BIOGRAPHICAL SKETCH

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NAME: Stevens, Reece

eRA COMMONS USER NAME (credential, e.g., agency login): REECE_STEVENS

POSITION TITLE: Graduate Research Assistant

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Rhodes College	BS	08/2015	05/2019	Biochemistry & Molecular Biology
University of South Alabama	PhD	08/2019	08/2024	Basic Medical Sciences (Lung Biology focus)

A. Personal Statement

My interest in the pulmonary endothelium and its role in cardiopulmonary diseases became apparent during my time as a high school and undergraduate researcher in the Center for Lung Biology at the University of South Alabama. I first worked for Dr. Adam Morrow under a sponsored research program for high school students, studying how bacterial infection alters the bioenergetic state of pulmonary endothelial cells. During my undergraduate studies, I returned to the University of South Alabama for two summers under a T32 grant for short term trainees (HL076125) to continue research pertaining to the lung endothelium. I trained under Dr. Ronald Balczon to investigate cytotoxic amyloids released from the lung endothelium during pneumonia. My studies demonstrated that disruption of the amyloid protein structure eliminates cytotoxicity, and these results were incorporated into Dr. Balczon's recent publication on lung amyloids.

After receiving a bachelor's degree in biochemistry and molecular biology from Rhodes College, I decided to attend the University of South Alabama's Basic Medical Science PhD program due to the program's focus on lung endothelial research and contribution of the endothelium to cardiopulmonary disorders. After completing the first year in the program I received the **Edwin R. Hughes Memorial Award**, an accord given to the first-year student with the strongest academic performance. Currently, I am training under the co-mentorship of Dr. Ji Young Lee and Dr. Ronald Balczon to study the role of carbonic anhydrase IX in pH homeostasis and lung endothelial injury during pneumonia. My previous research experience and current mentorship has allowed for efficient development of my project, as I plan to have a first authored-manuscript ready for submission by the summer of 2021. In addition to garnering technical skills, the proposed training plan will provide opportunities for me to learn CRISPR/Cas9 and work with summer research students to further develop my mentoring skills. Completion of my proposed studies will lead into future *in vivo* experiments involving my labs' incoming carbonic anhydrase IX knockout mice. Such a training plan will aid my career development and pursuit to become an academic professor.

1. **Stevens R**, Paudel S, Johnson S, Stevens T, and Lee J. Endothelial Metabolism in Pulmonary Vascular Homeostasis and Acute Respiratory Distress Syndrome. *Am. J. Physiol. Lung Cell Mol Physiol*, in submission, 2021.
2. Lee J, **Stevens R**, Migaud M, Stevens T. Salvaging the endothelium in acute respiratory distress syndrome: A druggable intersection between TLR4 and NAD+ signaling. *Eur Resp J*, accepted for publication, 2021.

3. Balczon R, Morrow K, Stevens TC, **Stevens R**, Agwaramgbo E, Langham G, Francis C, and Stevens T. Cystatin C regulates the cytotoxiity of infection-induced endothelial-derived beta amyloid. *FEBS Open Bio,* 10: 2464-2477, 2020.
4. Lee J, **Stevens R**, Kash M, Zhou C, Koloteva A, Renema P, Paudel S, & Stevens T. KD025 shifts pulmonary endothelial cell bioenergetics and decreases baseline lung permeability. *Am J Respir Cell Mol Biol*, 63: 519-530, 2020.

B. Positions and Honors
List scholarships, traineeships, fellowships, and development awards

Positions and Employment

Fall 2017, Fall 2018	Foundations of Chemistry Lab Teaching Assistant, Rhodes College
2019 – present	Graduate Research Assistant, University of South Alabama

Professional Memberships

2018 – present	Member, Sigma Epsilon Honor Society
2018 – present	Member, TriBeta Biology Honor Society
2018 – present	Member, Mortar Board National College Senior Honor Society
2019 – present	Member, American Thoracic Society
2020 – present	Member, American Heart Association

Honors and Awards

Fall 2015, Spring 2018, Spring 2019	Honor Roll, Rhodes College
Spring 2016	Dean's List, Rhodes College
2015, 2018	Cross Country Academic All-American, Rhodes College
2015 – 2019	Southern Athletic Association Academic Honor Roll, Rhodes College
2019 – 2020	**Edwin R. Hughes Award** for best academic performance in first year basic medical science courses, University of South Alabama

C. Contributions to Science

1. **Bacterial infection suppresses pulmonary endothelial bioenergetics:** During my time in high school, I had the opportunity to train in the lab under Dr. Adam Morrow at the University of South Alabama, supported by a NIH-sponsored high school research fellowship. Dr. Morrow investigated the mechanisms for inhibiting repair in pulmonary microvascular endothelial cells (PMVECs) following *Pseudomonas aeruginosa* infection. PMVECs utilize aerobic glycolysis to meet their bioenergetic demands during proliferation, therefore my research project was to determine if bacterial infection impairs aerobic glycolysis in PMVECs. I infected PMVECs with *Pseudomonas aeruginosa*, treated the cells with antibiotics, and then subjected the cells to single cell cloning. Aerobic glycolysis acidifies the medium and can be detected by a medium color shift. Two weeks after single cell growth, I analyzed cell colony media color and found bacterial infection reduces the number of PMVECs utilizing aerobic glycolysis. My results suggested that PMVECs have impaired bioenergetics post infection, contributing to reduced vascular repair.
    1a. Stevens R, Hartman L, Balczon R, Morrow A, and Stevens T. ExoY impairs the rapid growth of pulmonary microvascular endothelial cells. Research day, summer medical research program, University of South Alabama College of Medicine, July 31, 2015.

2. **The chemical and physical properties of cytotoxic amyloids derived from lung endothelium:** During my undergraduate studies, I joined Dr. Balczon's lab at the University of South Alabama to continue investigating endothelial dysfunction after bacterial infection. Dr. Balczon and collaborators had recently discovered bacterial infection elicits the release of cytotoxic amyloid proteins from PMVECs, which may cause secondary end-organ damage in pneumonia patients**.** The chemical and physical nature of these infectious amyloid proteins were not well understood, therefore, my first summer research

project focused on characterizing the chemical properties and fluorescent signatures of cytotoxic amyloids derived from the lung endothelium**.** I isolated lung endothelial amyloids and then treated the proteins with 1,1,1,3,3,3-hexofluoro-2-propanol (HFIP), a chemical known to disrupt amyloid protein structure. Amyloids treated with HFIP did not display any toxicity to naïve PMVECs and had reduced fluorescence when exposed to Thioflavin T (ThT), a fluorescent dye that specifically binds to amyloid proteins. Thus, my results demonstrated HFIP disrupts the complex structure of cytotoxic amyloid variants that are derived from the lung endothelium, and in doing so, eliminates their cytotoxicity. These results proved fruitful as they were incorporated into Dr. Balczon's recent publication (2020).

The following summer, I worked with Dr. Balczon to determine if ThT could be repurposed as a clinical test to detect cytotoxic amyloids in pneumonia patients. I treated pneumonia patient blood samples with ThT and then scanned for fluorescence over a range of excitation and emission wavelengths. Next, I immunoprecipitated cytotoxic amyloid proteins from pneumonia blood samples, treated them with ThT, and repeated the fluorescent scan. Points of overlap in fluorescence between the pneumonia blood samples and isolated amyloid proteins were detected. Thus, my results identified unique fluorescent signatures of amyloid proteins in pneumonia blood samples, suggesting that ThT has the potential to be developed into a rapid bedside diagnostic test.

2a. Balczon R, Morrow K, Stevens TC, **Stevens R**, Agwaramgbo E, Langham G, Francis C, and Stevens T. Cystatin C regulates the cytotoxiity of infection-induced endothelial-derived beta amyloid. *FEBS Open Bio,* 10: 2464-2477, 2020.

2b. Berrou M, **Stevens R**, Voth S, Williams C, Balczon R, and Stevens, T. *Pseudomonas aeruginosa* induced lung endothelial amyloid proteinopathy: characteristics and inhibitors. *Am J Respir Crit Care Med*, 197: A5724, 2018.

2c. Stevens R, Francis M, and Balczon R. Chemical properties of endothelial cytotoxic amyloids. Research day, summer medical research program, University of South Alabama College of Medicine, July 28, 2017.

2d. Stevens R, Cioffi E, Voth S, and Balczon R. Analysis of cytotoxic amyloids from human pneumonia patients. Research day, summer medical research program, University of South Alabama College of Medicine, July 27, 2018.

3. **KD025, a ROCKII specific inhibitor, causes a bioenergetic shift in pulmonary microvascular endothelial cells in a manner independent of ROCKII:** Upon joining Dr. Lee's lab, I had the opportunity to contribute to her studies on KD025, an oral selective ROCK2 inhibitor currently being tested in phase II clinical trials for treatment of fibrotic lung diseases, including idiopathic pulmonary fibrosis and chronic graft-versus-host disease. Previous research has demonstrated KD025 modulates the immune system, pro-fibrotic pathways, and fat metabolism, but its effect on PMVECs was unknown. Pulmonary endothelial barrier integrity is dependent upon the inter-related functions of cell metabolism, pH homeostasis, and migration, so we tested to see if KD025 alters these cellular processes. We found KD025 causes a bioenergetic shift from aerobic glycolysis to oxidative phosphorylation, while decreasing intracellular pH, migration, and pulmonary permeability. Our work identified novel mechanisms of action that contribute to the therapeutic effect of KD025 and may be repurposed to treat other disease conditions.

3a. Lee J, **Stevens R**, Kash M, Zhou C, Koloteva A, Renema P, Paudel S, & Stevens T. KD025 shifts pulmonary endothelial cell bioenergetics and decreases baseline lung permeability. *Am J Respir Cell Mol Biol*, 63: 519-530, 2020.

3b. Stevens R, Kash M, Stevens T, Lee J. KD025, a ROCK2 specific inhibitor, increases oxidative phosphorylation and inhibits aerobic glycolysis, intracellular pH, migration and network formation in pulmonary microvascular endothelial cells in a ROCK2 independent manner. Virtual presentation at the American Thoracic Society, 2020.

4. **CA IX domain specific function and shedding mechanism in pulmonary microvascular endothelial cells during infection:** PMVECs express carbonic anhydrase IX (CA IX), a membrane bound carbonic anhydrase isoform that promotes pH homeostasis, cell migration, metabolism, and angiogenesis. CA IX is comprised of four domains, but the functions of these individual domains are unknown. My first predoctoral project is to elucidate the functional role of each CA IX domain in pH homeostasis, cell migration, metabolism, and angiogenesis. Characterizing each CA IX domain will improve our understanding on how CA IX promotes PMVEC homeostasis and repair during pneumonia. These

studies may also provide evidence that CA IX acts as a receptor, regulating the PMVEC response to injury and pH changes.

Recently, our lab discovered PMVECs shed CA IX from the membrane in response to infection, potentially compromising capillary pH homeostasis and repair. My second predoctoral project will investigate if CA IX shedding acts to buffer systemic blood pH at the cost of increased injury to the lung endothelium. Understanding the consequences of CA IX shedding may identify that the pulmonary endothelium has a novel role in systemic blood pH buffering, while acting as a therapeutic target specific to preserving the lung capillary during pneumonia.

4a. Stevens R, Balczon R, Weintraub S, Zhou C, Koloteva A, Renema P, Gwin M, Stevens T, & Lee J. Carbonic anhydrase IX is shed from pulmonary microvascular endothelial cells and increases in the plasma of rat and human pneumonia subjects. Virtual Presentation at the American Thoracic Society, 2020.

D. Additional Information: Research Support and/or Scholastic Performance

1. National Institutes of Health-Sponsored High School Research Fellow, Center for Lung Biology, College of Medicine, University of South Alabama, summer: 2015.

2. National Institutes of Health T32 Training Grant (HL076125) short-term trainee, Center for Lung Biology, College of Medicine, University of South Alabama, summer: 2017.

3. National Institutes of Health T32 Training Grant (HL076125) short-term trainee, Center for Lung Biology, College of Medicine, University of South Alabama, summer: 2018.