BIOGRAPHICAL SKETCH

NAME: Headley, Colwyn Ansel

eRA COMMONS USERNAME: CAHEADLEY

POSITION TITLE: Post-Doctoral Fellow

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	COMPLETION	FIELD OF STUDY
The Ohio State University, OH	B. S	05/2014	Microbiology
The Ohio State University, OH	Ph.D.	05/2020	Biomedical Sci. - Immunology
Texas Biomedical Research Institute, TX	-	5/2021	Host-Pathogen Interactions
Stanford University, CA	-	-	Cardiovascular Medicine

A. Personal Statement

Aging-associated mitochondrial dysfunction affects every cell system in our body and contributes to a myriad of pathologies, including cardiovascular diseases, neurodegenerative diseases, and immune exhaustion and senescence. My passion lies in investigating the complex interplay between aging-associated mitochondrial dysfunction and immune dysregulation, which are both causes and consequences of various chronic pathologies in the elderly, including cardiovascular disease (CVD). My primary goal is to elucidate the contributions of mitochondrial dysfunction to immune dysregulation during aging and explore the therapeutic potential of mitochondrial transplantation for improving health outcomes in the elderly. I aim to pioneer the use of mitochondrial transplantation as a transformative health-span extension therapeutic.

The research I am proposing is informed by my extensive experience related to oxidative stress and mitochondria (11+ years) and aging and immunity (8+ years). Regarding oxidative stress, I have investigated the effects of reactive oxygen species (ROS) and antioxidants using in vitro disease models and developed novel protocols and probes to detect the subcellular compartmentalization of antioxidants delivered to cells **[1]**. My contributions collectively generated new insights into how cellular and mitochondrial dysregulation affects redox signaling in cells.

During my graduate training in immunology and host-pathogen interactions, I became particularly interested in mitochondrial dysfunction in T cells and their role in aging-associated low-grade chronic inflammation. Drawing on my background in oxidative stress research, I hypothesized that mitochondrial dysfunction could be a primary driver of age-related T cell dysregulation. I independently developed a research strategy to transplant healthy mitochondria into T cells from aged mice and demonstrated that this could effectively rescue adaptive immune responses *in vivo* **[2,3]**.

These results opened the possibility for future translational studies into the immunological and therapeutic implications of directed mitochondrial transfer in aged leukocytes (and beyond) to re-establish their function **[4]**. In my current position at Stanford University, I am expanding my perspective on how immune function influences cardiovascular diseases. I am investigating whether similar strategies I used to reinvigorate T cells from old mice can be adapted to different cardiovascular pathophysiologies that are strongly associated with aging. To this end, I have created antibody-mitochondria conjugates (Ab-*M*Cs), which use antibody-specific interactions with surface receptors on recipient cells to facilitate endocytosis of conjugated mitochondria. This project will examine whether targeted delivery to immune and immune precursor cells can significantly reduce aging-associated immune dysfunctions.

Ongoing or recently completed projects:

American Heart Association Career Development Award (24CDA1269111)
Headley (PI); *Rescuing aging-associated Cellular Dysfunctions in the Immune-Cardiovascular Axis Through Mitochondrial Transplantation*
1/1/25-12/31/27

Burroughs' Wellcome Fund Career Awards at the Scientific Interface (CASI)
Headley (PI); *Bioengineering Mitochondria for Targeted Delivery*
01/01/2025 – 12/31/2030

FY24 BID & PROPOSAL PAVIR Small Funds Grant
Tsao, Llorente & Headley (Co-PI); *Mitochondrial Transplantation for Improving Cerebrovascular Health*
07/2024 – 09/2024

Wu Tsai Human Performance Alliance Agility Grant
Yang & Ikeda (PI); Role: Co-Investigator; *Mitochondrial DNA Replenishment Therapy for Sarcopenia*
01/01/2024 – 12/31/2026

Burroughs' Wellcome Fund Post-Doctoral Enrichment Program
Headley (PI); *Mitochondrial Dysfunction and Transplantation in the Cardiovascular System*
09/01/2023 – 08/31/2026

Stanford's Propel Post-Doctoral Scholar Award
Headley (Awardee); *Mitochondrial Dysfunction and Transplantation in the Cardiovascular System*
01/01/2023 – 01/01/2025

Stanford University, Cardiovascular Institute Seed Grant
Ikeda (PI); Role: Co-Investigator; *Mitochondrial Transplantation Therapy for Mitochondrial Disease*
01/01/2023 – 12/31/2023

Stanford's Jump Start Award for Excellence in Research
Headley (PI); *Mitochondrial Dysfunction in Abdominal Aortic Aneurysm (AAA) Pathology*
09/20/2021 – 07/21/2022

NIH-NHBLI T-32 Mechanisms and Innovations in Cardiovascular Disease (1T32HL098049)
Tsao (PI); Role: Co-Investigator; *Mitochondrial Phoenix: The dysfunction, transplantation, and rejuvenation of mitochondria in CVD.*
05/01/2021 – 04/30/2023

Texas Biomedical Post-Doctoral Fellowship
Headley (PI); *Ameliorating Aging-Associated T Cell Dysfunction Through Mitochondrial Transplantation*
01/01/2021 – 12/31/2021

Citations:

1. **Headley CA,** Hoffman CM, Freisen JM, Han Y, Macklin JM, Zweier JL, Kuret J, Rockenbauerc A, Villamena FA. Membrane-specific spin trap, 5-dodecylcarbamoyl-5-N- dodecylacetamide-1-pyroline-N-oxide (diC_{12}PO): Theoretical, bioorthogonal fluorescence imaging and EPR studies. *Organic and Biomolecular Chemistry* (17): 7694-7705. 2019. PMCID: PMC6703941**.**
2. **Headley CA,** Gautam S, Olmo-Fontanez A, Garcia-Vilanova A, Dwivedi V, Akhter A, Schami A, Chiem K, Ault R, Zhang H, Cai H, Whigham A, Delgado J, Hicks A, Tsao PS, Gelfond J, Martinez-Sobrido L, Wang Y, Torrelles JB, Turner J. Extracellular Delivery of Functional Mitochondria Rescues the Dysfunction of CD4+ T Cells in Aging. *Advanced Science,* 2303664. 2023. PMCID: PMC10837346.
3. **Headley CA,** Guatam S, Olmo-Fontanez AM, Garcia-Vilanova A, Dwivedi V, Schami AA, Weintraub S, Tsao PS, Torrelles JB, Turner J. Mitochondrial transplantation promotes protective effector and memory CD4+ T cell response during *Mycobacterium tuberculosis* infection and diminishes exhaustion and senescence in elderly CD4+ T cells. (*In press, Advanced Science*)
4. **Headley CA**, Tsao P. Building the Case for Mitochondrial Transplantation as an Anti-aging Cardiovascular Therapy. *Frontiers in Cardiovascular Medicine*, 10, 707. 2023. PMCID: PMC10203246.

B. Positions, Scientific Appointments, and Honors

Positions

2021 – Present	Post-Doctoral Fellow, Stanford University, Stanford, California
2020 – 2021	Post-Doctoral Scientist I, Texas Biomedical Research Institute, Texas
2015 – 2020	Graduate Student, Immunology, The Ohio State University/Texas Biomed
2014 – 2015	Lab Technician, Biological Chemistry and Pharmacology, CardioX Corporation
2014 – 2015	Research Assistant, Dept. Biomedical Engineering, The Ohio State University, College of

Dentistry, Div. of Orthodontics**,** Columbus, Ohio
2011 – 2015 Research Assistant, Dept. Biological Chemistry & Pharmacology, The Ohio State University, Columbus, Ohio
2010 – 2011 Research Assistant, Dept. Plant Path., The Ohio State University/ USDA-ARS/ OARDC, OH

Scientific Appointments

2023 – Present Review Editor in Bioenergetics - Frontiers in Molecular Biosciences
2022 – Present Grant Coach/Teaching Assistant, The Grant Writing Academy, Stanford University, CA
2022 – Present Trainee/Member, Endocrine Society
2022 – Present Trainee/Member, American Heart Association (AHA)
2022 – Present Trainee/Member, American Aging Association (AGE)
2022 Mentor, Metascience Analyses and Explorations of Reproducibility in Cardiovascular Science, Stanford University, Palo Alto, California
2022 – Present VP of Diversity, Equity & Inclusion, Biotech Connection Bay Area, CA
2021 – Present Co-Organizer, Social Media Coordinator, Black In Immuno, Houston, Texas
2020 – 2021 Mentor, Post-Baccalaureate Research Education Program (PREP), UT-Health San Antonio
2020 – 2021 Team Lead, Diversity, Equity & Inclusion Committee, Texas Biomedical Research Institute
2019 – 2021 Committee Member, Community Outreach, Texas Biomedical Research Institute
2019 – 2020 Co-founder and Deputy Director, Enventure San Antonio (Non-profit), Texas
2018 – 2020 Co-founder and Executive Committee member, Texas Biomedical Research Institute Student Association (TBAT), Texas Biomedical Research Institute
2018 – 2020 Associate, The Initiative on Maximizing Student Development (IMSD), UT Health San Antonio
2016 – 2020 Mentor, Discovery Post-Bacc Research Education Program, The Ohio State University
2016 – 2020 Grad Student Ambassador, Biomedical Sciences Graduate Program, Ohio State University
2011 – 2015 Student Trainer, Department of Pharmacology, College of Medicine, The Ohio State University

Honors

2023 Judge Travel Award; Annual Biomedical Research Conference for Minoritized Scientists
2023 Post-Doctoral Leadership Institute, SACNAS
2023 NIA Summer Training Course Travel Award
2023 Stanford University Justice Equity Diversity & Inclusion (JEDI) Post-Doctoral Award
2023 – 2026 Burroughs' Wellcome Fund Post-Doctoral Enrichment Program Awardee
2023 Sanford Burnham Prebys Rising Star
2023 – 2025 Stanford University, Propel Post-Doctoral Award
2023 Future Leaders Advancing Research in Endocrinology (FLARE) 2023 Fellowship
2022 Best Post-Doctoral Poster, Stanford-Cornell Cardiovascular Research Symposium
2022 Vasculata Scholarship/Travel Award
2022 Rockefeller University Exceptional Scholar Award
2021 – 2022 Jump Start Award for Excellence in Research
2021 – 2023 Mechanisms & Innovations in Vascular Disease NIH T-32 – Stanford University
2020 – 2021 Texas Biomedical Post-Doctoral Forum Fellowship
2020 Stanford PRISM Scholar (Cohort 5)
2017 – 2019 Douglass Foundation Forum Scholarship
2017 Hodges Family Legacy Trainee Travel Award for Infectious Diseases
2014 The Ohio State University, College of Arts and Sciences Dean's list
2014 The Davis Heart and Lung Institute Research Day (Undergraduate Division – 2nd Place)
2012 – 2014 The Ohio State University Arts and Sciences Undergraduate Research Scholar
2011 The Ohio State University Scarlet and Gray Grant

C. Contributions to Science

1. Research in Mitochondrial Transplantation

In a proof-of-principle study, I demonstrated that transferring functional mitochondria into aged CD4+ T cells from mice and elderly humans rescues mitochondrial dysfunction and enhances cell functionality. This transfer improved mitochondrial metabolism, reduced ROS, enhanced TCR-signaling, increased IL-2 production, proliferation, and decreased markers of exhaustion and senescence. Notably, mito-transferred aged CD4+ T cells protected immune-deficient mice from influenza A and *Mycobacterium tuberculosis* infections **(a)**.

Further research showed that mitochondrial transfer promoted protective effector and memory T cells during *Mycobacterium tuberculosis* infection in mice and improved mitochondrial mass and cytokine production in elderly human T cells, reducing exhaustion and senescence markers. These findings suggest that mitochondrial transfer could enhance aged CD4+ T cell functionality, benefiting immune responses in the elderly and chronic TB patients, with broader implications for diseases involving mitochondrial dysfunction **(b)**.

Research also indicates that immune cells can naturally exchange mitochondria. Understanding these dynamics, especially in regenerative mitochondrial transfer, could transform perspectives on immune signaling and therapy development **(c)**. Additionally, my ongoing work on mitochondrial dysfunction in aging-associated cardiovascular diseases suggests that similar strategies could mitigate cardiovascular aging **(d)**.

a. Headley CA, Gautam S, Olmo-Fontanez A, Garcia-Vilanova A, Dwivedi V, Akhter A, Schami A, Chiem K, Ault R, Zhang H, Cai H, Whigham A, Delgado J, Hicks A, Tsao PS, Gelfond J, Martinez-Sobrido L, Wang Y, Torrelles JB, Turner J. Extracellular Delivery of Functional Mitochondria Rescues the Dysfunction of CD4+ T Cells in Aging. *Advanced Science,* 2303664. 2023. PMCID: PMC10837346.

b. Headley CA, Guatam S, Olmo-Fontanez AM, Garcia-Vilanova A, Dwivedi V, Schami AA, Weintraub S, Tsao PS, Torrelles JB, Turner J. Mitochondrial transplantation promotes protective effector and memory CD4+ T cell response during *Mycobacterium tuberculosis* infection and diminishes exhaustion and senescence in elderly CD4+ T cells. (*In press, Advanced Science*)

c. Headley CA. Mitochondrial Transplantation in the Immune Compartment. (*Book Chapter, in press*)

d. Headley CA, Tsao P. Building the Case for Mitochondrial Transplantation as an Anti-aging Cardiovascular Therapy. *Frontiers in Cardiovascular Medicine*, 10, 707. 2023. PMCID: PMC10203246.

2. Research in Aging Immunology & Host-Pathogen Interactions

My Dissertation research focused on understanding how aging affects the immune response to mycobacteria and the mechanisms that increase susceptibility to tuberculosis in the elderly. I hypothesized that 'inflammaging', or age-related changes in inflammatory pathways, leads to suboptimal reprogramming of pulmonary and systemic immunity, worsening infection outcomes in older individuals **(a)**. I led a groundbreaking study examining how mycobacteria affect cardiac function in aged mice, finding that pre-existing inflammation from aging may lead to cardiac dysfunction in *M. avium*-infected old mice. Our findings revealed dysrhythmia, increased recruitment of CD45+ leukocytes, cardiac fibrosis, and elevated expression of inflammatory genes in isolated heart tissue **(b, c)**.

Beyond mycobacteria, I assisted in validating the K18-hACE2 transgenic rodent model for studying SARS-CoV-2 and evaluating the therapeutic efficacy of human monoclonal antibodies against SARS-CoV-2 infections in these animals. These highly collaborative projects were sponsored by the Bill & Melinda Gates Foundation. One publication resulting from this work reported on the lethality of SARS-CoV-2 infection in K18-hACE2 transgenic mice, with more publications pending **(d).**

a. Headley CA. *Mycobacterial Heartbreak: Up in Inflammation & The Redox Opera of Mitochondria in Aged Lymphocytes.* 2020. Doctoral dissertation, Ohio State University. OhioLINK Electronic Theses and Dissertations Center.

b. Headley CA, Gerberick A, Mehta S, Ganesan LP, Wu Q, Yu L, Fadd P, Kha M, Turner J, Rajaram MVS. Nontuberculous Mycobacterium *M. avium* infection predisposes aged mice to cardiac abnormalities and inflammation. *Aging Cell,* 18(3):e12926. 2019. PMCID: PMC6516181.

c. Headley C, Turner J, Rajaram MV. Aging heart and infection. *Aging,* 11(14), 4781-4782. 2019. PMCID: PMC6682520.

d. Oladunni FS, Park JG, Pino PA, Gonzalez O, Akhter A, Allué-Guardia A, Olmo-Fontánez A, Gautam S, Garcia-Vilanova A, Ye C, Chiem K, **Headley C,** Dwivedi V, Parodi LM, Alfson KJ, Staples HM, Schami A, Garcia JI, Whigham A, Platt RN, Torrelles JB. Lethality of SARS-CoV-2 infection in K18 human angiotensin-converting enzyme 2 transgenic mice. *Nature communications,* 11(1), 6122. 2020. PMCID: PMC7705712.

3. Research in Oxidative Stress & Free Radical Biochemistry

My interest in oxidative stress has been a consistent theme throughout my biomedical research career, with over 11 years of experience in this area. This expertise is reflected in my other 'Contributions to Science' sections (1, 2, & 4). I have led as well as assisted in several projects focused on the role of reactive oxygen species (ROS) in biological systems and the cytoprotective effects of antioxidants.

For instance, I investigated the impact of nitrones on hyperglycemia-induced endothelial dysfunction in endothelial cells. My research demonstrated that the nitrones alpha-phenyl-n-tert-butylnitrone (PBN) and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) could reverse endothelial dysfunction induced by hyperglycemia in bovine aortic endothelial cells. This finding is significant as endothelial dysfunction is linked to cardiovascular

abnormalities, including atherosclerosis, coronary heart disease, diabetes, and abdominal aortic aneurysms (AAA) **(a).** In another collaborative project, I examined the antioxidant properties of seven novel hydroxycoumarin compounds and their ability to protect endothelial cells from ROS-induced cell death. Our findings indicated that two of the seven tested compounds effectively scavenged ROS and protected endothelial cells **(b)**.

I also assisted in designing oxidative stress-related experiments, standardizing protocols, analyzing data, and manuscript editing for a project comparing the immune responses of adult and elderly primates vaccinated with *Mycobacterium bovis bacillus* Calmette-Guérin (BCG) and challenged with the tuberculin skin test (TST). Our findings revealed that skin biopsies from elderly baboons were highly oxidized, and their peripheral blood mononuclear cells exhibited increased migration and functional responses to both antigen-specific and non-specific stimulation **(c)**.

a. **Headley CA,** DiSilvestro D, Bryant KE, Hemann C, Chen CA, Das A, Ziouzenkova O, Durand G, Villamena FA. Nitrones Reverse Hyperglycemia-Induced Endothelial Dysfunction in Bovine Aortic Endothelial Cells*. Biochemical Pharmacology,* 104, 108-117. 2016. PMCID: PMC5248535

b. Pérez-Cruz F, Villamena FA, Zapata-Torres G, Das A, **Headley CA**, Quezada E, Lopez-Alarcon C and Olea-Azar C. Selected hydroxycoumarins as antioxidants in cells: physicochemical and reactive oxygen species scavenging studies, *Journal of Physical Organic Chemistry.* 26:773-783. 2013.

c. Scordo JM, Piergallini TJ, Reuter N, **Headley CA,** Hodara VL, Gonzalez O, Giavedoni LD, Papin JF, Turner J. Local immune responses to tuberculin skin challenge in *Mycobacterium bovis* BCG-vaccinated baboons: a pilot study of younger and older animals. *Immunity & ageing: I&A*, *18*(1), 16. 2021. PMCID: PMC8024439.

4. Innovative Biotechnology Development

Bio-engineered Mitochondria: I have explored previously uncharted aspects of mitochondrial dysfunction and developed innovative bio-engineered mitochondrial therapies. These therapies show great promise in treating a range of complex health conditions, offering a new direction in the treatment landscape **(a)**.

In Situ Visualization of Nitrones: I developed a technique for the *in-situ* conjugation of nitrones to a fluorescently labeled alkyne, providing a simple and cost-effective method for determining their intracellular compartmentalization. This method enabled the visualization of nitrones in living cells, enhancing our understanding of their intracellular localization and therapeutic potential **(b)**.

Immune Correlates of TB Exposure: I assisted Dr. Russell Ault in identifying a gene signature comprising 250 genes in blood samples that correlates with recent *Mycobacterium tuberculosis* (M.tb) exposure and is reproducible across different species. Our findings demonstrated that this signature could predict recent tuberculosis exposure in mice, macaques, and humans, significantly impacting the development of non-invasive diagnostic tools for tuberculosis and advancing our understanding of host-pathogen interactions **(c)**.

Craniofacial Tissue Engineering and Regeneration: I gained hands-on experience in *in vivo* research on craniofacial tissue engineering and regeneration using stem cells in preclinical pig and athymic rat models. This work contributed to the development of advanced techniques for tissue repair and regeneration, highlighting the potential of stem cell therapies in clinical applications **(d)**.

a. **Headley CA**. Bio-engineered Mitochondria for Targeted Delivery to Cells – Prov. Patent Application No. 63/560,421.

b. **Headley CA,** Hoffman CM, Freisen JM, Han Y, Macklin JM, Zweier JL, Kuret J, Rockenbauerc A, Villamena FA. Membrane-specific spin trap, 5-dodecylcarbamoyl-5-N- dodecylacetamide-1-pyroline-N-oxide (diC_{12}PO): Theoretical, bioorthogonal fluorescence imaging and EPR studies. Organic and Biomolecular Chemistry. (17): 7694-7705. 2019. PMCID: PMC6703941.

c. Ault RC, **Headley CA**, Hare AE, Carruthers BJ, Mejias A, Turner J. Blood RNA signatures predict recent tuberculosis exposure in mice, macaques and humans. Scientific reports, 10(1), 16873. 2020. PMCID: PMC7547102.

d. Lloyd B, Tee B, **Headley CA**, Mallery S, Emam H, Sun Z. Similarities and Differences between Porcine Mandibular and Limb Bone Marrow Mesenchymal Stem Cells. *Archives of Oral Biology*, 77, 1–11. 2017. PMCID: PMC5366281.

Complete List of Publications: https://www.ncbi.nlm.nih.gov/myncbi/colwyn.headley.2/bibliography/public/