



Vascular Kinetics: Affect of Varying Insulin Levels on BMEC Metabolism



Christian Sun

College Park Scholars – Science & Global Change Program
Kinesiology
csun1235@terpmail.umd.edu
College Park Scholars Academic Showcase, May 6, 2022

Introduction

Endothelial cells are the cells that line all our blood vessels and their function is to regulate vascular homeostasis. When the endothelium is altered however, it can lead to anasthesia. My particular project goal this semester was to understand how increasing insulin levels changes primary vs. iPSC brain microvascular endothelial cell (BMEC) metabolism.

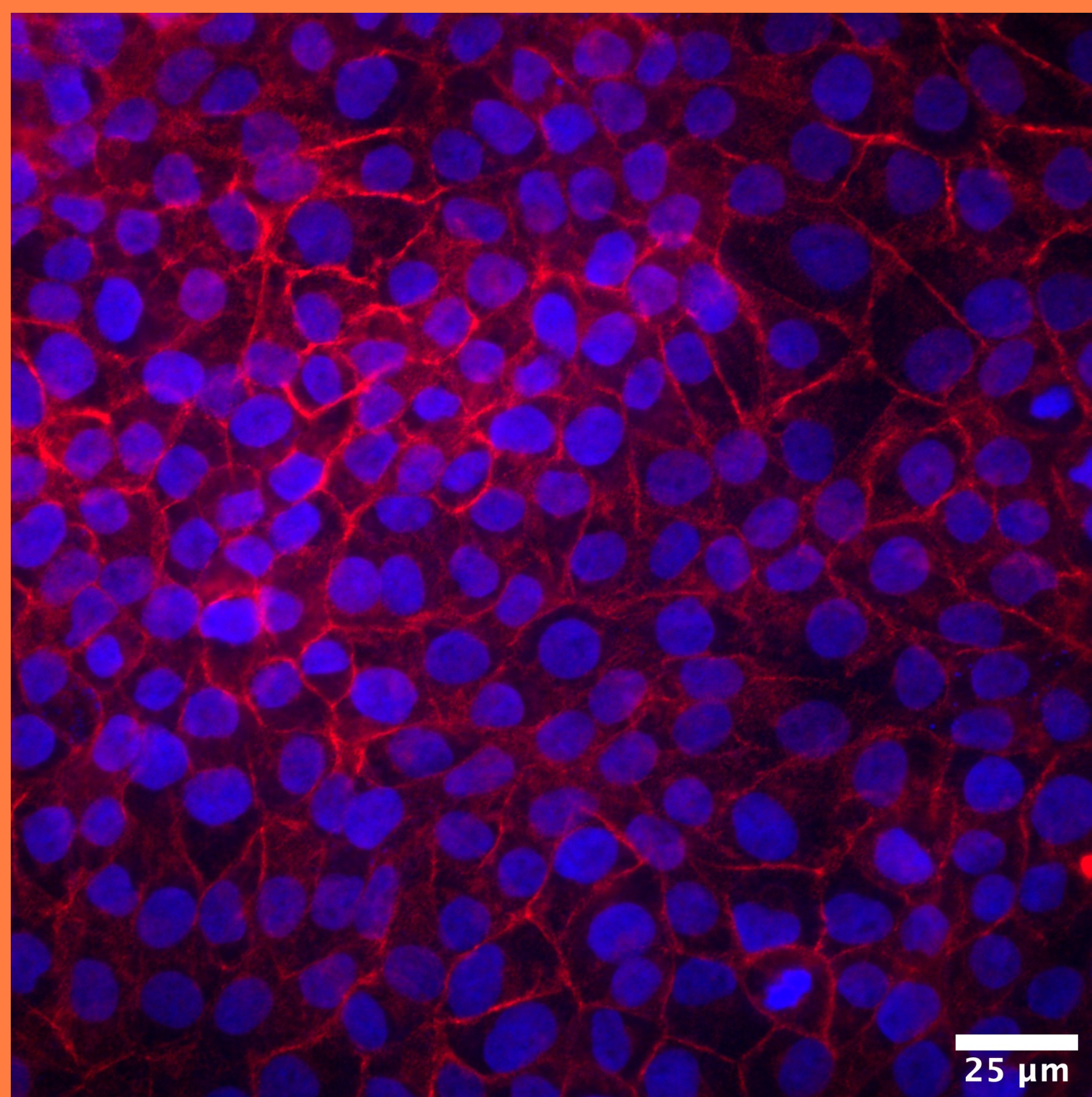


Image 1. Image taken on confocal in lab as I was analyzing localization of GLUT1, a glucose transporter responsible for glucose uptake into brain endothelial cells.

Methods:

- Brain endothelial cell culture
- YSI experiments: to find affect of insulin on glucose/ lactate/ glutamine/ glutamate uptake and production
- Western Blots: to analyze GLUT1 and Insulin receptor protein content
- Membrane Fractionation: to determine whether insulin changes GLUT1 membrane localization
- TEER: to determine insulin affect on blood-brain barrier strength
- Immunocytochemistry: does insulin change tight junction protein expression



Image 2. I am using a micropipette to treat cells with media for one of my experiments early on during the school year. Image taken in lab.

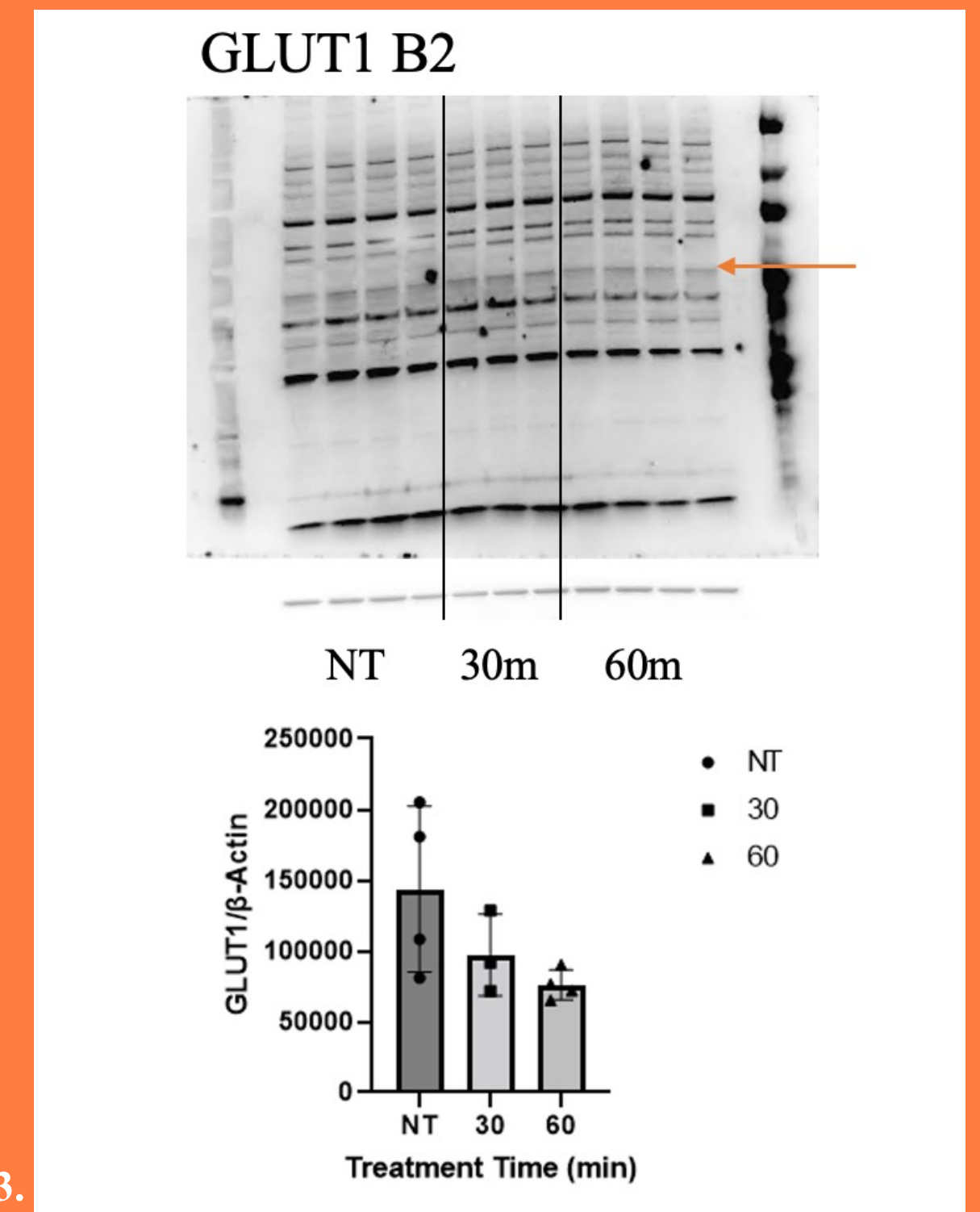


Image 3. Western blot probing for GLUT1 protein in response to insulin treatment also testing different treatment times.

Impact:

- I have learned a lot about how research is conducted, and the many techniques required in order to come to conclusions on the inquiry we pose
- I was able to contribute to the work of my own supervisor Callie as well as other lab members' research whether it was through assisting in shearing red blood cells or even voluntarily donating my own blood in order to be used for experiments

Future Work:

I hope to continue my work in Dr. Clyne's lab as I feel there is much more that I can contribute to this lab as well as this lab to me. I originally joined because I saw they dealt with the cardiovascular system, and with cardiovascular diseases being prevalent in my family, I wanted to learn more about what causes these diseases and how we may be able to treat or prevent them.

Results/Discussion:

Looking at the Western blot and analysis in image 3, we can see that with increasing insulin treatment duration, there was decreasing GLUT1 protein content in the cells. When conducting this experiment, I had to compare this data with YSI results to see if there was also decreased glucose uptake in cells with 24-hour insulin treatment. Typically, we should see increased glucose uptake with increased insulin treatment, however the opposite results were found here. Experiments are generally run at least 3 times before we are able to conclude that results found are reliable and replicable. I do not believe the results I found in this particular run of the experiment were reliable.

Some other relevant factors affecting endothelial cells, leading to potential cardiovascular disease:

- Glucose levels
- Insulin levels
- Shear stress

Site Information:

A. James Clark Hall – Vascular Kinetics Lab
University of Maryland, 8278 Paint Branch Dr,
College Park, MD 20742
Supervisor: Callie Weber
The site mission: To understand how integrated biochemical and biomechanical factors contribute to cardiovascular disease through the use of engineering methods.

Acknowledgments:

Thank you to my PI Dr. Alisa Clyne for giving me the opportunity to join the lab, and for connecting me with my supervisor Callie Weber who's been an amazing teacher in my lab techniques and research experience. Thank you also to Dr. Holtz & Dr. Merck for pushing my peers and I over the last three semesters to learn how to think critically as this skill has done nothing but contribute to my love for science and the many ways it benefits us to learn more about how our own bodies work and react to things.

