MCW
GRADUATE SCHOOL

Doctoral Dissertation Defense Announcement

Adaysha Williams, MSc

"Hyperphosphatemia and microRNA-21 as mediators of chronic kidney disease-related cardiac dysfunction"

Candidate for Doctor of Philosophy in Physiology

Basic & Translational Science Concentration

School of Graduate Studies

Medical College of Wisconsin

Committee in Charge:

Alison Kriegel, PhD (Mentor)

Andreas Beyer, PhD

Ranjan Dash, PhD

Christian Faul, PhD

Caitlin O'Meara, PhD

Date: Thursday, June 13, 2024

Time: 10:30AM (CST)

Defense Location: Kerrigan Auditorium

https://mcw-edu.zoom.us/j/91279554079?pwd=MzRqUFJkcmgxTE5BR3MrSk1kbDF3dz09

Zoom Meeting ID: 912 7955 4079 **Passcode:** KFZ4fyfS

Graduate Studies:

Foundations in Biomedical Sciences I

Foundations in Biomedical Sciences II

Foundations in Biomedical Sciences III

Foundations in Biomedical Sciences IV

Techniques in Molecular & Cell Biology

Professional Development I

Organ Systems Physiology

Understanding Cell Signaling – Therapeutics Drugs

Current Topics in Physiology

Special Problems in Physiology

Biostatistics Health Sciences

Ethics & Integrity in Science

Physiology Seminar

Complement to Graduate Human Physiology

Graduate Human Physiology

Functional Genomics

Fundamental Practice & Experience in Grant Writing

Current Concepts in Cardiovascular Biology

Research Ethics Discussion Series

Basic & Translational Science Seminar

Boundaries of Science and Medical Practice

Reading and Research

Doctoral Dissertation

Dissertation

“Hyperphosphatemia and microRNA-21 as mediators of chronic kidney disease-related cardiac dysfunction”

Chronic kidney disease (CKD) is a condition diagnosed by a drop in the glomerular filtration rate (GFR) below 60mL/min. This is a clinical indication that the ability of the kidneys to filter blood has become severely impaired. It results in a buildup of waste products known as “uremic toxins,” within the circulation. Individuals with kidney failure (GFR<15 mL/min) must either undergo dialysis to remove circulating waste products or receive a healthy transplanted kidney.

Approximately 90% of people with CKD will develop chronic cardiovascular impairment and eventually, cardiac disease. Individuals with CKD that develop chronic cardiac dysfunction are classified as having cardiorenal syndrome type 4 (CRS4). Our laboratory has utilized the 5/6 nephrectomy (5/6Nx) model in rats as a surrogate to study CRS4 pathology. In this model approximately 5/6 of the total kidney mass is surgically removed, significantly reducing GFR without causing renal failure. Significant pathological changes characteristic of CRS4 in humans are observed within the first 7 weeks following 5/6Nx surgery including uremia, proteinuria, vascular and myocardial calcification, dyslipidemia, left ventricular hypertrophy, and diastolic dysfunction.

A major focus of our work in the 5/6Nx model has been to understand molecular mechanisms that mediate the development of cardiac pathology and dysfunction in CRS4. Bulk RNA sequencing reveal changes in left ventricle expression of mRNAs and microRNAs from 5/6Nx rats, when compared to sham-operated controls. MicroRNAs are small non-coding RNAs that typically inhibit expression of M RNA's by complementary binding leading to either transcript degradation or translational repression. The upregulation of microRNA miR-21-5p and left kidney tissue coincided with diastolic dysfunction and cardiac remodeling in 5/6Nx rats 7-weeks after surgery. Analysis of mRNA expression changes led to the identification of mir-21-5p as an inhibitor of PPARα protein expression in left ventricle tissue. Subsequent work determined that miR-21-5p plays a role in switching cardiac metabolism from fatty acid oxidation to glycolysis through its regulation of peroxisome proliferator activated receptor alpha (Ppara), and possibly other mechanisms. While this increase in left ventricle miR-21-5p was clearly functionally important, the stimulus driving the upregulation of miR-21 in CKD was unknown.

We hypothesized that the upregulation of left ventricle miR-21 could be driven by high levels of uremic toxins with the advancement of CKD/CRS4. Hyperphosphatemia, or elevated serum phosphate, was of particular interest because of its prevalence in individuals with advanced CKD and its associations with left ventricular hypertrophy, atherosclerosis, hypertension, and even all cause morbidity and mortality. Phosphate is taken into the body from our diet and the kidneys play an important role in regulating its elimination through the urine. Additionally, chronic hyperphosphatemia has been linked to many cellular processes—including pathologically altered metabolism—which makes it an enticing target of study.

Therefore, the focus of this thesis was to determine if the elevation in circulating phosphate (hyperphosphatemia) in the 5/6Nx model would lead to cardiac pathology, particularly through inducing miR-21-5p expression in the heart. We studied this potential interaction via two aims, utilizing both in vitro and in vivo models. Aim 1 of this thesis was

to determine if miR-21-5p upregulation mediates hyperphosphatemia-induced changes on a variety of cellular phenotypes using a novel miR-21 knockout (miR-21$^{-/-}$) line of H9C2 rat cardiomyoblast cells generated in our laboratory. Wild-type (WT) H9C2 cells treated with high-phosphate (HP; 5 mM) medium for 24-hours exhibited impaired proliferation with a concordant upregulation of miR-21-5p. Conversely, miR-21$^{-/-}$ cells exhibited augmented proliferation in response to the same treatment. Hypoxia-inducible factor 1α (HIF1α), a well-known driver of miR-21 expression was increased in WT cells with as little as 60 minutes and 2.5 mM HP treatment, but not 24-hours post-treatment and was unlikely to explain the chronic elevation in miR-21-5p. HP treatment (5 mM, 24-hrs) increased cellular oxygen consumption in WT, but not miR-21$^{-/-}$ H9C2 cells, when compared to those treated with an osmotically controlled medium. Together these results suggest that augmented cellular respiration and reduced proliferation in WT cells treated with HP medium is mediated by miR-21-5p.

To understand the effects of these interactions at the whole animal level, we took two complementary strategies: 1) inducing hyperphosphatemia with a HP diet (CKD-independent) in WT and miR-21$^{-/-}$ rats, and 2) attenuating CKD-related hyperphosphatemia with delivery of a phosphate binder, ferric citrate. The goal of this second aim was to elucidate the roles of elevated serum phosphate and cardiac miR-21-5p on cardiac pathology in the left ventricle of sham and 5/6Nx rats. In the first animal study, adult male and female Sprague Dawley rats that were homozygous for a global deletion of miR-21 (miR-21$^{-/-}$), and WT littermates, were placed on either a normal phosphate (NP; 0.6%) or HP (2% Pi) diet for eight weeks while undergoing bi-weekly cardiac and renal ultrasound analysis. Blood biochemistry, renal function, and tissue pathology were examined at the end of the study. Wall thickness during both systole and diastole was increased in male and female miR-21$^{-/-}$ rats, compared to WT rats. Though clinical literature suggests associations with both miR-21 and phosphate and adverse cardiac outcomes in humans, we did not observe any significant differences in left ventricle function between groups compared by genotype, diet, or sex in rats. In the second study, adult male and female WT Sprague Dawley rats underwent either a sham or 5/6Nx operation. Sham animals and half of the 5/6Nx rats were maintained on a normal phosphate (NP) diet, while the other half of the 5/6Nx rats were fed the NP diet supplemented with 4% ferric citrate, a clinically used dietary phosphate binder. Phenotypic measurements described in the first animal study were also performed here. Ferric citrate dietary supplementation reduced renal cross-sectional area and phosphate levels in both serum and left ventricle tissue, when compared to 5/6Nx rats fed the NP diet. Male 5/6Nx rats that did not receive ferric citrate had significantly more LV phosphate than female rats. Despite the efficacy of ferric citrate in lowering phosphate levels, longitudinal echocardiography did not identify any measurable protection from CKD-related left ventricle remodeling or changes in function. Interestingly, male animals that underwent 5/6Nx had elevated LV miR-21 levels, while female rats given ferric citrate had significantly higher levels in comparison to 5/6Nx controls but comparable levels to sham animals.

Overall, this thesis highlights the complex nature of microRNA physiology with respect to secondary cardiac consequences as a result of primary renal injury. The results presented here have broadened our understanding of the effects of hyperphosphatemia, which is in part mediated via miR-21, highlight cardiorenal crosstalk at a molecular level. In our in-vitro model, miR-21 and phosphate interactions dictate changes in cellular metabolism

and proliferation. The animal studies revealed a sexual divergence in both miR-21 expression and phosphate handling; with female rats exhibiting apparent cardioprotection despite worsened renal function with HP diet. This finding may be of clinical importance because of differences in phosphate handling between men and women, with healthy women having higher phosphate excretion rates than men. Further, the prescription of phosphate binders at early disease stages, particularly in women, may be beneficial in reducing cardiac hypertrophy and renal damage.

Adaysha C. Williams, MSc
Curriculum Vitae
adcwilliams@mcw.edu

EDUCATION

July 2019 – Present **Medical College of Wisconsin, Milwaukee, WI**
PhD Candidate, Department of Physiology
Concentration in Basic & Translational Science

Sept 2018 – Aug 2019 **Dublin City University, Dublin, Ireland**
Masters in Business Management

July 2013 – May 2017 **Sacred Heart University, Fairfield, CT**
Bachelor of Science in Biology
Minor in Chemistry

TEACHING EXPERIENCE

Sept 2023 – Dec 2023 **Meharry Medical School, Nashville, TN**
Adjunct Instructor of Cardiovascular Section
Fundamentals of Human Physiology

Sept 2021 – Dec 2023 **Carroll University, Waukesha, WI**
Adjunct Lecturer and Instructor
General Biology I & II Laboratory
Genetics Lecture and Laboratory

PROFESSIONAL, LEADERSHIP, & COMMUNITY SERVICE

Aug 2021 - 2024 Medical College of Wisconsin – Graduate School
Academic Coach

June 2022 – 2023 Medical College of Wisconsin – Graduate School
Public Relations Officer – Graduate Student Association
Social Student Rep
Diversity, Inclusion, and Equity GSA Student Rep
Travel Award Policy Study Rep

May 2022 Medical College of Wisconsin
Staging Party Student Hooder
Representative for the Graduate School

June 2021 – 2022 Medical College of Wisconsin – Graduate School
President – Graduate Student Association
*Committee Involvement (*denotes chair position)*
Diversity, Inclusion, and Equity GSA
Mental Health Climate
GSA Symposium*
Student Assembly, GSA Student Rep

Assessment Oversight
Commencement Committee, GSA Student Rep
Graduate Studies Council
Program Evaluation
Spotlight on Science
Travel Award Policy*

June 2020 – 2021 Medical College of Wisconsin – Graduate School
Department of Physiology Student Rep
Committee Involvement
Diversity, Inclusion, and Equity
Assessment Oversight
Program Evaluation
Spotlight on Science
Curriculum Evaluation
Professionalism
Awards

Sept 2018 – May 2019 Sports Changes Life, Ireland
Victory Scholar

Aug 2018 – Present Black Leaders Acquiring Collective Knowledge (B.L.A.C.K.)
Trip Counselor (Southeastern U.S. – Aug 2018)
Youth Leadership Advisor (La Crosse, WI – Feb 2018)

STUDENTS MENTORED AT MEDICAL COLLEGE OF WISCONSIN

Aug 2023 – Feb 2024 Olivia Allen, IDP Student Mentee
Pursuing PhD in Biochemistry at MCW

Summer 2023 Joel Pan, Cardiovascular Center Trainee
Pursuing BS at University of Wisconsin-Madison
Bryn Gonzalez, ROADS (Research Opportunity in Academic Development in Science) Intern
Whitefish Bay High School Senior
Katherine Lizarrage Mazaba, ROADS Intern
St. Joan Antida High School Senior
John Obiefuna, SPUR Intern
Pursing BS at University of North Carolina-Chapel Hill

June 2022 – Dec 2022 Jeylan Zemaj, Medical Student
Former Medical Student at MCW

Summer 2022 Sophia Aliaga, SPUR Intern

Fall 2020 Alisha Ziegler, IDP Student Mentee
Former PhD candidate at MCW; defended Apr 2024

HONORS & AWARDS

Apr 2024	Best Poster Presentation Conference for Black Physiologists – Black in Physiology
June 2023	Underrepresented Minority Scholarship Keystone Symposia – Heart Failure: All Cells Considered
Apr 2023	Martin Frank Diversity Travel Award American Physiology Society (APS)
June 2022	Underrepresented Minority in Biomedical Research Scholar Award Medical College of Wisconsin – Graduate School
Mar 2022	Top Scored Abstract Award Medical College of Wisconsin – GSA Symposium
July 2021-24	NIH T32 Training Grant Appointee Medical College of Wisconsin – Department of Physiology
July 2020-21	National Research Service Award (NRSA) Training Grant Appointee Medical College of Wisconsin – CTSI
Aug 2018 – 2019	Full Athletic Scholarship, women's basketball player Dublin City University, Dublin, Ireland
July 2013 – 2017	Full Athletic Scholarship, NCAA Div. 1 women's basketball player Sacred Heart University, Fairfield, CT

PUBLICATIONS

Peer-Reviewed

1. Nasci, V.L., Liu, P., Marks, A.M., **Williams, A.C.**, and Kriegel, A.J., "Transcriptomic analysis identifies novel candidates in cardiorenal pathology mediated by chronic peritoneal dialysis," Sci Rep, 13(1), 2023, pp. 10051. doi: 10.1038/s41598-023-36647-7

2. **Williams, A.C**., Singh, V., Liu, P., and Kriegel, A.J., "Liquid Biopsies poorly mirror Renal Ischemia-Reperfusion Injury," Noncoding RNA, 9(2), 2023, pp. 24. doi: 10.3390/ncrna9020024.

3. Adam, R.J., **Williams, A.C.**, and Kriegel, A.J., "Comparison of the Surgical Resection and Infarct 5/6 Nephrectomy Rat Models of Chronic Kidney Disease," AJP Renal, 322(6), 2022, pp. F639-654. doi:10.1152/ajprenal.00398.2021

Abstracts

1. **Williams, A.C.**, Yunker, L., and Kriegel, A.J., “Cardiorenal Effects of Chronic High Phosphate Diet in miR-21 knockout rats” Proceedings of American Physiology Society Summit; Long Beach, CA. Apr 2024

2. **Williams, A.C**., Yunker, L., and Kriegel, A.J., “Cardiorenal Effects of Chronic High Phosphate Diet in miR-21 knockout rats” Conference for Black Physiologists; Long Beach, CA. Mar 2024

3. **Williams, A.C.** and Kriegel, A.J., “High inorganic phosphate treatment reduces rat embryonic cardiomyoblast viability and upregulates transcription of microRNA-21 expression in an in-vitro model of Cardiorenal Syndrome Type IV,” Proceedings of Keystone Symposia, Heart Failure: All Cells Considered; Santa FE, NM. June 2023

4. **Williams, A.C.**, Singh, V., Liu, P., and Kriegel, A.J., “Limited overlap between plasma, renal, and urinary microRNAs altered by ischemia reperfusion-induced Acute Kidney Injury (AKI)” Proceedings of American Physiology Society Summit; Long Beach, CA. Apr 2023

5. **Williams, A.C.**, Singh, V., Liu, P., and Kriegel, A.J., “Limited overlap between plasma, renal, and urinary microRNAs altered by ischemia reperfusion-induced Acute Kidney Injury (AKI)” Conference for Black Physiologists; Long Beach, CA. Apr 2023

6. **Williams, A.C.**, Marks, A.M., and Kriegel, A.J., “Apelinergic System: an in-vitro investigation of Inflammatory & Oxidative Stress in Chronic Kidney Disease,” Proceedings of Experimental Biology, Philadelphia, PA. Apr 2022.

7. **Williams, A.C.** and Kriegel, A.J., “Investigation of the Apelingeric System on Oxidative Imbalance within Cardiorenal Syndrome Type 4,” Proceedings of Translational Science Conference; Washington D.C., USA, Apr. 2021.

8.Sajdah, B.S., Salmon, A.E., **Williams, A.C.**, Merriman, D.K., Carroll, J., “Assessing seasonal changes of cone photoreceptor structure in the 13-lined ground squirrel,” Proceedings of ARVO Annual Meeting; Honolulu, HI, May 2018.