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2017 APS Fall Conference

Physiological and Pathophysiological Consequences of Sickle Cell Disease

Washington, DC • November 6–8, 2017

Special Conference Abstract Issue

Is Your Research a Clinical Trial? The Answer May Surprise You!

Elizabeth Barksdale

Elizabeth Barksdale

Background

In October 2014, the National Institutes of Health (NIH) announced a change in its definition of *clinical trial* (1), to take effect in January 2015. The new definition, which applies only to NIH-funded clinical trials, reads: "A research study in which one or more human subjects are prospectively assigned to one or more interventions (which may include placebo or other control) to evaluate the effects of those interventions on health-related biomedical *or* behavioral outcomes." At the time, few in the research community paid attention, but as the implementation begins, researchers are learning more about who will be affected.

NIH defines *intervention* as "a manipulation of the subject or subject's environment for the purpose of modifying one or more health-related biomedical or behavioral processes and/or endpoints," and *health-related biomedical* or *behavioral outcome* as "the pre-specified goal(s) or conditions(s) that reflect the effect of one or more interventions on human subjects' biomedical or behavioral status or quality of life."

These definitions both explicitly and implicitly encompass nearly all clinical, behavioral, cognitive, and educational research being conducted with human subjects, thus increasing the scope of research encompassed by the *clinical trial* moniker.

Continued on page 32

A Matter of Opinion

Reflections

When was the American Physiological Society founded? For most physiologists, the answer is 1887. After all, we celebrated its centennial in 1987. However, our APS was not the first APS. If you read the APS Centennial history (*History of the American Physiological Society: The First Century, 1887–1987*; https://link.springer.com/book/10.1007/978-1-4614-7576-7), you will discover that there was another APS founded in 1837. Its purpose was "to acquire and diffuse a knowledge of the laws of life, and of the means of promoting human health and longevity." The original APS dissolved in 1840 because of an overemphasis on diet at the expense of health. Perhaps one should view the original APS as a predecessor to the American Nutrition Society.

Continued on page 33

THIS ISSUE:

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Publications

Assessing the Outcomes of Introducing a Digital Image Quality Control Review into the Publication Process for Research Articles in APS Journals

Christina Bennett ***(APS Associate Publisher, Ethics and Policy)*** **and Rita Scheman** ***(APS Director of Publications and Executive Editor)***

In September 2017, Christina Bennett and Rita Scheman attended the Eighth International Congress on Peer Review and Publication in Chicago, Illinois. This meeting, held once every 4 years, shares new research on the "science" of peer review and facilitates open discussion on how all stakeholders in research can increase the quality and credibility of science. On the first day of the meeting, Bennett gave a podium presentation describing the results of a study, performed by Bennett and Scheman, titled "Assessing the Outcomes of Introducing a Digital Image Quality Control Review into the Publication Process for Research Articles in Physiology Journals." The study assessed whether the workflow for checking digital images for image manipulation in manuscripts submitted to APS journals (QC check) has been effective. The study also assessed whether corresponding authors involved in a QC query had submitted subsequent manuscripts without generating another QC query. The results of the study are shared below.

Articles published in APS journals undergo both a scientific and an ethics review. Peer reviewers are responsible for reviewing the merits of the science reported in each manuscript, and often they alert editors to ethics concerns. However, it is an APS staff responsibility to perform a number of checks before and after peer review to confirm that the information published in the journals is original and transparent. The QC check is one such process that screens photographs (gels, blots, histology, etc.) in all accepted manuscripts for manipulation.

APS is a member of the Committee on Publication Ethics (COPE) and addresses all ethics concerns according to COPE guidelines. This means that APS takes all ethics concerns seriously and seeks clarification from the authors when questions are raised. For especially difficult or serious concerns, the matter is referred to the author's institution for mediation or investigation. In terms of the QC check, each letter that is sent to the author identifies the concern, describes the presentation guideline that may be compromised, and requests a corrected figure along with the original capture that can verify that the data are valid. In this way, the QC check workflow serves as an educational tool for good practice in image presentation as well ensuring that the images presented better reflect the data collected.

The APS Art Department, managed by Eric Pesanelli, performs the QC check using forensic analysis tools that were developed by the Office of Research Integrity in the U.S. Department of Health and Human Services. Art staff review all photographic images for adherence to APS image presentation guidelines, including signs of splicing, extreme contrast adjustment, selective editing, and duplication. Initially, the QC check occurred when the images were prepared for final publication. However, where problems were identified and corrections for non-fraudulent manipulations needed, the published early view version of the article required a correction explaining why the figures had been revised. This was frustrating to the authors and staff. Thus the QC check for the AJP journals was moved to an earlier stage in the publication process: after acceptance but before early view publication, allowing authors to make revisions before publication of any version of the article rather than post corrigenda to the published early view article.

Implementation of this QC check into our publication workflow took several years and warranted a full evaluation of its impact on identifying and resolving the concerns that were raised. Thus the study findings presented at the Congress described the overall outcomes of the QC check process from 2009 through 2016, for the seven AJP titles, which collectively publishes between 1,500 and 2,000 articles per year. The dataset included numbers of QC cases queried per year, modifications identified, and manuscript outcomes (no revision, revision, corrigendum, rejection, or retraction).

The number of subsequent submissions of unique manuscripts to the journals by these corresponding authors was assessed, via the manuscript submission database, as were the outcomes of those QC checks.

Ethics concerns involving manipulated images had started to increase in 2009–2010, but because there was no formal process within the publication workflow to systematically review images, the overall number of cases analyzed was relatively low. In 2010, APS implemented the QC check workflow, adding a new AJP journal every 3–6 months. As shown in Figure 1A, the number of QC queries peaked in 2011. Since then, the number of QC queries, compared to the total number of articles published, has decreased year after year, even with all seven AJP journals in the workflow. The number of corrigenda has decreased every year since 2011, whereas corrections via image revisions prior to publication have increased (Figure 1B). These data show that the QC check is effective in identifying image presentation concerns and in eliminating post-publication corrections related to image manipulation, the latter attributed to moving the QC check to an earlier step in the publication process.

Figure 1

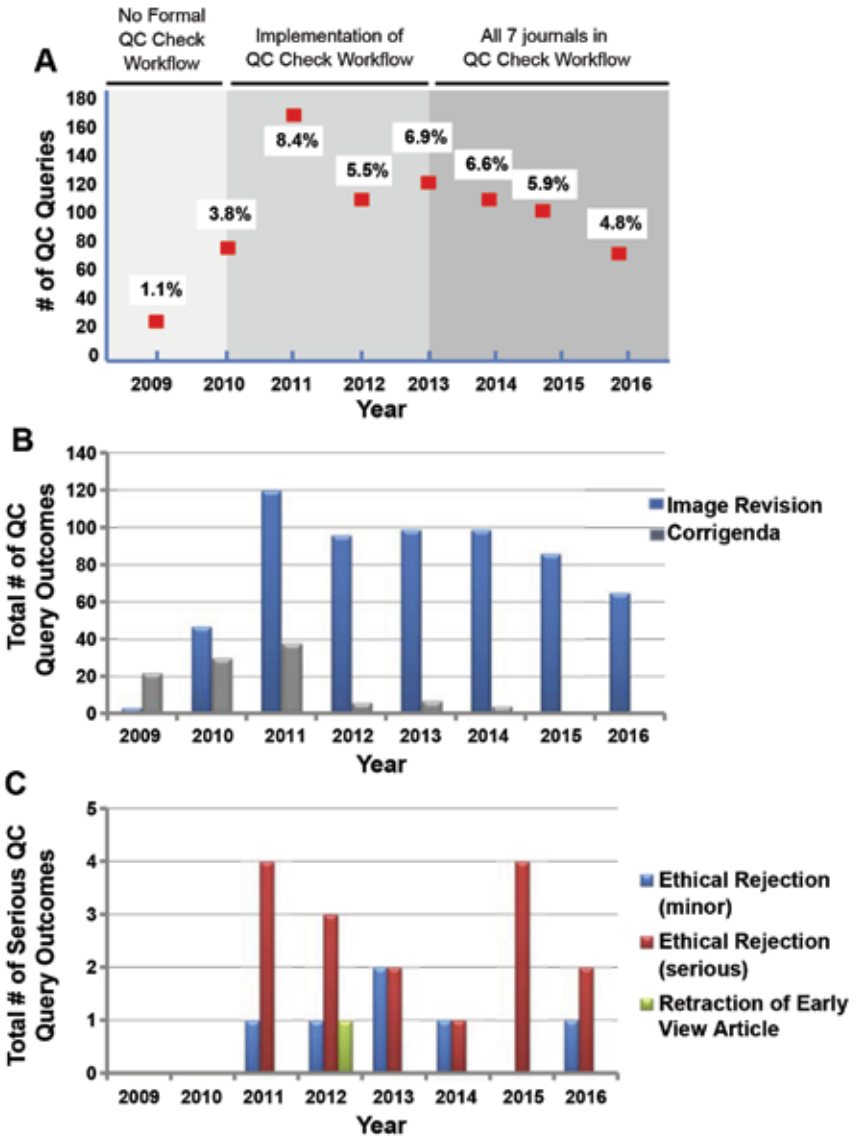


Figure 1. *Assessment of QC check query outcomes from 2009 to 2016. A: total number of QC queries per year before, during, and after the QC check workflow was implemented. The percentage of accepted manuscripts that underwent a QC query is noted. B: total number of QC query outcomes that resulted in correction prior to publication (image revision) and correction after early view publication (corrigenda) per year. C: total number of QC query outcomes that resulted in ethical rejections or retraction per year.*

Most of the image manipulation identified during QC check are relatively minor and can be readily corrected. However, the QC check has uncovered image falsification and fabrication. In these instances, corrections are not invited; rather, acceptance of the manuscript is rescinded, and a decision of ethical rejection is issued; if the manuscript is already a published article, it is retracted. In 2009–2010, before the QC check was implemented, no manuscripts were issued an ethical rejection, nor were any early view articles retracted for image concerns. Since then, several QC queries each year result in a decision of ethical rejection or a retraction of the early view publication due to image manipulation that changed the interpretation of the data, falsification, or fabrication (Figure 1C).

The study also assessed the types of image manipulations that the APS art staff identified during QC check. Figure 2 shows the types of

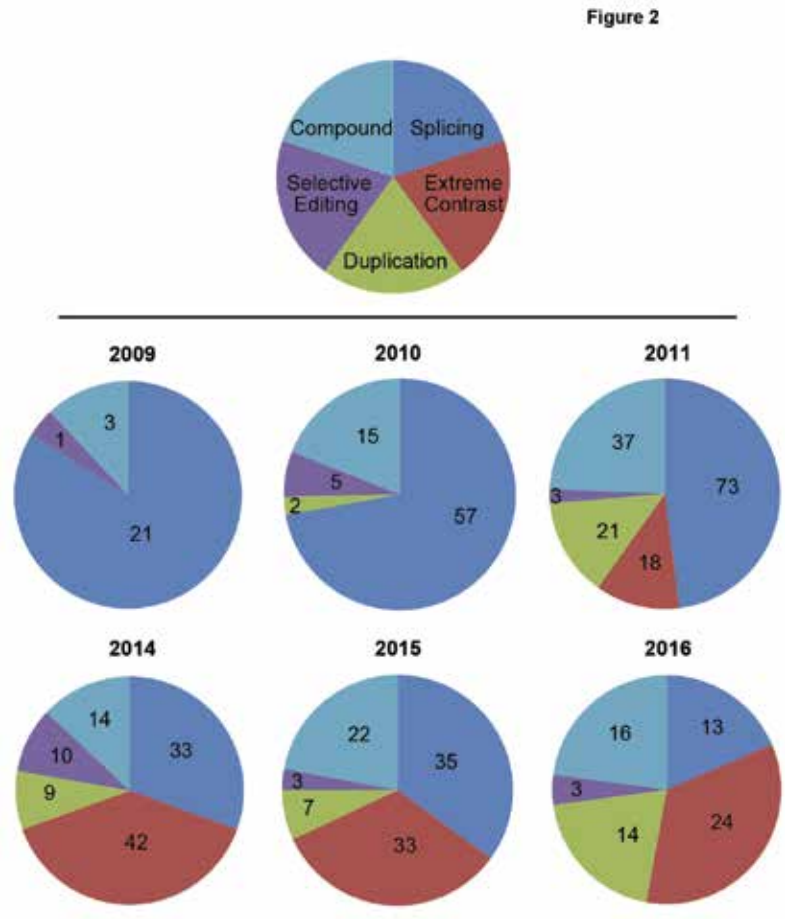


Figure 2. *The types of image manipulation identified during QC check in 2009–2011 and 2014–2016.*

manipulations [splicing, extreme contrast, duplication, selective editing, and a mix of issues (compound)] detected and how these have changed over time. Splicing was initially the major issue, but cases of extreme contrast adjustment, which can mask rearrangements and duplication, have increased. These changes in types of image manipulation detected may not reflect author behavior. Rather, the change is more likely due to the increased forensic expertise of APS staff.

Last, the study assessed whether authors who received a QC query once between 2013 and 2015 received another QC query during a subsequent submission. The data show that 58% of authors who received a QC check query submitted another manuscript for publication by December 2016. Of those authors, only eight (4%) received another query. We hope the low rate of "repeat offenders" is due, at least in part, to author awareness and increased vigilance in subsequent submissions to adhere to APS standards for digital image presentation.

In summary, results from the study show that the QC check is effective in identifying image manipulation and correcting the errors prior to publication, and may play a role in altering author behavior toward best practice in digital image presentation. ●

Our Mission
The SSPA is dedicated to identifying and disseminating vital scientific research, by scientists for science. Our not-for-profit member societies provide authors with the opportunity to have their work validated by peers in a fair manner for publication in a prestigious journal managed by working scientists. By reinvesting in the community, the members of the SSPA are committed to delivering important discoveries worldwide.

Our Vision
Promoting discovery by publishing the best research

Scientific Society Publisher Alliance

Rita Scheman
APS Director of Publications and Executive Editor

On November 6, 2017, the Scientific Society Publisher Alliance (SSPA) launched its website to raise the visibility of scientific journals published by societies, which "provide authors with the opportunity to have their work validated by peers in a fair manner for publication in a prestigious journal managed by working scientists" (*https://byscientistsforscience.org*). The founding societies, including APS, seek to promote the unique values of journals owned by mission-driven societies that emphasize quick yet robust peer review, working scientists who serve as the editors as well as the reviewers, and reinvestment in the communities from which the journals are generated.

SSPA started in the summer of 2015 as a discussion on the FASEB campus among editors, executive directors, and publication directors of 10 scientific societies. It was, in a sense, a call to arms by Mark Johnston, Editor-in-Chief of *Genetics* (Genetics Society of America), to raise the visibility of society publications through promoting awareness of the scientific principles and values that our journals represent. The idea was that this message could be communicated more effectively via the power of collaboration – and then taken to our individual scientific communities – supported by the larger group.

SSPA members (then self-identified as the "Collaborative") committed formally to exploring this concept with funds to retain consultants. A working group was formed, composed of Mark Johnston, Chair (GSA); Jennifer Pesanelli (FASEB), Barbara Goldman and Stacey Burke (ASM), Christopher Towne (APS), and me. In collaboration with DeltaThink consultants, we developed a mission statement (at top of article), surveyed scientists via focus groups, identified audience segments by career stage (Figure 1; career stage being a significant determinant of publishing motivation), and agreed on targeted messaging to engage each audience segment. The marketing plan that evolved is intended for use by each individual member society to reach out

to its own scientific community, as well as for use by the SSPA collaboration. Through continued planning and development, SSPA was branded, and a website was developed. A full meeting of all stakeholders in summer of 2017 supported the launch of the website.

The SSPA story is compelling – it is a journal managed by distinguished working scientists, not professional editors, of missions dedicated to fair and robust peer review, of disciplines that reflect the research profile of the community over metrics-driven topics, and of proceeds that are reinvested in the journals and in supporting the science community through endeavors such as education, awards and fellowships, and science policy advocacy. It is a story that is known less well, especially by the current generation of research scientists.

Assuming these things matter, the story of APS journals is particularly compelling. APS journals are edited by world-class working scientists, and they offer a level of support for authors and editors during the publishing process that is unparalleled. APS original research journals have citation profiles that keep on giving – many APS journals have a cited half-life of >10 (10 being the highest score displayed), which means that articles published in APS journals are likely to be cited for many years during the career of the scientist – not only primarily in the first 2 years following publication. (The Cited Half-Life of an article is the median age of the articles that were cited in the JCR year. Half of a journal's cited articles were published more recently than the cited half-life. See *http://ipscience-help.thomsonreuters.com/inCites2Live/indicatorsGroup/aboutHandbook/usingCitationIndicatorsWisely/citedHalfLife.html*.) APS review journals provide a spectrum of authoritative reviews: accessible short review articles, textbook-like state-of-the-art comprehensive review articles, and hot-topic state-of-the-art review articles, edited by the most distinguished researchers working in their fields of physiology and related sciences.

We are now a week on from the website launch, which has brought much wanted (and warranted) attention, and SSPA members are thrilled. Motivated by a desire to share our laudable publication principles, SSPA is a publicity effort, pure and simple. The research, publishing, and organizational sectors we sought to reach have already taken notice: as encouraged, other societies have requested information about membership in the SSPA. Mark Johnston has written an editorial in *Genetics* (*https://doi.org/10.1534/genetics.117.300427*) and has been interviewed by the Scholarly Kitchen (*https://scholarlykitchen.sspnet.org/2017/11/08/biological-science-societies-hope-to-convince-authors-to-stay-in-the-society-family*), and there has been considerable listserv and social media chatter.

Audience Segment	Goals	Primary Targets
Early Investigator – Pre-Tenure	Encourage and increase submissions. Ensure awareness of the benefits of publishing in APS journals. Promote a robust and fair publishing experience	• Postdocs • Investigators Pre-Tenure/Tenure Track • Authors and Members
Midcareer – Senior Investigator	Encourage and increase submissions. Influence others to submit. Engage with the journals as reviewers, board members, and/or associate editors. Recognize the value of publishing in a society journal.	• Post Tenure Investigators • Thought Leaders/Senior Investigators • Board Members, Reviewers • Authors and Members
Non-Engaged Investigator – Any Career Stage	Become engaged. Build awareness and encourage submissions. Understand the value of publishing in a society journal. Recommend the journals to others.	• Members • Meeting Attendees • Authors publishing in competing journals

Figure 1. Publication audience segment marketing plan

APS members can also help spread the word about SSPA – and the society journals it promotes. APS journals have remained relevant to serve APS members and the entire physiology and related discipline community with important discoveries, insights, and recognition for 120 years. We hope members will continue to support their society and its journals by submitting manuscripts. ●

Follow SSPA online:

Facebook: *https://www.facebook.com/Scientific-Society-Publisher-Alliance-SSPA-334302053704786/*

Twitter: *https://twitter.com/sspajournals*

LinkedIn: *https://www.linkedin.com/company/scientific-society-publisher-alliance/*

See the APS press release at: *http://www.the-aps.org/mm/hp/Audiences/Public-Press/2017/69.pdf*

Current Calls for Papers

Physiological Genomics

- Genetics of Metabolic Syndrome
  Submission deadline:
  June 30, 2018
- Single Cell Analysis
  Submission deadline:
  May 31, 2018

Journal of Neurophysiology

- Progress in Motor Control
  Submission deadline:
  June 30, 2018
- Neuroscience at the 38th World Congress of the International Union of Physiological Sciences
  Submission deadline:
  June 30, 2018
- The Role of Eye Movements in Perception, Cognition, and Action
  Submission deadline:
  June 30, 2018

Advances in Physiology Education

- Historical Perspectives and Living Histories

Journal of Applied Physiology

- Vascular Aging
  Submission deadline:
  January 31, 2018

American Journal of Physiology – Heart and Circulatory Physiology

- Novel Mechanisms of Myocardial Ischemia, Ischemia-Reperfusion, and Protection by Myocardial Conditioning
  Submission deadline:
  February 1, 2018
- Cardiac Regeneration and Repair
  Submission deadline:
  February 1, 2018
- Extracellular Matrix in Cardiovascular Pathophysiology
  Submission deadline:
  February 1, 2018

American Journal of Physiology – Regulatory, Integrative and Comparative Physiology

- Cardiovascular and Metabolic Consequences of Sleep and/or Circadian Disruption
  Submission deadline:
  May 1, 2018

For a complete list of current Calls for Papers, visit the APS website.

Experimental Biology

Experimental Biology 2018 Distinguished Lectures

Physiology in Perspective: The Walter B. Cannon Memorial Award Lecture

Ole H. Petersen

Cardiff University

The Roles of Ca^{2+} and ATP in Pancreatic Physiologi and Pathophysiology

Sunday, April 22, 2018, 5:30 PM

Supported by Sucampo

Henry Pickering Bowditch Memorial Award

Yatrik M. Shah

University of Michigan

Oxygen Sensing Pathways: A Critical Link Between Inflammation and Cancer

Monday, April 23, 2017, 5:30 PM

Robert M. Berne Distinguished Lectureship of the APS Cardiovascular Section

Jason Yuan

University of Arizona Health Sciences

Mechanisms of Pulmonary Vascular Disease: Pathogenic Role of Ion Channels

Tuesday, April 24, 2018, 3:30 PM

Hugh Davson Distinguished Lectureship of the APS Cell and Molecular Physiology Section

Paul Quinton

University of California, San Diego

Confessions of a Long-Term Extra-Marital Affair with Bicarbie

Sunday, April 22, 2018, 3:30 PM

Joseph Erlanger Distinguished Lectureship of the APS Central Nervous System Section

Wolfram Schultz

University of Cambridge

Getting the Best Reward: Neuronal Mechanisms for Utility Maximisation

Monday, April 23, 2018, 3:30 PM

August Krogh Distinguished Lectureship of the APS Comparative and Evolutionary Physiology Section

Stanley S. Hillman

Portland State University

Anuran Amphibians as Models for Understanding Extreme Dehydration Tolerance

Tuesday, April 24, 2018, 3:30 PM

Supported by Novo Nordisk Fonden

Solomon Berson Distinguished Lectureship of the APS Endocrinology and Metabolism Section

Erik A. Richter

August Krogh Institute

The BIG story: the Beautiful, Integrative, Glucose Metabolism and Exercise

Monday, April 23, 2018, 3:30 PM

Edward F. Adolph Distinguished Lectureship of the APS Environmental and Exercise Physiology Section

David Poole

Kansas State University College of Veterinary Medicine

Muscle Microcirculation: Gateway to Function and Dysfunction

Monday, April 23, 2018, 1:30 PM

Horace W. Davenport Distinguished Lectureship of the APS Gastrointestinal and Liver Physiology Section

Jerrold R. Turner

Brigham and Women's Hospital

Mucosal Barriers: Pathways and Pathologies

Tuesday, April 24, 2018, 3:30 PM

History of Physiology Group Lecture

Peter B. Raven

University of North Texas Health Science Center

Bengt Saltin, MD, DSci (1935–2014): Exercise is Medicine

Tuesday, April 24, 2018, 1:00 PM

Carl Ludwig Distinguished Lectureship of the APS Neural Control and Autonomic Regulation Section

David Paterson

University of Oxford

Heart Meets Brain: Brain Meets Heart: Therapeutic Opportunities

Monday, April 23, 2018, 1:30 PM

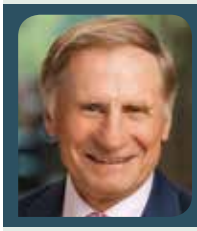
Carl W. Gottschalk Distinguished Lectureship of the APS Renal Section

Lisa M. Satlin

Icahn School of Medicine at Mount Sinai

In the Flow: Cell-Specific Expression and Regulation of BK Channels in the Distal Nephron

Monday, April 23, 2018, 3:30 PM



Julius H. Comroe, Jr. Distinguished Lectureship of the APS Respiration Section

Bert Forster

Medical College of Wisconsin

Interdependence of Neuromodulators in the Control of Breathing

Tuesday, April 24, 2018, 3:30 PM

Claude Bernard Distinguished Lectureship of the APS Teaching of Physiology Section

Jenny L. McFarland

Edmonds Community College

Transformations – Paths to Student-Centered, Evidence-Based Physiology Education

Sunday, April 22, 2018, 3:30 PM

Supported by ADInstruments

Ernest H. Starling Distinguished Lectureship of the Water and Electrolyte Homeostasis Section

David L. Mattson

Medical College of Wisconsin

Diet, Inflammation and Hypertension

Sunday, April 22, 2018, 3:30 PM

APS Nobel Prize Award Lecture

Leland Hartwell

The Biodesign Institute, Arizona State University

Wednesday, April 25, 2018, 3:30 PM

Experimental Biology 2018

April 21–25, 2018, San Diego, CA

PHYSIOLOGY PLATFORM SESSIONS

Saturday, April 21, 2018

Room	Times as Indicated		
20A	8:00 AM–12:00 PM *Education Committee Refresher Course* GI Physiology: Not Just the Gut Anymore **Sasser/Merritt**	2:15 PM–5:15 PM *WEH Section Award Session* WEH Trainee Award Finalists and Data Diuresis **O'Conner/Polichnowski**	
22			3:00 PM–5:00 PM *NCAR Section* Data NCARNation
24	9:30 AM–11:30 AM *MCS President's Symp*	1:00 PM–3:00 PM *MCS Abstract-based Symp*	3:30 PM–5:30 PM *MCS Abstract-based Symp* 6:00 PM–8:00 PM *MCS Poster Discussion*
25A	9:00 AM–5:00 PM ETG Pre-Meeting		
25B		1:00 PM–2:30 PM *ACE Committee Symp* Avoiding Common Pitfalls in Preclinical Animal Research Design **Michele/Uray**	
25C			3:00 PM–4:30 PM *Communications Committee Symp* Social Media for the Professional Scientist **Goodman**
26		1:00 PM–3:00 PM *Techniques Workshop* Sex and Age as Biological Variables in Physiology Research **Yosten/Kolar**	3:00 AM–5:00 AM *Techniques Workshop* Transformative Technologies Enabling Ecological Assessment of Human and Wildlife Physiology **Sandberg/Crossley**
27		1:00 PM–2:30 PM *AFMR Symp* The Role of TIEG1 in Disease Processes: From Bench to Bedside **Rajamannan/Hawse**	3:00 PM–4:30 PM *AFMR Symp* The Mevalonate Pathway: A Fundamental Player in Human Disease **Zeki/Ghavami**
28A		1:00 PM–5:15 PM *PGG Special Session* 5th Annual Physiological Genomics Conference	

Sunday, April 22, 2018

Room	8:30–10:00 AM	1:30–3:00 PM	3:30–5:00 PM
20A	*IPSS Symp* Ischemic and Hypoxic Conditioning: Potential for Protection of Vital Organs **Rickards/Sprick**	*APS President's Symp Series* Exosomes: The New Frontier. Cell Biology of Exosomes **O'Driscoll**	*MCS Lecture* MCS Landis Award Lecture and Business Meeting 5:30 PM–6:30 PM *APS Cannon Lecture* Supported by Sucampo AG **Petersen**
22	*MBG Symp* Maintenance and Remodelling of the Neuromuscular Junction in Health and Disease **Ljubicic**	*CV Section Symp* American Journal of Physiology Heart and Circulatory Physiology Editors Symp **Zucker/Lindsey**	3:30 PM–5:00 PM *CV Section FT* Kaley Award FT: Cerebral Vascular Dysfunction and Impaired Cognitive Function **Roman**
23	*CV Section Symp* Of Mice and Men: What Have We Really Learned About the Regulation of Coronary Vascular Function in Health and Disease? **Phillips/Goodwill**	*GIL Section FT* Cell Plasticity and Repair and Disease Mechanisms in the Stomach, Liver and Intestine **Powell**	3:30 PM–4:30 PM *WEH Section* *Starling Lecture* **Mattson** 4:30 PM–5:30 PM *WEH Section* New Investigator Award Lecture **Hinojosa-Laborde/Madhur**
24	*CV Section FT* Role of the Microbiome in Cardiovascular Disease **Buys**	*CV Section FT* Innate and Adaptive Immunity in Cardiovascular Physiology **Madhur/Cornelius**	
25A	7:00 AM–8:00 AM *TAC Symp* Do it Again: How to Achieve Rigorously Reproducible Research I **Downey/Obi** 8:30 AM–10:00 AM *ETG Symp* Building Epithelial Organs In Vitro to Study Physiology and Pathogenesis of Disease **Nørregaard/Dixon**	*Renal Section Symp* ENaC Proteins As Mechanosensors in Endothelial and Vascular Smooth Muscle Cells **Drummond/Ashley**	*Resp Section FT* Communication and Miscommunication in Lung Injury and Repair **Koval/Birukov**
25B	7:00 AM –8:00 AM *Careers Symp* 2018 Careers in Physiology Symp I **Brandauer/Becker** 8:30 AM–10:00 AM *Translational Physiology Interest Group FT* Translational Physiology Showcase: TBD **Young**	*EEP Section FT* Exploring Novel Mechanisms to Improve Exercise Tolerance in Health and Disease **Harris/Barnes**	*EEP Section Symp* Epigenetic Memory of Environmental Exposure: a Physiological Perspective **Murashov/Clanton**

Sunday, April 22, 2018, continued

25C	7:00 AM–8:00 AM *WIPC Symp* Recognizing and Responding to Implicit Bias in Science I **Al Alam/Wallace/Ho** 8:30 AM–10:00 AM *EEP Section Symp* Too Hot to Handle: Controversies in Exertional Heat Stroke Prevention and Treatment **Laitano/King**	*CEP FT* Comparative and Evolutionary Physiology Section Trainee-Driven FT **Crossley**	*NCAR Section FT* Psychological Stress Disorders: Novel Concepts and Mechanisms **Sabharwal**
26	*NCAR Section FT* NCAR Young Investigator Awards **Moraes/Poglitsch**	*CNS Section Symp* Sex Differences in Central Circuits **Wainford/Browning**	*Teaching Section* *Bernard Lecture* Supported by ADInstruments **McFarland**
27	*WEH SectionSymp* Inflammation and Sodium Reabsorption **Lee/Pai**	*Resp Section Symp* Non-Canonical Functions of the Lung in Immunity and Hemostasis **Kuebler/Juss**	*Cell Section Davson Lecture* **Quinton**
28A	*Renal Section FT* Renal Section Young Investigator Award FT: Novel Roles for Renal GPCRs **Pluznick/Caplan**	*Cell Section Symp* Organoids: Modelling Cell Physiology and Disease in 3D **Bradbury/Ameen**	*CNS Section Symp* Intersection of Central Pain and Reward Circuitry in CNS Disorders **Edwards/Roberto**
28B	*Teaching Section Symp* Addressing Higher Levels of Bloom's Taxonomy in the Teaching and Learning of Physiology **Clements-Jewery/Hopper**	*PGG FT* From Gene to Function of Complex Traits: Analysis of Genes Identified in Human GWAS and Animal Models **Solberg Woods**	*Resp Section FT* Microglia as Effectors of Respiratory Plasticity in Health and Disease **Kinkead/Powell**
28DE	*PGG Nonfunded FT* Physiological Genomics Trainee Highlights	*SfRBM Symp* Redox Biology: A Unifying Theme in the Etiology of Human Diseases **Case/Kevil**	*GIL Section* John Forte GIL Plenery Session **Uno/Frey**

Monday, April 23, 2018

Room	8:30–10:00 AM	1:30–3:00 PM	3:30–5:00 PM
20A	*IPSS Symp* Bioartificial Organs: Using Donor and Synthetic Scaffolds **Harrison-Bernard**	*President's Symp Series* Exosomes: The New Frontier. Pathophysiology of Exosomes **Théry**	
22	*Sex Group Symp* Impact of Sex-Specific Size of the Normal and Failing Left Ventricle: Studies in Humans and Mice **Kerkhof/Miller**	*CV Section Symp* CV Section – Young Investigator Symp **Goulopoulou/Belin de Chantemele**	*CV Section FT* Protective Mechanisms in the Vasculature: Wiggers Award Session **Sigmund**
23	*Hypoxia Group Symp* Novel Physiologic-Based Approaches to Treating Sleep Apnea **Dempsey/Bates**	*GIL Section Symp* Identification of Novel Drug Targets For the Modulation of Gastrointestinal Motility **Uray/Perrino**	*CV Section Symp* Brown Adipose Tissue and Cardiovascular Function: Insulin Resistance, Vascular Tone, and Cardioprotective Effects **Stanford/Scherrer-Crosbie**

Monday, April 23, 2018, continued

24	*NCAR Section FT* Novel Insights on Sympathetic Activation in Kidney Disease: From Animal Models to Clinical Trials **Park/Becker**	*NCAR Section Ludwig Lecture* **Paterson**	*Renal Section Gottschalk Lecture* **Satlin** 5:30 PM–6:30 PM *APS Bowditch Lecture* **Shah**
25A	7:00 AM–8:00 AM *TAC Symp* Do It Again: How to Achieve Rigorously Reproducible Research II **Downey/Obi** 8:30 AM–10:00 AM *PGG FT* Non-coding RNA Regulation of Inflammation in Cardiovascular, Kidney, and Respiratory Diseases **Kriegel/Rogers**	*ETG FT* Hans Ussing Lecture of the Epithelial Transport Group **Akiba**	*Resp Section FT* Molecular, Cellular and Systems-Level Mechanisms Driving Ventilation and CO2 Sensitivity during Acute and Chronic Hypercapnia **Hodges/Hawkins**
25B	7:00 AM–8:00 AM *Careers Symp* **Brandauer** 8:30 AM–10:00 AM *Renal Section Symp* New Concepts in JGA Physiology **Peti-Peterdi/Buckley**	*Renal Section FT* Advances in Renal Physiology I **Ortiz**	*Translational Group Symp* Altering Phenotype Without Genotype **Sones/Reijnders-Most**
25C	7:00 AM–8:00 AM *WIPC Symp* Recognizing and Responding to Implicit Bias in Science III **Al Alam/Wallace/Ho** 8:30 AM–10:00 AM *Cell Section FT* Ion Channels and Transporters in Health and Disease **Worrell**	*Cell Section Symp* Biophysical and Metabolic Regulation of Stem Cells **Rehman/Shin**	*CEP Section Symp* Comparative Perspectives on Maximal O2 and CO2 Transport in Animals **Hedrick**
26	*Nutrition Symp* The Physiology of Personalized Nutrition **Voy/Anthony**	*PIC Symp* Biosensors in Health and Disease **Bucher/Olver**	*CNS Section Erlanger Lecture* **Schultz**
27	*CV Section FT* Regulation of Blood Flow in Health and Disease **Ohanyan**	*EEP Section Adolph Lecture* **Poole**	*EM Section Berson Lecture* **Richter**
28A	*EM Section FT* Gut-Brain Interactions and Control of Feeding Behavior **Stein**	*EM Section Symp* The role of REDD1 in the Regulation of Skeletal Muscle Metabolism **Steiner**	*WEH Section FT* Impact of Diet on Blood Pressure Regulation **Greene/Stodola**
28B	*Teaching Section FT* Abstract-Driven FT **Osborne**	*History Group Symp* The Physiological Challenges of Escaping Extreme Environments: DIsabled Subs and Stratospheric Bailouts **Ryan/Dean**	*Teaching Section Symp* Synergizing Teaching and Scholarship **Harris**

Monday, April 23, 2018, continued

28DE	8:30 AM *Pubs Symp* Publishing 101: How to Get Your Work Published and Avoid Ethical Minefields **Sigmund**	1:30 PM *NCAR FT* Hot Topics in Autonomic Regulation **Mathis/Banek**	

Tuesday, April 24, 2018

Room	8:30–10:00 AM	1:30–3:00 PM	3:30–5:00 PM
20A	*IPSS Symp* Tissue-Distributed Control of Sex Differences in Diabetes and Cardiovascular Disease **Stafford**	*President's Symp Series Symp* Exosomes: The New Frontier. Exosomes in Diagnostics and Therapeutics **Jones**	5:30 PM–7:00 PM *APS Business Meeting*
22	*EM Section Symp* Mechanisms Underlying Skeletal Muscle Adaptation in Health and Disease **Lang/Molina**	*CV Section Symp* Steroid Receptor Signaling in Cardiovascular Health and Disease **Hamblin/Clayton**	*MBG FT* Exercise and Skeletal Muscle as Key Regulators of Whole Body Aging **Jackson/Brooks**
23	*NCAR Section Symp* Neuro-Immune Interactions in Inflammatory Homeostasis **Marvar**	*GIL Section FT* GI and Liver Physiology and Disease **TBD**	*GIL Section Davenport Lecture* **Turner**
24	*Cell Section Symp* Molecular Mechanisms for Salt-induced Cardiovascular Disease **Kirbo/Ruggeri Barbaro**	*Resp Section Comroe Lecture* **Forster**	
25A	7:00 AM–8:00 AM *TAC Symp* Do It Again: How to Achieve Rigorously Reproducible Research **Downey/Obi** 8:30 AM–10:00 AM *Renal Section FT* Advances in Renal Physiology II **Inscho**	*Cell Section FT* Epithelial Mechano-Sensitivity in Health and Disease **Beyder/Chebib**	*ETG FT* Hebert Lecture of the Epithelial Transport Group **Subramanya**
25B	7:00 AM–8:00 AM *Careers Symp* **Brandauer** 8:30 AM–10:00 AM *EEP Section FT* Getting Blood to Where it Needs to Go: Emerging Mechanisms Regulating Skeletal Muscle Blood Flow in Health and Disease **Romero/Hearon**	*Resp Section FT* Cell Plasticity: Calcium, cAMP and Beyond **Mehta**	*WEH SectionFT* Adaptations in Fluid Balance and Blood Pressure Regulation during Pregnancy **Denton/Veiras**

Tuesday, April 24, 2018, continued

25C	7:00 AM–8:00 AM *WIPC Symp* WIPC Symp 2018: Recognizing and Responding to Implicit Bias in Science **Al Alam/Wallace/Ho** 8:30 AM–10:00 AM *CEP Section FT* The Effects of Environmental Challenges on Performance and Metabolism **Williams/Hindle**	*EEP Section Symp* Molecular Transducers of the Physiological Adaptations to Exercise and Aging **Seals/Martens**	*EEP Section Symp* Respiratory and Limb Skeletal Muscle Weakness in Disease: Mechanisms and Treatments **Bowen/Ferreira**
26	*Resp Section Symp* Neuroplasticity of Airway Reflexes **Bolser/Pitts**	*Hypoxia Group FT* **Harris/Moya Cespedes**	*CEP Section Krogh Lecture* Supported by Novo Nordisk Fonden **Hillman**
27	*CV Section FT* Novel Discoveries in Vascular Physiology **Earley**	*WEH Section FT* Origins of Cardiovascular Disease: Does Metabolic Disease Always Come First? **Spradley/De Souza**	*CV Section Berne Lecture* **Yuan**
28A	*CNS Section FT* Interrogating Neuronal Circuits Mediating Body Fluid Homeostasis **Krause**	*CV Section Symp* The Vasculome: An Integrated Exploration of Vascular Reactivity, Lineage, and Specialization **Galis/Yin**	*Cell Section FT* Cell Signaling: Proteins, Pathways, and Mechanisms **Hamilton/Helms**
28B	*CV Section FT* Endothelial Cell Contraction or Retraction (Insights Into Barrier Function and Permeability) **Webb/Wenceslau**	*NCAR Section Symp* Sympathetic Neurovascular Transduction in Humans: Are we there yet? **Shoemaker**	*NCAR Section FT* Battle of the Reflexes: Chemo- vs. Baroreflexes during Physiological Stressors, Aging and Cardiovascular Disease **Kellawan**
28DE		*Renal Section Symp* Structure and Function of Renal Epithelial Cilia **Bell/Satlin**	
TBD		1:00 PM–2:00 PM *History Group Lecture* **Raven**	

Wednesday, April 25, 2018

Room	8:30–10:00 AM	1:30–3:00 PM	3:30–5:00 PM
20A	*IPSS Symp* Extracellular Matrix Remodeling and Integrin Signaling in Metabolic Diseases **Wasserman**		3:30 PM–4:30 PM APS Nobel Prize Award Lecture **Hartwell**
22	*CV Section Symp* Chemotherapy Induced Vascular Toxicity: Do Small Things Matter? Cosponsored by AJP - Heart and Circulatory Physiology **Beyer/Croce**	*WEH Section FT* Stress, Sleep, Circadian Rhythms and Blood Pressure Regulation **Gumz/Johnston**	

Wednesday, April 25, 2018, continued

23	*Physoc Symp* Epithelial Crosstalk and Innate Immunity **Garnett**	*APS/Physoc Symp* DAMPs and Inflammasomes: A Clear and Present Danger **Khan**	
24		*WEH Section FT* Immune Modulation of Blood Pressure and Vice Versa **Ryan/Itani**	
25A	*GIL Section Symp* Bile Acids in the Small Intestine and Colon, Physiology, Pathophysiology, and Therapeutic Opportunities **Keely/Lajczak**	*SFiB Pan-American Symp* Pan-American Symp **Campagnole-Santos**	
25B	*CV Section FT* Post-translational modifications in cardiovascular disease **Scott/Kohr**	*EEP Section FT* AMPK-mediated control of mitophagy **Hood/Yan**	
25C	*CEP Section FT* Comparative Models of Disease **Pamenter**	*EM Section Symp* Cardiac Metabolism Moving Center Stage: New Insights Enabling Metabolic Modulation Therapy **Wende/Glatz**	
26	*MBG FT* Role and Importance of Mitophagy in Skeletal Muscle in Health and Disease **Beaudry/Deldicque**	*CV Section FT* Mechanotransduction in Cardiovascular Function **Thodeti/Lindsey**	
27	*Resp Section FT* The Influence of State on Cardiorespiratory Control Mechanisms **Cummings/Dutschmann**		
28A	*EM Section FT* Brain - Gut Microbiota Interactions in Cardiovascular and Metabolic Control **Collister**	*CNS Section FT* The Gut-Brain Axis **Torres-Reveron/Appleyard**	
28B	*WEH SectionFT* Novel Approaches and Techniques in Water and Electrolyte Research **Smith**	*Resp Section FT* Resp Section Abstract-Driven FT **Prakash/Koval**	

Mentoring Forum

Where Academics Go to Die: Mentorship and "Alternative" Careers in Life Science

Emily J. Johnson
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Emily J. Johnson

Excess generally causes reaction, and produces a change in the opposite direction, whether it be in the seasons, or in individuals, or in governments.

– Plato, *Republic*

In 2002, mathematician and biologist Dr. Irakli Loladze argued that elemental changes in the earth's atmosphere could alter the nutrient composition of plants at the base of the food chain (15). The idea was not incredible: reports of altered growth, yield, and micronutrient-to-carbohydrate ratios in rice and cereal crops grown in high-CO_2 field conditions had been surfacing since the 1990s (5, 7, 8, 22). A rapid uptick in the pace of these reports has since removed any doubt that base food crops are susceptible to negative effects from excessive exposure to CO_2, a prerequisite for photosynthesis (9, 12, 16, 20). It is, literally, an example of total ecological shift resulting from too much of a good thing.

In many ways, this story of excess and its repercussions parallels the recent history of the science job market. It is a story of evolution, market pressure, and adaptation, which all mentors and students must know in order to navigate the new landscape of science jobs.

The Science Bubble

It is no secret that the familiar economy of academia – a vortex that sucks in students and keeps them forever as professors – is struggling to keep up with the sheer numbers of scientists emerging from institutes of higher education. Due, allegedly, to the ever-swelling ranks of their peers, young would-be scientists and professors are increasingly failing to find and keep long-term employment.

Of course, arguing that this is due to the change in our numbers would require accurate tracking of the number of scientists over time, which is nearly impossible. (For what it's worth, a hand-waving headcount of *The School of Athens* suggests that the original *Akademia* housed between four and five dozen scientists, not including Raphael, angels, and cherubs.) Regardless of how one might arrive at a serious baseline estimate, modern academia now has orders of magnitude more scientists. In 2015, U.S. institutions awarded 55,006 graduate degrees in science, technology, engineering, and math (STEM) fields, topping the previous record of 54,070 in 2014 (1, 2). Meanwhile, the number of academic positions has plateaued. By one calculation, the reproductive rate or R_0 of academic jobs tells us that this sector can employ just 12.8% of scientists (14). Put another way, 87.2% of scientists will have to find another home.

The public response to these numbers has been, at best, a little bit glum, and at worst, a dumpster fire of fear and indignation. *Why You Shouldn't Go To Grad School* and similar articles reflect deep anxiety rumbling in the ranks of current and future researchers (3, 17, 21). Voices from within the scientific community have tried to counter the angst by arguing that the problem is overstated, inaccurately presented, or even imaginary (18). However, telling students that the only thing they have to fear is fear itself does not seem to be working. The admonition that science is on a kind of employment precipice continues to appear. The *New York Times* stated the situation bluntly, telling readers, "The United States is producing more research scientists than academia can handle" (13). Even the National Public Radio Science Squeeze program admitted that "most postdocs are being trained for jobs that don't actually exist" (11).

These data cast a long shadow, and it is not pessimistic to ask questions about the future of science. Are there really too many of us? Will science be smothered by its own success?

As you might have guessed, I doubt it. There are indeed lots and lots of us, but I think the number of scientists itself is a red herring. No matter how you slice it, having too many scientists is not a problem. How could it be? An unprecedented number of scientists is a solution begging to be implemented.

There is another set of data, which receives less attention, and which very clearly points to a different problem. According to these figures, every Tom, Dick, and Harry scientist should have a job. From 2009 to 2015, the same period of time during which the U.S. awarded a record number of STEM graduate degrees, net domestic STEM-related employment grew twice as fast as non-STEM-related employment (10.5% vs. 5.2%), producing 817,260 new STEM jobs (10). Yet, in 2014, the proportion of PhD-trained individuals with "definite commitments for employment or postdoc study" declined, as it had for 4 of the 6 previous years (1). The same trend held for those who received doctoral degrees in the year 2014.

These data show that the problem with the academic job market is not just the number of scientists. The real problem, which the U.S. Bureau of Labor Statistics has précised – not without expressing some puzzlement – is that the U.S. has too much of three things at the same time: science jobs, scientists, and unemployment in science (24).

Figuring out how these problems can exist at the same time ought perhaps to be left to economists, who have been conducting naval-gazing evaluations of supply and demand in their own corner for quite some time, asking questions such as, *Does the Academic Labor Market Initially Allocate New Graduates Efficiently?* (the answer is no) (23). One hypothesis, which uses the analogy of taxi queuing, says that the fundamental problem is timing. That is, the asynchronous appearance of scientists compared to science employers creates bottlenecks that result in apparent oscillations in employment (24).

Regardless of the mechanism, the bottom line is that we are facing a problem that should not exist. Are there too many scientists for the traditional ecosystem of grants and professorships? Yes. Are tenure and grant funding withering? Maybe. But is the number of scientists the root of the science employment problem? No. The root of the problem is that new scientists are not, apparently, very good at getting those 817,260 new jobs.

To me, the solution to this problem starts and ends with mentorship – realistic, career-oriented mentorship. In my opinion, the biggest barrier that mentors have to overcome is embodied in three little words that make every non-academically employed scientist I know say, "Goosfraba."

The "Alternative" Career

In February of 2017, I left my postdoc for a position in clinical research at a community hospital. I love my new role, which is challenging, exciting, busy, and uses my education. But when I accepted it, many colleagues in academia thought I was making a bad choice by choosing an "alternative" career. "Once you leave, you can't come back," the apocryphal mantra went.

To this day, I am still not sure why this prodigal son narrative exists in academia. Calling every non-academic job "alternative" is so simplistic, it is almost meaningless. Imagine if the science of physiology recognized two types of species: zebra fish and non-zebra fish. The distinction is true, but useless for approximately 8,699,999 of the 8.7 million species of organisms on earth. Nevertheless, the idea of "normal" academic and "alternative" non-academic careers persists, and the future of life science may literally depend on how long we insist on approaching careers this way.

My argument is that good career-orientated mentorship is the answer to this problem. Certainly, it is the best chance we have to inspire the 87.2% of scientists who will not get academic jobs to break the industry ceiling.

First, the idea that the private sector is some kind of prison colony for people who are bad at Western blots must go. Obviously, the private sector is chock full of high-caliber scientists, but ex-academics still feel the need to defend against this prejudice.

"Regardless of not having an official faculty appointment, I consider myself a scholar, especially considering my training, my way of thinking, and how I approach and solve problems," says Dr. Vanessa Gonzalez-Perez, Assistant Dean for Diversity Initiatives in the Natural Sciences at Princeton University. In her role at Princeton, to which she transitioned from a faculty appointment in 2016, Gonzalez-Perez focuses on student access and retention across 13 natural science departments,

especially among historically underrepresented and first-generation students. Far from wasting her science education, Gonzalez-Perez feels that she is living her mission as a scientist every day. "I may not be in the lab designing experiments, but I am a still a scientist, and I definitely get to think of the problems we need to solve, design strategies, test them, and analyze the outcomes. I definitely have to use my critical thinking." And she is adamant in combating prejudice about leaving science. "People think administrators are frustrated people who just ended up in these positions. I had a choice to stay in science or do this, and I chose to do this, and its highly rewarding!"

Ryan Schindler, a Manufacturing Technical Specialist with Genentech whose work spans biology and engineering, agrees that the scientific method does not belong only to academia. Ryan was trained as a biologist and obtained a degree in biotechnology from Washington State University. "My friends in engineering used to tell me, you're basically an engineer." But it's all science, he says, and the application of scientific principles is more important than the specific facts he learned in his biology education. "My education helped me get the job, for sure," Schindler allows, "but the scientific mentality – the hypothesis testing – is something I apply a lot more often than my knowledge of PI3K signaling."

Some scientists actually leave academia to find inspiration. Dan Rodgers, founder and Chief Science Officer of AAVogen, Inc., ran a well-funded lab focusing on muscle-wasting diseases, but he left academia for an entrepreneurial venture inspired by his family. "My father died recently from cancer cachexia, and my nephew has Duchenne muscular dystrophy, two disease states directly related to my field of expertise," says Rodgers. "I personally love the academic mission," he explains, but eventually he felt that the private sector was a better fit for his mission. "I in no way regret my decision. Academia just wasn't rewarding anymore – it wasn't fun. Starting my own business? Now that's fun!"

Heidi Medford, a technology licensing associate at Washington State University, also left science to pursue a career with bigger impact. "It's becoming increasingly challenging to successfully fund an academic research laboratory," says Medford, a previous American Physiological Society Minority Fellow. For a scientist who wants to make an impact on her field, Medford believes, this is discouraging. "It has been my experience that very few scientists make a large impact on their chosen field." During her postdoc, Medford took a chance internship with her university's Office of Commercialization, which eventually offered her a permanent position. Far from leaving science, she feels that she has finally found a niche within science where she can make an impact. Besides publishing, she says, "many scientists have a hard time delivering their research to the greater good." But in her new job, she draws on her education to help scientists "bridge these gaps and deliver their discoveries to benefit mankind."

Gonzalez-Perez echoed these sentiments. "I am a scientist, but my motivation in life is to serve others," she said. Whether she does that by developing new therapies, pushing the boundaries of scientific knowledge, or helping students get access to higher education, she is living her goals. In fact, she sees unity between her science education and her current role. As a first-generation college student and a Latina woman, she sees her job as an exciting platform from which she can lift the next generation of scientists.

The private sector also pays well, although this can be an awkward conversation for academia, where a good salary is still something that should be killed with fire. Private sector careers offer a real and viable way for scientists to work in science and also, for example, pay off the average $18–36,000 in student loans that college-educated individuals acquire, depending on their state, by their senior year of college (4).

Failing to communicate this to STEM students is, in my opinion, an ethical issue. In the millennial workforce, a little guilt goes a long way: despite their debt, one of the distinguishing features of the millennial generation's job search is choosing meaningful causes and inspiration over paycheck size (19). In such a workforce, representing science as a bastion of (unpaid) holy stoicism might do more harm than good.

Even for successful professors, there is a pay gap between academia and industry. "I was a tenured full professor in an undergraduate department," says Rodgers. "I had a respectable salary and established responsibilities. My job was as stable as one can get in academia. Although

I now have much less job security, the prospect for financial success in particular is far greater."

Although industry definitely has the edge financially, working in private industry comes with less freedom compared with most faculty jobs. Compared with her previous faculty position, Gonzalez-Perez notes that her current job has "a lot of structure, and end goals are less flexible, but there is also room for being creative, innovative, and resourceful." A high level of individual freedom is one of the unique factors that makes academic jobs different from all other jobs. Scientists can expect a lower level of freedom when they join the industry workforce, where priorities are company-driven, compared with what they can do in faculty positions, she says.

Employees of a company like Schindler's are expected to function within the larger company mission. There is, however, comparative freedom for an individual like Rodgers, who runs his own company, although such freedom tends to come with risk. As the founder of his company, all decisions rest with him, as does "all of the good and bad credit" for every decision he makes.

Breaking the Industry Ceiling

Whether academia itself is an industry is a touchy subject. "Education is by definition an investment, with short-term costs and long-term gains. It is not, nor will it ever be, a business. Treating it as one debases the academic mission," says Rodgers. However, he acknowledges that the parallels between modern education and industry cannot be ignored, and the thin green line separating academia from industry is increasingly blurry.

"Both are driven by a bottom line," said Gonzalez-Perez, "but maybe they shouldn't be." Medford is unequivocal about it – when asked if she considers academia an industry, she says, "Absolutely."

Whether or not one considers academia an industry, since a transition out of academia is the likely career path for most scientists (14), breaking down barriers between academia and the private sector is essential for easing their way.

Mentors are uniquely poised to lead this change. Teaching students practical job-seeking skills, such as writing resumes rather than CVs, or even telling students that other careers exist, are good places to start. "I didn't know that the industry I'm working in existed," confessed Schindler.

Aimee Sutliff, a current graduate student in pharmaceutical sciences, expresses similar bewilderment. "During my time in graduate school, very little information has been provided about the variety of opportunities for a career outside of academia," says Sutliff. "I don't even know where to start looking for opportunities that are outside of strictly bench work in industry or faculty jobs in academia."

Discussing private sector jobs with students as a primary option rather than some back-alley alternative, and explaining the incredible variety of these jobs, will also help the next generation of scientists find employment. Encouraging students to seek internships and do activities outside of the curriculum is fundamental for their future success, although this is admittedly hard to do in laboratory cultures where 60-hour work weeks are the norm. In this area, life science could benefit from taking a page out of the playbooks of computer science and engineering, which have always partnered heavily with industry. University-hosted job fairs for life science companies, for example, would connect students with potential employers and smooth the path for private-sector collaborations.

Additionally, although technical skills and publications are the currency of academia, it is critical for students to know that soft skills are just as important as technical skills in the private sector. In this arena, mentors can promote their students' professional development by encouraging teamwork, collaboration, and communication skills in their lab groups. Above all else, networking may be the number one soft skill that academic programs can help students develop. "Knowing someone can help your resume get to the top of the stack," Schindler advised. "Networking can be critical to getting a job."

Networking also helps students stay abreast of market trends and current developments in their fields outside of the university environment, which can help these young scientists break into the private sector.

For students who are dedicated to their bench work, learning how to network can be an uphill battle. Sutliff says she is aware that some "invisible" jobs exist but

is unsure how to find them. "I have been told that most people find postdoctoral fellowships through unconventional means – for example, being offered a post that was never even advertised," she says. This gives the frightening impression that missed opportunities in grad school could ruin one's chances of obtaining a postdoctoral fellowship.

Including some non-traditional classes in graduate curriculums can also give students a leg-up in the private sector. Indeed, "diversifying a graduate education" is essential in modern science, according to Rodgers. "Running a biotech company requires formal training in a relevant life science as well as business management. Very few universities offer such training (for example, a combined PhD/MBA degree program), although this is exactly what's needed in the field." He also argues that students should be trained in practical aspects of non-academic science. "Students interested in a scientific career in industry should include business development and management courses in their formal course of study. Actually, I think this is critical. All other students should be encouraged to do this as well, because one can never predict the future."

The landscape of science jobs continues to change, but as physiologists, we can be prepared to adapt. By changing our vocabulary about "alternative" careers, reducing barriers in the academia-industry transition, and engaging in partnerships between academic institutions and life science industries, we can ensure that physiology survives and thrives. The stakes have never been higher: if we fail, the antiquated stigma about "alternative" jobs will be remembered as the meteor that killed the physiologists. ●

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Emily Johnson Biography

Emily Johnson is a scientific writer and project manager for Providence Medical Research Center at Sacred Heart Medical Center & Children's Hospital in Spokane, WA. During her PhD training in pharmaceutical sciences, Emily was a Graduate Fellow of the National Science Foundation, President of the Washington State University Spokane Graduate Research Student Association, and a trainee member of the American Physiological Society Communications Committee. Emily studied pharmacokinetic natural product-drug interactions during her post-doctoral training from 2016 to 2017. In her spare time, Emily is a freelance writer and illustrator.

Education

APS at SACNAS National Conference

The APS exhibited at and was a conference sponsor of the Society for the Advancement of Chicanos and Native Americans in Science (SACNAS) 2017 National Diversity in STEM Conference at the Salt Palace Convention Center (Salt Lake City, UT) October 18–20.

Christopher Mendias (Hospital for Special Surgery and Porter Physiology Development and Minority Affairs Committee Member) and Brooke Bruthers (Senior Program Manager, Diversity Programs) staffed the exhibit booth to promote APS's four summer research fellowship programs, undergraduate membership, and professional skills training opportunities, and to provide physiology career advice. Bruthers also staffed the physiology networking session. Over 100 conference attendees stopped by the APS booth, which was situated in the same row as several other FASEB societies.

APS was pleased to provide funding for awards for the first time this year. Judges awarded the best undergraduate poster presentations in physiology to:

- **Rufus Sweeney**, a Native American undergraduate student at the University of Utah. Sweeney's abstract title was "Islet Sphringolipids are Requisite for Maximal Insulin Secretion in Vivo."
- **Kenny Veliz**, a Hispanic/Latino undergraduate student at the University of California, Merced. Veliz's abstract title was "Activation of the Renin Angiotensin-Aldosterone System's Effect on Protein Disulfide Isomerase."

Each student received $1,000 and 1 year of APS membership.

The SACNAS National Conference is designed to motivate, inspire, and engage participants to achieve their highest goals in pursuing education and careers in all disciplines of science, technology, engineering, and mathematics from across the country. Conference programming is specifically tailored to support undergraduate and graduate students, postdoctoral researchers, and career professionals at each transition stage of their career as they move toward positions of science leadership.

The conference showcases cutting-edge science and features mentoring and training sessions for students and scientists at all levels. Over 4,000 students and professionals attended the conference, more than 1,000 oral and poster presentations were given, and more than 350 exhibits shared training, research, grad school, and job opportunities. SACNAS offers over 100 professional development and scientific sessions and gives out over 500 travel scholarships.

2017 SACNAS student awardees

Support for travel to this conference is supported by the National Science Foundation (IOS-1238831) and National Heart, Lung, Blood Institute/NIH (R25 HL-115473-01).

The 2018 annual conference will be held in San Antonio, TX, October 11–13. For more information about the SACNAS National Conference, visit *www.2017sacnas.org*. For more information on APS diversity programs, visit *www.the-aps.org/diversity*.

APS at Annual Biomedical Research Conference for Minority Students

The APS was an exhibitor and major conference sponsor at the 2017 Annual Biomedical Research Conference for Minority Students (ABRCMS) at the Phoenix Convention Center (Phoenix, AZ) November 1–4. ABRCMS is one of the largest professional conferences for underrepresented minority students in science, technology, engineering, and mathematics (STEM), attracting over 4,000 attendees.

The conference is designed to encourage underrepresented minority students to pursue advanced training in STEM disciplines and to provide faculty mentors and advisors with resources for facilitating students' success. More than 900 representatives from graduate programs at U.S. colleges and universities as well as scientists from government agencies, foundations, and professional scientific societies joined ABRCMS in the exhibitors program to share information about graduate school and summer internship opportunities. APS attends to encourage students to pursue physiology PhDs and to promote APS membership and awards.

The APS, represented by Adrienne Bratcher (University of Louisville and APS Porter Physiology Development and Minority Affairs Committee Member) and Lindsey Stavola (Yale University and APS Minority Outreach Fellow), staffed the exhibit booth.

During this 4-day conference, over 2,000 undergraduate students participated in poster and oral presentations in 12 disciplines in the biomedical and behavioral sciences, including mathematics. All undergraduate student presentations are judged, and those receiving the highest scores in each scientific discipline and in each educational level were given an award during the final banquet.

The APS was pleased to provide funding to help award 16 undergraduate students for the best oral and poster presentations in the physiological sciences. Students received $300 and 1 year of APS membership.

Support for APS to attend and exhibit at this conference is provided by APS, the National Heart, Lung, and Blood Institute/NIH (R25 HL-115473-01), and FASEB MARC (R25 GM-116706-01).

The 2018 annual conference will be held in Indianapolis, IN, November 14–17. For more information about ABRCMS, visit *www.abrcms.org*. For more information on APS diversity programs, visit *www.the-aps.org/diversity*. ●

2017 Physiology Awardees at ABRCMS

Oral Presentations

Student Name	Student Institution	Abstract Title
Eden Ramirez	Schoolcraft College	*Role of Obstructive Sleep Apnea in Nocturnal Heart Blocks*
Emily Valentin-Mendezw	University of Puerto Rico, Rio Piedras	*Cinnamic Aldehyde Encapsulated in PLGA Nanoparticles as a Potential Therapeutic to Improve Arterial Surgery Outcomes*
Reuben Hogan	Washington University in St. Louis	*Scavenger Receptor Class B Type I Mediates Coenzyme Q Levels in Metabolic Tissue*
Kailey Singh	City College of New York	*Protective Effects of Mitochondrial Targeted Peptide SS-20 on Renal Damage in the Unilateral Ureteral Obstruction Model*

Poster Presentations

Student Name	Student Institution	Abstract Title
Ha Brenda Luu	University of St. Thomas	*Determining the Correlation between Drosophila melanogaster Toluene Exposure and the Resulting Toxicity Effects on Fly Survival and Fecundity*
Kaitlyn Matthey	St. Edward's University	*Effects of Initial Pesticide Exposure on Habitat Choice in the Gulf Coast Toad*
Onoriode Ighofose	Prairie View A&M University	*Changes in Renal Lymphatic Vessels During Hypertension*
Cheysaliz Perez-Verdejo	University of Puerto Rico, Rio Piedras	*Cinnamic Aldehyde Conjugate-based Nanoparticles (NPs) for the Treatment of Arterial Restenosis*
Ricardo Navarro	University of Puerto Rico	*Nitrogen Mustard Modulates Cell Cycle Progression in Human Keratinocytes*
Judd Collado	University of California, Los Angeles	*Sarcospan Overexpression and its Effect on Duchenne Muscular Dystrophy-Associated Cardiomyopathy*
Ariella Saslafsky	University of Central Florida	*Sympathetic Innervation of Pancreatic Islets in Individuals with Type 1 Diabetes Innervation of Pancreatic Islets in Individuals with Type 1 Diabetes*
Steven Toro	University of Puerto Rico, Humacao	*Methionine Sulfoxide has no Hepatoprotective Effect against Acetaminophen-Induced Liver Injury*
Maribel Anguiano	University of California, Davis	*Mobile Elements Responsible for the Acquisition of Resistance Genes, dfrA21 and sul1, Found in an Environmental Raoultella ornithinolytica*
Emily Bryant	University of California, Irvine	*Environmental Escherichia coli Carries Extended Spectrum Beta-lactamase Antibiotic Resistance Genes*
Arren Simpson	University of Detroit Mercy	*Uterine Myometrial Preconditioning Facilitates Molecular Tocolytics Producing a Term Pregnancy*
Rebecca Zlatkin	Miami Dade College	*The Effects of Ocean Acidification on California Sea Hare (Aplysia californica)*

APS Promotes Physiology to K-12, Community, and 4-Year College Teachers at Fall Meetings

Association for Middle Level Education

APS promoted physiology for the seventh consecutive year to middle school educators at the annual Association for Middle Level Education (AMLE) Conference. The conference, held in Philadelphia, PA, November 5–8, was attended by over 3,500 teachers, administrators, and counselors from across the country.

Excited teachers kept APS staff busy with questions about the APS Life Science Teaching Resource Community, age-appropriate careers materials such as the career trading cards, and the Online Six Star Science Professional Development Fellowship. APS's booth is highly attended because of the limited opportunities to procure science resources at AMLE. Next year's conference will be held in Orlando, FL, October 25–28.

National Association of Biology Teachers

APS featured physiology resources and supported a speaker at the National Association of Biology Teachers (NABT) Conference in St. Louis, MO. The annual national conference, held the first week of November, attracts middle and high school teachers, as well as, 2- and 4-year college faculty from across the nation. APS booth materials highlighted the Undergraduate Research Fellowships, Life Science Teaching Resource Community (*www.LifeSciTRC.org*), Frontiers in Physiology teacher professional development program, career materials, and K-12 outreach opportunities.

This year's sponsored speaker was APS member Kathryn Johnson. Johnson presented "Horse Hormones: Predicting and Preventing Painful Lameness Initiated by Insulin Resistance." Johnson utilized her reaserch presentation as a platform for modeling effective teaching methods while presenting original data. Attendees were engaged in active learning pedogogies and followed the presentation with a series of lively questions. Johnson's presentation closed with an invitation to attendees to register for the APS Institute on Teaching and Learning to be held in Madison, WI in June 2018. (*www.the-aps.org/itl*)

Undergraduate programs were the focus of questions from community and 4-year college teachers who eagerly asked for information about the undergraduate research opportunities; discussion focused particularly on the variety of available locations, the inclusion of URM, especially students with disabilities, and the availability of stipends. Many of the attendees who stopped at the booth brought colleagues and friends back to the booth the following day.

Next year's conference will be held in San Diego, CA, November 8–11. For further information, please contact Margaret Shain Stieben, Program Manager, K-12 Education Programs (*mstieben@the-aps.org*) or Jessica Taylor, Sr. Manager, Higher Education Programs (*jtaylor@the-aps.org*). ●

Kathryn Johnson, PhD

Ring in the New Year with Award Opportunities from the Education Office!

To apply for any of these awards, go to *the-aps.org/awardapps*.

Barbara A. Horwitz and John M. Horowitz Undergraduate Research Awards

Application deadline: January 12, 2018

the-aps.org/eb-undergrad

The Horwitz/Horowitz Undergraduate Research Awards are presented annually to undergraduate students who are first authors on an EB abstract and presenting their research at the EB meeting. There are two types of awards that students can apply for through a single application. See the website for more details.

Barbara A. Horwitz and John M. Horowitz Outstanding Undergraduate Abstract Awards

This award provides $100 and a 2-year complimentary membership with APS. The student must be enrolled as an undergraduate at the time of the abstract submission, be the first author on a submitted abstract for the EB meeting, and be working with an APS member. Selection of awardees is based on the abstract, letter of application, and letter of support from the research host. Receipt of the award is contingent on presenting the research at EB.

Barbara A. Horwitz and John M. Horowitz Excellence in Undergraduate Research Awards

To be considered for this award, students must be a Horwitz/Horowitz Outstanding Undergraduate Abstract Awardee and must attend and present a poster at the EB meeting. The recipients receive $400 and a certificate. The highest-ranked awardee receives another $250. Selection of awardees is based on the quality of the poster and oral presentation of the poster to the Award Selection Committee.

Porter Physiology Development Fellowships

Application deadline: January 15, 2018

the-aps.org/porter

The goal of the Porter Physiology Development Program is to encourage diversity among students pursuing full-time studies toward the PhD in the physiological sciences and to encourage their participation in the Society.

The Porter program provides a full-time graduate fellowship ($28,300 during the academic year) to students in programs leading to a PhD in the physiological sciences at U.S. institutions. The program is open to underrepresented racial and ethnic minority applicants who are citizens or permanent residents of the U.S. or its territories and student members of the Society. The applicant's PI/mentor must also be a member of APS.

Porter Fellows

Dale J. Benos Early Career Professional Service Award

Application deadline: January 24, 2018

the-aps.org/benos

The Dale J. Benos Early Career Professional Service Award honors an early career stage (graduate student, postdoctoral fellow, assistant professor, or equivalent position) member of the APS who is judged to have made outstanding contributions to the physiology community and demonstrated dedication and commitment to furthering the broader goals of the physiology community. This can be by serving on professional committees, by participating in K-12 education outreach, by participating in scientific advocacy and outreach programs, or by otherwise strengthening and promoting the physiology community. See the website for more details and apply online at *the-aps.org/awardapps*.

Undergraduate Summer Research Fellowships

Application deadline: February 1, 2018

the-aps.org/summerresearch

APS is proud to offer four fellowship programs that allow undergraduate students to participate in research during the summer (IOSP, STRIDE, UGSRF, and UGREF). Recipients spend an average of 10 weeks in the laboratory of an established scientist and APS member. Each program recruits undergraduate students nationwide, two internationally. Two programs specifically target students from underrepresented backgrounds and students with disabilities. Each Fellow receives a stipend plus additional funds for travel to present his or her research at EB. Research hosts receive funds for student lab supplies. See the website for more details. ●

Science Policy

APS Members Advocate for Animal Research on Capitol Hill

Seventeen current and former members of the Animal Care and Experimentation Committee went to Capitol Hill on September 26 to provide the APS perspective on the importance of humanely-conducted animal research.

Dan Michele, Laura McCabe, and Harold Schultz stand outside Michigan Congressman Micheal Bishop's office before a meeting

Sonnet Jonker, Richard Auten, and Ken McKeever pose before Mountain and Clouds by Alexander Calder in the Hart Senate Office Building

Ed Dzialowski, Karen Uray, Gaylen Edwards, and Corey Reynolds show off their #ActualLivingScientist buttons in front of the Capitol dome

Lauren Stein, Timo Rieg, and Don Bolser break for a photo between meetings

Committee members sported #ActualLivingScientist buttons while on the Hill

Groups Urge Streamlining Animal Research Oversight

Prominent research organizations recently issued recommendations for promoting greater efficiency in the oversight of animal research. *Reforming Animal Research Regulations* was based on an April 17, 2017 workshop convened by the Federation of American Societies for Experimental Biology, Association of American Medical Colleges, Council on Governmental Relations, and the National Association of Biomedical Research. The goal of the workshop was to "provide actionable recommendations for promoting regulatory efficiency, animal welfare, and sound science." Participants included research scientists, laboratory animal veterinarians, institutional officials responsible for research compliance, and science policy experts. The report reflects their consensus on best approaches to improve on conflicting, outdated, or ineffective oversight policies.

The report includes numerous recommendations for simplifying regulations and policies issued by the National Institutes of Health (NIH) and the U.S. Department of Agriculture (USDA), which are the primary federal agencies involved in the oversight of federally funded biomedical research. It also contains several recommendations for dealing with regulatory processes that are directed toward the White House and its Office of Management and Budget (OMB). Other recommendations that would require changes to current law are directed toward Congress.

Reforming Animal Research Regulations recommends that both NIH and USDA establish a risk-based process for reviewing animal research protocols, similar to the one in place for reviewing research protocols involving human subjects. The goal of this change is to expedite Institutional Animal Care and Use Committees (IACUC) review of studies involving low-risk, noninvasive, or minimally invasive procedures.

The report points out that the *Guide for the Care and Use of Laboratory Animals* "is not a regulatory document" and urges NIH's Office of Laboratory Animal Welfare (OLAW) to "use the *Guide* as it was intended, namely, 'to assist institutions in caring for and using laboratory animals in ways judged to be professionally and humanely appropriate.'" The report notes that the *Guide* "allows facilities to produce welfare outcomes for animals in diverse and innovative ways by permitting alternative strategies to 'should' statements upon approval by the IACUC." Accordingly, the report recommends that OLAW revise its policies to state that IACUC-approved alternatives to "should" statements in the *Guide* will no longer be "deemed departures or deviations" from acceptable practice.

The report calls on the USDA to revise the language of its Animal Care Policy #12 regarding literature searches. It points out that the Animal Welfare Regulations give the IACUC the responsibility of ensuring that the principal investigator has "considered alternatives to procedures that may cause more than momentary or slight distress or pain," and that the regulations only require that the investigator provide "a written narrative description of the methods and sources" used. The report also urges USDA to revise its Animal Care Policy #14 to permit multiple survival surgeries at the discretion of the IACUC when justifiable for scientific and animal welfare reasons. This change reflects the intent of the language in both the Animal Welfare Act and the Animal Welfare Regulations. In addition to reducing regulatory burden, this change would also enable researchers to reduce the total number of animals needed for research.

Perhaps the most sweeping recommendation asks the White House and OMB to explore the possibility of "[harmonizing] existing federal requirements for those species currently covered by USDA and those covered by the Public Health Service Policy on Humane Care and Use of Laboratory Animals to conform to the least burdensome standard while maintaining animal welfare." The report also recommends that OMB consider requiring at least a 60-day comment period when federal agencies issue "interpretive rules" such as policies, guidance documents, or frequently asked-questions.

The report is available at *http://www.the-aps.org/ReformingRegulations*. ●

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Is Your Research a Clinical Trial? The Answer May Surprise You!

Subsequently, in September 2016, NIH introduced policies affecting the award (2), conduct (3), and reporting (4) of NIH-funded clinical trials. These policies were released through both internal (5) and external (6) channels, with the stated – and laudable – goals of improving transparency and public trust in the clinical trials process. But many people were unaware of the new policies' implications on studies previously not considered to be clinical trials.

First, the updated policy (7) on registering and reporting results from NIH-supported clinical trials on the website Clinicaltrials.gov requires investigators to register their trials within 3 weeks of enrolling volunteers, and to submit trial results within 1 year of completion; this policy took effect in January 2017.

Second, also effective January 2017, all investigators and staff involved in any capacity with clinical trials must complete Good Clinical Practice training. Finally, beginning in January 2018 (8), all grant applications proposing clinical trials must be submitted through clinical trial-specific Funding Opportunity Announcements (FOAs), as opposed to existing parent announcements.

Concerns from the Community

Because of the more inclusive language NIH employed in its definition, most human-subject research now qualifies as a clinical trial. However, if a researcher's work had not previously been categorized as a clinical trial, she or he likely would not have been attentive to a definition change or all the ensuing clinical trials policies. With two major policy changes in effect and implementation of another rapidly approaching, a lack of awareness still exists in the research community regarding these changes and their implications.

Some question whether the new requirements make sense for non-traditional (e.g., exploratory, mechanistic, or observational) trials. Another cause of concern is whether the Clinicaltrials.gov website is capable of handling the increased data uploads and traffic that will result from the new registering and reporting requirements. And some wonder how the FOA policy will apply to investigators in the Small Business Innovation Research (SBIR) program or those funded through grant mechanisms intended to replace a researcher's R01 portfolio (i.e., the R35). FASEB raised these various concerns with the NIH Office of Extramural Research.

NIH officials maintain that the community was given ample time to prepare for the changes, noting that the new clinical trial definition took effect in 2015, and the Notice of Proposed Rulemaking (NPRM) (9) regarding the new registration and reporting requirements was released in 2014. FASEB responded (10) to the NPRM but did not foresee the inclusion of "basic" research in the clinical trial reporting regulations as an outcome of the proposal. Based on its response (11) to an open sign-on letter (12) and meetings with community stakeholders, NIH seems unlikely to change or delay implementation of clinical trial-related policies.

What This Means for You

If you conduct research with human subjects, visit the NIH decision tool (13) to determine whether NIH considers your work to be a clinical trial – even if you do not. The answer has implications for your funding as well as for laboratory and administrative practices, as outlined above.

In addition to the decision tool, NIH has produced a video tutorial (14), Case Studies (15), and Frequently Asked Questions (16) to help investigators better understand how the clinical trials definition and policies apply to their research. Other useful resources and explanations can be found on the NIH clinical trials webpage (17).

Be in close contact with your program officer (PO), who understands the nuances of the NIH clinical trial definition and implications for your research, and should be able to answer questions that the case studies and FAQs cannot.

Finally, familiarize yourself with the FOAs that pertain to your work. NIH recently released the parent announcements (PAs) for clinical trial-eligible R01s (18) and R21s (19), and although many institutes are accepting applications through the PAs, some are not. Moreover, institutes have been clarifying through notices what kinds of research applications they will accept through the PAs, and will continue to release clinical trial-eligible, topical requests for applications.

Elizabeth Barksdale is a Science Policy Analyst in the Office of Public Affairs at the Federation of American Societies for Experimental Biology. ●

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3. *https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-148.html*
4. *https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-149.html*
5. *https://nexus.od.nih.gov/all/2016/09/16/clinical-trials-stewardship-and-transparency/*
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9. *https://www.gpo.gov/fdsys/pkg/FR-2014-11-21/pdf/2014-26197.pdf*
10. *http://www.faseb.org/Portals/2/PDFs/opa/2015/3.4.15%20FASEB%20Response%20to%20HHS%20NPRM.pdf*
11. *http://fabbs.org/wp-content/uploads/2017/10/Lauer-Response.pdf*
12. *http://fabbs.org/wp-content/uploads/2017/10/Letter-to-NIH-Collins-Re-Clinical-Trials-Signed.pdf*
13. *https://grants.nih.gov/ct-decision/index.htm*
14. *https://grants.nih.gov/policy/clinical-trials/tutorial/story_html5.html?lms=1*
15. *https://grants.nih.gov/policy/clinical-trials/case-studies.htm*
16. *https://grants.nih.gov/grants/policy/faq_clinical_trial_definition.htm*
17. *https://grants.nih.gov/policy/clinical-trials.htm*
18. *https://grants.nih.gov/grants/guide/pa-files/PA-18-345.html*
19. *https://grants.nih.gov/grants/guide/pa-files/PA-18-344.html*

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Reflections

Having been at APS for as long as I have, there are some who consider me to be the founder of APS, or at least the one constant at the Society that many of our members have experienced during their careers. In actuality, I came to APS long after the founding of the Society. I joined APS in July 1985 as Society's Executive Secretary-Treasurer, the historical title for my current position. I followed Milton O. Lee, who held the office from 1948 to 1956, Ray G. Daggs from 1956 to 1972, and Orr E. Reynolds from 1973 to 1985. Although I was hired as the Society's Executive Secretary-Treasurer, I soon discovered that a change was in the wind. During the Centennial held in 1987, the Council called me into an executive session to inform me that they had decided to fire the Executive Secretary-Treasurer. Not surprisingly, I was shocked and, uncharacteristically, had few words in response. After a short pause, Frank Knox informed me that they had decided to rehire me as Executive Director. I accepted, and I have had the pleasure of serving as the Society's Executive since 1985, working with the volunteer leadership, the membership, and the outstanding APS staff to advance the Society and the discipline of physiology.

When I arrived at APS in 1985, the programs of the Society were not as expansive as they are at present. Instead, the Society was focusing on the upcoming Centennial celebration that was held in Washington, DC in 1987. The Society was looking backward in order to honor its history, but my charge was to help the APS move forward. As part of its retrospective efforts, Orr E. Reynolds, my predecessor as Executive Secretary-Treasurer, worked with Toby A. Appel, an historian of science, and John R. Brobeck, a former President, to write the *History of the American Physiological Society: The First Century, 1887–1987*. Hopefully, each of you has had the opportunity to read the Centennial history, since it is freely available online to APS members as a result of our partnership with Springer.

In preparing the history and defining the origins of the Society, the authors took some literary license when they identified the founders of APS. As can be seen below, the invitation letter was signed by S. Weir Mitchell, Henry Newell Martin, and Henry Pickering Bowditch.

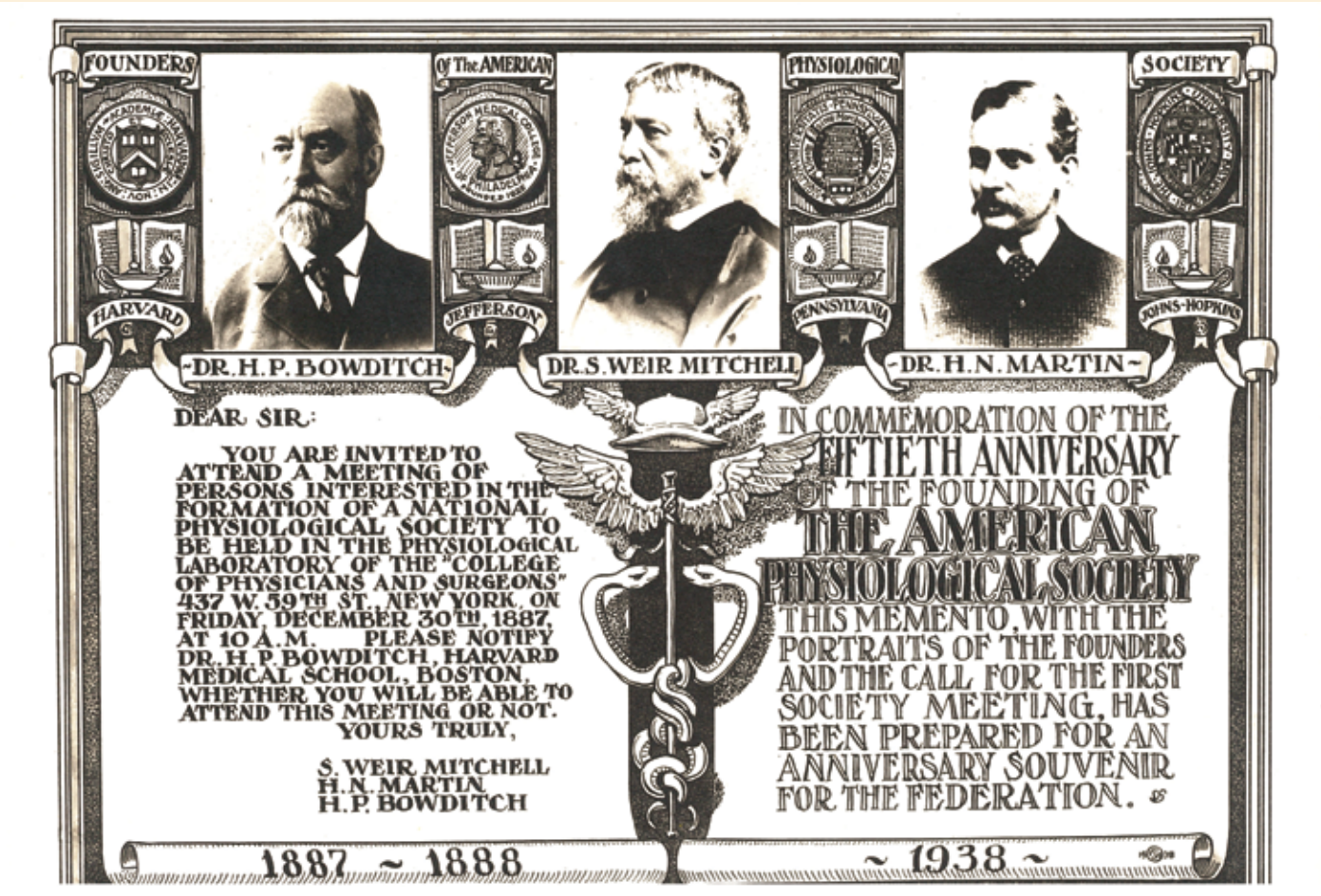

Figure 1. Commemorative invitation from the 50th anniversary celebration depicting the names of the three primary founders of APS: Henry Pickering Bowditch, S. Weir Mitchell, and Henry Newell Martin.

However, it was decided that two additional individuals should be identified as APS founders. It was relatively easy to include John Green Curtis as a founder since he hosted the Society's organizational meeting at the College of Physicians and Surgeons, Columbia University. The inclusion of Russell Henry Chittenden was a bit more difficult to understand. Chittenden held the distinction of serving as president of APS for the longest period of time, from 1896 through 1904, a total of 9 years. Scientifically, he was considered to be the dean of "physiological chemistry," or biochemistry in America, a field that was considered to be part of physiology at the time. However, in 1906, Chittenden became the first president of the American Society of Biological Chemists. Despite this, it was decided that Chittenden should also be considered an APS founder.

Over the years, I have heard concerns expressed about the decision to recognize five individuals as the Society's founders. It is clear that Mitchell, Martin, and Bowditch were founders because they did sign the letter of invitation. Whether or not Chittenden and Curtis should be considered founders is uncertain. However, at the time of the Centennial, and in the pages of the APS Centennial history, it was decided that APS had five founders who were memorialized on the medallion created for the event. Although some may disagree with the decision of the authors of the Centennial volume and the organizing committee to take editorial license to name the five as the Society's founders, I urge you to read the Centennial history and make your own decision. In the meantime, APS will continue to refer to the five as the Society's founders. ●

– Martin Frank

Membership

New Regular Members

***transferred from student membership**

Hasan Abdallah
Childrens Heart Inst., McLean, VA

Ryan John Adam
Univ. of Nebraska Med. Ctr., Omaha, NE

Shipra Agrawal
Nationwide Children's Hosp., Columbus, OH

Trent Anderson
Univ. of Arizona-Coll. of Med. Phoenix, Phoenix, AZ

A. Vania Vania Apkarian
Northwestern Univ., Chicago, IL

Prem Balaguru
Government Thiruvannamalai Med. Coll., Pondicherry, India

Albertino Bigiani
Univ. di Modena e Reggio Emilia, Modena, Italy

Hannah Justine Block
Indiana Univ. Bloomington, Bloomington, IN

Cord Michael Brundage
Cal Poly-Pomona, Pomona, CA

Vienna Elizabeth Brunt*
Univ. of Colorado-Boulder, Boulder, CO

Kanokwan Bunsawat
Univ. of Utah, Salt Lake City, UT

Roberto Carlos Burini
UNESP Med. Sch., Botucatu, Brazil

Marcelo Coertjens
Federal Univ. of Piaui, Porto Alegre, Brazil

Rosemary Alice Cowell
Univ. of Massachusetts-Amherst, Amherst, MA

Steven Allen Crone
Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Franziska Dengler
Univ. of Leipzig, Leipzig, Germany

Lucie Delphine Desmoulins
Tulane Univ., New Orleans, LA

Clayton Thomas Dickson
Univ. of Alberta, Edmonton, AB, Canada

Wei Dong
Loma Linda Univ. Hlth. Loma Linda CA

John Edwards
New York Med. Coll., Valhalla, NY

Wenguang Feng
Univ. of Alamaba at Birmingham, Hoover, AL

Michelle Brigid French
Univ. of Toronto, Toronto, ON, Canada

Frank Frisch
Chapman Univ., Orange, CA

Xiaoming Gong
Texas Tech Univ. Hlth. Sci. Ctr., El Paso, TX

Martin Grosell
RSMAS, Univ. of Miami, Miami, FL

Phil Hansbro
The Univ. of Newcastle, Callaghan, NSW, Australia

Ulrik Winning Iepsen
Univ. of Copenhagen, Copenhagen, Denmark

Kamlesh Jha
All India Inst. of Med. Sci. Patna, Khagul Patna, India

Prathamesh Haridas Kamble
All India Inst. of Med. Sci. Jodhpur, Jodhpur, India

Kazuyo Kegan
Johns Hopkins Univ., Baltimore, MD

Izhak Kehat
Technion-Israel Inst. of Tech., Haifa, Israel

Elizabeth Kim
Cedars-Sinai Med. Ctr., Los Angeles, CA

Christine Anne Klemens*
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Dudley Lamming
Univ. of Wisconsin-Madison, Madison, WI

Gabrielle Lapping-Carr
Univ. of Chicago, Chicago, IL

Meredith Luttrell
Drake Univ., Des Moines, IA

Lee Mitchell Margolis*
U.S. Army Res. Inst. of Environmental Med., Norfolk, MA

C Daniel Meliza
Univ. of Virginia Charlottesville VA

Claudio Molinari
Univ. Del Piemonte Orientale, Novara, Italy

Adrien Molinas
Tulane Univ., New Orleans, LA

Geoffrey G. Murphy
Univ. of Michigan, Ann Arbor, MI

Omaima Dahab Nasir
Taif Univ., Turabah Branch, Taif, Saudi Arabia

Atsushi Noritake
Natl. Inst. for Physiological Sci. Okazaki, Aichi, Japan

Everson Araujo Nunes
UFSC-Trindade-ccb-Centro Ciências Fisiológicas, Florianópolis, Brazil

Colleen Klocek Nye
Baldwin Wallace Univ., Berea, OH

Cristhiaan D. Ochoa
UT Southwestern Med. Ctr., Dallas, TX

Mark L. Ormiston
Queen's Univ., Kingston, ON, Canada

Rajan Pandit
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Arshad Rahman
Univ. of Rochester Sch. of Med., Rochester, NY

Matthew D. Regan
Univ. of Wisconsin-Madison, Madison, WI

David Ritter
Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Robert Steven Rogers
Kansas City Univ. of Med. and BioSci., Joplin, MO

Per Svenningsen
Univ. of Southern Denmark, Odense, Denmark

Shigekazu Takemura
Osaka City Univ., Osaka, Japan

Madras Venkatachalam
SV Med. Coll., Hyderabad, India

Christine Vohwinkel
Univ. of Colorado, Aurora, CO

Elani Fourie Wiest
Univ. of New Mexico, Albuquerque, NM

Justin L Wilson
Cleveland State Univ., Cleveland, OH

Michael Zawada
A.T. Still Univ., Mesa, AZ

New Graduate Student Members

Maiadah Nasser Alfares
Univ. of Bristol, Bristol, United Kingdom

Adrien Anthony Arias
Univ. of California, Irvine, Colton, CA

Hana Elisabeth Baker
IU Sch. of Med., Indianapolis, IN

Dakshnapriya Balasubbramanian
Texas A&M Univ., College Station, TX

Kris Camelio
Tennessee State Univ., Nashville, TN

Alexandra Chang-Graham
Baylor Coll. of Med., Houston, TX

Adam Thomas Corkery
Univ. of Wisconsin-Madison, Madison, WI

Manuel Alejandro Cornejo
UC Merced, Merced, CA

Khalid Elsaafien
The Florey Inst. of NeuroSci. and Mental Hlth., Melbourne, VIC, Australia

Anish Engineer
Western Univ., London, ON, Canada

Malika Felton
Bournemouth Univ., Bournemouth, United Kingdom

Roland Ferger
RWTH Aachen Univ., Aachen, Germany

Anabel Flores
Univ. of Michigan, Ann Arbor, MI

Barnita Haldar
Univ. of South Alabama, Mobile, AL

Ana Patricia Hernández García
Univ. Autónoma de San Luis Potosí, San Luis Potosí, Mexico

Eva Hernández-Navalón García
Univ. of Barcelona Manuel, Valencia, Spain

Michelle Herrera
Univ. of California-Irvine, Irvine, CA

Sile Hu
New York Univ. Sch. of Med., Elmhurst, NY

Victoria Noel Jensen
Univ. of Cincinnati, Cincinnati, OH

Shannon Kelly
St. Louis Univ., St. Louis, MO

Hyo Gun Kim
Pennsylvania State Univ., State College, PA

Benjamin Kramer
New York Inst. of Tech. Coll. of Osteopathic Med., East Northport, NY

Shin-Haw Lee
Univ. of Toronto, Toronto, ON, Canada

Vena Martinez
Baylor Coll. of Med., Houston, TX

Greg Lawrence McKie
Wilfrid Laurier Univ., Waterloo, ON, Canada

Christopher L. Melendez
California State Univ., San Marcos Escondido, CA

Keyshla M. Melendez-Rosado
Univ. of Massachusetts Boston, Riverside, RI

J. Grant Mouser
Univ. of Mississippi, University, MS

Francesca Elisabeth Mowry
Auburn Univ., Auburn, AL

Cahuê Murat
Univ. of São Paulo, Ribeirão Preto, Brazil

Jalissa Lovenia Nguyen
Univ. of Wisconsin-Madison, Madison, WI

Brandon Edward Pike
North Carolina State Univ., Littleton, NC

Elizabeth Puente
Baylor Coll. of Med., Houston, TX

Christine Dayanara Rodriguez
Pontifical Catholic Univ., San German, PR

T. Alex Ruwe
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Medha K. Satyal
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Nicole M. Sauls
Univ. of Wyoming, Laramie, WY

Richard Steven Severin
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Mirian de Almeida Silva
Auburn Univ., Auburn, AL

Nicholas Talley
Midwestern Univ., Glendale, AZ

Sundus Tariq
Univ. Med. and Dental Coll., The Univ. of Faisalabad, Faisalabad, Pakistan

Rany Vorn
Chung Ang Univ., Seoul, Republic of Korea

Alyssa Weinrauch
Univ. of Alberta, Edmonton, AB, Canada

Undergraduate Student Members

Uttpal Anand
Shuats, Allahabad, Uttar Pradesh, India

Jayson Bakonyi
Texas A&M Univ. Kingsville, New Braunfels, TX

Adetula Blessing
Univ. of Med. Sci., Ondo State, Ondo, Nigeria

Megan Lee Boniface
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Ryan Griffin
Michigan State, Macomb, MI

Hunter M. Hesslink
Portland State Univ., Damascus, OR

Abe Dwayne Johnson
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William Wade McAdams
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Krystal Bianca Puente
Texas A&M Univ.-Kingsville, Kingsville, TX

Cheyenne Unterbrink
Texas A&M Univ. Kingsville, Riviera, TX

Albert Uzzle
Old Dominion Univ., Norfolk, VA

Affiliate Members

Magnus Michael Chukwudike Anyakudo
Univ. of Med. Sci. Ondo State Nigeria, Ondo City, Nigeria

Maya Goapalakrishnan
Jawaharlal Inst. of Postgraduate Med. Education & Res., Pondicherry, India

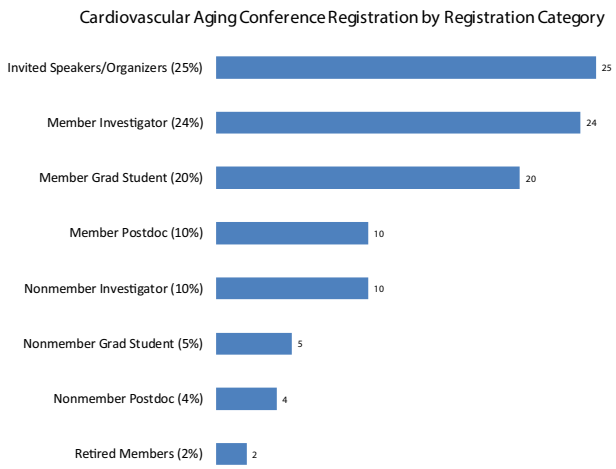
Conference Reports

Cardiovascular Aging: New Frontiers and Old Friends

Conference Report

The 2017 APS Conference on *Cardiovascular Aging: New Frontiers and Old Friends* was held August 11–14 in Westminster, CO. The conference was organized by Andreas Beyer (co-chair), Anthony Donato (co-chair), Prasad Katakam, Amanda Jo Leblanc, Lisa Lesniewski, Judy Muller-Delp, and Douglas Seals. The purpose of the conference was to investigate novel developments in the field of cellular and arterial aging and the mechanisms that contribute to cardiovascular disease through the mitochondria, free radical signaling, immune cell responses, and cellular inflammation. Sessions were developed to discuss the implications of aging vascular systems in a host of different physiological systems, organs, and tissues. The sessions emphasized the translation of cell and molecular observations to ex vivo and intact mammalian systems. Topics included age-related arterial disease states such as atherosclerosis, hypertension, and heart failure, as well as age-related tissue and systems dysfunction that are not typically thought to be influenced by vascular functions such as cancer, metabolic syndrome, and autoimmune disease.

A total of 100 registrants attended the conference. The majority of registrants (83%) were from within the U.S. Other countries represented were Canada (5%), Japan (2%), South Korea (2%), Thailand (2%), Saudi Arabia (1%), The Netherlands (1%), Israel (1%), Italy (1%), Singapore (1%), and the United Kingdom (1%). Figure 1 provides the breakdown of registration by registration category.



The conference program consisted of a welcome address and opening reception, 11 symposia sessions, 1 trainee competition oral session, and 2 poster sessions incorporating all volunteered abstract submissions.

A total of 45 volunteered abstracts were submitted for presentation at the conference. All volunteered abstracts were scheduled as posters. In addition, six abstracts were selected to present orally in a trainee competition session. Thirty-six percent (36%) of abstract authors were female, and 2% were submitted by authors outside the U.S.

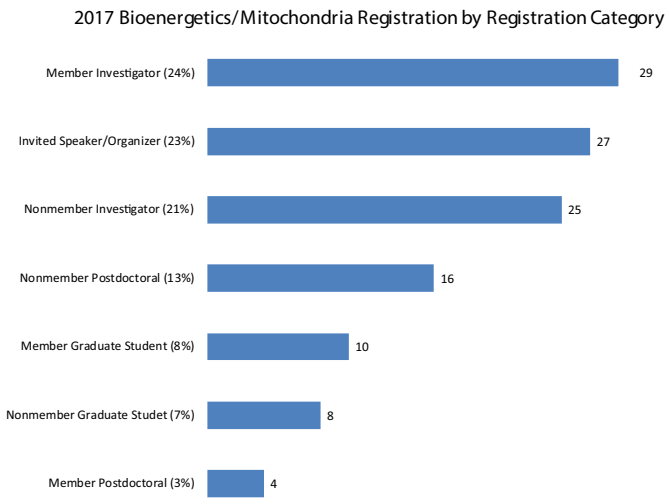
Trainee abstract authors had the opportunity to apply for a competitive abstract presentation award. The organizers reviewed the pool of candidates by reading the abstract submissions and judging presentations at the conference. The following individuals received best abstract presentation awards: First Place, Martine DeBoer (Erasmus MC); Second Place, Karima Ait-Aissa (Medical College of Wisconsin); Third Place, Matthew Racine (Colorado State University); and Fourth Place, Matthew J. Rossman (University of Colorado, Boulder). The *American Journal of Physiology—Heart and Circulatory Physiology* provided funds for a travel award based on abstract presentation to Anna Pedrinolla (University of Verona). Paola Rosas (Texas A&M University) was selected for the Minority Travel Fellowship Award, which is provided to encourage participation of underrepresented minority individuals in the physiological sciences. The fellowship provides reimbursement of all expenses associated with travel and participation in the workshop. Jill Badin (Indiana University School of Medicine) was awarded a Microsoft Surface Pro 4 drawn from the pool of names of attendees who registered early for the conference.

The American Physiological Society and the Organizing Committee gratefully acknowledge the financial support provided though generous educational grants from Amgen, DMT, and the *American Journal of Physiology – Heart and Circulatory Physiology*.

Physiological Bioenergetics: Mitochondria from Bench to Bedside

The 2017 APS Conference on *Physiological Bioenergetics: Mitochondria from Bench to Bedside* was held August 28–30 at the U.S. Grant Hotel in San Diego, CA. The conference was organized by Sruti Shiva (co-chair), Robert Gottlieb (co-chair), Shannon Bailey, Andreas Beyer, Paul Brooks, Janine Santos, Russell Swerdlow, and Yisang Yoon. The purpose of the conference was to bring together experts studying varied facets of bioenergetics across disciplines and in the context of different pathologies to share the most recent findings and discuss strategies to advance the field of mitochondriology into translational and clinical studies. The conference was designed for researchers at any career stage interested in studying mitochondrial function or integrated bioenergetics.

A total of 119 registrants attended the conference. The vast majority of registrants (92%) were from within the U.S. Other countries represented were Canada, Belgium, Denmark, Japan, Sweden, and the United Kingdom. Figure 1 provides the registration breakdown by registration category.



The conference program consisted of an opening reception on the first evening, where arriving registrants could mingle in an informal setting. The scientific schedule included a keynote lecture, two didactic *Energy School* sessions, five symposia, and poster presentations of all contributed abstracts.

A total of 68 volunteered abstracts were submitted for presentation at the conference. All volunteered abstracts were scheduled as posters. In addition, five abstracts were selected to present. Forty-six percent (46%) of abstract authors were female, and 13% were submitted by authors outside the U.S.

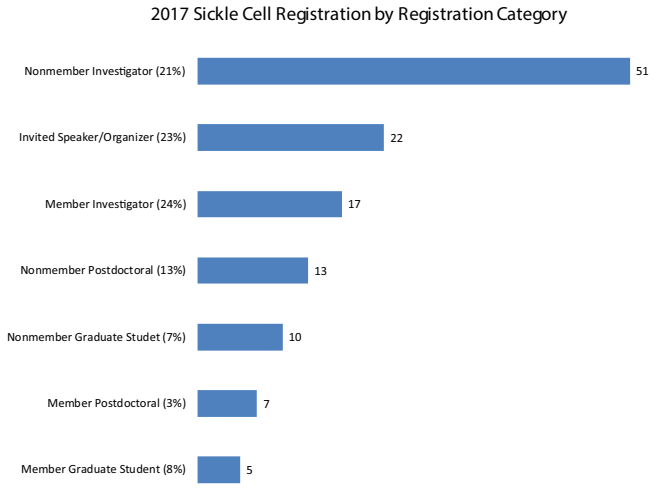
Trainee abstract authors had the opportunity to apply for a competitive abstract presentation award. The organizers reviewed the pool of candidates by reading the abstract submissions and judging presentations at the conference. The following individuals received best abstract presentation awards: Amanda Bundgard, Andrew Gibbs, Marthe Ludtmann, and Mahendra Singh. In addition, Laura Corrales-Diaz and Maria Torres were selected for the Minority Travel Fellowship Award, which is provided to encourage participation of underrepresented minority individuals in the physiological sciences. The fellowship provides reimbursement of all expenses associated with travel and participation in the workshop.

The American Physiological Society and the Organizing Committee gratefully acknowledge the financial support provided though generous educational grants from the National Heart, Lung and Blood Institute of the National Institutes of Health and from donations provided by the Society for Redox Biology and Medicine, Agilent Technologies, and the University of Pittsburgh.

Physiological and Pathophysiological Consequences of Sickle Cell Disease

The 2017 APS Conference on *Physiological and Pathophysiological Consequences of Sickle Cell Disease* was held November 6–8 at the Embassy Suites Washington, DC Convention Center Hotel. The conference was organized by Dexter Lee (chair), Sergei Nekhai (co-chair), Doug Engel, Felicity Gavins, Kalpna Gupta, Karen Lang, Harvey Luksenburg, Betty Pace, and David Pollock. The purpose of the conference was to provide a premier forum for physiologists and clinicians to present and discuss their research findings about the effects of sickle cell disease (SCD) on physiology, with the intent that these discoveries will help researchers and clinicians better understand the disease and its consequences, and to devise strategies that prevent and better treat SCD-related pathophysiology. The meeting supported the momentum created by the NHLBI Excellence in Hemoglobinopathies Research Award (EHRA) program to aid in developing studies that should accelerate high-impact multi-disciplinary basic and translational research in the hemoglobinopathies and facilitate maximal collaborations among basic and translational scientists and clinical hematologists located throughout the U.S.

A total of 124 registrants attended the conference. The vast majority of registrants (91%) were from within the U.S. Other countries represented were Australia, France, Ghana, Italy, Jamaica, Japan, and Switzerland. Figure 1 provides the breakdown of registration by registration category.



The conference program consisted of an opening reception, one educational workshop, seven symposia sessions, two *Data Blitz* sessions featuring rapid-fire talks from abstract presenters, two poster sessions, a welcome address from Harvey Luksenburg of the National Heart, Lung and Blood, Institute (National Institutes of Health), and an address by Wayne Franklin, President of Howard University.

A total of 63 volunteered abstracts were submitted for presentation at the conference. Twenty (20) abstracts were selected for presentation in symposia sessions. The remaining 43 abstracts were scheduled as posters. Thirty-seven percent (37%) of abstract authors were female, and 9% were submitted by authors outside the U.S.

Trainee abstract authors were enrolled in a competition for best abstract presentation award. The organizers reviewed the pool of candidates by reading the abstract submissions and judging presentations at the conference. The following individuals received best abstract presentation awards: Mary Lee, Scott Ferguson, Gabrielle Lapping-Carr, Malgorzata Kasztan, Anton Ilich, and Junaid Ansari. In addition, Adam Bush, Derick Okwan, Eric Rivas, and Crystal Taylor were selected for the Minority Travel Fellowship Award, which is provided to encourage participation of underrepresented minority individuals in the physiological sciences. The fellowship provides reimbursement of all expenses associated with travel and participation in the workshop

The American Physiological Society and the Organizing Committee gratefully acknowledge the financial support provided though generous educational grants from the National Heart, Lung and Blood Institute of the National Institutes of Health and from donations provided by Howard University's Center for Sickle Cell Disease, Imara, Inc., Prolong Pharmaceuticals, LLC, and Global Blood Therapeutics, Inc.

Positions Available

Assistant Professor: The Aerospace Physiology Program (*https://daytonabeach.erau.edu/degrees/bachelor/aerospace-physiology/index.html*) in the Department of Human Factors and Behavioral Neurobiology (*http://daytonabeach.erau.edu/college-arts-sciences/human-factors/* at Embry-Riddle) Aeronautical University (ERAU) is seeking a cellular physiologist to join the faculty at the assistant professor level. We are seeking an individual who uses modern molecular techniques in both teaching and research. Applicants should be capable of teaching both general microbiology and cell and molecular biology. Experience in microbiology, human physiology, radiation biology, or pharmacology, as it pertains to space and flight, is desired. The BS in Aerospace Physiology at ERAU is the first in the nation and concentrates on all aspects of aerospace physiology, including aerospace medicine. The Department of Human Factors and Behavioral Neurobiology offers BS, MS, and PhD degrees in Human Factors, in addition to the BS in Aerospace Physiology. An established research program in healthcare human factors within the department gives candidates broad opportunities to develop an applied program of research. The successful candidate will hold a PhD in Biology, Physiology, Microbiology, or a closely related field. Experience in microbiology, human physiology, radiation biology, or pharmacology, as it pertains to space and flight, is desired. ERAU is committed to diversity and inclusion, and encourages underrepresented groups, especially women and minorities, to apply. For more information on this position, please contact Eric Vaden, Committee Chair (*vaden9d9@erau.edu*). To be considered please complete an online application at *https://embryriddle.taleo.net/careersection/002/jobdetail.ftl?job=170353&tz=GMT-04%3A00*. Applications will be reviewed until the position is filled. However, first review will begin Nov. 1, 2017.

Assistant Professor: The Department of Exercise Science in the School of Education at Syracuse University seeks a full-time, tenure-track faculty member at the assistant professor level. We encourage creative, dynamic, and passionate candidates with an earned doctorate in exercise science, kinesiology, or a related area to apply. Preference will be given to candidates with postdoctoral training and a strong research agenda as supported by peer-reviewed publications and evidence of grant funding. All areas of expertise within the general field of exercise science will be considered. A collaborative multi-disciplinary approach to research that transects faculty interests in exercise/physical activity, applied physiology, chronic disease, and/or disability is desirable. The ideal candidate must also demonstrate a strong commitment to teaching, student mentoring/advising, and professional service. Salary is competitive and commensurate with qualifications and experience. The Department of Exercise Science comprises an interdisciplinary faculty with expertise in the broad areas of adapted physical activity, urban inclusive physical education, dance, physical activity measurement, exercise endocrinology, muscle biology, cardiovascular exercise physiology, genetics, respiratory physiology, and environmental physiology. Our faculty are also active members of campus-wide research groups such as the Neuroscience Program, Aging Studies Institute, Burton Blatt Institute on Disability, and Institute for Veterans and Military Families. Current research funding comes from the National Science Foundation, National Institutes of Health, American College of Sports Medicine, American Heart Association, The Robert E. Leet and Clara Guthrie Patterson Trust, and a variety of other foundation and industry collaborators. The Department of Exercise Science currently has one teaching lab and five research labs (The Muscle Biochemistry Lab, the High Altitude Simulation Lab, the Human Performance Lab, Human Behavior Measurement Lab, and the Exercise Prescription Lab) and maintains strong clinical and basic research collaborations with investigators around campus in chemistry, biology, psychology, nutrition, public health and at neighboring hospitals (SUNY Upstate Medical University, Joslin Diabetes Center). The department currently has 6 tenured/tenure-track faculty, 2 full-time non-tenure track faculty, and 2 full-time administrative assistants. The department houses one of the largest undergraduate programs in the School of Education with ~275 Health & Exercise Science and Health & Physical Education students. Our I-MOVE program offers a variety of physical activity and exercise courses to over 3,500 students, faculty, and staff each semester, serving as the applied arm of Exercise Science and helping our university meet its health and wellness needs. Our doctoral program was ranked among the top 20 programs in the nation by the National Academy of Kinesiology, and our doctoral students are regionally and nationally recognized for their scholarship, receiving multiple grants and awards from the National Institutes of Health, the American

College of Sports Medicine, NASA, the American Heart Association, the North American Artery Society and the American Physiological Society. *Responsibilities and Required Expectations*. *1*) Establish an active and extramurally funded research program at Syracuse University that will enhance the department's research portfolio and augment department strengths. *2*) Seek and obtain external research funding from federal and state agencies, foundations, and industry partnerships. *3*) Teach undergraduate and graduate courses in Exercise Science. *4*) Recruit, advise, and mentor graduate students at the masters and doctoral levels. *5*) Oversee MS theses and PhD dissertations, undergraduate independent studies, honors/capstone projects. *6*) Academically advise undergraduate students. *7*) Serve on departmental, college, and university committees as assigned or based on interest. For a detailed position description and online instructions, go to *www.sujobopps.com* (#073491). All applicants must attach a current CV, cover letter explaining your interest in the position, teaching and research interests, and contact information for three (3) letters of recommendation. Syracuse University is an Affirmative Action/Equal Opportunity Employer. Questions about the position should be sent by e-mail to the Search Chair: Kevin S. Heffernan, Ph.D., Dean's Associate Professor of Exercise Science, *ksheffer@syr.edu*; 315-443-9801. PI99665577

Assistant Professor: The Department of Physiology in-vites outstanding scientists with a PhD, or professional degree with a research focus, to apply for a tenure-track faculty position at the level of assistant professor. Didactic lecture experience in physiology courses is highly desired. Candidates who bring innovative and integrative approaches to physiology research questions and whose research complements existing strengths in the department are encouraged to apply. The successful candidate will have a track record of both research and teaching excellence. Midwestern University is dedicated to the highest standards of academic excellence to meet the educational needs of the healthcare community. This includes exposure of students to research in productive laboratory settings, training the next generation of healthcare professionals, and public outreach to the local community. The position will be supported by laboratory space, competitive salaries, state-of-the-art core facilities, a start-up package, and a shared research technician. Additional information about the Department of Physiology can be found at (*http://www.midwestern.edu/programs_and_admission/az_osteopathic_medicine/physiology.html*). Applicants should submit a cover letter, a CV, a statement of current and proposed research (1 page), copies of two significant publications, a statement of teaching philosophy (1 page), and the contact information for three references who are able to provide letters of recommendation on the applicant's behalf. All items should be submitted in a single pdf file online at *https://www.midwestern.edu/about/employment_at_mwu.html*. Completed applications will be reviewed starting December 15, 2017. Please contact Michael Quinlan (Department Chair) at (*mquinl@midwestern.edu*) for further information about the position.

Assistant Professor: The Biology and Health Sciences Departments at the University of Colorado Colorado Springs are seeking applications for three faculty positions at the assistant professor level for the joint Exercise Science degree program. Applicants must have an earned PhD in hand by start date (August 13, 2018), a strong commitment to undergraduate teaching, and a research agenda focusing on human subjects that will complement current faculty and integrate into the new William J. Hybl Sports Medicine and Performance Center. The Hybl center is a new, 104,000-sq.-ft., state-of-the-art facility that will incorporate UCCS's exercise science degree program with medical and performance clinics. Construction of this one-of-a-kind facility is scheduled to open on the campus in 2019 (*http://pressreleases.uccs.edu/?p=3340*). Applicants are sought who have expertise the following areas: integrative/applied physiology; anatomy; biomechanics; motor learning/control; adapted physical activity; or exercise testing and prescription. Each position is a 9-month, tenure-track appointment with 40% teaching, 40% research, 20% service work distribution to begin August 2018. Faculty are expected to teach 15 credit hours/academic year of undergraduate and graduate-level courses in their area of expertise and other undergraduate courses in health sciences and/or biology; advise undergraduate and graduate students; develop a successful line of research and scholarly record; seek external funding that strengthens/broadens current areas of research; participate in both internal and external professional service activities; and demonstrate collaborative interests and collegiality in all aspects of faculty engagement. The Bachelor of Science (BS) in Exercise Science is a joint degree offered

by the departments of Biology and Health Sciences that combines UCCS faculty with allied interests, as well as clinical faculty from our healthcare partner, Centura Health Systems. The departments offer Master of Sciences (MSc) degrees with options in exercise science and strength and conditioning. These programs will be consolidated and housed within the William J. Hybl Sports Medicine and Performance Center. More information on the programs can be found at *www.uccs.edu*. The University of Colorado offers a comprehensive benefits package. Information on benefits programs can be found at: *https://www.cu.edu/employee-services/benefits*. The University of Colorado is committed to providing a safe and productive learning, living, and working community. To assist in achieving this goal, we conduct background investigations for all prospective applicants being considered for employment. UCCS is an equal opportunity and affirmative action employer. In compliance with applicable laws and in furtherance of its commitment to fostering an environment that welcomes and embraces diversity, the University of Colorado does not discriminate on the basis of race, color, creed, religion, national origin, gender, disability, age, veteran status, sexual orientation, gender identity or expression, genetic information, political affiliation, or political philosophy in its programs or activities, including employment, admissions, and educational programs. Alternative formats of this ad can be provided upon request for people with disabilities by contacting the Office of Human Resources at (719) 255-3372. The Immigration Reform and Control Act requires that verification of employment eligibility be documented for all new employees by the end of the third day of work. *Minimum Required Qualifications*. PhD in exercise or health science, integrative/applied physiology, kinesiology, or related field. *Special Instructions to Applicants*. Applications must be submitted through CU Careers. Applications submitted through e-mail or surface mail will not be considered. Applications received by October 31 will receive full consideration. Review of candidates will begin November 1, 2017 and continue until the positions are filled or the search is terminated. For more information about this position, contact Dr. Steve Johnson, search committee chair (*sjohns28@uccs.edu*) or Karen Short, Helen and Arthur E. Johnson Beth-El College of Nursing & Health Sciences HR (*kshort@uccs.edu*), 719-255-4415. *Application Materials Required*. Cover letter, resume/CV, list of references, unofficial transcript(s), statement of teaching philosophy. *Additional Attachments*. Refer to Application Materials Instructions below. *Application Materials Instructions*. Applicants must submit *1*) cover letter; *2*) curriculum vitae; *3*) statement of teaching philosophy; *4*) research agenda; *5*) three representative publications; *6*) names and contact information of five professional references willing to provide a confidential letter of recommendation; and *7*) unofficial transcripts for consideration. In order for an application to be considered, applicants must label each required attachment with the relevant title, attach the required documents for this position announcement to the submission, and check the Job Specific Attachments box next to each document in the submission. If you have technical difficulties with your application, please contact the CU Careers help desk at 303-860-4200 #5 or *cucareershelp@cu.edu*. Job-related inquiries should be directed to the posting contact. Job Category: Faculty. Primary Location: Colorado Springs. Department: C0001 – Colorado Springs Campus. Schedule: Full-time. Posting Date: Sep 14, 2017. Closing Date: Ongoing. Posting Contact Name: Stephen Johnson. Posting Contact E-mail: *sjohns28@uccs.edu*. Position Number: 00480513. PI99419590

Assistant Professor: The Exercise Science Division in the School of Health Sciences at Central Michigan University (CMU) is recruiting for a full-time, 9-month, tenure-track assistant professor, beginning August 2018. The CMU School of Health Sciences offers undergraduate and graduate degrees and is located in The Herbert H. and Grace A. Dow College of Health Professions. The undergraduate exercise science major is recently COAES accredited and a new MS in exercise physiology program is launching Fall 2017. Starting salary is commensurate with qualifications and experience. Attractive benefits package and research support is available. *Responsibilities*. Faculty members are expected to demonstrate a commitment to excellence in teaching, student advising, and university service, and to participate in graduate and undergraduate research. *Teaching*. Teach undergraduate and graduate courses in clinical exercise physiology, exercise testing, and prescription and exercise across the lifespan, etc. Possible teaching opportunities exist with the College of Medicine. *Research*. Conducting research and scholarly endeavors that lead to securing extramural funding to support an ongoing research agenda; directing undergraduate and graduate research. *Advising and*

Service. Advise undergraduate and graduate students; involvement in campus and community service. *Qualifications*. Required: earned doctorate in Clinical Exercise Physiology or related field; ABD considered if it is clear that the degree will be completed by the start of the appointment. Demonstrated clinical exercise physiology experience and successful college teaching. Demonstrated commitment to diversity; effective oral/written communication skills. Ability to perform the essential functions of the job with or without reasonable accommodations. Preferred: certification (CES, RCEP); experience or demonstrated interest in securing extramural grants and contracts; postdoctoral experience; demonstrated research agenda based on published works and/or external funding. Evidence of involvement with professional associations and organizations, including ACSM and AACVPR; and demonstrated leadership skills. *General Information*. The School of Health Sciences offers programs in exercise and health science, community health education, and health service administration. The departmental faculty is dedicated to student-centered teaching, innovative delivery methodology, collaborative work projects, and applied research. The teaching space and labs are state of the art and extensive; research labs are available and include opportunities in biochemistry, metabolism, cell culture, applied, sports performance, environmental physiology, body composition (DEXA), biomechanics, neuroscience, and animal research. *Application Procedure*. Review of applications will begin immediately and will continue until the position is filled. Starting date of the position will be August 2018. Applications must be submitted through an online process beginning at *www.jobs.cmich.edu*. Electronically attach a letter of application, curriculum vita, evidence of teaching ability, statement of teaching philosophy, statement of research interests, copy of academic transcripts, and a list of three professional references, including telephone numbers and e-mail addresses. Inquiries may be sent to Dr. Jeff Edwards at *edwar4je@cmich.edu* or Dr. Jeff Betts at *betts1jj@cmich.edu*. CMU, an AA/EO institution, strongly and actively strives to increase diversity within its community (see *http://www.cmich.edu/aaeo*).

Assistant Professor: The Department of Biological Sciences at Colorado Mesa University invites applications for a tenure-track Assistant Professor of Biology-Vertebrate Physiology. Teaching responsibilities will include human anatomy and physiology, and/or pathophysiology, general human biology, and potential upper-level courses based on the applicant's areas of specialization. We seek applications from candidates who display evidence of excellence and innovation in teaching, and whose research and teaching expertise complements the strengths and expertise already present in the department. The standard teaching load is 12 credit hours per semester; some assigned courses may be taught online, hybrid, or via distance delivery modalities. Scholarship in the discipline, involvement with advising, and service to the department and the university are expected. Additional responsibilities include providing research opportunities for undergraduates, and seeking extramural funding. To perform this job successfully, an individual must be able to perform each essential duty and responsibility satisfactorily. The requirements listed below are representative of the knowledge, skill, and/or ability required. *Required Education and Experience*. PhD in Biology/Zoology with a vertebrate physiology emphasis; ABD considered, but degree must be granted prior to the start date. Effective communication and organizational skills. *Desired Education and Experience*. Experience teaching college level biology courses. Postdoctoral experience. Demonstrated enthusiasm for teaching and innovation in the classroom. Experience mentoring undergraduates in research. Colorado Mesa University is particularly interested in candidates who have experience working with students from diverse backgrounds and who have a demonstrated commitment to improving the levels of access and success for underrepresented students within higher education. Colorado Mesa University is committed to providing a safe and productive learning and living community. To achieve that goal, we conduct background investigations for all final applicants being considered for employment. Background investigations include reference checks, a criminal history record check, and, when appropriate, a financial and/or motor vehicle history. Applicant must be able to verify U.S. employment eligibility. Colorado Mesa University is an Equal Opportunity Employer, committed to a culturally diverse faculty, staff, and student body. Women and minorities are encouraged to apply. Reasonable accommodations may be made to allow individuals with disabilities to perform the essential duties of the position. *Salary*: $50,000.00–$55,000.00. Commensurate with education and experience. Excellent health and retirement benefits package. *Application*. Submit a cover

letter, resume or CV, statement of teaching philosophy, plan for research involving undergraduates, three letters of recommendation, and a copy of transcripts for all degrees completed (official transcripts will be required upon hire). *Application Deadline*. Open until filled. To ensure consideration, complete applications must be received by January 13, 2018. *Contact for Direct Inquiries*: Denise McKenney – Professor of Biology, *dmckenne@coloradomesa.edu*. For more information, please see the complete job posting at *https://coloradomesa.csod.com/ats/careersite/JobDetails.aspx?id=49&site=1*.

Assistant/Associate Professor: The University of Toledo College of Medicine and Life Sciences has designed and recently implemented an innovative and clinically integrated foundational science curriculum. This 18-month curriculum is organized into four curricular threads and requires active participation of foundational and clinical faculty working collaboratively across the integrated threads to deliver medical student education. In collaboration with the Department of Medical Education, the Department of Physiology & Pharmacology is currently recruiting tenure-track faculty positions at the rank of assistant or associate professor. The ideal candidate will be a multidiscipline educator with a primary responsibility to take a leadership role in leading the horizontal and vertical integration of the Physiology & Pharmacology curriculum at UTCOM. Additional information about the department can be found at the following website: *https://www.utoledo.edu/med/depts/physpharm/index.html*. The successful candidate must possess a PhD in a related field and/or one of the following: MD, DO, or the equivalent. Previous or established experience teaching in a medical school environment is preferred, with the ability to organize a curriculum related to physiology and pharmacology for medical students. This person will have strong interpersonal skills to motivate, encourage, and lead faculty as a system or thread director. Expertise and interest in cardiopulmonary and renal systems physiology/pharmacology is preferred. Knowledge and/or understanding of LCME core competencies, is desirable. The University of Toledo offers competitive salaries and excellent retirement options. The city of Toledo is within the Northwest Ohio region, which has very reasonable housing and living costs and excellent school districts. Applicants should submit curriculum vitae and names of three referees to *https://jobs.utoledo.edu/applicants/jsp/shared/Welcome_css.jsp* (posting no. 0600434). Questions can be directed to Dr. Bina Joe at *Bina.Joe@utoledo.edu* or Dr. Imran Ali at *Imran.Ali@utoledo.edu*. Applications will be considered until the positions are filled. The University of Toledo is an equal access, equal opportunity, affirmative action employer and educator.

Assistant/Associate Professor: The Department of Nutritional Sciences seeks applications for two tenure-track positions with a focus on the physiological regulation of metabolism and/or the interface of nutrition and the microbiome. Both positions are open at the assistant professor (tenure-track) or associate professor (tenure-eligible) rank. This position will complement the research of existing faculty, which includes research related to prevention of cardiovascular disease; chemosensation; mechanisms of obesity; studies of diet, physical activity, and cancer prevention; research on genetic models of inflammation; micronutrient metabolism in animal models or humans; epidemiology; and/or global health nutrition. The research areas of the successful applicant could include one of the "-omics," (including nutrigenomics and metabolomics) and/or kinetic models in the domain of cancer or other chronic diseases. The successful applicant's research can be basic using cell and animal models; translational using basic and clinical models; and/or clinical with a focus on human metabolism and its physiological regulation or the nexus of nutrition and the microbiome. The successful applicant's research is expected to be synergistic with core areas of the strategic plans of the Department of Nutritional Sciences, the College of Health and Human Development, and Penn State, of which Enhancing Health is one of five thematic priorities (*http://strategicplan.psu.edu/thematic-priorities/*). The positions are in the Department of Nutritional Sciences, one of eight departments in the College of Health and Human Development (*http://hhd.psu.edu/*). Both positions are tenure-track research, teaching, and service positions. Penn State takes pride in faculty who integrate these three aspects of academic activity. The National Research Council ranked Penn State's Graduate Program in Nutritional Sciences among the nation's best. More information is available at *http://nutrition.hhd.psu.edu/*. The Department has 24 full-time faculty and 27 research scientists. Current student enrollments are 240 undergraduate students and 34 PhD candidates. The Department provides a supportive environment for interdisciplinary translational research spanning

applied and basic sciences. Collaborative opportunities abound in the Department of Nutritional Sciences; in other departments in the College of Health and Human Development; as well as in other Penn State Colleges (e.g. Agriculture, Engineering, Medicine, Science), Centers, and Institutes across campus, including but not limited to the newly formed Microbiome Center, the Huck Institutes for the Life Sciences, Penn State Clinical and Translational Institute (CTSI), and the Penn State Cancer Institute. Furthermore, depending on the research area of the candidate, this position may be part of a co-hire with the Huck Institutes for the Life Sciences (*https://www.huck.psu.edu/*). Applicants should have strong academic training in nutritional sciences, physiology, microbiology, or a closely related field. A doctoral degree (PhD, DrPH, MD, and/or equivalent) is required. The successful candidate will be appointed in a tenure-track position at the appropriate level commensurate with experience. For applicants applying at the assistant professor level, 2 or more years of postdoctoral research experience is required. Candidates will be expected to establish/maintain an extramurally funded research program and to teach in the Department's undergraduate and graduate programs. Service expectations will be appropriate to Penn State's mission as a land grant university. Qualified candidates should provide evidence of original research published in peer-reviewed journals and are expected to have obtained or show strong potential to obtain external support for their independent research program. Applicants should also have experience that demonstrates proficiency in both teaching and potential for mentoring students. To apply, interested candidates should complete an online application (*https://psu.jobs/job/74859*) and upload *1*) a cover letter of application; *2*) a personal statement addressing interests and vision (future goals and plans) in *a*) research and *b*) teaching; *3*) a curriculum vitae, along with *4*) names, titles, and complete contact information for three professional references who may be contacted. Please address materials to Assistant/Associate Professor of Nutritional Sciences (Physiology, Metabolism, and Microbiome) Search Committee and direct questions or informal inquiries to the search committee chair, Connie J. Rogers, PhD, MPH, Associate Professor (*cjr102@psu.edu*). Please indicate "Position in Nutritional Sciences: Physiology, Metabolism and the Microbiome" in the subject line of e-mail correspondence and cc: the message to Julie Brenneman (*jqk7@psu.edu*). Review of applications will begin immediately and continue until the position is filled. Salary is competitive, commensurate with background and experience. An attractive benefits package is available. *Campus Security Crime Statistics*. For more about safety at Penn State, and to review the Annual Security Report, which contains information about crime statistics and other safety and security matters, please go to *http://www.police.psu.edu/clery/*, which will also provide details on how to request a hard copy of the Annual Security Report. Penn State is an equal opportunity, affirmative action employer and is committed to providing employment opportunities to minorities, women, veterans, disabled individuals, and other protected groups.

Assistant/Associate Professor: Reports to: Chair, Department of Science and Mathematics. Grace College invites applications and nominations for the position of assistant professor in biology, with emphasis in anatomy and physiology. Responsibilities include teaching, advising, department and college service, scholarly activity, and professional development. Grace College is a Christ-centered Liberal Arts College informed by pietist and evangelical traditions. Located in the resort community of Winona Lake, near Warsaw, Indiana (36 miles west of Ft. Wayne), Grace College offers 52 academic majors (38 minors). Central to the mission is developing character, sharpening competence, and preparing for service. Our goal in Christian living and teaching is to make Christ preeminent in all things. The programs of the college, as well as its community lifestyle commitment, are designed to encourage serious academic inquiry, a biblical worldview, spiritual understanding, and social conscience, all in the context of God's grace. The candidate will be expected to teach courses in general biology, microbiology, and anatomy and physiology throughout the academic year, as well as advise students. The successful applicant will have a strong commitment to liberal arts undergraduate education and will help support the Department's Faith, Science, and Reason Grace core course. Also, the candidate will be expected to develop a reasonable research plan, appropriate to the candidate's training and expertise, which includes undergraduate students in their program, as part of their scholarly growth. *Qualifications*. This position offers a challenging opportunity to a faculty member with both academic and research experience who seeks to grow the biology program. The successful candidate will: have

a PhD in biology, preferably in the area(s) anatomist or physiologist; assist in the growth of the biology program and coordinate with the nursing program by developing two streams of Anatomy and Physiology, one for the biology and pre-health majors, a second for the nursing program; have a research plan that can involve undergraduate students appropriate for a 4-yr college; have evidence of excellence in teaching; have a desire to integrate faith with learning in all areas of life; have a willingness to collaborate with colleagues and students; have evidence of relevant scholarship. *Application Process*. Applications may be found at *www.grace.edu/about/employment/faculty/staff-applications*. Review of applications will begin immediately and will continue until the position is filled. The start date for this position is August 2018. Grace College and Theological Seminary seeks a diverse work environment by encouraging women and minorities to apply.

Course Director: The Touro College of Osteopathic Medicine (TouroCOM) is looking for an experienced medical educator to be hired at the associate or full professor level to lead the instruction of Medical Physiology on the Middletown, NY campus. *Job Responsibiltiies*. The Physiology Associate Course Director position specifically involves collaborating with faculty on the sister Harlem campus in the development, organization, and delivery of the first-year Medical Physiology curriculum presented simultaneously on both campuses. The successful candidate will have experience teaching multiple physiological systems, such as renal, pulmonary, cardiovascular, and gastrointestinal physiology. Although a commitment to excellence in teaching is the primary responsibility of the position, faculty members also serve on the administrative committees of the college and are expected to develop their own research programs and participate in scholarly activity. *Requirements (Education, Preparation, and Training)*. Applicants must have a PhD in Physiology or a related field or have a DO or MD degree. The successful candidate will have a proven track record of exceptional teaching at the medical school level in the topics mentioned in the job responsibilities section, have experience designing medical physiology curricula, and work well with the rest of faculty team. *Skills*. The ideal candidate will be proficient in the following areas: relevant content expertise, research techniques, instructional design, delivery and assessment, course management, sychometrics/statistics, learning theory, public speaking, effective communication, conflict management. *Physical Demands*. Standing or walking for a long period of time while teaching in a classroom or laboratory setting. Computer work, requiring fine motors skills for typing and good vision at close distances. *Computer Skills*. Basic computer skills supporting the proficient use of the Microsoft Office Suite, including Powerpoint, Word, Outlook, and Excel, as well as software used to prepare and administer written exams electronically. *Travel*. Infrequent travel to the branch campus location in Manhattan, NY. *Qualifications*. To perform this job successfully, an individual must be able to perform each essential function satisfactorily. The requirements listed above are representative of the knowledge, skill, and/or ability required. Reasonable accommodation may be made to enable individuals with disabilities to perform the essential duties. Touro College is committed to the principles of equal employment opportunity. Our practices and employment decisions regarding employment, hiring, assignment, promotion, compensation, and other terms and conditions of employment are not be based on an employee's race, color, sex, age, religion, national origin, disability, ancestry, military discharge status, sexual orientation, marital status, genetic predisposition, housing status, or any other protected status, in accordance with applicable law. Our policies are in conformance with Title IX, 1972 Education Amendments.

Lecturer: The Center for the Study of Human Health (CSHH) at Emory University is a relatively young and rapidly growing program that seeks a Lecturer in Nutrition Science to begin fall semester of 2018. CSHH is an interdisciplinary liberal arts program that supports a Bachelor of Arts (BA) in Human Health and minors in Global Health, Culture and Society; Nutrition Science; and Predictive Health through innovative courses and scholarly endeavors with broad perspectives on health and the human condition. We seek an individual trained in nutrition science with additional broad expertise in any of the following: immunology, cancer, integrated physiology, epigenetics, development, policy, or health disparities. Experience teaching innovative, evidence-based nutrition science courses at an undergraduate level is necessary. Successful candidates should be capable of teaching courses on, but not limited to, the following topics: introduction to nutrition science; mechanistic

pathways by which nutrition influences health; and sociocultural influences on nutrition. Successful candidates should demonstrate the ability to integrate their discipline with a broad liberal arts perspective and should have experience mentoring students outside the classroom. Candidates with a history of nutrition science research and an interest in exposing students to research are encouraged. This is a full-time, lecture-track position requiring teaching of five courses per academic year, including large lecture courses and small seminars. The initial appointment will be for a period of 3 years, with renewals and promotions possible within the lecture-track system, as detailed in the Emory College of Arts and Sciences Guidelines for Appointment of Lecture-Track Faculty (*http://college.emory.edu/home/administration/policy/lecturer.html*). Applicants who hold a relevant doctorate should submit a curriculum vitae, a personal statement describing teaching experience and teaching philosophy, two (2) syllabi from representative courses taught, a description of previous and current leadership efforts and contributions, transcripts, and contact information for three (3) references. Along with these materials, include a brief statement that addresses your past activities and future plans to advance equity, inclusion, and diversity in your professional career. Submit your application and references by December 28, 2017. Reference letters will be requested at a later date. Please direct questions to the Human Health Search Committee, *Human.HealthSrch@emory.edu*. Submit your application at *http://apply.interfolio.com/46827*. Emory University, Atlanta, GA is an equal opportunity/affirmative action/disability/veteran employer. Women, minorities, persons with disabilities, and veterans are encouraged to apply. ●

Meetings & Congresses

2018

January 14-18

Keystone Symposia: Heart Failure–Crossing the Translational Divide, Keystone, CO. *Information*: e-mail: *info@keystonesymposia.org*; Internet: *http://www.keystonesymposia.org/18A1*

January 14-18

Keystone Symposia: State of the Brain–Genetic Dissection of Brain Circuits and Behavior in Health and Disease, Keystone, CO. *Information*: e-mail: *info@keystonesymposia.org*; Internet: *http://www.keystonesymposia.org/18A2*

February 17-21

Biophysical Society 62nd Annual Meeting, San Francisco, CA. *Information*: Internet: *http://www.biophysics.org/2018meeting/Home/tabid/7117/Default.aspx*

February 25-March 1

Keystone Symposia: Vascular Biology and Human Diseases–From Molecular Pathways to Novel Therapeutics, joint with the meeting on Uncomplicating Diabetes: Reducing the Burden of Diabetes Related End-Organ Injury, Santa Fe, NM. *Information*: e-mail: *info@keystonesymposia.org*; Internet: *http://www.keystonesymposia.org/18J8*

February 25-March 1

Keystone Symposia: Endoderm Development and Disease–Cross-Organ Comparison and Interplay, Taos, NM. *Information*: e-mail: *info@keystonesymposia.org*; Internet: *http://www.keystonesymposia.org/18C3*

March 25-29

Keystone Symposia: iPSCs–A Decade of Progress and Beyond, Olympic Valley, CA. *Information*: e-mail: *info@keystonesymposia.org*; Internet: *http://www.keystonesymposia.org/18C7*

April 21-25

Experimental Biology, San Diego, CA. *Information*: Internet: *http://experimentalbiology.org/2018/Home.aspx*

June 13-16

Keystone Symposia: Novel Aspects of Bone Biology, Snowbird, UT. *Information*: e-mail: *info@keystonesymposia.org*; Internet: *http://www.keystonesymposia.org/18E3*

June 18-22

APS Institute on Teaching and Learning, Madison, WI. *Information*: Internet: *http://www.the-aps.org/itl*; #ITLPhysiology

July 7-11

11th FENS Forum of Neuroscience, Berlin, Germany. *Information*: Internet: *http://forum2018.fens.org/*

September 5-8

8th International Congress of Pathophysiology, Bratislava, Slovakia. *Information*: internet: *http://www.icp2018.com*

September 30-October 3

Cardiovascular, Renal and Metabolic Diseases: Gender-Specific Implications for Physiology on Sex and Gender, Knoxville, TN.

October

The 17th International Biochemistry of Exercise Conference (IEBC), Beijing, China. *Information*: Organized by the Chinese Association of Exercise Physiology and Biochemistry

October 18-21

34th World Congress of Internal Medicine, Cape Town, South Africa. *Information*: internet: *http://www.wcim2018.com*

October 25-28

Intersociety Meeting. Comparative Physiology: Complexity and Integration, New Orleans, LA.

2019

April 6-10

Experimental Biology, Orlando, FL.

Physiological and Pathophysiological Consequences of Sickle Cell Disease

Washington, D.C. • November 6-8, 2017

Conference Abstracts

the-aps.org/SickleCell

#SickleCell17

2017 APS CONFERENCE: PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL CONSEQUENCES OF SICKLE CELL DISEASE

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4.0 Poster Session: Neural Circuits/Neurovascular Physiology

4.1

NEURAL NETWORK ANALYSIS OF SICKLE CELL DISEASE PATIENTS USING GRAPH THEORY

Michelle Case[1], Huishi Zhang[1], Yvonne Datta[2], Stephen Nelson[3], Kalpna Gupta[2], Bin He[1]

[1]Biomedical Engineering, University of Minnesota, 7-105 Hasselmo Hall, 312 Church St. SE, Minneapolis, MN, 55455, [2]Department of Medicine, University of Minnesota, 401 East River Parkway, Minneapolis, MN, 55455, [3]Hematology and Oncology, Children's Hospitals and Clinics of Minnesota, 2530 Chicago AVE S STE 175, Minneapolis, MN, 55404

Background: Sickle cell disease (SCD) is a lifelong disease that negatively impacts patients' lives. It is not well understood how the long term effects of SCD impacts the brain and the natural dynamics of neural connectivity. In order to gain a better understanding of alterations in neural behavior of SCD patients, we recorded resting state activity of both patients and healthy controls using functional magnetic resonance imaging (fMRI), an imaging modality that measure brain activity. Assessing the natural dynamic of patients and controls was done on a network level using graph theory.

Methods: A total of 15 SCD patients and 15 healthy controls were recruited for the study. Resting state fMRI data was recorded on a 3T scanner where sessions lasted for 8 minutes. The CONN toolbox was used to create a connectivity matrix for each subject. Each matrix contains the correlation coefficient between all 136 brain regions used in this study. The connectivity matrices were used to make undirected graphs, which consist of nodes and edges. The graphs were assessed across different sparsity values ranging between 5% and 60%. The characteristic path length, node density, clustering coefficient, global efficiency, and small worldness were calculated to assess the network of each subject.

Results: Based on medical history, 2 groups of patients [i] with more severe and [ii] less severe symptoms were examined. The patients with a more severe case of SCD exhibited lower small world values than controls ($p < 0.01$). The more severe patients also had significantly different clustering coefficient values ($p<0.03$) and global efficiency ($p<0.01$). The small world value of patients and clustering coefficient of patients also showed a trend associated with the number of hospitalizations in the past 2 years.

Conclusions: These results suggest that graph theory can be used as a tool to assess global network connectivity between patients and controls. It was found that patients with more severe SCD tend to have lower small world values and have higher global efficiency. The small world value represents short path lengths and a numerous amount of clusters in the graph. A decrease in small worldness indicates that patients with more severe symptoms lose the natural organization of neural networks. Graph theory could be utilized as a tool to measure the progression of SCD on neural activity through long term follow up. This work was supported by NIH U01-HL117664 and NSF IGERT DGE-1069104.

4.2

EEG CLASSIFICATION OF SICKLE CELL PATIENTS AND CONTROLS USING EEG POWER DURING RESTING STATE

Michelle Case1, Vishal Vijayakumar1, Huishi Zhang1, Yvonne Datta2, Stephen Nelson3, Karim Sadak4, Kalpna Gupta2, Bin He1

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Background: A genetic mutation causes sickle cell disease (SCD) which causes red blood cells to mutate and form a sickle shape. This mutation causes many symptoms including ischemia, pain, inflammation and other comorbidities. Recently, neuroimaging techniques have been applied to patients to gain a better understanding of how SCD affects the natural neural dynamics of patients. The goals of this study were 1) to use electroencephalography (EEG) to differentiate between SCD patients and healthy controls using EEG power and 2) to differentiate two levels of severity in patients. Classifying between patients and controls and classifying severity in patients are important steps to objectively finding neural biomarkers of SCD.

Method: Resting state EEG was recorded in 20 patients and 14 controls. EEG sessions lasted 10 minutes and subjects had their eyes open during recordings. The EEG spectral power was found for common frequency bands. The theta and beta bands were found to be significantly different between patients and controls and were used for classification. Two classifiers were implemented, one to discriminate between patients and controls, and the other to discriminate between more severe patients and less severe patients. Patients were grouped as more severe if the number of hospital visits and emergency department visits over the past two years were greater than eight. Several models of each classifier were implemented utilizing independent component analysis to ensure all electrode data was incorporated.

Results: The receiver operating characteristic curve was found to determine the performance of the classifiers. The average area under the curve (AUC) for determining

patients from controls was 0.84, where a value of 1 is perfect classification. The best performing model had an AUC of 0.97. The average AUC for determining patient severity was 0.76, and the best performing model had an AUC of 0.86.

Conclusions: The classification results show that patients can be differentiated from controls using EEG power. This shows that theta and beta band power are relevant and applicable to SCD patients. Furthermore, these bands were able to distinguish between disease severity in patients indicating that these EEG bands could be used as a potential biomarker of SCD severity. An objective way to measure cognitive health could help improve treatment in patients. This work was supported by NIH U01-HL117664 and NSF IGERT DGE-1069104.

4.3

MICROVASCULAR PERFUSION IS A PHYSIOLOGIC BIOMARKER OF MENTAL STRESS AND FEAR OF PAIN IN SICKLE CELL SUBJECTS AND NORMAL CONTROLS

P Shah[1], M Khaleel[1], W Thuptimdang[2], S Veluswamy[1], J Sunwoo[2], P Chalacheva[2], R Kato[1], J Detterich[1], J Wood[1], J Tsao[3], L Zeltzer[3], R Sposto[1], M Khoo[2], T Coates[1]

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INTRODUCTION: Sickle cell disease (SCD) is a genetic disorder in which abnormal hemoglobin-S polymerizes upon deoxygenation and forms rigid sickle shaped red blood cells that can occlude the microvasculature leading to the sudden onset of painful vaso-occlusive episodes (VOC). Thus any trigger that decreases microvascular blood flow (MBF) can promote vaso-occlusion and progression to VOC. We previously showed that SCD subjects have an augmented autonomic mediated vasoconstriction response to sigh and pain. Our subjects also showed a decrease in MBF when instructed of upcoming pain, suggesting that neural induced vasoconstriction might be the physiologic link between mental stress triggers and vaso-occlusion.

OBJECTIVES: To study the effect of mental stress on autonomic parameters – MBF and heart rate variability (R to R interval; RRI) in SCD.

METHODS: 19 SCD and 16 controls performed two standard mental stress tasks with graded levels of difficulty (N-back and Stroop).We also exposed them to a pain anticipation task where they were instructed about upcoming pain, but no pain was applied. We measured MBF using photo-plethysmography (PPG) on the left thumb and calculated the average drop in PPG during the tasks compared to baseline. From the electrocardiogram we extracted RRI and its spectral index: high frequency power (HFP) ≈ parasympathetic activity.

RESULTS: There was a significant decrease in mean MBF, RRI and HFP during N-back and Stroop compared to the baseline ($p<0.01$), indicating vasoconstriction. While MBF decreased during all the sublevels of N-back and Stroop compared to baseline, there was no difference in MBF between the task sublevels. During pain anticipation task there was a significant decrease in MBF compared to baseline ($p<0.001$) and N-back ($p<0.01$). The parasympathetic withdrawal in response to mental tasks and pain anticipation followed a similar pattern.

CONCLUSIONS: Mental stress and fear of pain causes significant decrease in regional blood flow and parasympathetic withdrawal in SCD and normal controls. The pattern of responses were not significantly different between the two groups however the consequences of decreased blood flow can be quite different because of the resultant entrapment of sickle cells in the microvasculature in SCD. This could explain how mental stress precipitates VOC in SCD by causing neural mediated vasoconstriction and thus increasing the likelihood of vaso-occlusion. Supported by NIH Grant (U56 HL117718)

4.4

DERIVING PHENOTYPIC MARKERS OF THE PERIPHERAL VASOCONSTRICTION RESPONSE TO HEAT-INDUCED PAIN IN SICKLE CELL DISEASE

Patjanaporn Chalacheva[1], Thomas Coates[2], Michael Khoo[1]

[1]Biomedical Engineering, University of Southern California, 1042 Downey Way, DRB 140, Los Angeles, CA, 90089, [2]Division of Hematology, Oncology and Blood & Marrow Transplantation, Children's Hospital Los Angeles, 4650 Sunset Blvd, Los Angeles, CA, 90027

In sickle cell disease (SCD), pain is generally thought to be a consequence of vaso-occlusive crises (VOC), but pain itself could trigger a cascade of events that leads to full-blown VOC by promoting regional peripheral vasoconstriction. Thus, knowledge of the magnitude and time-course of the reduction in peripheral blood flow (PBF) in response to pain may be useful for predicting risk for VOC. In this study, we employed our recent model (Chalacheva et al., PLoS One 12(5), 2017) to derive and further investigate the neurogenic response to a standardized pain stimulus in individual SCD and control subjects.

The standardized neurogenic response from each subject was decomposed into the weighted sum of several "wavelets", each representing different time-frequency characteristics of the response. The largest contributions were derived from 2 wavelets: 1) one with a rapid initial drop in PBF followed by relatively slower recovery towards baseline; and 2) one showing a small rapid initial drop in PBF followed by a small rebound and subsequently a more

prolonged drop. As such, the weighted sum of both wavelets took the form of a relatively more sustained ("tonic") response, while the weighted difference of these 2 wavelets yielded a "phasic response" to each pain pulse. The sum of the 2 wavelet weight coefficients (Cs) was correlated with the mean drop in PBF during pain from baseline ($r = -0.47$, $p = 0.015$), while the difference between the wavelet coefficients was correlated with the slope between PBF change and temperature ramp accompanying each pain pulse ($r = 0.48$, $p = 0.014$). Cs was also correlated with the normalized high frequency power of heart rate variability (HFPn) during baseline (before pain was applied) in only SCD ($r = 0.68$, $p = 0.014$), but not controls. This result implies that SCD subjects with low baseline parasympathetic activity, as reflected by HFPn, have stronger tonic vasoconstriction patterns; but this association was absent in controls.

In summary, we have introduced a model-based approach for phenotyping the peripheral vasoconstriction response to acute pain in SCD using only noninvasive measurements. Based on our preliminary findings, we speculate that SCD subjects who are autonomically imbalanced are more likely to display prolonged peripheral vasoconstriction, potentially exposing them to higher risk for VOC.

Funding: National Institutes of Health National Heart, Lung, and Blood Institute grant U01 HL117718

4.5

INCREASED EXPRESSION OF CELLULAR STRESS PROTEIN,A-SYNUCLEIN(SNCA) WITHIN NORMOXIC AND HYPOXIC BRAINS OF SCD MICE: IMPLICATIONS FOR NEUROCOGNITIVE DYSFUNCTION IN SICKLE CELL DISEASE PATIENTS

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Background: The aggregation of the protein α-synuclein (SNCA) is linked to neurocognitive dysfunction in Parkinson's disease and Alzheimers Disease.. Neurocognitive dysfunction is also prevalent within sickle-cell disease (SCD) patients including those with strokes, silent and overt. however it is not found within all SCD patients with neurocognitive dysfunctions. SNCA has been found to be elevated in reticulocytes and pro-inflammatory states and peripheral blood mononuclear cells of hypoxic SCD patients(Zhang et al 2014). Since SCD patients have high retic counts and are in an chronic pro-inflammatory state, we investigated SNCA expression within the Brains of our Wild type and SCD mice under normoxic conditions and hypoxic conditions that would represent an acute pro-inflammatory state. Materials & Methods: SNCA qRT-PCR mRNA expression within Brains of Normoxic Wild type(WT) Control Mice (n = 5, Mean Age=5.5m/o) was compared to expression within the Brains of Normoxic SCD mice (n=5 Mean Age 5.7 m/o). Additionally, in a separate experiment, Brain SNCA expression of Normoxic SCD Mice(N=4, Mean 5.1m/o) controls was compared to sickle-cell mice, under hypoxic conditions (n = 4, Mean 5.5 m/o),. To simulate hypoxic conditions mice were placed in a chamber for 8 hours, that was infused with nitrogen to lower the oxygen levels to FiO2 of 7%. The cDNA from normoxic and hypoxic mice brains were synthesized and then subject to qRT PCR to determine the expression level of SNCA in Normoxic WT mice, Normoxic SCD mice, and Hypoxic SCD mice. The fold expression of SNCA was normalized in triplicates was compared to expression of GAPDH.The cDNA samples for each mouse were done in triplicates and the relative expressions of the three samples were averaged. Results: SNCA expression were significantly higher in the Brains of Normoxic SCD mice(mean 9.95, sd 3.06) compared to Normoxic WT Control mice(mean 5.04, sd 1.09) p= 0.01. Further, SNCA Brain expression was significantly higher within the SCD Normoxic Brain(8.75) vs the SCD Hypoxic Brain(15.25). Conclusion: There are significant differences in SNCA expression between the brains of wildtype mice and sickle-cell mice. Sickle-cell mice have a higher expression of SNCA in the brain, even more so when subject to hypoxic conditions. SCD complications and associated comorbidities that result in acute and chronic hypoxia could lead to an increased expression of SNCA in the brain, possibly contributing to neurocognitive dysfunction seen in non-stroke patients. Funding: NHLBI: K01 HL-03-011

5.0 Poster Session: Sickle Cell Disease Gene Therapy, Gene Editing and Pharmacological Treatment

5.1

A STUDY OF THE GEOGRAPHIC DISTRIBUTION AND ASSOCIATED RISK FACTORS OF LEG ULCERS WITHIN AN INTERNATIONAL COHORT OF SICKLE CELL DISEASE PATIENTS: THE CASIRE GROUP ANALYSIS

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The **aim** was to determine the Geographic Distribution and associated clinical and demographic risk factors associated with leg ulcers (LU) in an international cohort of SCD patients. The CASIRE group is an international multi-institutional collaborative group evaluating the clinical severity of adults and children with SCD Sites included U.S., Italy, and Ghana. **Results:** 585 subjects enrolled: 261, 236 and 88 recruited from US, Ghana and Italy respectively. 57(9.6%) had LU. The majority of LUs occurred in adult (>18y/o), (98.2%, p<0.001) and within the Severe Genotype (96.5% vs. 3.5% in Mild Phenotype, p<0.001). Demographically, LU patients were more of Males (62.7%, p=0.010) and from Ghana (82.5% N=47 vs. 17.5% N=10 from US/Italy, p<0.001). Clinically, LU patients were hypoxic (O2 Saturation Room Air: 95.5% vs. 97.4%, p=0.025), underweight (BMI <5th%tile : 33.3% vs. Non-underweight 8.2%, p<0.001), more anemic (Hemoglobin 7.6 vs 9.3g/dL, p<0.001), more hemolytic (TBili 3.6 vs. 2.4, p= 0.036, AST 54 vs. 42 p=0.035) with more leukocytosis(12.9 vs.10.4 1000/ul), more thrombocytosis (438 vs. 367 1000/ul, p=0.004). Reported higher Creatinine levels (0.79 vs. 0.52, p=0.003) and more urine acidosis (Urine pH=5.7 vs. 6.0, p=0.002) suggests some associated related renal dysfunction. There was no significant relationship between LUs and microalbuminuria, age, stroke, pain crises patterns, or priapism. **Conclusions:** This is the 1st comprehensive analysis of LUs prevalence and SCD demographic and clinical risk factors within a Cohort in International SCD Patients. West African background, male gender, leukocytosis, thrombocytosis, severe anemia, lower oxygen saturation, and hemolysis, Renal Acidosis and higher creatinine and being an Adult are risk factors for LUs

5.2

ERYTHROCYTE HYPOMAGNESEMIA IN PATIENTS WITH SICKLE CELL ANEMIA IS ASSOCIATED WITH INCREASED FREQUENCY OF VASO-OCCLUSIVE CRISES.

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The role of magnesium (Mg), if any, in the management of patients with sickle cell anemia (SCA) remains unsettled. Previous studies using oral Mg supplementation showed improvement in the hematological and biochemical markers of RBC in patients with sickle cell disese (SCD) but with no definite conclusion on therapeutic efficacy. Previous single arm trial with historical controls showed that intravenous Mg deceased the length of hospital stay of children with vaso-occlusive crises (VOCs) but a subsequent multicenter randomized controlled trial of intravenous Mg for VOCs in children showed no beneficial effects. However, serum and red blood cell (RBC) Mg levels in these trials were not determined. In this retrospective study, RBC and serum Mg levels were measured in 50 patients with SCA when in the steady sate as outpatients between 2002 and 2010. Twelve of the 50 patients had normal RBC Mg level of 4.7 ± 0.59 mg/dL (normal range: 4.0 – 6.4 mg/dL). The remaining 38 patients had significantly lower RBC Mg level of 2.7 ± 0.81 mg/dL ($p < 0.001$). The serum Mg level was not significantly different in the two groups of patents: 1.79 ± 0.12 mEq/L versus 1.81 ± 0.19 mEq/L ($p > 0.5$). Normal range of serum Mg level is: 1.3 – 2,1 mEq/L. Nine of the patients with normal RBC Mg levels had 2.3 ± 1.00 VOCs per year that required treatment in the Emergency Department and/or the hospital. Likewise, 28 of the patients with low RBC Mg level had 4.9 ± 4.70 VOCs per year that required treatment in the ED and/or hospital ($p < 0.05$). Recently, patients with fibromyalgia were reported to have deficiencies in RBC Mg levels.

Together, the data suggest that patients with SCA and RBC hypomagnesemia may be at risk to have frequent VOCs. Further studies including larger number of patients are needed.

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5.3

HAPTOGLOBIN AND HEMOPEXIN INHIBIT INFLAMMATION AND VASO-OCCLUSION IN MURINE SICKLE CELL DISEASE THROUGH RAPID INDUCTION OF HEME OXYGENASE-1 AND CO PRODUCTION

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Cell-free hemoglobin and heme promote inflammation and vaso-occlusion in murine models of sickle cell disease (SCD). Plasma haptoglobin and hemopexin scavenge plasma hemoglobin and heme, respectively, thwarting these clinical sequelae. Chronic hemolysis depletes plasma haptoglobin and hemopexin in SCD; studies in SCD mice suggest beneficial effects of supplementation. To explore mechanisms mediating this protection and provide a basis for supplementation in patients, dorsal skin-fold chambers were implanted onto Townes-SS mice and stasis (% non-flowing venules) was measured after hemoglobin challenge. Human haptoglobin, hemopexin, or albumin was co-infused with hemoglobin or 1 hour (h) after hemoglobin at equimolar concentrations. SS-mice co-infused with hemoglobin + haptoglobin or hemoglobin + hemopexin had less stasis 1-4h after infusion, compared to hemoglobin or hemoglobin + albumin. Haptoglobin or hemopexin given to SS-mice 1h after hemoglobin, decreased stasis 2-3h after infusion, while venules of SS-mice given albumin remained static. The combination of haptoglobin + hemopexin was similar to either scavenger alone. Plasma hemoglobin and heme levels were unchanged 3-4h after supplementation. Haptoglobin or hemopexin increased hepatic Nrf2 and decreased pro-inflammatory NF-κB phospho-p65 relative to hemoglobin or hemoglobin + albumin. Notably, haptoglobin or hemopexin increased heme oxygenase-1 (HO-1) within 1h in liver and dorsal-skin. Inhibition of HO-1 activity with tin protoporphyrin reversed haptoglobin/hemopexin-mediated inhibition of stasis and NF-κB. Protection was restored by administering the HO-1 reaction product CO, which blocked hemin-mediated Weibel-Palade body P-selectin expression on cultured endothelial cells and hepatic NF-kB activation. Haptoglobin or hemopexin in unchallenged SS-mice induced HO-1 and inhibited stasis for 48h. These data suggest a link between haptoglobin/hemopexin, HO-1, and CO in alleviating SCD vaso-occlusion.

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5.4

A MACROPHAGE-STIMULATING PROTEIN RECEPTOR INHIBITOR CAUSES A GREATER REDUCTION IN INTERFERON-GAMMA EXPRESSION IN THE HEART OF FEMALE MICE WITH SICKLE CELL DISEASE

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Interferon-gamma (INF-γ has been shown to have adverse and beneficial effects during various pathological conditions. Very little information is known about the role of INF-γ during human sickle cell disease (SCD). Interleukin-17 (IL-17) has been shown to be increased during vaso-occlusive crisis of sickle cell patients; however little information is known about IL-17 expression in the hearts during SCD. We utilized the Townes mouse model of homozygous sickle cell disease to determine the role INF-γ and IL-17 in the hearts of male and female groups. We hypothesized that the Macrophage-stimulating protein receptor inhibitor (RON-inh) would decrease INF-γ expression in male and female T-homozygous mice. Male and female Townes mice (8 – 10 weeks of age) were divided into T-homozygous and T-controls and injected with the Ron-inh inhibitor (5 mg/kg in 2% DMSO) or DMSO for 14 days. Western-blots were performed on whole heart homogenates to determine IF-γ and IL-17 expressions. When comparing male and female INF-□□expression in control mice, female controls had a 6 ± 4% increased expression during RON-inh treatments. The Thomo females during baseline conditions had a 4.6 ± 2% increase in INF-γ when compared to Thomo males. The RON-inh decreased INF-γ expression by 13.7 ± 7% and 13.1 ± 5% in the Thomo male and Thomo female groups, respectively. IL-17 expression in the hearts were similar between all groups. There were no differences in body weights of Thomo males and females treated with the RON-inh for 14 days. In summary, our results suggest that RON-inh treatment caused a greater reduction in INF-γ in female Thomo mice. There were no significant changes in IL-17 expression in male or female groups treated with or without RON-inh. Future studies are needed to determine if macrophage stimulating protein receptor inhibition is more effective in reducing INF-γ expression in female hearts with SCD.

5.5

EFFECT OF THE LSD1 INHIBITOR RN-1 ON HBF, GENE EXPRESSION, AND ERYTHROID DIFFERENTIATION

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Sickle cell disease (SCD) is caused by a mutation of the β-globin gene that results in polymerization of deoxygenated sickle hemoglobin (HbS). Elevated levels of Fetal Hemoglobin (HbF) inhibit polymerization of HbS and are associated with less severe illness and longer survival. Hydroxyurea, the only approved drug for SCD, increases HbF but is effective in only 50% of patients. Therefore additional strategies to increase HbF are needed. Repression of the β-globin gene is mediated by epigenetic modifications catalyzed by DNMT1, LSD1, and HDACs present within corepressor complexes recruited to the gamma-globin gene by the site-specific DNA binding proteins TR2/TR4 and BCL11A. Our laboratory showed that DNMT inhibitors increased HbF in baboons, long considered as the best animal model to test HbF-inducing drugs because the structure and developmental regulation of genes within the β-globin gene complex is conserved among all simian primates. The effectiveness of DNMT inhibitors has been demonstrated in multiple clinical trials. Following studies that identified LSD1 as an additional therapeutic target (Shi et al, Nat Med 19:291, 2013), we showed that the LSD1 inhibitor RN-1 dramatically increased HbF, F cells and F retics in baboons (Rivers et al, Haematol 101:698, 2015) and these effects were sustained upon long-term treatment (>265d; Ibanez et al, Blood 129:260, 2017). ChIP analysis showed increased levels of Histone H3 di and tri-methyl K4 at the β-globin gene, consistent with LSD1 inhibition. RN-1 increased β-globin mRNA in BM subpopulations enriched in CFUe, but not BFUe, suggesting the CFUe is the earliest cell in the erythroid differentiation pathway "targeted" by the drug. Flow cytometry analysis showed increased proerythroblasts and decreased orthochromatic and polychromatic precursors in BM of RN-1 treated compared to untreated baboons. Increased expression of 120 genes and decreased expression of 18 genes was observed in FACS-purified BM proerythroblasts from RN-1 treated baboons. Among genes with increased expression were GATA-2 and GFi-1b that regulate erythroid differentiation, and BNIP3L (NIX), a key gene in mitochondrial clearance. Preliminary experiments have shown that RN-1 is active when administered orally. The ability of LSD1 inhibitors to increase HbF in non-human primates strongly suggests that further studies be performed to evaluate these drugs for therapy of SCD.

5.6

RNA TRANS-SPLICING REPAIR OF ENDOGENOUS B-GLOBIN PRE-MRNA IN HUMAN ERYTHROID CELLS

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Sickle cell disease is caused by a single nucleotide mutation in exon 1 of the beta-globin gene. We are developing an RNA trans-splicing approach that corrects all mutations within the coding region of human beta-globin messenger RNA. In our initial proof of principle experiments, we converted ~0.1% of endogenous beta-globin pre-mRNA by trans-splicing, reprogramming the product mRNA to encode for gamma-globin in human erythroid cells. This was accomplished by transducing human CD34+ cells with a lentivirus expressing an RNA trans-splicing molecule (RTM), which is an RNA that consists of the coding sequence of gamma-globin, a 5' splice site, and an anti-sense binding domain to beta-globin intron 2. Upon binding to a beta-globin pre-mRNA, the RTM is able to induce an alternative splicing reaction in *trans-*, between the 5' splice site of the RTM and the 3' splice site of beta-globin. Exons 1 and 2 of beta-globin are replaced by the entire coding sequence of gamma-globin and a stop codon provided by the RTM, followed by beta-globin exon 3. Since it follows a stop codon, beta-globin exon 3 is not translated. Alternatively, the RTM may be designed to deliver beta-globin exons 1+2 to produce corrected beta-globin. Further development of beta-globin targeted RTMs is ongoing. Initial results suggest that higher and more clinically relevant levels of trans-splicing are achievable. There are now nearly sixty publications that have reported RTMs targeting other disease genes with efficiencies reaching 40%. High through-put screens have been developed and should lead to the identification of more efficient RTMs.

RNA trans-splicing offers several advantages over gene editing techniques such as CRISPR-Cas. 1) Trans-splicing is mediated by spliceosomes which are abundant in the nucleus of human cells, no enzymes or other components need to be delivered. 2) Cell division is not necessary for RNA trans-splicing, but is required for gene editing repair 3) Since mRNA turns over, off target trans-splicing is transient whereas off-target gene editing leads to permanent mutations. Because gene editing repair requires co-delivery of additional components, requires cell division and the consequences of off-target edits are unknown, it is likely to remain an ex-vivo approach. Generally RTMs are just a few hundred nucleotides longer than the coding sequence they deliver, thus enabling delivery by clinically relevant vectors such as AAV. RTMs can also be delivered as plasmids or as RNA.

5.7

'THE CASE OF ERADICATION OF SICKLE CELL ANEMIA DEATHS IN AFRICA'

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Abstract Title: 'The Case of Eradication of Sickle Cell Anemia Deaths in Africa'

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Sickle Cell Disease (SCD) causes the greatest burden to both survival of children under five years (U5Y) and to the Public Health Management (PHM) in the endemic areas of Africa, India and other developing areas of the world. It is the most dangerous – in terms of rate of morbidity and mortality, of all known hemoglobinopathies.

Several other confounding factors (both natural and inflicted), in the milieu of 'hypoxia' contribute to the expression of symptoms in SCD patients.

"In the late 1960s and early 1970s, as sickle cell anemia was caught up in the torrent of U.S. congressional and presidential politics, the malady became widely characterized as a "neglected disease," a disease of a people whose "pain and suffering" had been ignored for too long, and a disease finally achieving its moment of national recognition". [1], [3].

A neglectful healthcare policy enabled the disease to reach present epidemic proportions to the tune of 150,000 babies with SCD recorded annually in Nigeria – the highest recorded incidence of SCD in the world. [5].

Sickle Cell Disease patients have compromised immunity. There is an increased incidence of meningitis and septicemia and a high mortality. [6]. Serious infections are common in SCD than in other hemoglobinopathies and occur more frequently in patients younger than 5 years of age. [7].

WHO indices for Nigeria showed she suffers a 10 to 40% carrier state, with a prevalence of 2% [9]. A further 75% infant cases, and 80% share of the mortality in the whole of Africa. [11].

It is nearly impossible to eradicate SCD because of the pathophysiology of the disease, but we can ameliorate the scourge or the rate of death due to complications of stroke during the crisis moments.

Transcranial Doppler (TCD) scanning, is the technique of choice to evaluate and diagnose the probable onset of stroke in SCD patients. TCD is a unique technology with many positive attributes - it is non-invasive, portable, relatively cheap, and easy to apply in the hands of experts. It is just one part of a comprehensive health management for TCD cannot be used in isolation. Early intervention based on the TCD evidence of continuing embolism can prevent stroke from occurring. [16]

A major aim of this project therefore, is to emphasize implementation of TCD scanning in the management and care of SCD patients in an endemic region with co-morbidities that trigger sickle cell disease crisis and stroke. This exercise, in the milieu of a comprehensive PHM, I hope, will help ameliorate the morbidity and mortality. Diabetes is a major and dangerous comorbidity leading to adverse cardiovascular complications and blindness in this geopolitical setting (enclave).

Unlike Ebola - a deadly disease with a quick swift decimating rocket style, Malaria and SCD are silent killers with a 'chameleon' tactics and seeking the most vulnerable of all – our children, especially the U5Y age group.

Peter Piot, the Belgian microbiologist who discovered Ebola in 1976 said: "we shouldn't forget that this is a disease of poverty, of dysfunctional health systems – and of distrust." [17].

5.8

GBT440 IMPROVES RHEOLOGICAL PROPERTIES OF SICKLE CELL BLOOD BY INCREASING HEMOGLOBIN OXYGEN AFFINITY

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In sickle cell disease (SCD), polymerization of deoxygenated hemoglobin S (HbS) leads to the formation of rigid, non-deformable sickled red blood cells (SS RBCs). Loss of RBC deformability causes abnormal blood rheology which contributes to vaso-occlusion of capillaries and reduced blood flow. HbS polymerization increases blood viscosity, sickling and irreversible membrane damage; all these are major contributors to the pathophysiology of SCD. GBT440 is an allosteric modulator of Hb-oxygen (O_2) affinity. When bound to HbS, GBT440 maintains HbS in an oxygenated state, prevents HbS polymerization and inhibits RBC sickling.

We monitored the effect of GBT440 on SCD patient blood rheology, *ex vivo*, to determine effects on the viscosity and deformability of SS blood and SS RBCs under hypoxic conditions (2-3% O_2 for 2 h for viscosity experiments and 30 min for deformability experiments). Blood viscosity was measured in a cone-plate viscometer at shear rates ranging from 60 s^{-1} to 415 s^{-1}. SS RBCs deformability was measured in three independent systems which tested: 1) the ability of SS RBC to migrate through a gel filtration column 2) the pressure required to enable SS RBCs to pass through a 5 µm polycarbonate pore filter and 3) the SS

RBCs membrane elasticity module by micropipette aspiration.

We report that GBT440 maintains the deformability of SS RBCs under hypoxic conditions, enabling the unobstructed migration of SS RBCs through a gel filtration column. In addition, GBT440 reduces the SS RBCs elastic modulus during aspiration and the pressure required to pass SS RBCs through the filter under deoxygenated conditions. Moreover, GBT440 dose-dependently reduces the viscosity of SS blood under deoxygenated conditions. Together, these data suggest that inhibition of HbS polymerization by GBT440 helps to maintain SS RBC deformability and improves blood rheological properties. Thus, GBT440 has the potential to reduce the likelihood of vaso-occlusion and preserve microvascular flow in SCD patients.

5.9

ENUCLEATION AND BETA-GLOBIN EXPRESSION IN INDUCED RED BLOOD CELLS: A PLATFORM TO MODEL SICKLE CELL ANEMIA

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Human induced pluripotent stem cells (hiPSCs) hold promise for both disease modeling and the development of novel therapeutic treatments for sickle cell anemia (SCA). Such models are practical systems to screen new drug therapies and to examine the effects of gene editing. hiPSCs can theoretically produce all cell types including erythroid cells. However, in vitro modeling of SCA with reprogrammed cells has been limited by their inability to differentiate into beta globin-expressing, enucleated erythroid cells. Here, we propose strategies to produce improved in vitro and in vivo models of SCA using these cell types. We derived hiPSCs from sickle cell patients with hemoglobin SS disease seen at our hematology clinic at Boston Children's Hospital. Using a cocktail of transcription factors promoting self-renewal and multi-potentiality expressed under the control of a doxycycline-regulated promoter (Erg, HoxA9, RORa, Sox, Myb) we generated conditionally immortalized hematopoietic cell lines that serve as a renewable source of robust erythroid progenitors in vitro. Erythroid progenitors differentiated from these lines underwent globin-switching once engrafted into immune-radiated mice with a 27% induction of beta globin expression. Concurrently, we further improved the in vitro differentiation protocols described to generate 30-40% beta-globin-expressing, erythroid cells with an enucleation rate of 20-50%. In future studies, we hope to employ hiPSCs to test the therapeutic hypothesis that genetic manipulation of BCL11A, a master regulator of fetal hemoglobin (HbF) expression, will ameliorate sickling. The generation of hiPSC-SCA models will be critical in broadening the current understanding of the molecular mechanisms of this disease, the development of improve pharmacological treatments and a future of autologous cell therapy for the cure of SCA. **Funding**: Howard Hughes Medical Institute, Doris Duke Charitable Foundation.

5.10

HUMANIZED SICKLE MICE ARE SENSITIVE TO HYPOXIA/ISCHEMIA-INDUCED STROKE, BUT RESPOND TO TISSUE PLASMINOGEN ACTIVATOR TREATMENT.

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Stroke, a devastating complication of Sickle Cell Anemia (SCA), consists of silent cerebral infarct (SCI) and large overt stroke. As high as 37% of this population showed SCI by age 14, and 11% develop large overt stroke by age 20 without prophylactic therapy. The current management relies on blood transfusion without the use of thrombolytic agents. However, a recent study showed that co-existent SCA does not impact the safety of tissue plasminogen activator (tPA) treatment. This finding calls for systemic analysis of the effects of thrombolysis in experimental stroke and there is also a need for predictive markers of SCA-associated SCI for preventive interventions. Here we test the hypothesis that Townes humanized sickle mice (knock-in/out mice that express the human α, γ, and sickle-β hemoglobin genes) are sensitive to hypoxia-ischemia (HI)-induced stroke, but respond to tPA-thrombolytic therapy.

We report three sets of results. First, three-month-old sickle mice of the SS genotype (βS/βS) have a higher resistive index (RI), but normal flow velocity in the common carotid artery, than with AA (βA/βA) or AS (βA/βS) mice. SS mice were also prone to repetitive-mild HI (rmHI)-induced cerebral infarct and mortality, whereas AA mice were resistant to rmHI. Second, 6-month-old SS mice developed elevated flow velocity and greater RI without stenosis of the carotid artery akin to those previously implicated in large overt stroke in SCA. Further, SS mice showed ectopic P-Selection and plasminogen activator inhibitor (PAI-1) expression in cerebral blood vessels, suggesting a hyper-coagulation state. Finally, six-month-old SS mice endured 20-min transient hypoxia-ischemia (tHI), but showed enhanced leukocyte and platelet adherence to the cerebral blood vessel, as well as, extensive vascular perfusion deficits and fibrin deposition at 4 h post-injury, followed by greatly increased mortality

than AA and AS mice at 24 h recovery ($p<0.0001$). Importantly, intravenous tPA administration at 0.5 h post-tHI markedly improved vascular reperfusion, mitigated fibrin deposition, and cut the mortality of SS mice by nearly 60%.

These results indicated that humanized sickle mice develop hyper-coagulation and hypersensitivity to HI-induced stroke without large-vessel obstructive vasculopathy at up to 6 months of age. Elevated resistive index may be an early ultrasonic marker for sickle cell vasculopathy and the risk of SCI in SCA. Future studies are warranted to confirm the therapeutic benefits of thrombolytic stroke therapy in SCA.

5.11

GENETIC TESTING FOR ALPHA THALASSEMIA USING DROPLET DIGITAL PCR

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In America, 1 in 500 African-American children are born with sickle cell disease. Many develop acute or chronic vascular problems such as stroke, pulmonary hypertension, kidney damage and leg ulcers. These vascular complications of sickle cell disease are prevented or delayed by co-inheritance of one or more alpha globin gene deletions. In order to begin studying how alpha globin gene deletions confer this protective effect, we needed to develop a robust quantitative genotyping method.

Genotyping the alpha globin locus is challenging because each chromosome has two alpha globin genes (*HBA1* and *HBA2*) with nearly identical sequences. The most common alpha globin mutation is a -3.7 kb deletion that reduces gene copy number by one. Therefore, we approached this deletion as a copy number variant, and designed droplet digital polymerase chain reaction (ddPCR) assays to quantify alpha globin gene copy number.

We designed two ddPCR assays, one which targeted exon 2 of the *HBA1* and *HBA2*, and a second assay that targeted a unique (single copy) sequence in the intergenic region between *HBA1* and *HBA2*. We obtained saliva or blood samples from volunteers (NHLBI protocol 03-H-0015) and extracted genomic DNA. For validation, we sent 14 samples representing different alpha globin gene copy numbers to the Mayo Clinic Lab for analysis by multiplex ligation-dependent probe amplification (MLPA). The presence or absence of the -3.7 kb deletion determined by our assays was 100% in agreement with the MLPA gold standard. Statistical comparison our two assays across hundreds of DNA samples revealed that targeting the intergenic sequence which is present in 0, 1, or 2 copies provided greater precision than targeting exon 2 which is present in 2, 3, or 4 copies. This comparison highlights a real-world performance limitation of ddPCR and provides a technical solution for accurate alpha globin genotyping.

5.12

DEVELOPMENT OF NOVEL LSD1 INHIBITORS AS A STRATEGY TO TREAT SCD

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Development of Novel LSD1 Inhibitors as a Strategy to Treat SCD

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β-globinopathies, including β-thalassemia and Sickle Cell Disease (SCD), are the most common hereditary monogenic diseases. Both clinical and experimental evidence indicates that increasing the levels of fetal hemoglobin (HbF; the α2γ2 tetramer) alleviates the symptoms of β-globinopathies. Specifically, Hereditary Persistence of Fetal Hemoglobin (HPFH) is a natural human genetic variant in which high levels of fetal γ-globin synthesis aberrantly persist into adulthood. It has been observed clinically that when an HPFH mutation is co-inherited with β-thalassemia or SCD, the elevated production of γ-globin significantly mitigates the symptoms of the disease. To date, hydroxyurea (HU) is the only current FDA-approved drug for HbF induction and SCD treatment, but only about half of all SCD patients are responsive. Therefore, new therapeutics are required. Lysine-Specific Demethylase 1 (LSD1, KDM1a), an enzyme that removes activating histone H3 (H3K4) methylation marks from chromatin, has been identified as one component of a large multi-protein complex, DRED, that represses the human γ-globin genes. Importantly, pharmacologic inhibition of LSD1 leads to dose-dependent increases in γ-globin synthesis. We have also reported that *in vivo* inhibition of LSD1 by a second chemical inhibitor (RN-1) in SCD model mice induced HbF synthesis and led to dramatic improvement in many pathological features normally associated with SCD; we collaboratively demonstrated that RN-1 is also able to significantly stimulate HbF synthesis in baboons, animals that, like

humans, exhibit a fetal to adult switch in γ-type globin synthesis. These findings all strongly underscore the possibility that LSD1 might constitute an outstanding molecular target for therapeutic intervention in treating SCD. We report the synthesis and tests for novel HbF-inducing LSD1 inhibitors as potentially safer and more effective γ-globin inducers in CD34 cells. One novel compound induces γ-globin synthesis up to 24% of total β-type globin and in a dose-dependent manner with mild side effects at 10-fold lower concentrations than RN-1. These data suggest that the novel LSD1 inhibitors might serve as lead candidates for further in vivo studies.

6.0 Poster Session: Small Molecules to Treat Sickle Cell Disease

6.1

TREATMENT OF THE FIRST SICKLE CELL DISEASE PATIENTS WITH ANTAGONIST OF N-METHYL D-ASPARTATE RECEPTOR MEMANTINE: BIOLOGICAL OUTCOME OF THE MEMSID TRIAL

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We have reported pathologically high abundance of NMDA receptors in red cell (RBC) membranes of sickle cell disease (SCD) patients several years ago. Hyperactivated state of these receptors results in Ca^{2+} overload, which in turn causes dehydration, and increased proteolytic activity and adhesiveness of RBCs to each other. A pilot clinical trial was initiated in 2015 in which memantine, the antagonist of NMDA receptors, was applied to a limited number of adult patients to estimate safety and tolerability of the compound and the potential benefits of a new treatment (NCT02615847). Four patients have successfully completed the trial that was closed in March 2017. While statistical analysis of the outcome is in preparation, we may share the biological findings related to the effects of memantine of RBCs of the patients. We have observed the changes in membrane stability and an increase in RBC longevity. These effects were associated in rehydration and substantial reduction in K^+ loss from the cells. Improvement of morphological appearance indicated decrease in proteolytic destruction of cytoskeletal proteins. Ca^{2+} levels in RBCs were decreasing, as were the numbers of active receptors at the RBC membrane of patients. These changes in RBC properties observed during the treatment were reverted upon the suspension of therapy (challenge). These findings were underlying the substantial improvement of life quality of patients and low number of consultations and hospitalizations.

7.0 Poster Session: Coagulation/Thrombosis

7.1

ASSAY-DEPENDENT RESULTS OF ADAMTS13 ACTIVITY IN SICKLE CELL DISEASE

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Background: von Willebrand Factor (VWF) is an adhesive multimeric plasma protein that is acutely elevated in VOC and may play a mechanistic role in the pathogenesis of vaso-occlusion. However, discrepant findings concerning the functionality of ADAMTS13, the VWF-cleaving plasma protease, have been reported in sickle cell disease.

Objectives: To characterize ADAMTS13 activity in adult sickle cell patients using multiple *in vitro* assays.

Methods: Plasma samples were obtained from adult sickle cell patients undergoing regular exchange transfusion (n=20) and healthy control patients (n=15). Plasmatic ADAMTS13 activity was determined by two VWF A2 domain peptidyl-based assays (FRETS VWF73 and VWF73 GST) and a shear-based assay employing the full length VWF molecule. The level of thrombospondin-1 in plasma was determined by ELISA.

Results: In peptidyl-based assays, sickle cell disease plasma displayed significantly lower ADAMTS13 activity relative to healthy controls (mean activity 69.5 vs. 1.11 IU/mL, respectively, for VWF73 GST ELISA, $P < 0.0001$). By contrast, the cleavage potential against the full length VWF molecule was normal or enhanced in sickle cell disease patient plasma. The level of plasma thrombospondin-1, an inhibitor of VWF cleavage which correlates with disease activity in SCD, was not significantly elevated in this study population.

Conclusions: Our findings demonstrate assay-dependent results of ADAMTS13 activity measurements in sickle cell disease, and imply the possible existence of alternative blood proteases capable of VWF cleavage. These findings may have implications for the interpretation of ADAMTS13 activity in sickle cell disease and for the monitoring of ADAMTS13 activity in clinical trials.

7.2

ACCELERATED VENOUS THROMBOSIS WITH ENHANCED FIBRIN DEPOSITION AND PLATELET ACCUMULATION IN SICKLE CELL MICE

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Patients with sickle cell disease (SCD) are at increased risk for thrombotic complications, such as venous thromboembolism and stroke. Previous studies in mouse models have revealed accelerated thrombus formation in the cerebral microvessels of sickle (SS) mice compared to wild type (AA) controls. The objective of this study was to evaluate if SCD alters the dynamics of venous thrombosis in large vessels using a mouse model of electrolytic injury-induced thrombosis in the femoral vein. Anesthetized male AA and SS mice (12 weeks old) were infused with fluorescent antibodies to fibrin (59D8) and platelets (rhodamine 6G) 5 min before positive current was applied to the femoral vein. In the standard injury model, 1.5 V was applied for 30 s. In the severe injury model, 3.0 V was applied for 90 s. Fluorescence was monitored for 60 min after injury using intravital microscopy.

In the standard injury model, AA mice developed thrombi with a fibrin core and platelet cap that peaked at 20 minutes then began to resolve. In SS mice, there was no distinct border between platelets and fibrin in the thrombus, and both continued to accumulate throughout the monitoring period, leading to significantly more fibrin and platelet deposition within the clot compared to AA controls. There was no occlusion of flow in the vein around the developing thrombi in either AA or SS mice. In the severe injury model in AA mice, platelet accumulation began within 10 minutes and was sustained, whereas fibrin accumulation steadily increased over 60 min. In SS mice, there was a rapid increase in both platelet and fibrin accumulation by 15 and 25 min, respectively, which plateaued for the remainder of the experiment. Moreover, the fluorescence intensities of both platelets and fibrin deposition were nearly 2-fold higher in SS mice compared to AA controls starting at the times mentioned above, and remained significantly elevated throughout the monitoring period. In summary, we have established a model of venous thrombosis that can be used to study the dynamics of venous thrombosis in sickle mice. Future studies will use this model to elucidate the mechanisms of increased risk of venous thromboembolism in SCD, with a focus on the clot stability and rate of fibrinolysis.

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9.0: Neural Circuits and Neurovascular Physiology

9.1

TARGETING PAIN AT ITS SOURCE IN SICKLE CELL DISEASE

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Pain, a hallmark of sickle cell disease (SCD), can start in infancy and continue throughout life, leading to increased morbidity and mortality. Yet, how sickle pathobiology evokes pain is not understood. Hemin released upon hemolysis contributes to vascular sickle pathobiology via toll-like receptor 4 (TLR4)-mediated mechanisms. We examined whether hemin contributes to hyperalgesia via TLR4 mediated peripheral and central mechanisms of pain in SCD, using a humanized model of transgenic HbSS-BERK sickle mice. We found that hemin induced hyperalgesia in sickle and control mice. However, genetic deletion of TLR4 reduced hemin-induced chronic hyperalgesia in sickle and control mice, and attenuated hypoxia/reoxygenation (H/R)- evoked acute hyperalgesia. Pharmacological blockade of TLR4 decreased mast cell activation, neurogenic inflammation, IL-6, and substance P in the periphery and spinal cord, as well as p38/MAPK phosphorylation in the spinal cord. TLR4 inhibition attenuated hemin-evoked spinal microglial cell activation by reducing endoplasmic reticulum (ER) stress in vitro. Either inhibiting TLR4 with TAK242 or reducing ER stress with salubrinal ameliorated chronic hyperalgesia in sickle mice in a time-dependent manner. TAK242 pretreatment also reduced the severity of acute pain evoked by H/R and accelerated recovery from H/R-induced hyperalgesia in sickle mice. Collectively, our data demonstrate the pivotal role of TLR4 in evoking chronic and acute pain in SCD. It suggests potential therapeutic benefit of limiting hemin, TLR4 inhibition and ER stress reduction in ameliorating pain and inflammation in SCD. NIH R01HL103773 and U01 HL117664. Reference: Aich A, Beitz AJ, and Gupta K. Mechanisms of pain in sickle cell disease. In, Sickle Cell Disease, Inusa B (Ed). November 2016. InTech Publishers, Croatia, EU.

9.2

ENDOTHELIN TYPE A RECEPTORS MEDIATE SICKLE CELL DISEASE-ASSOCIATED PAIN BY UP-REGULATING NAV1.8 IN PRIMARY SENSORY NEURONS

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Sickle Cell Disease (SCD), a hemoglobinopathy resulting in a mutated β globin gene, is associated with acute painful episodes and persistent intractable pain. Endothelin-1, a known pain inducer, is elevated in the blood plasma of both SCD patients and SCD mouse models. Endothelin-1 binds to Endothelin-type A (ETA) receptors and Endothelin-type B receptors. In dorsal root ganglion (DRG), ETA receptors are found in the neurons, while ETB receptors are expressed in the surrounding satellite cells. We hypothesize that ET-1 binding to ETA receptors in DRG contributes to SCD-associated pain through increased Nav1.8 channel expression. Mechanical, thermal, and cold sensitivity were assessed in 6 months old Townes HbSS and HbAA control mice before and after exposure to hypoxia. The effect of ABT-627, an ETA receptor-specific antagonist, on evoked pain hypersensitivity was also analyzed. Additionally, DRG-specific ETA receptor knockout mice ($ETA^{flox/cre}$) underwent total body irradiation and bone marrow transplantation (BMT) using HbSS and HbAA bone marrow, resulting in $ETA^{flox/cre}$ mice expressing human sickle β globin (HbSS marrow recipients) or normal human β globin (HbAA marrow recipients). Pain behavior was assessed in these mice before BMT, after BMT, and after hypoxia. Our results show that HbSS mice possess basal evoked mechanical and thermal pain hypersensitivity and basal spontaneous pain. Subcutaneous injection of ABT-627 attenuated basal and post-hypoxia evoked mechanical and thermal pain hypersensitivity in HbSS mice. Additionally, $ETA^{flox/cre}$ mice transplanted with HbSS bone marrow displayed less basal and post-hypoxia evoked mechanical and thermal pain hypersensitivity compared to $ETA^{flox/flox}$ mice transplanted with HbSS bone marrow. Electrophysiology recording of HbSS DRG neurons showed an increase in Nav1.8 current and nociceptor spontaneous activity. ABT-627 blocked the increases in Nav1.8 channel current, protein levels, and mRNA levels in the DRG of HbSS mice. Our findings indicate that ABT-627 may be beneficial for the treatment of SCD-associated pain. This work was supported by NIH grants (R01NS072206, R01DA033390, F31NS092310, and U01HL117684) .

9.3

PROSTAGLANDIN GLYCEROL ESTERS CONTRIBUTE TO HYPERALGESIA IN A HUMANIZED MOUSE MODEL OF SICKLE CELL DISEASE

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Sickle Cell Disease (SCD) is a hereditary chronic hemolytic anemia with numerous clinical consequences including ongoing and episodic pain. HbSS-BERK mice which express human sickle hemoglobin (HbSS) and mirror symptoms of human SCD were used to explore the contribution of the prostaglandin glycerol ester, PGE_2-G, the cyclooxygenase (COX)-2 oxidative metabolite of 2-arachidonylglycerol (2-AG), to mechanical and cold hyperalgesia. Mechanical hyperalgesia was defined as an increase in the frequency of withdrawal evoked by a von Frey monofilament with a force of 3.9 mN applied to the plantar surface of the hind paws. A cold preference test was used to determine cold hyperalgesia. The level of the endocannabinoid 2-AG in dorsal root ganglia (DRG) was determined by HPLS-MS. Hyperalgesia in HbSS mice was accompanied by increased activity of COX-2 in DRGs and decreased levels of 2-AG, the natural substrate for COX-2. Considering that COX-2 oxygenates 2-AG to form PGE_2-G, we investigated the contribution of PGE_2-G to the development of mechanical and cold hyperalgesia. Intraperitoneal administration of PGE_2-G to control HbAA mice produced acute bilateral mechanical hyperalgesia that was dose-dependent. Intraplantar injection of PGE2-G produced local hyperalgesia, suggesting a peripheral mechanism of action. *R*-Flurbiprofen is a slow reversible inhibitor of COX-2 that preferentially inhibits the production of PGE_2-G. Systemic administration of *R*-Flurbiprofen reduced cold and mechanical hyperalgesia in HbSS mice, and this was also dose-dependent. Collectively, our results suggest that COX-2-mediated oxidation of 2-AG results in production of PGE_2-G in DRGs that may contribute to nociceptor sensitization and to pain in SCD.

This work was supported by: NIH grants R01 HL135895 and U01 HL117664.

9.4

DIMINISHED CEREBRAL OXYGEN EXTRACTION IN ANEMIC SUBJECTS USING VENOUS MRI OXIMETRY: IS TRUST OXIMETRY CALIBRATION RELIABLE IN SICKLE CELL DISEASE?

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MRI oximetry techniques have opened the door for noninvasive, non-irradiating investigations of cerebral oxygen supply and demand. The most widely used MRI oximetry technique is T_2 Relaxation Under Spin Tagging (TRUST). TRUST works by magnetically isolating blood water in order to determine the blood transverse relaxation (T_{2b}). T_{2b} is proportional to deoxyghemoglobin and can be used to derive venous oxygen saturation (S_vO_2) using predetermined T_{2b} calibrations.

To date, studies of TRUST in SCD have reported elevated cerebral oxygen extraction. Some have even concluded that the increased oxygen extraction in SCD exposes patients to increased stroke risk due to diminished oxygen reserve. Unfortunately, these conclusions were made based on T_{2b} calibrations developed in bovine blood and derived over a non-anemic hemoglobin range. In order to assess the accuracy of recent conclusions, we derived a SCD specific T_{2b} calibration ex vivo and performed TRUST in healthy subjects (CTL), SCD patients and chronic, non-SCD related anemia subjects (ACTL).

Methods: Ex vivo, 83 T_{2b} measurements from 11 SCD subjects were performed to derive an ex-vivo SCD specific T_{2b} calibration. TRUST of the sagittal sinus was performed on 84 subjects (37 CTL, 33 SCD, 14 ACTL) to measure S_vO_2 and OEF.

***Results**: Ex-vivo*, SCD blood demonstrated a distinct T_{2b} behavior compared to previously reported bovine and HbA T_{2b} calibrations. This difference resulted in diametrically opposed in-vivo oximetry predictions between T_{2b} calibrations. For instance, the HbS model predicted higher Y_v (73±5%) in SCD subjects compared to CTL (61±6%), the HbA model predicted equitable Y_v (64±4%) values and the bovine calibration predicted lower Y_v lower values in SCD subjects (60±8% vs 65±5%). In both the SCD and ACTL subjects, use of the appropriate human T2b calibration produced extraction estimates than were lower than CTL subjects (table 1).

	CTL	SCD	ACTL	Dunnett (p value)
Bovine Model	0.34±0.5	0.38±0.08	0.32±0.05	SCD(p=0.06)
HbA model	0.38 ±0.06	0.34 ±0.06	0.30±0.04	SCD (p<0.0085), ACTL (p<0.0001)
HbA and HbS model	0.38 ±0.06	0.24 ±0.04	0.30±0.04	SCD (p<0.0001), ACTL (p<0.0001)

Conclusion: These finding demonstrate that specific T_{2b} models are critical in SCD subjects, suggesting TRUST conclusions in SCD using bovine blood are spurious. Additionally, we report here that cerebral oxygen is actually decreased in SCD and ACTL subjects, suggesting hyperemic shunting and/or cerebral oxygen supply demand uncoupling.

10.0 Poster Session: Renal and Vascular Physiology

10.1
ACTIVATION OF RENAL HEPCIDIN EXPRESSION IN SICKLE CELL DISEASE MOUSE MODEL

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Chronic hemolysis and blood transfusions can lead to iron overload and organ iron accumulation in patients with red blood cells disorders. The pattern of iron accumulation within different organs is disease specific. Renal cortical iron deposition is characteristic for sickle cell disease (SCD) but not for β-thalassemia patients; and it does not correlate with the levels of iron overload and number of blood transfusions. SCD mice (Townes) accumulate iron in the epithelial cells of proximal tubules, and can be used to study renal iron metabolism.

The project objective was to measure expression levels of renal iron-regulating proteins in SCD mice. The animal protocol was approved by the Institutional Animal Care and Use Committee at the Children's National Health System. Both SCD and control mice do not express mouse hemoglobin (Hgb). Townes sickling mice express human Hgb S and Hgb F, whereas control mice express human Hgb A1. Kidneys were collected from 4 months old mice. Renal cortexes were used for RNA and protein isolation. Real time RT-PCR, ELISA and Western Blot and immunostaining were performed for characterization of iron-regulating proteins expression.

We detected a significant accumulation of iron in the epithelial cells of proximal tubules in SCD mice. Renal expression of hepcidin was not detected in controls. In contrast renal hepcidin expression was detected in the epithelial cells of proximal tubules of SCD mice. The mRNA levels of FPN, TFR1, DMT1, ferritin and hephaestin were decreased in SCD mice kidney compared to controls. In contrast, protein levels of TFR1, ferritin, and CP were increased. Protein levels of FPN were similar in SCD and control animals. We also observed significant renal macrophages infiltration in SCD mice.

In conclusion, activation of hepcidin expression in renal proximal tubular epithelial cells may induce partial degradation of FPN in SCD mice. Increased levels of iron importers (TFR1 and DMT1) without significant change in FPN levels can saturate iron storage in ferritin and lead to the accumulation of intracellular iron. Activation of renal hepcidin expression in SCD mice may be associated with renal inflammation.

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10.2

HEMOGLOBIN INHIBITS UPTAKE OF FILTERED PROTEINS BY PROXIMAL TUBULE CELLS: IMPLICATIONS FOR SICKLE CELL DISEASE

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Proximal tubule (PT) dysfunction, including tubular proteinuria, is a significant complication in sickle cell disease (SCD) that can eventually lead to chronic kidney disease. The PT is especially susceptible to cytotoxic damage, and tubular dysfunction in SCD is thought to result from prolonged exposure to hemoglobin (Hb) released from damaged red blood cells. Filtered Hb dimers are internalized into PT cells upon binding to the multiligand receptors megalin and cubilin. These receptors bind to numerous filtered proteins, including albumin and vitamin D binding protein, and are important for maintaining vitamin D homeostasis and protein-free urine. We found that concentrations of Hb predicted to enter the tubule lumen during hemolytic crisis profoundly inhibit the uptake of other megalin/cubilin ligands (albumin and vitamin D binding protein) by PT cells. These effects were independent of heme reduction state, occurred in the absence of a cytotoxic response, and appear to be due to direct competition for megalin/cubilin binding. The Glu7Val Hb mutant that causes SCD was equally effective at inhibiting albumin uptake compared with wild type Hb. Haptoglobin restored albumin uptake in the presence of Hb, suggesting that haptoglobin binding to the Hb αβ dimer interferes with Hb binding to megalin/cubilin. BLAST searches and structural modeling analyses revealed regions of similarity between Hb and albumin that map to this region and may represent sites of Hb interaction with megalin/cubilin. Using these data, we established a robust, scalable assay that enables us to screen for selective inhibitors of Hb uptake that preserve PT function. Our studies suggest that the primary cause of tubular proteinuria in SCD is impaired endocytosis of megalin/cubilin ligands due to competition from filtered Hb. Our results have therapeutic implications for SCD, as preventing Hb uptake is predicted to slow the progression of kidney disease. Additionally, our data suggest a potential explanation for the vitamin D deficiency commonly observed in sickle cell patients. Ongoing studies include quantitation of vitamin D metabolites in PT cells to assess the impact of Hb inhibition of vitamin D binding protein uptake, and experiments to refine our screen for inhibitors of Hb uptake. Sources of support: National Institutes of Health R01 DK101484, R01 DK100357, P30 DK079307T32; Pittsburgh Heart Lung and Blood Vascular Medicine Institute P3HVB pilot grant.

10.3

REGULATION OF RENAL HEPCIDIN IN SICKLE CELL DISEASE MICE

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Hemolysis and frequent blood transfusions lead to the iron overload and organ iron accumulation in patients with red blood cells disorders. The pattern of iron accumulation within different organs is disease specific. Sickle cell disease (SCD), unlike β-thalassemia, is characterized by abnormalities of renal iron metabolism. Renal iron deposition does not correlate with iron overload and blood transfusion. Transgenic SCD mice accumulate iron in the epithelial cells of proximal tubules and represent a suitable model to study iron metabolism in SCD patients. To characterize proteins of the renal iron metabolism in SCD mouse model, RNA and proteins were isolated from the kidney of 5 months old transgenic SCD (Townes) and control mice. Western blot, ELISA, and quantitative RT-PCR

were used to measure levels of renal hepcidin, ferroportin, transferrin receptor (TFR1), divalent cation receptor (DMT1), ferritin, and hephaestin in the renal cortex. Immunostaining was used for detection of renal iron accumulation on paraffin-embedded sections. Significant accumulation of iron was found in the epithelial cells of proximal tubules in SCD mice. Also, we found an increased expression of renal hepcidin in SCD mice compared to controls and, surprisingly, decreased mRNA levels of all other proteins involved in renal iron metabolism (ferroportin, TFR1, DMT1, ferritin, and hephaestin). In contrast, increased levels of transferrin receptor, ferritin, and ferroportin were observed alongside with a significant renal macrophages infiltration in SCD mice. These findings suggest that increased levels of renal hepcidin expression in SCD mice may be associated with renal inflammation. Also high levels of locally expressed hepcidin may lead to the partial degradation of ferroportin and significantly impair iron export from renal epithelial cells thus leading to the intracellular iron accumulation.

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10.4

WHICH ALPHA GLOBIN GENE IS PRIMARILY EXPRESSED IN THE VASCULAR ENDOTHELIUM?

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People with Sickle Cell Disease (SCD) who co-inherit one or two deletions of the alpha globin gene (a condition called alpha thalassemia) are protected against cerebrovascular and renal complications of SCD. Alpha globin was recently discovered in the endothelium of resistance arteries, where it interacts with endothelial nitric oxide synthase (NOS3) to regulate the diffusion of nitric oxide, raising the question of how endothelial alpha globin may modulate vascular pathophysiology in SCD.

While it is known that alpha globin locus *HBA2* contributes 65% of total alpha globin in red blood cells in humans, the expression profile of each locus in the vasculature is currently unknown. Determining total and relative locus-specific expression of alpha globin in resistance arteries is critical to understand the consequences of *HBA1* or *HBA2* locus deletion. To measure the expression of alpha globin across major organ systems, perfused vessels and whole blood were collected from C57Bl/6J mice. Conduit arteries (thoracic aorta and carotid arteries) and resistance arteries (middle cerebral arteries, skeletal muscle arterioles, mesenteric arteries, and renal arterioles) were dissected and placed in RNAlater. Total mRNA was isolated from homogenized vessels (RNeasy, Qiagen) and whole blood (RiboPure Blood Kit, Ambion), and converted to cDNA (SuperScript IV VILO, Invitrogen).

Absolute gene expression of *Hba-a1* (mouse homolog of *HBA2*), *Hba-a2* (mouse homolog of *HBA1*), *Nos3*, and *Ae1* (erythrocyte anion exchanger) was quantified by digital droplet PCR (BioRad). *Ae1* was highly expressed in whole blood, but not in vascular tissue, confirming that vessels were clear of red cells. Conversely, *Nos3* was expressed in all vessels, but not in whole blood. Abundant expression of *Hba-a1* and *Hba-a2* was observed in whole blood, as well as in all vascular tissues. In whole blood, the *Hba-a1/Hba-a2* ratio was (2.55:1), consistent with expression in human blood. However, in all arteries and arterioles, the *Hba-a1/Hba-a2* ratio was inverted (0.60:1).

We report robust, locus-specific expression of alpha globin in six anatomically distinct arteries. The expression ratio of *Hba-a1* and *Hba-a2* is inverted between vascular tissue and whole blood in mice, suggesting differential regulation of alpha globin transcription. Further investigation into the physiologic role of endothelial alpha globin may provide new insights into how alpha thalassemia modulates vascular pathologies associated with SCD.

10.5

ENDURANCE TRAINING DOES NOT CORRECT METABOLIC ABNORMALITIES RELATED TO ISCHEMIA - REPERFUSION IN SICKLE CELL DISEASE MICE

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Sickle cell disease (SCD) is a genetic hemoglobinopathy characterized by the occurrence of painful vaso-occlusive crises (VOC) in many tissues including skeletal muscle. Yet, the muscle metabolic abnormalities related to VOC are still unknown in SCD. Our first aim was to evaluate the impacts of ischemia/reperfusion cycle on muscle energetic metabolism in SCD. We aimed secondly at determining whether endurance training could alleviate some of these possible metabolic defects.

Ten control (HbAA), 13 heterozygous (HbAS), 10 sedentary SCD (HbSS-SED) and 9 endurance-trained SCD (HbSS-END) mice were submitted to a standardized rest – ischemia (30 min) – reperfusion (25 min) protocol during which ATP and phosphocreatine (PCr) concentrations, as well as intramuscular pH were measured using 31-phosphorus magnetic resonance spectroscopy. Forty-eight hours later, skeletal muscles were sampled. While the time-courses of

ATP (that was fairly stable throughout the protocol) and PCr (that decreased linearly) concentrations were similar among groups during the ischemic period, both HbSS-SED and HbSS-END displayed a larger acidosis as compared to the HbAA and HbAS groups ($p < 0.01$) during the same period, with no difference between HbSS-END and HbSS-SED mice. During the reperfusion period, the initial rate of phosphocreatine resynthesis was slower in HbSS-SED and HbSS-END compared to HbAA ($p < 0.05$) and HbAS ($p < 0.01$) animals. The total hindlimb muscles weight was lower in the hindlimb submitted to the ischemia/reperfusion protocol as compared to the control hindlimb ($p < 0.001$). In conclusion, SCD mice displayed an exacerbated intramuscular acidosis in response to ischemia, while the subsequent reperfusion disclosed an impaired skeletal muscle oxidative capacity. Interestingly, these metabolic defects were not improved as a result of endurance training.

10.6

TRANSIENT DESATURATION CHALLENGE REVEALS WHITE MATTER MICROVASCULAR DISEASE IN PATIENTS WITH SICKLE CELL DISEASE.

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Introduction: Patients with sickle cell disease (SCD) have chronic, progressive, white matter disease that impairs cognitive function, quality of life, and employment. Patients with sleep apnea also suffer from similar neurologic sequelae. Since many sickle cell disease patients also exhibit sleep apnea, we devised a MRI imaging protocol to study the impact of transient hypoxia on cerebral blood flow and saturation.

Methods: We performed Blood Oxygen Level Dependence(BOLD) MRI and cerebral near infrared spectrometry(NIRS) in 28 SCD patients, 12 patients with non sickle anemia syndromes and 31 age and race matched control subjects. Hypoxia was induced by changing the inhaled gas mixture from room air to 100% nitrogen for five breaths (20-25 seconds), resulting in transient desaturations of 10% - 30%.

Results: Changes in peripheral oxygen saturation were mirrored by increased NIRS deoxyhemoglobin, decreased NIRS oxyhemoglobin, and decreased BOLD signal. NIRS total hemoglobin concentration increased post hypoxia, suggesting a small compensatory hyperemic response. BOLD and NIRS changes were temporally concordant with one another but began prior to detectable changes in pulse oximetry, reflecting shorter transit times to the brain compared with the finger. Peak pulse oximetry, BOLD, and NIRS changes were all inversely related to hemoglobin concentration, consistent with higher cardiac output and cerebral blood flow in anemic subjects. In the BOLD signal, the induced desaturation behaved like a contrast bolus. Anemic subjects had more rapid desaturation wash in, shorter time to peak and more rapid recovery over most of the brain. However, time-to-peak and recovery half-time were pseudonormal (not decreased relative to controls) in white matter regions at greatest risk for stroke.

Conclusions: Taken together, patients with chronic anemia had more rapid and severe cerebral desaturation in response to transient hypoxia because of their increased pulmonary and cerebral blood flow. The induced desaturation pulse exposed subtle differences in time-to-peak and recovery half-life consistent with microvascular damage in watershed areas. BOLD changes were mirrored by changes in cerebral NIRS, supporting BOLD as a metric of tissue oxygenation. Subsequent studies will explore the relative importance of hemoglobin S levels compared with changes in total hemoglobin concentration.

10.7

TRANSFUSION THERAPY IMPROVES MULTILEVEL VASCULAR DYSFUNCTION IN SICKLE CELL DISEASE: ARE MULTIPLE LEVELS OF THE VASCULATURE PATHOPHYSIOLOGICALLY LINKED?

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Objective: To determine whether chronic transfusion therapy improves multiple levels of vascular dysfunction in patients with sickle cell anemia.

Sickle cell disease (SCD), the most common inherited disease in the world, is a model of diffuse vascular disease. The etiology of vascular dysfunction is multifactorial, including decreased nitric oxide bioavailability, chronic inflammation, increased erythrocyte adhesion to the endothelium, and decreased erythrocyte deformability. Together, these affect multiple levels of the vasculature; however, multi-level vascular assessment has not been systematically evaluated. Using a forearm ischemia model, we simultaneously assessed microcirculatory post occlusive hyperemia (PORH) using laser Doppler flowmetry of the nailbed, tissue oxygenation using near infrared spectroscopy (NIRS) over the dorsum of the hand and flow mediated dilation (FMD) of the brachial artery. We evaluated blood viscosity, erythrocyte deformability and erythrocyte aggregation in addition to blood cell counts, markers of hemolysis and inflammation. The Children's Hospital Los Angeles review board approved this protocol. We enrolled 18 healthy, 75 non-transfused SCD and 26 chronically transfused SCD patients. All three levels of the vasculature were diseased in non-transfused SCD patients compared to healthy, with lower FMD (P=0.04), lower PORH (P=0.003) and lower NIRS (P<0.0001). Chronic transfusion improved FMD and NIRS, by 23% (P=0.08) and 32% (P<0.0001) respectively, but not microcirculatory PORH. Consistent with our previously published data, plasma free hemoglobin was an independent predictor of FMD (P<0.0001) and we also found it was an independent predictor of NIRS (P=0.003) in non-transfused SCD patients. Further, there is a strong positive association between tissue oxygenation and FMD after controlling for transfusion status (P=0.0009). There is no association between microcirculatory PORH and FMD or NIRS, nor did markers of rheology, inflammation or hemolysis predict PORH. Baseline resting laser Doppler flow was the strongest predictor of PORH.

Chronic transfusion therapy improves tissue oxygenation and FMD but not microcirculatory post occlusive hyperemia. FMD and tissue oxygenation have similar pathophysiologic effectors, such as plasma free hemoglobin, while microcirculatory PORH is primarily determined by baseline resting flow.

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10.8

EXERCISE (IN)TOLERANCE IN SICKLE CELL DISEASE: POTENTIAL DISRUPTIVE ROLE OF FREE HEMOGLOBIN ON SKELETAL MUSCLE OXYGEN DELIVERY/UTILIZATION MATCHING AND FUNCTIONAL CAPACITY

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Sickle cell disease (SCD) reduces exercise tolerance likely due to vascular and skeletal muscle abnormalities stemming from increased free hemoglobin (Hb, a known scavenger of nitric oxide). However, there has been little advancement in our understanding of the precise mechanisms responsible for the reduction in physical functionality. Therefore, the purpose of this investigation was 1) determine the degree by which exercise tolerance is impaired in mice expressing human HbSS (BERK) and 2) examine the impact of free Hb on the skeletal muscle microvascular PO_2 (PO_2mv, the principal driving force that facilitates blood-muscle O_2 flux) at rest and during contractions in rats. We hypothesized that exercise capacity would be lower in BERK relative to wild-type mice (WT) with a lower PO_2mv observed following Hb infusion. Twenty female mice (WT, n=10 and BERK, n=10) performed 4 constant-speed treadmill tests that resulted in fatigue within 1.5 to 20 min. Time to fatigue vs. treadmill speed were fit to a hyperbolic model to determine critical speed (CS). PO_2mv was measured during 180 s of electrically induced muscle contractions during control, following free Hb infusion (Hb, 50mg), and L-nitro arginine methyl ester superfusion (L-NAME, 1.5 mM) conditions in 9 rats. Speed and time to exhaustion for WT and BERK conformed to a hyperbolic relationship (WT: r^2 = 0.93 ± 0.02, BERK: r^2 = 0.97 ± 0.01, p>0.05). CS was significantly lower in BERK when compared to WT (WT: 33.1 ± 1.5, BERK: 25.2 ± 0.7 m/min, p<0.05). Following the onset of contractions, Hb and L-NAME significantly increased the amplitude of the fall in PO_2mv when compared to control, with no significant differences between Hb and L-NAME conditions ($\Delta 1PO_2$mv: control: 9.5±0.7, Hb: 11.7±1, L-NAME 10.4±0.8 mmHg, p<0.05). The increased $\Delta 1PO_2$mv resulted in a significantly lower PO_2mv during the steady-state of muscle contractions in both Hb and L-NAME conditions, with no differences between Hb and L-NAME (PO_2mv(steady-state): Control: 24.1±0.9, Hb: 21.3±0.7, L-NAME: 19.6±1 mmHg, p<0.05). To summarize, exercise tolerance, as measured via CS, was significantly lower in BERK mice relative to WT. Furthermore, the lower $PO_2mv_{(steady\text{-}state)}$ in Hb and L-NAME represents a compromised blood-myocyte O_2 driving force during muscle contractions. Collectively, these data suggest that SCD impacts physical capacity via a disruption in the tight

matching between oxygen delivery and utilization within the skeletal muscle.

10.9

ASSOCIATIONS BETWEEN CARDIORESPIRATORY FITNESS AND ARTERIAL FUNCTION IN ADOLESCENTS WITH SICKLE CELL ANEMIA

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Introduction: Sickle cell anemia (SCA) results in consequences to the cardiovascular system, due to chronic inflammation and endothelial dysfunction. Individuals with SCA have been shown to have a decreased peak aerobic capacity (VO_{2peak}); however, the physiological basis for this limitation has yet to be fully understood. Proposed factors include chronic anemia and poor cardiopulmonary function. Endothelial dysfunction, assessed via flow mediated dilation (FMD), may also contribute to reduced VO_{2peak}. **Purpose:** To explore relationships between VO_{2peak} and markers of endothelial function in adolescents with SCA. Also, to compare indices of arterial health in adolescents with and without SCA. **Methods:** Eleven adolescents with SCA (13±1yrs, 19±4kg/m^2) and 11 ethnicity-, age-, and sex-matched controls (13±1yrs, 27±6kg/m^2) underwent a standardized FMD protocol. VO_{2peak} was measured in SCA using indirect calorimetry on a cycle ergometer. Associations between fitness and arterial function were determined with bivariate correlations, and the Mann Whitney Wilcoxon rank sum test was used to explore potential differences in endothelial function between adolescents with and without SCA. **Results:** Adolescents with SCA tolerated FMD without adverse events. VO_{2peak} was not associated with FMD % or FMD area under the curve (R^2 of 0.011 and 0.058, respectively). Comparisons of endothelial function between those with and without SCA are shown in Table 1. Baseline as well as peak velocity and shear during FMD testing were significantly higher in the brachial arteries of adolescents with SCA. **Conclusion:** Despite having a greater artery wall stimulus both at baseline as well as during FMD, adolescents with SCA exhibited a similar % change in brachial artery diameter compared to controls. Also, VO_{2peak} was not associated with endothelial function. Further research with a larger sample size is warranted in adolescents and adults with SCA. **Funding:** NHLBIK23HL094376 (Liem)

Table 1

	Group		
Variable	**Sickle Cell**	**Control**	***P*-Value**
n	11	11	
SBP (mmHg)	113±9	110±7	0.62
DBP (mmHg)	59±9	56 ± 5	0.26
Baseline Diameter (mm)	3.3±0.004	3.3±0.003	0.76
Baseline Velocity (m/sec)	1.08±0.25	0.58±0.11	<0.001
Baseline Shear (sec^{-1})	331±84	181±35	<0.001
FMD (%)	7±3	9±5	0.44
FMD AUC	681±379	913±635	0.72
Peak Shear (sec^{-1})	561±72	326±70	<0.001
Peak Velocity (m/sec)	1.77±0.23	0.97±0.14	<0.001

Data are mean ± SD. *P*-value < 0.05 demonstrates significant differences between means.

10.10

ESTIMATION OF GFR IN ADULT PATIENTS WITH SICKLE CELL DISEASE: SERUM CREATININE AND CYSTATIN-C BASED ESTIMATION EQUATIONS OVER-ESTIMATE AND ARE POORLY PREDICTIVE OF TRUE GFR.

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Epidemiological studies of chronic kidney disease (CKD) in adults with sickle cell disease (SCD) show a high prevalence of abnormal albuminuria but relatively low rates of low GFR, based on serum creatinine (Scr) or creatinine-based eGFR equations. This gap could result from the lower Scr values observed in SCD vs non-SCD patients, possibly related to lower muscle mass and/or differences in tubular creatinine handling. Recently, Cystatin C and cystatin-C based eGFR equations have been suggested to better estimate CKD in non-SCD populations, but they have not been validated in SCD.

Our goal was to compare the ability of Scr and Cystatin-C based eGFR to estimate true GFR in SCD and to analyze the implications of the performance of the different eGFR equations in sickle cell patients.

Eighty-five adult SCD patients (18-65 years , male n=41, female n=44, Hb SS n=69, other sickle Hb n=16) had the GFR measured by the gold-standard urinary clearance of inulin or iohexol on 159 occasions, and were compared with eGFR derived from Scr or Cyst-C based formulas: 1) Cockroft-Gault, 2) MDRD, 3) CKD-Epi-creat, 4) CKD-Epi-Cysc, and 5) combined CKD-EPI Cr/Cysc equations. Scr ranged from 0.30 to 7.65 mg/dL, serum Cystatin C from 0.45 to 3.4 mg/L, and GFR between 5 and 165 ml/min/1.73m2.

All equations overestimated true GFR, ranging between 35% and 60% for different equations. The lowest overestimation was with the CKD-Epi-Cys equation, but still was 35%, on average. We determined the accuracy of

the equations as the percentage of estimated values within 10% (P10), 30% (P30) and 50% (P50) of the true values. All equations lacked accuracy: only about 10-15%, 30-45% and 48-65% of the estimated values were within P10, P30 and P50%, respectively. When restricting the GFR to > 90 ml/min/1.73, the equations performed numerically slightly better, but P10 was only 20-30% of values and P30 ranged between 35-75%.

We conclude that none of the current estimation GFR equations based on Scr or cystatin C accurately estimate true GFR in adults with SCD. Moreover, all of them significantly overestimate the true GFR (35-60% on average). Only patients with advanced renal insufficiency are identified with current equations. We conclude that measurement of GFR is needed when accurate determination of GFR is required, and that new clinical markers or biomarkers of CKD are needed to assess CKD in adults with SCD.

10.11

CIRCULATING EXOSOMES FROM PATIENTS WITH SICKLE CELL REGULATE PATHWAYS OF INFLAMMATION AND ENDOTHELIAL INTEGRITY

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Abstract; Sickle Cell Disease (SCD) causes intermittent, cumulative endothelial damage, which results in significant end organ damage.(1) We have recently demonstrated that exosomes isolated from patients with SCD cause disruption in endothelial monolayers *in vitro* as measured via electric cell-substrate impedance sensing and as visualized with immunofluorescence.(2,3) The exact pathways regulated by exosomes from patients with SCD remain unknown. Given the critical role of the endothelium in SCD, we hypothesized that exosomes from patients with SCD change behavior of endothelial cells via gene expression. We collected platelet-free plasma when patients were clinically well from patients with SCD and control patients, HgbAA. Patients with SCD were excluded if they had asthma, obesity or a history of Acute Chest Syndrome, as we know that those exosomes also have unique characteristics. Exosomes were isolated from plasma using the Total Exosome Isolation Kit. We then treated HMVEC-d cells for 24 hours with exosomes followed by isolation of RNA for microarray analysis to examine gene expression. Bioinformatic analysis of microarrays was performed including gene ontology and differential pathway analysis, leading to the identification of 364 genes with a q-value <0.05 and fold-change >1.2 that are differentially regulated by SCD-derived exosomes compared to control-derived exosomes. The identified genes are in pathways of innate immune response, cell structure, nitric oxide signaling, and inter-cellular junctions. In conclusion, we provide evidence that exosomes from patients with SCD regulate a unique set of pathways important in maintaining endothelial integrity. Further analysis will be done to validate these genes (including qRT-PCR). Funded by NIH CTSA, Grant numbers: 5K12HL119995, UL1TR000430.

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10.12

IDENTIFICATION OF URINARY HGFL PROTEIN AS A POTENTIAL MARKER FOR THE DEVELOPMENT OF CHRONIC KIDNEY DISEASE IN SICKLE CELL DISEASE PATIENTS

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Chronic kidney disease (CKD) is common in patients with sickle cell disease (SCD). However, the progression of CKD in SCD and factors associated with such progression remain poorly defined. The purpose of this study was to identify the potential markers associated with CKD progression in patients with SCD. Since glomerular hyperfiltration is an early stage of renal dysfunction, we performed label-free quantitative proteomic analysis for urine samples collected from SCD patients with hyperfiltration (N=3) and normal (N=3). Hepatocyte growth factor-like (HGFL) protein was found to be significantly downregulated (5.52-fold, p=8.05×10^{-5}) in samples with glomerular hyperfiltration compared to

normal group. Next, we developed a high resolution/selected ion monitoring (HR/SIM) method by measuring the HGFL peptide (*m/z* 585.79) with isotope labeled-HGFL peptide (*m/z* 590.80) as internal standard (IS). HR/SIM quantification was performed for 19 urine samples from SCD patients and 12 urine samples from healthy controls. HGFL levels were found to be significantly downregulated (p=0.0084) in the SCD urine samples compared to samples from healthy controls. To further assess the correlation between HGFL level and CDK stage, we expanded the analysis to SCD patients with different CKD stages ranging from 0 to 5 and 19 healthy individuals by ELISA. The result confirmed the finding of HR/SIM quantification, moreover, showed that urinary HGFL level correlated with CKD stage (R=0.17) and showed high sensitivity and specificity by ROC analysis (AUC=0.78). HGFL protein has been identified as a negative regulator of phosphatidylinositol 3-kinase (PI3K), and PI3K/Akt pathway was found to be activated in the progress of CKD. Therefore, the decrease of HGFL level in urines from SCD patients may indicate the development of CKD. A limitation of our study is the small number of samples from high stage (4 or 5) of CDK patients used for the correlation analysis.

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10.13

THE PLASMA PROTEOME OF SICKLE CELL PAIN CRISIS

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People living with sickle cell disease experience severe episodic pain crises that require hospitalization for pain management and supportive care. Hydroxyurea, blood transfusion, and emerging anti-adhesive therapies have been used to reduce the frequency or severity of pain crises; however, questions remain regarding the etiology, diagnosis and optimal treatment of sickle cell pain crisis. We used proteomics to quantify changes in the abundance of plasma proteins that occur during sickle cell pain crisis.

We obtained EDTA plasma from 7 adults with sickle cell disease within 36 hours of admission to the NIH Clinical Center for treatment of pain crisis and at a follow up visit after resolution of pain crisis (clinicaltrials.gov #NCT01568710). We also studied plasma from two independent reference groups: 7 adults with sickle cell disease in steady state, and 6 healthy African American adults. Samples were TMT-labeled and analyzed by high performance liquid chromatography followed by surface-enhanced laser desorption/ionization time of flight mass spectrometry. Proteins were identified by mass of peptide fragments using Proteome Discovery 2.0beta. We calculated fold change for each protein comparing pain crisis against recovery in paired analyses. In addition, we analyzed quantitative differences between crisis, steady state and healthy groups.

1334±128 proteins were quantified. Cell regulation proteins, metabolic proteins and cell organization proteins accounted for 19.6%,17.5% and 10.4% respectively of the identified proteins. Haptoglobin was significantly lower in plasma from patients with sickle cell disease. We identified changes in the concentrations of proteins involved in immunity, coagulation, and erythrocyte functions. The changes in these proteins, as well as proteins with incompletely defined functions, provide novel insight into the pathogenesis of sickle cell pain crisis.

10.14

URINE PROTEOMIC ANALYSIS IDENTIFIES CERULOPLASMIN AS A BIOMARKER OF CHRONIC KIDNEY DISEASE IN SICKLE CELL DISEASE PATIENTS

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Chronic kidney disease (CKD) is a complication of sickle cell disease (SCD) that is associated with early mortality. We used high resolution mass spectrometry to analyze urine samples collected before the onset or in the early stage of kidney disease. Urine from patients with late stages of CKD contains large quantities of plasma proteins that overwhelm and complicate mass-spectrometry analysis. As hemoglobinuria is associated with CKD progression in SCD patients and glomerular hyperfiltration is an early stage of renal dysfunction, we compared urine samples from patients with normal GFR and hemoglobinuria (n=2), with glomerular hyperfiltration but not hemoglobinuria (n=12) and with normal GFR but not hemoglobinuria (n=6) to identify biomarkers of the early stages of CKD. Label-free quantitative proteomic analysis showed greater ceruloplasmin (CP) levels in the samples with hemoglobinuria. To test whether urine ceruloplasmin and other proteins of iron metabolism correlate with CKD stage, we measured urine ceruloplasmin, transferrin (TF), ferritin (FT) and free hemoglobin (Hb) concentrations by

ELISA in these patients plus 34 additional SCD patients with CKD stage ranging from 0 to 5 and in 19 healthy individuals. The urinary levels of CP, TF, FT and Hb were all significantly higher in all tested SCD patients comparing to healthy controls. CP concentrations demonstrated a strong correlation with urinary Hb, and both CP and Hb concentrations correlated with CKD disease stage and showed high sensitivity and specificity by ROC analysis. Abnormal renal iron metabolism including cortical iron deposition is characteristic of SCD nephropathy. CP facilitates cellular iron export by ferroportin and iron binding by TF. While TF-bound iron is reabsorbed in renal tubules in healthy individuals, increased urinary TF is found in type 2 diabetes patients along with increased urinary CP. Increased urinary CP may reflect increased intra-glomerular hydraulic pressure. While we found significantly increased urinal TF levels in SCD patients, TF did not correlate with CKD stage. In conclusion, urinary CP may represent a non-invasive biomarker for CKD in SCD patients.

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10.15

EFFECTS OF SICKLE CELL DISEASE ON THE RIGHT VENTRICLE AND PULMONARY VASCULATURE

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Sickle cell disease (SCD) red blood cells (RBC) are more rigid than healthy RBCs, are frequently trapped in the microcirculation, and lyse easily, leading to chronic anemia. Recent studies demonstrated that coronary microvascular disease, myocardial fibrosis, decreased right ventricular (RV) ejection fraction, RV dilatation and RV hypertrophy are associated with SCD [1,2]. Patients with SCD-related pulmonary hypertension (PH) have low survival -- 50% within 2 years -- and a substantially increased risk of sudden death compared to those with SCD alone [1]. The goal of this study was to quantify how SCD affects RV function, the interactions between the RV and the pulmonary vasculature, and RV afterload. We hypothesized that SCD mice would have poor RV function, impaired ventricular-vascular coupling (VVC) measured with pressure-volume loops, and increased pulmonary vascular resistance as measured by pulmonary vascular impedance (PVZ) compared to healthy control mice, which would be exacerbated in SCD mice after exposure to acute hypoxia.

Methods: 12 male C57Bl6 mice (CTL) and 12 Berkeley SCD mice (SCD) aged 20-24 weeks were cannulated with a pressure-volume (PV) catheter. After initial RV PV measurements were obtained, the inferior vena cava was briefly occluded to calculate end-systolic and end-diastolic pressure relations. In separate CTL and SCD mice, a pressure catheter was advanced into the main pulmonary artery (PA) while echocardiography simultaneously measured PA flow velocity and PA diameter for PVZ. Measurements were obtained under normoxic ventilation (21% oxygen) and, subsequently, after 5 minutes of acutely hypoxic ventilation (10% oxygen) in both studies. It is worth noting that 10 of 12 SCD mice did not survive hypoxia, while all CTL animals survived.

Results and Discussion: Cardiovascular hemodynamics in SCD mice were not significantly different from CTL mice under baseline conditions, however pulmonary vascular resistance and RV volume tended to be elevated in SCD mice and VVC tended to be lower. Despite apparently normal ventricular and vascular function absent of development of PH, SCD mice could not tolerate the insult of acute hypoxia.

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10.16

URINARY OROSCOMUCOID CONCENTRATION CORRELATES WITH CHRONIC KIDNEY DISEASE IN SICKLE CELL DISEASE PATIENTS

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Chronic kidney disease (CKD) is a prevalent complication of sickle cell disease (SCD) and associated with early mortality. Discovery and validation of non-invasive biomarkers for early stage renal disease is needed identify and facilitate CKD treatment in SCD. Here, we performed comparative proteomic analysis of urine samples collected from SCD patients with hemoglobinurea (N=2), hyperfiltration (N=3) and normal (N=3). We observed upregulation of orosomucoid in samples with hemoglobinurea versus control (49.93-fold, $p=1.9x10^{-10}$).

We next validated presence of oroscomucoid in urine by ELISA and also expanded the analysis to additional SCD patients with CKD stage ranging from 0 to 5 and in 19 healthy individuals. The urinary level of oroscomucoid was significantly higher in all tested SCD patients comparing to healthy controls. Oroscomucoid concentrations correlated with CKD disease stage and showed high sensitivity and specificity by ROC analysis. Moreover, oroscomucoid concentrations showed strong correlation with urinary free hemoglobin concentrations (R=0.45), an established marker of CKD in SCD. Oroscomucoid concentations also correlated well with ceruloplasmin (R=0.62) that we recently identified as a potential biomarker of CKD in SCD. Oroscomucoid is involved in inflammation, and its increased levels were found in urine of type 2 diabetes patients. Orosomucoid is also an independent factor for diabetic microvascular complication. Microvascular complications and vaso-occlusive crisis are hallmarks of SCD. Taken together, urinary oroscomucoid may represent a non-invasive biomarker for CKD in SCD patients.

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10.17

HEME AND FREE IRON- MEDIATED OXIDATION OF PLASMA LIPIDS IN SICKLE CELL DISEASE PATIENTS UNDERGOING REGULAR EXCHANGE BLOOD TRANSFUSION

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Background: Red blood cells of SCD patients are prone to sickling, hemolysis and heme release. Increased plasma heme is linked to vasculopathy. The intercalation of heme into plasma lipoproteins promotes LDL oxidation in vitro and may define heme-to-lipid transfer in plasma. Transient free iron is also common in regularly transfused patients. Together heme and intermittent iron exposures may be relevant contributors to vascular dysfunction in transfused SCD patients.

Objectives: (1) characterize heme, iron, cholesterol, LDL, HDL and endothelial dysfunction markers in human plasma; (2) quantify levels of lipid peroxidation in purified HDL and LDL; (3) define relationships between lipid peroxidation to heme and iron levels; (4) evaluate histological markers in vascular tissues.

Methods: Plasma samples obtained from SCD patients undergoing regular exchange transfusion and healthy controls were evaluated for heme, iron, lipid levels, and endothelial dysfunction markers. Extraction of HDL and LDL from plasma was performed by density gradient centrifugation. In purified HDL and LDL, oxidized lipids were measured by MDA and Western blotting. Vascular tissue was evaluated for histopathological markers of injury.

Results: SCD plasma showed low cholesterol, HDL and LDL levels as well as an increase in heme, iron and markers of endothelial dysfunction. MDA was increased in purified LDL and HDL form SCD patients relative to control. Western blotting revealed bands of oxLDL. MDA found in purified LDL and HDL correlated with plasma heme and free iron concentrations. Individual SCD patients demonstrated a similar extent of oxidation in both LDL and HDL. Purified control LDL and HDL spiked with different heme-albumin concentrations resulted in a dose dependent increase in MDA development. Histological markers demonstrated evidence of injury in vascular tissue.

Conclusions: Chronic hemolysis and release of heme into the circulation triggers oxidation of lipoproteins. The oxidation of LDL and HDL is thereby dependent on heme exposure. In regular exchange transfused patients increased free iron levels may further enhance lipid oxidation. These findings may have implications in SCD progression.

Abbreviations: Sickle cell disease (SCD), Low density lipoprotein (LDL), High density lipoprotein (HDL), Malondialdehyde (MDA), Oxidized LDL (oxLDL)

11.0 Poster Session: Lung Physiology/Pathophysiology

11.1

EXPOSURE TO MODERATE ALTITUDE ENHANCES PULMONARY VASCULAR DISEASE IN BERKLEY SICKLE CELL MICE

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A number of sickle cell disease (SCD) patients live at moderate altitude with a mean elevation of 5,900 ft. However, it is unclear if lower barometric pressure affects the disease process. Herein we investigated the impact of disease progression in wild type and Berkley sickle cell mice (BERK-SS) over 2.5 months of altitude exposure associated with sea level, Denver (5280 ft) and moderate (8,000 ft) elevations to assess cardiopulmonary dysfunction. Study end points for hemolysis, pulmonary hypertension, right heart function and pulmonary vascular remodeling were assessed. The adaptive balance between pulmonary vascular endothelial nitric oxide synthase (eNOS) and endothelin (ET-1) was studied as to assess differences in lung vasculature adaptation. Mortality of animals was assessed throughout the study. We hypothesized that BERK-SS mice would demonstrate significant deterioration in the defined parameters of morbidity and mortality when compared to WT mice and to BERK-SS exposed to differing altitudes. As expected BERK-SS mice were significantly different from WT mice in all parameters tested, supporting our hypothesis differences within the BERK-SS cohort demonstrated changes associated with increasing altitude. The primary changes observed were increased pulmonary hypertension and evidence of right heart failure in BERK-SS mice exposed to 8,000 ft. consistent with this were differences in the adaptive response to eNOS/ET-1 balance observed in WT mice. We conclude that exposure to moderate and physiologically relevant altitude enhances the progression of pulmonary hypertension in BERK-SS mice compared to healthy wild type cohorts.

11.2

SCOPING REVIEW OF THE LITERATURE ON SICKLE CELL LUNG DISEASE ACROSS THE LIFESPAN

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Background: Despite a high and growing global burden of sickle cell disease (SCD) with an estimated 400,000 births per year by 2050, evidence-based interventions against its pulmonary complications are limited. Pulmonary complications account for much of the accelerated mortality observed in the SCD population, yet we know little about their natural history. While practically every structure and cell type of the lung is affected in SCD patients, the inter-relationship between the airways and vasculature in this population is not well described. One of the limitations in SCD research has been a focus on either the adult or pediatric patient group without recognition of the early life origins of cardiopulmonary morbidity and mortality in adulthood. We know that patients with SCD are at significantly increased risk of lung function abnormalities, respiratory symptoms with progressive dyspnea, and sleep disordered breathing, yet we do not understand how each of these phenomena is associated with overall disease pathogenesis and the increased risk of early mortality. Furthermore, the roles that chronic hemolytic anemia, left-sided cardiac dysfunction, intermittent hypoxia, and thrombosis play in modulating the underlying SCD process are not well understood. Therefore, we undertook a scoping review of the literature with systematic search criteria in order to assess the published literature.

Methods: A systematic literature search was performed in four main areas of sickle cell lung disease: 1) Acute chest syndrome 2) Airways disease 3) Sleep-disordered breathing and hypoxia 4) Pulmonary vascular disease and thromboembolic disease. Common search parameters used for each topic included the following: human subjects research in both children and adults in the PubMed and Cochrane Library databases from 1970 to April 20, 2017. In addition, search terms specific to each of the 4 areas of sickle cell lung disease were used to conduct the search strategy.

Results: A total of 448 articles met the initial search parameters (acute chest [230], pulmonary vascular disease and thromboembolic disease [102], airways disease [78], and sleep-disordered breathing and nocturnal hypoxia [38]).

Conclusions: In conclusion, the number of published articles on 4 specific topics of pulmonary sickle cell disease remains low. More high-quality, multicenter studies are needed in order to evaluate clinical outcomes and treatment interventions in pulmonary sickle cell disease.

12.0 Poster Session: Red Cell Physiology

12.1

HOW DOES IRON DEFICIENCY REDUCE INTRACELLULAR HEMOGLOBIN IN MICE?

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Sickle cell disease (SCD) involves polymerization of hemoglobin-S within the red blood cell (RBC). Cells with a lower mean cell hemoglobin concentration (MCHC) of hemoglobin-S exhibit longer delays between deoxygenation and polymerization.

Sickling delay time is dependent on $[HbS]^{30}$. Iron-deficient erythropoiesis results in RBC with lower MCHC, which could reduce sickling and improve red cell survival. One strategy for lowering MCHC in patients is the induction of iron deficiency anemia. Several clinical case reports and one study in a SCD mouse model suggest that low iron availability is associated with less hemolysis, longer cell survival, and improved symptoms of SCD. However, the mechanisms governing chronic induction of iron deficiency anemia in SCD are not currently well understood.

Data from a four-week pilot study shows iron deficiency anemia induces a reduction in RBC count, hemoglobin, and hematocrit. For this pilot, half of the mice received a single dose of recombinant erythropoietin (EPO, 1000 IU/KG) to accelerate RBC production. EPO effectively stimulated erythropoiesis but did not increase platelets count. At four weeks, MCH and MCV of mature RBCs haven't had changed but reticulocyte hemoglobin content (CHr) was decreased.

To that extent, the purpose of the present study is to characterize the timing of dynamic hematologic changes, that occur within the bone marrow and blood during the onset of iron deficiency anemia, using an iron-restricted diet in 6-week old male and female C57Bl/6J mice over a period of twelve weeks. Blood samples and bone marrow are collected at two-week intervals; blood is processed for CBC and ELISA for serum iron markers, and bone marrow is evaluated by flow cytometry to study erythroblast maturation.

This study will provide a clear picture of the hematologic and physiological changes that occur in the bone marrow and blood during the onset of iron deficiency anemia. This data will help inform a strategy for using iron deficiency anemia to modify MCHC in RBC as a potential treatment for SCD.

12.2

TR4 HAPLOINSUFFICIENCY RESULTS IN DECREASED PROLIFERATION AND MATURATION DURING ERYTHROPOIESIS.

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The orphan nuclear receptors TR4 (NR2C2) and TR2 (NR2C1) are the DNA binding components of the repressor complex, direct repeat erythroid-definitive (DRED), which represses the transcription of ε- and γ- globin during adult definitive erythropoiesis. Previously, several studies have implied that TR2 and TR4 can act largely in a redundant manner during erythroid differentiation; however, our laboratory has observed variably penetrant phenotypes in the *Tr4* mutants, suggesting that indirect effects of the genetic deletion might be masked by multiple modifying genes. In order to test this hypothesis, *Tr4*heterozygous mutantmice were bred into a congenic C57BL/6 background and their phenotypes were reexamined. Surprisingly, the homozygous *Tr4* null mutant mice expired early during embryogenesis, at approximately embryonic day (E) 7.0 well before erythropoiesis commences. Further examination found that $Tr4^{+/-}$erythroid cells failed to fully differentiate and exhibited diminished proliferative capacity and no changes in apoptosis. Furthermore, reduced TR4 abundance resulted in decreased expression of genes required for heme biosynthesis and erythroid differentiation (*Alad* and *Alas2*), but led to significantly increased expression of the proliferation inhibitory gene, cyclin dependent kinase inhibitor 1c, *Cdkn1c*. The cellular differentiation and abundance defects were only observed within the erythroid cell populations. These studies support a vital role for TR4 in promoting erythroid maturation and proliferation, and demonstrate that TR4 and TR2 execute distinct, individual functions during embryogenesis and erythroid differentiation.

12.3

HAPTOGLOBIN GENOTYPE IS ASSOCIATED WITH INCREASED MORBIDITY IN ADULTS WITH SICKLE CELL DISEASE

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Objective: Chronic hemolysis is a defining feature of sickle cell disease (SCD), and intensifies during times of illness. This process leads to the release of cell free hemoglobin, which has been associated with an increased odds of developing acute chest syndrome (ACS) in patients with SCD. Haptoglobin, the major endogenous hemoglobin-binding protein, has two alleles in humans (1 and 2) leading to three possible genotypes: HP1-1, HP1-2, and HP2-2. The affinity of haptoglobin for free hemoglobin is genotype dependent with the HP2-2 protein having the lowest binding affinity and increased propensity for oxidative damage due to cell free hemoglobin. We hypothesize that among adults with SCD, those participants with the HP2-2 genotype will have an increase in occurrence of disease specific complications.

Methods: Haptoglobin genotype was assayed on an adult cohort of participants with SCD, ages 19-55 years, from the Vanderbilt DNA repository (BioVU), which links DNA samples to de-identified medical record data. We developed an ICD9/10 code based algorithm to identify patients. This was refined through repeated sampling and manual review to achieve >95% sensitivity and specificity. All records in the final cohort were reviewed to collect basic demographic information, lab data at baseline health, and incidence of SCD related complications. Recorded complications included vaso-occlusive pain, ACS, stroke, retinopathy, nephropathy, pulmonary hypertension, and priapism.

Results: A total of 58 adults with SCD were included with a mean age of 33.6 years (SD 9.4). Of these 58 participants, 25.9% (N=15) had the HP1-1 genotype, 55.2% (N=32) had the HP1-2 genotype, and 19.0% (N= 11) had the HP2-2 genotype. A total of 90.9% of participants with the HP2-2 genotype had 2 or more complications as opposed to 46.7% of those with the HP1-1 genotype and 56.3% of the HP1-2 genotype. After adjusting for age, sex, SCD genotype, and baseline hemoglobin, bootstrapped logistic regression demonstrated increased odds of having 2 or more complications in those with the HP2-2 genotype as compared to the HP1-1 and HP1-2 genotypes (OR= 8.6, 95% CI= 1.4-52.2, p=0.019).

Conclusion: These findings suggest adults with the HP2-2 genotype are at increased risk for SCD complications. Further research is needed to confirm this finding in a larger, prospective fashion and to determine the role of the haptoglobin genotype and the oxidative effect of cell free hemoglobin in the pathophysiology of SCD.

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13.0 SCD Gene Therapy, Gene Editing and Pharmacological Treatment

13.1

GENE THERAPY FOR HEMOGLOBINOPATHIES: THE CHALLENGE TO FIND A CURE

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Sickle cell disease (SCD) and ß-thalassemia major are congenital anemias caused by mutations in the ß-globin gene, resulting in either abnormal hemoglobin structure or reduced/absent production of ß-globin chains. They are the most common monogenic disorders associated to morbidity and mortality. Treatment of these diseases is essentially supportive, requiring transfusions, iron chelation and use of hydroxycarbamide in SCD. At present, the only curative approach is represented by allogeneic hematopoietic stem cell transplantation, with a probability to find a well-matched donor of <25%.
Ex vivo gene therapy, using autologous genetically modified hematopoietic stem cells, potentially represents a cure applicable to all patients regardless of donor availability and free from transplant related immunological complications such as graft rejection and GVHD. The development and large scale production of clinical grade lentiviral vectors expressing human globins, and the optimization of gene transfer protocols in hematopoietic stem/progenitor cells have progressed this field to the pioneering clinical trials in France and in U.S.A., and more recently in Italy. The first results of clinical benefit, including early engraftment, hemoglobin expression and transfusion independence were reported for some patients and are proving the potential efficacy of this therapeutic approach.
Although these encouraging results, early clinical studies showed the safety and potential efficacy of this therapeutic approach, as well as the hurdles still limiting its general application. These are the nature and source of hematopoietic stem cells, the suboptimal transduction efficiency and gene expression levels, the toxicity and

efficacy of bone marrow conditioning, and the overall cost and complexity of vector and cell manufacturing. In addition, for both beta-thalassemia and sickle-cell disease, an altered bone marrow microenvironment might reduce the efficiency of stem cell harvesting as well as engraftment.
Our contribution to this field in the last 10 years was devoted to the clinical development of a safe gene therapy approach for ß-thalassemia using the GLOBE lentiviral vector GLOBE. The crucial steps leading to the recent start of TIGET BTHAL clinical trial (NCT02453477), as well as preliminary data on treated patients, will be presented.

13.2

COMBINED HYDROXYUREA AND ET_A RECEPTOR BLOCKADE REDUCES RENAL INJURY IN THE HUMANIZED SICKLE CELL MOUSE

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Sickle cell disease (SCD) is a monogenetic hemoglobinopathy associated with an increasing incidence of kidney disease, for which the underlying etiology has yet to be elucidated. Recent data from our lab and others have shown that the vasoactive peptide endothelin-1 (ET-1) plays a critical role in the pathophysiology of sickle cell nephropathy (SCN) and that blockade of its ET_A receptor improves renal function and protects against renal injury. Hydroxyurea (HU), the only FDA approved therapy for patients with SCD, is commonly prescribed for the treatment of SCN due to its ability to increase fetal hemoglobin levels. Therefore, we hypothesized that combined ambrisentan (ET_A selective antagonist) and HU treatment has a synergistic effect on renal injury in SCN when compared to HU treatment alone. Male 12 week old humanized sickle mice (HbSS) and their genetic controls (HbAA) were treated with vehicle, HU (50mg/kg/day), ambrisentan (10mg/kg/day), or HU plus ambrisentan. After 2 weeks of treatment, mice were placed in metabolic cages to assess renal function and then euthanized for blood and tissue collection. Vehicle treated HbSS mice exhibited significant proteinuria compared to vehicle treated HbAA mice (3.4 ± 0.4 vs 2.1 ± 0.2 mg/day, respectively, p=0.03). HbSS mice also displayed elevated plasma ET-1 concentration (1.09 ± 0.08 vs. 0.49 ± 0.01 pg/ml, p=0.04) and decreased urine osmolality (1225 ± 67 vs. 1966 ± 46 mOsmol/kg, p=0.008) compared to HbAA controls. Proteinuria was significantly attenuated in the HU treated animals compared to vehicle treated HbSS mice (2.1 ± 0.3 vs. 3.4 ± 0.4, p<0.05); however, there was no additional improvement in HbSS mice treated with combined ambrisentan and HU. Ambrisentan also produced a similar decrease in proteinuria (1.9 ± 0.2 vs. 3.4 ± 0.4 mg/day, p=0.02). HU alone also reduced nephrinuria (4.3 ± 0.7 vs. 12.3 ± 1.0 ng/ml, p<0.0001) and albuminuria (13.4 ± 1.9 vs. 25.4 ± 4.4 µg/day, p=0.01) similar to what we have previously reported for ambrisentan. However, KIM-1 excretion, a marker of tubular injury, was not attenuated with HU treatment alone. The absence of further attenuation of renal injury with combined treatment suggests that the mechanism of action for both treatments may converge on the same mechanistic pathway.

13.3

NRF2 GENE KNOCKOUT EXACERBATES TISSUE PATHOPHYSIOLOGY IN THE SICKLE CELL DISEASE TRANSGENIC MOUSE MODEL

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Abstract

The basic leucine zipper transcription factor, nuclear factor (erythroid-derived 2)-like 2 (NRF2) plays a critical role in the cellular antioxidant response to control oxidative stress levels. We and others previously demonstrated that NRF2 activation enhances γ-globin gene and fetal hemoglobin expression in human primary erythroid progenitors. Herein we show the role of NRF2 function in the pathophysiology of sickle cell disease (SCD) in a novel Townes SCD mouse/NRF2 knockout (SCD/NRF2-KO) transgenic model. Loss of NRF2 function reduced γ-globin gene expression during erythroid differentiation from the E13.5 and E18.5 fetal liver to adult spleen and bone marrow stages of hematopoiesis. In peripheral red blood cells, the level of reactive oxygen species was increased 33% (p<0.05) and under in vitro hypoxic conditions, the level of sickling was significantly increased by 38%. We next characterized the effect of NRF2 knockout on organ pathophysiology. For the SCD/NRF2-KO mouse, by 8-10 weeks of age, we observed greater splenomegaly and significant inflammation in spleen, lung and liver tissue when compared to SCD/NRF2 wild-type mice. Protein expression profiling by western blotting using adult spleen whole protein extracts, demonstrated downregulation of the antioxidant proteins heme oxygenase 1 (HMOX1), NADPH: quinone oxidoreductase 1 (NQO1) and catalase by 31%, 60%, and 48% respectively. The expression of cellular adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) were significantly increased by 1.7-fold and 2.3-fold (p<0.05) while the expression of vascular endothelial growth factor (VEGF) was not changed obviously. In addition, the expression levels of pro-inflammatory molecules interleukin 1β (IL-1β), IL-6, tumor necrosis

factor α (TNF-α), monocyte chemoattractant protein (MCP-1) and macrophage migration inhibitory factor (MIF-1) were elevated in SCD/NRF2-KO mice. These data corroborate a critical role of NRF2 in protecting against the pathophysiology of SCD including red blood cell sickling/oxidative stress and tissue inflammation. Furthermore, the ability of NRF2 to mediate fetal hemoglobin induction provides a rationale for the development of therapeutic agents that activate NRF2 expression. This work was supported by funding from the National Heart, Lung, and Blood Institute to XZ through the Hemoglobinopathy Translational Research Skills Core component of U01 grant HL117684 and R01 grant HL069234 to BSP.

13.4

DISCOVERY OF PHARMACOLOGIC FETAL HEMOGLOBIN INDUCING AGENTS FOR TREATMENT OF SICKLE CELL DISEASE

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During adult development, production of fetal hemoglobin (HbF) ameliorates the clinical severity of sickle cell disease (SCD) by inhibiting hemoglobin S polymerization. To develop new drugs that induce HbF in erythroid cells, we tested the heme precursor δ-aminolevulinate (ALA). We demonstrated the ability of 2 mM ALA to activate HbF synthesis by 23-fold ($p<0.05$) in KU812 erythroid cells. Subsequent studies in primary erythroid progenitors confirmed γ-globin activation and robust HbF induction whereas β-globin gene transcription was not altered. Our lab previously demonstrated the ability of sodium butyrate (NaB) to induce HbF via the p38 MAPK signaling and CREB binding to the Gγ-globin cyclic AMP response element. Although butyrate induces HbF in adults with SCD by intravenous administration, oral dosing was ineffective. Therefore to address this barrier, we investigated oral acyloxyalkyl (AN233) or butyrylalkyl (AN908) ester prodrugs of ALA and butyric acid. These prodrugs are activated through intracellular esterase-dependent hydrolysis and oral dosing in anemic Balb-c mice increases total hemoglobin and reticulocyte count. Treatment of K562 cells with AN233 and AN908 (0.25 mM) produced up a 1.5-fold increase in γ-globin mRNA and induced HbF synthesis 10-fold ($p=0.029$). By flow cytometry analysis, the %HbF positive cells increased from 45% to 71% and mean fluorescence intensity from 15% to 25%. Studies in normal and sickle primary erythroid progenitors are in progress to confirm the ability of both prodrugs to induce HbF and the effects on intracellular heme levels, oxidative stress and red blood cell sickling. Support: NIH RO1 HL069234. References: Sangerman J, Lee MS, Yao X, Oteng E, Hsiao CH, Li W, Zein S, Ofori-Acquah SF, Pace BS (2006). Mechanism for fetal hemoglobin induction by histone deacetylase inhibitors involves gamma-globin activation by CREB1 and ATF-2. Blood 108:3590-9. Liu L, Karmakar S, Dhar R, Mahajan M, Choudhury A, Weissman S, Pace BS (2013). Regulation of Gγ-globin expression by ATF2 and its associated proteins through the upstream cAMP-response element. PlosOne, 8(11): e78253.

14.0 Cell Therapy

14.1

CONTROL OF HBF SILENCING: IMPLICATION FOR GENETIC AND PHARMACOLOGIC INDUCTION OF HBF FOR THERAPY

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A premise underlying our work has been that an improved understanding of the mechanisms involved in silencing of HbF will provide new avenues for induction of HbF at the adult stage for therapy of the major hemoglobinopathies, beta-thalassemia and sickle cell disease (SCD). Over the past several years we have shown that BCL11A serves as a major quantitative regulator of HbF and collaborated with T. Maeda to identify LRF as a second potent repressor. Both proteins function in concert with the NuRD complex. At this time, these componets (BCL11A, LRF, and NuRD) constitute the central factors for HbF silencing. Our hypothesis is that direct targeting of these components, either genetically or through pharmacological means, offers the best hope of effective HbF reactivation. Through near-saturating CRISPR/Cas9 mutagenesis of the erythroid enhancer of BCL11A, we have identified a remarkably discrete region (of < 20 bp) that provides the major activity of the entire 12kb enhancer. Disruption of this vulnerable region by a single cleavage and non-homologous end-joining markedly impairs BCL11A expression, but only in erythroid lineage cells, and represents an attractive therapeutic gene editing target that is actively being investigated. As a genetic therapy will by nature be “low throughput” due to reliance on bone marrow transplantation, a pharmacological approach is greatly needed to meet the clinical need. To achieve this end, we are focusing on BCL11A as a therapeutic target for development of small molecules.

14.2

GUT MICROBIOME ANALYSIS REVEALS MAJOR DYSBIOSIS IN SICKLE CELL DISEASE PATIENTS WITH A PREVALENCE OF VEILLONELLA STRAINS

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Gut microbiome analysis reveals major dysbiosis in Sickle Cell Disease patients with a prevalence of *Veillonella* strains

Background: Sickle cell disease (SCD) is an inherited blood disorder that occurs primarily in patients of African descent and generally associates with frequent pain crises. It has been suggested that the gut microbiome structure and function may have a major impact on host health. Currently, microbiological studies are typically based on cultivable bacteria. Here we used high throughput sequencing technologies to explore the gut microbiome specifics in SCD patients.

Aim: To characterize the gut microbiome in patients with sickle cell disease.

Materials & Methods: Stool samples from 14 controls and 14 SCD patients were used for DNA extraction. Among the SCD patients, 7 had mild pain crises (< 3 hospitalizations/year) while 7 had severe pain crises (≥ 3 hospitalizations/year). The 16S rRNA gene V4 variable region was PCR amplified, purified using calibrated Ampure XP beads and used to prepare illumina DNA library. Sequencing was performed on a MiSeq following the manufacturer's guidelines. Sequences were joined, and depleted of barcodes. Sequences less than 150bp or ambiguous base calls were then removed. OTUs clustering was performed after the sequences were denoised, and chimeras removed. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). The final OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI. A LeFSe analysis was used to determine differential bacteria.

Results: A major dysbiosis was noticed in the SCD gut microbiome. Several bacterial groups have been depleted from the SCD patients when compared with controls. The SCD gut microbiome has been defined by the prevalence of *Bifidobacteria , Campylobacter , Veillonella , Actinomyces , Scardovia* and *Atopobium* . A major shift towards anaerobic bacteria was noted. The analysis among the two SCD groups of revealed a higher prevalence of *Veillonella* and O*xalobacter* species among SCD patients with severe pain crises.

Conclusion: We report a major dysbiosis in SCD patients' microbiota that seems to be driven by local acidosis and hypercapnia that are prevalent condition in these patients. *Veillonella*, a normal oral and colon inhabitant, is known for its ability to form biofilms and as a facilitator of Streptococcus strains pathogenesis. Its high prevalence in SCD patients might exacerbate pain crises primarily due to blood vessels occlusion as a consequence of sickle shaped blood cells. Indeed, *Veillonella* biofilms might block blood vessels as well and increase *Streptococcus* strains virulence.

14.3

THE THERMODYNAMIC HYPOTHESIS OF SICKLE CELL DISEASE PATHOPHYSIOLOGY

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Since 1979 we have studied the biophysics and cell biology of the intracellular

polymerization of sickle hemoglobin to better understand the pathophysiology of this disease. Using then new solid-state NMR methods, with Dennis Torchia, we found to our surprise that polymerization is detectable almost to full oxygen saturation of sickle cells and that these results were explicable due to the non-ideal behavior of hemoglobin at red cell concentrations, as modeled by Allen Minton. On the basis of these and many related studies we developed a model of pathophysiology based on the approximate amounts of polymer in red cells in various tissues, as determined primarily by oxygen saturation and intracellular hemoglobin concentration and composition. Polymer amounts are likely close to equilibrium conditions since growth and melting are both very rapid as oxygen levels change in the body. We expect that most impairment of blood flow occurs in the pre-capillary arterioles where resistance to flow is maximal. Variations in the severity of the sickle syndromes and responses to therapies, such as hydroxyurea to elevate fetal hemoglobin, can be quantitatively accounted for by this model.

"Polymerization tendency" can be calculated based on average properties of sickle red cell populations and corresponding hemoglobin solubility at a any given oxygen saturation. Further refinement of this model can be accomplished by detailed data on heterogeneities of intracellular red cell hemoglobin compositions and concentrations but are unlikely to change the overall results greatly. Studies of sickle red cells in various blood vessels as oxygen levels and other physiological parameters vary are equally important in better understanding the relative roles of intracellular polymerization and other factors, such as hemolysis and adhesive interactions, in overall pathophysiology.

15.0 Small Molecules to Treat SCD

15.2

ORAL TETRAHYDROURIDINE AND DECITABINE FOR NON-CYTOTOXIC EPIGENETIC GENE REGULATION IN SICKLE CELL
DISEASE: A RANDOMIZED PHASE 1 STUDY

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Background: Sickle cell disease (SCD) is driven by polymerization of mutated sickle hemoglobin (HbS) in red blood cells (RBC). Fetal hemoglobin (HbF) interferes with this polymerization, but HbF is epigenetically silenced from infancy onward by DNA methyltransferase (DNMT1).

Methods and Findings: To pharmacologically re-induce HbF by DNMT1 inhibition, this first-in-human clinical trial (NCT01685515) combined two small molecules, decitabine to deplete DNMT1, and tetrahydrouridine (THU) to inhibit cytidine
deaminase (CDA), the enzyme that otherwise rapidly deaminates/inactivates decitabine, severely limiting its half-life, tissue distribution, and oral bioavailability. Oral decitabine doses, administered after oral THU 10 mg/kg, were escalated from a very low starting level (0.01, 0.02, 0.04, 0.08 or 0.16 mg/kg), to identify minimal doses active in depleting DNMT1 without cytotoxicity. Patients were SCD adults at risk of early death despite standard-of-care, randomized 3:2 to THU-decitabine versus placebo in 5 cohorts of 5 patients treated 2X/week for 8 weeks, with 4 weeks of follow-up. Adverse events were not significantly different in THU-decitabine- versus placebo-treated patients. At the decitabine 0.16 mg/kg dose, plasma concentrations peaked at ~50 nM (Cmax) and remained elevated for several hours. This dose decreased DNMT1 protein in peripheral blood mononuclear cells by >75% and repetitive element CpG methylation by ~10%, and increased HbF by 4-9% ($p<0.001$), doubling HbF-enriched RBC (Fcells) up to ~80% of total RBCs. Total hemoglobin increased by 1.2-1.9 g/dL ($p=0.01$) as reticulocytes simultaneously decreased; that is, better quality and efficiency of HbF-enriched erythropoiesis elevated hemoglobin using fewer reticulocytes. Also indicating better RBC quality, biomarkers of hemolysis, thrombophilia and inflammation (LDH, bilirubin, D-dimer, CRP) improved. As expected with non-cytotoxic DNMT1-depletion, platelets increased and neutrophils concurrently decreased, but not to an extent requiring treatment holds. As an early phase study, limitations include small patient numbers at each dose level and narrow capacity to evaluate clinical benefits.
Conclusion: Administration of oral THU-decitabine to patients with SCD was safe and by targeting DNMT1 upregulated HbF in
RBC. Further studies are warranted.

18.0 Renal and Vascular Physiology

18.2
RESISTANCE ARTERIES OF HUMANIZED SICKLE CELL DISEASE MICE DISPLAY SIMILAR SENSITIVITY TO A_1-ADRENERGIC AND ENDOTHELIN-1 VASOCONSTRICTION

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Sickle cell disease (SCD) patients at baseline exhibit lower diastolic blood pressure, decreased systemic vascular resistance, and reduced pulse wave velocity compared to controls. In contrast to these cardiovascular alterations in patients, investigation of the vascular reactivity of isolated aorta from SCD mice has demonstrated markedly enhanced α_1-adrenergic vasoconstriction and has contributed to the hypothesis that enhanced sensitivity to vasoconstrictors plays a role in vaso-occlusive processes in SCD. The aim of this study was to examine vasoconstrictor sensitivity in resistance arteries of SCD mice, as this is directly related to systemic vascular resistance and diastolic blood pressure and has greater relevance to vaso-occlusion in SCD. Humanized SCD mice (HbSS) and humanized hemoglobin A control mice (HbAA) were utilized for all experiments. Vascular reactivity to phenylephrine (PE), endothelin-1 (ET-1), and potassium chloride (KCl) was examined in resistance mesenteric arteries (100-150μm diameter) as well as aortic reactivity to PE and KCl. Resistance mesenteric arteries from HbSS mice displayed similar EC50 (-5.81 ± 0.12 vs. -5.80 ± 0.07 log[PE, M], $p>0.05$) and Emax (133.9 ± 13.4 vs. 118.3 ± 6.7 %KCl, $p>0.05$) in response to PE compared to HbAA mice. In contrast and consistent with previous findings, aorta from HbSS mice displayed lower EC50 (-6.22 ± 0.14 vs. -6.90 ± 0.04 log[PE, M], $p=0.002$) and elevated Emax (118.7 ± 3.2 vs. 155.9 ± 5.2 %KCl, $p<0.001$) in response to PE compared to HbAA mice. ET-1 is also an important vasoconstrictor in resistance arteries and enhanced ET-1 signaling has been implicated in the pathophysiology of SCD. In response to ET-1, resistance mesenteric arteries from HbSS mice displayed similar EC50 (-7.85 ± 0.07 vs. -7.82 ± 0.05 log[ET-1, M], $p>0.05$) and Emax (104.2 ± 10.2 vs. 90.9 ± 16.5 %KCl, $p>0.05$) compared to HbAA mice. Graded concentration responses to KCl were similar

between genotypes in both resistance and conduit arteries. These data suggest regional differences in arterial sensitivity to vasoconstriction, with enhanced α_1-adrenergic vasoconstriction isolated to the aorta. Additionally, HbAA and HbSS mice have similar sensitivity to multiple vasoconstrictors in resistance arteries, suggesting that enhanced vasoconstriction may not participate in vaso-occlusion and resultant tissue hypoxia in SCD.

Acknowledgment

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18.3

IMPAIRED POST-ISCHEMIC NEOVASCULARIZATION IN SICKLE CELL DISEASE

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Aims: The appropriate physiologic response to vascular insult is the development of functional collateral vessels to preserve organ function. To that end, first responder immune cells (neutrophils, macrophages) must be tightly recruited to the site of injury, promote repair and resolve inflammation to maintain vascular integrity. Repeat ischemia is the hallmark of sickle cell disease (SCD), leading to many clinical complications such as stroke, pain crises, proliferative retinopathy and sudden cardiac death. However the fundamental response to vascular injury in SCD is unknown, and the roles of first responder immune cells are poorly understood.

Methods: We used the hind limb ischemia (HLI) model to determine collateral vessel formation in the humanized Townes sickle cell mice (SS) in comparison to wildtype (AA). Perfusion recovery was measured weekly using LASER Doppler perfusion imaging (LDPI). The voluntary running wheel test was used to measure motor function recovery. Flow cytometry and immunostaining were used to characterize phenotype of neutrophils and macrophages after HLI. Anti-Ly6G antibody was used to deplete neutrophils *in vivo*. Resolvin D1 was encapsulated in liposome and delivered weekly after HLI.

Results: Collateral vessel formation was significantly impaired in SS; by day 28, AA mice showed 76 ± 13% perfusion recovery, compared to 34 ± 10% in the SS mice, $p < 0.001$, n=8 per group). Spontaneous motor recovery was significantly impaired in SS mice after HLI (98% in AA, vs 36% in SS mice. $p < 0.001$). Whereas all neutrophils were cleared in AA mice by day 5, SS mice demonstrated persistent neutrophils that remained in the ischemic hind limb for weeks. SS neutrophils were highly inflammatory and produced a 2.45 fold increase in hydrogen peroxide. Importantly, *in vivo* depletion of neutrophils reduced oxidative stress and improved collateral vessel formation in SS mice. Administration of resolvin D1, which promotes neutrophil clearance and resolution of inflammation, also significantly improved collateral vessel formation in SS mice after HLI.

Conclusions: Our data identify impaired post-ischemic neovascularization as an underlying etiology of the numerous vascular complications in SCD, driven largely by maladaptive inflammation and oxidative stress. Our results also provide potential therapeutic targets to improve vascular function after ischemic injury in SCD.

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18.4

ROLE OF MACROPHAGE STIMULATING PROTEIN 1 (MSP1) IN THE DEVELOPMENT OF ENDOTHELIAL INJURY IN SICKLE CELL DISEASE

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Patients with Sickle Cell Disease (SCD) have an approximately three-fold higher risk of developing chronic kidney disease (CKD) compared to the general population. However, the current standards of diagnosis and care do not prevent and simply delay progression of this disease. The majority of renal complications in SCD are believed to result from vasculopathy and endothelial injury. Hemolysis inside the renal tissue stimulates macrophages infiltration and endocytosis of the products of hemolysed red blood cells (RBCs) by those infiltrating macrophages leads to MT-SP1 protease activation. Macrophage Stimulating Protein 1 (MSP1) is a circulating non-active substrate of MT-SP1.

The objective of this project was to demonstrate a role of MSP1 in the development of renal endothelial injury.

Human renal glomerular endothelial cells (HGEC) and a mouse model of SCD (Townes) were used in this investigation. The animal protocol was approved by the Institutional Animal Care and Use Committee at the Children's National Health System. Townes sickling mice express human Hgb S and Hgb F and control mice express

human Hgb A1. Renal glomerular permeability was measured by a determination of albumin permeability.

MSP1 treatment induced RON receptor activation and downstream signaling evidenced by increased phosphorylation of ERK and AKT kinases. MSP1 also increased motility of HGEC. RON inhibitor (RONi) significantly reduced RON activation and ERK and AKT phosphorylation. We demonstrated a significant accumulation of MSP1 in the glomeruli of SCD mice. Glomerular capillary enlargement and endothelial injury was characterized by PAS staining and immunostaining of ICAM, vWF and CD34. Endothelial injury was significantly increased in SCD mice compared to controls. MSP1 increased glomerular permeability in the mouse whole glomeruli assay. RONi prevented the induction of renal glomerular permeability by MSP1. Treatment of mice with RONi (s.c. daily injections for 14 days) significantly reduced renal endothelial injury in young SCD mice. In conclusion, renal glomerular accumulation of MSP1 is one of the factors involved in the induction of renal endothelial injury in SCD mice. Inhibition of MSP1 receptor RON kinase activation significantly reduced endothelial injury and development of kidney disease.

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18.5

IRON ACCUMULATION IN THE KIDNEY – POTENTIAL NEW ROLE FOR ENDOTHELIN SYSTEM

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Elevated endothelin-1 (ET-1) levels reported in sickle cell disease are associated with microalbuminuria and renal iron deposition early in sickle nephropathy. In humanized sickle cell mice (HbSS), long-term ET_A receptor antagonism provides robust protection from diverse renal pathologies, including significant attenuation of renal tubular iron deposition in the proximal tubules. These observations led us to hypothesize that ET-1 regulates renal iron trafficking in iron overload-associated sickle nephropathy. We first determined the effect of ET-1 on the expression of iron trafficking mediators in mouse primary proximal tubular epithelial (PT) cells. Expression of the iron importer transferrin receptor 1 (TfR-1) and the iron storage protein, H-ferritin, were increased in a concentration-dependent manner by ET-1. The ET-1-induced decrease in the iron exporter ferroportin-1, FPN-1 (65% reduction), was associated with a doubling in expression of hepcidin, a key regulator of FPN-1 and iron removal from the cell. In keeping with these observations, cellular iron uptake in response to ET-1 was significantly increased. Addition of plasma from HbSS mice to PT cells increased cellular iron uptake compared to plasma from control HbAA mice (0.098±0.017 vs. 0.004±0.001 ng/µl). Pre-incubation with the selective ET_A receptor antagonist, BQ123, completely prevented ET-1 induced alterations in all iron import, storage and export mediators, suggesting a likely involvement of the ET_A receptor in iron trafficking mechanisms. Interestingly, neither selective ET_B nor combined (ET_A+ET_B) receptor antagonism affected ET-1-induced changes in iron trafficking mediators, supporting the hypothesis that the ET_A receptor mediates ET-1-dependent changes in renal iron handling. Simultaneously, *in vivo* studies showed increased expression of TfR-1, H-ferritin and decreased expression of FPN-1 in cortical tissues of HbSS mice. These effects were prevented by long-term treatment with a selective ET_A receptor antagonist. Overall, these results reveal a novel role for ET-1 in proximal tubule iron trafficking and provide rational for the use of selective ET_A receptor blockade as a potential therapeutic approach in iron overload disorders, specifically sickle cell disease.

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19.0 Lung Physiology and Pathophysiology

19.2

DIFFERENTIALLY EXPRESSED LNCRNAS IN LUNGS AND PLASMA EXOSOMES OF SICKLE CELL MICE

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Rationale: Sickle cell disease (SCD)-associated pulmonary hypertension (PH) causes significant morbidity and mortality. We recently demonstrated that Townes humanized sickle cell (SS) mice spontaneously develop PH and vascular remodeling. Emerging evidence indicates that long non-coding RNAs (lncRNAs), transcripts longer than 200 nucleotides, play a pivotal role in cellular proliferation, differentiation, and apoptosis. Exosomes, nano-sized extracellular vesicles contain lncRNAs and play an important role in intercellular communication. However, the role of lncRNAs released from lungs and plasma exosomes in SCD-PH pathogenesis has not been defined. To further examine the pathogenesis of SCD-PH, we

hypothesized that lncRNAs are differentially expressed in SS mouse lungs and plasma exosomes.

Methods: lncRNAs were isolated from lungs and plasma exosomes of SS mice and littermate control (AA) mice at age 15-17 weeks. Isolated plasma exosomes were characterized for levels of exosomal markers (CD9, CD45, and CD63), and exosomes and lung samples were subjected to measurements of the expression profiles of 90 lncRNAs, 5 housekeeping genes, and one negative control using the LncProfiler™ lncRNA qPCR array (System Biosciences).

Results: We found that the levels of four novel lncRNAs (GAS5, Foxn2-as, AK007836-upstream of PCNA, and Adapt33) were increased in the lungs and plasma exosomes of SS mice compared to AA mice. The fold changes ranged from 1.3 to 4.5. Nine lncRNAs (Rmst, Vax2os1, Zeb2NAT, Neat1 v1/MEN epsilon, Six3os-clone9, Kcnq1ot1, lincENC1, linc1242 LINC-Enah, and linc1368) were decreased in both the lungs and plasma exosomes of SS mice.

Conclusions: Collectively, these studies establish that lncRNAs are differentially expressed in SS mice. Further characterization of these lncRNAS may determine new mechanisms for SS pathologies and identify a novel therapeutic approach in SCD pulmonary vascular dysfunction and PH. Functional investigation of SS specific lncRNAs should consider the effects of different etiologies of SCD-PH.

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19.3

LONGITUDINAL CHANGES IN DIFFUSE MYOCARDIAL FIBROSIS IN SICKLE CELL ANEMIA

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Background: We recently demonstrated that diffuse myocardial fibrosis is a common and novel mechanism of heart disease in sickle cell anemia (SCA) that is strongly associated with diastolic dysfunction (Niss et al. Blood 2017). Diffuse myocardial fibrosis can be detected noninvasively by cardiac magnetic resonance imaging (CMR).The temporal evolution of diffuse myocardial fibrosis in SCA has not been studied before.

Methods: This is an ongoing, prospective, longitudinal study to characterize the cardiomyopathy of SCA. We used CMR measurements of native T1 and extracellular volume fraction (ECV) to quantify diffuse myocardial fibrosis. ECV was calculated from pre- and post-gadolinium T1 measurements of blood and myocardium. Focal myocardial fibrosis was detected by late gadolinium enhancement (LGE). Diastolic function was assessed by echocardiography. We compared paired CMR studies from study entry and 1-year follow-up.

Results: We studied 25 individuals with SCA who were 23±11 years of age (mean±SD). All had markedly increased ECV at baseline compared to controls (0.44±0.08 vs. 0.26±0.02, P<0.0001), indicating the presence of diffuse myocardial fibrosis; 1 also had LGE-defined focal myocardial fibrosis. At baseline, 8 (32%) had normal diastolic function, 17 (71%) had diastolic abnormalities, and 7 (29%) met the definition for diastolic dysfunction. The second (1-year follow-up) CMR was performed 12±0.4 months after the baseline. For all participants, the mean difference (year 1 - baseline) in ECV was -0.03 (95% CL: -0.08, 0.03; P=0.34), indicating general stability of the phenotype over 1 year. Similarly, the mean difference in native T1 values was 19 ms (95% CL: -10, 48; P=0.19). None developed new focal fibrosis. Participants with normal systolic and diastolic function at baseline (N=7) had median increases of 23% and 6% in ECV and native T1, respectively, at 1-year follow-up, compared to those with diastolic dysfunction at baseline (N=7) who had a median -0.46% decrease in ECV (P=0.32) and 2% increase in native T1 (P=0.45).

Conclusions: Diffuse myocardial fibrosis is a common feature of SCA. Overall, the CMR measurements of diffuse fibrosis, native T1 and ECV, are stable over 1 year in SCA, indicating their utility to monitor anti-fibrotic or disease-modifying therapy for the cardiomyopathy of SCA. Individuals with normal heart function, however, may be at risk of progressive myocardial fibrosis.

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20.0 Red Cell Physiology

20.1

DEVELOPMENTAL REGULATION OF ERYTHROID SELF-RENEWAL

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More than 80% of all the cells in the adult human are red blood cells (RBCs) and maintenance of their steady state level requires a staggering production of 2.5x106 new RBCs every second. Over 15 million units of RBCs are transfused yearly in the United States to treat patients following trauma or major surgery, or with hereditary anemias, including sickle cell disease, requiring chronic transfusion therapy. Donations, projected to decrease over the coming decades, are the sole current source for RBCs and are associated with infectious risks, high costs of screening, and sporadic shortages, especially for rare blood types required for alloimmunized patients. Ex vivo-generated human RBCs could offer a potential solution by serving as an alternative RBC source. A major obstacle has been generating enough RBCs to constitute even one unit of blood because erythroid precursors from adult sources are only capable of limited self-renewal when cultured in vitro with erythropoietin, stem cell factor, and dexamethasone. We discovered that erythroblasts derived from the murine embryo have a unique ability to self-renew extensively (>1060-fold) ex vivo, all the while retaining the ability to terminally mature into reticulocytes. We subsequently identified Bmi-1, a member of the polycomb repressive complex-1, as a critical regulator of ex vivo erythroid self-renewal. Bmi1-induced extensively self-renewing murine erythroblasts terminally mature both in vitro and in vivo. Our recent studies indicate that Bmi-1 may also regulate human adult and fetal erythroblast self-renewal. Expanding the proliferative capacity of human erythroblasts lays the groundwork for the in vitro generation of reagent RBCs for improved blood typing of sickle cell patients and ultimately of cultured RBCs for transfusion therapy. Support: NIH grants HL130670 and HL134696, and NYSTEM C030134.

20.2

PATHOBIOLOGY OF SICKLE RED CELLS: IMPLICATIONS FOR PATHOPHYSIOLOGY OF SICKLE CELL DISEASE

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Sickle cell anemia is a common inherited red cell disorder with high morbidity. Chronic severe hemolytic anemia, episodic vasoocclusive crisis and end organ damage are clinical features of the disease. Current treatment options are limited and as such the development of new and effective therapies is urgently needed. Our current understanding of the pathobiology of sickle red cells includes: 1) decreased cellular deformability as a consequence of sickling induced structural changes in membrane organization and cell dehydration and 2) increased adhesion of sickle red cells to vascular endothelial cells and other blood cells as a consequence of increased surface expression of adhesion receptors and plasma factors. Altered rheological properties and increased cellular adhesion of sickle red cells are key contributors to the pathophysiology of the disease. Currently, several treatment options are being explored to minimize or prevent the cellular alterations responsible for clinical manifestations. These include approaches for preventing in vivo sickling by either reactivating fetal hemoglobin synthesis or preventing sickle red cell dehydration and by decreasing the adhesive interactions of sickle red cells with endothelial cells and other blood cells.

20.3

RED BLOOD CELL RHEOLOGY AND VASCULAR DYSFUNCTION IN SICKLE CELL DISEASE

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Sickle cell anemia (SCA) is a genetic disease characterized by the presence of abnormal hemoglobin (HbS) that polymerizes under deoxygenated conditions causing a mechanical distortion of red blood cells (RBC). SCA patients have decreased RBC deformability and increased RBC aggregates strength. We recently investigated the contribution of blood rheology and vascular dysfunction in vaso-occlusive crises (VOC). Our findings demonstrated that SCA patients have blunted microvascular reactivity during local thermal heating tests compared to controls. The lower microvascular reactivity was negatively associated with the levels of plasma advanced oxidation protein products and nitrotyrosine suggesting a key role of oxidative/nitrosative stress in vascular dysfunction in this disease. Moreover, we recently observed that circulating exosomes in SCA, originating mainly from RBCs, were able to promote monocytes adhesion to endothelial cells through an increase in P-selectin expression and alter in vitro endothelial cells barrier permeability and the topographic distribution of the tight junction protein ZO-1 in a SCA severity-dependent manner compared to healthy children. These new data suggest that exosomes originating from RBCs could be one of the sub-cellular elements involved in the endothelial dysfunction associated with SCA. Finally, multivariate analyses recently performed in SCA cohorts showed that increased blood viscosity and decreased microcirculatory oxygenation are independently associated with a higher risk to develop frequent VOC episodes. In conclusion, vascular dysfunction and blood hyperviscosity emerge as key factors involved in the severity of SCA and the occurrence of frequent VOC events.

20.4

THE EFFECT OF HEMIN ON HUMAN RED BLOOD CELL TRANSFORMATION

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Background. Lysis of red blood cells (hemolysis) is associated with pathological states such as Sickle Cell Disease (SCD), ischemia reperfusion injury, and malaria, resulting in high levels of free hemoglobin (Hb). Hb degrades and formats hemin, a highly toxic molecule that triggers increased oxidative reactions, leading to cell damage and increased membrane permeability. Naturally in plasma, during mild to moderate hemolysis, extracellular Hb and hemin are neutralized by a number of pathways scavenger proteins, receptors, and enzymes. At the same time in case of pathological states such as anemia (SCD), the level of hemin exceeds the neutralization capacity and thus leads to different health complications and even death. Thus, the aim of the current study was to ascertain the effect of hemin on RBC transformation.

Materials and methods. Washed human RBCs (0.5x10^12/L) were exposed to a standard saline solution. Hemin concentrations less than 20 µM (described as fatal) were used. RBC membrane transformations and micropartical production (MPs) were assessed using flow cytometry (Navios, BeckmanCoulter, USA) and low angle laser scattering (Lasca-TM, BiomedSystems, Russia). EC50 of hemin was used as the marker for the description of RBCs membrane transformations triggered by hemin.

Results. We found that hemin action on RBCs induced a biphasic dose-dependent transformation in: i) membrane transformation from biconcave to spherocyte under low concentrations of hemin (EC50 [hemin] ~ 120nM), and ii) MP formation and hemolysis (EC50 [hemin] ~ 5µM) with MPs sized ~ 0.6 micron.

Conclusions. Our results demonstrate the effects of hemin on RBCs, which can be distinguished into two stages, those being: 1) spherisation, and 2) MP formation and hemolysis. The established effects of hemin on RBC transformation may be used in clinical practice to elucidate a current state of RBCs in SCD patients.

20.5

MOLECULAR RESOLUTION OF AN ACTIVE VASO-OCCLUSIVE CRISIS IN VOC PATIENTS TREATED WITH SANGUINATE®.

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SANGUINATE® (PEGylated carboxyhemoglobin bovine) is a gas transfer agent that can deliver oxygen directly to the hypoxic (sickled) RBCs of sickle cell disease (SCD) patients. Importantly, the transfer of oxygen also promotes rapid conversion of the sickled RBCs to a normal morphology. SANGUINATE® is currently being investigated as a clinical candidate for the treatment of acute severe Vaso-Occlusive Crisis (VOC) in SCD patients (NCT02411708).

Consenting SCD patients in acute severe VOC were treated with a single IV infusion of SANGUINATE® (8ml/Kg) with blood samples collected pre-treatment and at two post-treatment points; VOC resolution (discharge time) and 72hrs later. Image-based multi-parameter flow cytometry was performed on all samples (including placebo controls) and the extent of sickled/unsickled cell populations was determined across all enrollees at these time points.

Patient samples treated with SANGUINATE® showed a shift towards a more normal morphology when the pre- and post-VOC resolution samples were compared for each individual. In contrast, the placebo control patient samples showed worsening or no improvement of the sickled/unsickled ratios. Additionally, only patients treated with SANGUINATE® exhibited extended unsickling profiles at 3 days following treatment.

SANGUINATE® was capable of promoting the rapid depolymerization of sickled hemoglobin within peripheral blood samples of severe VOC patients treated with this novel gas transfer agent. Despite a half-life of 18-24hrs SANGUINATE® treated patients continued to exhibit fewer sickled cells at the 72hr sample point. SANGUINATE® can quickly oxygenate patient sickled RBCs effectively reducing both the short-term and long-term sickled RBC levels. Collectively these results support the continued clinical evaluation of SANGUINATE® as a rescue agent for rapid resolution of SCD patients in severe VOC in the ambulatory setting.

20.6

INTRINSIC CELLULAR FACTORS MODULATE HIV-1 REPLICATION IN SICKLE CELL DISEASE

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Intrinsic Cellular Factors Modulate HIV-1 Replication In Sickle Cell Disease

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Human immunodeficiency virus type 1 (HIV-1) is challenged by intrinsic antiviral restriction factors which it has to counteract with its own viral proteins. We recently showed that in Sickle Cell Disease (SCD), anti-viral restriction factor SAMHD1 is activated and inhibits HIV-1 infection. SAMHD1 activation was due to its reduced phosphorylation by CDK2, which activity was inhibited by reduced intracellular iron levels in SCD peripheral blood mononuclear cells (PBMC). The present study was aimed at identifying additional restriction factors that might be induced in SCD PBMC. This was paralleled by the HIV-1 infection analysis in PBMC from healthy donors treated with hemin and iron chelators to mimic SCD conditions. We used customized array that included known anti-HIV-1 restriction factors to measure mRNA expression of anti-viral factors. The identified factors were further validated with shRNA-mediated knockdowns of the identified genes and their effect on HIV-1 replication in cultured and primary cells. **We observed** upregulation of 19 genes in SCD PBMC including APOBEC3A, APOBEC3C, TRIM5α, TRIM22, MX2 and RSAD2 which were significantly upregulated and were further validated using real-time q-PCR. In PBMC treated with hemin, ABOBEC3A, RASD2 and MX2 mRNAs were overexpressed, whereas TRIM5α expression was increased in PBMC treated with either hemin or iron chelators. ShRNA-mediated knockdown of, APOBEC3C and TRIM5α, but not APOBEC3A or TRIM22 induced HIV-1 replication. In conclusion, in addition to SAMHD1, restriction factors APOBEC3A, APOBEC3C, TRIM5α, TRIM22, MX2 and RSAD2 may also contribute to HIV-1 restriction in SCD. Similar to SAMHD1, these factors are likely to be modulated by hemolysis and intracellular iron levels. Our findings further point to the importance of hemolysis and deregulation of iron metabolism as an underground cause for HIV-1 restriction in SCD.

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21.0 Coagulation and Thrombosis

21.0

PROMOTING THE RESOLUTION OF INFLAMMATION IN SICKLE CELL DISEASE

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Patients with Sickle Cell Disease (SCD) are more susceptible to thrombotic events, in which leukocytes, platelets and erythrocytes have all been implicated in the pathogenesis. However, the underlying mechanisms promoting a pro-coagulant and pro-thrombotic phenotype in SCD (especially in the brain) are still unknown. The causes for the SCD phenotype may relate to a well-established link between thrombosis and inflammation i.e. inflammation can beget local thrombosis, and thrombosis can amplify inflammation. Accumulating data linking inflammation and thrombosis supports the hypothesis that anti-inflammatory therapies may limit thrombosis and that anti-thrombotic therapies may reduce vascular inflammation. One such target is the anti-inflammatory and pro-resolving endogenous mediator Annexin A1 (AnxA1) and its interaction with the Formyl Peptide Receptor (FPR) family. In humans three FPRs (FPR1, FPR2, and FPR3) regulate innate inflammatory responses. Their function has been most extensively investigated in the context of leukocyte recruitment and activation, where FPRs promote cell motility (chemotaxis) and microbicidal respiratory burst. Here we have tested the effect of targeting the AnxA1-Fpr2/ALX pathway as a therapeutic strategy for SCD with a special focus on thrombo-inflammation. Data presented during the talk adds to the current literature suggesting a proinflammatory and pro-thrombogenic cerebral microcirculation is found in SCD mice. Furthermore, targeting the AnxA1-Fpr2/ALX pathway is effective in resolving SCD associated thrombo-inflammation.

21.1

DE-CLOTTING SICKLE CELL DISEASE

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Sickle cell disease (SCD) is a hematological disorder caused by a single nucleotide mutation in the β-globin gene. Hemolytic anemia and vaso-occlusive crises, resulting from the sickling of red blood cells, are the primary pathologies of the disease that ultimately result in irreversible damage of multiple organs. Furthermore, the contribution of vascular inflammation and the hypercoagulable state to the pathology of SCD has been recently recognized. This hypercoagulable state is mediated by activation of both the intrinsic and extrinsic coagulation pathways. Consistent with the increased markers of coagulation activation, the incidence of venous thromboembolism is higher in SCD patients than matched controls and is associated with increased mortality. In mouse models of SCD, tissue factor expressed on leukocytes activates coagulation and contributes to inflammation via microvascular thrombosis, whereas the non-coagulant form of tissue factor expressed by endothelial cells mediates factor Xadependent activation of protease activated receptor-2 and contributes to inflammation via IL-6 expression. Inhibition of coagulation also protectes sickle mice from developing multi-organ pathologies and improves survival. Together, these data point to the coagulation system as an important mediator of end-organ damage in a mouse model of SCD, and suggest that long-term anticoagulation might lead to improved clinical outcomes in sickle cell patients. The question is how to reduce the prothrombotic state in SCD without compromising hemostasis. Concerns about hemorrhagic transformation during ischemic stroke or increased risk of hemorrhagic stroke has limited the use of currently available anticoagulants in SCD. There is growing interest in targeting components of the intrinsic coagulation pathway (factor XII and XI) to prevent thrombotic disorders, primarily because this goal may be attainable without incurring any significant bleeding risk. Preliminary data from our group indicate that FXIIa contributes to the prothrombotic state during both steady state and vaso-occlusive crises. Targeting of FXII(a) could eliminate risk of serious hemorrhage yet still provide benefits associated with reduced thrombosis, vascular inflammation and end-organ damage in SCD. This research was funded by NIH U01 HL117659

21.2

SICKLE RED BLOOD CELLS ALTER CLOT RETRACTION IN MICE AND HUMANS

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A chronic hypercoagulable state and increased risk for venous thromboembolism are prominent features of sickle cell disease (SCD). Red blood cells (RBC) were thought to be merely trapped within a fibrin-rich clot without affecting thrombus formation. However, recent evidence suggests that the retention of RBC within clots by factor XIII directly affects thrombus size. It has been proposed that clot contraction-mediated packaging of RBC is significantly altered in SCD and may affect clot stability. We investigated the cellular mechanism of clot retraction in SCD.

An *ex vivo* clot retraction assay was performed with blood from sickle patients and from Townes sickle mice. Clot formation was initiated in citrated blood by $CaCl_2$ (10 mM) and tissue factor (1 pM), and after 2 hours the number of RBC extruded from the clot were counted in the serum and expressed as a percentage of initial RBC number.

Dramatically fewer sickle (SS) RBC were extruded into the serum during clot contraction than wild type (AA) RBC (0.8±0.8% vs. 19.4±0.8%, $p<0.0001$) in sickle mice; a similar result was observed for human RBC. Mixing the platelet free plasma, platelets, and RBC of AA and SS mice demonstrated that the entrapment of SS RBC within the clot is entirely mediated by yet unknown properties of SS RBC. Since SCD is associated with a lower hematocrit (Hct), we investigated if the initial number of RBC affects the extrusion of these cells during clot retraction. Indeed, lowering the Hct of AA mouse blood reduced RBC extrusion, yet normalizing the Hct in SS mouse blood had no effect on the number of SS RBC extruded from the clot, suggesting that the entrapment of SS RBC within the clot is not simply caused by lower RBC number.

Clots formed from AA blood had a soft gel-like structure, and electron microscopy revealed that these normal RBC had polyhedral shapes tightly packed in the clot core. In contrast, SS clots were more firm and stiff, while many SS RBC had undergone sickling and were not compacted within the clot. RBC sickling was also observed in clots formed in the inferior vena cava of SS mice after vessel stenosis. Our data indicate that SCD alters the structure and dynamics of clot formation. The effect of these differences on the increased risk of pulmonary embolism and stroke in SCD is currently being investigated.

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21.3

SICKLE CELL ANEMIA: HYPER- OR HYPOFIBRINOLYSIS STATE?

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Background. Sickle cell disease (SCD) is caused by hemoglobin polymerization in hypoxic erythrocytes, due to $Glu_6 \rightarrow Val$ mutation in the β-globin gene. Numerous studies have been done to evaluate coagulation in SCD; however, fibrinolysis in SCD remains poorly researched. Cellular components play an important role in fibrinolysis, and therefore we used two separate assays, utilizing either whole blood (WB) or the platelet free plasma (PFP) samples.

Aims. To evaluate resistance to t-PA-mediated fibrinolysis in PFP and WB of patients with SCD.

Methods. *Turbidity Assay.* Citrated PFP from 13 SCD patients (SS genotype) at baseline and 9 controls (race-, age- and sex-matched) was mixed with tissue factor (TF, 1pM), $CaCl_2$ and t-PA (2.5, 1.25 or 0.625nM) and optical density changes were read spectrophotometrically. Clot lysis time (CLT–the time from half clotting to half lysis) was used to evaluate fibrinolysis.

Global Assay in WB. Citrated WB in transparent tubes from 15 patients with SCD and 11 matched controls was mixed with TF, $CaCl_2$ and t-PA (0.6, 1.2, 2.5, 5 and 10nM). When the clot was formed, a steel ball (d=2mm, w=0.13g) was placed on its surface. The time from coagulation/fibrinolysis activation to the ball reaching the bottom of the tube was measured by capturing time-lapse video.

Sample collection was approved by IRB. Informed consents were obtained.

Results. In the PFP assay, we found that CLT was significantly shorter in SS patient samples compared to the AA group with all three t-PA concentrations (CLT mean±SEM SS *vs*. AA and 'p' value for 2.5, 1.25, 0.625 nM t-PA, respectively – 60.7±2.7 *vs*. 136.7±12.3, p<0.00001; 92.9±5.9 *vs*. 182.7±9.2, p<0.00001; and 138.5±12.9 *vs*. 226.7±0.7, p<0.0001).

In the WB ball sedimentation assay, no significant difference was noted between AA and SS clots at high t-PA concentrations (>2.5nM). However, SS clots challenged with lower tPA concentrations (0.625-1.25nM) were more resistant to fibrinolysis than AA clots (CLT mean±SEM SS *vs*. AA and 'p' value for 1.25 and 0.625 nM t-PA, respectively – 401.9±59.3 *vs*. 157.9±57.9, p=0.0085; 651.5±33.9 *vs*. 369.9±75.4, p=0.001).

Conclusion. Plasma clots from SCD patients are more susceptible to t-PA challenge compared to healthy controls. In contrast, in WB, where cell components likely play a role, SS patient samples showed resistance to fibrinolysis (at low t-PA concentrations). Therefore, patients with SCD appear to have a hypofibrinolytic state, which is present in WB but cannot be detected with plasma assays.

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21.4

ANNEXIN A1 AFFORDS PROTECTION IN SICKLE CELL DISEASE

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Introduction: Neutrophils have recently been implicated in the pathogenesis of sickle cell disease (SCD) vaso-occlusion potentially by their ability to capture sickle red blood cells and, by the production of neutrophil extracellular traps (NETs). Endogenous anti-inflammatory mediator Annexin A1 and its N-terminal derived peptide ($AnxA1_{Ac2-26}$) have been shown to mitigate cerebrovascular inflammation by engaging with formyl peptide receptors (FPRs). Here we aimed to-determine whether $AnxA1_{Ac2-26}$ is able to attenuate SCD vaso-occlusion via a reduction in NET formation.

Materials and Methods: All patients and healthy donors gave written consents and Institutional Review Board of the LSUHSC-S approved the study. Following blood collection, neutrophils were isolated and incubated (1 x 10^5) with $AnxA1_{Ac2-26}$ (30μM), the FPR pan-antagonist Boc2 (N-tert-butoxycarbonyl-L-Phe-D-Leu-L-Phe-D-Leu-L-Phe)(10μM) or $AnxA1_{Ac2-26}$+Boc2 for 30 minutes followed by NET stimuli (Ionomycin, 4μM) for a period of three hours. Cells were then stained with Sytox green or fixed, permeabilized and blocked for immunocytochemistry with NET specific antibodies; mouse anti-H3Cit, rabbit anti-NE, followed by species-specific secondary antibodies. NETs were quantified by measuring the percentage of $CitH3^+/NE^+$ stained DNA fibers and percentage of Sytox green stained DNA fibers. Extracellular DNA levels were also quantified by analyzing Sytox green intensity.

Results: Neutrophils from SCD patients at baseline exhibited enhanced $H3Cit^+$ NET production versus healthy controls. These effects were further exacerbated by stimulation with Ionomycin (p<0.0001). $AnxA1_{Ac2-26}$ was able to significantly reduce the $H3Cit^+$ NETs (p<0.001), which was abrogated by the addition of Boc2 (p<0.05). Interestingly, there was no change in extracellular DNA levels in stimulated SCD neutrophils compared to controls, and $AnxA1_{Ac2-26}$ had no effect in Ionomycin stimulated extracellular DNA production.

Conclusion: Our study demonstrates that $AnxA1_{Ac2-26}$ is able to inhibit SCD-associated NET production via an engagement with FPRs. Furthemore, these results support the notion of targeting the AnxA1-FPR pathway as a potential therapeutic strategy to prevent neutrophil driven thrombo-inflammation in SCD.

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