

Polycystic Ovary Syndrome (PCOS) is a common endocrinopathy that affects about 5-10% of all women of reproductive age. PCOS is the leading cause of infertility in women [1]. The defining feature of the syndrome is hyperandrogenemia. PCOS is also associated with a number of reproductive and metabolic symptoms including oligo- or amenorrhea, excess LH secretion, cystic ovaries, hirsutism, insulin resistance, hyperinsulinemia, and visceral adiposity. The metabolic and reproductive phenotypes are thought to both be the results of hyperandrogenism as was first discovered in a 1921 study by Achard and Thiers [2]. The causes of PCOS are not fully understood, but recent studies suggest that prenatal exposure to androgens (PNA) can lead to PCOS symptoms in animal models leading to the hypothesis that PCOS is a disease that results from endocrine disorders early in development [3]. Levine and colleagues have recently shown that rats exposed to excess androgen *in utero* exhibit insulin resistance in adulthood [4, 5]; similar results have been observed in monkeys [6, 7]. Through the course of my research, I hope contribute to understanding the mechanism by which the exposure to androgens *in utero* leads to insulin resistance in adulthood.

It is well known that exposure to androgen early in life influences sexual differentiation of the brain in mammals [8]. Levine and colleagues hypothesize that actions of androgens *in utero* in the brain, specifically the hypothalamus, may also contribute to insulin resistance in adulthood [5]. Understanding the connection between androgen-mediated organizational programming of the brain and the manifestation of the metabolic phenotype of insulin resistance will be an important tool in eventually discovering the ultimate mechanisms and causes behind PCOS.

Insulin resistance is one of the symptoms of PCOS that is shown to be induced by prenatal exposure to excess androgens in rodents. However, the pathway between PNA and insulin resistance is not fully understood. A recent study by Kreier et al. [9] demonstrating the presence of distinct neural pathways connecting the hypothalamus to the liver and visceral adipose tissues, both targets of the hormone insulin, may explain the correlation between the androgen environment *in utero* and the subsequent manifestation of particular phenotypes. This evidence combined with the results of the PNA studies suggest that androgens may be acting on a direct neural pathway connecting the hypothalamus and liver, a major target of insulin action, leading to the metabolic symptoms observed in PCOS.

Over the course of the summer, I plan to test the hypothesis that prenatal androgenization alters the neural input to the liver thereby inducing hepatic insulin resistance. By concentrating on the liver's response to prenatal androgenization, I hope to isolate a potential connection between the hypothalamus and liver tissues and look at how that connection is affected by hormones in the prenatal environment. I also will attempt elucidate the liver's contribution to the general insulin resistance that has already been observed in PNA animals. In exploring a potential connection between the organizational effects of androgens on the brain and hepatic insulin resistance, I hope to further elucidate the causes of the PCOS phenotype.

The main technique I will be using to address my research question is the hyperinsulinemic-euglycemic and pancreatic clamp procedure [10]. This technique was recently used in an experiment by Pocai et al. [10] to test the hypothesis that the activity of hypothalamic K_{ATP} channels regulates hepatic glucose production. A physiological "clamp" refers to a procedure in which one or more hormone(s) or metabolic substance(s) are infused into an animal to maintain constant levels of those substances, so the effect of other variables can be observed without the confounds of variation in other substances. Because changes in blood glucose level alter pancreatic insulin secretion, we must control, or clamp, glucose levels and insulin secretion

to observe the responses by the liver. The method proved effective in isolating the connection between the hypothalamus and the liver and thus fits well with my project aims.

First, I will create a group of prenatally androgenized as well as control female rats with IP injections of the mothers with either testosterone dissolved in benzyl benzoate or vehicle. When these rats are 60-80 days old, they will be ovariectomized and provided with hormone replacement. One week before the clamping procedure, rats will be fitted with two catheters, one in the carotid artery and the other in the jugular vein. On the day before the experiment, food will be removed from the rats' cages to generate an overnight fast. The clamping will take place over 4 hours. At time 0, the rats will be injected with radioactively labeled glucose with continuous infusion through the carotid catheter. Two hours after this injection, insulin, somatostatin, and glucose (as needed to prevent hypoglycemia) will be infused. During this time, whole blood glucose measurements will be made on blood samples drawn from the jugular vein to ensure stable blood glucose levels are maintained. Rates of whole-body glucose appearance and uptake will be determined by comparing the rate of the radioactive glucose clearance and the plasma glucose production by measuring the amount of radiolabelled glucose in additional blood samples taken at the same time. Hepatic glucose production will be calculated by subtracting the rate of glucose infusion from the rate of glucose appearance during the final 30 minutes of the experiment.

I plan on beginning the preparation for this project this spring by learning how to perform the catheterization on the rats. This procedure is fairly difficult and requires a lot of practice. Ultimately, I would like to start performing the actual clamp procedure by the end of the summer and continue data collection into the fall.

By the time I start this project, I will have taken several relevant courses including Biology 210-1,2,3, Biology 320 (Animal Behavior), and a complete year of Biology 398-399 (Independent Study). Furthermore, I have spent the last two summers at St. Luke's Hospital in Milwaukee, WI and the Medical College of Wisconsin working on endocrine and molecular biology research projects. I have completed all of the animal training and certification courses required to work in the Northwestern animal facilities and labs. I am very familiar with aseptic technique and I have learned how to perform several rodent survival surgeries. In working with some of the other people in the lab, I have also learned how to perform serial bleeds as well as glucose monitoring. In addition, I have been attending lab meetings and seminars all year long that have provided me with sufficient background information for understanding the larger context of my project.

Since this procedure is fairly new, I will require some help from my faculty advisors, Dr. Horton and Dr. Levine. I plan on working with them to learn the catheterization technique as well as the clamp procedure. Also, when I obtain results, I will likely need help from Dr. Horton in how to best analyze and present my data.

I am very excited to be involved in this project. I hope that this experience will help me refine my understanding of endocrinology as well as my surgical technique. I plan on using this project as my senior honors thesis in biology. I hope that the results that I obtain will point me to other questions regarding PCOS that could direct further research. Depending on the results, I would also like to present my findings as either a presentation or a poster at an academic conference. Eventually, my goal is to publish my results in a scholarly journal.

In a larger context, I hope that this experience will help me on my way to earning an MD/PhD in the coming years. Eventually, I hope to find a career that allows me to use my research-based knowledge and apply it in a clinical setting.