



# Racial Differences in the Effect of Influenza Vaccine on eNOS Expression and Regulatory MicroRNAs

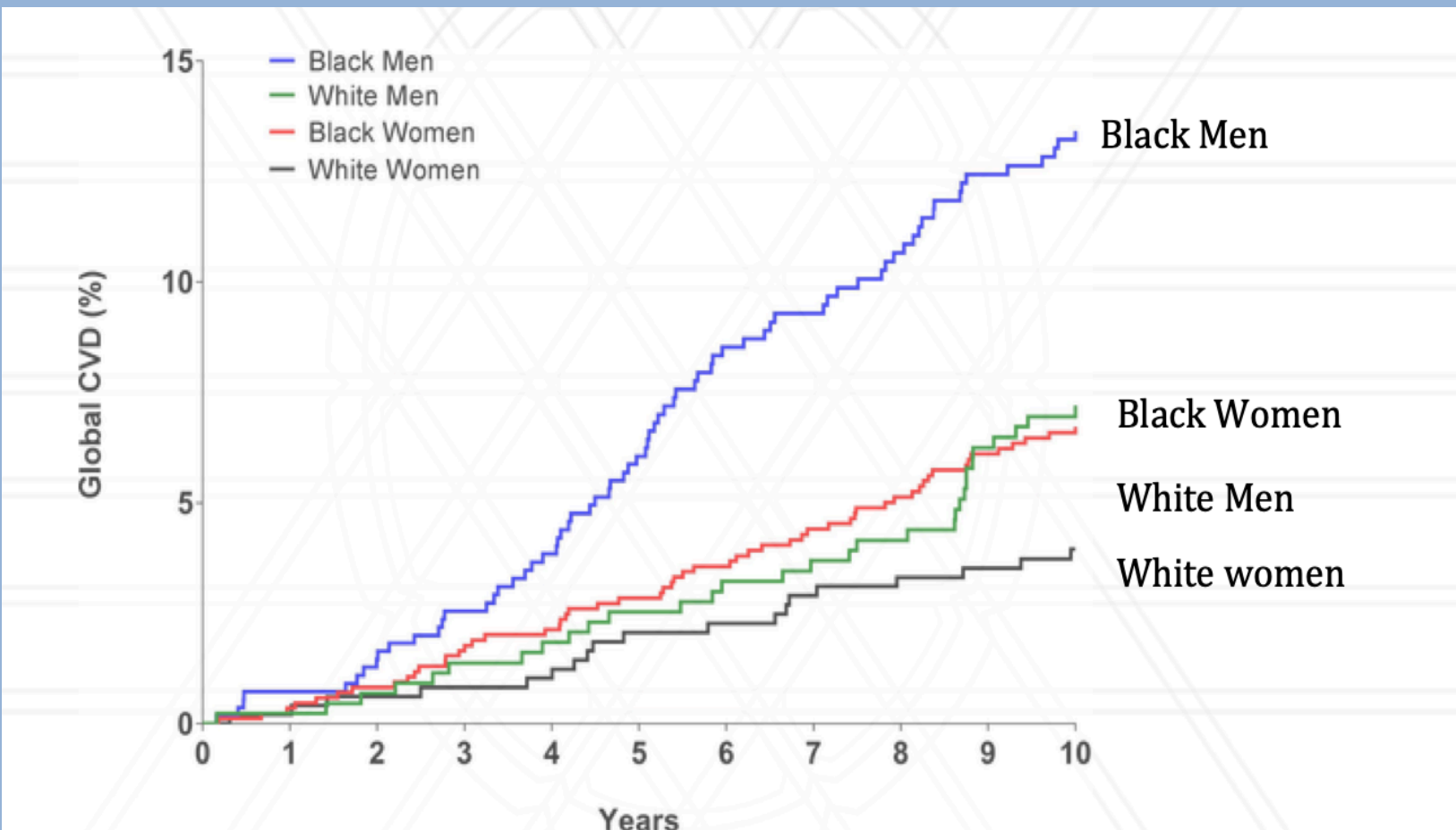
Matthew Laskowski  
matthewr.Laskowski@gmail.com

College Park Scholars - Science and Global Change Program  
Department of Chemistry



## Site information

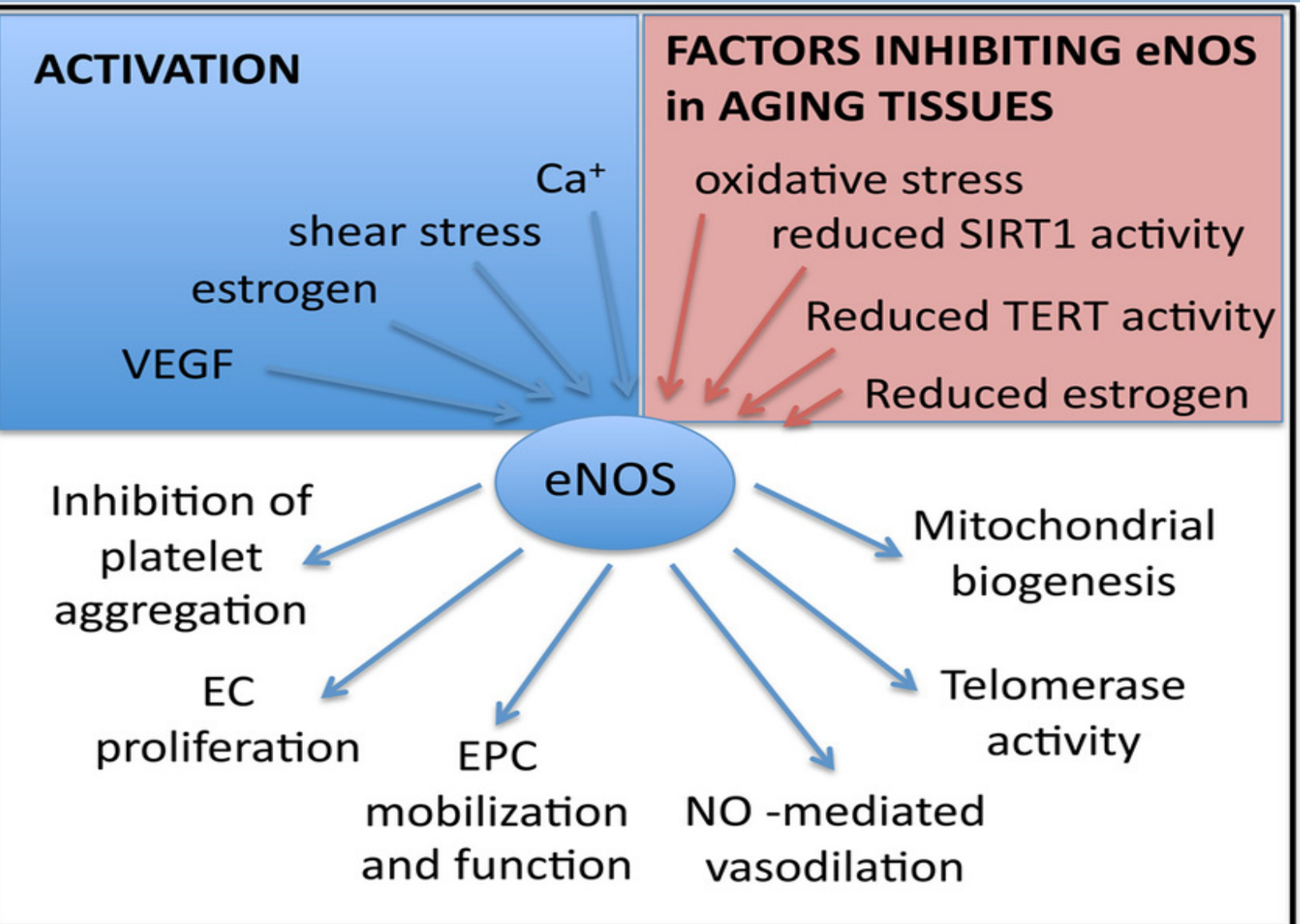
- I worked for the Cardio Metabolic lab, on the top floor of the University of Maryland's School of Public Health, in the lab of Dr. Steven Prior and directly under the supervision of two PhD students, Bill Evans and Ryan Sapp. I worked here during both summer terms of 2019
- The focus of this lab was to examine how a wide variety of factors can effect the cardiovascular system. The lab consisted of typical lab benches as well as exercise equipment for human subjects to perform. All my work was done in a hood, especially growing and applying specific conditions to endothelial cells.
- The work I did was one part of a larger study which was attempting to examine similarities and differences in the cardiovascular system between races.



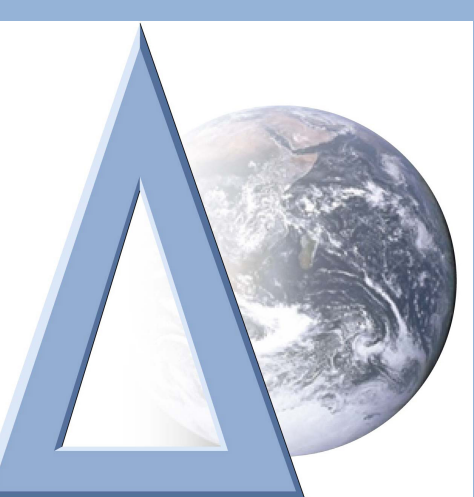
**Cardiovascular disease in various populations over time:**  
It is clear to see that African American population is at higher risk that Caucasian population. Why are there such significant differences?

## Introduction

- There are disparities in cardiovascular function between different races. One way to look examine the health of the cardiovascular system is to look at endothelial cells.
- Endothelial cells have varying responses to inflammation; for example, people with hypertension or diabetes, the inflammatory response is much greater.
- One of the most important proteins in an endothelial cell is endothelial nitric oxide synthase (eNOS) which plays many roles in these cells. Previous studies have shown racial differences in the expression of eNOS.
- The basis of this experiment is adding influenza vaccine to endothelial cells, which causes an inflammatory response, and then measuring specific microRNAs (miRNA) that have some relationship to the inflammatory response in endothelial cells.



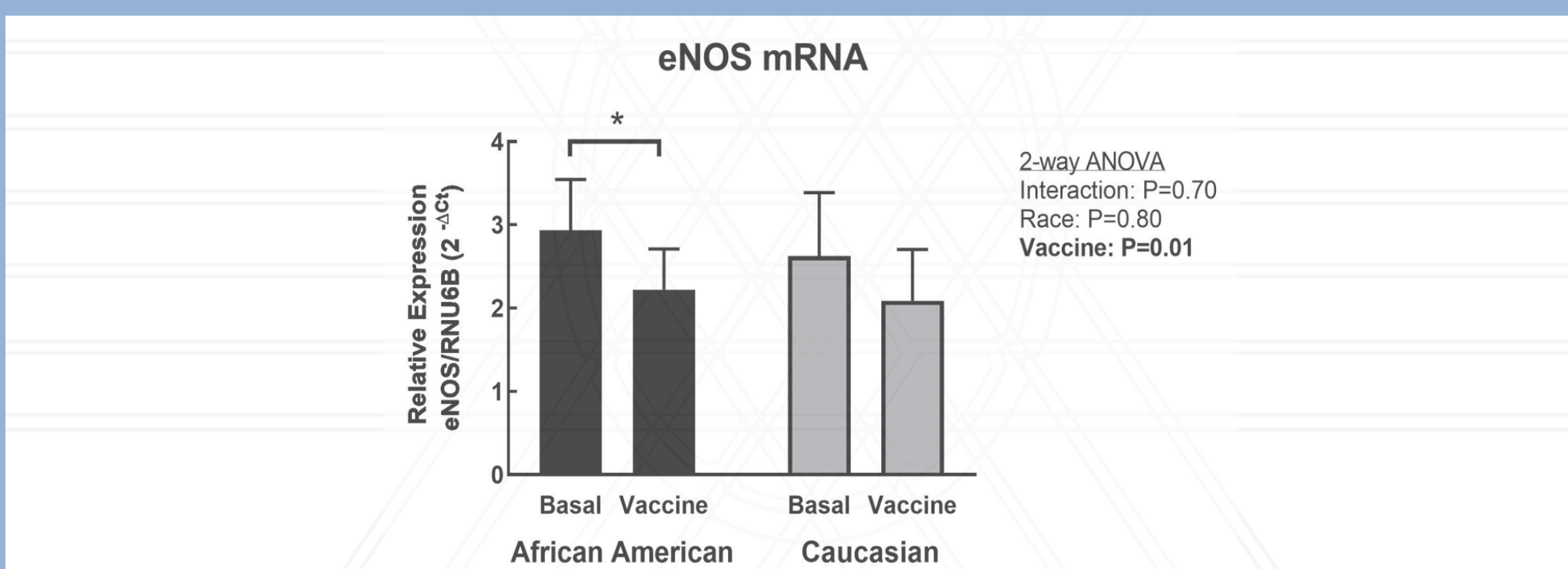
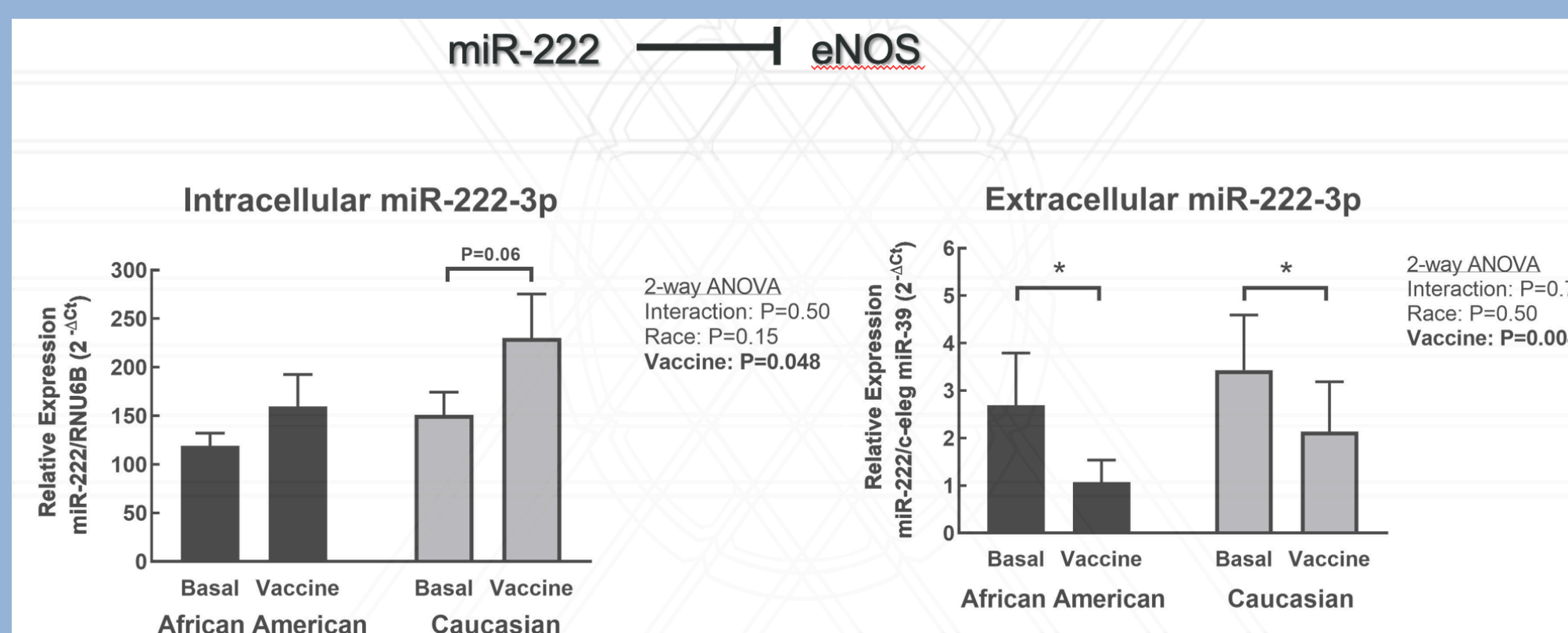
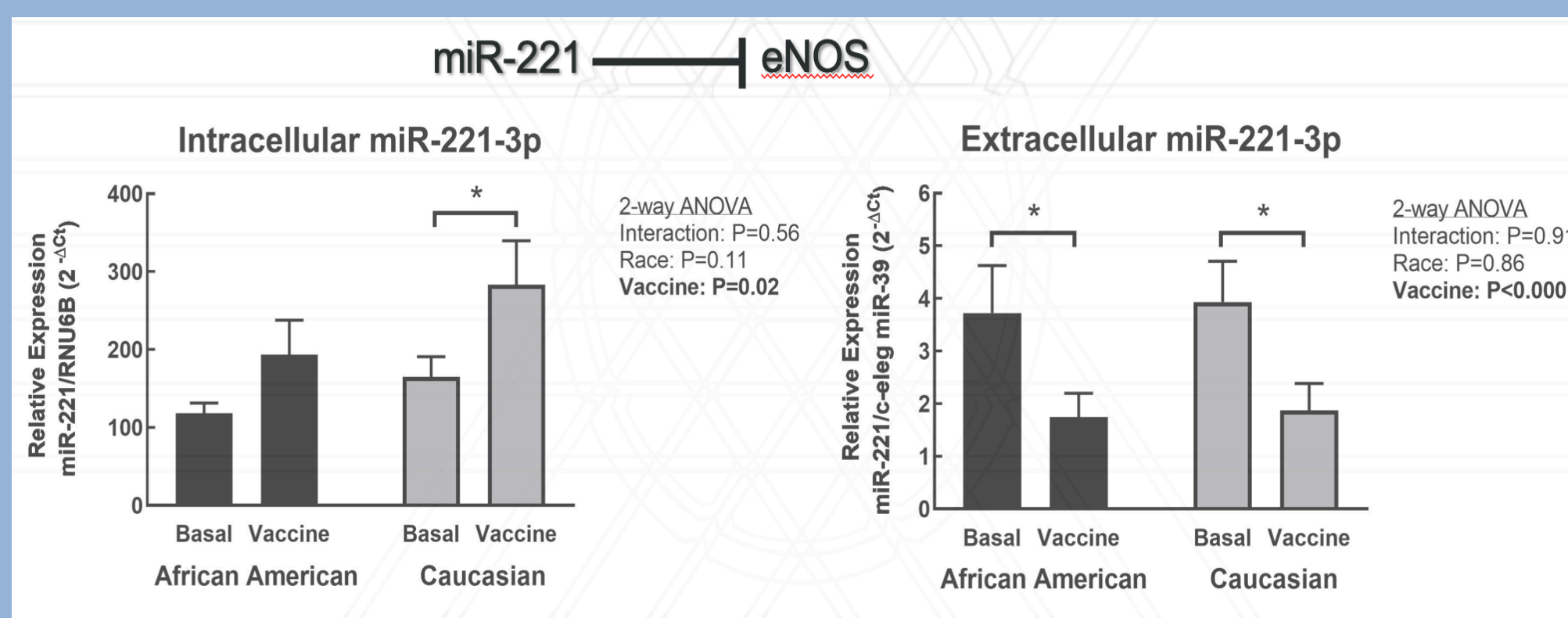
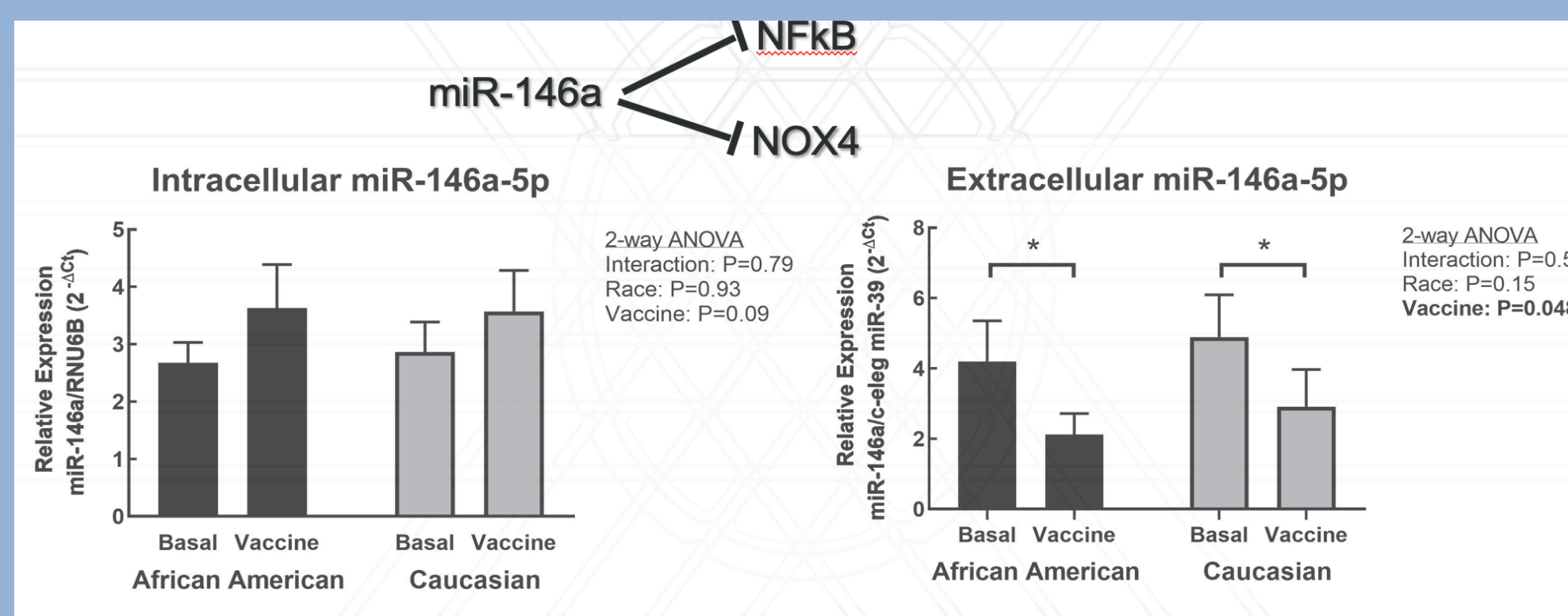
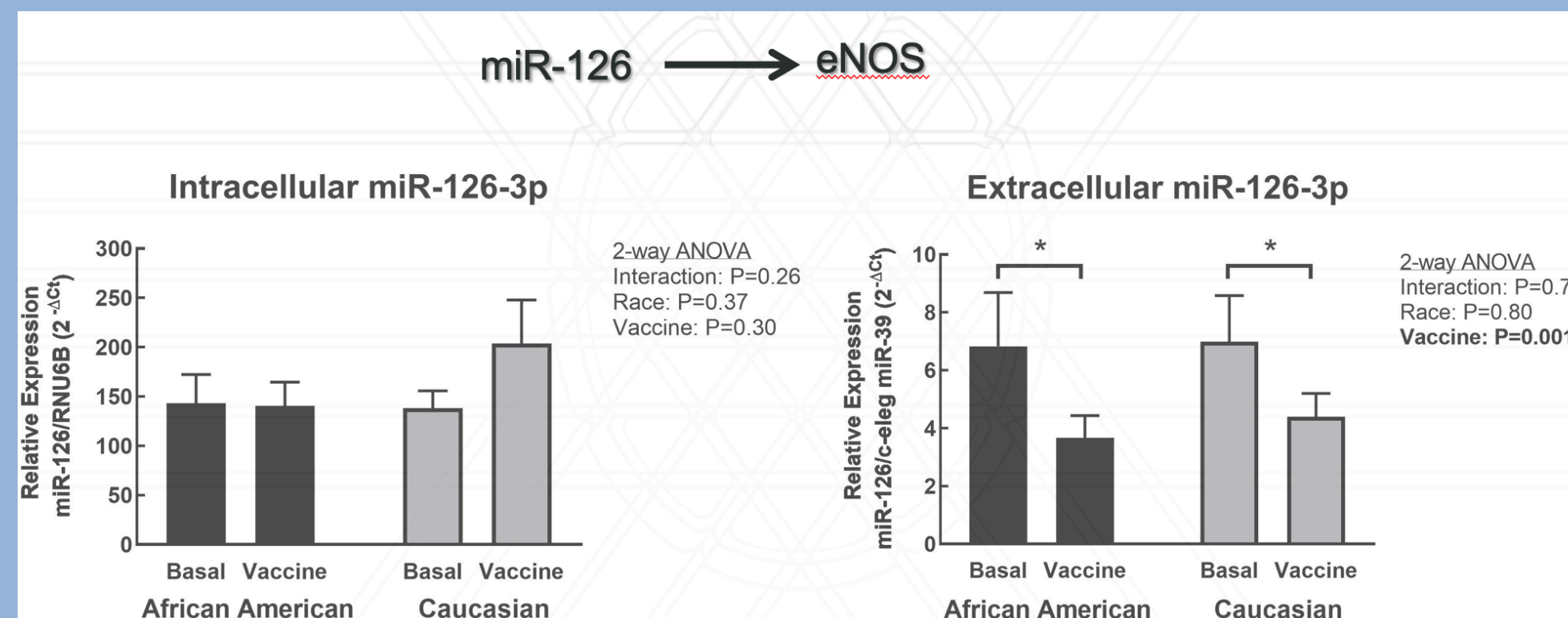
**The multi-functionality of Endothelial Nitric Oxide Synthase (eNOS) throughout the endothelium:**  
eNOS is an enzyme whose presence is extremely important in many mechanism within endothelial cells. What would happen if its concentration changed?



Thank you to Dr. Holtz and Merck for their advising and support through this whole process, Dr. Steven Prior, Dr. Jim Hagberg, and Dr. Sushant Ranadive for their research insight, as well as Ryan Sapp, Bill Evans, and Daniel Singer for their day to day help working in lab and analyzing the results.

## Methods

- I would culture Human Umbilical Vein Endothelial Cells (HUVECs) of healthy and young African American and Caucasian American individuals. After they had been grown to confluency, I would apply 250 ng/mL of the influenza vaccine.
- After exactly 24 hours, I would isolate the proteins and miRNA's separately from inside the cells (intracellular) and the medium where the cells resided(extracellular)
- I would set miRNA samples ready for RT-qPCR where specific nucleotides could be recognized, and a fellow graduate student would analyze the protein through Western Blotting.
- I was able to practice using the software, Prism, to create very in-depth figures, like the ones below.
- When time allowed, I would help others with their work, by completing scratch wound assays, Bradford assays, or just help set up equipment and reagents.



**Comparing miRNA Expression in both races before and after the addition of influenza vaccine.**

Many miRNA: 126,133,146a,221,222,and eNOS miRNA as well as eNOS protein concentrations were measured inside and outside of the cell (cellular medium). Only significant changes are shown above.



**The shape and structure of mircoRNAs (miRNA):**  
miRNA are short non-coding nucleotides that will prevent the formation (transcription) of proteins. This is accomplished by destroying the corresponding mRNA for that specific protein.

## Findings

- There was a decrease in the expression of eNOS mRNA between African American while no response in Caucasian American HUVEC cells
- miRNA 221, an inhibitor of eNOS and inflammation, had a significant increase in the cells in Caucasian Americans, but not African Americans
- Anti-inflammatory miRNAs decrease in extracellular media during induced inflammation
- Even with all these changes in miRNA, there was no significant difference in the expression of eNOS between the vaccine and basal conditions.

## Conclusions

- None of the miRNAs that we tested showed a significant difference in the decrease of eNOS expression after the vaccine was added
- There are racial differences in the release of miRNAs in induced inflammation in endothelial cells
- In the future, pro-inflammatory proteins and miRNAs can be analyzed in a similar ways to look at other mechanisms, other than eNOS, that regulate endothelial function

## Impact

- This research is important because it can play a role in what treatment is given for cardiovascular diseases, such as diabetes or even more recently COVID-19, and where the underlying differences may be coming from.
- On a more personal level, I really began to see science as a story rather than just pulling facts out of thin air. From asking a question, to researching previous knowledge, completing the lab work, and finally analyzing the results and making conclusions.
- I was really given a lot of freedom and space to work; as if I was doing graduate level research. When something did not work, such as my first samples in the RT-qPCR machine, I fixed this problem by thinking what might be wrong, changing something small, and eventually it worked.
- In November of 2019, I presented a PowerPoint at the Mid-Atlantic Regional Conference of the American College of Sports Medicine in Harrisburg PA.
- As somebody who is pursuing a career in scientific research and inquiry, and hopefully going to graduate school one day, this work was a very positive experience by transforming how I approach science in my mind but also my future classes.

## References

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- Kurian, A. K., & Cardarelli, K. M. (2007, December). Racial and ethnic differences in cardiovascular disease risk factors: A systematic review. *Ethnicity and Disease*, Vol. 17, pp. 143–152. <https://doi.org/10.13016/rsqw-zt1s>

