Doctoral Dissertation Defense Announcement

**Gopika SenthilKumar**

“The Yin and Yang of Ceramide’s Role in the Human Microcirculation”

Candidate for Doctor of Philosophy in Physiology
Department of Physiology
Department of Anesthesiology
Graduate School of Biomedical Sciences
Medical College of Wisconsin

Committee in Charge:
**Julie K. Freed MD PhD (Thesis Advisor)**
David Gutterman MD
Melinda Dwinell PhD
Hershel Raff PhD
Jennifer Pollock PhD

**Wednesday, February 28, 2024**
1:00 PM – 2:00 PM CST
Bolger Auditorium

**Zoom Meeting ID:** 973 0548 4680 **Passcode:** F1Yjx2qD
https://mcw-edu.zoom.us/j/97305484680?pwd=S3oxZ0Fyb1orRmsrMnBOR1k4cGJIUT09
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Dissertation Summary

The Yin and Yang of Ceramide’s Role in the Human Microcirculation

Microvascular endothelial dysfunction, in both the coronary and peripheral circulation, is an independent and strong predictor of poor cardiovascular outcomes. Microvascular dysfunction can also precede the development of large vessel diseases, and thus strategies that protect the microvessels can greatly reduce cardiovascular disease (CVD) morbidity and mortality. Prior work from our lab showed that chronic exposure to ceramides, a family of bioactive sphingolipids that when elevated in the plasma are associated with increased risk of future adverse cardiac events, can induce microvascular endothelial dysfunction in ex vivo arterioles collected from otherwise healthy adults. This presents as a switch from the endothelial production of the vasoprotective dilator nitric oxide (NO) to pro-oxidative hydrogen peroxide (H_2O_2) in response to shear stress. NO dependent dilation to shear stress is a physiologically critical phenomenon for maintaining proper organ perfusion as well as for an overall healthy surrounding tissue environment. As such, our work, along with ample preclinical studies linking ceramides with endothelial damage, has sparked great interest in developing therapeutics that lower ceramide formation in an attempt to prevent and treat CVD. As such, the primary objective of my PhD thesis was to evaluate whether inhibition of neutral sphingomyelinase (NSmase), a critical shear-sensitive source of ceramide formation in endothelial cells, can promote beneficial human microvascular health and therefore be utilized for preventing CVD.

Surprisingly, our results revealed that inhibition of NSmase triggers microvascular endothelial dysfunction in ex vivo human arterioles collected from otherwise healthy adults and in an isolated human endothelial cell model. Specifically, inhibition of NSmase diminished endothelial NO production during shear stress, and acutely upregulated NADPH-oxidase 2 (NOX-2) dependent H_2O_2 production. Further, our data showed that ceramides can acutely exert beneficial NO producing effects on the endothelium via its conversion to sphingosine-1-phosphate (S1P) and subsequent activation of S1P-receptors 1 and 3 (S1PR1/S1PR3). Together, this work shows that acute ceramide production and subsequent activation of S1P signaling is actually necessary for proper microvascular endothelial functioning. Significantly lowering ceramide formation via NSmase may thus propagate, rather than prevent, CVD.

I next asked whether activation of this NSmase/Ceramide/S1P/NO pathway can help maintain beneficial endothelial health. Estrogen, a well-known steroid hormone that is linked with protective cardiovascular effects in preclinical studies, has been shown to activate and increase expression of the enzymes in the sphingolipid pathway. As such we evaluated the influence of estrogen on human microvascular endothelial function. Our results showed that acutely, estrogen induced NO mediated dilation of arterioles from otherwise healthy females via the NSmase/Ceramide/S1P signaling pathway. However, chronic exposure to estrogen resulted in the accumulation of ceramides within endothelial cells. This was associated with microvascular dysfunction in ex vivo arterioles from both biological males and females. Inhibition of ceramide formation via NSmase as well as treatment with a mitochondrial antioxidant MitoTempol, protected
against estrogen-induced endothelial dysfunction in both sexes. To explain these seemingly paradoxical effects of ceramides, we hypothesized that while acute ceramide signaling may promote beneficial NO production via S1P signaling, their chronic accumulation may trigger endothelial dysfunction via the upregulation of mitochondrial oxidative stress.

To evaluate this, we selected a well-established model of endothelial dysfunction – arterioles from patients with CAD – which are known to rely on mitochondrial H$_2$O$_2$ for dilation during shear and have high accumulation of endothelial ceramides. Chronically inhibiting ceramide formation in these arterioles has also been shown to reverse endothelial dysfunction – similar to what was seen in arterioles chronically exposed to estrogen. Using this model, we demonstrated that acute ceramide/S1P signaling results in the production of H$_2$O$_2$ as opposed to NO, and this was associated with the activation of S1PR3 rather than S1PR1. This shift in S1P-receptor signaling was not driven by protein expression changes. Further, our data showed that acute ceramide production via NSmase in response to shear, directly activated the compensatory mitochondrial H$_2$O$_2$ production in arterioles from patients with CAD – independent of ceramide’s conversion to S1P. This work sheds light on critical ceramide/S1P signaling changes that are seen in arterioles with high intracellular ceramide accumulation, that then contribute to detrimental H$_2$O$_2$ production as opposed to the beneficial NO production seen in healthy arterioles.

In conclusion, our work challenges the paradigm that ceramides are solely detrimental to the vasculature and created the foundation that ceramides are critical for maintaining a healthy endothelium. Therefore, preventative therapies that aim to significantly reduce ceramide formation may in reality be detrimental to the cardiovascular system. Furthermore, we shed light on why ceramides are paradoxically linked with poor cardiovascular outcomes in patients, by showing that their chronic accumulation results in altered sphingolipid signaling as well as mitochondrial damage. Preventing these S1P signaling changes and protecting the mitochondria may thus be a better therapeutic strategy for protecting the microvasculature. Finally, these acute versus chronic ceramide-signaling differences provide needed insight into why estrogen has been linked with acute NO-producing effects in the preclinical setting but can also increase cardiovascular disease risk with chronic exposure (e.g. oral contraceptives, gender affirmation). Inhibiting NSmase may be a potential strategy for protecting cardiovascular health in patients undergoing chronic estrogen therapy.
Curriculum Vitae
Gopika Senthilkumar
gsenthilkuma@mcw.edu

EDUCATION
June 2019 – Present
Medical College of Wisconsin, Milwaukee, WI
MD-PhD Candidate, Medical Scientist Training Program.

Aug 2015 – May 2019
University of Wisconsin at Madison, College of Engineering, Madison, WI
Bachelor of Science in Biomedical Engineering.

GRANT FUNDING
Jan 2022 – Dec 2023
Title: The necessary role of ceramide within the human microvascular endothelium.
Source: American Heart Association Pre-Doctoral Fellowship
Mentors: Julie K Freed MD PhD, David D. Gutterman MD
Amount: $64,072

AWARDS AND HONORS
Jan 2024
2024 Cardiovascular Section Outstanding Graduate Student Trainee Award Finalist, American Physiology Society

Nov 2023
Elaine W. Raines Early Career Investigator Award Competition Winner, American Heart Association

Sept 2023
Diversity Equity & Inclusion Conference Award 2023, Association of Women Surgeons and Society of Asian Academic Surgeons

July 2023
2023 Best Poster Award, National MD-PhD Student Conference

July 2023
2023 Diversity Travel Award, National MD-PhD Student Conference

Feb 2023
2023 Cardiovascular Section Research Recognition Award, American Physiology Society

Feb 2023
Association for Academic Surgery Outstanding Medical Student Presentation Award, Academic Surgical Congress 2023

Nov 2022
32nd Annual Graduate School Research Poster Competition Winner, MCW

Sept 2022
2022 MCW Innovations in Healthcare Education Research Annual Conference Poster Competition Winner

Sept 2022
2022 American Heart Association Scientific Sessions ATVB Travel Grant for Early Career Investigators

April 2022
Graduate Student Association Symposium Top Oral Presentation Award, MCW

Feb 2022
Education Research Session Winner – Advancement of Women in Science and Medicine 2022 Symposium Poster Competition, MCW

Feb 2022
Laboratory/Clinical Research Session Winner – Advancement of Women in Science and Medicine 2022 Symposium Poster Competition, MCW

Feb 2022
2022 Experimental Biology Travel Stipend, Wiggers Award Featured Topic Speaker

Dec 2021
2021 MCW Cardiovascular Center Retreat Top 3 Abstract Stand Out Honor

Nov 2021
31st Annual Graduate School Research Poster Competition Winner, MCW

Nov 2021
New Trends in Sex and Gender Medicine Diversity Conference Scholarship, American Physiological Society

Feb 2021
American College of Physicians National Abstract Competition Winner

Oct 2020
Women in Science Student Award, Advancement of Women in Science and Medicine

Nov 2019
Smart Cities Smart Futures Foxconn Innovation Competition Round 1 Winner

April 2019
American Association of Cancer Research Undergraduate Award 2nd place

Aug 2018
Donald W. Novotny Excellence in Engineering Scholarship, UW Madison

April 2018
Biomedical Engineering Design Excellence Award 2nd Place Winner, UW Madison

Aug 2017
Grotophorst, Carl and Henry Scholarship in Engineering BME, UW Madison
Aug 2017  Berbee Walsh Foundation Prototype Pathway Scholarship
Aug 2016  University of Wisconsin Madison Dean’s Scholarship
Aug 2016  University of Wisconsin Madison Dean’s Engineering Scholarship
Aug 2016  Society of Women Engineers Scholarship
Aug 2016  Madison Education Start-Up Weekend 2nd Place Winner
Aug 2015  Wisconsin Alumni Association Scholarship, Waukesha County Chapter
Aug 2015  Emerging Leaders Inaugural Philanthropic Youth Award, United Way of Greater Milwaukee and Waukesha County

NATIONAL ELECTED/ APPOINTED LEADERSHIP AND COMMITTEE POSITIONS

2023 – Present  Women's Leadership Committee Early Career Member, Council on Arteriosclerosis, Thrombosis and Vascular Biology, American Heart Association
2023 – Present  International Committee Member, American Physiology Society
2022 – Present  Cardiovascular Section Trainee Committee Member, American Physiology Society
2023 – Present  Vice Chair, Association of Women Surgeons National Medical Students Committee
2023 – Present  Communication Committee Associate Member, Society of Asian Academic Surgeons
2022 – Present  Conference Planning Committee, Association of Women Surgeons
2021 – 2023  Mentorship Coordinator, Association of Women Surgeons National Medical Students Committee
2022 – 2023  Executive Director, F1 Doctors (National student-led volunteer mentorship organization for international students pursuing medicine in US)
2021 – 2022  Research and Quality Improvement Coordinator, F1 Doctors

EDITORIAL BOARDS AND JOURNAL/ABSTRACT/GRANT REVIEWS

Editorial Board:
2023 – Present: Assistant Editor, American Journal of Surgery

Journal Review:
Journal of Applied Physiology
American Journal of Physiology-Heart and Circulatory Physiology
Microcirculation
The American Journal of Surgery
Clinical Interventions in Aging
Journal of Inflammation Research
Journal of International Students
Journal of Vascular Research (ad hoc)
Circulation Research (ad hoc)
Circulation (ad hoc)
Nature Communications (ad hoc)
Journal of American Heart Association (ad hoc)

Conference Abstract Review:
American Heart Association Scientific Sessions National Conference, 2023
American Physiology Summit, 2023 - Endocrinology & Metabolism Section
Advancement of Women in Science and Medicine Research Symposium, 2023
American Heart Association Scientific Sessions National Conference, 2022

PROFESSIONAL DEVELOPMENT

2019 – Present  4C Coaching Program for Character, Caring, and Competence, Kern Institute for the Transformation of Medical Education
2023  Reviewer Certificate Program, American Physiology Society
2021  Center for the Improvement of Mentored Experiences in Research (CIMER) – Mentoring Up Training for Trainees
2021  Focus on Peer Review, Nature Masterclasses, Nature Research
2020  Cultivating the Entrepreneurial Mindset, Center for Professional Development, Stanford University
2019  Adult & Pediatric CPR/ AED & FIRST AID
2018  Human Centered Design Summer Intern, Massachusetts Institute of Technology Integrated Design and Management program.

LOCAL INVOLVEMENTS, SERVICE, TEACHING AND MENTORSHIP

2023 – Present  Women’s History Month Symposium Planning Committee, Center for the Advancement of Women in Science and Medicine
2021 – Present  Association of Women in Medicine Research Group, Center for the Advancement of Women in Science and Medicine
2022 – Present  Medical Scientist Training Program Admissions Interviewer, MCW
2021, 2022, 2023  Instructor, SUPREMES (Students Understanding Principles of Research Education through Medicine, Engineering, and Science), Marquette University, Milwaukee WI
2022 – 2023  Clinical Continuity Track Volunteer for Saturday Clinic for the Uninsured
2023  Oral Presentation Competition Judge, Innovations in Healthcare Education Conference
2022 – 2023  International Graduate Health Student Org Co-Founder and Co-Lead
2022 – 2023  G2 Student Council Representative, Medical Scientist Training Program
2022, 2023  Organized free Information and Q&A sessions via zoom for students from first-generation immigrant families throughout the US interested in medicine. Attendance ranges from 30-80 students and family members.
2022, 2023  Poster Competition Judge, Advancement of Women in Science and Medicine Research Symposium
2021 – 2023  Medical School Admissions Interviewer, MCW
2019 – 2023  Instructor/small group facilitator and grader for writing assignments, Student Health Initiative Pipeline Program, MCW
2022  Volunteer COVID Vaccinator
2021 – 2022  Community Service Chair, Association of Women Surgeons MCW Chapter; Established a partnership with Riverside High School, Milwaukee - monthly journal clubs and bi-yearly biomedical careers Q&A sessions.
2021 – 2022  Tutor, Academic Enhancement USMLE-STEP1 MCW
2020 – 2021  Co-President for Research and Development, Student Leadership Development Initiative Student Organization
2020 – 2021  Co-Chair of Kern Institute Student Leadership Committee. Robert D. and Patricia E. Kern Institute for the Transformation of Medical Education
2020  Transformational Ideas Initiative Seed Grant, Developing Innovative Methods to Overcome the Under-representation of Women in Healthcare Leadership. Robert D. and Patricia E. Kern Institute for the Transformation of Medical Education.
2020  M2 Student Representative, Discovery Curriculum Exploration Committee
2020  Small group facilitator, REACH (Recognize, Empathize, Allow, Care, Hold each other up) for first year medical students
2020  Small group facilitator, Medical School Orientation
2020  Science Fair small group instructor, Wauwatosa STEM Middle School
2020  Communication Co-Chair, Milwaukee COVID Sitters
2019 – 2020  M1 Liaison, Executive Board for Student Health Initiative Pipeline Program.

Undergraduate Students Mentored
2023 – Present  Zachary Zirgibel, University of Wisconsin Oshkosh, Program for Undergraduate Research Experience.
Summer 2022, 2023  Carolyn Shult, University of Wisconsin Eau Claire, Summer Program for Undergraduate Research, MCW. Received Cardiovascular Center SPUR Award for funding student
2022 – 2022 Elise Raschke, Marquette University Biomedical Engineering, Program for Undergraduate Research Experience.

2021 – 2022 Nicole Buiter, Wisconsin Lutheran College, Undergraduate Thesis Student – Program for Undergraduate Research Experience, MCW. Current MCW PhD student.

**Panel Memberships**

Aug 2023 Medical and Graduate Student Panel. Student-Centered Pipeline to Advance Research in Cancer Careers (SPARCC), MCW.

June 2023 My Journey Luncheon - American Heart Association’s Supporting Undergraduate Research Experience Program, MCW.

Nov 2022 Tips for Navigating the USMLE Step 1 and Step 2 CK Exams. MCW Student Health Sciences Conference, MCW. Moderator.

Oct 2022 Students of MCW: Exuding Professionalism in Medical Education. Professionalism Week, MCW.

July 2022 Career exploration panel. MEDAL (Medicine, Engineering, Dentistry, Architecture, and Law) Program for MKE area middle schoolers, MCW.

July 2022 Medical Careers Panel. American Indian Science Scholars Program for high school students, MCW.

June 2022 Medical Student Panel. American Heart Association’s Supporting Undergraduate Research Experiences.

April 2022 Career exploration panel for Biomedical Engineers. Marquette Biomedical Engineering Student Association.

Feb 2022 The Do’s and Do Not’s of Mentorship for Medical Students. Association of Women Surgeons.

Nov 2021 Pre-health advising for international students. Brown University, Rhode Island.

Sept 2021 Helping students become influencers: Promoting intentional involvement and building on strengths. Professionalism Week, MCW. Moderator.

April 2021 Presentation and Q&A for International pre-med applicants. University of Toledo, Ohio.

July 2019 Q&A panel session for international MD-PhD applicants. UCLA Med X International, California.

2020 – 2021 MCW Admissions Office. MCW Interview Day Q&A, International Students Q&A, Community Engagement and Volunteering in Milwaukee Q&A sessions. MCW.

Aug 2020 Career exploration panel for Student-Centered Pipeline to Advance Research in Cancer Careers (SPARCC), MCW.

**PEER-REVIEWED BIBLIOGRAPHY FROM PHD**

*Full Peer-Reviewed Bibliography – [Google Scholar]*

**PUBLICATIONS**


**Currently Undergoing Peer-Review**
1. **SenthilKumar G**, Freed JK. Role of Sphingolipids in Human Microvascular Acetylcholine-Induced Dilation. *Atherosclerosis, Thrombosis, and Vascular Biology.*

**Currently Under Preparation**

**ABSTRACTS**

**International**

**National**


Regional/Local


4. Gopika SenthilKumar, Boran Katunaric, Henry Bordas-Murphy, Julie K. Freed. Exogenous Sphingosine-1-Phosphate Restores Nitric Oxide-Mediated Flow-Induced Dilation During Acute Inhibition of Ceramide Formation. (1) 32nd Annual Graduate School Research Poster