in neural connections that help explain why AN is hard to recover from, as the formation of habits cause a patient to fall into anorexia-like behaviors unintentionally. If the struggle of recovery from AN has neurological ties, then it is not a large jump in logic to believe there could be neurological reasons why patients maintain AN. My thesis aimed to better understand the neurobiological mechanisms underlying the anxiety-reducing effects of chronic food restriction in rodents.

CHAPTER II: Stress

In order to understand how the persistence of AN in individuals may have neurological causes, it is important to understand how organisms respond to stress. Stress is defined as a state of real or perceived threat to homeostasis (Smith & Vale, 2006). When a stressor is sensed by an organism, the nerves throughout the body send that stimulus to the brain. The brain then recognizes that message and activates a stress response. There are two systems that mediate stress responses: the nervous system, and the endocrine system.

To understand how the nervous system works, I will give the example of a dog that starts to chase you. Your eyes perceive the dog starting to charge you and that message is sent to the brain. Once the brain and spinal cord recognize the message from the stressor, it activates the nervous system in a series of steps. The first step is the brain sends a message to the peripheral nervous system (cranial and spinal nerves). The peripheral nervous system then stimulates efferent effectors to transmit the message to muscles and glands (Moyes & Schulte, 2016). That message can either go to the autonomic nervous system (ANS) or the somatic nervous system. The ANS is in charge of involuntary functions such as your heart rate while the somatic nervous system deals with voluntary functions such as moving your hand. Thus, the message regarding the stressor goes to both the ANS and the somatic nervous system. You start to run away (somatic nervous system response) but to maintain enough energy and alertness to stay ahead of the dog, your body needs to go through some internal changes as well (ANS response). The ANS is broken up into two more separate systems. The parasympathetic nervous system (PNS) conserves energy and promotes housekeeping functions such as growth, digestions, reproduction, and immune response (Moyes & Schulte, 2016). The sympathetic nervous system (SNS) mobilizes the body so that it can respond through fight or flight.

The SNS and the PNS have opposite responses in the body. For example, the SNS dilates your pupils so that you can see more, while the PNS constricts your pupils because you are in a state of rest. Your SNS increases cardiovascular activity so that you can run away, while the PNS decreases your heart rate (Moyes & Schulte, 2016). Since you are currently in survival mode, the message from the brain activates the SNS and deactivates the PNS. This is because your main concern is to get away from the dog. In the moment of running from the dog, using energy to digest the food in your stomach takes away from the energy you need to run away. Thus, the SNS is activated to increase glucose, epinephrine, and your heart rate and decrease reproduction, immunity, and digestion. It takes seconds for the message from the perceptions of the stressor to reach the adrenal medulla to stimulate the release of epinephrine into the blood stream.

The endocrine system increases heart rate and glucose levels like the nervous system does, but instead of using the spinal cord, the message from the brain stem gets transferred via hormones and neurohormones (Moyes & Schulte, 2016). One system by which hormones can be transferred is through the hypothalamic-pituitary adrenal axis (HPA). The hypothalamus receives the message from the brain stem and secretes corticotropin-releasing hormone (CRH). CRH travels through the blood vessels that connect the hypothalamus to the anterior pituitary. The anterior pituitary secretes adrenocorticotropic hormone (ACTH). ACTH travels through the blood to the adrenal cortex. The adrenal cortex then releases cortisol into the blood stream where it stimulates target cells to increase blood glucose. The increase in blood glucose enables muscles to convert more sugar into energy which gives you the ability to run from the dog. The hypothalamic-pituitary adrenal axis works in a matter of minutes which is slower acting than the nervous system (Moyes & Schulte, 2016).

Why do the stress response mechanisms matter to people who have anxiety and anorexia nervosa? People who have anxiety are constantly under a lot of stress. As described earlier, they can often feel as if they do not have control in their own lives which is very stressful even if you are not someone who needs to feel total control. That chronic stress keeps both the SNS and HPA axis activated. If the body is constantly in a state of unrest (fight or flight), developing a way to decrease feelings of stress is advantageous for the individual's body. For some individuals with anxiety, having total control over what you can put into your body decreases stress. Once anorexia-like behaviors develop, the body may feel less stress at first and is able to return to the restful state by activating the PNS. Individuals with AN have more anxiety towards eating compared to healthy individuals (Steinglass et al., 2010); therefore, once a person starts decreasing their food intake in order to feel more control, eating becomes a stressor. At some point, the starving of oneself causes more stress on the body, but it is easy to see why decreasing food intake appears to be beneficial in the short run for someone who has an anxiety disorder.

In comparison to humans, rodents display a similar reduction of anxiety-like behavior when deprived of food (Genn et al., 2003; Heiderstadt et al., 2003; Maniscalco & Rinaman, 2013). Periods of caloric deficit significantly decrease responses to acute stress, specifically the deactivation of the neuroendocrine HPA axis in rodent models (Maniscalco & Rinaman, 2013). This correlation may indicate that there are underlying metabolically tuned neural substrates that receive feedback signals in response to energy levels (Maniscalco & Rinaman, 2013). The neural reasons behind why this is observed are not well understood. Dr. Maniscalco has conducted studies examining two types of neurons, GLP-1 and PrRP, as possible metabolic tuners in the system that decreases stress following decreased caloric intake.

CHAPTER III: GLP-1 and PrRP Neurons

Glucagon-like peptide 1 (GLP-1) is a thirty amino acid peptide hormone (Holst, 2007). It is produced by processing of the proglucagon gene in the intestinal epithelial endocrine L-cells (Holst, 2007). GLP-1 is released when there is food intake, and it is rapidly metabolized by an enzyme before the hormone leaves the gut (Holst, 2007). The proglucagon gene is also found in the nucleus of the solitary tract of the brain stem in a number of organisms, in particular rats and humans (Holst, 2007; Maniscalco & Rinaman, 2017). The gene codes for a neurotransmitter that is released in many different areas of the brain, including the nucleus of the hypothalamus (Holst, 2007), where it can elicit release of corticotropin-releasing hormone (CRH) from the hypothalamus as part of the HPA axis stress response (Ghosal, Myers & Herman, 2014).

Prolactin-releasing peptide (PrRP) is a twenty amino acid member of the phenylalanine family (Brunton & Russell, 2015). It stimulates prolactin release both *in vitro* and *in vivo* (Maruyama et al., 2001). Prolactin is a hormone that is made by the pituitary gland and stimulates milk production after a baby has been born. PrRP-producing cells can be found in the dorsomedial hypothalamic nucleus. The PrRP neurons extend into the paraventricular hypothalamic nucleus where PrRP receptors can be found. This indicates that PrRP plays an important role in the neuroendocrine system namely by stimulating ACTH via CRH which is also a part of the HPA axis that is used in stress response (Maruyama et al.).

Both GLP-1& PrRP neurons are present in the caudal brainstem (Maniscalco & Rinaman, 2017). The caudal brainstem nucleus is the initial central nervous system terminus for gut peptide signals as well as gastrointestinal mechanical signals which work to control food intake limits while eating (Schwartz, 2006). Both are also stress-related neurons that play important roles in the HPA axis. By focusing on these two specific neural groups in the brainstem of rats,

we can experimentally determine if chronically decreasing food intake also decreases their activation. The hypothesis is that the chronic food restriction will decrease their neural activation because food intake is not high enough to stimulate the neurons to limit consumption. If the neurons are not stimulated, they will not "trigger" a response within the HPA axis which will, in essence, prevent a stress response from occurring. Male rats who were overnight fasted for twenty-four hours showed a significant decrease in activation of both GLP-1 and PrRP neurons of the brainstem (Maniscalco, Zheng, Gordon & Rinaman, 2015). The reason behind focusing on these two specific neurons within the brainstem is to see if the same results occur in chronically food restricted animals and those modelling anorexia. Due to the unique connection GLP-1 and PrRP have with both the gastrointestinal system and stress response, they are ideal neurons to focus on for this specific study.

It is important to mention that rats are not humans. While that is an obvious statement, it is crucial to recognize in order to understand the relevancy of this study. Rats share a lot of neural structure and neural connectivity with humans which makes them a good model for neuroscience studies (Institute of Medicine, 2012). The GLP-1 and PrRP neurons within rats are found in the same region as they are found in humans (Holt & Rinaman, 2021). Rats also exhibit anorexia-like phenotypes (the activity-based anorexia model) which makes them useful in foundational studies where human clinical relevance is the future goal.

CHAPTER IV: Methods

Just like any plan in life, what we went in thinking we would do for the experiment changed as time went on. The original plan was to slowly build upon Dr. Maniscalco's previous research. This meant that this study was originally designed as a bridge between what he had found previously in overnight fasted animals and what we were hoping to find in chronically food restricted animals. The reason behind this was to create a more solid foundation of support before we moved to models that resembled more human forms of AN. I find it important to recount our original general methods as they do help to better understand how prior research done with the GLP-1 and PrRP neurons prior to this study relates to why they are also important in an AN based model.

The original plan was to have two groups of male Sprague-Dawley rats. One group would be fed *ad libitim* for two weeks while the other group would undergo chronic food restriction (CFR) for two weeks. The food in the CFR group would be reduced so that the rats maintained 80% of their baseline body weight. After this, half of the rats from each group would be exposed to stress by being placed on an elevated platform for five minutes (this is stressful for rats because they are a prey species, and being isolated in an open, exposed environment increases their risk of predation). The other half of the rats from each group would remain in their home cages, as they represented nonhandled controls. After the animals were either stressed or not stressed, they would be sacrificed, and their brains would be preserved. Immunohistochemistry would be performed for cFos (a marker for neural activity) within GLP-1 and PrRP neurons and brightfield microscopy would be used to image brain sections and quantify percentages of GLP-1 and PrRP neurons which became activated by the stressor to display cFos.

After a couple of months of running a trial experiment, we began to question why we needed a "bridge" study at all. The research Dr. Maniscalco had done previously was only related to the AN model we were interested in with regards to the neurons we wanted to focus on. Doing the original method would have provided important information to build later studies on, but the real interest for the both of us was how anorexia could be maintained in a group that most accurately reflected the demographic of people who have AN: adolescent females. We then decided to shift to an activity-based anorexia model with young female rats. Most of the original procedure was kept the same, but some other variables were added so that the results would be more applicable to what is observed in patients who have AN. This change in methods allowed us to better replicate how anorexia presents itself in human individuals without taking too big of a leap that prior studies could not support it.

The new method required two groups of sixteen female rats each. One group was *ad libitim* fed while the other was chronically food restricted to up to 75% of their baseline body weight. Those two groups were then divided into two subgroups. One subgroup had access to a running wheel, while the other group did not (Figure 1). In total, there were four distinct groups: activity-based anorexia (ABA), chronic food restricted (CFR), exercise (EX), and sedentary (SED). ABA rats (n=8) had access to a running wheel and only had access to food for 1.5 hours a day. CFR rats (n=8) did not have access to a running wheel and only had access to food for 1.5 hours a day. EX rats had access to a running wheel and were fed *ad libitum.* SED rats did not have access to a running wheel and were also fed *ad libitum* (Figure 2).

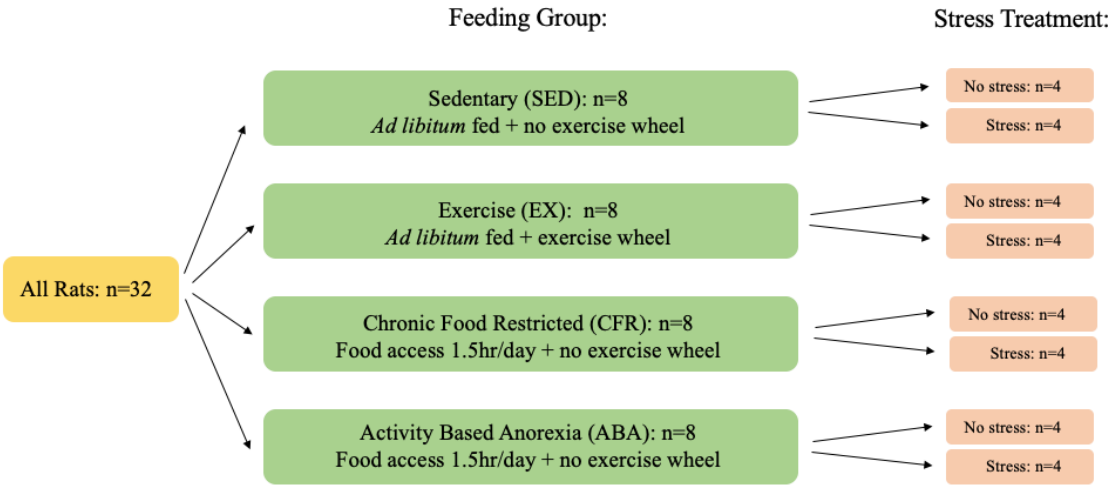

Figure 1: Feeding groups and stress treatment for rats.

There were three phases within the experiment (Figure 2). The first one was colony acclimatation. This phase lasted seven days, although we only collected quantitative data on four of those seven days. This is because we elected to do a liquid diet (to make measuring the amount of food the rats consumed easier) and the supplies needed to hold the liquid diet came in late. We did not want to put any of the rats on a chow diet as that would require some time of liquid-diet acclimatization after the supplies came in. Thus, we had to pair-house the rats and could not collect data when they were together. Pair-housing decreases the level of stress rats feel which was helpful within the colony acclimation period since the rats were under a lot of stress due to moving to their new environment (Westenbroek, 2005).

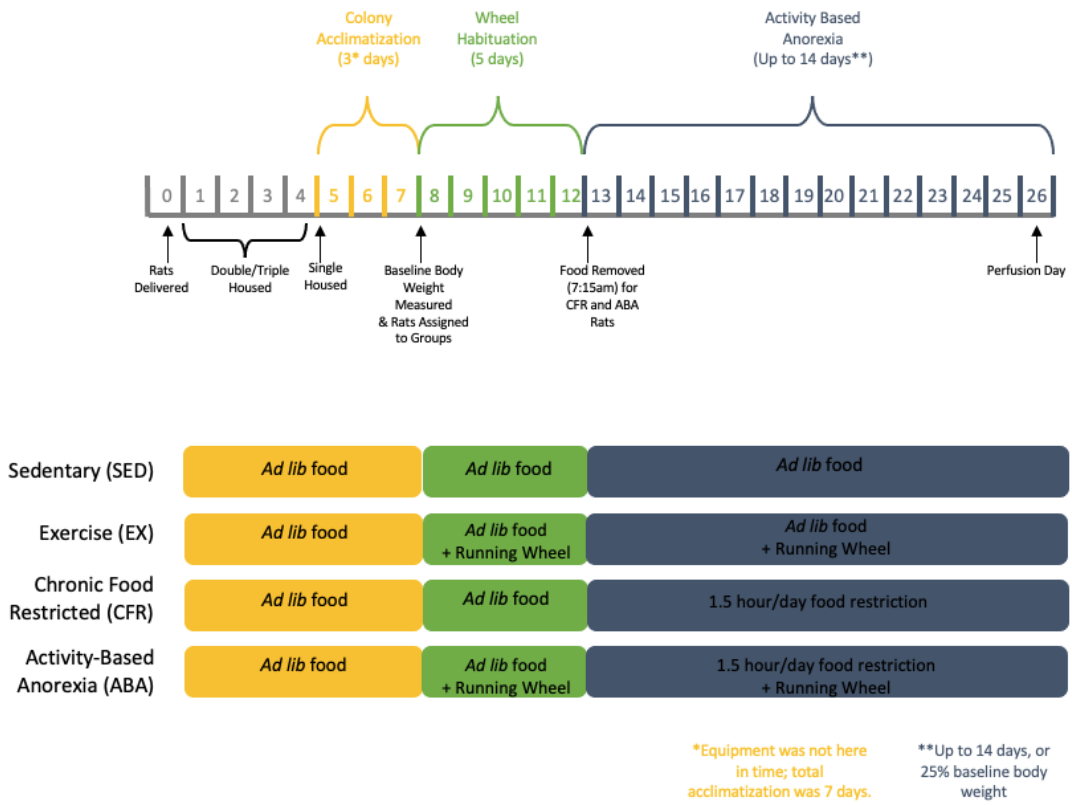

Figure 2: 26-day schedule from delivery of the rats to perfusion

Once we got the supplies in and all the rats single housed, every day at 7:30 in the morning we measured the amount of food the rats consumed over the 24-hour period, took their body weights, and the refilled the liquid food to 90mL. We collected data at 7:30 in the morning because the rats were on a light/dark cycle that was opposite of ours. Rats are nocturnal animals and are more active in the dark periods. We wanted to collect data regarding running, food, or overall behavior as they were becoming more active. The time in the morning was set to fit around our own schedules better. When we were in the colony room, all lights were off, and we used a red light. A recent study observed that rats are not red-light blind, contrary to the long-held belief that they were (Niklaus, 2020). Rats do lack red-sensitive cones, so in order to take measurements in the dark part of their cycle, we still used red light. This did not completely

reduce the stress of having light, but it was less than the stress that might have been caused due to standard white light. After the seven days, the wheel habituation phase began.

The wheel habituation phase lasted five days and the purpose was to introduce the wheels to the ABA and EX rats as well as get a baseline running count before food was restricted. We placed a running wheel with a rotation counter in each ABA and EX rat cage and the rats were able to run whenever they wanted for 24 hours. From 7:30-9:15 A.M., we recorded the amount of food consumed, body weights, and how much they had run over the past day. We refilled the food and then the rats were left for the rest of the day undisturbed.

After the five days of wheel habituation, the ABA phase of the study began. The day before the start of ABA (day five of wheel habituation), we did not return the food to the CFR and ABA rats. This began the 22.5 hour fast. The next day, day one of the ABA phase, we locked all the wheels, we recorded the number of rotations from the day prior and took all body weights. Once we weighed all the rats, we took the food out of the SED and EX cages and placed 40mL of the liquid diet back into the CFR and ABA cages. Once we placed the food, we left the colony room undisturbed for 1.5 hours. During this time, the SED and EX rats did not have access to food and none of the rats were able to run. The cages were locked with wooden dowls so that the ABA rats could only eat and not run. Once the 1.5 hours were up, we took the food out of the ABA and CFR cages and placed 90mL of food back into the SED and EX cages. We then unlocked all the wheels. This same process repeated itself until an ABA rat reached 75% of their baseline body weight.

Once an ABA rat reached criteria, it along with a CFR, EX, and SED rat were sacrificed. Half of the rats in each group were stressed. They were stressed by being restrained in a small cage for 30 minutes. Once the 30 minutes were up, they were placed back in the colony room for

one hour and then sacrificed. The rats who were not stressed were just sacrificed. We injected each rat with 0.3 mL of Euthasol. Euthasol was used to achieve strong analgesia and knocked them unconscious. Before the rats were perfused we tested their reflexes to ensure the Euthasol was active. Once we perfused the rats, we removed their brains and placed them in paraformaldehyde for twenty-four hours in the refrigerator. After the twenty-four hours, we transferred the brains to a 20% sucrose solution and left them in the refrigerator until they were ready to be cut. We cut the brains using a microtome. Once the brains were cut, we performed immunohistochemistry for cFos within GLP-1 and PrRP neurons. We used brightfield microscopy to image brain sections and quantify percentages of GLP-1 and PrRP neurons.

CHAPTER V: Results

Once we had sacrificed the rats we began to review the data regarding the liquid diet intake, wheel rotations, and body weight. We used three separate mixed factor ANOVAs for body weight, liquid diet intake, and wheel rotations. Each mixed factor ANOVA made comparisons between treatment groups and also within each group over time. In addition to body weight loss, we also ran a mixed ANOVA for percent change in body weight loss. There are two main reasons why these four groupings of data were important to look at separate from the neural data. The first is to ensure that we were able to create an anorexia phenotype within the adolescent female rats and the second is to ensure that there was a significant difference between the groups prior to sacrificing.

An AN phenotype in humans is characterized by a decrease in food intake which results in a drop in body weight, as well as an increase in exercise. Previous studies have achieved this phenotype within rats through similar methods to ours (Chowdhury, Chen & Aoki, 2015; Dixon, Ackert & Eckel, 2003). This is called activity-based anorexia and it models AN in humans because rats with wheel access and food restriction, run more than rats with wheel access and *ad libitum* fed. Based on the liquid diet intake data, all four cohorts had about the same intake of food prior to wheel access and food restriction (Figure 3). While this seems like a small achievement, it is exciting that our data verified this as an unequal initial consumption amount would have influenced our data throughout the rest of the experiment. The SED group maintained about an equal food consumption throughout the study, which was expected as they were not exercising or in need to increase food intake to replace lost calories. The EX group increased liquid diet intake, especially towards the end of the study. This is most likely because they were exercising and increasing food intake to replace those expended calories. The CFR and

AN rats both dropped in food consumption on day nine which is expected as we only gave the access to food for ninety minutes. It is important to note that the CFR and ABA rats did not have a large difference in food consumption. This means that the ABA rats did not eat way less than the CFR rats. A possible reason for this could be that while the food restricted animals had food access, all running wheels were locked. This helped prevent the ABA rats from running instead of eating. In addition, when one rat began to run, all the other rats within that colony with wheel access would begin to run. The reason behind this would be interesting to look into, but we expected that the ABA rats would consume less than the CFR rats since they were also exercising. This specific relationship between the CFR and ABA cohorts would be an interesting future study.

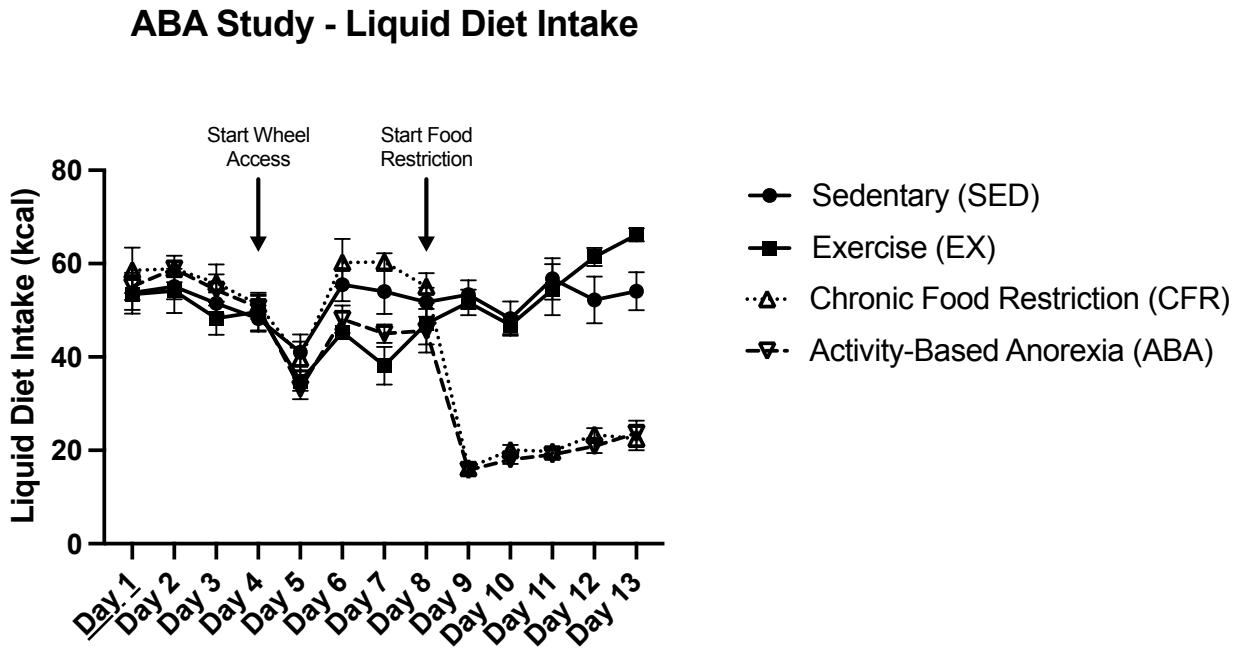

Figure 3: Average liquid diet intake in mL of all four cohorts between days 1-13 of the study. Error bars represent standard deviation.

Prior to the start of food restriction, both the EX and ABA rats ran similar amounts (Figure 4). Once food restriction started, ABA rats began to run more than the EX rats. On day

thirteen, both groups ran about the same. Day eleven is the day after food restriction was implemented to the CFR and ABA rats. It is not clear why food restriction of ABA would have affected the running activity of the EX rats, since all rats were housed separately. From day twelve to fourteen, the ABA rats ran significantly more rotations per day compared to the EX rats. The ABA group ran about 5000 more rotations than the EX group on day thirteen which was what we wanted to see, but also seems to be opposite of what one would expect. This was also seen in other studies, in that the ABA rats ran more than the rats who had wheel access but *ad libitim* fed (Chowdhury, Chen & Aoki, 2015). It is important to note that the rats did not *have* to run, they chose to run despite having their food limited to ninety-minutes a day. This specific behavior is observed in humans. Patients with AN do tend to increase their exercise while decreasing their food intake, causing severe calorie deficiency (Calogero & Pedrotty, 2010).

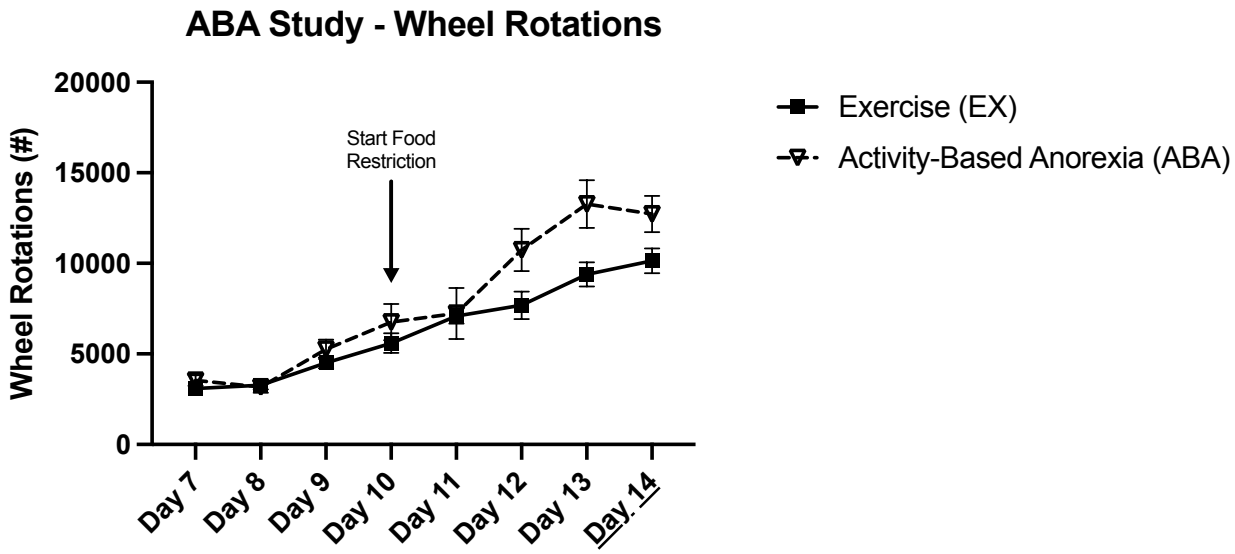

Figure 4: Average number of wheel rotations of EX and ABA cohorts from day 7 (the first day of wheel access) to day 14 of the study. Error bars represent standard deviation.

The body weight data shows that from days one to nine, body weight of all four cohorts was increased at about the same rate (Figure 5). The values for the body weight were about the same for EX, CFR, and ABA groups. The SED rats were a little higher, but that was consistent

throughout the duration of the study. Starting on day ten, both the CFR and ABA rats began to drop in body weight with the ABA rats losing more weight than the CFR. Both groups continued to lose weight until day fourteen, but the ABA rats lost weight at a higher rate than the CFR rats. This was also expected as the ABA rats were exercising in addition to decreasing food intake which increased calorie deficiency.

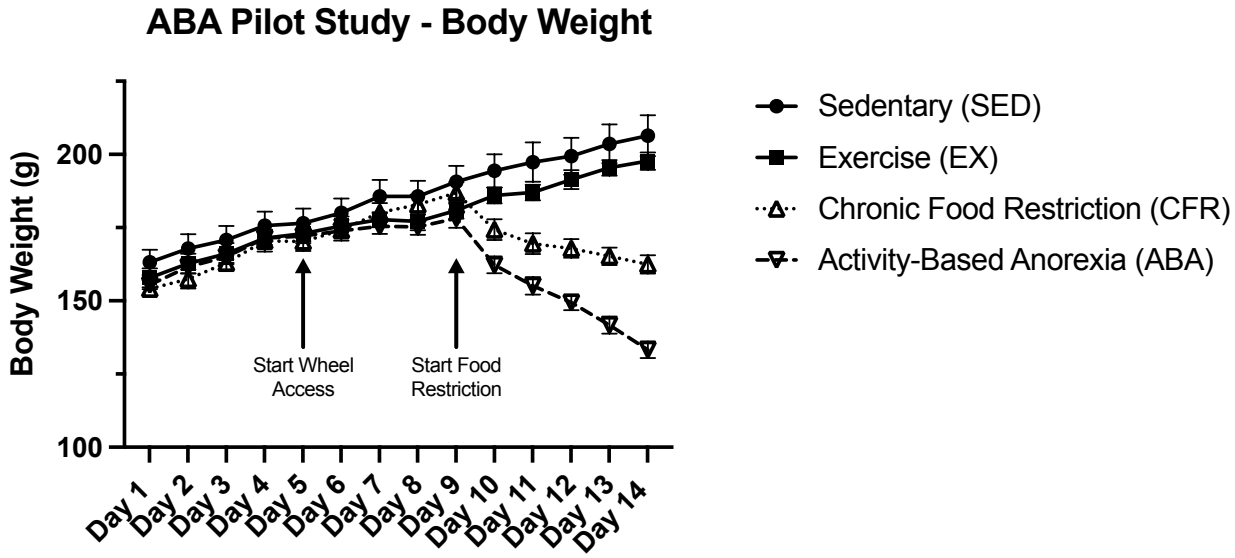

Figure 5: Body weight in grams of all four cohorts between days 1-14 of the study. Error bars represent standard deviation.

The cut off for weight loss was set at 25% of the baseline body weight. 25% body weight loss is a little more than what an anorexia diagnosis in humans is (Dukarm, 2007). Due to availability to perfuse the rats, one rat did lose around 35% of the average baseline body weight (Figure 6). We were not anticipating the rats to lose weight as quickly and drastically as they did as the study went on. As seen in Figure 6, the body weight percentage change from day thirteen to fourteen was almost 10%. In the future, this observation of how quickly they lose body weight percentage will be helpful in making sure that rats do not exceed 25% body weight loss by perfusing even if the rats are not quite to 25%. This will ensure that we keep them in a humane body weight loss range. The dotted line indicates the average body weight in grams at the start of

the food restriction. This is why all four groups start *below* the dotted line for the first nine days. After day nine, the sedentary and exercise groups increased in weight whereas the CFR and ABA rats decreased weight. The ABA rats lost weight at a quicker pace than the CFR rats which was expected since the ABA rats also had access to the running wheel.

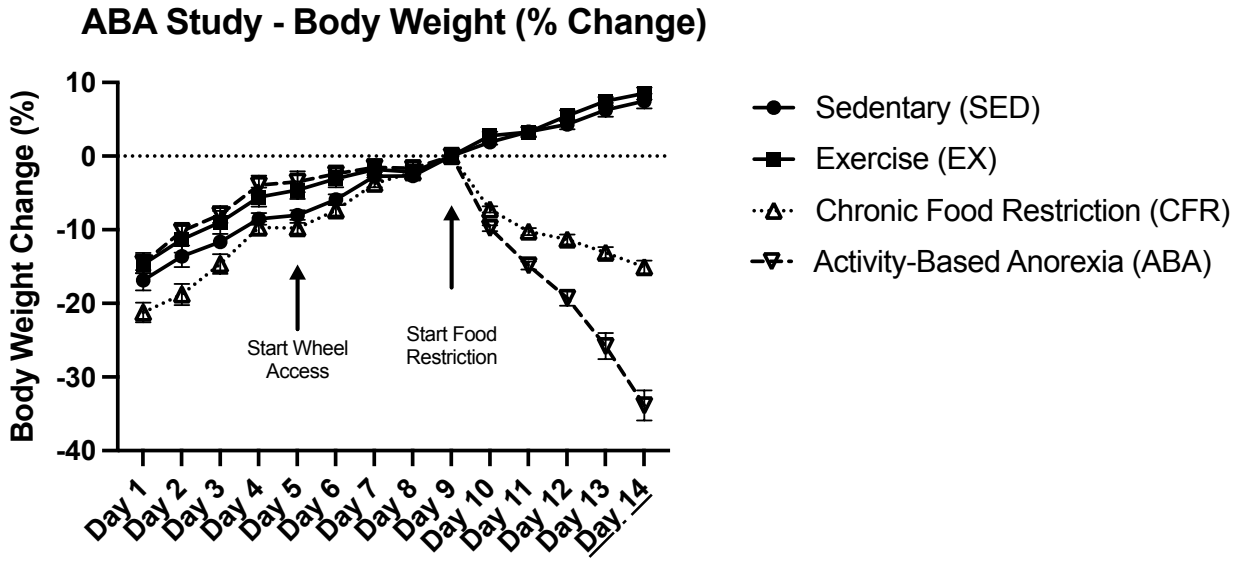

Figure 6: Body weight loss in percentage change of all four cohorts between days 1-14 of the study. The dotted line at 0 indicates the average baseline body weight in grams. Error bars represent standard deviation.

All four of those groupings of data indicate that we were successful in achieving the ABA phenotype. This was one of the main concerns with the methods of this study, because in order to see if there are any differences neurologically between the cohorts, we had to first establish an ABA phenotype. Since we were successful in reaching the ABA criteria, the data we collect regarding the neurons will provide relevant information regardless of whether we support or reject our hypothesis.

A possible future study could be getting the rats to 25% BBW (baseline body weight) and then having them recover. In other words, generate an ABA phenotype in a cohort of female

adolescent rats, and then let them recover. Recovery would have to be defined, but it could be anywhere from returning back to baseline body weight, or perhaps a certain percentage above the baseline value. This might be beneficial because recovery from AN in humans is a long and oftentimes treatment options are not very effective. By looking into what recovery looks like neurologically in rats, we will better understand the effects of anorexia on the brain after an individual is said to have “recovered” could be achieved. It would also be interesting to see what the running wheel activity looks like in a “recovered” anorexic rat.

CHAPTER VI: Discussion

Anorexia appears in written history during the Middle Ages. During that time, it was called anorexia mirabilis which was practiced for spiritual purity (Dell'Osso et al., 2016). This then developed into a group called the "miraculous maids" which was characterized with developing extraordinary starving abilities. It was not until 1694 that it was seen as a mental illness. During the seventeenth century, it was called nervous atrophy and it was defined as fasting caused by an ill and morbid sense of the spirits. In the late 1800s, the term anorexia nervosa was used, and the disorder was associated with a condition of romanticizing a pale and languid body. It was at this point that the drive for thinness emerged as a large contributing factor for the eating disorder (Dell'Osso et al., 2016). Anorexia began as a religious practice, but it is now seen as a disorder that is caused, in part, by societal pressures imposed upon both women and men.

As discussed in the beginning of this thesis, patients with anxiety disorders can begin to use food control to regain feelings of power within their life. In some cases, patients find that decreasing food intake helps decrease stress in a sort of feedback manner. Eventually that individual begins to lose weight as they, essentially, starve themself. There is no current evidence of neurological causes of AN; however, the goal of this study was to begin to investigate if there is a possible relationship between neural activity and the development and maintenance of AN. Once a person with AN feels decreased stress, a habit forms that makes AN hard to overcome. Due to the formation of a neurological habit, treatment for AN is very difficult.

There are currently very few effective treatment options for people who develop AN. The most common treatment is for patients to be hospitalized in order to ensure that they intake

enough calories (Mayo Clinic, 2021). Another promising treatment method is psychotherapy which can be family or individual based. With family-based psychotherapy, parents are directed with how best to support and nourish their child until he/she is able to take responsibility of their own food intake. This is the only evidence-based treatment option for teenagers. Individual psychotherapy is more aimed at normalizing eating patterns and providing support for weight gain. It then aims to change thoughts that maintain anorexic behaviors. There are no medications to treat anorexia specifically. Usually, anxiety medications are prescribed as the two are commonly co-developed (Mayo Clinic, 2016).

When looking at the treatment of AN through a religious lens, as the history of anorexia has its origin in religious practices, there is a strong focus on meditation/mindfulness. In Buddhism, mindfulness meditation is a common practice (Slyter, 2012). Mindfulness entails enhancing one's self-awareness within the environment around them as well as controlling emotions (Levine, 2000). The sole purpose of this form of meditation (Mindfulness-Based Stress Reduction) is to bring one's attention to the present moment instead of dwelling on the past. It aims to increase acceptance of the current reality without being goal or process oriented (Rapgay & Bystrinsky, 2009).

Treating AN within the Islamic religion, relies upon the concept of *tawwakul*, the reliance on God or trusting in God's plan (Haque & Keshavarzi, 2013). The purpose of this practice is to encourage patients to bring aspects of one's life that are out of their control to God. Instead of using food to cope with feelings of anxiety, *tawwakul* encourages followers to bring it to God first. The end goal is to replace beliefs that contribute to one's anorexic state with beliefs that align with Islamic belief (Musleh, 2017). This practice enables patients to deepen their faith while helping them cope with both anorexia and anxiety.

The Christian religion utilizes the same redirection of thought. James Keenan, SJ, uses the teachings on moral theology of Thomas Aquinas to emphasize the importance of our everyday choices. Thomas focused on the idea that we become what we repeatedly do; therefore, what we consume plays into the way we think, act, and react (Keenan, 2010). If we take time to scroll through social media, and all the posts we see have people who are fit in a way we idolize, we will give into thoughts that we are not "healthy" enough. We will eventually create a moral standard regarding what we believe to be the ideal physique which will cause us to compare ourselves with others. This consciousness about what we consume is not to say that we have to stay away from magazines, movies, or posts where people look a certain way. Instead, it means that we must be more mindful about how what we are consuming can affect who we become.

All three major world religions aim to draw one's attention back to the present in order to treat various anxiety-based disorders such as AN. In many ways I do think this is a good method of treatment, but I also believe that it is mostly reliant upon the patient themselves. It is hard to be mindful all the time, and in some instances, patients with anorexia struggle because their understanding of reality is distorted. Treating AN through psychotherapy methods is good and it is important, but I would offer that using mindfulness as a *daily* practice before any issues develop could be a preventative way of keeping various mental health disorders from developing to begin with.

An ethical issue arises, however, when patients who have AN see it as a lifestyle choice and not an illness. When this is the case, there is a moral question of, if someone does not want to be treated, how much bodily autonomy can a medical team grant someone if that choice will end in death? From a purely medical standpoint, medical personnel hold no power in helping a person "get better" if they do not desire to. In some cases of terminal cancer, patients sometimes

elect to not receive treatment and oncologists respect that decision. When that same solution is applied to patients with AN, I feel a moral tension. AN is not a terminal illness. Recovery might not happen for different reasons, but an essential factor is that the patient *desires* to get better. In many cases, AN is a disorder that a patient *can* recover from. There are not cancerous cells, slowly harming the person from the inside. There is no known physiological system that is causing a patient to maintain AN. Patients with AN have a distorted body image which leads to them starving themself. While AN and terminal cancer are similar in some ways, the bottom line is that AN is something that can be treated if the person decides to get better, terminal cancer is not. Recovery from AN is not like recovery from a viral infection or a broken arm. Recovery from AN looks different to different people. For some it can be building a relationship with food that enables the patient to eat the calories needed to sustain their body processes without forced intervention from others. For others, it may also require the patient to keep thoughts about food consumption from dominating their daily life.

If this is the case, then it seems reasonable to hold medical personnel responsible to treat patients with AN regardless of if they desire to be treated or not. As I mentioned earlier, the treatment options are very limited, and they only work if the patient themselves want to get better. So, by stating that I believe it is the duty of medical personnel to treat AN patients even if they do not want to be treated, I mean it is their responsibility to keep the patient alive until they can survive on their own. In many cases this looks like hospitalization. While I understand the gravity of this statement, there is no harm that medical personnel do by keeping the person alive, aside from the financial side of the issue. In some cases, patients are administered feeding tubes and in most cases they are given intravenous fluids, laboratory tests, and vital monitoring (Cowden, 2020). Acknowledging that hospitalization of a patient with AN is expensive, I still

think that keeping the person alive should take priority to either their personal desires or the cost for treatment.

Jenkins and Ogden (2011) interviewed women who were either recovered or in recovery for AN, and they found that a shared feeling was having a split personality. Women stated that they were in denial and could not act upon the rational side of their brain that urged them to eat. Instead, the women commented that they felt an "anorexic voice" that would dominate their rational side, causing them to starve themselves (Jenkins & Ogden, 2011). While this is only one study, it presents a compelling argument for why patients who suffer from extreme AN should be treated even if they do not consent to wanting to be helped. It seems to be a moral responsibility of health care workers to keep the patient alive until they can rationally decide to get better on their own. If forced hospitalization is ethically permissible, then treatment has to be more effective than it is currently. Simply keeping someone alive but not giving them the resources to overcome the core issue is not as morally wrong as not doing anything in the first place, but it does little good for the patient themselves.

Both secular and religious methods of treatment attempt to encourage the patient to see AN for what it is, a manipulative disorder that distorts the way a person sees themselves. While both types of methods are effective in some cases, overall, these treatment options only truly work if the person who has AN wants to get better. Despite AN being recognized as a disorder for over three hundred years, little has been done to change the way it is treated; therefore, it has not been researched to the extent I believe it should be. A possible reason for the limited treatment options could be due to the limited understanding of what AN is and how it is caused.

The long-term vision for why we examined the possible neurological underpinnings of AN within rats for this study is to better understand neural changes and dysfunction in models of

anorexia to create a more complete foundation of the causes and consequences of anorexia. This could lead to a better way to treat and prevent AN through developing a better understanding of what is happening neurologically. By better understanding how neural activity contributes to the formation and maintenance of AN, we can combat it early on by finding ways to prevent food from becoming a coping mechanism for stress.

While important, the purpose of our research study and this thesis, is not to just fill a research gap within the neuroscience and medical world. The main purpose for me is to start a conversation surrounding a mental health disorder that has harmed individuals since the mid-1600s. While this is just a foundational study, the question we are asking, regardless of the neural results, is creating a conversation surrounding a mental health disorder that is generally not talked about. Eating disorders do carry with them a certain stigma that they should not be discussed; however, when we silence a group of people, we become unable to help them. Throughout my research writing this thesis, something that kept coming up was the lack of understanding of how and why eating disorders like AN develop. It is hard to improve or better a situation if one does not understand what is wrong in the first place. This thesis is aiming to be more contemplative about AN in order to create conversation that may lead to better treatment, and better care for those who do have AN.

As a Jesuit institution, the value of cura personalis is central to the way in which we are taught to see those around us. We are called to not only respond to the needs of those we see but aim to care for them in such a way that they are able to live better lives. When it comes to medicine, and treatment of anorexia in particular, simply treating the individual people is not enough to combat the disease. The core issue, for me, is why people develop eating disorders like AN in the first place. Societal pressures which lead to comparison with others is a main

social trigger for their development. Why is being thin an ideal in our society and why is it such a pressure that men and women, more commonly girls and boys, starve themselves to the extreme point that 5% of people who develop AN die (Hamilton, 2018)? That is a question that is not easily answered, but it is one that needs to be asked more in order to address the issue of AN at its core.

Social media is not the direct cause for the development of eating disorders in young people, but it is a trigger. A trigger starts something that is predisposed to occur whereas a cause is the reason for something developing (Stöppler, 2021). From both personal experience and survey data, scrolling on Instagram leaves people feeling just as inadequate as having someone you love criticize your appearance (Witmer, 2020). On the surface level, social media seems to be a place where people can share real life updates about their lives. They do not seem like the fashion magazines or tabloids where you can tell that all the cover models are photoshopped slightly. On social media, posts give the illusion that they are real. For example, you see a fellow classmate sitting on a boat in a two-piece bathing suit and you make a mental note about how fit she looks. You know she does not have a fancy camera, so you reason that she took the photo with her iPhone. You marvel at how perfectly candid it looks and you wish you could take more pictures that look that effortless. Yes, there are imperfections in the lighting and angle, but it makes you feel even worse that you do not look like her and she is not even a professional model. You start tearing your own physical appearance apart and before you know it you are scrolling through an Instagram influencer's page with 10K followers, and you wonder why you cannot be just like her. The reason, you deduce, is because you do not look like her.

What is not obvious are the hours your classmate spent manipulating the picture to look perfect. Yes, the picture was shot on an iPhone, but companies have developed apps to target

girls by making photoshopping their Instagram posts super simple, anyone can do it. In fact, a study done by a phone case company revealed that only 29% of people who responded in the survey would post an unedited picture to a social media platform (Aspinall, 2020). Almost everyone does it. Social media does not show reality. That statement might seem obvious to many, but there is a huge difference in knowing something to be true and actually believing it. When the people you are comparing yourself to are actors, singers, or models, it is easier to remind yourself that photoshop is real. When the people are instead your friends, classmates, and teammates, it is harder to simply dismiss a picture-perfect image as fake.

With the above exploration into how easy it is to fall into comparing yourself to others, it seems difficult to figure out a way to make social media less controlling for the individual. Social media platforms are privately owned and are not subject to the same laws that news platforms are subjected to. In recent years, there have been calls for social media platforms such as Facebook and Instagram to pull advertisements for products such as dieting pills for accounts that belong to people who are younger than eighteen (BBC, 2019). This, among other steps, was in response to rising data that social media has become the new trigger for many to develop eating disorders (Saul & Rodgers, 2018). Despite the efforts of social media companies, I do not believe that change will come from actions taken by the companies themselves. Prior to the age of social media, printed forms of mass media were one of the only ways girls could compare themselves to others. In 2018, 93% of teenagers had Internet access at home within the United States which shifted the means for young girls to feel the pressure of “looking” a specific way (Saul & Rodgers, 2018). The main focus for combatting eating disorder formation in young girls and boys should be more education regarding the distorted reality that social media, or mass media in general, creates.

Teaching the "magic" of photoshop in the health education system for high school aged students could better educate young men and women on the deception of any image that is either posted to a social media site or released in the mass media. Explaining that a majority of the content that we as a society consume has been filtered to look picture perfect could alleviate some of the unrealistic pressures that young people put on themselves to look an ideal way. Other potential solutions include suggesting to parents and teenagers that social media accounts should not be created until the young adult understands that what they see on the apps is not reality. I chose to take a break from social media for about six months and in that time I realized how twisted the apps can make reality. Once I went back on, I was better able to separate feelings of comparison since I knew what I was seeing was most likely filtered. That realization came through maturing and being able to love and accept myself for who I was and not putting my value in what society views as beautiful.

Overall, the best way to care for the whole person who has anorexia is to teach them how uniquely beautiful and loved they are. Nothing can be done if people continue to put their worth in what the world says about them; therefore, taking small actions to reverse that way of thinking could go a long way in decreasing the prevalence of eating disorders in general. One of the easiest ways of doing this is truly seeing the person who is in front of you. Being conscious of who is physically next to you and taking the time to talk and interact with them, instead of being on one's phone, is a small step but one that is absolutely effective in building acceptance. In order to be men and women for each other, we have to be men and women *with* each other, and this requires us to be present with those around us. I acknowledge that many of my ideas for combatting anorexia sound too small after the gruesome realities that were discussed surrounding the disorder. The truth is that there might never be an ideal treatment for anorexia which means

that the changes we make in our everyday life towards the people we interact with are more important in the long run. One of the most prevalent triggers of AN is societal based, so we have a responsibility to work towards changing that.

For the time being, we can aim to combat eating disorders at their source in order to end the cycle of anorexia before they begin. While that sounds like a daunting and futile task, it is one that has been a long time coming. AN began being recognized as a mental health disorder in the seventeenth century. Since then, little has been done until recently to challenge the unrealistic expectations society had regarding body figures for both men and women. Now, there is a push for more inclusivity of all body types within most forms of mass media. As society as a whole continues to move forward in terms of seeing all people as uniquely beautiful, we can continue to do our part and care for the people in front of us. While finding more effective treatment options for AN is important, if we combat AN at the start of the cycle while also finding new ways to break the cycle once it has started, we may be closer to ensuring that all people feel loved and accepted for who they are and what they look like.

CHAPTER VII: Conclusion

Anorexia nervosa is a dangerous mental health disorder due to its quick habit-forming syndrome. While controlling the food one consumes is not bad, taking it to the extreme and using it to both combat stress and achieve a desired figure, is incredibly destructive. What may start out as a way to control stress and anxiety can quickly turn into a compulsive action. Once an individual begins AN behaviors, they become hard to overcome. Social factors such as social media, advertisements, and beauty standards all contribute to triggering AN in people or cause those who are trying to recover, to relapse. The causes of AN are not well understood which makes treatment difficult for individuals with it. The research we conducted aimed to fill the knowledge gap of possible neurological causes for the formation and maintenance of the disorder, as well as initiate conversation surrounding the destructive disorder. While we have not yet analyzed the neurological data in terms of the GLP-1 and PrRP neurons, the results we do have so far indicate that we were able to get the rats to an anorexic phenotype. This means that our next step of looking at those specific neurons will either provide evidence that there is some correlation, or there is not. Both outcomes will be helpful in the larger conversation surrounding AN.

Just doing a quick poll of my personal friends, three have diagnosed AN, and four have admitted to some type of eating disorder. AN is not a rare disorder and the age group it targets is one with young adults who are easily influenced and swayed by what they see from those around them. There is a rising need to address the causes for development of AN before it becomes a disorder that claims more lives than it spares. My friend who inspired this thesis was one of the lucky ones who made a full recovery. She is currently running cross country in college and plans to become a first-grade teacher. After talking with her about my thesis and what I have found

both in the study and just through my research of triggers, she offered that she has seen a rise in a cultural push that seems to encourage obsessive thoughts about eating. She said that videos with "what I eat in a day," by an Instagram influencer can subconsciously make people wonder if what they eat in a day is acceptable, which, if not checked, can give way to disordered eating. Steps need to be taken to investigate more fully the psychological impacts of social media on AN individuals as well as young adults in general in terms of how they encourage thinking about food in an obsessive way. Restrictions on the types of advertisements and messages companies can use to draw in certain age groups should be discussed. More studies like this one need to be conducted to try and better understand how AN develops and ways in which health professionals can intervene to increase chances of recovery. Most of all, if this thesis has taught me anything, we must find ways to love the people in front of us better. Studies, restrictions, and awareness take quite a bit of time. So, in the meantime, we can be more accepting of all body types, encourage healthy but not obsessive eating patterns, and be open to conversation with either those who have AN, or with those we love who could develop anorexic-like behaviors. We are called to care for each person we encounter, and the easiest way to do so is to pay attention to those in front of us, start up a conversation with the student eating by themselves, and find small ways to show great love.

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