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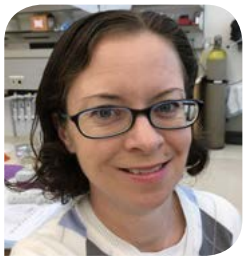
Special Abstract Issue

Questions to Ask Before Playing on the Tracks: Job Security and Salary Considerations for Tenured and Non-Tenured Faculty Positions

Erika I. Boesen



Erika I. Boesen

University of Nebraska Medical Center, Omaha, NE

Tenure: a six-letter word for security, which simultaneously strikes fear into the hearts of new and prospective assistant professors. But should it? The concept of tenure was originally proposed in part as a means to ensuring academic freedom while guaranteeing secure employment. Along with keeping your job for as long as you want it, this has generally been taken to mean employment at full pay. But does tenure still mean job security and full salary coverage, forever? Aren't universities increasingly doing away with tenure? Does having tenure or the opportunity to get it really matter to your career? Rather than trying to distill the secrets to securing tenure, this article will discuss what kinds of models are currently offered in terms of tenure status and salary, and some questions to consider if you are newly on the job hunt for a faculty position.

First, some basics, and a disclaimer: The information and commentary below focuses on full-time faculty positions at academic institutions in the United States. Academic ranks, career structures, and whether tenure or something equivalent even exists varies considerably between countries. Interested readers are encouraged to explore the European University Institute's website for an excellent and growing summary of such information for Europe and beyond (1).

What is Tenure?

Tenure, as most people think of it, is essentially a guaranteed ongoing employment contract at an academic institution, continuing in perpetuity. Unless, of course, your contract is terminated for cause (yes, you can still be fired!), or if an extraordinary circumstance arises such as a fiscal crisis for the university. The criteria and expectations to be met to secure tenure differ between institutions and between types of positions (e.g., research-focused vs. educator tracks). There may even be nuances in expectations between different schools or colleges within an institution. Whether and how frequently your institution has an opportunity to reevaluate its commitment to you once you are tenured, in the form of post-tenure review, varies between institutions. Detailed information regarding tenure and post-

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Gawad Kalinga e.V.: Supporting Self-Help for the Poorest of the Poor in the Philippines

Peter Scheid

Gawad Kalinga, a word in the Philippine language Tagalog, means "to bestow care." And this is indeed pressingly necessary in that country. Let me give you a brief background from which you may understand why the rate of very poor people, those who live on less than $1 a day, is so extremely high in the Philippines.

Background

"Paradise lost" is an epithet frequently applied to the Philippines. Four hundred years of colonization deeply hurt this Southeast Asian archipelago. Since obtaining unrestricted independence in 1986, the country has made considerable efforts to alleviate the incredible poverty of its people. But still, joblessness and life in temporary corrugated-iron huts in the slums of the big cities makes survival for millions of people a daily challenge. Natural catastrophes also take a heavy toll. Each year, the country is hit by a number of tropical cyclones. One of the strongest ever recorded was typhoon Haiyan, which cost about 10,000 lives and devastated the homes of more than 4 million people, depriving them of their meager goods. And those were, and regularly are, the poorest whose shelters are built in high-risk areas.

2012: The poor at the irrigation canal

The Organization Gawad Kalinga

Land for the landless, homes for the homeless, and food for the hungry—those are the main weapons with which to fight poverty. And this is what the national project Gawad Kalinga is aimed at. Gawad Kalinga builds villages in various regions of the country. However, in this project of help by self-help, the goal is not only to provide safe shelter—basic and higher education for children and young people, medical care, jobs for the families, and a peaceful and friendly social coexistence of the inhabitants in their villages are of equal importance. Each village in the project is supported by one or more sponsors. Depending on the local situation, an amount of only 3,000–4,000 USD is required to build a house, largely by the future inhabitants themselves; and the government helps in providing infrastructure such as water and electricity, access roads, and the like.

2012: Children whose mother recently died

The Ateneo de Manila University

The Jesuit University Ateneo de Manila is co-founder and a major supporter of Gawad Kalinga. The university is committed both materially and ideologically to supporting the project, and both professors and students participate in helping to achieve the goals of Gawad Kalinga. In the first months after the initiation of the project, the university pledged to establish Blue Eagle Village. It has helped to build 185 homes there for about 1,000 people who had lived in corrugated-iron huts on the big waste dump of the Payatas in the suburbs of Manila. With its preschool, health, and youth centers, it is truly a paradise for its

inhabitants. The university has also helped the villagers find jobs, as well as assisting in organizing a homeowners' association. Since then, several other new villages have been built under the auspices of the university. To bestow care is more than building houses; the ultimate goal is to create pleasant living conditions for the poor. No longer will life be dominated by a daily struggle for survival. Instead, it will be characterized by trust in a better future for all, especially the children.

2017: Visit to the new German village, GK St. Joseph

The Association Gawad Kalinga e.V.

In 2005, I visited the Ateneo de Manila University as member of the International Advisory Committee of a Japanese Foundation. Father Bienvenido Nebres S.J., then rector of the university, introduced us to the project Gawad Kalinga and guided us through its new Blue Eagle Village. I was so deeply touched by the project and moved by the people who were proud of their new homes and happy in their safe environment that I decided to help the university in its social endeavors. Together with my wife and some friends, we founded the charitable association Gawad Kalinga e.V., registered in Germany. Its statutory goal is to support the Ateneo de Manila University in its care for the socially disadvantaged population in the Philippines. The major goal is to assist the university in achieving its goals in the national project Gawad Kalinga. We enjoy excellent personal relationships with the university and its senior administrative officers, who ensure that funds collected by our association and transferred to the university are passed to the project organizers with minimal overheads. On our website (*www.gawad-kalinga.org*), we regularly inform our supporters about how their donations are spent (tab *Projects*).

2017: Cheerful children

In the first years, we transferred funds to the Ateneo University to build 13 houses in two villages sponsored by them. In 2013, we started a project with the university to build a new village with houses for about 80 families who had spent a miserable life at an old irrigation canal. My wife Cornelia and I had visited these families in 2012, and we were shocked by their situation. When we returned to Germany, we started a project to build, together with Ateneo, houses for these people in a village, now called German Village GK St. Joseph. This project was generously supported by the German Federal Ministry for Economic Cooperation and Development (BMZ).

When we last visited the Philippines in March 2017, we were most impressed to see how happy the residents in their new homes appeared to be. They told us of the excitement not only to move into a solid house but also to have become part of a social community. We found the children happy and cheerful.

Currently, we are building a new village, German Village Dasmariñas, south of Manila with houses for about 70 families, whom we had visited in 2017 in their most primitive shelters.

Although our contributions are only moderate, it gives us great pleasure to see that at least some of the very poor people are enabled to start a new and better life. And this is, of course, particularly true for the children.

If you want to know more, please visit our homepage at *www.gawad-kalinga.org*, where you can find information on the association and its projects. ●

Education

APS Promotes Physiology to K-12 Teachers at Fall Meetings

NABT invited speaker Tatum Simonson

The APS highlighted physiology for middle school science teachers and administrators at the annual Association for Middle Level Education (AMLE) Annual Conference for Middle Level Educators held in Orlando, FL from October 25 to 27. This was the ninth year for an APS presence at the AMLE conference, which is attended by over 4,000 teachers, administrators, and counselors from across the country.

Teachers were as excited as ever for a national science society's presence, since so few opportunities are available for science-related materials at this meeting. The APS exhibit booth was extremely busy and well received, with many questions about the Life Science Teaching Resource Community (*www.lifescitrc.org*), age-appropriate career materials such as the APS career trading cards, and the Online Six Star Science Professional Development Fellowship. Patricia Halpin (Teaching Section Liaison to the Education Committee) and Margaret Steiben (APS Program Manager, K-12 Education Programs and Professional Skills Training) assisted in the booth promoting PhUn Week and science careers strategies with teacher attendees. Next year's conference will be held in Nashville, TN from November 7th to 9th.

APS also promoted physiology for K-12 biology teachers at the National Association of Biology Teachers (NABT) 2018 Professional Development Conference in San Diego, CA. The annual national conference, held the second week of November, attracts middle and high school teachers as well as 2- and 4-year college faculty from across the nation.

The APS exhibit booth highlighted the Undergraduate Research Fellowships, Life Science Teaching Resource Community, Frontiers in Physiology teacher professional development program, physiology career materials, and K-12 outreach resources.

This year's sponsored speaker was APS member Tatum Simonson. Simonson presented "Cross-Population Insights into Hypoxia Adaptation and Maladaptation." Her presentation focused on human populations at high altitude challenged by low oxygen for hundreds of generations and showing unique physiological responses to this environmental stress, some of which are associated with genomic signatures of adaptation. Simonson applies integrative physiological genomics approaches to understand systems-level responses to hypoxia in highland populations. More than 70 attendees were engaged during Simonson's presentation, which was followed by a lively question-and-answer session.

Interest in undergraduate programs was a highlight at the exhibit booth throughout the 3-day conference as resources and program materials were shared and discussed with community college attendees. The 2- and 4-year college faculty were also excited about the undergraduate research opportunities, particularly the location availability, programs for underrepresented students, especially students with disabilities, and the available stipends. Booth traffic increased as attendees brought their colleagues and friends to the booth to learn more about APS offerings.

Next year's conference will be held in Chicago, IL from November 14th to 17th. For further information, please contact Margaret Stieben (Program Manager, K-12 Education Programs and Professional Skills Traning; *mstieben@the-aps.org*) or Allison Hood (Program Manager, Higher Education Programs; *ahood@the-aps.org*). ●

APS at SACNAS National Diversity in STEM Conference

Annelyn Torres-Reveron (left) and Crystal Taylor (right) at the APS booth in San Antonio

The APS sponsored and hosted an exhibit at the 2018 SACNAS National Diversity in STEM Conference at the Henry B. Gonzalez Convention Center in San Antonio, Texas from October 11th to 13th.

The APS exhibit booth was staffed by Annelyn Torres-Reveron (University of Texas Rio Grande Valley and Porter Physiology Development and Minority Affairs Committee Member) and Crystal Taylor (University of Alabama at Birmingham and 2018 Outreach Fellow). At the exhibit booth, they provided information about the many benefits of APS membership, such as access to summer research fellowship programs, professional development opportunities, and various funding opportunities. Over 50 conference attendees, many of them undergraduate students, visited the APS booth and requested information regarding membership.

The APS was pleased to provide funding to recognize students with the best poster presentation in the physiological sciences. The 2018 award recipients are:

- **John Tworek** (Augustana College). John's abstract title was "Effects from Airborne Metal-Enriched Particulate Matter from an Abandoned Uranium Mine."
- **Aleksis Ramirez-Pedroza** (University of the Incarnate Word). Aleksis' abstract title was "Calcium-Dependent Dual Oxidase 2 is a Novel Source of Reactive Oxygen Species Implicated in Glomerular Mesangial Cell Fibrotic Response to Angiotensin II."

Each student received $1,000 and a 1-year complimentary undergraduate membership. A total of 105 students received awards for their research and presentations during this year's conference.

The SACNAS National Diversity in STEM 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. Nearly 4,000 attended the conference, and more than 300 exhibits shared training, research, graduate school, and job opportunities. The next conference will be held in Honolulu, Hawaii from October 31 to November 2, 2019. For more information about SACNAS, visit *www.sacnas.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 2018 Annual Biomedical Research Conference for Minority Students (ABRCMS) at the Indiana Convention Center (Indianapolis, IN), November 14–18. ABRCMS is one of the largest professional conferences for underrepresented minority students in science, technology, engineering, and mathematics (STEM), attracting over 4,400 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 exhibitor's program to share information about graduate school and summer internship opportunities.

The APS, represented by Adrienne Bratcher (University of Louisville and Porter Physiology Development and Minority Affairs Committee Member) and Anberitha Matthews (University of Tennessee Health Science Center and APS Outreach Fellow), staffed the APS exhibit booth to promote student membership, awards, and career resources.

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.

2018 Physiology Awardees at ABRCMS

The APS was pleased to provide funding to award 11 undergraduate students for the best oral and poster presentations in the physiological sciences. Each student received $300 and a 1-year complimentary student membership.

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

The 2019 annual conference will be held in Anaheim, CA, from November 13th to 16th, 2019. For more information about ABRCMS, visit *www.abrcms.org*. For more information on APS diversity programs, visit *www.the-aps.org/diversity*. ●

Table 1. 2018 Physiology Awardees at ABRCMS

Student Name	Student Institution	Abstract Title
Dominik Aylard	University of California, Davis	The Role of the Brahma Chromatin Remodeling Complex in Maintaining Circadian Physiology and Healthspan
James Ball	University of Missouri	The Regulatory Effects of Diet and Exercise on Glucose Metabolism and Vascular Insulin Signaling in Obese Mice
Christian Checkcinco	University of California, Irvine	Mobile Class 1 Integron Containing Antibiotic Resistant Genes is Newly Described in Environmental *Enterobacter kobei*
Dalia El-Desoky	Xavier University of Louisiana	Taste Buds to the Brain: The Effects of Dietary Fat Intake on Serotonin Signaling in Rat Tongue
Jenee Farrell	Howard University	Downregulation of Hepatic Stem Cell Factor by Vivo-Morpholino Treatment Inhibits Mast Cell Migration and Decreases Liver Damage in a Murine Model of Primary Sclerosing Cholangitis
Jaresley Guillen	University of California, Davis	Engineering Enzymatic Alkane Hydroxylation: Exploring Phenol Hydroxylase's Secondary Coordination Sphere
Shekinah Hernandez	Louisiana State University Health Science Center	Effect of Binge Eating of Dietary Fat on Lingual Markers of Fat Sensing and Inflammation
Jose Sandoval	University of California, Davis	Glioblastoma Multiforme (GBM) Stem Cell Killing Using mTORC1 Inhibitors
Talia Seymore	The Pennsylvania State University	The Effect of Prenatal Exposure to an Environmentally Relevant Phthalate Mixture on Testosterone Levels in Adult Male Mice
Jack Shanahan	UCI	A Novel Combination of Plasmid Mediated Antibiotic Resistant and Heavy Metal Resistant Genes in Environmental *Klebsiella pneumoniae*
Malcolm Williams-Green	University of Pittsburgh	Can Febuxostat Therapy Protect Kidney Function in Mice with Sickle Cell Disease?

Ring in the New Year with the Following Award Opportunities!

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, 2019

the-aps.org/eb-undergrad

The Barbara A. Horwitz and John M. Horowitz Undergraduate Research Awards are presented annually to undergraduate students who submit a first-author abstract to the APS annual meeting at EB and present their research at the meeting. There are two types of Horwitz/Horowitz Undergraduate Awards that students can apply for through a single application.

Outstanding Undergraduate Abstract Awards

These awards provide $100 and a 2-year complimentary undergraduate membership. The student must be enrolled as an undergraduate at the time of the abstract submission, be the first author on an abstract submitted to the APS annual meeting at EB, 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 upon presenting the research at EB.

Excellence in Undergraduate Research Awards

To be considered for these awards, students must be an Outstanding Undergraduate Abstract Awardee and must attend and present a poster at the APS annual meeting at EB. The recipients receive $400 and a certificate. The highest-ranked awardee receives an additional $250, thanks to the generous contribution of an APS member. Selection of awardees is based on the quality of the poster and poster presentation to the Award Selection Committee.

Porter Physiology Development Fellowships

Application deadline: January 15, 2019

the-aps.org/porter

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

The program is open to underrepresented racial and ethnic minority graduate students who are citizens or permanent residents of the U.S. or its territories and are student members of APS. Applicants must be enrolled in a graduate program in the physiological sciences at a U.S. institution. The applicant's mentor/PI must also be an APS member.

Dale J. Benos Early Career Professional Service Award

Application deadline: January 24, 2019

the-aps.org/benos

The Dale J. Benos Early Career Professional Service Award honors an early career APS member (graduate student, postdoctoral fellow, assistant professor, or equivalent position) who has 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, participating in K-12 education outreach, participating in scientific advocacy and outreach programs, or by otherwise strengthening and promoting the physiology community. The awardee receives a $1,000 honorarium plus complimentary advanced registration to attend the APS annual meeting at EB.

Undergraduate Summer Research Fellowships

Application deadline: February 1, 2019

the-aps.org/summerresearch

The APS undergraduate summer research fellowships offer undergraduate students the opportunity to participate in a summer immersed in research. Fellows spend an average of 10 weeks in the laboratory of an APS member-researcher. Each fellow receives a $4,000 stipend plus additional funds for travel to present his or her research at the APS annual meeting at EB. Research hosts receive funds for student lab supplies. See the website for more details about eligibility requirements, application instructions, and program benefits. ●

Science Policy

NIH Revises Plans for Moving Elderly, Frail Chimpanzees

On October 18, 2018, NIH Director Francis Collins announced that NIH would implement new criteria for decisions about transferring chimpanzees from their current homes in research facilities to the Chimp Haven federal sanctuary. The focus of these changes is to protect frail and elderly animals. "NIH remains fully committed to retiring all chimpanzees it owns or supports to the Federal sanctuary unless relocation would severely or irreversibly accelerate deterioration of the chimpanzee's physical or behavioral health," Collins said in a statement.

Collins further noted that, "Contrary to what is often portrayed in the media, chimpanzees at research facilities do not live individually in cages or in laboratories." Rather, "chimpanzees at all NIH supported facilities have long been housed in social groups with the ability to roam indoors and outdoors, with access to large structures that allow for species-specific behavior such as climbing and swinging."

Under the plan developed in 2016 when NIH first began the process of moving chimpanzees to Chimp Haven, concerns about health status were "second only to space availability" in relocation decisions, according to Collins. Nevertheless, there were a significant number of deaths: in one cohort of 13 animals sent to Chimp Haven in 2014–2015 from the MD Anderson Keeling Center for Comparative Medicine and Research, 9 died within 18 months of their arrival.

"The point is coming soon, however, when NIH will need to decide on transfer of the final population of the frailest chimpanzees," Collins said, noting that some may "benefit from remaining at their current facilities, where they receive high-quality care and have longstanding relationships with their caretakers."

NIH is seeking "a path forward that is consistent with existing laws and regulations," according to Collins, "but in some instances those are conflicting." The Chimpanzee Health Improvement, Maintenance and Protection Act mandated that chimpanzees no longer needed in research should be transferred to sanctuaries. However, the Animal Welfare Act prohibits transporting sick, injured, or physically distressed animals. Because of the challenges of balancing these conflicting mandates, in February 2018, Collins asked a working group of the NIH Council of Councils (COC) to "provide recommendations on factors to be considered by attending veterinarian staff when deciding to relocate at-risk chimpanzees." The working group's report, *Assessing the Safety of Relocating At-Risk Chimpanzees* (*https://dpcpsi.nih.gov/sites/default/files/CoC_May_2018_WG_Report_508.pdf*), was published May 18, 2018 and received more than 4,000 public comments.

"Based on the CoC recommendations and the public comments, NIH will oversee the development and implementation of standardized approaches for assessing each chimpanzee based on its health, behavior, social, and environmental requirements," Collins said. "All four of the NIH-supported facilities housing NIH-owned or -supported chimpanzees, which includes the Federal sanctuary, will use these standardized approaches to assess chimpanzees for relocation." In the event that either the sending or the receiving facility refuses to approve the transfer of an animal, NIH "will enlist the advice of a panel of independent veterinarians to inform the final decision." ●

What to Expect in the 116th Congress

The midterm elections held on November 6, 2018 will bring change to Washington, DC, although, as of this writing, it is unclear exactly how much. Democrats will take the majority in the House of Representatives, whereas Republicans are expected to maintain a slim majority in the Senate. (See Table 1 for preliminary election results in both chambers.)

Two weeks after the election, newly elected House Republicans chose their leaders for the 116th Congress. Rep. Kevin McCarthy was elected House minority leader, and Rep. Steve Scalise moved up to the second-ranking Republican position as minority whip. House Democrats were scheduled to select the new Speaker at the end of November. The vote to ratify the Democrats' choice for Speaker will take place on January 3, 2019 when the 116th Congress convenes. Soon thereafter, both parties will formally make committee assignments. House Democrats are then expected to focus on conducting oversight investigations of the Trump administration and advancing their priority issues such as health care, gun control, and infrastructure spending. However, passage of any legislation will require the support of the Republican-held Senate and the White House.

Rep. Eddie Bernice Johnson, currently the ranking member on the House Science Committee, is in line to become the chair of that committee, which oversees the NSF and NASA. She would replace retiring Republican Chair Lamar Smith, who was a vocal critic of climate change and other politically charged fields of science. Leadership of the House Appropriations Committee will also be handed over to the new Democratic majority, but that will likely produce less of a change, since members of that committee from both parties have demonstrated an ongoing commitment to federal funding for scientific research.

Although Republicans are expected to see a slight increase in their majority in the Senate, they will still fall short of the 60 votes needed to invoke cloture, meaning that they will need the support of Democrats to pass significant legislation. Senate leadership will remain unchanged in both parties with the reelection of Senator Mitch McConnell as majority leader for the Republicans and Senator Charles Schumer as minority leader for the Democrats.

The new Congress will also include seven new members with STEM or health care backgrounds. ●

Table 1. 2018 mid-term election results as of mid-November

	Democrats	Republicans	Undecided/TBD
House of Representatives	228	199	8
Senate	47 (includes 2 independents)	51	2

Publications

Current Calls for Papers

Physiological Genomics

- Physiology of Cell State Transitions
  Submission deadline: June 30, 2019

Journal of Neurophysiology

- 50 Years of Modeling Neural Activity: Celebrating Jack Cowan's Career
  Submission deadline: June 30, 2019
- International Motoneuron Society
  Submission deadline: June 30, 2019
- Modularity and Compositionality in Motor Control: Acknowledging Emilio Bizzi
  Submission deadline: June 30, 2019

Advances in Physiology Education

- K–12 Outreach
- Curricular Integration of Physiology

Journal of Applied Physiology

- Mechanisms of Respiratory Modulation of Cardiovascular Control
  Deadline: January 31, 2019

AJP—Gastrointestinal and Liver Physiology

- The Gut Microbiome: A Virtual Organ
  Deadline: April 30, 2019
- Brain-Gut Interactions
  Deadline: April 30, 2019
- Translational Human Physiology and Pathophysiology
  Deadline: April 30, 2019
- The Physiology of Immune Therapies and Their Application in Treating Gastrointestinal Cancers
  Deadline: April 30, 2019

AJP—Heart and Circulatory Physiology

- Adaptive Immunity in Cardiovascular Disease
  Submission deadline: *January 31, 2019*
- Many Avenues to Cardiac Cell Death
  Submission deadline: *January 31, 2019*
- Chromatin and Epigenetics in Cardiovascular Disease
  Submission deadline: *February 15, 2019*

AJP—Regulatory, Integrative and Comparative Physiology

- Cardiovascular and Neural Adjustments to Exercise in Chronic Disease States
  Deadline: April 1, 2019

AJP—Renal Physiology

- Epigenetics and MicroRNAs in Kidney Physiology and Pathophysiology
  Deadline: June 30, 2019
- Circadian Rhythms or Time-of-Day Effects in Renal Physiology, the Urinary System, Blood Pressure or Volume and Electrolyte Regulation
  Deadline: June 30, 2019

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

Membership

New Regular Members

***transferred from student membership**

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APS News

Physiology Majors Interest Group 2018 Second Annual Meeting

Valerie S VanRyn, Erica A Wehrwein, and Claudia I Stanescu

History

The Physiology Majors Interest Group (P-MIG) was founded in 2014 by individuals at the University of Oregon, the University of Arizona, Gonzaga University, and Michigan State University. The first official gathering of P-MIG was at EB in 2015 at an APS teaching section symposium. In 2016 and 2017, P-MIG hosted networking sessions at EB sponsored by the APS education office. With these sessions being well attended, P-MIG hosted its first stand-alone meeting dedicated to Physiology Undergraduate Programs in May 2017.

Second Annual Meeting Planning Committee

The Second Annual Meeting Planning Committee is composed of Erica Wehrwein (Michigan State University), Claudia Stanescu (University of Arizona), Jennifer Rogers (University of Iowa), Jeff Osborn (University of Kentucky), Valerie VanRyn (Michigan State University), Lisa Carney-Anderson (University of Minnesota), and James Davis (Indiana State University).

Second Annual Meeting

The second annual meeting of P-MIG was hosted on June 28–29, 2018 at the University of Arizona in Tucson, Arizona.

Due to generous sponsorship, we were able to run the event, including registration and meals, at no cost to participants (see acknowledgements below). Participants had only to cover travel and lodging expenses. Without this funding, our meeting could not have taken place since the majority of the attendees stated their presence was contingent on this funding.

The meeting had registrants from 6 different countries (United States, Canada, India, Saudi Arabia, Nigeria, and Australia), totaling 47 individuals from 34 institutions. We had representation from collaborating societies, including The American Physiological Society, Human Anatomy and Physiology Society, Society for the Advancement of Biology Education Research, and Association of Chairs of Departments of Physiology.

BS Degree Characteristics of Represented Programs

- 40% of programs self-classified as Systems/Integrative, 26% Human Physiology, 13% Exercise Physiology, 7% Cellular/Molecular Physiology, 7% Animal Physiology, and 7% other.
- 67% of participating programs had the word physiology in their program title; 33% were named Physiology, 14% Human Physiology, 10% Physiological Sciences, 5% Exercise Physiology, and 5% Physiology and Exercise Science.
- 35% of programs were housed in liberal arts and sciences colleges, 25% in colleges of medicine, and 40% in a variety of other colleges.
- Total enrollment in majors for spring 2018 varied; however, the majority of programs (73%) have enrollments greater than 300 students, and some programs (7%) have more than 1,500 students enrolled. Enrollment size: 0–50 was 27%, 301–400 was 33%, 401–500 was 13%, 501–1,000 was 20%, 1,501+ was 7%.
- Graduating class size for 2017–2018: 0–50 was 25%, 51–100 was 59%, 101–150 was 8%, and 301–400 was 8%.

Meeting Agenda

All oral sessions were recorded and are available on the P-MIG website. The meeting commenced at 12 PM noon with a welcome from the University of Arizona Physiology Department Chair Nicholas Delamere and an introduction of Erica Wehrwein, founding member of P-MIG. The goals of the meeting were presented:

- Create national curricular guidelines for physiology programs
- Share resources on programmatic learning outcomes and assessments
- Develop the P-MIG mission statement and group organizational structure

- Discuss student perspective by sharing data from 2018 student surveys
- Discuss undergraduate career trajectory and employability skills development
- Discuss how participating programs teach professional skills in their curricula
- Build a community and network with other professionals invested in undergraduate physiology
- Share resources and best practices for teaching physiology
- Discuss the role of professional societies in our efforts
- Collect data about the use of published physiology core concepts

Day 1 Sessions

Erica Wehrwein (current director of P-MIG) of Michigan State University introduced the organization's mission and structure, the new P-MIG website (www.physiologymajors.org), and thanked sponsors before her main talk titled "Creating National Guidelines for Physiology Undergraduate Programs." Here, she discussed the current state of the major and how other similar disciplines, such as kinesiology, have approached establishing national curriculum guidelines.

Jessica Taylor of the American Physiological Society (APS) presented a talk full of helpful information to share with undergraduates and other educators titled "Physiology Undergraduate Students and APS: Past, Present and Future." Here, she discussed awards and funding that are available related to undergraduate research and the impact APS provides related to undergraduate education across the world.

Dee Silverthorn of the Human Anatomy and Physiology Society (HAPS) informed the P-MIG attendees of the mission, goals, and current projects of HAPS in a talk titled "Physiology Learning Outcomes and Competency Exam; a HAPS Initiative." Here, she specifically discussed the HAPS exam and the physiology-only learning outcomes and petitioned volunteers to get involved in their development.

Nicholas Delamere of the Association of Chairs of Departments of Physiology (ACDP) conveyed how ACDP utilizes teamwork to flourish as an organization in a talk titled "Building a Useful Partnership." Here, he discussed the status of medical school physiology curriculum and how ACDP can partner with physiology educators.

Jenny McFarland of Edmonds Community College challenged P-MIG attendees to think differently about their teaching with her keynote lecture titled "Physiology Core Concepts in the Curriculum: Aligning Concepts, Conceptual Frameworks and Assessments." Here, she discussed the value of teaching based on physiology core concepts to promote enhanced learning.

Claudia Stanescu of the University of Arizona shared her experiences with surveying the University of Arizona faculty on physiology core concepts in a talk titled "Collecting Data on Core Concepts from Participating Institutions." She discussed how data represents not only what is taught but what is valued, and petitioned volunteers to gather the information at their institutions and to use the information gathered to develop national guidelines.

The first day concluded with full introductions by each individual in attendance and breakout session roundtable discussions related to the identification of two to three desired physiology programmatic outcomes, followed by a large group discussion on finding consensus for undergraduate physiology programmatic outcomes and the formation of working groups.

Day 2 Sessions

The second day focused on student career development, research skills, and professional skills. The day began with a poster session titled "Innovations in Physiology Undergraduate Education," where 16 posters were presented. The poster session was very well attended and lively. Active discussions at the session helped participants connect with each other and share ideas about teaching and assessment. Following the poster session, there were oral sessions.

Jennifer Rogers of the University of Iowa shared data from the extensive 2018 P-MIG student survey in an illuminating talk titled "A Snapshot of Undergraduate Physiology—From the Students Themselves." Here,

she presented data captured from 667 students across 9 institutions related to demographics, careers, activities, perceptions of programs, and much more.

Erica Wehrwein of Michigan State University shared published national and international data from a number of outside sources regarding various aspects of our student population in a talk titled "Teaching Beyond the Facts: What Else Do We Need to Teach Our Students?" Here, she presented facts related to the importance of educating our students on nonacademic skills so that they can be more competitive applicants for future postgraduate programs or careers.

Julia Choate of Monash University inspired P-MIG attendees with her keynote talk titled "Using a Professional Development Program to Enhance Undergraduate Career Development and Employability." Here, she provided a detailed account of the five professional development modules embedded into courses and the importance of assessment in the realm of professional skills traning.

Michelle French of the University of Toronto discussed the importance of teaching students to appreciate all of the steps that go into finding scientific knowledge early on in college in her talk titled "Why Wait? Helping Freshman Develop Scientific Research Skills." Here, she revealed how she flipped the traditional model of education, where textbook knowledge is the focus during the introductory years of college prior to research experiences later on, and how that process improved student knowledge, skills, and enthusiasm.

The second day concluded with roundtable discussions related to translating desired course/program goals into specific course-related and other activities, and an in-depth large group discussion on P-MIG organization: its mission, structure, and by-laws moving forward.

Data Collection

Extensive information was collected through pre- and post-meeting surveys from all attendees as well as a national physiology undergraduate student survey. The post-meeting survey data from 2017 along with the pre-meeting survey data for 2018 were used as a guide for meeting planning. The 2018 post-survey data is being reviewed. The data, where relevant for broader dissemination, will be published or shared freely on our website (*https://www.physiologymajors.org/*). The meeting planning committee would gladly share any data with interested parties before publication.

Summary

The meeting was well attended, informative, and energizing. The gathering allowed for dissemination of ideas, relevant discussion among all attendees, networking among peer programs, and focused breakout discussions. As a result of the intense work at the meeting, excellent progress has been made on writing national guidelines for physiology undergraduate programs. Three working groups were formed for the 2018–2019 academic year: two were focused on writing of national guidelines for physiology undergraduate programs based on core concepts and professional skills, and another was focused on advising. These committees will be gathering resources, sourcing and developing program self-assessment tools, and drafting curricular guidelines. Based on the post-meeting survey, the attendees overwhelmingly expressed a desire to meet again, indicating a successful meeting; therefore, planning has started for the next meeting at The University of Minnesota on June 18–20, 2019. To stay up to date on P-MIG communication, sign-up for the listserv at *https://www.physiologymajors.org/communication*. For more information regarding P-MIG and/or to get involved, contact Erica Wehrwein at *wehrwei7@psl.msu.edu*. ●

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Continued from page 1: Questions to Ask Before Playing on the Tracks: Job Security and Salary Considerations for Tenured and Non-Tenured Faculty Positions

tenure review can usually be found on the individual college or school's webpage, or in the institution's faculty handbook. Whether tenure also guarantees 100% of your full salary is a different matter. More will be said below on implications for salary, regardless of tenure status.

The Tenure Track and the Dreaded "Tenure Clock"

The terms "tenure-track," "tenure-eligible," and "tenure-leading" all indicate that someone employed in such a position could one day apply for tenure. And the default expectation is that they will indeed apply. If tenure-track faculty do not apply for and are not granted tenure in a timely fashion (i.e., before the clock runs out), usually their contract will be terminated, and they will need to find another job.

Typically, tenure-track faculty are expected to have amassed the appropriate research/scholarship, teaching, and "service" experiences, and to have achieved a level of productivity to warrant being tenured within approximately 5–7 years of their initial faculty appointment. This 5–7 years to prove why your institution should want to keep you around forever represents the time on your "tenure clock." Often, people submit their application for tenure at the same time as their application for promotion to associate professor, although not always. At my institution, for example, you may apply for promotion and tenure at separate times; the two have overlapping but slightly different criteria. Some institutions reserve tenure for full professors only. If you are an assistant professor who is recruited by another institution as an associate professor, tenure might form part of the recruitment package but more likely won't be granted straight away. In that case, you may be both eligible and expected to apply for tenure in a shorter period of time than if you had started there as an assistant professor (e.g., 3 years). At other institutions, tenure is not offered at all, regardless of rank.

At institutions with an "up or out" promotion and tenure policy, if you do not successfully apply for and are not granted promotion and tenure by the end of the allotted period on your clock, your contract is not renewed. People hired at the associate professor level who don't make tenure in their abbreviated time frame may also be let go. Such all-or-nothing scenarios can make that clock tick very loudly. But before you break into a cold sweat at the thought, ask your prospective employer some questions. Is there a hard-and-fast timeline, and, if so, in what year do you need to apply and succeed? Is there flexibility on this deadline at the discretion of your department chair? Does your institution use the classical 5- to 7-year clock or has it been extended to 10 years under the more challenging funding conditions of modern times? Will you even be hired on a tenure-track basis in the first place, or does this designation occur later? If you join the tenure track later, how does that affect how long you have before you must secure tenure?

Career progress isn't always smooth, and life can throw unexpected challenges your way. Ask whether your institution has a policy that allows you to "stop the clock" and be granted more time, if, for example, your career progress is interrupted by having a family, major illness, or other unexpected life events. A variation of this idea is to allow faculty to switch from tenure track to non-tenure track, with the option of returning to the tenure track if the situation improves. Anecdotally, such offers tend to be made if funding is the main concern. If you ever find yourself considering this track-switching option, make sure you ask a lot of questions first. Just because it is theoretically possible to get back on the tenure track doesn't mean that it is likely, and it could be easier to cut your salary and/or terminate your employment in the interim. Are there other consequences, such as loss of eligibility for intramural pilot grants or certain extramural awards if you go off the tenure track? Where would your salary coverage come from? How would your independence and general job duties be affected? What are the new expectations and what requirements must be met to restore your tenure eligibility?

Okay, So What's the Deal With Non-Tenure Track Positions?

The defining characteristic of non-tenure-track faculty positions is that they explicitly do not come with the potential for a lifetime appointment. That isn't to say that job security is necessarily poorer than for tenure-track positions. Indeed, non-tenure-track faculty may well enjoy more longevity in their positions than tenure-track faculty who don't make tenure! Contracts may be for fixed terms or renewable indefinitely, provided that the need for the position and funds to support it remain available. This can mean very stable employment at institutions with consistent levels of student enrollment, healthy finances, and the like. Faculty positions at institutions that do not offer tenure are all non-tenure track by default, but different types of contracts may still exist within the same

institution. Sometimes the opportunity for presumptive annual contract renewals or multi-year contracts follows a probationary period (e.g., 3 years). Advancement in rank can also be possible, provided you meet the relevant criteria set forth by your institution, although not all institutions allow this. Consult the faculty handbook, the fine print in your contract, and your department chair for details on expectations for contract renewal, procedures regarding non-renewal, and what level of due process you can expect. Frankly, this is prudent for faculty in tenure-track appointments too!

At institutions employing both tenure- and non-tenure-track faculty, expectations and job duties assigned to the two tracks are often different. For example, non-tenure-track research faculty typically aren't regarded as fully independent, won't be offered a startup package or their own dedicated lab space, and may not be expected to teach. Rather, they often work with an established investigator, who usually provides at least some salary support. In contrast, tenure-track research faculty are expected to develop an independent, extramurally funded research program, as well as teach (the amount varies widely), as well as provide service to the university, peers, and the public. Whether non-tenure-track faculty are afforded the same level of autonomy and respect, and the same privileges as tenure-track faculty, or included to the same degree in department-level decision-making depends on concrete factors such as institutional policies and potentially more flexible factors such as departmental culture and management philosophy.

As a faculty member rather than merely a staff member, some amount of service is typically expected, regardless of track. The amount may be minimal if you are research faculty exclusively paid for by a senior colleague's grants. That being said, my service activities as a non-tenure-track research faculty member were not all that different in nature and scope to my current commitments as a tenure-track faculty member. Service opportunities abound, and where there is a willingness, someone will find a way to make use of you!

How Common Is It to Have Tenure?

Not as common as it used to be. Based on National Center for Education Statistics (3), of all degree-granting post-secondary institutions with a tenure system in the United States, there has been a gradual decline in the percentage of full-time faculty with tenure, from 56.2% in 1993–1994 to 47.3% in 2015–2016. According to the Association of Chairs of Departments of Physiology 2017 Survey Results (2), of a total number of 945 faculty, 64% were tenured, a further 17.5% were tenure-eligible, with an almost identical percentage who were not tenure-eligible. This hefty proportion of tenured or tenure-eligible faculty may reflect the top-heavy nature of the academic ranks represented: 74% were associate or full professors (or chairs). Although these numbers are relatively high, the picture will undoubtedly change as institutions reconsider their approach to tenure, and as current tenured faculty retire. Some institutions are doing away with tenure altogether for new faculty hires; it's not just private or for-profit institutions that are doing this but state-funded institutions too.

What is the Advantage of Having Tenure?

In this brave new world of the gig economy, tenure may seem like a dusty relic of a distant past to many people. But it has its benefits. Tenure bestows a far higher level of security in your ongoing employment than what is offered in most other professions. For many in academia, there will always be a sense of achievement and prestige associated with being tenured. Once you have tenure, it can serve as a bargaining chip of sorts if you explore an employment offer at another institution. For teaching faculty, tenure allows you to try out new approaches without fear that negative student evaluations will impact your contract renewal. For most research-focused faculty, tenure isn't prized so much for its implied freedom to express controversial ideas or to speak truth to power. Rather, the key advantage is that it buys you time to turn your funding fortunes around if your grants run out. This has undoubtedly been a boon to many mid-career and more senior scientists, but while your job might stay safe, your full salary might not.

So, Tell Me More About Salary

Many institutions have a system to reward faculty for covering part or all of their salary with grant dollars, although such rewards may only be offered to tenure-track faculty. Rather than focusing on possible bonuses, let's focus on your regular salary level. Here are four factors to think about, regardless of tenure status or eligibility:

1. **What proportion of the year does your contract cover?** Is it 12 months, or does the institution guarantee somewhere in the 9- to 11-month range? If less than 12 months of salary is guaranteed, are you expected to provide the balance through extramural grant funding,

if research is part of what you do? For educators on 9-month contracts, is there an option for you to participate in teaching over the summer to make up the difference, should you want to?

2. **Is 100% of your full salary guaranteed, or just some portion?** If it is just a portion, is that a percentage or a fixed dollar amount? If it is a fixed dollar amount, is it the same for everyone or is it tiered by academic rank? Given your personal financial situation and the local cost of living, how comfortable would you feel about taking home only that fixed amount or only that percentage of your total salary? Seriously consider this question. Finances are tough in most academic environments, so dropping to that base at some point of your career is a very real possibility. One argument I have heard in favor of guaranteeing a fixed base amount and adding a flexible amount determined by extramural funding is that the total amount of money you are eligible to earn might be higher than if your salary was guaranteed but fixed. I should point out that the person putting this argument to me was a financial administrator rather than a scientist. Although a compensation plan including both fixed and variable components could be advantageous for very successful, often well-established investigators, would it be a good deal for you? And even if it is a good deal right now, will it still be a good deal if a current grant ended and it took a while to get the next one?

3. **If you are in a research-oriented position, what percentage of your salary are you expected to cover through extramural funding?** Is that expectation put in writing in your contract, or is it more of a friendly guide to keeping the dean happy? In medical schools and tier 1 research universities, expectations of upwards of 50% salary coverage are common. That being said, you might be interested to learn that the Association of Chairs of Departments of Physiology 2017 Survey Results also indicated that, for reporting departments, on average only 34% of total faculty salary was derived from research grants (excluding fringe benefits cost) (2). Ask what the expectation is at your prospective institution and find out how rigorously it is enforced. Has this been a major factor in cases where faculty contracts were not renewed or people were not granted tenure? Be realistic about your chances for success in measuring up to the standard, whatever that might be. Less drastic consequences of not meeting extramural salary support targets could be an increase in teaching or clinical duties, or a pay cut, which brings me to our next point.

4. **Can the institution decrease your salary?** There used to be a presumption that tenure meant you kept 100% of your salary no matter what. Rules are being written or rewritten to change this. Indeed, lawsuits have been brought over whether a tenured faculty member's salary can be involuntarily reduced for not meeting external funding requirements (4). Institutions of several faculty I spoke to already have procedures in place to reduce salary if targets for salary coverage are not met, for both tenured and non-tenured faculty. Do ask prospective employers whether salary reductions could occur, and if so, how rapidly and by how much.

How Secure is a Tenured Position, Really?

Most tenured faculty I have spoken to actually don't view their positions as fully secure, especially if there is a loss of grant funding. The thinking goes, you might be tenured, but your work situation may become untenable. Incentives for faculty to investigate employment opportunities elsewhere include salary cuts, taking away lab space, being moved into a smaller office, and increasing unenjoyable administrative assignments or teaching duties. Under-performing "deadwood" is an often-cited downside to allowing tenure at all, with the larger salaries of senior faculty gobbling up funds that could be used to support other initiatives or younger, hungrier (and cheaper) faculty. Many institutions do have a process of reevaluating tenured faculty, called post-tenure review. How frequently or rigorously post-tenure review is applied varies. It might be on the books, but seldom used. At least until now. This is an area that university administrators are increasingly looking to as a means to provide more flexibility in how they handle tenured faculty who do not meet expectations, whatever those expectations are. Dissolving a department or program can also allow your institution to divest itself of tenured faculty assigned to that department. Tenure might not really be forever.

What About Losing Your Position if You Aren't Tenured Yet, or Aren't in a Tenure-Track Position?

As much as no one wants to think about being let go, do educate yourself on the circumstances under which this might occur and find out what the timeline and process would be. Untenured tenure-track faculty may be on a

fixed contract or yearly renewal that would allow for 12-months' notice prior to termination. Employment contracts of faculty who are not tenure track may allow for a much more rapid severance process, especially if the funding supporting the position runs out (90 days' notice at my institution). Other faculty might be on 9- or 12-month contracts that their institution may decline to renew without explanation, and with much less notice.

Coming back to the "nicest" of these termination scenarios, having 12 months to find a new job is extraordinarily generous compared with most industries. However, if you are trying to find a new faculty position, this can be a slow process with limited openings and fierce competition. Depending on what time of year it is, there could be a long delay before a start date timed to coincide with the new academic year, or you might have missed the window to apply for positions commencing in the coming year. Community colleges running on quarter systems might offer shorter lead times on start dates. Of course, you could well use non-renewal of your academic contract as an opportunity to explore the world of possibilities outside the ivory tower!

If Tenure Isn't on the Table, What Should You be Thinking About?

Several faculty indicated that factors such as how supportive the environment is are more important than tenure per se. If research is your primary focus, the ability to maintain funding for your lab is a far more practical concern than tenure in any case. Other practical concerns are the length of contract, expectations to be met for having it continued, and how much notice you will be given if the institution decides not to renew. If you are comparing similar offers from one institution that does offer tenure and another that doesn't, are there any financial or other perks available to offset the lack of a possible lifetime appointment?

With the demise of tenure at some institutions, and implementation of stricter post-tenure review policies at others, institutions will need to consider the challenges that these pose to recruitment and retention of faculty, at least for as long as tenure remains on the table elsewhere and is perceived as valuable. Best of luck to all faculty who are navigating this shifting landscape, now and into the future. ●

Acknowledgments

I thank the many friends and colleagues out there in faculty-land for sharing their insights and experiences with me during the writing of this article.

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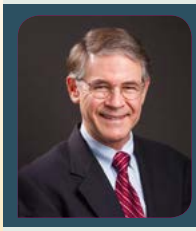
Erika Boesen Biography

Erika Boesen received her BS (Hons.) and PhD in physiology from Monash University, Australia, before moving to the then Medical College of Georgia in Augusta, GA in 2005 to continue her research career in renal physiology and pathophysiology. After completing her postdoctoral training and serving as a research faculty member for 4 years, Erika was recruited to the University of Nebraska Medical Center as a tenure-track assistant professor in 2012. Currently an associate professor, Erika enjoys the juggling act of research, teaching graduate and health professions students, and providing service within and outside her institution. A past member of the American Physiological Society's Career Opportunities in Physiology Committee, Erika is the current Renal Section representative on the Committee on Committees.

Experimental Biology

Experimental Biology 2019 Distinguished Lectures



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

Peter Aronson

Yale University School of Medicine

From Salt to Stones to CKD: Anion Transporters in Health and Disease

Sunday, April 7, 2019, 5:30 PM

Henry Pickering Bowditch Award Lecture

Jennifer Pluznick

Johns Hopkins University

Functional Roles for Orphan GPCRs in the Kidney

Monday, April 8, 2019, 5:30 PM

Robert M. Berne Distinguished Lectureship of the APS Cardiovascular Section

Rong Tian

University of Washington

Cardiac Metabolism: An Odyssey of Energy and Beyond

Monday, April 8, 2019, 8:30 AM

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

David Dawson

Oregon Health Sciences University

Membranes, Music and Economics: What I Learned from CFTR, Bob Dylan and Adam Smith

Sunday, April 7, 2019, 3:30 PM

Joseph Erlanger Distinguished Lectureship of the APS Central Nervous System Section

Tracy Bale

University of Maryland School of Medicine

Parental Stress and Germ Cell Epigenetic Contributions to Offspring Development

Monday, April 8, 2019, 3:30 PM

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

Terrie Williams

University of California, Santa Cruz

On the Cardio-Neural Trail of Stranded Whales and Wounded Warriors: An August Krogh Investigation

Monday, April 8, 2019, 3:30 PM

Supported by Novo Nordisk Fonden

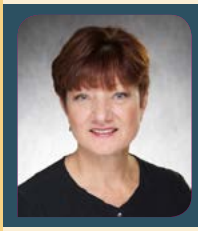
Solomon Berson Distinguished Lectureship of the APS Endocrinology and Metabolism Section

Juleen R. Zierath

Karolinska Institutet

Epigenetic Control and the Circadian Clock: Turning Back Time on Diabetes Pathogenesis

Monday, April 8, 2019, 3:30 PM



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

Sue Bodine

University of Iowa

Skeletal Muscle Atrophy: Multiple Pathways Can Achieve the Same Outcome

Monday, April 8, 2019, 1:30 PM

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

Nicholas LaRusso

Mayo Clinic

Monday, April 8, 2019, 3:30 PM

History of Physiology Lecture

James Bassingthwaighte

University of Washington

Ancel Keys Lambasted at the Faraday Society Meeting of 1937!

Tuesday, April 9, 2019, 1:00 PM

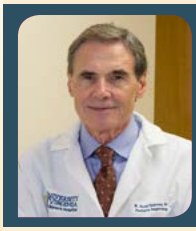
Carl Ludwig Distinguished Lectureship of the APS Neural Control and Autonomic Regulation Section

Susan Barman

Michigan State University

What Can We Learn about Neural Control of the Circulation by Studying Rhythms in Sympathetic Nerve Activity?

Monday, April 8, 2019, 1:30 PM



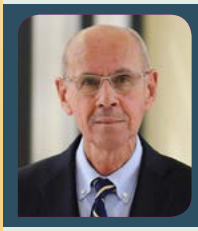
Carl W. Gottschalk Distinguished Lectureship of the APS Renal Section

R. Ariel Gomez

University of Virginia School of Medicine

Regulation of Renin Cell Fate in Homeostasis and Disease

Monday, April 8, 2019, 3:30 PM



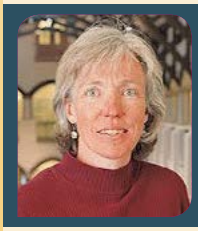
Julius H. Comroe, Jr. Distinguished Lectureship of the APS Respiration Section

Sadis Matalon

University of Alabama, Birmingham

Heme as a Central Mediator of Acute and Chronic Lung Injury

Tuesday, April 9, 2019, 1:30 PM



Claude Bernard Distinguished Lectureship of the APS Teaching of Physiology Section

Mary Pat Wenderoth

University of Washington

Evidence Based Teaching: So That All Students May Learn

Monday, April 8, 2019, 8:30 AM

Supported by APS Strategic Partner ADInstruments

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

Jennifer S. Pollock

University of Alabama at Birmingham

There's NO Place Like Home(ostasis)

Sunday, April 7, 2019, 3:30 PM

APS Nobel Prize Award Lecture

Peter Agre

Johns Hopkins School of Public Health

Tuesday, April 9, 2019, 5:30 PM

Experimental Biology 2019

April 6–9, 2019, Orlando, FL

www.apsebmeeting.org

We are excited to present the tentative educational program for the APS annual meeting held in conjunction with Experimental Biology (EB) 2019 in Orlando.

NEW this year: The 2019 annual meeting features a compressed, 4-day meeting schedule from Saturday, April 6 to Tuesday, April 9. The daily schedule for APS will be:

7:00 AM to 8:00 AM	Professional development sessions
8:30 AM to 10:00 AM	Oral scientific sessions
10:15 AM to 12:15 PM	Poster presentations
12:15 PM to 1:30 PM	Free time for lunch and visiting exhibits
1:30 PM to 3:00 PM	Oral scientific sessions
3:30 PM to 5:00 PM	Oral scientific sessions
5:30 PM to 6:30 PM	Plenary named lectures

We hope you find the new meeting format valuable and that you take advantage of the many collaborative, cross-society offerings, such as the plenary Tang Award Prize lecture, the EB-wide Opening Reception, and the joint APS/ASPET President's Symposium Series held daily throughout the meeting. Submit an abstract, register, reserve your housing, and more on the EB 2019 website (*www.experimentalbiology.org*). We can't wait to see you in Orlando!

PHYSIOLOGY PLATFORM SESSIONS

Saturday, April 6, 2019

Morning	Afternoon	Afternoon/Evening
8:00 AM–12:00 PM *Education Committee Refresher Course* Beyond the Weight Room: The Importance of Skeletal Muscle in Health and Disease *(Supported by APS Strategic Partner ADInstruments)* **Merritt/Durocher**	12:00–1:00 PM *MCS Mentoring Lunch*	2:00–5:00 PM *WEH Section* Trainee Award Finalists and Data Diuresis
9:00 AM–5:00 PM *ETG Pre-EB Meeting Conference* **TBD**	1:00–2:30 PM *AFMR Symp* Alzheimer's Disease: Many Failed Trials so Where Do We Go from Here? **Reiss/Stecker**	3:00–4:30 PM *AFMR Symp* New Method and Models to Study Human Metabolism with Stable Isotope Tracers **Cree-Green/Parks**
9:30 AM–11:30 AM *MCS President's Symp* Clinical Perspectives on the Microcirculation **Pierce-Cottler/Mendelson**	1:00–3:00 PM *Science Policy Committee Symp* A Role for Professional Societies in Addressing and Preventing Sexual Harassment in the Sciences **Goulopoulou/Wilson**	3:00–5:00 PM *NCAR Section Awards Session* Data NCARnation
	1:00–3:00 PM *APS-ASPET Presidential Symp Series on Microbiome* Workshop on Microbiome Research: What You Need to Know **Patterson/Hullar**	3:15–5:15 PM *Techniques Workshop* Writing Good Multiple Choice Questions: A Hands-on Workshop **Silverthorn**
	1:00–5:00 PM *Physiological Omics Group 6th Annual PG Conference*	3:30 PM–5:30 PM *MCS Symp*
	1:00–3:00 PM *MCS Symp*	6:00–7:00 PM *Tang Prize Award Lecture* **Druker**
		6:00–8:00 PM *MCS Poster Discussion and Reception* **Pierce-Cottler**

Sunday, April 7, 2019

7:00–8:00 AM Professional Development Hour	8:30–10:00 AM	1:30–3:00 PM	3:30–5:00 PM
Trainee Advisory Committee Symp Marketing Yourself for a Successful Career I **Obi/Zarate**	*CV Section FT* Kaley Award FT: The Microcirculation and Its Many Cells that Contribute to Tissue Repair **Kubes**	*Physiological -Omics Group Symp* Metagenomic and Metabolomic Studies of Host-Microbiotal Contributions to Health and Disease **Joe/Abais-Battad**	*MCS Landis Award Lecture and Business Meeting*
Women in Physiology Committee Symp Career Planning: No Scientist Left Behind **Nichols/Maura Porta**	*Resp Section Symp* Central Control of Breathing: Gliocentric Mechanisms **Funk/Mulkey**	*Hypoxia Group Symp* The Lahari Cherniak Lecture Symp **Wilson**	*EB Symp Series* Carotid Body Sensing—More than Just an O2 Sensor **MacFarlane**
Career Opportunities in Physiology Committee Symp The Hidden Job: Aspects of a Physiology Career You May not be Aware of **Becker/Trimby**	*Pubs Committee Workshop* Publications 101 Workshop **Sigmund/Scheman**	*CNS Section Symp* Interaction of Cardiovascular Disease and Neuro-cognitive Impairment. **Wallace**	*GIL Section Symp* Innovative Imaging Approaches in Gastrointestinal Epithelial Biology **Edelblum**
	Renal Section FT Young Investigator Award: At the Nexus of Circadian Biology and Renal Physiology **Gumz**	*Cell Section FT* pH Homeostasis and Acid-Base Transport **Parker/Romero**	*EM Section Symp* Hormonal Influences on Tissue Remodeling **Lindsey/Gohar**
	NCAR Section FT Young Investigator Awards **TBD**	*PIC Symp* SGLT2 Inhibitors: From Basic Physiology to Clinical Success **Gonzalez Villalobos/Pati**	*NCAR Section Symp* Autonomic Control of Metabolism **Aubert**
	EEP Section Symp Exercise and Heat Therapy: Shared Molecular Targets and Cardiometabolic Benefits **Minson**	*ETG Ussing Lecture FT* **Keely**	*Nutrition Group Symp* Nutrition as a Biological Variable: Considerations for the Future of Physiology Research **Anthony/Baum**
	WEH Section Symp Translational Models of Renal and Cardiovascular Disease: Informing Human Health or Not? **Harrison-Bernard/Osborn**	*NCAR Section FT* Autonomic Anti-inflammatory Mechanisms: Which Branch Trumps? **Harwani/Pham**	*Renal Section Symp* Mechanism of Hypertension-induced Kidney Damage **Li/Prieto**
	CV Section FT Cardiac ECM Niche in Health and Disease **Dixon/Griffiths**	*TPIG FT* Translational Physiology Highlights **Young**	3:30–4:30 PM *Cell Section Davson Lecture* **Dawson**
	CV Section Symp Neurodegeneration and Heart Failure: An Intimate Connection **del Monte/Wold**	*CV Section Symp* Survived a Complicated Pregnancy? Maternal, Postpartum, and Fetal Cerebrovascular Risks **Warrington/Cipolla**	*Respiration Section FT* Sex and Gender in Respiratory Physiology **Silveyra/Prakash**

Sunday, April 7, 2019, continued

	CEP Section FT Trainee session **Crossley**	*EM Section FT* Sex Specific Differences in Obesity Induced Hypertension **Barnes/Primeaux**	*Sex/Gender Symp* Sex and Metabolic Health: Emerging Challenges and Discoveries **Alexander/Rice**
	Teach Section FT Innovative Techniques for Teaching Health Sciences to Increase Retention and Mastery Learning **Jones/ElSayed**	*CV Section FT* Aging, Exercise, and Heart Failure: Common Connections and New Targets **Emter/Wrann**	*PanAm Symp* Common Pathways of Angiotensin on Cardiovascular and Respiratory Responses Induced by High-Fat-Diet, Hypoxia, and Renal **Hypertension** **Colombari/Del Rio**
	EB Symp Series Aquaporins – More than Water under the Bridge **Mamenko/Ilatovskaya**	*Teaching Section Symp* Using Writing to Teach and Assess Undergraduate Physiology Students **Anderson**	*EEP Section Symp* Nrf2 as a Promising Target for Increasing Healthspan and Diminishing Aging-Related Chronic Diseases **Hamilton**
	APS/ASPET President's Symp Series on Microbiome Gut Microbiome and Metabolic Disorders **Sands/Morgan**	*EEP Section Symp* The Role of Skeletal Muscle Damage in Adaptive Remodeling **McCarthy/Murach**	3:30–4:30 PM *WEH Section Starling Lecture* **Pollock**
			4:30–5:30 PM *WEH Section Lecture* New Investigator Award
			5:30–6:30 PM Physiology in Perspective: The Walter B. Cannon Award Lecture **Aronson**

Monday, April 8, 2019

7:00–8:00 AM Professional Development Hour	8:30–10:00 AM	1:30–3:00 PM	3:30–5:00 PM
Trainee Advisory Committee Symp Marketing Yourself for a Successful Career *II* **Obi/Zarate**	8:30–9:30 AM *CV Section Berne Lecture* **Tian**	1:30–2:30 PM *NCAR Section Ludwig Lecture* **Barman**	3:30–4:30 PM *EM Section Berson Lecture* **Zierath**
Women in Physiology Committee Symp Career Planning: No Scientist Left Behind (Monday) **Nichols/Maura Porta**	*EEP Section FT* EEP Impact Award FT: Modulation of Systemic and Tissue Metabolism via Differences in Activity and Fitness **Morris/Rector**	1:30–2:30 PM *EEP Section Adolph Lecture* **Bodine**	3:30–4:30 PM *CEP Section Krogh Lecture* *Supported by Novo Nordisk Fonden* **Williams**

Monday, April 8, 2019, continued

Careers in Physiology Committee Symp The Hidden Job: Aspects of a Physiology Career You May not be Aware of **Becker/Trimby**	8:30–9:30 AM *Teaching Section Bernard Lecture* Supported by APS Strategic Partner ADInstruments **Wenderoth**	*GIL Section FT* Gastrointestinal and Liver Physiology and Disease **Zachos**	3:30–4:30 PM *GIL Section Davenport Lecture* **LaRusso**
	Cell Section FT Molecular Mechanisms of Cellular Signaling and Transduction **Blazer-Yost/Teal**	*CAC Symp* APS Chapters: Why, How, What of Starting New Chapters **Hopper**	*Cell Section Symp* New Frontier in Direct Effects of Lipids and Lipid Metabolic Defects on Membrane Proteins **Jiang/Greenberg**
	WEH and ETG FT Hot Topics in Water and Electrolyte Homeostasis and Epithelial Transport **TBD**	*History Symp* Updating the History of Insulin: Novel Insights into Regulation of Release, Delivery, and Functions **Molina/Souza-Smith**	*WEH Section FT* Environmental and Epigenetic Contributions to Disease Origin **TBD**
	TPIG Symp Type 2 Diabetes: A Metabolic Karma **Sepulveda/Chen**	*EM Section Symp* Crosstalk between Metabolic Disorders and Mitochondrial Oxidative Stress **Dikalov/Sack**	*CV Section FT* Wiggers Award Featured Topic **Chien**
	CNS Section FT Effects of Diet on Emotion and Motivated Behavior **Silberman**	*Renal Section FT* Advances in Renal Physiology I **Ellison**	*Respiration Section Symp* ENaC and Fluid Transport across Airway Epithelia **Althaus/Butterworth**
	EB Symp Series Oxidative Stress and Post-translational Modification of Protein Thiols in Cell Signaling **Helms**	*EB Symp Series* Brown Adipose Tissue – The Fat that Makes you Thin **Symonds**	3:30–4:30 PM *Renal Section Gottschalk Lecture* **Gomez**
	NCAR Section Symp Neural Circulatory Mechanisms Linking Sleep Loss to Hypertension **Limberg/Somers**	*CV Section FT* CV Section Young Investigator Competition **Faulkner/Dorrance**	3:30–4:30 PM *CNS Section Erlanger Lecture* **Bale**
	Respiration Section FT Mapping The Lung: Leveraging Emerging Technologies To Better Understand Pulmonary Disease **Beers/Bastarache**	*WEH Section FT* Therapeutic and Adverse Effects of Pharmacological Agents on Integrative Physiology and Pathophysiology **TBD**	*EEP Section FT* Mechanisms underlying the Greater Propensity for Cardiovascular Disease in Populations with Elevated Risk **Keller/Brothers**
	Muscle Biology Group FT Hot Topics in Muscle Biology **Wang/Mitch**	*CV Section Symp* Thoracic and Abdominal Aortic Aneurysm: From Models and Mechanism to Clinical Trials and Therapy Updates **Kassiri/Liu**	*CV Section FT* Cardiac and Peripheral Vasculopathies: Emerging Biomarkers, Imaging and Treatments **LeBlanc/Kaufman**

Monday, April 8, 2019, continued

	Respiration Section FT Perinatal reprogramming of respiratory control **Huxtable/Baker**	*CEPS Symp* From The Brainstem to the Heart: Cardio-Respiratory Control in Ectothermic Vertebrates **Janes/Conner**	*Respiration Section FT* Emerging Therapeutics for Respiratory Dysfunction **Turner/Falk**
	APS/ASPET President's Symp Series on Microbiome Gut Microbiota: A Chemical Factory **Jeong/Pluznick**		5:30–6:30 PM Henry Pickering Bowditch Award Lecture **Pluznick**

Tuesday, April 9, 2019

7:00–8:00 AM Professional Development Hour	8:30–10:00 AM	1:30–3:00 PM	3:30–5:00 PM
Trainee Advisory Committee Symp Marketing Yourself for a Successful Career III **Obi/Zarate**	*Muscle Biology Group Symp* Muscle-Derived Extracellular Vesicles in the Regulation of Metabolic Health and Disease **Lark**	1:00–2:00 PM *History Lecture*	*EM Section* Awardees Session **Romero/Sidossis**
Women in Physiology Committee Symp Career Planning: No Scientist Left Behind **Nichols/and Maura Porta**	*Physoc/APS Joint Symp* Physiology of Obesity: From Mechanisms to Medicine **Aldiss/Lewis**	1:30–2:30 PM *Respiration Section Comroe Lecture* **Matalon**	*Physoc Symp* The Clinical and Metabolic Benefits of Exercise for People with Type 1 Diabetes **Wagenmakers**
Careers in Physiology Committee Symp The Hidden Job: Aspects of a Physiology Career You May not be Aware of **Becker/Trimby**	*CV Section Symp* Protein Degradation Mechanisms: A Compartmentalized Affair in Cardiac Muscle and Disease? **Sheik/Liang**	*GIL Section FT* *Control of cellular organelle function, metabolism, and injury in liver and intestinal diseases* **Li**	*ETG Hebert Lecture* **McDonough**
	Cell Section FT Ion Channels, Solute and Molecular Transporters In Heath and Disease **Baines/Althaus**	*Cell Section Symp* *Emerging Roles of the Cytoskeleton in Striated Muscle* **Hawke/Perry**	*NCAR and CNS FT* Hot Topics in Neuroscience **TBD**
	NCAR Section FT Autonomic Function in Normal and Preeclamptic Pregnancies **Stachenfeld**	*WEH Section FT* Hormones and Receptors in Homeostatic Control of Physiology and during Allostasis in Pathophysiology **TBD**	*Nutrition Group FT* Hot Topics in Nutrition **Thalacker-Mercer/Borsheim**
	CV Section FT Cardiovascular Metabolism in Diabetes **Hill/Harmancey**	*CNS Section FT* New Advances in CNS Physiology **Wainford**	*WEH Section FT* Effect of Diet on Metabolism, Cardiovascular and Renal Physiology and Pathophysiology **TBD**

Tuesday, April 9, 2019, continued

	EEP Section FT Examining Physiological Mechanisms Using Environmental Stressors **Johnson/Schlader**	*NCAR Section FT* Regulation of Muscle Sympathetic Outflow during Exercise **Floras/Millar**	*CV Section FT* Inflammation and Leukocyte Biology in Cardiovascular Disease **Halade/de Castro Brás**
	Renal Section FT Advances in Renal Physiology II **Layton**	*EB Symp Series* Metabolism, Organ Crosstalk, Microbiome and Mechanisms **Sun**	5:30–6:30 PM Nobel Prize Award Lecture **Agre**
	Respiration Section Symp Musculoskeletal Afferents and the Control of Breathing **Streeter/Fuller**	*Renal Section Symp* Mighty Concepts in Mitochondrial Biology in the Kidney **Mallipattu/Li**	
	GIL Section FT John Forte Plenary Session **Frey**	*CV Section Symp* AJP-Heart Editors Symposium **Zucker/DeLeon Pennell**	
	EB Symp Series Crosstalk among Myofibers, Microvessels and Motor Nerves during Skeletal Muscle Regeneration **Segal**	*EM Section FT* Noncoding RNAs: Emerging Functions in Metabolism and Diseases **Huang/Zhang**	
	Resp Section FT Inflammasome Activation in Diseases of the Lung **Waters/Yeligar**	*Hypoxia Group FT* Hot Topics in Hypoxia **TBD**	
	APS/ASPET President's Symp Series on Microbiome Microbiota in Action: The Gut and Beyond **McCabe/Cui**	*CEP Section Symp* Causes and Consequences of Inter-individual Physiological Variation **Rees**	

New Session Offered for EB 2019—Come Learn How to Start an APS State Chapter

In support of strengthening and growing the chapter program, APS has granted the Chapter Advisory Committee (CAC) a brand new session for EB 2019. With a rich history and strong contribution to achieving the mission of the APS, a chapter in every state makes sense! To learn more about how to initiate a chapter or share ideas with other active Chapters, please plan to attend an informational workshop on Monday, April 8 from 1:30 to 3:00 PM in room W311A of the Orange County Convention Center, Orlando, Florida.

The APS Chapter Program was launched over 23 years ago—yet many members remain unaware of the opportunities and excitement generated by chapters. At the "grass roots" level, chapters strive to achieve the mission of the APS and are in many ways exemplars of success in doing so. For example, in 2017, 11 chapters held APS-supported annual meetings, with over 1,150 attendees. During these meetings, 100 trainees gave oral talks, and 430 presented posters. There were a total of 83 awards given. Just imagine all those proud young scientists holding up their certificates during the photo op!

Hosting a chapter within your state provides the opportunity for physiology-minded folks to engage and energize. In addition to regular conference programming, chapter meetings are innovative and include "minute to win it," "great debates," and a variety of breakout sessions that address diverse topics including social media, job hunting, and teaching tips. Many chapter meeting attendees are folks unable to attend larger meetings requiring greater travel and expense. For those who do attend larger meetings, chapters are a safe place to develop presentation skills and get feedback from the scientific community. In addition to annual meetings, chapters have the opportunity to apply for APS grants and put the money to work advancing physiology in a number of creative ways. To sum it up, chapters are involved in career development and networking, advocacy and outreach, and development of trainees and faculty of all levels—including high school!

Chapters are a wonderful place to establish a physiology family. State chapter members include students, faculty, and industry partners who look forward to meeting with their peers from within the state. After attending chapter meetings in five different states, I think it is safe to say that the food and fellowship are beyond compare.

If your state does not currently have an active chapter, I would personally invite you to consider starting one. For trainees and new faculty, time and energy applied to beginning a chapter will help cultivate personal and professional connections, and allow you to develop organizational and professional skills—all while exerting HUGE impact in your chosen discipline. For more senior folks, helping to establish a chapter will provide opportunities for trainees and early career faculty close to home. You can be assured that, in time, you will be able to move to a mentor role as the baton is passed (often to your former students!). If your state already has an established chapter, consider getting involved—your students will thank you! (See APS website for full list of chapters.)

APS chapters offer many opportunities for professional growth and engagement. Consider attending the EB chapter session—mark your calendar now—and come see for yourself how energized folks are about chapters! ●

People and Places

Erica Wehrwein

Erica Wehrwein Named Associate Professor at Michigan State University

APS member Erica Wehrwein has been promoted to associate professor in the department of physiology at Michigan State University. Wehrwein is an active member of the Teaching of Physiology section, past chair of the Trainee Advisory Committee, and co-founder of the Physiology Majors Interest Group. ●

Jeff Sands

APS President Jeff Sands Receives Honorary Degree from Aarhus University

APS President Jeff Sands was awarded an honorary doctorate at Aarhus University in Denmark in September 2018. Sands, a leader in experimental renal research, has collaborated with Aarhus University and Aarhus University Hospital for more than 20 years. Sands is the Juha P. Kokko Professor of Medicine and Physiology and director of the renal division at Emory University School of Medicine. Learn more at *http://www.emorydailypulse.com/2018/09/17/jeff-sands-honored-with-honorary-doctorate-from-aarhus-university/.* ●

News from Distinguished Physiologists

Letter to Luis G. Navar

Peter Scheid writes: "Thank you very much indeed for your kind e-mail and your wishes on the occasion of my getting very old. Actually, I don't really feel like 80, I must admit. My four daughters and the 13 grandchildren feel that they have a quite vivid and "young" father/grandfather. I am truly grateful!

"I am now rather remote from active physiology, having had to shut down my lab when I retired some years ago. Rather, I have focused on a social activity: Gawad Kalinga (see, if you have a minute, *www.gawad-kalinga.org*), a charitable society with which we build houses to re-socialize the very poor in the Philippines. We work with the renowned Jesuit University *Ateneo de Manila*. This s very demanding but even more rewarding. [For more, see "Gawad Kalinga e.V.: Supporting Self-Help for the Poorest of the Poor in the Philippines" on p. 3.]

"I wish you and your colleagues at APS all the best in your wonderful endeavors. I have always been very proud of being able to calling myself a member of this wonderful Society." ●

Reports

2018 APS Conference: Cardiovascular, Renal and Metabolic Diseases: Sex-Specific Implications for Physiology

Knoxville, Tennessee, September 30–October 3, 2018

APS Travel awardees

The 2018 APS Conference: Cardiovascular, Renal and Metabolic Diseases: Sex-Specific Implications for Physiology was the sixth in a series of conferences inaugurated in 2001 related to the impact of sex differences on physiological and clinical research. This 3-day meeting focused on sex-specific implications in the physiology of brain, heart, and kidneys. The conference structure—in keeping with the goal of the APS Conference program—provided a forum to promote the exchange of ideas and future collaborations across disciplines that do not otherwise meet. Researchers from multiple areas of physiology and clinical research met to focus on how biological sex impacts physiology and pathology. In addition, trainees and early career investigators were encouraged to design studies that were included for both oral and poster presentation.

The meeting venue, Crowne Plaza Knoxville Downtown Hotel, was located next to Knoxville's famed Market Square, established in 1854 as a market place for regional farmers, now hosts shops, restaurants, and also an outdoor event venue. The meeting schedule allowed time for attendees to experience true Appalachian hospitality and cuisine offered in Market Square and the surrounding area.

Jennifer Sullivan (Augusta University) and Michael Ryan (University of Mississippi Medical Center) were the Conference Organizing Committee Chairs. The Organizing Committee members were Eric Belin de Chantemele (Augusta University), Christopher DeSouza (University of Colorado), Delisa Fairweather (Mayo Clinic), Louise Kenny (University College, Cork, Ireland), Franck Mauvais-Jarvis (Tulane University), David Pollock (Unviersity of Alabama at Birmingham), Jane Reckelhoff (University of Mississippi Medical Center), and Gina Yosten (Saint Louis University).

The conference was attended by 108 total registrants: 37 (34%) of registrants were represented by invited chairs, speakers, and members of the organizing committees, 31 (29%) were students, and 8 (7%) were postdoctoral fellows. Moreover, 27 (25%) of attendees identified themselves as APS members, and the remaining 5 (5%) registered as nonmembers. Table 1 (below) shows the breakdown of the different registration types. The conference attracted 12 (11%) registrants from outside the United States. The international attendees represented countries from Canada, Dominica, Germany, Hungary, India, Ireland, Norway, South Africa, and Spain. A total of 72 volunteered abstracts were submitted for the conference. Of the first authors on submitted abstracts, 23 (32%) were graduate students, 2 (3%) were medical students, 18 (25%) were postdoctoral fellows, 21 (29%) were investigators, and 3 (4%) were undergraduate or undefined.

Marjorie Jenkins presents a national update on sex- and gender-based medicine, education, and policy

The conference program consisted of eight symposia, two poster sessions, and one plenary lecture. The plenary lecture was presented by Marjorie Jenkins of Texas Tech University Health Science Center and FDA Office of Women's Health, and provided an overview of national initiatives in sex- and gender-based medicine. All sessions had lively Q&A time for

the audience to share ideas and dig deeper into the issues specific to their area of research. All of the symposia included young-investigator and trainee presentations selected from the submitted abstracts. The conference schedule included an opening reception, daily group breakfasts, and a closing banquet, each designed to give attendees a chance to enrich their professional networks and receive some respite and refreshment during the 3 days of intense learning.

Poster presentation

The conference organizing committee selected 31 trainee travel awardees who were recognized during the closing banquet. The following individuals were presented with a certificate and cash prize: Mahmoud Abdelbary (Augusta University), Hijab Ahmed (University of North Texas, Health Science Center), Yasir Alsiraj (University of Kentucky), Reem Atawia (Augusta University), Kasey Belanger (Augusta University), Wael Eldahshan (University of Georgia), Jessica Faulkner (Medical College of Georgia at Augusta University), Anna Gams (The George Washington University), Olivia Gannon (Albany Medical College), Ellen E. Gillis (Augusta University), Eman Y. Gohar (University of Alabama at Birmingham), Autumn N. Harris (University of Florida), Teri Hreha (Washington University in St. Louis), Preethi Krishnan (Indiana University), Jessica Leete (Duke University), Eli Louwagie (Sanford School of Medicine-University of South Dakota), Stéphane Nemours (Vall d'Hebron Institut de Recerca), Ahmed Oloyo (College of Medicine, University of Lagos), Marissa Pier (University of Arizona), Dennis Pollow Jr. (University of Arizona), Lindsey Ramirez (Augusta University), Sarah Ray (Augusta University), Ibrahim M. Salman (Case Western Reserve University), Purnima Singh (University of Tennessee Health Science Center), Reham Soliman (University of Alabama at Birmingham), Megan Sylvester (University of Arizona), Lia Taylor (Augusta University), Marta Tejedor (Universidad Complutense Madrid), Edgar Torres Fernandez (University of Mississippi Medical Center), Joshua Uhlorn (University of Arizona), and Victoria Wolf (University of Mississippi Medical Center).

The APS Minority Travel Fellow Award, which is provided to encourage participation of underrepresented minority students in the physiological sciences, was awarded to the following recipients: Chelsee Holloway (Rutgers University), LaDonya Jackson (Medical College of Georgia), and Aline Souza (Georgetown University). With generous support from the APS, the fellowship provides reimbursement of all expenses associated with travel and participation in the conference.

The American Physiological Society and the Organizing Committee gratefully acknowledge the financial support provided though generous educational grants from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Heart, Lung and Blood Institute (NHLBI), University of Mississippi Medical Center Women's Health Research Center, *Clinical Science*, and the American Heart Association Councils on Kidney and Cardiovascular Disease, Basic Cardiovascular Sciences, and Hypertension. ●

Table 1. Registration statistics

Registration Type	Total	Percent
APS Member	27	25%
Nonmember	5	5%
Postdoctoral Fellow	8	7%
Graduate Student	31	29%
Invited Speaker/Chair	37	34%
Total	**108**	

The 2018 APS Intersociety Meeting: Comparative Physiology: Complexity and Integration

New Orleans, LA, October 25–28, 2018

The 2018 APS Intersociety Meeting: Comparative Physiology: Complexity and Integration was held in the bustling city of New Orleans, Louisiana, at the Astor Crown Plaza Hotel-New Orleans French Quarter. The meeting was well attend and was sponsored in part by 10 guest societies and other organizations including National Science Foundation, Society for Experimental Biology (SEB), Journal of Experimental Biology, Loligo Systems, Hannah Carey, Comparative Biochemistry and Physiology, Bioelectricity-Mary Ann Liebert Inc. Publishers, Physiological and Biochemical Zoology, Society for Integrative & Comparative Biology (SICB), and Sable Systems.

The meeting was organized and chaired by Dane Crossley (University of North Texas), along with an organizing committee composed of comparative physiologists with diverse research and organizational experience. Included in the organizing committee is Michael Hedrick (Cal State East Bay, Chair of the CEPS Steering Committee) and Scott Kirkton (Union College, a co-organizer of the 2014 APS Intersociety Meeting in Comparative Physiology). The other members of the Organizing Committee are:

- Lynn Hartzler (Wright State University)
- Allyson Hindle (Harvard University)
- Bridget Martinez (University of California, Merced)
- Tobias Wang (Aarhus University)
- Daniel Warren (St. Louis University)
- Caroline Williams (University of California, Berkeley)
- Cassondra Williams (University of San Diego)
- Arthur Woods (University of Montana)

The meeting program had a packed schedule of dynamic plenary lectures, concurrent symposia, workshops, interactive poster sessions, and networking opportunities that made the meeting a valuable experience for all attendees.

The meeting was attended by 273 total registrants, of whom 54 (20%) identified themselves as APS members. Table 1 shows the breakdown of the different registration types.

figure 1. Congratulations to the APS Abstract-based Travel Award winners. Each winner receives a certificate and travel reimbursement stipend.

Table 1. Registration statistics

	No. of Attendees	No. of Attendees
Invited Speakers/ Session Chairs	81	30%
APS Members	54	20%
APS Postdoc Members	8	3%
APS Student Members	51	19%
SEB Members	13	5%
SEB Postdoc Members	2	1%
SEB Student Members	15	5%
Nonmembers	22	8%
Postdoc Nonmembers	5	2%
Student Nonmembers	22	8%
Total Attendees	273	100%

This meeting mainly attracted comparative scientists and students from the United States, however, of the 273 registrants, 99 (36%) represented many countries from around the world. Table 2 depicts the breakdown of the different geographical regions that attendees came from.

Table 2. Registration geographic region statistics

Country	No. of Attendees	% of Attendees
Australia	6	2%
Austria	1	0%
Brazil	7	3%
Canada	57	21%
Chile	1	0%
China	1	0%
Columbia	1	0%
Denmark	10	4%
Germany	1	0%
Israel	2	1%
Italy	1	0%
Japan	1	0%
New Zealand	1	0%
Nigeria	1	0%
Norway	3	1%
Poland	1	0%
Taiwan	2	1%
United Kingdom	2	1%
Total Foreign Attendees	99	36%

The meeting program consisted of opening and closing plenary lectures, 16 concurrent symposia sessions, numerous oral presentation sessions based on submitted abstracts and the exciting Scholander Competition Finals, where 8 attendees participated. The audience was encouraged to share their ideas and thoughts with the speakers at the end of their talks. Regrettably, the trainee workshop was cancelled due to the lack of funding available for the trainees who proposed the session. The meeting also had several social activities, including a Welcome and Opening Reception, which was designed to give attendees a chance to meet with colleagues, create new friendships, and enjoy some hors d'oeuvres, desserts, and beverages after the opening plenary lecture. There were two afternoon poster sessions, which included the Best Poster Competition, where scientists presented their work and discussed their comparative work with other attendees. Snacks and a cash bar were available during the poster sessions as well. During the two poster sessions, there were three companies exhibiting: Sable Systems, Star-Oddi, and The Company of Biologists (JEB), as well as a material tables for the sponsoring companies to display a piece of literature. And last, there was a banquet and an awards ceremony at the conclusion of the meeting, which also included the closing plenary lecture.

A total of 252 abstracts were submitted for the meeting, with 181 of these abstracts programmed as either a poster or an oral presentation. The remaining 71 abstracts were submitted by invited speakers. Of the 181 programmed abstracts, 93 were programmed as oral presentations, and 88 were programmed as poster presentations.

Rosario A. Marroquin-Flores (Illinois State University) was the recipient of the APS 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. Thank you to Jose Vasquez-Medina (University of California, Berkeley) for mentoring Rosario A. Marroquin-Flores during the meeting.

The grand finale of the successful meeting was during the Closing Banquet and Awards Ceremony. During the banquet, Dane Crossley and Scott Kirkton thanked the attendees and all of the individuals involved in the various parts of planning the meeting. Specifically, Lynn Hartzler (Wright State University) was thanked for her efforts in coordinating the judging of all of the awards that were to going to be presented. Excitement mounted among the attendees as the 15 APS Abstract-based Travel Awardees were announced and congratulated.

Figure 1 shows the Abstract-based Travel Awardees. Next, Crossley and Kirkton announced the winners of the Poster Award Competitions. Catherine Ivy (McMaster University) was awarded the American Physiological Society Intersociety Meeting Best Poster Award; Timothy Healy (University of California at San Diego) was awarded the AJP Regulatory, Integrative and Comparative Physiology Poster Award; and Sulayman Lyons (McMaster University) was awarded the Sable Systems Poster Award. Winners of the poster awards received a certificate and a cash prize. The winners of the Poster Awards can be seen in Figure 2.

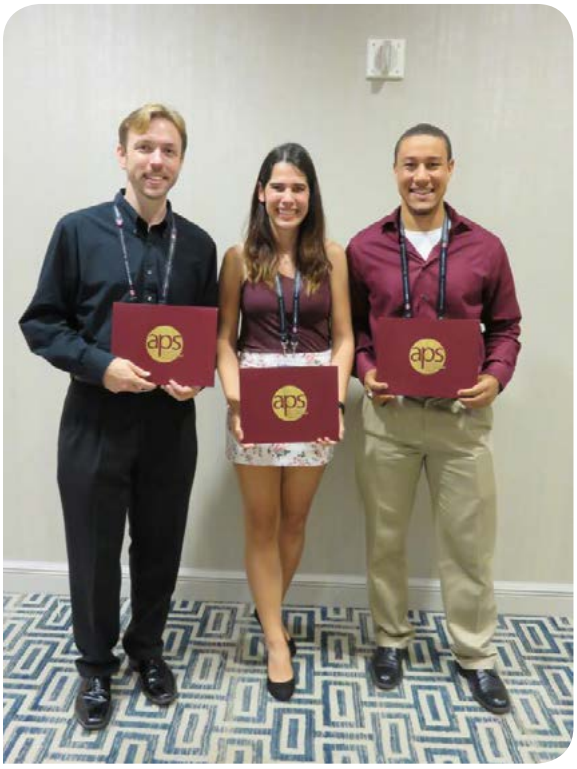


Figure 2. Congratulations to the Best Poster Awardees. From left to right: Timothy Healy (University of California at San Diego, AJP Regulatory, Integrative and Comparative Physiology Poster Award), Catherine Ivy (McMaster University, the American Physiological Society Intersociety Meeting Best Poster Award), and Sulayman Lyons (McMaster University, Sable Systems Poster Award). Each winner received a certificate and a cash prize.

Figure 3. Congratulations to the 2018 Comparative and Evolutionary Physiology Section (CEPS) Scholander Award Winners. From left to right: Alexander Clifford, Sinya Benyajati, Elizabeth Johnston, and Till S. Harter. Each awardee receives a cash prize and a certificate. The first-place winner also receives a 1-year complimentary membership to APS, reimbursement of advance registration fee, and a copy of the Per Scholander Biography Enjoying a Life in Science (Univ. of Alaska Press, 1990).

Finally, the time had come to announce the winner of the 2018 Comparative and Evolutionary Physiology Section (CEPS) prestigious Scholander Award. Sinya Benyajati (Chairperson of the APS Awards Committee) praised the excellent work presented by the eight competitors during the Scholander Award Competition, which was the final session of the meeting. After the deliberations of the Scholander judges, Elizabeth Johnston (University of Guelph, Canada) was awarded first place, followed by Alexander Clifford (University of Alberta, Canada) with second place, and Till S. Harter (University of British Columbia, Canada) with third place. As the 2018 Scholander Award recipient, Elizabeth Johnston receives a cash award, 1-year complimentary membership to APS, reimbursement of advance registration fee, and a copy of the Per Scholander Biography Enjoying a Life in Science (University of Alaska Press, 1990) in addition to a certificate. The second- and third-place winners also receive a cash prize and a certificate.

The American Physiological Society and the Organizing Committee gratefully acknowledge the financial support provided though generous educational grants from the National Science Foundation, Society for Experimental Biology (SEB), Journal of Experimental Biology, Loligo Systems, Hannah Carey, Comparative Biochemistry and Physiology, Bioelectricity-Mary Ann Liebert Inc. Publishers, Physiological and Biochemical Zoology, Society for Integrative & Comparative Biology (SICB), and Sable Systems. ●

Positions Available

Assistant Professor: The Arkansas College of Osteopathic Medicine in Fort Smith, AR is seeking an Assistant Professor of Physiology who will be responsible for developing interactive and didactic osteopathic medical education curricula centered around clinically relevant, systems-based physiological mechanisms of health and disease. Faculty duties will also include maintaining a viable scholarly/research agenda and engaging in institutional, community and professional service. *Essential functions and responsbilities*: Work in harmony within an interdisciplinary team of biomedical scientists and clinicians to develop novel interactive medical educational materials and curricula; develop didactic and interactive team-based learning activities in systems-based physiology within and outside primary areas of expertise; supervise, interact, and mentor osteopathic medical students in a variety of learning activities; develop and maintain a productive ongoing scholarly research agenda; functions as a faculty member of ARCOM including teaching, research, and service. *Qualifications and credentials*: Education and experience: *Minimum qualifications*: terminal degree (PhD) in Physiology or a closely related field; for Assistant Professor, minimum of 2 years of successful teaching experience as an instructor or course coordinator/director of a relevant scientific discipline at the undergraduate or graduate level; demonstrated productivity and contribution (e.g., presentations, publications, patents, etc.) to a relevant professional field; possess skills necessary to contribute to the establishment and formation of a Department of Physiology, Pharmacology, and Pathology at a new medical school. *Preferred qualifications*: postdoctoral experience in a biomedical research setting; established track record of directing/overseeing student trainees at the undergraduate or graduate level within a research setting; terminal degree (PhD) in Physiology or a closely related field; for Assistant Professor, 2 years academic experience as a full-time faculty member at a College of Osteopathic Medicine, College of Allopathic Medicine, College of Health, or Allied Sciences; excellent leadership and administrative skills, a demonstrated record of faculty mentorship and involvement in faculty development, and experience in medical and/or graduate education; experience in the development and implementation of courses in pharmacology, physiology, and pathophysiology and related topics, and demonstrated excellence in delivering course content to medical students; experience in team-based learning approaches to instruction; experience in teaching neurophysiology, renal physiology, and/or pathophysiology. Required knowledge, skills, and abilities: demonstrate proficiency in computer skills, i.e., Microsoft Office; display professionalism for the college in all communication and interaction; ability to maintain confidentiality and privacy; ability to prioritize and organize numerous and varied assignments; high-energy, versatile, self-directed. To learn more or to apply, visit *http://acheedu.org/employment-opportunities/* and click on this position title. If you need assistance in the application process because of a disability, or for any other reason, please contact Barbara Jetton, Director of Human Resources, at 479-308-2291 or *barbara.jetton@acheedu.org*.

Assistant Professor: Stetson University seeks applications for the position of tenure-track Assistant Professor of Biology. This is a full time, 9-month position, beginning in August 2019. *Qualifications*. Position 1: Organismal biology, physiology, or developmental biology. A PhD in biological sciences with expertise that could include, but is not limited to, organismal biology, physiology, or developmental biology. The successful candidate will have demonstrated excellence in teaching undergraduates, an ability to mentor undergraduate research projects, and a record of scholarly publication in area of expertise. Position 2: Microbiology, molecular biology, microbial genetics, or similar. A PhD in biological sciences with expertise that may include, but is not limited to, microbiology, molecular biology, virology, microbial genetics, host-microbe interactions, or microbial ecology. The successful candidate will have demonstrated excellence in teaching undergraduates, an ability to mentor undergraduate research projects, and a record of scholarly publication in area of expertise. We seek a dedicated teacher-scholar who will be an excellent teacher, engaged undergraduate research mentor, and productive scholar. Postdoctoral experience is strongly recommended but not required. Candidates should have a strong commitment to innovative teaching and academic advising of undergraduates. We are particularly interested in candidates with an interest in or record of scholarship in engaging students from diverse backgrounds. *Responsibilities*. As a liberal learning institution, Stetson focuses on education of the whole student through a strong foundation in general education, deep disciplinary training, and experiential learning. Thus this tenure-track faculty position has a strong focus on teaching, advising, and mentoring of undergraduate research. Primary teaching responsibilities for position 1 could include an upper-division physiology course and associated lab

sections, an introductory biology course, and mentoring of capstone senior research projects. Primary teaching responsibilities for position 2 could include an upper-division microbiology course and the associated lab section, genetics and the associated lab section, an introductory biology course, and mentoring of capstone senior research projects. Other courses will be based on the candidate's expertise, and may include new or existing upper-division biology and/or general education courses. The combined teaching load is equivalent to three course units per semester, which typically ranges from 9 to 12 contact hours. All majors in the College of Arts and Sciences complete a senior project, and therefore mentoring undergraduates as researchers is a key responsibility. As teacher-scholars, candidates will be expected to be excellent teachers and pursue a vibrant research program. The candidate will also provide academic advising for undergraduates each semester. University service and or leadership are also expected commensurate with rank. For more information on our expectations in teaching, scholarship, and service our tenure policies, and our support for faculty development, please see the Office of the Provost and Academic Affairs website: *http://www.stetson.edu/administration/provost/index.php*. *The Department*. The Biology Department (*http://www.stetson.edu/other/academics/programs/biology.php*) consists of 10 full-time faculty members with areas of expertise in molecular biology, ecology, botany, microbiology, neurobiology, developmental biology, evolutionary biology, and aquatic and terrestrial organismal ecology. We emphasize small classes, investigative research in and out of the classroom, and service- learning/community based research. For examples of collaborative research with students across the University see "Stetson Showcase" at *http://www.stetson.edu/other/research/showcase.php*. *College of Arts and Sciences*. Stetson's College of Arts and Sciences (*http://www.stetson.edu/portal/artsci/*) is the largest and most diverse of the University's colleges and schools; it includes the humanities, social sciences, natural sciences, education, and creative arts. With 19 academic departments and 8 interdisciplinary programs, the College is the liberal arts core of the university. A great strength of the College is its faculty of approximately 140 full-time teacher-scholars. The College offers 31 undergraduate majors and 34 undergraduate minors; six graduate degrees are offered in Education, Counselor Education, and Creative Writing. Every undergraduate major requires a senior research project, giving students the opportunity to design and carry out independent research under faculty mentorship. Our academic programs are shaped by the values of social justice and civic responsibility. All of our courses and programs of study promote engaged learning, whereby students create connections between the classroom or lab and the local, regional, and global community. The academic programs of the College of Arts and Sciences prepare students to live significant lives, to interact with and positively affect the world around them. *The University*. Founded in 1883, Stetson University (*http://www.stetson.edu*) is a private, selective university comprised of a rich array of liberal arts and professional academic programs. Collectively, Stetson's faculty works with nearly 4,000 students in undergraduate, graduate, and professional programs. The university's historic main campus, located in DeLand, enrolls more than 3,000 students in undergraduate programs in the College of Arts & Sciences, the School of Business Administration, and the School of Music. Stetson University College of Law, Florida's first law school, moved from the main campus to Gulfport in 1954, and, with the addition of the Tampa Law Center, serves approximately 1,000 students working full-time or part-time toward JD or LLM degrees. Graduate programs offered at the main campus and at Stetson University Center in Celebration include Business, Accounting, Elementary Education - Education for Social Justice, Educational Leadership, Counseling, and Creative Writing. Florida's oldest private institution of higher learning, Stetson has regularly been ranked among the best regional universities in the Southeast and was the first private college in Florida to be granted a chapter of Phi Beta Kappa. Stetson University provides an inspiring education that engages students with rigorous academic and creative study grounded in liberal learning and promotes civic values of personal and social responsibility. Working closely with faculty and with one another, students cultivate abilities to explore issues deeply, think critically, reason empirically, speak persuasively, and connect ideas creatively. Firmly committed to inclusive excellence, our vibrant community of teacher-scholars nurtures the potential of individual students to lead lives of significance and prepares each to meet the challenges of shaping the future—locally, nationally, and globally. *The Community*. DeLand is a picturesque residential community of 32,000 located 20 miles west of Daytona Beach and 35 miles northeast of Orlando. The area offers extensive cultural as well as recreational activities. *Salary*: Salary is competitive. *Starting Date*: August 12, 2018. *Application*: Applicants must send the following materials for consideration: *1*) a cover letter that includes the

candidate's philosophy of teaching, advising, and mentoring of undergraduate research in a liberal arts university, 2) a research plan, 3) a curriculum vitae, and 4) three letters of recommendation, at least two of which include an evaluation of teaching ability. Candidates are encouraged to highlight skills and experiences that demonstrate a commitment to diversity and inclusion. Review of applications will begin immediately and continue until the position is filled, with preference given to applications received on or before November 15, 2018. Application materials may be submitted electronically to Dr. Kirsten Work at *kwork@stetson.edu*, or mailed to the following address: Dr. Kirsten Work, Department Chair, Department of Biology, Stetson University, 421 N. Woodland Blvd. Unit 8264, DeLand, FL 32723. Stetson University is an Equal Opportunity Employer that affirms cultural diversity and inclusion as a core value of academic excellence at Stetson University. We are committed to achieving equal access in education, employment, and participation through the recruitment and retention of outstanding faculty, staff, and students from diverse backgrounds, and to meaningful academic and intellectual transformation in curriculum, research, and service. We are dedicated to actions and policies that foster a community in which individuals with various identities, cultures, backgrounds, and viewpoints work together to create opportunities for engagement through rewarding and fulfilling careers and personal experiences in a culturally and racially diverse society and a globalized world. We strongly encourage members of historically underrepresented and economically disadvantaged groups and women to apply for employment. Stetson University is an EEO, ADA, ADEA, and GINA employer.

Assistant Professor: The University of Dallas Constantin College of Liberal Arts invites applications for a full-time, tenure-track faculty position in biology at the rank of Assistant Professor. The ability to teach, enhance, and create undergraduate lecture and laboratory courses and to develop a research program that engages undergraduate students in a manner to help them prepare for careers or graduate school in the health sciences or related fields is required. Service responsibilities will include service on committees, advising, and activities that are a service to the candidate's field. Start date is August 2019. *Primary Responsibilities*. Teach anatomy, physiology, and other health-related courses; development of upper-level electives; development and maintenance of research that engages undergraduate students; professional, departmental, and university service. *Minimum Requirement*. PhD and/or MD or DO; background in health sciences and/or anatomy/physiology. *Preferred Qualifications*. Postdoctoral experience; demonstrated commitment to undergraduate teaching, research, and mentorship. *Application*. Candidates should submit a letter of interest clearly indicating the areas of teaching and research expertise and interest, a curriculum vita detailing all academic and professional experience (references will be requested from candidates invited for an interview) electronically. All applications should be submitted online at: *https://hr.udallas.edu/apply/*. Inquiries regarding a posting can be made via fax, e-mail or in person at Office of Human Resources, Cardinal Farrell Hall, 1845 E. Northgate Dr., Irving, TX 75062; fax: (972) 721-4095; e-mail: *hr@udallas.edu*. *Who We Are*. The University of Dallas is a Catholic university that seeks to educate the whole person, encouraging its students to pursue wisdom, truth and virtue as the proper and primary ends of education (*http://www.udallas.edu/about/mission.php*). All faculty members are expected to understand and support the mission. *Benefits*. UD provides competitive pay and benefits, including eligibility to participate in medical, dental, life, and disability insurance; employee events; a health and wellness program; a free fitness center; competitive leave programs; free tuition for employees and their families; and matching retirement plan contributions. The University of Dallas is an Equal Opportunity/Affirmative Action employer. The university does not discriminate against individuals on the basis of race, color, religion, sex, national origin, genetic information, protected veteran status, age, or disability in the administration of its employment practices.

Assistant Professor: The Universidad de los Andes, a top university in Latin America, invites applications for a full time, tenure track faculty position in Medical Physiology, at the rank of Assistant Professor. Start date: July 2019. *Required qualifications*: Professional degree in medicine or a related health/biomedical science, valid in Colombia; doctoral degree in a biomedical science with emphasis in human or cell physiology, valid in Colombia. The program granting the Doctoral degree must be on-site and last 4 years or longer. Applicants not having received their Doctoral degree, but having successfully defended their thesis and expecting only the graduation ceremony, will be considered (the applicant must be able to prove this status). *The qualified applicant is expected to*: teach or coordinate biomedical science courses (Cell and

Molecular Biology, Physiology, Final Research Project or advanced biomedical courses) at the undergraduate and graduate levels at the School of Medicine; coordinate research projects from undergraduate and graduate students of Universidad de los Andes; lead a successful research program in cell or integrative physiology at Universidad de los Andes; attract competitive research funding from national and international funding bodies. *Professional experience*: Experience teaching/coordinating undergraduate or graduate courses in biomedical sciences; publication of original research articles in translational medicine, human or cellular physiology; at least 2 original research publications in journals Q1 or Q2 of ISI Web of Science at any time; the applicant must be one of the two first authors or the corresponding author in at least one original research paper within the last 2 years; proven experience in the writing and submission of research projects to funding bodies, application to research scholarships, and/or application to funding for research projects; excellent command of Spanish and another language. *Salary and benefits*: Salary will depend on qualifications, publications, and prior experience. The Universidad de los Andes offers a competitive salary and benefits versus other Universities in Colombia. *Application*: Candidates should submit: *1*) CV, including publication list, teaching experience, and professional references; *2*) intent letter explaining the academic and research objectives that the candidate aims to accomplish at the School of Medicine during the next 2 years; *3*) two (2) recommendation letters, addressed to the School Dean, Dr. Natalia Mejia. All applications should be submitted to the following e-mail (*decanaturamedicina@uniandes.edu.co*). *Deadline*: February 16, 2019.

Assistant Professor: Boston University's College of Health and Rehabilitation Sciences: Sargent College invites applications for a full-time, renewable, non-tenure track faculty position at the Clinical Assistant Professor level in the Human Physiology Program within the Department of Health Sciences. This is a 9-month appointment with the possibility of summer instruction. The position will start in September 2019. We are looking for a colleague who will serve as Program Director for the Undergraduate Program in Human Physiology. This position will be a strategic hire that will allow us to build upon the College's tradition of excellence in the health sciences and its commitment to diversity and inclusion. Clinical faculty at Boston University are expected to teach undergraduate and graduate students and participate in advising, service, and scholarly activities. This faculty member will be responsible for teaching Gross Human Anatomy and Human Physiology courses, and will be responsible for directing the Undergraduate Program in Human Physiology. A successful candidate will have demonstrated excellence in classroom instruction at the college/university level, expertise in STEM pedagogy, and experience with curriculum development. Qualifications include: strong leadership, communication, mentoring, and advising skills; a doctoral degree (PhD or equivalent) in Human Physiology or a closely related field; a minimum of 5 years teaching experience at the college/university level is required; demonstrated competence in teaching cadaver-based gross human anatomy is required; an established record of productivity in research and scholarship; prior experience with academic advising, specifically pre-medical advising, is highly desired. The Department of Health Sciences is part of a vibrant Boston University academic and research community that includes 17 schools and colleges across the Charles River and Medical campuses, including the Boston University School of Medicine as well as many other highly regarded medical and educational institutions in the Boston area that allow for collaborative and interdisciplinary activities. We invite you to join our interdisciplinary faculty team and become involved with our network of collaborations within Boston University and the greater Boston community. Boston University is an AAU institution with a rich tradition dedicated to diversity, inclusion, and social justice. The College of Health & Rehabilitation Sciences: Sargent College is dedicated to increasing the participation of all talented students and scholars. We are committed to cultivating an environment that fosters the pursuit of excellence in research and education by underrepresented groups at BU and beyond. Review of applications will begin immediately and continue until the position is filled. Please send applications (letter of intent including statement of teaching and clinical experience, curriculum vitae, and three letters of recommendation from individuals qualified to speak on your behalf regarding your qualifications) to Danka Charland, *charland@bu.edu*, Assistant to the Program Director, Department of Health Sciences, Boston University, College of Health and Rehabilitation Sciences: Sargent College, 635 Commonwealth Avenue, Boston, MA 02215 (electronic submission is preferred: *charland@bu.edu*). We are an equal opportunity employer, and all qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin,

disability status, protected veteran status, or any other characteristic protected by law. We are a VEVRAA Federal Contractor.

Assistant/Associate Professor: The Zucker School of Medicine at Hofstra/Northwell *(http://medicine.hofstra.edu)* seeks a qualified medical educator to participate in the delivery of our integrated physiology and pathophysiology curriculum. The successful applicant will also participate in the facilitation of our case-based learning curriculum and will be expected to engage in scholarly activities related to medical education research. Applicants must possess an MD/DO and/or PhD degree, and must have experience instructing medical students in clinically oriented physiology and pathophysiology. Candidates must demonstrate willingness to contribute to multidisciplinary biomedical instruction in both small-group and large-group settings. The successful candidate must possess the organizational skills that would enable him/her to assume the role of course director for the block course that covers cardiac, pulmonary, and renal physiology, pathophysiology, and related pharmacology. Applicants are invited to submit a cover letter that describes the applicant's interest in the position, current curriculum vitae, statement of teaching philosophy, and the names and contact information for three professional references to *SOMRecruit@hofstra.edu*.

Assistant/Associate Professor: Tufts University School of Medicine is seeking to Bll a full-time (12 month) faculty position at the rank of Assistant or Associate Professor in the Department of Medical Education (DME). We are seeking an individual with expertise in teaching medical physiology. The DME is a newly formed basic science department providing instruction to students in the MD, DMD, PA, and MS programs. The DME is home to basic science and clinical faculty members who have medical education as a primary role. Advancement in DME is along a recently instituted Educator Track (non-tenure). The medical school is currently developing a new, highly integrated curriculum that will emphasize active learning (to be implemented in the fall of 2019). Applicants with experience in small group, problem-based learning and other interactive teaching strategies are preferred. The successful candidate will be expected to engage in innovative scholarship in medical education and to be active in student advising/mentoring and serving on committees. *Qualifications*: Applicants must have a doctoral degree in the biological sciences and evidence of outstanding teaching in professional degree programs. *Application Instructions*: Applicants should submit only complete applications electronically through Interfolio. Please address questions to Dr. Jeffrey K. Marchant at *jeffrey.marchant@tufts.edu*. *Application Process*: This institution is using Interfolio's Faculty Search to conduct this search. Applicants to this position receive a free Dossier account and can send all application materials, including confidential letters of recommendation, free of charge. Apply now (*https://dossier.interfolio.com/apply/57266*); Powered by *https://www.interfolio.com/*. *Equal Employment Opportunity Statement*: Tufts University shall abide by the requirements of 41 CFR §§ 60-1.4(a), 60-300.5(a), and 60-741.5(a). These regulations prohibit discrimination against qualified individuals based on their status as protected veterans or individuals with disabilities, and prohibit discrimination against all individuals based on their race, color, religion, sex, or national origin. Moreover, these regulations require that covered prime contractors and subcontractors take affirmative action to employ and advance in employment individuals without regard to race, color, religion, sex, national origin, protected veteran status, or disability.

Assistant/Associate Professor: The Philadelphia College of Osteopathic Medicine (PCOM), Department of Biomedical Sciences, invites applications for a full-time academic appointment, tenure-track position at the rank of Assistant or Associate Professor. In this position, based at our Philadelphia, PA campus, you will be instrumental in establishing and conducting an independent, extramurally funded research program, aid in course administration, and contribute to institutional and departmental governance and service. Applicants at the Associate Professor level are expected to have funding and a recognized reputation of innovative research excellence and productivity. Rank will be commensurate with experience. *Requirements*: DO, MD, or PhD degree in the biological sciences and demonstrated excellence in graduate biomedical education; 3 years' experience in teaching any biomedical science, however, preference will be given to candidates with expertise in physiology or pathophysiology; bringing innovative approaches into the classroom, and the ability to work collaboratively with other faculty and staff in team taught courses; investigators using state-of-the-art techniques, including animal models, with research programs that leverage molecular and cellular insights to inform physiological and pathophysiological functions, are highly desired.

Competitive lab space and start-up funds are available. Please apply online. Applicants must be authorized to work in the U.S. PCOM adheres to a policy that prohibits discrimination on the basis of race, color, sex, sexual orientation, gender identity, religion, creed, national or ethnic origin, citizenship status, age, disability, veteran status, or any other legally protected class.

Assistant/Associate Professor: The School of Dental Medicine at LECOM (Bradenton, FL campus) is seeking candidates for a full-time non-tenure track, 12-month faculty position at the Assistant/Associate Professor of Physiology level. Successful candidates will have a desire to teach and mentor students at the graduate/professional level, including facilitation of Problem-Based Learning (PBL) groups and the delivery of traditional lectures. In addition, faculty members are expected to develop an active and independent program of scholarship. All areas of expertise will be considered. The successful applicant will have a doctorate level of achievement (PhD, DO, MD, or EdD) and prior experience of 2–5 years teaching physiology to medical or dental students. Preference will be given to individuals with a progressive and innovative teaching philosophy and previous experience in a small group teaching or facilitating environment. Additional qualifications to strengthen the applicant include a strong desire to foster learning in an innovative curriculum, advisement and mentorship of dental students, a progressive teaching philosophy, and the ability to contribute to the development of the college through research and service. Faculty rank and salary are dependent on qualifications and experience. *About the LECOM School of Dental Medicine*: The School of Dental Medicine provides investigators and teacher-scholars an exciting environment to develop collaborative and innovative research projects. We value diversity in our faculty, staff, and students. The SDM is located in Bradenton, FL, only minutes from beautiful beaches to the west and less than 1 hour from the Tampa-St. Petersburg metropolitan area. *To Apply*: Complete an online application at *www.lecom.edu/jobs*. At the time of application please be prepared to submit the following: *1*) cover letter, *2*) current CV, *3*) teaching/mentoring philosophy, and *4*) contact information for three professional references. The applicant must also e-mail to *hr@lecom.edu* the following: a cover letter outlining how he/she would enhance the LECOM family, a statement of teaching philosophy, and contact information for three professional references. EEO/AA/M/F/Vets/Disabled.

Leadership Position: The Medical College of Georgia at Augusta University invites applications and nominations for the position of Chair of the Department of Physiology. This is an exciting opportunity for a distinguished scientist to lead a physiology department with a long legacy of innovation and excellence in research. The next Chair will have an opportunity to build on the Department's strong foundation and craft a vision that will continue to foster discovery and excellence in basic science, translational research and education. The Chair will report to the Dean and will serve as the department's academic and administrative leader, providing direction in educational programs; faculty recruitment, retention, and development; budget management and oversight; research; and strategic planning. *Minimum Qualifications*. The successful Chair must hold a PhD and/or MD as well as meet the academic accomplishments that merit tenured appointment at the rank of professor. In addition, the successful candidate will have: an accomplished research portfolio; evidence of leadership and administrative experience in an academic health sciences or equivalent setting; a successful track record of recruitment, retention, and development of faculty, trainees, staff, and students; service on national grant review study sections; service to national scientific professional organizations; proven skills in mentoring young researchers; a commitment to advancing the research initiatives within the department; excellent communication and human relation skills. *About the Medical College of Georgia at Augusta University*. The Medical College of Georgia (MCG) is the flagship medical school of the University System of Georgia, the state's only public medical school and one of the top 5 largest medical schools in the U.S. Founded nearly 200 years ago in 1828 as the nation's 5th public medical school, the 3rd medical school in the Southeast, and the 13th in the nation, MCG has risen to its current role optimizing health care in Georgia and beyond through education, discovery, and service. The Medical College of Georgia educational experience is anchored by the main campus in Augusta, as well as three regional clinical campuses for third-and fourth-year students and a second 4-year campus in Athens, Georgia, in partnership with the University of Georgia. The state's medical school also offers clinical training in more than 200 sites across the state, providing students experience in the full spectrum of medicine, from tertiary/quaternary care hospitals to small-town solo practices. MCG and its teaching hospitals provide postgraduate education to nearly 500 residents in 44 different programs. *About the Department of Physiology*. The Department of Physiology

at the Medical College of Georgia at Augusta University consists of 14 full-time tenure-track faculty members and four research professors. The primary research interests of the department are cardiovascular, renal, endocrine, and neurovascular physiology. Thirteen graduate students, nine postdoctoral fellows, and two medical residents are currently homed in Physiology. Visiting professors and visiting students from various countries including China, Japan, and Brazil frequently come to work or perform research fellowships in the Department of Physiology. Physiology faculty currently enjoy robust funding from the NIH, the AHA, and the Veterans Affairs Department, including a program project and generous trainee support from the American Heart Association. Members of the Department teach in the Medical and Graduate Schools, including Medical Physiology and Integrated Systems Biology, Neuroscience, and advanced courses in cardiovascular and endocrine physiology. *How to Apply*. Review of applications will begin immediately and continue until the position is filled. Candidate materials should include a letter of interest and complete curriculum vitae. On behalf of the Search Committee Chair, Neal Weintraub, MD, applications, nominations, and inquiries will be received electronically by Sharon Wingard, Executive Search Consultant, Augusta University, at *executivesearch@augusta.edu*. The final candidate will be required to provide proof of completed academic degree in the form of an original transcript. Those candidates trained by a foreign institution will also be required to provide an educational/credential evaluation. Augusta University is an AA/EEO/Equal Access/ADA employer.

Leadership Position: The Tulane University School of Medicine invites applications from outstanding scientists with strong and robust research programs funded by multiple major grants. Applicants should hold a MD or PhD or both and have a record of excellence in research and history of a rigorous sustained externally funded research program. Applicants must also have a history of academic service in medical and graduate education. Research areas targeted for development can involve basic, translational, and or clinical levels determining cardiovascular, renal, endocrine, and neural mechanisms of hypertension and associated cardiovascular diseases including diabetes. The THRCE Director will have responsibility for managing the Cores including the Molecular, Analytical and Imaging Core, and the Phenotyping Core. Further responsibilities include the recruitment and nurturing of new faculty. Required application materials include a curriculum vitae, a brief statement of research interests, copies of 3 major scholarly papers, and 3 letters of recommendation. Applicants must apply via *Interfolio* at *http://apply.interfolio.com/53517*. Applications will be accepted until the position is filled. Tulane University is an equal opportunity/affirmative action/person with disabilities/veterans employer committed to excellence through diversity. Tulane will not discriminate against individuals with disabilities or veterans. All eligible candidates are encouraged to apply.

Postdoctoral Fellow: The laboratory of Dr. Schwingshackl in the Division of Pediatric Critical Care (UCLA David Geffen School of Medicine) is searching for a highly motivated postdoctoral fellow to study the role of stretch-activated ion channels in the development of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). The ideal candidate should have a combined interest in ion channel physiology, molecular biology, and in vivo lung injury models. The project will provide training and mentorship in electrophysiological, genetic, pharmacologic, and immunofluorescence imaging techniques, as well as in vivo models of ALI/ARDS. The Schwingshackl laboratory closely collaborates with Dr. Riccardo Olcese's group in the Division of Molecular Medicine (UCLA, Anesthesiology). Dr. Olcese will co-supervise the candidate in the electrophysiology aspects of the project, whereas Dr. Schwingshackl will provide primary mentorship for the molecular biology and in vivo lung injury studies. Applicants should have *1*) prior experience in voltage-clamp electrophysiology, *2*) a first author peer-reviewed publication, and *3*) excellent English oral and writing skills. Highly motivated PhD and MD graduates please e-mail a detailed CV with bibliography, the names of three referees, and a short (1/2 page) paragraph outlining research interests and career goals to Dr. Schwingshackl (*aschwingshackl@mednet.ucla.edu*).

Postdoctoral Fellow: A postdoctoral fellowship position is open in the Gewin lab at Vanderbilt University Medical Center. The Gewin lab uses basic science approaches to investigate how epithelial cell responses to chronic renal injury lead to repair versus tubulointerstitial fibrosis. The lab focuses on how growth factors (TGF-beta, Wnt/beta-catenin) modulate the epithelial response to injury through cell cycle changes, altered metabolism, matrix production, and mitochondrial injury. Competitive candidates should have a recent PhD degree with a background in molecular biology, murine models of injury, and cell

culture techniques. Experience in confocal microscopy, metabolism (Seahorse), and mitochondrial injury are preferred but not required. Interested candidates should send a CV with references and research interests to: Leslie Gewin, MD, *l.gewin@vumc.org* or *leslie.gewin@vanderbilt.edu*; *http://www.vckd.org/vckd-members/leslie-gewin*; *https://wag.app.vanderbilt.edu/PublicPage/Faculty/Details/31010*.

Postdoctoral Fellow: A postdoctoral position is available immediately in the laboratory of Dr. William Mayhan in the Division of Basic Biomedical Sciences at the University of South Dakota (USD). Applicants should have a PhD with experience in vascular biology as well as a willingness to work with animal model systems. Experience with animal surgery/surgical techniques, RT-PCR, Western blot, and/or immunohistochemical approaches is also desirable. This is a grant-funded position with funds available for 2–4 years, with extensions contingent on future funding. In addition to research experience (developing and conducting experiments, analyzing data, writing peer-reviewed manuscripts), opportunities for professional development (mentor training, opportunities to teach, etc.) will also be provided. Interested applicants must include a cover letter summarizing research experience, research interests and career goals, a curriculum vitae, and contact information for three professional references. All materials should be posted to USD's online employment website at *https://yourfuture.sdbor.edu*. Posting number: NFE00380P. Review of applications is ongoing and will continue until position is filled. Women and individuals from diverse ethnic, religious, cultural, and social backgrounds are especially encouraged to apply. AA/EOE.

Postdoctoral Fellow: Jason H. Mateika PhD is an Associate Chair for Research in the Department of Internal Medicine and a Professor in the Department of Physiology at Wayne State University, and a Veterans Affairs Research Career Scientist. Dr. Mateika is presently funded by the U.S. Department of Veterans Affairs and the National Institutes of Health for two primary proposals. He is seeking postdoctoral fellows interested in working on one of the proposals listed below. The first proposal is designed to investigate the impact of genetically or spinal cord injur- induced reductions in central nervous system serotonin on mechanisms that influence breathing stability and cardiovascular/autonomic function during sleep in mice. He is seeking postdoctoral fellows interested in this area of research. Ideally, the postdoctoral fellow will have expertise in one or more of the following methods: telemetry implantation, plethysmography, echocardiography, dissection, sectioning, and immunohistochemistry. *Desired Qualifications*: *1*) Ability to work independently and as part of a collaborative team. *2*) Excellent communication skills, both verbal and written. *3*) Willing to perform in vivo experiments in an animal research laboratory. *4*) Reliable and willing to work flexible hours. *5*) Prior experience with telemetry implantation, plethysmography, echocardiography, dissection, sectioning, and immunohistochemistry. The second proposal is designed to explore whether repeated daily exposure to mild intermittent hypoxia enhances the impact of continuous positive airway pressure on co-morbidities linked to sleep apnea in humans with intact or injured spinal cords. He is seeking postdoctoral fellows interested in this area of research. Ideally, the postdoctoral fellow will have expertise in one or more of the following methods: microneurography, non-invasive measures of respiratory, cardiovascular and autonomic function during wakefulness and sleep. *Desired Qualifications*: *1*) Ability to work independently and as part of a collaborative team. *2*) Excellent communication skills, both verbal and written. *3*) Willing to perform experiments in a human research laboratory. *4*) Reliable and willing to work flexible hours. *5*) Prior experience in microneurography and non-invasive measures of respiratory, cardiovascular and autonomic function during wakefulness and sleep. *Contact Information*: Jason H. Mateika PhD, Associate Chair for Research (Internal Medicine) & Professor of Physiology, Wayne State University School of Medicine, Research Career Scientist, John D. Dingell VA Medical Center; Phone: 313-576-4481; e-mail: *jmateika@med.wayne.edu*.

SYRACUSE UNIVERSITY
Department of Exercise Science
Assistant/Associate Professor - Exercise Science

The Department of Exercise Science in the School of Education at Syracuse University seeks to fill a full-time position for a Tenure-Track Assistant Professor or an Associate Professor with Tenure as part of the Invest Syracuse Signature Hires Initiative. All areas of expertise within the general field of exercise science will be considered. A collaborative multidisciplinary approach to research that transects faculty interests in exercise/physical activity, applied physiology, genetics, chronic disease and/or disability is desirable. Salary is competitive and commensurate with qualifications and experience.

Department
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 the 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 5 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 approximately 260 Health & Exercise Science and 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.

Qualifications
An earned Doctorate in exercise science, kinesiology or a related area. The successful applicant will have demonstrated effective teaching practices that can be applied across our undergraduate, Master's and Doctoral programs, provision of effective individual and group supervision, previous mentorship of students, and demonstrated professional service. At the Assistant Professor level, highest preference will be given to candidates with Postdoctoral training and a strong research agenda as supported by peer-reviewed publications and funding from grants and/or fellowships. To be considered as an Associate Professor with Tenure, applicants must evidence development of a sustained research trajectory and record of extramurally funded research.

Responsibilities and Required Expectations:
1. Establish an active and extramurally funded research program at Syracuse University that will enhance the departments 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 Master's and Doctoral levels
5. Oversee MS theses and Ph.D. 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

The School/Department seeks candidates whose research, teaching or service has prepared them to contribute to our own commitment to diversity and inclusion in higher education.

For a detailed position description and online instructions, go to **www.sujobopps.com** (job #074113). All applicants must attach a current CV, cover letter explaining your interest in the position along with teaching and research interests, and contact information for three (3) letters of recommendation. To receive full consideration applicants are encouraged to submit completed materials by November 16, 2018; although applications will be accepted until the position is filled.

Questions about the position should be sent by email to the Search Chair:
Keith C. DeRuisseau, Ph.D.
kcderuis@syr.edu

Syracuse University is an equal opportunity/affirmative action employer with a strong commitment to equality of opportunity and a diverse work force. Women, military veterans, individuals with disabilities, and members of other traditionally underrepresented groups are encouraged to apply.

Meetings and Congresses

2019

March 20–23

23rd Annual Regenerative Medicine Workshop, Charleston, SC. *Information:* Internet: *https://regenerativemedicineworkshop.com/*

March 25–26

8th Edition International Conference on Internal Medicine, 2019, Rome, Italy. *Information:* Internet: *https://internalmedicine.euroscicon.com/*

April 5–6

European Meeting on Adult Congenital Heart Disease (EuroGUCH), Zagreb, Croatia. *Information:* Internet: *http://wp1.euroguch.com/*

April 6–9

Experimental Biology, Orlando, FL. *Information:* Internet: *http://apsebmeeting.org/eb2019/*

April 22–23

4th International Heart Conference, Dubai, UAE. *Information:* e-mail: *advancedheart@pulsusevents.org*; internet: *https://heart.cmesociety.com/*

May 27–June 3

PanAm 2019, Havana, Cuba. *Information:* Internet: *http://panam2019.com/*

June 10–13

14th FELASA Congress, Prague, Czech Republic. *Information:* Internet: *http://www.felasa2019.eu/*

June 23–29

APS/ASN Conference: Control of Renal Function in Health and Disease, Charlottesville, VA.

September 11–14

APS Conference: Interface of Mathematical Models and Experimental Biology: Role of the Microvasculature, Scottsdale, AZ

October 2–5

APS Conference: 9th Annual International Conference of Aldosterone and ENaC in Health and Disease: The Kidney and Beyond, Estes Park, CO

October 29–November 1

31st World Congress of the International College for Maxillo-Facial-Surgery, Tel Aviv, Israel. *Information: http://icmfs2019.com/*

2020

June

APS Conference: 11th International Conference on Heme Oxygenase & Related Enzymes: From Physiology to Therapeutics

2018

Cardiovascular, Renal and Metabolic Diseases: Sex-Specific Implications for Physiology

Knoxville, TN • September 30–October 3, 2018

Special Abstract Issue

MONDAY, OCTOBER 1, 2018

1.0 **Welcome and Introduction from Conference Chairs**
Mon., 7:50-8:00 AM, Summit I

Jennifer Sullivan, *Augusta Univ.*
Michael Ryan, *Univ. of Mississippi Med. Center*

2.0 **SESSION 1: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE BRAIN AND NERVOUS SYSTEM**

Mon., 8:00-9:45 AM, Summit I

Chairs: **Gina Yosten,** *St. Louis Univ. School of Med.*
Taylor Schlotman, *US Army Institute of Surgical Research*

8:00 AM **2.1** Adipokines and the Central Regulation of Cardiovascular Function
Gina Yosten *St. Louis Univ. School of Med.*

8:25 AM **2.2** Abstract 15: Comparing Time to Presyncope during Simulated Hemorrhage Across Menstrual Cycle
Taylor Schlotman *US Army Institute of Surgical Research*

8:35 AM **2.3** Abstract 48: Estrogen Receptor Alpha Contributes to the Inhibitory Action of Central Cytochrome P4501B1 Generated 17β Estradiol Metabolite 2-Methoxyestradiol to Angiotensin II-Induced Hypertension
Purnima Singh *Univ. of Tennessee Health Science Center*

8:45 AM **2.4** The Role of Sensitization and Neuroplasticity in Sex Differences in Blood Pressure and Hypertension
A. Kim Johnson *Univ. of Iowa*

9:10 AM **2.5** Abstract 89: Central Acetylcholinesterase Inhibitor, Galantamine, Prevents Lipid-Induced Oxidative Stress in African American Women
Cyndya Shibao *Vanderbilt Univ. Med. Center*

9:20 AM **2.6** Brain Blood Flow Control: Sex Differences and Sex-Specific Conditions
Jill Barnes *Univ. of Wisconsin-Madison*

3.0 **POSTER SESSION I**

Mon., 9:45-11:00 AM, Tennessee Ballroom

Board #

1 **3.1** Diabetes Promotes a Sexual Dimorphic Expansion of Circulating and Cerebrovascular Th17 Cells in Female Rats
LaDonya Jackson, Weiguo Li, Yasir Abdul, Guangkuo Dong, Babak Baban, Susan C. Fagan, Adviye Ergul. *Medical College of Georgia*

2 **3.2** Sexual Dimorphism in Central Modulation of Baroreceptor Afferent Input in Hypertension
Ibrahim M. Salman, Omar Z. Ameer, Sheridan McMurray, Arun Sridhar, Stephen J. Lewis, Yee-Hsee Hsieh. *Case Western Reserve Univ.; Galvani Bioelectronics*

3 **3.3** Central Leptin Receptor Antagonism Attenuates the Development of Menopausal-Induced Hypertension in the Rat
Maria Barnes, Sarah Clayton. *Des Moines Univ.*

4	**3.4**	Time Course Assessment of Acute and Chronic Hypotonic Brain Edema by Dynamic NMR: Topographical and Gender Differences **Marta Tejedor, Alberto Lázaro, Alejandro Rojo, Lorena Cussó, Marian González-Nicolás, Jorge .J García Seoane, Meritxell López, Alberto Tejedor.** *Universidad Complutense Madrid;Hospital General Gregorio Marañón*
5	**3.5**	Estrogen Receptor Alpha Contributes to the Inhibitory Action of Central Cytochrome P4501B1 Generated 17β Estradiol Metabolite 2-Methoxyestradiol to Angiotensin II-Induced Hypertension **Purnima Singh, Chi Young Song, Shubha Ranjan Dutta, Scott A. Heldt, Kafait U. Malik.** *Univ. of Tennessee Health Science Center*
6	**3.6**	Sex-Dependent Effects of Prediabetes in Mouse Models of Alzheimer's Disease and Mixed Dementia **Olivia Gannon, Lisa Robison, Abigail Salinero, Melissa Thomas, Alya Tyson, Kristen Zuloaga.** *Albany Medical College*
7	**3.7**	DOCA-Salt Hypertension Leads to Neurocognitive Deficiencies in Female SD Rats **Kasey Belanger, Ellen Gillis, Jennifer Sullivan.** *Augusta Univ.*
8	**3.8**	Sex-Specific Hormones Produced During Proestrous and Estrous Exert a Regulatory Effect on the Nesfatin-1 mRNA Levels and Autonomic Function in Female Cycling Rats **Alicia Pate, Gina Yosten.** *St. Louis College of Pharmacy; Saint Louis Univ.*
9	**3.9**	Central Acetylcholinesterase Inhibitor, Galantamine, Prevents Lipid-Induced Oxidative Stress in African American Women **Cyndya Shibao, Jorge Celedonio, Shahram E, Sachin Paranjape, Andre Diedrich.** *Vanderbilt Univ. Medical Center*
10	**3.10**	Angiotensin Type 2 Receptor Stimulation With Compound 21 Improves Stroke Outcome in Female Rats: Possible Role For Peroxisome Proliferator-activated Receptor Gamma **Wael Eldahshan, Bindu Pillai, Mohammed Sayed, Abdulrahman Alwhaibi, Abdelrahman Fouda, Tauheed Ishrat, Adviye Ergul , Susan Fagan.** *Univ. of Georgia;Augusta Univ.;The Univ. of Tennessee Health Science Center*
11	**3.11**	Ovariectomy induces anxiety-like behavior and short-term recognition memory impairment **Glenda Campos, Aline De Souza, Crystal West, Hong Ji, Rodrigo De Menezes, Kathryn Sandberg.** *Georgetown Univ.;Federal Univ. of Ouro Preto*
12	**3.12**	Sex Differences in Healthy Human Heart Revealed by Cap Analysis Gene Expression (CAGE) **Anna Gams, Ndeye Rokhaya Faye, Ruslan Deviatiiarov, Aaron C. Koppel, Igor R. Efimov.** *The George Washington Univ.; Kazan Federal Univ.*
13	**3.13**	Comparison of Measures of Compensatory Reserve in Differentiating Tolerance to Simulated Hemorrhage in Males versus Females **Taylor Schlotman, Victor Convertino.** *US Army Institute of Surgical Research*
14	**3.14**	Comparing Time to Presyncope during Simulated Hemorrhage Across Menstrual Cycle **Winfred Stacey, Carmen Hinojosa-Laborde, Taylor Schlotman, Victor Convertino.** *US Army Institute of Surgical Research*
15	**3.15**	Stress Hormone inhibition of Estrogen Transcriptional Regulation of the Serotonin Signaling in Cardiomyocytes is deleterious for the Female Heart in Myocardial Infarction **Natalie Burford, Lilly Kamberov, Wayne Orr, John Cidlowski, Diana Cruz-Topete.** *LSU Health Sciences Center Shreveport;National Institute of Environmental Health Sciences*

16 **3.16** Heart Failure with Preserved Ejection Fraction (HFpEF) is Augmented in Obese Mice with an XX Sex Chromosome Complement
Sean Thatcher, Yasir AlSiraj, Lisa Cassis. *Univ. of Kentucky*

17 **3.17** AMP-activated Protein Kinase and Estrogen-Dependent Mechanisms Underlying Increased Susceptibility to Cardiovascular Disease During Menopause
Marissa Pier, John Konhilas. *Univ. of Arizona*

18 **3.18** Beta-Carotene Metabolism in the Maternal Heart During Pregnancy
Chelsee Holloway, Youn-Kyung Kim, Loredana Quadro. *Rutgers Univ.*

19 **3.19** Responses in Cardiac Stroke Volume Index and Cardiac Index Do Not Differ Between Genders During Moderate Central Hypovolemia
Nathalie Linn A. Holme, Martin Andreas Lehre, Signe Søvik, Maja Elstad, Maria Skytioti. *Univ. of Oslo*

20 **3.20** Sex Differences in the Effects of Prediabetes on Vascular Contributions to Dementia
Abigail Salinero, Lisa Robison, Olivia Gannon, David Riccio, Alya Tyson, Kristen Zuloaga. *Albany Med. Coll.*

21 **3.21** Interaction between GLP-1 Receptor Agonists and Renin Angiotensin System in the Metabolic Syndrome in a Model of Postmenopausal PCOS
Edgar Torres Fernandez, Damian Romero, Licy Yanes Cardozo. *Univ. of Mississippi Med. Center*

22 **3.22** Angiotensin II Induces a Pro-Inflammatory Shift in the Splenic $CD4^+$ T Cell Proteome in Menopausal Mice
Dennis Pollow Jr, Nathaniel Husband, Jill Romero-Aleshire, Joshua Uhlorn, Caitlin Moffett, Jennifer Uhrlaub, Janko Nikolich-Zugich, Heddwen Brooks. *Univ. of Arizona*

23 **3.23** Sex-Specific Regulation of Sirtuin-3 Mediates Differences in Ischemia-Reperfusion Kidney Injury
Jenny Pan, Vincent Yu, Qingtian Li, David Sheikh-Hamad. *Michael E. DeBakey VAMC; Baylor College of Medicine*

24 **3.24** Group IV Cytosolic Phospholipase $A_2\alpha$ is Required for 6β-Hydroxytestosterone Mediated Angiotensin II Induced Hypertension in Male Mice
Ajeeth Pingili, Purnima Singh, Chi Young Song, Ji Soo Shin, Joseph Bonventre, Kafiat Malik. *Univ. of Tennessee HSC; Brigham and Women's Hospital, Harvard Medical School*

25 **3.25** Sex Difference in T Regulatory Cell Expansion After Adoptive Transfer From Hypertensive Donors Leads to Protection Against T Cell-Mediated Hypertension in Premenopausal Mice
Megan A. Sylvester, Dennis P. Pollow, Nathaniel Husband, Joshua Uhlorn, Jill Romero-Aleshire, Jennifer Uhrlaub, Janko Nikolich-Zugich, Heddwen L. Brooks. *Univ. of Arizona*

26 **3.26** Sex Differences in the Diurnal Natriuretic Response to Benzamil in Sprague Dawley Rats
Reham Soliman, Jermaine Johnston, Eman Gohar, David Pollock. *Univ. of Alabama at Birmingham*

27 **3.27** Increased Severity of Renal Ischemia Reperfusion Injury in Male vs. Female Mice is Associated with Greater Expression of Choline Acetyltransferase in Tubules
Shannon Allen, Jacob Zalewski, Jacqui Potter, Conor Miles, Donald Hoover, Aaron Polichnowski. *East Tennessee State Univ.*

28 **3.28** Gender Differences in Human Skin Na^+ and Monocyte Salt-Sensitivity
Annet Kirabo, Natalia Barbaro, Jason Foss, Fernando Elijovich, Justin Van Beusecum, Cheryl Laffer, Mingfang Ao, Aseel Alsouqi, Alp Ikizler, David Harrison, Annet Kirabo. *Vanderbilt Univ. Medical Center*

29 **3.29** Greater Aortic Inflammation in Male SHR Corresponds to Vascular Dysfunction
Lindsey Ramirez, Jacqueline Musall, Jennifer Sullivan. *Augusta Univ.*

30 **3.30** Pretreatment with Low Dose Lipopolysaccharide Attenuates Medullary Congestion in Male WKY Following Acute Kidney Injury
Sarah Ray, G. Ryan Crislip, Riyaz Mohamed, Bansari Patel, Katie Wilson, Jinging Sun, Paul O'Connor. *Augusta Univ.*

31 **3.31** Immune Cell Influx in Cisplatin-Induced Acute Kidney Injury: Sex Differences
Lisa M. Curtis, Chunlan Fan, Ravindra Boddu. *Univ. of Alabama at Birmingham*

32 **3.32** Sex Differences in Renal Ammonia Metabolism
Autumn N. Harris , Hyun-Wook Lee, Gunnar Osis, Kierstin L. Webster, Jill W. Verlander, I. David Weiner . *Univ. of Florida*

LB32A **3.33** Sex Differences in the Metabolic and Physiological Effects of a High Fat Diet in the 3xTg-AD Mouse Model of Alzheimer's Disease
Lisa Robison, Olivia Gannon, Abigail Salinero, Kristen Zuloaga. *Albany Med. Coll.*

LB32B **3.34** Sex Differences in the Cerebral Vascular Function and K Channel Role in Adult Sprague Dawley (SD) Rats
Sumit Sontakke. *Univ. of Mississippi Med. Center*

LB32C **3.35** Cerebrovascular Dysfunction in the Dahl S Rat Model of Superimposed Preeclampsia
Kenji Maeda, Daniel McClung, Junie Paula Warrington, Michael Garrett, Michael Ryan, Jennifer Sasser. *Univ. of Mississippi Med. Center*

4.0 SESSION 2: PHYSIOLOGY AND GENDER: AGING AND SENESCENCE

Mon., 11:00 AM-12:45 PM, Summit I

Chairs: **Christopher DeSouza,** *Univ. of Colorado*
Jennifer DuPont, *Tufts Med. Center*

11:00 AM **4.1** Sex Differences, Aging and Vascular Function
Christopher DeSouza *Univ. of Colorado*

11:25 AM **4.2** Abstract 6: Sex Differences in the Effects of Prediabetes on Vascular Contributions to Dementia
Kristen Zuloaga *Albany Med. Coll.*

11:35 AM **4.3** Abstract 9: Sex Differences in the Role of the Smooth Muscle Cell Mineralocorticoid Receptor in Cardiovascular Aging
Jennifer DuPont *Tufts Med. Center*

11:45 AM **4.4** Maternal Aging and Cardiovascular Dysfunction
Sandra Davidge *Univ. of Alberta*

12:10 PM **4.5** Abstract 32: Central Leptin Receptor Antagonism Attenuates the Development of Menopausal-Induced Hypertension in the Rat
Maria Barnes *Des Moines Univ.*

12:20 PM **4.6** Bioenergetic and Metabolic Consequences of the Loss of Ovarian Function in Women
Wendy Kohrt *Univ. of Colorado*

5.0 SESSION 3: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE HEART

Mon., 3:00-4:45 PM, Summit I

Chairs: **Zdenka Pausova,** *Hospital for Sick Children*
Eli Louwagie, *Univ. of South Dakota-Sanford School of Med.*

3:00 PM **5.1** Sex and Gender Differences in Cardiac Function
Zdenka Pausova *Hospital for Sick Children*

3:25 PM **5.2** Abstract 7: Sex Differences in Healthy Human Heart Revealed by Cap Analysis Gene Expression (CAGE)
Anna Gams *The George Washington Univ.*

3:35 PM **5.3** Abstract 39: Heart Failure with Preserved Ejection Fraction (Hfpef) is Augmented in Obese Mice with an XX Sex Chromosome Complement
Sean Thatcher *Univ. of Kentucky*

3:45 PM **5.4** Estrogen Modulation of the Cardiac RAS in Diastolic Dysfunction
Leanne Groban *Wake Forest School of Med.*

4:10 PM **5.5** Abstract 71: Prenatal Diabetes and High-Fat Diet Exposure Impair Mitochondrial Function in Adult Rat Cardiomyocytes
Eli Louwagie *Univ. of South Dakota-Sanford School of Med.*

4:20 PM **5.6** Understanding the Unique Considerations for the Treatment of Heart Disease in Women vs. Men
Martha Gulati *Ohio State Univ. Wexner Med. Ctr.*

6.0 CAREER DEVELOPMENT AND TRAINEE NETWORK SESSION

Mon., 5:00-6:00 PM, Summit I

5:00 PM **6.1** Get the Job You Want: Tips for Interviewing and Negotiating
Kelly Hyndman *Univ. of Alabama at Birmingham*

TUESDAY, OCTOBER 2, 2018

7.0 SESSION 4: PHYSIOLOGY AND GENDER: OBESITY AND METABOLISM

Tue., 8:00-9:45 AM, Summit I

Chairs: **Franck Mauvais-Jarvis,** *Tulane Univ.*
Jessica Faulkner, *Med. Coll. of Georgia at Augusta Univ.*

8:00 AM **7.1** Sex and Sex Steroids Regulate of Metabolic Dysfunction
Franck Mauvais-Jarvis *Tulane Univ.*

8:25 AM **7.2** Abstract 29: Progesterone Upregulates Endothelial Mineralocorticoid Receptor Expression which Predisposes Female Mice to Obesity-induced Endothelial Dysfunction
Jessica Faulkner *Med. Coll. of Georgia at Augusta Univ.*

8:35 AM **7.3** Abstract 55: Identification of Sex-Specific miRNA and mRNA Expression Patterns in Type 1 Diabetes
Preethi Krishnan *Indiana Univ.*

8:45 AM **7.4** Sex Differences in Kidney Injury in a Rat Model Of Prepubertal Obesity
Jan Michael Williams *Univ. of Mississippi Med. Ctr.*

9:10 AM **7.5** Abstract 67: Female Rats Offered Free Access to Lard, Sucrose, and Chow Developed Features of Metabolic Syndrome and Periuterine Adipose Tissue Expansion
Hijab Ahmed *Univ. of North Texas Health Science Center*

9:20 AM **7.6** X Marks the Spot: Sex Chromosomes Regulate Hypercholesterolemia and Atherosclerosis
Lisa Cassis *Univ. of Kentucky*

8.0 POSTER SESSION 2
Tue., 9:45-11:00 AM, Tennessee Ballroom

Board #

33 **8.1** Long Term Consequences of Food Restriction on Body Composition and Angiotensin System
Aline Souza, Crystal West, Glenda Campos, Amrita Pai, Hong Ji. *Georgetown Univ.; Universidade Federal de Ouro Preto*

34 **8.2** Progesterone Upregulates Endothelial Mineralocorticoid Receptor Expression which Predisposes Female Mice to Obesity-induced Endothelial Dysfunction
Jessica Faulkner, Simone Kennard, Galina Antonova, Zsolt Bagi, Iris Jaffe, Vijay Patel, Eric Belin de Chantemele. *Medical College of Georgia at Augusta Univ.; Tufts Medical Center*

35 **8.3** CD8 T-cells Isolated from Female Mice have Increased in vitro Cell Activation in Response to Nutrient Deprivation
Merry Lindsey, Joshua Clayton, Elizabeth Flynn, Donald Menick, Kristine DeLeon-Pennell. *Univ. of Mississippi Medical Center; Medical Univ. of South Carolina*

36 **8.4** Arginase 2 is Involved in Diet-Induced Obesity and Metabolic Dysregulation in Male and Female Mice
Reem T. Atawia, Haroldo A. Toque, Jijun Chen, Tyler W. Benson, Neal L. Weintraub, Ruth B. Caldwell, R. William Caldwell. *Augusta Univ.*

37 **8.5** Identification of Sex-Specific miRNA and mRNA Expression Patterns in Type 1 Diabetes
Preethi Krishnan, Farooq Syed, Raghavendra Mirmira, Carmella Evans-Molina. *Indiana Univ.*

38 **8.6** Sex Specific Upregulation of Hepatic FGF21 Expression in Metabolic States Contrast by Nutrient Availability
Nadezhda Bazhan, Tatyana Yakovleva, Natalia Sitnikova, Elena Makarova. *Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia*

39 **8.7** Female Rats Offered Free Access to Lard, Sucrose, and Chow Developed Features of Metabolic Syndrome and Periuterine Adipose Tissue Expansion
Hijab Ahmed, Johanna Hannan, John Apolzan, Styliani Goulopoulou. *Univ. of North Texas Health Science Center; Brody School of Medicine; Pennington Biomedical Research Center, Louisiana State Univ. System*

40 **8.8** Prenatal Diabetes and High-Fat Diet Exposure Impair Mitochondrial Function in Adult Rat Cardiomyocytes
Eli Louwagie, Tricia Larsen, Michelle Baack. *Sanford School of Medicine - Univ. of South Dakota; Sanford Research*

41 **8.9** Withdrawn

42 **8.10** His and Her Computational Models of Long-term Blood Pressure Regulation
Jessica Leete, Anita Layton. *Duke Univ.*

43 **8.11** Sex Differences in the Role of the Smooth Muscle Cell Mineralocorticoid Receptor in Cardiovascular Aging
Jennifer DuPont, Seung Kim, Qing Lu, Rachel Kenney, M. Elizabeth Moss, Zhe Sun, Mark Aronovitz, Gregory Martin, Wendy Baur, Gerald Meininger, Michael Hill, Iris Jaffe. *Tufts Medical Center; Univ. of Missouri*

44 **8.12** Gender and Ageing Influence on Vascular Responses in a Pulmonary Hypertensive Rat Animal Model
Jesus Prieto-Lloret, Elena Olea, Angela Gomez-Niño, Ana Obeso, Asuncion Rocher. *Univ. of Valladolid. IBGM. CIBERES ISCiii*

45 **8.13** The Role of Sex in Oxidative Stress and Implications on Vascular Function and Blood Pressures
Rebecca Kappus, Anna Ruth Carmichael, Caroline Blackman, Jessica Yomano. *Appalachian State Univ.*

46 **8.14** Estrogen Determines the Sex-Differences in Adrenergic Vessel Tone Regulation
Kristin Riedel, Irakli Kopaliani, Zatschler Birgit, Müller Bianca, Carmen Friebel, Andreas Deussen. *Technische Universität Dresden, Med. Faculty Carl Gustav Carus*

47 **8.15** Inhibition of Neprilysin Attenuates Angii-Induced Abdominal Aortic Aneurysms (AAAs) and Atherosclerosis in Hypercholesterolemic Male Mice
Yasir Alsiraj, Sean Thatcher, Mark Ensor, Lisa Cassis. *Univ. of Kentucky*

48 **8.16** Withdrawn

49 **8.17** WIthdrawn

50 **8.18** Sex Differences in Vascular Reactivity and Biomarkers of Inflammation in Offspring of Dams Exposed to Perinatal High Salt Diet
Ahmed Oloyo, Santan Olley, Esther Ohihoin, Abdulahi Adejare, Khadijat Ismail-Badmus, Olusoga Sofola. *Coll. of Med., Univ. of Lagos*

51 **8.19** Nitric Oxide Helps Maintain the Buffering Capacity of Perivascular Adipose Tissue in Female Dahl SS in response to a High Fat Diet despite Increases in Blood Pressure and Vascular Inflammation
Lia Taylor, Babak Baban, Jennifer Sullivan. *Augusta Univ.*

53 **8.21** Sex-Specific Differences in Primary Neonatal Lung Fibroblasts and Microvascular Endothelial Cells Exposed to Hyperoxia in Vitro: Implications for Bronchopulmonary Dysplasia (BPD)
Xiaoyu Dong, Yuhao Zhang, Jason Gleghorn, Swati Balaji, Krithika Lingappan. *Baylor College of Medicine;Univ. of Delaware*

54 **8.22** Serelaxin Infusion Does Not Attenuate the Development of Hypertension in a Mouse Model of Systemic Lupus Erythematosus
Victoria Wolf, Jennifer Sasser, Michael Ryan. *Univ. of Mississippi Med. Center*

54A **8.20** Afferent Arteriolar Responsiveness to Endothelin Receptor Activation: Does Sex Matter?
Eman Y. Gohar, Anthony K. Cook, Edward W. Inscho, David M. Pollock. *Univ. of Alabama at Birmingham*

55	**8.23**	Purinoceptor-dependent Regulation of Sodium Excretion is Sexually Dimorphic **Eman Y. Gohar, Malgorzata Kasztan, Shali Zhang, Edward W. Inscho, David M. Pollock.** *Univ. of Alabama at Birmingham*
56	**8.24**	Differential Protein Expression of Renal Dopamine Receptors but Similar AT_1R Activity in Salt-Sensitive Male and Female C57Bl/6J Mice **Xiaoyan Wang, Laureano Asico, Xiaobo Ma, Pedro Jose.** *George Washington Univ.*
57	**8.25**	Renal Ischemia Reperfusion Injury in a Pig Model Reveals Gender Specific Expressed Genes as Potential New Biomarkers of Renal Injury/Regeneration Processes Driving to Chronic Kidney Disease **Stéphane Nemours, Luis Castro, Didac Ribatallada, Miguel Aranda, Marina Ferrer, Juan Morote, Anna Meseguer.** *Vall d'Hebron Institut de Recerca (VHIR)*
58	**8.26**	Female Rats with Preexisting CKD Exhibit Impaired Recovery From AKI and the Subsequent Development of Proteinuria **Jacqui Potter, Shannon Allen, Conor Miles, Aaron Polichnowski.** *East Tennessee State Univ.*
59	**8.27**	Oral L-Arginine Treatment Significantly Increases Renal Tregs in Female DOCA Salt Hypertensive Rats **Ellen E. Gillis, G. Ryan Crislip, Jennifer C. Sullivan.** *Augusta Univ.*
60	**8.28**	Necrox-5 Abolished Maturation Induced Sex Differences in Blood Pressure (BP) in Spontaneously Hypertensive Rats (SHR) **Mahmoud Abdelbary, Gene Crislip, Ellen Gillis, Jacqueline Musall, Micahel Brands, Jennifer Sullivan, Mahmoud Abdelbary.** *Augusta Univ.*
61	**8.29**	Androgen Influence on Renal Fibrosis Associated with Pyelonephritis **Teri Hreha, Christina Collins, Allyssa Daugherty, David Hunstad.** *Washington Univ.*
62	**8.30**	Collecting Duct NOS1, Specifically NOS1β, is Critical for Maintaining Fluid-Electrolyte Balance in Both Males and Females **Kelly Hyndman, Jennifer Pollock.** *Univ. of Alabama at Birmingham*
63	**8.31**	Tissue-Specific Estrogen Receptor Profiling Using Droplet Digital PCR **Dillion Hutson, Rakesh Gurrala, Ricardo Mostany, Ryosuke Sato, Sarah Lindsey.** *Tulane Univ.*
64	**8.32**	A Study on the Incidence and Types of Twinning in the South Indian Population **Shakthi Kumaran Ramasamy.** *Aarupadai Veedu Med. Coll., Puducherry*
65	**8.33**	T Cell Specific Knockdown of Estrogen Receptor-α Does Not Eliminate Premenopausal Protection from Angiotensin II-Induced Hypertension, but Does Impact Renal T Cell Expression of CD28 and CTLA-4 **Joshua Uhlorn, Nathaniel Alexander Husband, Melissa Jill Romero-Aleshire, Dennis Pollow, Jennifer L Uhrlaub, Janko Nikolich-Zugich, Heddwen Brooks.** *Univ. of Arizona*
66	**8.34**	Sex Shapes Cancer Cachexia and the Response to Therapeutic Blocking of ACVR2B Ligands in the Genetically Engineered KPC Mouse Model of Pancreatic Ductal Adenocarcinoma **Xiaoling Zhong, Jianguo Liu, Ashok Narasimhan, Leonidas Koniaris, Teresa Zimmers.** *Indiana Univ.*
LB67	**8.35**	Effect of Oleanolic Acid on Lipid Metabolism in Neonatal Rats with Metabolic Syndrome **Molefhi Moirapula Abotseng, Emmanuel Mukwevho, Ademola Ayeleso, Trevor Nyakudya.** *North West Univ.; Adeleke Univ., Ede,; Univ. of Johannesburg*

LB68 **8.36** Effects of Tender Coconut Water on the Coronary Artery of Male Diabetic Wistar Rats
Churchill Inneh, Oghenakhogie Momodu, Vanessa Oigbochie, Eseosa Adaniwmowan. *Univ. of Beniin*

LB69 **8.37** Therapeutic Role of Intrapartum PDE-5 Inhibition on Blood Pressure and Renal Injury in Offspring of Preeclamptic Rats
Hannah Turbeville, Sean Didion, Michael Garrett, Jennifer Sasser. *Univ. of Mississippi Med. Center*

LB70 **8.38** Effects of Prenatal Sildenafil Treatment On Long-term Cardiovascular Function in Offspring From Dahl Salt-Sensitive Rats
Fieke Terstappen, Frank Spradley, Sinead Clarke, Ying Ge, Courtney Ross, Michael Garrett, Jaap Joles, Titia Lely, Jennifer Sasser. *Univ. Medical Centre Utrecht; Univ. of Mississippi Medical Center*

LB71 **8.39** Testosterone Supplementation in Postmenopausal Hypertensive Rats
Rodrigo Maranon, Jane Reckelhoff. *UMMC*

LB72 **8.40** Hypogonadal Hypertension in Male Sprague-Dawley Rats is Reversed by Testosterone Replacement Therapy, Which Down-Regulates Renin-Angiotensin System Message Expression
Andrea Hanson, Nikolas Garcia, Joshua McKenna, Mercedes Perusquia, John Stallone. *Texas A&M Univ.; Universidad Nacional Autonoma de Mexico*

9.0 SESSION 5: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE KIDNEY

Tue., 11:00 AM-1:05 PM, Summit I

Chairs: **David Pollock,** *Univ. of Alabama at Birmingham*
Ellen Gillis, *Augusta Univ.*

11:00 AM **9.1** Sex Differences in Renal Function: Lessons From the ET-1 System
David Pollock *Univ. of Alabama at Birmingham*

11:25 AM **9.2** Abstract 5: Sex-Specific Regulation of Sirtuin-3 Mediates Differences in Ischemia-Reperfusion Kidney Injury
Jenny Pan *Michael E. DeBakey VAMC*

11:35 AM **9.3** (Patho)Physiological Consequences of Sex Differences in Renal Sodium Transporters
Alicia McDonough *Univ. of Southern California*

12:00 PM **9.4** Abstract 68: Oral L-Arginine Treatment Significantly Increases Renal Tregs in Female DOCA Salt Hypertensive Rats
Ellen Gillis *Augusta Univ.*

12:10 PM **9.5** Sex Differences in Renal Ischemia-Reperfusion Injury
Attila Szabó *Univ. of Budapest*

12:30 PM **9.6** Abstract : 92Sex Differences in Renal Ammonia Metabolism
Autumn Harris *Univ. of Florida*

12:40 PM **9.7** Sex Differences in the Regulation of Blood Pressure by the Circadian Clock Proteins PER1 and BMAL1 in C57BL/6J Mice
Michelle Gumz *Univ. of Florida*

10.0 PLENARY LECTURE

Tue., 3:00-3:45 PM, Summit I

3:00 PM National Initiatives In Sex And Gender-Based Medicine (SGBM)
Marjorie Jenkins *Texas Tech Univ. Health Sci. Ctr.*

11.0 SESSION 6: FEMALE-SPECIFIC CARDIOVASCULAR, RENAL AND METABOLIC COMPLICATIONS

Tue., 4:00-6:10 PM, Summit I

Chairs: **Jennifer Sasser,** *Univ. of Mississippi Med. Center*
Dennis Pollow Jr., *Univ. of Arizona*

4:00 PM **11.1** Clinical and Basic Science Considerations of Cardiovascular Health in Pregnancy
Jennifer Sasser *Univ. of Mississippi Med. Center*

4:25 PM **11.2** Abstract 59: Beta-Carotene Metabolism in the Maternal Heart During Pregnancy
Chelsee Holloway *Rutgers Univ.*

4:35 PM **11.3** Vascular Changes in the Postmenopausal Female
Sarah Lindsey *Tulane Univ.*

5:00 PM **11.4** Abstract 64: Female Rats with Preexisting CKD Exhibit Impaired Recovery From AKI and the Subsequent Development of Proteinuria
Jacqui Potter *East Tennessee State Univ.*

5:10 PM **11.5** Long-term Sequelae of Preeclampsia: A Clinical Perspective
Michelle Hladunewich *Stonybrook Health Sci.s Centre, Toronto*

5:35 PM **11.6** Abstract 93: Angiotensin II Induces a Pro-Inflammatory Shift in the Splenic CD4+ T Cell Proteome in Menopausal Mice
Dennis Pollow Jr. *Univ. of Arizona*

5:40 PM **11.7** Leptin Accelerates Disease Progression and the Development of Hypertension in an Experimental Model of Autoimmune Disease
Erin Taylor *Univ. of Mississippi Med. Ctr.*

WEDNESDAY, OCTOBER 3, 2018

12.0 SESSION 7: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE VASCULATURE

Wed., 8:00-10:10 AM, Summit I

Chairs: **Eric Belin de Chantemele,** *Augusta Univ.*
Eman Y. Gohar, *Univ. of Alabama at Birmingham*

8:00 AM **12.1** Impact of Sex on Vascular Function in Cardiovascular and Metabolic Disorders
Eric Belin de Chantemele *Augusta Univ.*

8:25 AM **12.2** Abstract 28: Estrogen Determines the Sex-Differences in Adrenergic Vessel Tone Regulation
Kristin Riedel *Technische Universität Dresden, Med. Faculty Carl Gustav Carus*

8:35 AM **12.3** Sex Differences in Endothelial Cells of the Microvasculature
Virginia Huxley *Univ. of Missouri*

9:00 AM **12.4** Abstract 78: Nitric Oxide Helps Maintain the Buffering Capacity of Perivascular Adipose Tissue in Female Dahl SS in response to a High Fat Diet despite Increases in Blood Pressure and Vascular Inflammation
Lia Taylor *Augusta Univ.*

9:10 AM **12.5** Using Mathematical Modeling to Understand the Basis for Sex Differences in Vascular Function
Anita Layton *Duke Univ.*

9:35 AM **12.6** Abstract 86: Afferent Arteriolar Responsiveness to Endothelin Receptor Activation: Does Sex Matter?
Eman Y. Gohar *Univ. of Alabama at Birmingham*

9:45 AM **12.7** The Impact of Sex and Diabetes on Vascular Function in Diabetes
Adviye Ergul *Augusta Univ.*

13.0 SESSION 8: MALE-SPECIFIC CARDIOVASCULAR, RENAL AND METABOLIC COMPLICATIONS

Wed., 10:40AM-12:30PM, Summit I

Chairs: **Jane Reckelhoff,** *Univ. of Mississippi Med. Ctr.*
Teri Hreha, *Washington Univ.*

10:40 AM **13.1** Androgens in Cardiovascular Health and Disease
Jane Reckelhoff *Univ. of Mississippi Med. Ctr.*

11:05 AM **13.2** Abstract 12: Group IV Cytosolic Phospholipase A2 is Required for 6B-Hydroxytestosterone Mediated Angiotensin II Induced Hypertension in Male Mice
Ajeeth Pingili *Univ. of Tennessee HSC*

11:15 AM **13.3** Abstract 79: Pretreatment with Low Dose Lipopolysaccharide Attenuates Medullary Congestion in Male WKY Following Acute Kidney Injury
Sarah Ray *Augusta Univ.*

11:25 AM **13.4** Abstract 82: Androgen Influence on Renal Fibrosis Associated with Pyelonephritis
Teri Hreha *Washington Univ.*

11:35 AM **13.5** Testosterone Deficiency in the Aging Male
Licy Yanes Cardozo *Univ. of North Carolina*

14.0 HIGHLIGHTS & CLOSING REMARKS

Wed., 12:00-12:30 PM, Summit I

ABSTRACTS OF INVITED AND VOLUNTEERED ABSTRACTS

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2: SESSION 1: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE BRAIN AND NERVOUS SYSTEM

2.1

ADIPOKINES AND THE CENTRAL REGULATION OF CARDIOVASCULAR FUNCTION

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Adipokines such as leptin and nesfatin-1 act as important metabolic signals that inhibit food intake in the presence of nutrient excess. These hormones also act in brain to modulate cardiovascular function by stimulating sympathetic nervous system activity and increasing blood pressure. Our recent studies suggest that female sex hormones protect against nesfatin-1-induced elevations in blood pressure, and that expression of nesfatin-1 is altered across the rat estrous cycle. We hypothesize that nesfatin-1 may play an important role in etiology of hypertension, particularly in postmenopausal women, who have lost the protective effect of ovarian steroid hormones against nesfatin-1 action. Future studies will investigate the function and expression of nesfatin-1 in aging females, particularly in the setting of obesity.

2.2

COMPARING TIME TO PRESYNCOPE DURING SIMULATED HEMORRHAGE ACROSS MENSTRUAL CYCLE

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Background: Several studies indicate that females have lower orthostatic tolerance compared to males. The mechanisms that contribute to the evident sex differences are not well understood. Multiple characteristics including sex hormones appear to contribute to low tolerance. Menstrual cycle and concurrent hormonal fluctuations are particularly relevant to understanding why females have lower tolerance to central hypovolemia. A number of studies designed to investigate contributions of ovarian hormones (particularly estradiol) to cardiovascular function have generated conflicting findings. Furthermore, there is paucity in the literature on contribution of the anterior pituitary hormones, luteinizing (LH) and follicle-stimulating hormones (FSH), which are elevated during the ovulatory phase of the menstrual cycle.

Objective: In our current study, we induced central hypovolemia similar to hemorrhage using progressively stepwise lower body negative pressure (LBNP) in women during various phases of the menstrual cycle. We tested the hypothesis that less time would be required to manifest presyncope in women during the follicular and luteal phases of the menstrual cycle when estradiol is at its highest levels compared to the ovulatory phase.

Methods: In this cross-sectional experimental design, LBNP application was induced on healthy volunteer female subjects (n=22, mean age 26.8 ± 6 years; mean weight 63.9 ± 9 kg; mean BMI 23.7 ± 2.7; mean height 164.3± 8.2 cm). All subjects were not taking oral contraceptives. Time to presyncope was calculated from start of baseline to termination of LBNP at three menstrual cycle phases; early follicular (Days 1 – 7; n=10), ovulatory (Days 12 – 16, n=5) and mid luteal (Days 20-26, n=7). Data are presented as mean ± SEM. The probability that any differences in LBNP tolerance across menstrual cycle did not exist by greater than chance were determined by ANOVA and expressed as exact 'p' values.

Results: The average time to presyncope was lower for females in the ovulatory phase (1254.2 ± 92 seconds) compared to those in early follicular (1613 ± 48 seconds; $p = 0.013$) and mid luteal (1496 ± 80 seconds; $p = 0.099$) phases. Time to presyncope for females in early follicular and mid luteal phases were marginally similar.

Conclusion: A major finding in this study is the lower tolerance to LBNP in the ovulatory phase of the menstrual cycle. Contrary to our hypothesis, the ovulatory phase of the menstrual cycle that is characterized by elevated LH and FSH with reduced levels of estradiol and progesterone is associated with lower tolerance to central hypovolemia. As such, our findings indicate that estradiol may not contribute to compromised tolerance to central hypovolemia in women given that its highest levels are before the ovulatory phase and during the luteal phase of the menstrual cycle. Such a hypothesis may be tested by subsequent cross-sectional comparisons of females with and without use of contraceptives.

Funding: Funding was provided by an appointment to the Post-doctoral research fellowship program, administered by the Oak Ridge Institute for Science and Education and a grant from the US Army Combat Casualty Care Research Program (D-009-2014-USAISR).

2.3

ESTROGEN RECEPTOR ALPHA CONTRIBUTES TO THE INHIBITORY ACTION OF CENTRAL CYTOCHROME P4501B1 GENERATED 17B ESTRADIOL METABOLITE 2-METHOXYESTRADIOL TO ANGIOTENSIN II-INDUCED HYPERTENSION

Purnima Singh[1], Chi Young Song[1], Shubha Ranjan Dutta[1,2], Scott A. Heldt[2], Kafait U. Malik[1]

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Hypertension in postmenopausal females is attributed to diminished levels of 17β-estradiol (E2). It has been documented that in various experimental models of hypertension including angiotensin (Ang) II, the protection against hypertension is lost in females following ovariectomy (OVX). Ang II produces hypertension by its action in the brain which is minimized by E2 through its action on estrogen receptor alpha (ERα). Cytochrome P4501B1 (CYP1B1) and catechol-O-methyltransferase (COMT) that sequentially metabolize E2 to 2-methoxyestradiol (2-ME) are expressed in the brain. This study was performed to test the hypothesis that 2-ME mediates the inhibitory effect of E2 on Ang II-induced hypertension via ERα in the brain of female mice. Intracerebroventricularly (ICV) administered E2 (1.5µg/2µL/every 2nd day) in OVX wild-type (*Cyp1b1*$^{+/+}$) mice attenuated the Ang II (700 ng/kg/min, 14 days)-induced increase in mean arterial pressure (MAP) measured by radiotelemetry, but not in the mice injected with COMT siRNA (ICV, 0.4 nmol) (108±2 vs. 144±3, n=5-6, P<0.05). ICV injections of 2-ME (1.5 µg/2µL/every 2nd day) but not E2 attenuated the increase in MAP by Ang II in OVX *Cyp1b1*$^{-/-}$mice (112±1 vs 142±5, n=5-6, P<0.05); this effect was minimized in the mice by ICV injected ERα siRNA (0.4 nmol; 137±9). Power spectral analysis of the data on day 12 showed that Ang II-infusion increased the low to high frequency ratio of heart rate variability, index of sympathetic outflow modulation in ICV E2 injected OVX *Cyp1b1*$^{-/-}$ compared to OVX *Cyp1b1*$^{+/+}$ mice (2.6±0.1 vs 1.6±0.1) or ICV 2-ME injected OVX *Cyp1b1*$^{-/-}$ mice (1.6±0.1); these effects were blunted by ICV injected COMT siRNA (3.0±0.4) or ERα siRNA (2.6±0.4). Administration of ganglionic blocker hexamethonium (30 mg/Kg, IP) on day 14 of Ang II infusion resulted in greater reduction in MAP in OVX *Cyp1b1*$^{-/-}$ than OVX *Cyp1b1*$^{+/+}$ mice injected ICV with E2 (Δ89±7 vs Δ64±3, mmHg) or 2-ME in OVX *Cyp1b1*$^{-/-}$ mice (Δ60±3 mmHg); these effects were attenuated in mice by ICV injected COMT siRNA (Δ84±7 mmHg) or ERα siRNA (Δ84±9, mmHg). Furthermore, in the intact *Cyp1b1*$^{+/+}$ female mice, adenovirus (Ad)-CYP1B1 shRNA but not Ad-scrambled (scr) shRNA (ICV, 2µL, 1.3X10^{13}particles/mL), potentiated Ang II-induced increase in systolic blood pressure (SBP, mm Hg)) measured by tail-cuff method (170±4 vs 141±3, n=8-9, P<0.05). In the intact *Cyp1b1*$^{-/-}$, but not OVX *Cyp1b1*$^{-/-}$ mice, reconstitution of CYP1B1 in the brain by transduction with Ad-CYP1B1-DNA (ICV, 2µL, 1.0 X 10^{12}particles/mL) reduced Ang II-induced increase in SBP (135±1 vs 167±6, n=7-10, P<0.05). These data provide the first evidence that 1) central effect of E2 to attenuate Ang II-induced hypertension is dependent on brain CYP1B1 and is most likely mediated via generation of 2-ME, and 2) 2-ME protects against Ang II induced hypertension by acting through ERα in the brain by reducing sympathetic outflow. Also, deoxycorticosterone acetate (DOCA, 50 mg/kg) salt (1% NaCl in drinking water) in uni-nephrectomized *Cyp1b1*$^{-/-}$ female mice at 4 weeks caused greater increase in SBP than in *Cyp1b1*$^{+/+}$ mice (165±6 vs. 133±4, n=4, P<0.05). In the intact *Cyp1b1*$^{-/-}$ female mice, reconstitution of CYP1B1 in the brain by transduction with Ad-CYP1B1-DNA but not its control Ad-GFP-DNA reduced DOCA salt-induced increase in SBP (133±3 vs 169±7, n=5, P<0.05). These data suggest that the brain CYP1B1 also protects against DOCA salt-induced hypertension in female mice. Whether this protection is mediated via DOCA-salt effect on brain Ang II, metabolism of E2 or ERα remains to be determined. The significance of this study is that the hormone replacement therapy in the USA have failed to lower BP or decrease cardiovascular disease in postmenopausal females. Therefore, 2-ME could be useful in treating hypertension in postmenopausal and hypoestrogenemic premenopausal females or in those with menstrual irregularities because of ovarian failure. This study was supported by NIH-HLBI Grant 19134-43.

2.4

THE ROLE OF SENSITIZATION AND NEUROPLASTICITY IN SEX DIFFERENCES IN BLOOD PRESSURE AND HYPERTENSION

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The nervous system has the capacity to not only control reflex responses, but also can be modified by experience to alter the magnitude of the responses of systems it controls and then maintain these changes over long periods of time. These types of adaptive changes in the control of systemic responses are characteristic of processes involving memory and implicate a mediating brain neuroplasticity. In recent years we have demonstrated under several experimental conditions that the hypertensive response can be sensitized in response to challenges (stressors) presented earlier in the lifetime of an animal (rat or mouse). Sensitization is a simple form of non-associative learning. To demonstrate Hypertensive Response Sensitization (HTRS), we employ an Induction-Delay-Expression (IND-

DEL-EXP) experimental paradigm. Adult males readily show HTRS even when IND of sensitization is conducted as early as during the perinatal period. Intact females do not show HTRS. This talk will focus on the mechanisms responsible for induction of HTRS, why it occurs only in males and why males and females display such a sexual dimorphism.

Funding Sources: NIH HL14388, HL098207, HL073986, HL84027

2.5

CENTRAL ACETYLCHOLINESTERASE INHIBITOR, GALANTAMINE, PREVENTS LIPID-INDUCED OXIDATIVE STRESS IN AFRICAN AMERICAN WOMEN

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African American women (AAW) have one of the highest prevalence of hypertension in the US. Obese AAW have decreased parasympathetic (PNS) activity compared to whites. Continuous lipid infusion that causes cardiovascular autonomic imbalance (decrease in PNS and increase in sympathetic activity) induces a greater increase in oxidative stress in AA compared to whites. Considering that PNS protects against oxidation and that central acetylcholinesterase inhibitors have been shown to suppress oxidative stress in animal models. We tested the hypothesis that the central acetylcholinesterase inhibitor, galantamine attenuates oxidation in response to lipid infusion in obese AAW compared to white women. We randomized 14 healthy obese AAW (39.5±10.7 yo, BMI 38.8±3.4) and 10 (35.9±8.3 yo, BMI 36.3±2.1) white women. All subjects underwent 4-h infusions of Intralipids and heparin. On separate days subjects received either 16 mg galantamine or placebo in a crossover fashion. Lipid-induced oxidative stress and inflammation were assessed with plasma F2-isoprostanes and cytokines at baseline, 2 and 4-h post-intralipid infusion. In AA, 16 mg of galantamine significantly suppressed the increase in lipid-induced oxidative stress (10±18 vs. -3.0±12 pg/mL with galantamine, P=0.014). No effect was noted in whites. Galantamine tended to increase IL10 (4.8±7.58 vs. 17.3±20.7 pg/mL with galantamine, P=0.06). We did not observe any effect on blood pressure or heart rate. **Conclusion**: Increased parasympathetic tone with central acetylcholinesterase inhibitor, galantamine, suppressed lipid-induced oxidative stress in African American women.

2.6

BRAIN BLOOD FLOW CONTROL: SEX DIFFERENCES AND SEX-SPECIFIC CONDITIONS

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Despite sexual dimorphism present in risk of stroke and cognitive decline, sex differences in brain blood flow regulation are understudied. Premenopausal women demonstrate higher basal brain blood flow and middle cerebral artery blood velocity (MCAv) compared with age-matched men. However, the brain blood flow response to a stimulus (such as hypercapnia), termed cerebrovascular reactivity, may be more relevant for determining future risk of cerebrovascular disease. Sex differences in cerebrovascular reactivity will be discussed using several different methodological approaches. In addition, conditions unique to women, such as menopause and pregnancy, may further affect brain blood flow regulation. We will discuss the effect of age and menopause in women, the influence of previous use of menopausal hormones, and impact of pregnancy history in postmenopausal women on cerebrovascular reactivity. Collectively this work suggests there are sex-differences in cerebrovascular reactivity and sex-specific conditions that may place postmenopausal women at greater risk of stroke or cognitive decline.

Support: NIH HL118154, Alzheimer's Association

3: POSTER SESSION I

3.1

DIABETES PROMOTES A SEXUAL DIMORPHIC EXPANSION OF CIRCULATING AND CEREBROVASCULAR TH17 CELLS IN FEMALE RATS

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Diabetes is a sexually dimorphic disease. Women not only experience more severe diabetes but also suffer from a higher rate of diabetic complications including stroke and cognitive impairment. Yet, experimental data on sex-differences in cerebrovascular complications of diabetes is limited. We recently showed that diabetes negates the cerebrovascular protection typically seen in adult female rats. It has been reported that a high salt diet promotes expansion of IL17-producing T cells (Th17) in the gut microbiome and contributes to cerebrovascular dysfunction and cognitive impairment in male mice. Based on these grounds, we postulated that 1) circulating and/or cerebrovascular IL17 are elevated in high fat diet (HFD)-induced diabetic female rats, and 2) diabetes promotes Th17 expansion in the gut.

Methods: Diabetes was induced in male and female Wistar rats by a HFD and low dose streptozotocin combination. After 8-11 weeks of diabetes, cell suspensions prepared from freshly harvested blood, brain and small intestine specimens were analyzed by flow cytometry using antibodies against cell surface markers (CD3, CD4, γδTCR) and intracellular markers (IL4, IL10, IL17, IFNγ and FOXP3) of T cells. Plasma IL17 concentration was measured through ELISA.

Results: (Table) Although HFD did not alter Th17 cells within the gut, it differentially increased both circulating ($p<0.05$) and cerebral Th17 cells ($p<0.05$), as well as plasma IL17 concentrations in females, but not males. Non-diabetic females also had significantly more circulating and cerebral inflammatory cells than their males counterparts (Th17 blood and brain $p<0.05$,0.01, Th1 blood and brain, $p<0.05$,0.05, δTCR brain $p<0.05$). This separation is further expanded in the presence of diabetes (Th17 blood and brain $p<0.001$,0.001, Th1 blood $p<0.001$, δTCR blood $p<0.001$).

Conclusion: Expansion of Th17 cells may contribute to the sexual dimorphic differences that exists in diabetes-related complications. Whether diabetes-mediated expansion of Th17 cells and IL17 concentrations contributes to cerebrovascular and cognitive dysfunction in females remain to be determined.

Male

Protein Activation	NON-DIABETIC *(N=6)*			DIABETIC *(N=6)*		
Plasma IL17	1.7±0.752			3.984±2.119		
T Cell Type						
	NON-DIABETIC *(N=6)*			DIABETIC *(N=6)*		
	Gut	Blood	Brain	Gut	Blood	Brain
Th17$_{(CD4+,IL17+)}$	5.2±1.5	7.0±1.528	0.3±0.1	5.5±1.7	8.5±1.0	0.5±0.1
γδTCR$_{(CD3+,γδTCR+)}$	9.3±3.3	5.7±0.8	0.6±0.0	5.3±2.3	12.2±1.0***	0.8±0.1*
Th1 $_{(CD3+,IFNγ+)}$	5.8±2.6	4.8±0.7	0.5±0.1	9.2±4.3	4.6±0.6	0.3±0.1***
Th2 $_{(CD3+,IL4+)}$	4.3±0.7	5.7±0.8	0.7±0.1	7.0±0.8	5.2±0.5	0.3±0.1*
TREG$_{(CD4+,FOXP3+)}$	5.7±0.6	5.5±0.4	0.3±0.1	6.3±0.8	7.7±0.6	0.3±0.1

Female

Protein Activation	NON-DIABETIC *(N=6)*			DIABETIC *(N=6)*		
Plasma IL17	1.79±0.8459			5.2229±1.537		
T Cell Type						
	NON-DIABETIC *(N=6)*			DIABETIC *(N=6)*		
	Gut	Blood	Brain	Gut	Blood	Brain
Th17$_{(CD4+,IL17+)}$	4.7±1.3	#10.0±1	##0.7±0.1	7.2±2.5	###13.0±1.2*	###0.9±0.1*
γδTCR$_{(CD3+,γδTCR+)}$	7.8±2.0	4.2±0.9	#0.9±0.1	5.7±2.7	###8.0±0.9**	0.9±0.0
Th1 $_{(CD3+,IFNγ+)}$	6.0±2.7	#7.5±0.4	#0.7 ±0.1	9.5±4..3	###9.0±0.8	0.5±0.1*
Th2 $_{(CD3+,IL4+)}$	5.0±1.0	8.7±0.9	0.9±0.1	7.2±0.8	##8.8±0.7	##0.6±0.1***
TREG$_{(CD4+,FOXP3+)}$	6.0±1.5	5.5±0.6	0.35±0.1	5.0±1.4	5.8±0.8	0.4±0.1

p<0.05 *vs non-diabetic; p< 0.01** vs non-diabetic; p< 0.001*** vs non-diabetic
p<0.05 # vs male; p< 0.01## vs male; p< 0.001### vs male

3.2

SEXUAL DIMORPHISM IN CENTRAL MODULATION OF BARORECEPTOR AFFERENT INPUT IN HYPERTENSION

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Baroreflex activation therapy (BAT) reduces blood pressure in patients with resistant hypertension; however, it remains unknown if hemodynamic responses to this novel approach are influenced by sex. Electrical activation of the baroreceptor afferents within the aortic depressor nerve (ADN) of spontaneously hypertensive rats (SHRs) may bring new insights into the mechanisms underlying BAT in males and females. Accordingly, the differences in cardiovascular responses triggered by stimulation of the left and right ADN were studied in male versus female SHRs. Pentobarbital-anesthetized SHRs of either sex (25-29 weeks, n=5-9) were instrumented for left and right ADN stimulation (1-40 Hz, 0.2 ms, 0.4 mA for 20s) and recording of mean arterial pressure (MAP), heart rate (HR) and mesenteric (MVR) and femoral (FVR) vascular resistance. Female rats were also matched for the diestrus phase of the estrus cycle. Male SHRs had greater resting MAP (177±7 vs. 141±6 mmHg, $P<0.01$) but comparable resting HR (357±7 vs. 333±11 bpm), FVR (207±32 vs. 225±19 mmHg.min.ml^{-1}) and MVR (35±3 vs. 30±4 mmHg.min.ml^{-1}) compared to females. Irrespective of sex, both left and right ADN stimulation evoked frequency-dependent drops ($P<0.01$) in MAP, HR, FVR and MVR. Left ADN stimulation mediated greater reflex reductions in MAP, HR and FVR but not MVR in male SHRs relative to females. Stimulation of the right ADN resulted in similar drops in MAP, HR and FVR but greater drops in MVR in female versus male SHRs. Thus, left ADN stimulation in males evokes a greater depressor response than stimulation of the right ADN, whereas in females the depressor response mediated by left and right ADN stimulation is comparable. Our data shows a differential central modulation of left but not right baroreceptor afferent neurotransmission in male versus female SHRs. Enhanced hypotensive responses to left ADN stimulation in male SHRs are likely driven by more potent baroreflex-mediated reductions in HR and FVR relative to females. The minor reductions in MVR in females in response to right ADN stimulation do not seem to contribute major differences in overall hemodynamic control in either sex. Collectively, this indicates that sexual dimorphism in baroreceptor afferent control of cardiovascular function

should be considered when developing novel therapeutic strategies targeting hypertension in both sexes.

3.3

CENTRAL LEPTIN RECEPTOR ANTAGONISM ATTENUATES THE DEVELOPMENT OF MENOPAUSAL-INDUCED HYPERTENSION IN THE RAT

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The fact that estrogen plays a protective role against hypertension in pre-menopausal women is well established. Additionally, the rapid increase in the rate of hypertension among post-menopausal female provides additional support for beneficial effects of estrogen. However, the events that occur in the absence of estrogen to augment the development of hypertension in post-menopausal women have not been fully elucidated. One event that occurs in menopausal women that may contribute to the development of hypertension is the significant increase in body weight in the form of adipose tissue. Increased adiposity is associated with an increase in the levels of circulating adipokines, substances released from adipose cells. One such adipokine is leptin, which is known to increase with increasing adiposity and play a role in the regulation of cardiovascular function, energy homeostasis and autonomic tone. Therefore, in the current study, using a post-menopausal rat model, we hypothesize that leptin, acting through central leptin receptors, augments menopausal-induced hypertension. Bilateral ovariectomized (OVX) female rats were used in these experiments. On the day the ovaries were removed, rats were instrumented with a telemetry probe for continuous monitoring of blood pressure and heart rate and an osmotic pump to deliver the leptin antagonist (LAN-6) (3µg/day, n=6) or vehicle (saline, n=6) into the lateral ventricle for four weeks. Sham animals were subjected to the same surgeries as OVX animals; however, the ovaries were not excised after opening the abdominal cavity. These animals received only vehicle infusion (SHAM-saline, n=4). Four weeks after the surgery, OVX-saline and OVX-antagonist daily food intake (58 grams) and average weight gain after 4 weeks (74 grams) were significantly greater than SHAM-saline (23 and 21 grams respectively; $p<0.05$). Four (4) weeks following the OVX surgery, the blood pressure of OVX-saline rats was significantly higher than SHAM-saline (106±2 mmHg vs. 96±2 mmHg respectively, P=0.0017), confirming the menopausal-induced hypertension model. The blood pressure of OVX-antagonist rats was similar to SHAM-saline (102±1 mmHg, $P>0.05$). To determine if the change in blood pressure in this model was due to differential activation of the sympathetic nervous system, we tested the blood pressure response to an acute injection of hexamethonium (30ug/kg, IP). The drop in blood pressure was significantly greater in OVX-saline animals, as compared to OVX-antagonist (-41±2 mmHg vs. -30±3 mmHg, P=0.021), which suggests an enhanced basal sympathetic tone in menopausal-induced hypertension due to leptin receptor activation. Taken together, these data suggest leptin, acting on its receptors in the central nervous system, modulates sympathetic nervous system activity and augments blood pressure in menopausal-induced hypertension.

The Iowa Osteopathic Educational Research Foundation supported this work

3.4

TIME COURSE ASSESSMENT OF ACUTE AND CHRONIC HYPOTONIC BRAIN EDEMA BY DYNAMIC NMR: TOPOGRAPHICAL AND GENDER DIFFERENCES

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Introduction and Aims: Hypotonic brain edema has not been studied in depth with imaging techniques such as dynamic NMR. Aims: to assess spatial, temporal and gender differences in brain responses to hypotonicity following acute and chronic hyponatremia induction.

Methods: Chronic hyponatremia was induced in Wistar rats and BALBc mice by intraperitoneal (ip) injection of desmopressin (0.4 ug/kg/d), hyposodic liquid diet and free access to water for 7 days (G1). A group of control normonatremic animals was fed with pellet based diet (G2). Brain edema was studied in real time with NMR at baseline and after ip injection of either 10% of body weight in water (both G1 and G2) or 2 mL of NaCl3% for every 100 grams of body weight (G1) over 120 minutes. ADC (apparent diffusion coefficient) assessed the degree of intracellular water content in different regions of interest: cortex, hypothalamus, nervous fibers, extra-pyramidal system.

Results: Baseline [Na] were 136,75±1.59mmol/L (G2) and 136±0,94mmol/L (G1); after acute water load: 112±5mmol/L and 119±5mmol/L respectively. Baseline ADC values were lower in G1, indicating relevant brain edema. After acute water load, a further drop in the ADC levels was observed in both control and SIADH group. Such ADC reduction started earlier in females. A transient period of cellular defense where water was actively pumped outside the cells was observed, being more efficient in G1, becoming the ADC levels similar in both groups at 60 min, but worsening again at 90 and 120 min. This defense was less intense in females and the final

ADC value was lower than that observed in males. Lateral hypothalamus was the first region to become edematous, followed by the cortex, and then, at different time points, by the extra-pyramidal system and myelinated fibers. Response to edema appeared with different time delays from the maximum degree of edema, but it seemed to follow a structural order: hypothalamus, cortex, extrapyramidal system and fibers. Treatment of G1 with hypertonic saline (NaCl3%) induced a correction of the edema that was three times faster than the spontaneous one, and extremely delayed in neural fiber regions. Sodium concentrations went from 136±0,94mmol/L to 140±7mmol/L.

Conclusions: Brain response to hypotonicity is not homogeneous, and edema develops at different time points in different regions. The time course of the response to brain edema is not homogeneous either. Different speed in the response to brain edema in adjacent areas suggests that damage leading to central pontine myelinolysis could be earlier than observed in clinical practice. Male and female response to brain edema is significantly different and should be considered when assessing this condition in the clinical practice.

3.5

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Hypertension in postmenopausal females is attributed to diminished levels of 17β-estradiol (E2). It has been documented that in various experimental models of hypertension including angiotensin (Ang) II, the protection against hypertension is lost in females following ovariectomy (OVX). Ang II produces hypertension by its action in the brain which is minimized by E2 through its action on estrogen receptor alpha (ERα). Cytochrome P4501B1 (CYP1B1) and catechol-O-methyltransferase (COMT) that sequentially metabolize E2 to 2-methoxyestradiol (2-ME) are expressed in the brain. This study was performed to test the hypothesis that 2-ME mediates the inhibitory effect of E2 on Ang II-induced hypertension via ERα in the brain of female mice. Intracerebroventricularly (ICV) administered E2 (1.5µg/2µL/every 2nd day) in OVX wild-type (*Cyp1b1*$^{+/+}$) mice attenuated the Ang II (700 ng/kg/min, 14 days)-induced increase in mean arterial pressure (MAP) measured by radiotelemetry, but not in the mice injected with COMT siRNA (ICV, 0.4 nmol) (108±2 vs. 144±3, n=5-6, P<0.05). ICV injections of 2-ME (1.5 µg/2µL/every 2nd day) but not E2 attenuated the increase in MAP by Ang II in OVX *Cyp1b1*$^{-/-}$mice (112±1 vs 142±5, n=5-6, P<0.05); this effect was minimized in the mice by ICV injected ERα siRNA (0.4 nmol; 137±9). Power spectral analysis of the data on day 12 showed that Ang II-infusion increased the low to high frequency ratio of heart rate variability, index of sympathetic outflow modulation in ICV E2 injected OVX *Cyp1b1*$^{-/-}$ compared to OVX *Cyp1b1*$^{+/+}$ mice (2.6±0.1 vs 1.6±0.1) or ICV 2-ME injected OVX *Cyp1b1*$^{-/-}$ mice (1.6±0.1); these effects were blunted by ICV injected COMT siRNA (3.0±0.4) or ERα siRNA (2.6±0.4). Administration of ganglionic blocker hexamethonium (30 mg/Kg, IP) on day 14 of Ang II infusion resulted in greater reduction in MAP in OVX *Cyp1b1*$^{-/-}$ than OVX *Cyp1b1*$^{+/+}$ mice injected ICV with E2 (Δ89±7 vs Δ64±3, mmHg) or 2-ME in OVX *Cyp1b1*$^{-/-}$ mice (Δ60±3 mmHg); these effects were attenuated in mice by ICV injected COMT siRNA (Δ84±7 mmHg) or ERα siRNA (Δ84±9, mmHg). Furthermore, in the intact *Cyp1b1*$^{+/+}$ female mice, adenovirus (Ad)-CYP1B1 shRNA but not Ad-scrambled (scr) shRNA (ICV, 2µL, 1.3X10^{13}particles/mL), potentiated Ang II-induced increase in systolic blood pressure (SBP, mm Hg)) measured by tail-cuff method (170±4 vs 141±3, n=8-9, P<0.05). In the intact *Cyp1b1*$^{-/-}$, but not OVX *Cyp1b1*$^{-/-}$ mice, reconstitution of CYP1B1 in the brain by transduction with Ad-CYP1B1-DNA (ICV, 2µL, 1.0 X 10^{12}particles/mL) reduced Ang II-induced increase in SBP (135±1 vs 167±6, n=7-10, P<0.05). These data provide the first evidence that 1) central effect of E2 to attenuate Ang II-induced hypertension is dependent on brain CYP1B1 and is most likely mediated via generation of 2-ME, and 2) 2-ME protects against Ang II induced hypertension by acting through ERα in the brain by reducing sympathetic outflow. Also, deoxycorticosterone acetate (DOCA, 50 mg/kg) salt (1% NaCl in drinking water) in uni-nephrectomized *Cyp1b1*$^{-/-}$ female mice at 4 weeks caused greater increase in SBP than in *Cyp1b1*$^{+/+}$ mice (165±6 vs. 133±4, n=4, P<0.05). In the intact *Cyp1b1*$^{-/-}$ female mice, reconstitution of CYP1B1 in the brain by transduction with Ad-CYP1B1-DNA but not its control Ad-GFP-DNA reduced DOCA salt-induced increase in SBP (133±3 vs 169±7, n=5, P<0.05). These data suggest that the brain CYP1B1 also protects against DOCA salt-induced hypertension in female mice. Whether this protection is mediated via DOCA-salt effect on brain Ang II, metabolism of E2 or ERα remains to be determined. The significance of this study is that the hormone replacement therapy in the USA have failed to lower BP or decrease cardiovascular disease in postmenopausal females. Therefore, 2-ME could be useful in treating hypertension in postmenopausal and hypoestrogenemic premenopausal females or in those with menstrual irregularities because of ovarian failure. This study was supported by NIH-HLBI Grant 19134-43.

3.6

SEX-DEPENDENT EFFECTS OF PREDIABETES IN MOUSE MODELS OF ALZHEIMER'S DISEASE AND MIXED DEMENTIA

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Alzheimer's Disease (AD) and vascular dementia are the two most common forms of dementia, and it has been estimated that 60% of individuals with AD have underlying cerebrovascular pathology/mixed dementia (MxD). Diabetes increases the risk for both vascular and non-vascular dementia (including AD). Less is known about the effects of prediabetes, insulin resistance and glucose intolerance in the absence of hyperglycemia. This is a critical gap in knowledge, as prediabetes is 3x more common than diabetes and affects 38% of Americans. Prediabetes has been linked to early indicators of dementia, including hippocampal atrophy and memory deficits. Despite strikingly high prevalence and rates of comorbidity, much less is known about the effects of prediabetes on vascular and AD pathology and symptomology, particularly when these pathologies co-occur. In addition, since females have higher rates of dementia and faster rates of decline in cognition, sex differences must be explored. Therefore, the aim of the current study was to determine whether sex differences in the effects of prediabetes (modeled by chronic high fat diet) exist in mouse models of AD and MD. Male and female 3xTg-AD mice received either sham (AD model) or right unilateral common carotid artery occlusion surgery (MxD model) at ~2.5 months of age; these were compared to wild-type (WT)/sham surgery (control) mice of both sexes. Mice were then placed on either low fat (LF; 10% fat) or high fat (HF; 60% fat; prediabetes model) diet for 3 months, then subjected to a glucose tolerance test (GTT), and used for either assessment of neurogenesis or behavior/cognition. Additional aspects of neuropathology (e.g. cerebral blood flow, amyloid, inflammation, cell death, and integrity of white matter, vasculature, and blood brain barrier integrity) are also being assessed. MxD surgery resulted in ~20% reduction in blood flow to the right hemisphere immediately after surgery. HF diet increased body weight gain and visceral fat mass, and impaired glucose tolerance, across groups. These metabolic deficits appear to be greater in females compared to males, and in 3xTg-AD mice regardless of surgery (AD and MxD models) compared to WT mice. HF diet did not affect novel object recognition performance in WT mice; however, most AD and MxD groups were impaired on this task. HF diet generally impaired spatial memory in the Morris water maze across mouse models, and MxD (regardless of diet) also hindered performance. This study will be critical for understanding sex differences in the effects of prediabetes on Alzheimer's disease and mixed dementia, and may point to pathways that could be targeted to enhance functional outcomes.

3.7

DOCA-SALT HYPERTENSION LEADS TO NEUROCOGNITIVE DEFICIENCIES IN FEMALE SD RATS

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Current publications report younger hypertensive women have a greater risk of dementia later in life compared to their hypertensive male counterparts. To investigate the impact of hypertension in cognitive function in females, we measured neurocognitive function in a rat model that mimics the salt-sensitive hypertension observed in humans, the deoxycorticosterone acetate (DOCA)-salt hypertension model. In the current study, we tested the hypothesis that DOCA-salt hypertension results in neurocognitive deficiencies in females. Female Sprague Dawley (SD) rats (10-11 wks of age, n=6) were uni-nephrectomized and a subset of rats (n=3) were subcutaneously implanted with a DOCA pellet (200 mg/rat, 60-d time release) and given 0.9% NaCl to drink ad libitum. Uni-nephrectomized control rats did not receive a DOCA pellet and were given tap water to drink. Blood pressure (BP) was measured weekly via tail cuff plethysmography and cognitive function was assessed by the Y Maze Alternation test, prior to the implanting DOCA pellets as well as following a 4 week DOCA treatment. DOCA-salt treatment resulted in a significant increase in BP from baseline (Baseline: 133 ± 4 mmHg vs Week 4 183 ± 3 mmHg; p= <0.0001). BP did not change in UNX controls (Baseline: 133 ± 2 mmHg vs Week 4: 134 ± 4 mmHg). During the Y Maze Alternation test, rats are exposed to A and B arms for 10 minutes followed by a 15 minute break. Following the break, all rats are then allowed 3 minute access to arms A, B, and the novel arm C. During those 3 minutes, the number of times they consequently enter a different arm, and the total number of arm entries are used to calculate the percent of alternation, a commonly applied measure to quantify cognitive function. Percent of alternation significantly decreased in DOCA-salt rats (Baseline: 50% ± 10 vs Week 3: 30% ± 6; p=.027), but remained unchanged in UNX controls (Baseline: 48% ± 6 vs Week 3: 51% ± 5), suggesting that DOCA-salt hypertension led to cognitive decline. In conclusion, DOCA-salt hypertensive female rats display cognitive deficiencies in relation to the increase in BP. Future studies will assess the mechanisms by which increases in BP alter cognitive function, including assessment of neuroinflammation, blood brain barrier permeability and the activated brain renin angiotensin system. Exploring sex specific mechanisms mediating hypertension and cognitive function could lead to novel therapy options to

reduce the risks of cognitive impairments in early hypertensive populations.

3.8

SEX-SPECIFIC HORMONES PRODUCED DURING PROESTROUS AND ESTROUS EXERT A REGULATORY EFFECT ON THE NESFATIN-1 MRNA LEVELS AND AUTONOMIC FUNCTION IN FEMALE CYCLING RATS.

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Nesfatin-1/ NUCB2 is a protein derived from the nucleobindin-2 precursor originally localized in different appetite controlling areas of the brain, such as the hypothalamic PVN, ARC, SON, LHA and NTS, and is thought to be regulated in a sex-specific manner. It has recently been suggested that nesfatin-1 might play an important metabolic role during pregnancy and fetal development (2) and the expression of nesfatin-1/ NUCB2 is regulated by progesterone and 17β-estradiol in mouse pituitary gland (1). We investigated Nesfatin-1 mRNA expression in female, cycling rats across the estrous cycle and observed a decrease in Nesfatin-1 mRNA during Proestrous, corresponding to a period of increased levels of Estradiol. We therefore hypothesized that the ability of centrally administered Nesfatin-1 to raise mean arterial pressure (MAP) in conscious, cycling, freely moving female rats would no longer be seen during the Proestrous day of the estrous cycle. We monitored MAP changes across estrous cycle with central Nesfatin-1 administration. We found that the ability of Nesfatin-1 to increase MAP is no longer prevalent in female, cycling rats on proestrous and estrous days, suggesting that sex-specific hormones produced during proestrous and estrous exert a regulatory effect on the Nesfatin-1 mRNA levels and autonomic function.

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3.9

CENTRAL ACETYLCHOLINESTERASE INHIBITOR, GALANTAMINE, PREVENTS LIPID-INDUCED OXIDATIVE STRESS IN AFRICAN AMERICAN WOMEN

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African American women (AAW) have one of the highest prevalence of hypertension in the US. Obese AAW have decreased parasympathetic (PNS) activity compared to whites. Continuous lipid infusion that causes cardiovascular autonomic imbalance (decrease in PNS and increase in sympathetic activity) induces a greater increase in oxidative stress in AA compared to whites. Considering that PNS protects against oxidation and that central acetylcholinesterase inhibitors have been shown to suppress oxidative stress in animal models. We tested the hypothesis that the central acetylcholinesterase inhibitor, galantamine attenuates oxidation in response to lipid infusion in obese AAW compared to white women. We randomized 14 healthy obese AAW (39.5±10.7 yo, BMI 38.8±3.4) and 10 (35.9±8.3 yo, BMI 36.3±2.1) white women. All subjects underwent 4-h infusions of Intralipids and heparin. On separate days subjects received either 16 mg galantamine or placebo in a crossover fashion. Lipid-induced oxidative stress and inflammation were assessed with plasma F2-isoprostanes and cytokines at baseline, 2 and 4-h post-intralipid infusion. In AA, 16 mg of galantamine significantly suppressed the increase in lipid-induced oxidative stress (10±18 vs. -3.0±12 pg/mL with galantamine, P=0.014). No effect was noted in whites. Galantamine tended to increase IL10 (4.8±7.58 vs. 17.3±20.7 pg/mL with galantamine, P=0.06). We did not observe any effect on blood pressure or heart rate. **Conclusion**: Increased parasympathetic tone with central acetylcholinesterase inhibitor, galantamine, suppressed lipid-induced oxidative stress in African American women.

3.10

ANGIOTENSIN TYPE 2 RECEPTOR STIMULATION WITH COMPOUND 21 IMPROVES STROKE OUTCOME IN FEMALE RATS: POSSIBLE ROLE FOR PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA

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Introduction: The angiotensin type 2 receptor (AT2R) agonist, compound 21 (C21), has been shown to be neurovascular protective after stroke in male rats. Here we aimed to study the effect of C21 treatment on female rats after stroke.

Methods: Young female intact Wistar rats (10-12 weeks) in the metestrus/diestrus phases of estrus cycle (low endogenous estrogen) were subjected to 3 h middle cerebral artery occlusion (MCAO) using a silicone-coated monofilament and treated at reperfusion with IP C21 0.03 mg/kg. Bederson and paw grasp tests were performed at 24h and animals were sacrificed for infarct size analysis. Another cohort of ovariectomized (OVX) and intact female rats were subjected to 1 h of MCAO and treated with IP C21 0.03 mg/kg at reperfusion and daily for 72 h. Bederson test was performed at 24h and 72h and animals were sacrificed and whole brain collected for infarct size analysis and western blotting.

In vitro, primary male and female brain endothelial cells (ECs) were isolated and grown in culture and the expression of the AT2R was compared between males and females using at the mRNA level and protein level.

Results (mean±SE): At 24h, C21 treatment in females resulted in significant decrease in infarct size (22±4% vs 34±2%, p value= 0.03) and improvement in Bederson (2.1±0.1 vs 2.6±0.1, p value= 0.03) and Paw grasp (2.7±0.3 vs 1.5±0.3, p value= 0.01) scores. Interestingly, C21 treatment showed a trend toward increased expression of the transcription factor peroxisome proliferator-activated receptor γ (PPARγ) at 72 h in sham and stroked animals suggesting a novel crosstalk between the AT2R and PPARγ after ischemic stroke. In addition, the expression of the AT2R was significantly higher in female ECs compared to male ECs.

Conclusions: Compound 21 improves stroke outcome in female rats probably through increased expression of PPARγ and the AT2R.

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3.11

OVARIECTOMY INDUCES ANXIETY-LIKE BEHAVIOR AND SHORT-TERM RECOGNITION MEMORY IMPAIRMENT

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Introduction: Low levels of ovarian hormones as a result of ovarian follicle failure in menopause decrease the estrogen supply in the brain. Important brain areas involved in the modulation of mood behavior and cognition, including amygdala and hippocampus, express a high density of estrogen receptors, suggesting estrogen deficiency could lead to anxiety development and memory impairments. Taking that into account, the aim of this study was to assess the effect of ovarian hormone loss on anxiety-like behavior, and spatial and recognition short-term memory in ovariectomized rats.

Methods: Three month old (mo) female Long Evans rats were ovariectomized (OVX group) or sham-operated (Sham group). Five weeks after surgery, the animals were exposed to a battery of behavioral tests including elevated plus maze (EPM), open field (OF), novel object recognition (NOR) and 12 arms radial maze (RAM) tests. The behavior protocol was performed during 18 days with two days of interval between each test.

Results: Our results found that OVX group exhibited increased anxiety-like behavior showing a lower percentage of time spent in the open arms (p=0.0278, Student *t* test) and the number of entries in the open arms (p=0.0044, Student *t* test) of EPM in comparison to Sham group. Additionally, OVX group spent less time exploring the center area of the OF than Sham group (p=0.0044, Student *t* test), reinforcing the anxiety response in OVX group. Also, OVX group showed reduced time exploring the novel object in the NOR test compared to Sham group (p=0.0131, Student *t* test), indicating an impairment of short-term recognition memory. In contrast, no difference was found in the short-term spatial memory in the RAM test.

Conclusion: Estrogen deprivation caused by ovariectomy induces anxiety-like behavior and decreased short-term recognition memory, but not short-term spatial memory.

3.12

SEX DIFFERENCES IN HEALTHY HUMAN HEART REVEALED BY CAP ANALYSIS GENE EXPRESSION (CAGE)

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Background: Cardiovascular diseases remain the primary cause of death worldwide. Several epidemiologic and investigative studies have shown the evidence of sex hormones affect in cardiac electrophysiology through genomic effects (ion-channels expression) and non-genomic effects (ion-channels function modulation). However, very little is known about the molecular basis for gender-related discrepancies in cardiac electrophysiology. Due to physiologically distinct functions of atria (electrical impulse initiation) and ventricles (blood pumping), there is a difference in ion-channel expression within a heart that causes different disease susceptibilities between both sexes.

Objective: Compare ion channel gene expression associated with sex differences using CAGE analysis on left atrial (LA) and left ventricular (LV) human donor hearts.

Materials and Methods: Total RNA was extracted from left atria (LA) and left ventricle (LV) of human donor hearts n=4 males (mean age = 53.75) and n=3 females (mean age = 58.7), the cause of death was determined to be non-cardiogenic. Samples were analyzed with CAGE, which is high throughput method for transcriptome analysis that utilizes 'cap-trapping'. The number of tags gives a frequency of usage that provides information about transcription start sites as well as transcript expression levels. Normalization of raw CAGE tag count was performed as counts per million. The two-sample t-test was used to determine statistical significance. **Results and Discussion:** Our results confirmed higher expression of SCN5A in ventricles compared to atria. SCN5A gene encodes Nav1.5 channel α-subunit (I_{Na}) which is more abundant in working myocardium compared to nodal cells. Loss of sodium channel function is associated with Brugada phenotype that is more predominant in males. Indeed, males had a lower expression of the gene that might indicate on their higher predisposition to the disease. KCND3 that encodes for outward current potassium voltage-gated channel (I_{to1}) that is the main contributing current to repolarizing phase 1 of the cardiac action potential had a significantly higher expression in female atria. KCNIP2, voltage-gated potassium channel ($I_{to,f}$) maintains early repolarization. During heart failure it augments $Ca_v1.2$ (CACNA1C) and $K_v4.3$ (KCND3). Since the hearts were healthy, high expression of KCNIP2 in atria did not affect the expression of KCND3 and CACNA1C. Estrogen is known to up-regulate CACNA1C which was indeed found in female hearts although not statistically significant for our postmenopausal female group. Mutations in potassium-channel genes, KCNH2 (I_{Kr}) and KCNQ1 (I_{Ks}), have been associated with Long QT syndrome type 2 and 1 respectively. Our results for these two ions channels did not show a statistically significant difference in expression across sexes. Interestingly, gene for inward rectifier potassium channel (I_{K1}), KCNJ3, that plays an important role in heartbeat generation is up-regulated in atria, the region with pacemaker cells. Another inward-rectifier potassium channel (I_{KACh}), KCNJ2 is up-regulated in ventricles. That demonstrates ion channel specificity to the anatomical areas of the heart and might carry a unique electrophysiological function.

Conclusion: In the past decade there has been a push towards sex-specific drug development since it has been established that medications affect males and females differently. Our study reveals that there are sex-dependent gene expression differences in cardiac ion channels and that CAGE approach allows high-throughput gene expression profiling which can be beneficial for gender-specific drug development and personalized medicine overall. Supported by NIH 5R01HL114395.

3.13

COMPARISON OF MEASURES OF COMPENSATORY RESERVE IN DIFFERENTIATING TOLERANCE TO SIMULATED HEMORRHAGE IN MALES VERSUS FEMALES

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Background: The physiological response to hemorrhage includes significant vasoconstriction to shunt blood to the heart and brain and subsequent cardiovascular collapse (shock). Lower body negative pressure (LBNP) induces central hypovolemia similar to hemorrhage. Healthy humans can either be high tolerant (HT) or low tolerant (LT) to hypovolemia during LBNP. The compensatory reserve (CRM) measures the sum total of all mechanisms that compensate for relative blood volume deficit by analyzing changes in photoplethysmographic (PPG) arterial waveform features. Decreased CRM with progressive reductions in central blood volume can provide a sensitive and specific status of an individual patient. The time to hemodynamic decompensation can be defined as tolerance, where decompensation occurs at 0% CRM. We previously reported that women have less responsiveness during reductions in central blood volume than men and that the female sex was a predictor of low tolerance to blood loss based on time to presyncope.

Objective: We tested the hypothesis that the higher tolerance to progressive central hypovolemia observed in males can be explained by a slower rate of reduction in CRM compared with females.

Methods: Continuous, noninvasive measures of CRM were collected from 208 healthy volunteer subjects (110 male, 98 female; mean age 27.3 ± 8 years; mean height 171 ± 11 cm; mean weight 73.3 ± 16 kg; mean BMI 24.9 ± 4.4; 143 HT, 65 LT) before and during a progressively stepwise LBNP protocol to the point of presyncope. Tolerance group was defined by those subjects who did (HT) or did not (LT) complete the stepwise protocol to 60 mmHg LBNP. Data were analyzed at equal CRM (60%, 30%, and 0%) using generalized estimating equations (GEE) to account for the repeated measures design.

Results: Comparisons of equal compensatory reserve in male and female subjects revealed mean ± 1 SE times were slower in male subjects compared to female subjects at 60% CRM (13.4 ± 0.4 vs. 13.2 ± 0.3 min; $p = 0.660$), 30% CRM (21.3 ± 0.4 vs. 19.7 ± 0.4 min; $p < 0.01$), and 0% [decompensation] (30.1 ± 0.6 vs. 26.4 ± 0.5 min; $p < 0.01$). HT female subjects (n = 61) had slower times than LT male subjects (n = 28) required to reach 0% CRM [decompensation] (29.0 ± 0.4 vs. 23.6 ± 0.6 min; $p < 0.01$).

Conclusion: Consistent with our hypothesis, male subjects had higher overall tolerance than female subjects, coincident with a significantly slower rate of reduction in CRM at 30% and 0% CRM. Additionally, HT female subjects required more time to reach 0% CRM (decompensation) compared to LT males, highlighting that the difference in tolerance between these gender cohorts can be explained by the rate of compensatory reserve depletion. While sex differences have been previously reported as a predictor of LBNP tolerance, we report for the first time the comparison of differentiating tolerance in males compared to females based on measures of the capacity to compensate. The results of the current study indicate that differences between males and females in tolerance to central hypovolemia can be explained by a more rapid depletion of the compensatory reserve in women.

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3.14

COMPARING TIME TO PRESYNCOPE DURING SIMULATED HEMORRHAGE ACROSS MENSTRUAL CYCLE

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Background: Several studies indicate that females have lower orthostatic tolerance compared to males. The mechanisms that contribute to the evident sex differences are not well understood. Multiple characteristics including sex hormones appear to contribute to low tolerance. Menstrual cycle and concurrent hormonal fluctuations are particularly relevant to understanding why females have lower tolerance to central hypovolemia. A number of studies designed to investigate contributions of ovarian hormones (particularly estradiol) to cardiovascular function have generated conflicting findings. Furthermore, there is paucity in the literature on contribution of the anterior pituitary hormones, luteinizing (LH) and follicle-stimulating hormones (FSH), which are elevated during the ovulatory phase of the menstrual cycle.

Objective: In our current study, we induced central hypovolemia similar to hemorrhage using progressively stepwise lower body negative pressure (LBNP) in women during various phases of the menstrual cycle.We tested the hypothesis that less time would be required to manifest presyncope in women during the follicular and luteal phases of the menstrual cycle when estradiol is at its highest levels compared to the ovulatory phase.

Methods: In this cross-sectional experimental design, LBNP application was induced on healthy volunteer female subjects (n=22, mean age 26.8 ± 6 years; mean weight 63.9 ± 9 kg; mean BMI 23.7 ± 2.7; mean height 164.3± 8.2 cm). All subjects were not taking oral contraceptives. Time to presyncope was calculated from start of baseline to termination of LBNP at three menstrual cycle phases; early follicular (Days 1 – 7; n=10), ovulatory (Days 12 – 16, n=5) and mid luteal (Days 20-26, n=7). Data are presented as mean ± SEM. The probability that any differences in LBNP tolerance across menstrual cycle did not exist by greater than chance were determined by ANOVA and expressed as exact 'p' values.

Results: The average time to presyncope was lower for females in the ovulatory phase (1254.2 ± 92 seconds) compared to those in early follicular (1613 ± 48 seconds; $p = 0.013$) and mid luteal (1496 ± 80 seconds; $p = 0.099$) phases. Time to presyncope for females in early follicular and mid luteal phases were marginally similar.

Conclusion: A major finding in this study is the lower tolerance to LBNP in the ovulatory phase of the

menstrual cycle. Contrary to our hypothesis, the ovulatory phase of the menstrual cycle that is characterized by elevated LH and FSH with reduced levels of estradiol and progesterone is associated with lower tolerance to central hypovolemia. As such, our findings indicate that estradiol may not contribute to compromised tolerance to central hypovolemia in women given that its highest levels are before the ovulatory phase and during the luteal phase of the menstrual cycle. Such a hypothesis may be tested by subsequent cross-sectional comparisons of females with and without use of contraceptives.

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3.15

STRESS HORMONE INHIBITION OF ESTROGEN TRANSCRIPTIONAL REGULATION OF THE SEROTONIN SIGNALING IN CARDIOMYOCYTES IS DELETERIOUS FOR THE FEMALE HEART IN MYOCARDIAL INFARCTION.

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Exposure to chronic stress is considered a risk factor for myocardial infarction (MI). Despite the fact that estrogen exerts cardioprotective effects, pre-menopausal women (25 to 40 years of age) are more susceptible to stress-induced MI than similarly aged men. These observed gender-specific effects of stress in MI may be partly attributed to the interactions between estrogen and glucocorticoids (the primary stress hormones). The goal of the present study is to investigate the effects of the combined actions of estrogen and glucocorticoids in cardiomyocytes. Our genome-wide studies show that glucocorticoids inhibit estrogen-mediated regulation of genes with established roles in cardiomyocyte homeostasis, including the cardiac serotonin receptor 5-HT2BR. Serotonin signaling via cardiac 5-HT2BR is critical to prevent mitochondrial dysfunction and cardiomyocyte apoptosis in the adult heart. Selective serotonin reuptake inhibitors (SSRIs) block the reabsorption of serotonin in the brain, making more serotonin available to other organs, including the heart. The use of SSRIs is associated with reduced risk of MI; however, the mechanisms underlying this protection are unknown. Immunohistochemistry for human heart slides of patients who died of MI shows higher expression of 5-HT2BR in females than in males. Therefore, we hypothesize that estrogen cardioprotective effects in the heart are mediated in part by serotonin signaling via the cardiomyocyte 5-HT2BR, and this protection is blocked by glucocorticoids. Our data show that treating cardiomyocytes with estrogen significantly up-regulated 5-HT2BR gene expression and protein levels, whereas co-treatment with glucocorticoids inhibited these effects. Supporting these results, treatment with glucocorticoids *in vivo* repressed estrogen up-regulation of 5-HT2BR expression in hearts from wild-type mice. Using siRNA, gene expression, and chromatin immunoprecipitation (ChIP) assays, we found that 5-HT2BR is a primary target of the glucocorticoid receptor (GR) and the estrogen receptor (ER)-α at the level of transcription. Ligand-bound GR blocks the recruitment of ER-α to the promoter of the 5-HT2BR gene, which may contribute to the adverse effects of stress in the heart of pre-menopausal women. In the context of MI, our data show that glucocorticoid co-treatment with estrogen depressed the expression of 5-HT2BR which lead to a poorer outcome in hearts of female mice challenged with ischemia-reperfusion injury. These findings suggest that increased stress levels in pre-menopausal women promote a negative outcome in MI by blocking estrogen up-regulation of the 5-HT2BR, which results in the inhibition of serotonin signaling cardioprotective effects. Funding for this study was provided by the Research Council and Center for Cardiovascular Diseases and Sciences LSU Health Shreveport.

3.16

HEART FAILURE WITH PRESERVED EJECTION FRACTION (HFPEF) IS AUGMENTED IN OBESE MICE WITH AN XX SEX CHROMOSOME COMPLEMENT

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Objective: Heart failure with preserved ejection fraction (HFpEF) accounts for at least 50% of cardiomyopathies associated with left ventricular dysfunction. HFpEF is associated with diastolic hypertension, atrial fibrillation, obesity, age, and female sex. Alarmingly, given its high prevalence, there are no effective therapies for HFpEF and few experimental models that exhibit features of the human disease. Since female sex is a risk factor for HFpEF, we used a novel murine model (the four core genotypes) that enables dissection of the relative contributions of sex hormones and sex chromosomes. We also incorporated diet-induced obesity and advancing age as risk factors for HFpEF into the experimental design.

Methods and Results: Male (M, XY and XX) and female (F, XX and XY) mice (6 months of age) were fed a 60% high-fat (HF) diet for 6 months. Carotid artery catheters and radiotelemeters were implanted at week 22, mice were allowed to recover for 1 week, and then blood

pressure was recorded for 7 days. Echocardiography was performed by ultrasound under anesthesia at study endpoint. There was a significant effect of sex (M>F) and genotype (XX>XY) on systolic (SBP) and diastolic blood pressures (DBP) of HF-fed mice. Within a sex, SBP and DBP were higher in XY than XX males, but a surprising opposite effect was observed in females (XX>XY SBP and DBP). Notably, left ventricular diastolic diameter (XX: M, 3.9 ± 0.1; F, 3.9 ± 0.1 mm; XY: M, 4.1 ± 0.08; F, 4.2 ± 0.06 mm; P<0.05) and volume were significantly decreased in XX compared to XY obese mice regardless of gonadal sex (P<.05). Consistent with indices of HFpEF, ejection fraction and fractional shortening were not different between groups. However, stroke volume was decreased in XX compared to XY obese mice, regardless of gonadal sex (XX: M, 35.3 ± 2; F, 35.9 ± 3 µl; XY: M, 44.7 ± 4.4; F, 39.6 ± 2.8 µl; P<0.05). Assessment of baroreceptor activity from blood pressure records indicated that baroreceptor slope (gain) and activity were impaired in XX mice, regardless of gonadal sex. Measurements of potential HFpEF biomarkers demonstrated increased serum total TGF-β in female mice with an XX sex chromosome complement (XX, 166 ± 20; XY, 116 ± 11 ng/ml; P<.05). Studies evaluating cardiomyocyte size and assessing cardiac collagen are underway in hearts from XX versus XY mice.

Conclusions: Using mice with differing sex chromosome complement, we have generated a murine model of HFpEF that exhibits features of the human disease, including obesity, female (XX) sex, advancing age, hypertension, diastolic stiffness, an impaired baroreceptor response, and higher serum TGF-β concentrations. Future studies will use this model to define potential therapeutic targets for HFpEF.

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3.17

AMP-ACTIVATED PROTEIN KINASE AND ESTROGEN-DEPENDENT MECHANISMS UNDERLYING INCREASED SUSCEPTIBILITY TO CARDIOVASCULAR DISEASE DURING MENOPAUSE

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Introduction: Premenopausal women are protected against heart and cardiovascular disease(CVD) compared to age-matched men. The cellular and molecular mechanisms underlying the transition from *premenopause/perimenopause*(CVD-resistance) to *postmenopause*(CVD-susceptible) in women is unknown and is the focus of this project. The critical barrier impeding translational progress is the lack of appropriate models to study menopause. Most studies have used surgical removal of ovaries as a model of menopause. We overcome this barrier with the 4-vinylcyclohexene diepoxide(VCD) model of menopause, which mirrors progressive ovarian failure and preserve the critical "perimenopause" transitional period. Using this model, we demonstrate that *perimenopausal*, like cycling(premenopausal) females, are protected from pathological angiotensin II(Ang II)-induced cardiac remodeling, while *menopausal* females are not. Multiple molecular, genetic and cellular mechanisms have been suggested to underlie protection against CVD in non-cycling females, many of which put estrogen as the key mediator. We discovered and interaction between adenosine monophosphate-activated kinase(AMPK) signaling axis and estrogen that is potentially responsible for this cardioprotection before onset of menopause. *We hypothesized that the loss of AMPK signaling is responsible for the exacerbated pathological cardiac remodeling in menopause.*

Methods: A gradual transition to menopause was induced by repeated daily injections of VCD(160 mg/kg). Ang II (800ng/kg*min) was infused into perimenopausal and menopausal females for 14 days. A separate cohort of mice received AMPK activator A769662 (30mg/kg) during perimenopause and menopause. We aimed to identify whether AMPK is necessary to mitigate pathological cardiac remodeling in menopausal females. Left ventricle heart tissue was flash frozen and processed for westerns, histology and quantitative proteomics. For proteomics, gene ontology and expressional changes were quantified using mus musculus(mouse) genome libraries in Perseus software. DAVID was utilized to discover pathway specific changes across treated groups. To validate proteomic across pre-, peri-, and menopausal females, biomarkers from the identified KEGG pathways were validated with immunoblots of AMPK signaling proteins. Additionally, histological staining validated fibrosis and structure of the heart during menopause.

Results: Quantitative proteomics provided a 5,370 proteome. Using a 3-way-ANOVA and arcsine function transformation, 348 proteins revealed unique alterations in expression. A series of heat maps determined cycling and perimenopausal females to have similar expressional profiles, while menopausal females had the opposite expression levels. Proteins expressed in menopausal females were further identified in DAVID and revealed that significant (p*<0.05) max-fold changes in the KEGG pathways "complement coagulation cascade" and "AMPK" were changing. Perimenopausal females also had increased levels of theronine 172 and acetyl-coa-carboxylase phosphorylation compared to menopausal females, indicating that AMPK activation and signaling is playing a crucial role in mitigating susceptibility to heart disease during menopause. This was further validated by the quantification of fibrosis in the menopausal hearts.

Conclusions: Using proteomics and westerns we determined potential cellular and molecular mechanisms

in premenopausal and perimenopausal females that prevents associated cardiovascular morbidities; and started to reveal the underlying shift that gives rise to increased susceptibility to CVD in menopausal females.

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3.18

BETA-CAROTENE METABOLISM IN THE MATERNAL HEART DURING PREGNANCY

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Pregnancy-related deaths associated with cardiovascular diseases have recently increased in the U.S. with a higher occurrence among African American and Hispanic women. Low intake of fruits and vegetables, the main source of vitamin A, is a hallmark of poor nutrition that is manifested in these populations that are mainly affected by gestational cardiac complications. Many signaling pathways have been associated with the physiological hypertrophy (remodeling) of the heart that occurs during pregnancy [1]. However, how these pathways are activated and influenced during pregnancy has yet to be fully understood. Retinoic acid, the active form of vitamin A that functions as a transcriptional regulator, has been implicated in cardiac remodeling in the adult [2], but whether or not retinoids (vitamin A and its derivatives) are essential during the cardiac hypertrophy of pregnancy is still unknown. Preliminary data from our laboratory revealed a small but significant decrease in *Dhrs3* (*Dehydrogenase reductase 3*) and *Lrat* (Lecithin:retinol acyltransferase) in the heart of pregnant wild-type mice at 14.5 dpc, suggesting that during gestation, heart retinoid metabolism may be shunted towards retinoic acid formation rather than storage. Thus, if cardiac retinoid acid synthesis is favored in wild-type mice during pregnancy, the active vitamin A metabolite may play a potential role in sustaining maternal cardiac hypertrophy (remodeling). Beta-carotene, the most abundant dietary precursor of vitamin A, can be cleaved asymmetrically by the mitochondrial beta-carotene 9',10'-oxygenase (BCO2) to generate beta-apo-10'carotenal, which can serve as a precursor of retinoids, but may also antagonize retinoid acid action *per se* [3]. Preliminary observations in our lab indicate that cardiac mRNA levels of *Bco2,* which is the only carotenoid cleavage enzyme expressed in the adult mammalian heart [4], were elevated in wild-type pregnant mice at mid-gestation. Moreover, in the absence of BCO2 (*Bco2-/-* mice) the maternal heart failed to enlarge during pregnancy. Notably during pregnancy, the heart of *Bco2-/-* mice showed significantly reduced retinyl ester levels compared to wild-type pregnant mice. Based on these premises, we hypothesized that BCO2 may contribute to the physiological hypertrophy of the maternal heart during pregnancy. Current studies aim at further understanding the effects of the lack of murine BCO2 on retinoid metabolism and cardiac function in the maternal heart. Understanding the role of carotenoid and retinoid metabolism in this process will allows us to ultimately design dietary preventative measures to potentially decrease adverse cardiac function during pregnancy

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3.19

RESPONSES IN CARDIAC STROKE VOLUME INDEX AND CARDIAC INDEX DO NOT DIFFER BETWEEN GENDERS DURING MODERATE CENTRAL HYPOVOLEMIA.

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Introduction: Gender-specific differences in physiological regulation are of growing interest within both research and clinical treatment. Men have been found to have a greater cardiovascular reserve than women, and female sex has been proposed as a predictor of lower tolerance to hypovolemia (Hinojosa-Laborde et al. 2015). In this study, we hypothesized that females have a greater relative decrease in cardiac stroke volume index (SVI) and in cardiac index (CI) during simulated hypovolemia, compared to men.

Methods: Seventeen females (median (range) age 21 yrs (21–22)) and Body Surface Area (BSA) (=0.000975482xweight$^{0.46}$xheight$^{1.08}$) (Schlich et al. 2010) 1.7 m^2 (1.5 – 2) and fourteen males (age 23 yrs (21–27) and BSA (=0.000579479xweight$^{0.38}$xheight$^{1.24}$) 1.9 m^2 (1.6 – 2.1) underwent experimental central hypovolemia

induced by lower body negative pressure (LBNP) to −30 mmHg. The study was approved by the regional ethics committee (ref.no: 2012/2251 and 2014/2228) and conformed to the Declaration of Helsinki. Heart rate (HR) was obtained from the duration of the R-R interval from a three-lead ECG signal. Non-invasive finger arterial pressure was recorded continuously from the left middle finger, providing beat-to-beat mean arterial pressure (MAP) and beat-to-beat left cardiac stroke volume (SV) (Finometer, FMS, the Netherlands). Cardiac output (CO) was calculated from HR and SV. SVI and CI were calculated by dividing SV and CO by BSA. Medians and 95% confidence intervals were calculated by Hodges-Lehmann's estimates. Wilcoxon signed rank sum test for paired samples was used to test differences between conditions. Unpaired Wilcoxon test was used to assess gender differences. $P<0.05$ was considered significant.

Results: In the female group, HR increased by 10% from rest (56 bpm (51, 59)) to LBNP (62 bpm (57, 65)); SVI decreased by 18% from rest (45 ml/m^2 (40, 48)) to LBNP (37 ml/m2 (31, 41)); CI decreased by 9% ($p<0.001$) from rest (2.5 l/(minxm^2) (2.1, 2.8)) to LBNP (2.3/(minxm^2) (1.8, 2.5)); MAP did not change from rest (73 mmHg (68, 75)) to LBNP (74 mmHg (71, 77)). $P<0.001$.

In the male group, HR increased by 13% from rest (58 bpm (52, 62)) to LBNP (66 bpm (57, 70)); MAP increased by +6% from rest (70 mmHg (66, 73)) to LBNP (75 mmHg (70, 78)); SVI decreased by 19% from rest (52 ml/m^2 (47, 56)) to LBNP (42 ml/m^2 (36, 47)); CI decreased by 10 % from rest (3.1 l/(minxm^2) (2.8, 3.3)) to LBNP (2.8 l/(minxm^2) (2.5, 3)). $P<0.001$.

Percentage change in HR, MAP, SVI and CI did not differ between female and male groups ($P>0.3$).

Discussion and Conclusions: We could not detect gender differences in the relative cardiovascular responses to moderate hypovolemia in these 31 subjects. Others have reported differences in cardiovascular responses between men and women experiencing decompensated hypovolemia. Thus, other factors (difference in body size, blood volume, hormonal differences) may explain why women reach decompensated hypovolemia sooner than men.

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3.20

SEX DIFFERENCES IN THE EFFECTS OF PREDIABETES ON VASCULAR CONTRIBUTIONS TO DEMENTIA

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Background: Diabetes causes endothelial dysfunction and is a major risk factor for vascular contributions to cognitive impairment and dementia (VCID). Diabetic women have increased risk of VCID compared to diabetic men. Data on the effects of prediabetes, which is 3 times more common than type 2 diabetes, are lacking. Prediabetes can be modeled in mice via long-term administration of a high fat (HF) diet, which we have shown causes cognitive deficits. Young females are protected from the metabolic effects of the diet. We hypothesized the middle-aged females would lose their protection, relative to males, from the metabolic and cognitive effects of a HF diet in a mouse model of VCID.

Methods: Middle-aged (8.5 month old) male and female C57BL/6J mice were placed on a HF or control diet for 6.5 months (15 months old at the end of the study). After 3 months on the diet, they received either VCID surgery (unilateral carotid artery occlusion) or sham surgery. Body weight and glucose tolerance were monitored. After 6 months on the diet, blood flow through the left carotid was measured via ultrasound and then cognitive function was assessed in the Morris water maze. Finally, blood flow was measured via laser speckle contrast imaging. Brain were also collected and we will be performing histology to examine neuroinflammation, white matter damage, and blood brain barrier damage.

Results: HF diet caused greater increases in body weight and glucose intolerance in females than males. Blood flow through the left carotid was reduced in HF-fed mice of both sexes. Cerebral blood flow was reduced in the right hemisphere following VCID surgery, with reductions being more severe in the HF female VCID mice. Spatial memory was impaired in VCID males, regardless of diet; however, in females, either HF diet or VCID (or a combination of the two) impaired spatial memory.

Conclusions: The data show that HF-fed/prediabetic middle-aged-females have more severe metabolic and cognitive deficits than prediabetic males. This is in line with the increased risk of VCID in diabetic women compared to men. Our data suggests that prediabetes might also be a greater risk factor for VCID in women.

3.21

INTERACTION BETWEEN GLP-1 RECEPTOR AGONISTS AND RENIN ANGIOTENSIN SYSTEM IN THE METABOLIC SYNDROME IN A MODEL OF POSTMENOPAUSAL PCOS

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Polycystic Ovary Syndrome (PCOS), the most common endocrine disorder in women, is characterized by androgen excess and ovarian dysfunction. PCOS is often associated with components of metabolic syndrome (MS) such as obesity, dyslipidemia, insulin resistance (IR) and increased blood pressure (BP). Small clinical trials showed that liraglutide, a glucagon-like peptide-1 receptor agonist (GLP-1RA), caused significant weight loss in young women with PCOS. Additionally, liraglutide decreased IR and BP in reproductive age-PCOS rats. Postmenopausal women who have had PCOS and chronic androgen excess may be at greater risk for MS and cardiovascular disease than normo-androgenemic postmenopausal women. It was also reported that Liraglutide decreases BP in simple obesity, but does not in obese women with PCOS. We have previously characterized a rat model of postmenopausal PCOS (PM-PCOS) in which chronic androgen excess causes features of MS: increased body weight, fat mass, dyslipidemia, IR, BP and activation of the renin-angiotensin system (RAS). In the present study, we tested the combination of GLP-1RA with RAS blockage as an effective therapeutic tool to treat the MS observed in PM-PCOS.

Methods: Female SD rats, 4 weeks-old, were randomized to chronically receive DHT (dihydrotestosterone, 7.5 mg/90 days) or placebo (n=18/group). At 17 months of age, PM-PCOS and age-matched placebo rats were randomized to consecutively receive: first, liraglutide (0.3 mg/kg/day SC) for 3 weeks, enalapril for 1 week and finally liraglutide+enalapril in combination for 1 additional week. Washout periods of 2 weeks were allowed between treatments. Mean arterial blood pressure (MAP) was recorded by radiotelemetry throughout the experiment. Before and after liraglutide treatment, body composition (by Echo-MRI) and components of the MS were measured.

Results: PM-PCOS rats exhibited significantly higher food intake, body weight, fat mass, total cholesterol, triglycerides, leptin and MAP compared to age-matched placebo rats. After 3 weeks of treatment, liraglutide caused greater reduction in cumulative food intake, body weight (-38.4 ± 4.7 vs -28.8 ± 2.4 g, $p<0.05$), fat mass/body weight (-7.7 ± 0.6 vs -4.8 ± 0.7, $p<0.01$), HOMA-IR index (-4.5 ± 0.6 vs -1.4 ± 1.1, $p<0.05$), leptin, cholesterol, and triglycerides levels in PM-PCOS. Liraglutide decreased MAP in age-matched placebo rats (108 ± 1 vs 101 ± 1 mmHg, p<0.0001); however, it did not lower MAP in PM-PCOS animals (123 ± 2 vs 126 ± 3 mmHg, p=0.56). Enalapril completely abolished differences in MAP (96 ± 2 vs 99 ± 2 mmHg, p=0.47) between groups. Liraglutide+enalapril in combination further decreased MAP in age-matched placebo rats (96 ± 2 vs 86 ± 3 mmHg, p=0.03), but did not in PM-PCOS animals (102 ± 3 vs 100 ± 3 mmHg, p=0.65).

Summary: Although liraglutide exhibited beneficial effects in several components of the MS, it failed to lower MAP in PM-PCOS model. Enalapril completely abolished the differences in MAP between groups. Enalapril+liraglutide in combination did not further decrease MAP in PM-PCOS, in contrast to age-matched placebo. Our results suggest that RAS blockage in combination with liraglutide could be an effective therapeutic tool to treat all components of MS in PM-PCOS, including hypertension.

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3.22

ANGIOTENSIN II INDUCES A PRO-INFLAMMATORY SHIFT IN THE SPLENIC $CD4^+$ T CELL PROTEOME IN MENOPAUSAL MICE

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Premenopausal female mice are protected against Angiotensin II-induced hypertension, however this protection against Ang II-induced hypertension is lost following the onset of menopause. T cells are required for the development of Ang II hypertension in male mice and we have shown that premenopausal females are protected from T cell-mediated hypertension. This protection is also lost in menopausal mice. The purpose of this study was to utilize a bottom-up shotgun proteomics-based approach to examine how $CD4^+$ T cell activation and pro-inflammatory signaling is modified in Ang II-infused menopausal mice compared to premenopausal counterparts. 10-week-old C57BL/6J female mice received intraperitoneal injections of 4-vinylcyclohexene diepoxide (VCD; sesame oil vehicle) for 20 consecutive days to induce menopause. Cyclicity was monitored daily to determine the day of onset of

menopause. Ang II (800ng/kg/min) was infused for 14 days via osmotic minipump into VCD-treated menopausal (Meno/Ang II) or vehicle-treated premenopausal (Ang II) mice. After 14 days of Ang II infusion, splenic $CD4^+$ T cells were isolated and purified via negative immunomagnetic selection. $CD4^+$ purity was measured via flow cytometry and protein was obtained from these cells. Splenic $CD4^+$ T cell protein samples were fractioned via SDS PAGE prior to trypsin digestion and Zip Cleaning on C18 columns. These peptide samples were analyzed via label-free MS/MS tandem mass spectrometry and were subsequently identified and quantified using Mascot and Progenesis software. 7,123 proteins were identified from the peptide samples. From this protein list, 5,857 proteins were identified by more than one unique peptide sequence and were used for subsequent analysis. 964 proteins were differentially expressed between control, Ang II and Meno/Ang II groups ($p < 0.05$). Of the 964 differentially expressed proteins, 350 were significantly different between Ang II and Control, while 639 proteins were differentially expressed between Meno/Ang II and Control, and 248 between Meno/Ang II and Ang II. Gene Ontology (GO) enrichment of the 964 differentially expressed proteins was assessed using Perseus software and the DAVID database. Ang II infusion resulted in the overexpression of 220 GO biological pathways ($p < 0.05$), including positive regulation of cell adhesion (5.1-fold enrichment), negative regulation of interleukin-6 production pathway (4.5-fold enrichment), and negative regulation of cell cycle arrest (4.1-fold enrichment). Overall, expression of proteins positively regulating cell adhesion and negatively regulating interleukin 6 were decreased in the Ang II group versus control and were further decreased in the Meno/Ang II group (see attached table). Proteins associated with negative regulation of cell cycle arrest were equally increased by Ang II in premenopausal and menopausal female mice, suggesting an increase in $CD4^+$T cell proliferation. These results demonstrate that Ang II induces a significantly greater shift in the splenic $CD4^+$ T cell proteome in female mice after menopause. This shift results in a proteomic profile favoring the proliferation and migration of pro-inflammatory T cells in postmenopausal females.

Protein Name	Control	Ang II	Meno+Ang II
Positive Regulation of Cell Adhesion			
Intercellular adhesion molecule 1	1.00 ± 0.04	0.79 ± 0.06*	0.73 ± 0.08*
Intagrin subunit alpha-2	1.00 ± 0.10	0.80 ± 0.08	0.63 ± 0.03*
Tyrosine-protein kinase SRC-1	1.00 ± 0.13	0.80 ± 0.11	0.51 ± 0.03*#
Platelet 12-LOX	1.00 ± 0.06	0.80 ± 0.07	0.54 ± 0.07*#
Negative Regulation of Interleukin 6 Production Pathway			
Beta-arrestin 1	1.00 ± 0.10	0.81 ± 0.07*	0.69 ± 0.05*
Interleukin-1 receptor-associated kinase	1.00 ± 0.34	0.55 ± 0.08*	0.30 ± 0.08*#
Toll-like receptor 9	1.00 ± 0.15	0.71 ± 0.10	0.58 ± 0.08*
Zinc finger CCCH-type containing 12A	1.00 ± 0.08	0.91 ± 0.14	0.52 ± 0.08*#
Negative Regulation of Cell Cycle Arrest			
Cyclin dependent kinase 4	1.00 ± 0.10	1.87 ± 0.20*	1.55 ± 0.20
JunD	1.00 ± 0.04	1.33 ± 0.12*	1.36 ± 0.19
Ras associated domain-containing protein 1	1.00 ± 0.04	1.24 ± 0.06*	1.28 ± 0.13
Nucleosomal binding protein 1	1.00 ± 0.01	1.17 ± 0.10	1.38 ± 0.08*

*p<0.05 vs Control; #p<0.05 vs Ang II

3.23

SEX-SPECIFIC REGULATION OF SIRTUIN-3 MEDIATES DIFFERENCES IN ISCHEMIA-REPERFUSION KIDNEY INJURY

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Background: While the pathogenesis of ischemic acute kidney injury (AKI) is better defined, the therapeutic options remain limited. Sex influences susceptibility to kidney ischemia-reperfusion injury (IRI), and sex hormones play a crucial role. We have previously shown that a pathway from stanniocalcin-1 (STC1) mediated activation of AMPK to induction of mitochondrial sirtuin-3 (SIRT3) suppresses ROS generation and confers resistance to kidney IRI. Our observations reveal increased baseline kidney expression of STC1, activated AMPK, and SIRT3 in female mice vs. males, and we hypothesize that SIRT3 protects from IRI and mediates the observed sexual dimorphism in response to injury.

Methods: We subjected wild-type (WT) and SIRT3 transgenic (Tg) male or female mice to bilateral kidney IRI (clamping of renal pedicles for 30 minutes). A group of male or female WT mice were treated with testosterone by subcutaneous implantation of a 200 mg (21-day release) testosterone pellet for 2 weeks. Cultured HEK 293T cells were treated with 17β-estradiol, testosterone or vehicle.

Results: We observed higher kidney expression of STC1, mitochondrial SIRT3 and activity of AMPK in WT female mice compared with males. While there was age-dependent decline in kidney SIRT3 and AMPK activity, differential expression in males and females persisted. Aged 6 months-old SIRT3 Tg male mice display less tubular vacuolization vs. similarly aged WT male mice. Compared with WT male mice, SIRT3 Tg male mice demonstrated resistance to 30-minutes of kidney IRI

characterized by: improved survival; preserved creatinine clearance (CrCl); decreased morphological damage and ROS production. SIRT3 Tg male mice tolerated IRI with survival and kidney function impairment similar to WT females. WT or SIRT3 Tg female mice display no measurable change in kidney function with 30-minutes of kidney IRI. In WT female mice, kidney mitochondrial SIRT3 expression correlates with both plasma estradiol and testosterone levels; in WT male mice, kidney mitochondrial SIRT3 expression correlates with only plasma testosterone level. Testosterone administration to aged 6 months-old WT male mice increased plasma testosterone ~4-fold, caused kidney injury (decreased CrCl and increased tubular vacuolization), and decreased kidney mitochondrial SIRT3 expression (with no effect on whole cell SIRT3). Testosterone treatment to WT female mice caused no measurable kidney injury, but increased whole cell and mitochondrial SIRT3 expression; possibly due to an associated increase in plasma estradiol level. In cultured HEK cells, estradiol increased whole cell and mitochondrial SIRT3 protein expression, and SIRT3 mRNA in a dose-dependent manner. Testosterone decreased mitochondrial SIRT3 protein expression in a dose-dependent manner, but had no effect on whole cell SIRT3 protein expression and SIRT3 mRNA. Estradiol also increased estrogen receptor-β and estrogen related receptor-α mRNA expression.

Conclusion: The data suggest that: 1) SIRT3 ameliorates kidney IRI, and decreased SIRT3 expression in males mediates the increased susceptibility to ischemic injury; 2) sex steroids regulate mitochondrial SIRT3 expression; estrogen via transcriptional regulation and testosterone via inhibition of mitochondrial targeting.

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3.24

GROUP IV CYTOSOLIC PHOSPHOLIPASE A_2A IS REQUIRED FOR 6B-HYDROXYTESTOSTERONE MEDIATED ANGIOTENSIN II INDUCED HYPERTENSION IN MALE MICE

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Previously we showed that cytochrome P450 (CYP) 1B1-testosterone derived metabolite 6β-hydroxytestosterone (6β-OHT), by acting as a permissive factor, contributes to the development of angiotensin II (Ang II)-induced hypertension in male mice. Also, we reported that Ang II-induced hypertension is mediated by group IV cytosolic phospholipase A_2α (cPLA$_2$α) activation, resulting in arachidonic acid (AA) release, and generation predominantly of eicosanoids with pro-hypertensive effects. This study was performed to investigate the interaction of CYP1B1 and cPLA2α/AA system by testing the hypothesis that 6β-OHT contributes to Ang II-induced hypertension by promoting cPLA$_2$α activation and generation of eicosanoids with pro-hypertensive effects. Male intact or castrated (Cas) *Cyp1b1*$^{+/+}$/cPLA$_2$α$^{+/+}$, *Cyp1b1*$^{-/-}$, and cPLA$_2$α$^{-/-}$ mice (8 weeks old, n=4-5) were infused with Ang II (700 ng/kg/min) and injected with 6β-OHT (15 μg/g, i.p. every 3rd day), for 2 weeks, and systolic blood pressure (SBP) was measured by tail cuff. In *Cyp1b1*$^{+/+}$/cPLA$_2$α$^{+/+}$ mice, castration or CYP1B1 gene disruption minimized the Ang II-induced increase in SBP (127±3 and 148±3 vs. 188±3 mmHg, respectively, P<0.05). Ang II infusion in 6β-OHT, but not its vehicle (DMSO, 50ml) treated *Cyp1b1*$^{-/-}$mice increased SBP (189±5 vs.148±3 mmHg, P<0.05); this increase was minimized by the AA metabolism inhibitor, 5,8,11,14-eicosatetraynoic acid (25 mg/kg, i.p. every 3rd day) (140±4 mmHg, P<0.05). Ang II infusion with 6β-OHT treatment increased SBP in Cas cPLA$_2$α$^{+/+}$mice, but not in Cas cPLA$_2$α$^{-/-}$mice (176±7 vs. 122±2 mmHg, P<0.05). Treatment with antagonists of prostaglandin (PG) E2 receptors EP1 (SC19220) and EP3 (L-798106) (28 μg/g, s.c. every 2nd day) attenuated the Ang II-induced increase in SBP in 6β-OHT treated Cas cPLA$_2$α$^{+/+}$ mice (123±4, 123±6 vs. 189±5 mmHg, respectively, P<0.05). These data suggest that 6β-OHT contributes to Ang II-induced increase in SBP via cPLA$_2$α activation, the release of AA and generation of eicosanoids, most likely PGE2 that exerts pro-hypertensive effects by stimulating EP1 and EP3 receptors. Therefore, the development of agents that selectively inhibit the cPLA$_2$α activity or block EP1 and EP3 receptors could be useful in treating hypertension and its pathogenesis.

3.25

SEX DIFFERENCE IN T REGULATORY CELL EXPANSION AFTER ADOPTIVE TRANSFER FROM HYPERTENSIVE DONORS LEADS TO PROTECTION AGAINST T CELL-MEDIATED HYPERTENSION IN PREMENOPAUSAL MICE

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Activation of T cell-dependent pro-inflammatory responses are required for Ang II hypertension in male

mice. However, females are protected from T cell-mediated hypertension and may suppress hypertension by directly preventing Ang II-induced pro-inflammatory T cell activation. Here we sought to determine whether transferring T cells from hypertensive donor mice eliminates female protection against T cell-mediated hypertension. Splenic $CD3^+$ T cells were transferred from normotensive (NT) or Ang II-hypertensive (HT) C57BL/6J male donors to female Rag-$1^{-/-}$ (NT T cell female-NTF; HT T cell female-HTF) or male Rag-$1^{-/-}$ (HT T cell male-HTM) recipient mice. Blood pressure was monitored (tail cuff) for 5 weeks post-transfer. Ang II (490ng/kg/min) was infused into recipient mice for 14 days during weeks 4 and 5 post-transfer (NTFA; HTFA; HTMA). Ang II significantly increased MAP in donor male mice (NT 114 ± 3 mmHg vs HT 157 ± 3 mmHg, *p<0.05). Transfer of T cells from HT donors did not induce HT in female or male recipients. Similarly, T cell donor environment did not affect Ang II-induced blood pressure in female recipients, which remained protected compared to male recipients (MAP: NTF 83 ± 4 mmHg*, HTF 88 ± 6 mmHg*, NTFA 101 ± 5 mmHg*, HTFA 103 ± 5 mmHg*, HTMA 138 ± 3 mmHg, *p<0.05 vs HTMA). Flow cytometry demonstrated similar splenic T cell frequency across all groups (CD3: NTF 18%, NTFA 16%, HTF 17%, HTFA 14%, HTMA 18%, p>0.05). However, T regulatory cells were significantly reduced in male recipients compared to all female groups (Foxp3: NTF 21.6%*, NTFA 22.2%*, HTF 22.8%*, HTFA 22.6%*, HTMA 15.3%, *p<0.05 vs HTMA). Females had significantly less renal T cell infiltration compared to males and infiltration was not impacted by Ang II infusion or T cell donor status (CD3: NTF 12,083*, NTFA 11,317*, HTF 12,656*, HTFA 8,997*, HTMA 22,405, *p<0.05 vs HTMA; CD4: NTF 6,411*, NTFA 4,702*, HTF 5,831*, HTFA 4,579*, HTMA 9,914, *p<0.05 vs HTMA; CD8: NTF 5,397*, NTFA 6,123*, HTF 6,362*, HTFA 3,792*, HTMA 11,727, *p<0.05 vs HTMA). These results demonstrate that female mice prevent T cell-mediated hypertension and renal T cell infiltration regardless of previous T cell exposure to a hypertensive environment, suggesting a direct preventive mechanism in females against pro-hypertensive T cell responses.

3.26

SEX DIFFERENCES IN THE DIURNAL NATRIURETIC RESPONSE TO BENZAMIL IN SPRAGUE DAWLEY RATS

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The epithelial sodium channel (ENaC) is expressed in a circadian manner, but time of day differences in activity are not known. Recent data also showed that ENaC has higher expression (Western blot) in female rats consistent with the well-established sex differences in renal sodium handling. However, we do not know whether there are time of day differences or sex differences in ENaC activity even though we know that circadian clock genes control ENaC expression and diurnal patterns in sodium excretion. Therefore, the aim of this study was to determine if the diuretic response to ENaC inhibition (benzamil) is different between sexes and if it follows a diurnal rhythm as well.

Sprague Dawley Rats (12-16 wk old) were placed in metabolic cages, where 12-hour urine collections were obtained to measure baseline urine volume and sodium excretion as well as water and food intake. On day 3, benzamil was given at a dose of 1mg/kg (i.p.) either at the beginning of their inactive period/lights on (Zeitgeber Time 0, ZT0) or their active period/lights off (ZT12). Following treatment, urine was collected at 2, 6 and 12 hours.

Both male and female rats had a significant increase in sodium excretion and urine volume in response to benzamil at the beginning of both active and inactive periods. The natriuretic response to benzamil was significantly greater in male compared to female rats (909 ± 302 vs 523 ± 83 µEq/kg/hr n=8) at ZT0 and (934 ± 94 vs 714 ± 151 µEq/kg/hr n=8) at ZT12. The diuretic response followed natriuresis being more prominent in male than female rats regardless of time of day (4.2 ± 0.7 vs 3.3 ± 0.5 ml/kg/hr n=8) at ZT0 and (3.6 ± 0.6 vs 2.5 ± 0.3 ml/kg/hr n=8) at ZT12. However, the larger response to benzamil given at the beginning of the inactive period (ZT0) compared to active period (ZT12) was not statistically significant (311 ± 98 vs 354 ± 30 µEq/hr and 120 ± 17.75 vs 174 ± 37 µEq/hr n=8) in male and female rats respectively. Given that endothelin-1 (ET-1) is an upstream inhibitor of ENaC, we measured urinary ET-1 levels to assess the effects of channel blockade and increased urine flow on intrarenal ET-1. ET-1 levels significantly increased following benzamil administration in both sexes but was significantly greater in females. ET-1 excretion increased from 0.06 ± 0.01 to 0.29 ± 0.05 pg/hr in males (n=8) and from 0.10 ± 0.01 to 0.57 ± 0.28 pg/hr in females (n=8) at ZT0. At ZT12, ET-1 increased from 0.09 ± 0.02 to 0.23 ± 0.06 pg/hr in males (n=8) and from 0.14 ± 0.05 to 0.34 ± 0.06 pg/hr in females (n=8). The ET-1 excretory response was significantly different between males and females at ZT0, but not ZT12, demonstrating a sex and time-related difference in ET-1 release.

These results demonstrate that the response to ENaC inhibition is less prominent in females independent of renal ET-1. Since ENaC expression is greater in female rat kidneys, this could be related to having less ENaC activity independent of expression in females or differences in non-ENaC related effects of benzamil.

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3.27

INCREASED SEVERITY OF RENAL ISCHEMIA REPERFUSION INJURY IN MALE VS. FEMALE MICE IS ASSOCIATED WITH GREATER EXPRESSION OF CHOLINE ACETYLTRANSFERASE IN TUBULES

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Activation of the cholinergic anti-inflammatory reflex has been reported to protect against renal ischemia-reperfusion (IR) injury in mice. Recent studies have documented the presence of a non-neuronal renal cholinergic system primarily within tubules, implying it could be directly targeted to reduce the incidence and severity of acute kidney injury (AKI). The goal of this study was to assess the severity of IR-induced AKI and the associated changes in the renal cholinergic system in male vs. female transgenic mice expressing green fluorescent protein (GFP) under control of the choline acetyltransferase (ChAT) promoter. AKI was induced in mice via clamping both renal pedicles for 20 minutes (bilateral IR) with body temperature precisely controlled at 37° C. First, we assessed the severity of AKI by assessing plasma creatinine (P_{Cr}) and renal injury at 3 days post IR in one group of male (n=8) and female (n=8) mice and by assessing mortality rate over 7 days post IR in another group of male (n=16) and female (n=13) mice. Males exhibited greater ($P<0.05$) levels of P_{Cr} 3 days post IR as compared to females (1.7±0.2 vs. 0.4±0.1 mg/dl) with parallel differences observed in the severity of tubular necrosis and protein cast formation. The mortality rate over 7 days post IR in males was 50% while no mortality was observed in females. The renal cholinergic system was assessed in separate groups of naïve control mice and in mice at 3 days post IR by detecting GFP expression via Western blot and immunohistochemistry. When male and female data were combined, GFP expression in renal tissue lysates (normalized to GAPDH) was higher ($P<0.05$) in the 3 day post IR (0.6±0.1, n=13) group vs. naïve control group (0.3±0.1, n=9). The immunohistochemistry data corroborated the Western blot data with high levels of GFP expression observed in injured tubules. In naïve control mice, there was a strong tendency ($P=0.07$) for GFP expression in renal tissue lysates to be higher in males (0.4±0.1, n=5) vs. females (0.2±0.03, n=4). In mice examined at 3 days post IR, GFP expression in renal lysates was higher in both males (0.7±0.1, n=6, $P=0.09$) and females (0.4±0.04, n=7, $P<0.05$) when compared to their respective naïve control groups. GFP expression in males at 3 days post IR was higher ($P<0.05$) vs. females. We conclude that male mice exhibit greater levels of renal ChAT expression at baseline vs. females and this difference is further amplified at 3 days post IR-induced AKI. The greater levels of renal ChAT expression in males 3 days post IR is likely due, in part, to greater levels of AKI-induced tubular injury. The question of whether increased tubular ChAT expression in male mice contributes to their increased severity of AKI as compared to females requires further investigation.

3.28

GENDER DIFFERENCES IN HUMAN SKIN NA^+ AND MONOCYTE SALT-SENSITIVITY

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Salt sensitivity increases cardiovascular mortality. Studies have focused on the role of the kidney and vasculature in salt sensitivity, however, recent data indicate that sodium accumulates in tissues and can activate immune cells. We recently found that in murine dendritic cells amiloride sensitive channels sense salt and trigger NADPH oxidase-dependent formation of isolevuglandin (IsoLG)-adducts. We tested the hypothesis that sex differences in tissue sodium accumulation affect activation of immune cells in humans. We recruited 67 subjects including pre- and post-menopausal women and men. We non-invasively quantified their skin Na^+ usingmagnetic resonance imaging (MRI) and examined their peripheral blood mononuclear cells (PBMCs), obtained the same day, using flow cytometry. We found that increased skin accumulation of Na^+ in humans is associated with a parallel increase in IsoLG-adducts (24±6 *n=33, low* Na^+ vs 38±6%, n=*37, high* Na^+, $p<0.05$) and expression of the activation marker CD83 (0.04 ± 0.009 vs 0.12± 0.04%, $p=0.04$) in circulating monocytes. In addition, we found that men exhibit higher Na^+ content in the skin than women *(*16.59 ± 1.693, n=13 men vs 12.92 ± 0.6623, n=42 women **p<0.05)*. Our preliminary findings also suggest that men have more IsoLG-adducts in their monocytes than women, and this seems to increase with age. These results suggest that elevated skin Na^+ is associated with increased activation of human monocytes via IsoLG-adduct formation and that this is greater in men than women. Such differences in sensitivity to salt by antigen-presenting cells may explain gender disparities in hypertension.

3.29

GREATER AORTIC INFLAMMATION IN MALE SHR CORRESPONDS TO VASCULAR DYSFUNCTION

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Hypertension is a prevalent disease and an estimated 1 in 3 adults will have high blood pressure in their lifetime. Vascular dysfunction is commonly associated with hypertension and our lab has previously reported that aortas from male Spontaneously Hypertensive Rats (SHR) have enhanced vascular contraction and impaired endothelial dependent vasodilation when compared to female SHR and normotensive male and female Wistar Kyoto rat (WKY), however the mechanism mediating altered vascular function in male SHR is unknown. Based on studies implicating a role for inflammation in mediating vascular dysfunction, the goal of the current study was to test the hypothesis that indices of inflammation are greater in male SHR compared to female SHR and WKY of both sexes. In the current study, aortic T-cell profiles and HMGB1 were assessed in male and female WKY and SHR using flow cytometry (n=6 in each group) and extracellular matrix and adhesion molecules expression were measured using a PCR array (n=4). HMGB1 is a damage associated molecular pattern (DAMP) that when released from the cell triggers an inflammatory response. CD3+ and CD4+ aortic T cell counts were comparable across both sex and strain (CD3+ T cell: effect of sex p=0.55, effect of strain p=0.09, interaction p=0.55; CD4+ T cell: effect of sex p=0.45, effect of strain p=0.62, interaction p=0.56). However, SHR had fewer Tregs compared to WKY (effect of strain p<0.001), and males had fewer Tregs than females (effect of sex p=0.0002; interaction p=0058). Consistent with greater vascular dysfunction, male SHR had more Th17 cells (effect of strain p<0.0001; effect of sex p=0.0045; interaction p=0.05) and HMGB1 compared to all other groups (effect of strain p<0.0001; effect of sex p<0.0001; interaction p=0.0015). We also found the RNA expression of numerous extracellular matrix and adhesion molecules to be differentially upregulated by sex and strain (Table 1). Of interest, matrix metallopeptidase 12 was significantly upregulated in a sex- and strain-dependent manner. In conclusion, vascular dysfunction in male SHR is associated with increased pro-inflammatory mediators (Th17 and HMGB1). Based on the central role of HMGB1 to mediate an inflammatory response, future studies will focus on establishing a primary endothelial cell culture to directly study the effects of HMGB1 on adhesion molecule expression.

UPREGULATED GENES

	Female SHR vs Female WKY		Male SHR vs Male WKY		Male SHR vs Female SHR		Male WKY vs Female WKY	
	p-value	fold change	p-value	fold change	p-value	fold change	p-value	fold change
ADAM metallopeptidase w/ thrombospondin type 1 motif, 1	0.55	1.13	0.42	0.54	**0.05**	**2.99**	0.32	1.29
Connective tissue growth factor	0.08	1.71	0.64	1.04	0.13	2.42	0.48	1.31
Ectonucleoside triphosphate diphosphohydrolase 1	0.65	1.08	0.19	0.42	0.06	2.74	0.72	0.95
Hyaluronan and proteoglycan link protein 1	0.49	0.84	0.97	1.03	**0.04**	**1.87**	0.23	2.05
Integrin, beta 1	1.00	1.03	0.14	0.37	0.09	2.82	0.45	0.90
Matrix metallopeptidase 12	**0.04**	**3.66**	0.38	0.69	**0.05**	**3.98**	0.37	0.67
Thrombospondin 2	0.08	1.36	0.14	0.46	**0.00**	**3.66**	0.66	1.10
TIMP metallopeptidase inhibitor 1	**0.03**	**1.38**	0.43	0.61	0.09	2.23	0.42	0.88
Versican	0.14	1.39	0.43	0.61	**0.04**	**2.55**	0.91	0.98

Table 1. Upregulated extracellular and adhesion molecules in SHR and WKY.

3.30

PRETREATMENT WITH LOW DOSE LIPOPOLYSACCHARIDE ATTENUATES MEDULLARY CONGESTION IN MALE WKY FOLLOWING ACUTE KIDNEY INJURY.

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Acute kidney Injury (AKI) is a sudden loss of renal function which can result in future complications or mortality, and prevalence of AKI continues to increase, specifically in hospitalized patients. Ischemia-reperfusion is a well-accepted experimental technique of AKI in rodents. Renal medullary congestion has been demonstrated to augment ischemia reperfusion (IR) in rodent models (1) and we have recently reported a sex difference in recovery from AKI, in which medullary congestion and indices of kidney function are worse in males than females at 7 days post-IR. As low grade inflammation can promote rouleaux, we hypothesized that prior exposure to LPS would worsen medullary congestion and augment AKI following IR. To test this hypothesis, we examined the effect of pretreatment with incrementing doses of lipopolysaccharide (LPS) on renal congestion following ischemia reperfusion. Male Wistar-kyoto rats (WKY, 9wks) were treated with 10 (n=3), 100 (n=3), 1000 (n=3) µg/kg LPS or control (saline, n=3) for 7 days (i.p), and then subjected to a 30 minute warm, bilateral ischemia reperfusion. Rats were allowed 24h to recover then anesthetized and humanely sacrificed. The right kidney was taken for histology and stained using Gomori's Trichrome Stain (Thermo 87020). Blood was collected by tail vein at baseline, at days 2 and 7, and at sacrifice. C-reactive protein (CRP, Thermo ERCRP), a marker of inflammation, increased for all groups from

baseline to post-IR (p_{time} <0.0001), with control treatment having the greatest increase and highest levels of CRP, and 1000ug/kg LPS demonstrating the lowest levels CRP ($p_{interaction}$=0.09), at sacrifice. In difference to our hypothesis, outer Medullary peritubular congestion (blinded scoring) showed LPS pre- treatment reduced congestion when compared to saline treated controls (% congestion: control=80%, 10 and 100ug/kg LPS=40%, 1000ug/kg LPS= 20%). We conclude that, despite promoting inflammation, paradoxically, prior low dose LPS exposure prevents red blood cell congestion in the outer-medulla following IR. As we have recently reported that peristaltic contractions of vasa recta pericytes may prevent RBC congestion in the renal outer-medulla (2), we speculate that LPS activation of toll-like receptors of vasa recta pericytes may prime pericytes to contract preventing congestion. Further investigation of these mechanisms may lead to novel therapeutic approaches to prevent AKI.

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3.31

IMMUNE CELL INFLUX IN CISPLATIN-INDUCED ACUTE KIDNEY INJURY: SEX DIFFERENCES

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A sex-defined difference in response to cisplatin-induced acute kidney injury (Cp-AKI) has been described to include differences in the presence of systemic pro- and anti-inflammatory cytokines. Female mice demonstrate a significant resistance to kidney functional decline in the presence of morphological damage in a model of Cp-AKI. A stress enzyme, heme oxygenase-1 (HO-1), has been shown to be upregulated differently in males and females in response to Cp-AKI, and is known to alter the inflammatory milieu. In order to better understand the role of HO-1 in conferring a sex-based protection via modulation of inflammation, we sought to identify the lymphoid and myeloid cell populations within the kidney after Cp-AKI in male and female mice that are replete (wild-type littermates, WT) or systemically lacking HO-1 (KO) (n=1-5 mice/group). All mice were treated with 20mg/kg cisplatin or saline as vehicle and observed on day 1 or day 3 following the injections. Serum creatinine levels were obtained to confirm kidney functional decline with Cp; and weight loss was monitored over the time course. Kidney tissues were harvested and processed for histology or to obtain single cell preparations. The latter were subjected to flow cytometry to detect cells of lymphoid [B cell, NK cell, T cells ($CD3^+$, $CD4^+$, $CD8^+$)] and myeloid [neutrophils, and renal resident and infiltrating immune cell populations ($CD11b^+Ly6C^{hi}$, $CD11b^+Ly6C^-$, $CD11b^+F4/80^+$, $MHCII^+CD11c^+$, $MHCII^-$)] lineages (each expressed as a proportion of total live $CD45^+$ cells). Proportions of neutrophils and $CD8^+$, but not $CD4^+$ or $CD3^+$, T cells showed distinct differences across groups; all other cell populations were not different. By 1-way ANOVA across all groups, on day three, neutrophils were significantly higher in male KO mice relative to WT mice of both sexes. While not significant in the analysis across all groups, analysis by within group ANOVA demonstrated that male KO mice had a significant rise in proportions of neutrophils at both time points. No significant elevation was seen in neutrophils in KO females by either statistical analysis. $CD8^+$ T cell proportions at baseline (saline-treated mice) in the different groups were higher in male KO mice relative to male and female WT mice and trended toward significance (p=0.0524) relative to female KO mice by 1-way ANOVA across all groups. $CD8^+$ cells appeared to decrease with injury only in male WT mice by within group 1-way ANOVA; all other declines were not significant. Immune cell influx is a hallmark of Cp-AKI. Neutrophils and T cell influx are detrimental to kidney function since elimination of the cells leads to decreased measures of injury and preserved kidney function in models of AKI. These current findings highlight sex-defined differences in the immune cell complement at baseline and after injury that are altered by the presence of HO-1 and may be important modifiers of Cp-AKI leading to preferential protection in young females. We acknowledge support from the UAB-UCSD O'Brien Core Center for Acute Kidney Injury Research (NIH/NIDDK P30-DK079337) and the Interdisciplinary Training in Kidney-Related Research Grant (NIH/NIDDK T32 DK007545 (to R.B.)) for this project.

3.32

SEX DIFFERENCES IN RENAL AMMONIA METABOLISM

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Renal ammonia excretion is a critical component in maintaining acid-base homeostasis. Sex differences are well recognized as an important biological variable in many aspects of renal function. However, sex differences in renal ammonia metabolism have not been previously reported. This study's objective was to investigate sex differences in renal ammonia metabolism. We compared

4-month-old C57/BL6 male (M) and female (F) mice fed a normal diet, with measurement of plasma electrolytes, urinary ammonia excretion, morphometric analysis of renal structure, and evaluation of changes in key proteins involved in ammonia metabolism using immunoblot analysis and immunohistochemistry. Despite similar level of food intake (F, 8.9±0.9; M, 9.6±1.2 gram/day; P=NS), and thus protein intake, which is the primary determinant of endogenous acid production, female mice excreted significantly more ammonia (F, 72±23; M, 46±19 µmol/day; P<0.01) than did male mice. This difference in ammonia excretion was not due to differences in urine acidification, as urine pH did not differ significantly (F, 6.40±0.18; M, 6.36±0.14; P=NS). Titratable acid excretion (F, 53±26; M, 74±20 µmol/day; P=NS) another component of net acid excretion, did not differ significantly. Serum Na^+, K^+, and HCO^-_3 did not differ significantly. There are fundamental structural differences between the female and male kidney. In the female kidney, proximal tubules account for a lower percentage of the renal cortical parenchyma than the male kidney (F, 42±3; M, 60±3%; P<0.01), whereas the collecting ducts account for a greater percentage of the renal parenchyma (F, 15.4±2.0; M, 9.6±1.6%; P<0.001). Phospho*enol*pyruvate, a major proximal tubule (PT) ammonia generating protein, was significantly greater in female mice than male mice. Expression of glutamine synthetase, which recycles ammonia, was significantly greater in the PT of female mice. Expression of NBCe1, a basolateral PT transporter, recently shown to regulate PT ammonia metabolism did not differ significantly between the sexes. Expression of NHE3, which is believed to be the major mechanism of PT ammonia secretion, did not differ significantly between the sexes. Expression of NKCC2, which mediates thick ascending limb ammonia reabsorption, was significantly greater in the female kidney than the male kidney. The collecting duct secretes the majority of urinary ammonia and the Rhesus glycoproteins, Rhbg and Rhcg are the primary collecting duct ammonia transporting proteins. Rhbg was significantly greater in connecting segment cells and intercalated and principal cells in the collecting duct in the cortex and inner stripe of the outer medulla (ISOM) in female mice. Expression of Rhcg was significantly greater in female mice in connecting segment cells and in the basolateral membrane of intercalated and principal cells in the collecting of the ISOM. Thus, there are sex differences in basal ammonia metabolism that involves both renal structural differences and differences in expression of critical proteins involved in ammonia metabolism. These studies were supported by funding from the National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-045788 (IDW), RO1-DK-107798 (IDW and JWV) and 5T32-DK-10472.

3.33

SEX DIFFERENCES IN THE METABOLIC AND PHYSIOLOGICAL EFFECTS OF A HIGH FAT DIET IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER&RSQUO;S DISEASE

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Obesity and factors associated with metabolic syndrome are strongly correlated to an increased risk for dementia. Specifically, over 80% of individuals with Alzheimer's disease (AD) have either diabetes or prediabetes; therefore, it is of critical importance to understand the effects of this common comorbidity. Beta-amyloid deposits have been found in the pancreas of both humans and transgenic AD mouse models, thought to contribute to observed metabolic impairments. Additionally, high fat-diet induced obesity aggravates AD pathology, confirming the feedback loop that exists between these two conditions. In the current study, we used a high fat (HF) diet in a genetic mouse model of AD to induce prediabetes, insulin resistance and glucose intolerance in the absence of hyperglycemia, which is 3x more common than diabetes and affects 38% of Americans. In addition, since females have higher rates of dementia and faster rates of decline in cognition, sex differences in the metabolic and physiological effects of prediabetes in AD mice were explored. At three months of age, male and female wild-type (WT; B6129SF2/J) and 3xTg-AD mice were placed on either low fat (LF; 10% fat) or high fat (HF; 60% fat) diet for 3 months, then subjected to a glucose tolerance test (GTT), followed by blood and tissue collection. HF diet increased body weight gain and fat mass, and impaired glucose tolerance, to a greater degree in females compared to males, and in 3xTg-AD mice compared to WT mice. These differences in metabolic outcomes were not attributable to differences in food intake normalized to body weight. While heart mass was unaffected by diet in WT mice, HF diet increased heart mass in 3xTg-AD mice, though this effect reached significance in females only. Additionally, HF diet reduced reproductive organ weight in 3xTg-AD females only. Liver steatosis and inflammatory cytokine expression are also being assessed. Results thus far suggest that AD females are particularly sensitive to the detrimental effects of HF diet, in line with greater cognitive impairments observed in our other studies. This study will be critical for understanding sex differences in the effects of high fat diet on metabolic and physiological outcomes that may contribute to cognitive decline in AD.

3.34

SEX DIFFERENCES IN THE CEREBRAL VASCULAR FUNCTION AND K CHANNEL ROLE IN ADULT SPRAGUE DAWLEY (SD) RATS

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The incidence of cerebrovascular disease is lower in adult women compared to adult men but the sex differences in cerebral vessel function and associated mechanisms are not clear. Using a combination of vascular, electrophysiological and biochemical approaches we explored the hypothesis that "sex differences in the cerebral vascular function in adult Sprague Dawley (SD) rats is associated with differential large conductance potassium (BK) channel subunit function". Middle cerebral arteries (MCA) isolated from adult female rats exhibited attenuated myogenic response (decrease in vascular diameter with increase in lumen pressure). In contrast, MCA of adult male rats exhibited myogenic response (% change in diameter from 40 to 140mmHg: Females 16±8, Males -25±4, $p<0.05$, n=5-8). Percent myogenic tone (%MT) (calculated from active and passive diameters) is ~3.4 fold higher in females compared to their male counterparts (% MT at 40mm Hg: Females 51±6, Males 15±5, n=5-8, $p<0.05$). Total potassium, BK and Kv channel currents were significantly larger in female vascular smooth muscle cells (VSMCs) compared to males. Spontaneous transient outward currents (STOC) amplitude that represent BK channel function are ~1.73 fold higher in VSMCs isolated from female rats compared to males (pA: Females 90±6, Males 53±5, n=5, $p<0.05$). While BKα protein content is higher, BKβ1 subunit protein content as well as mRNA is lower in cerebral vessel homogenates yielding a lower BKβ1/α ratio in females than males. Endothelium-independent (Sodium nitro prusside (SNP)) relaxation is ~2.3 fold higher in female MCA compared to males (1μM SNP: Females 88±10%, Males 39±5 %, n =3-5, $p<0.05$). Together these results suggest that adult female MCAs exhibit attenuated myogenic response that may be associated with higher BK channel function. Also, higher BK channel function can result in greater endothelium-independent vasodilation in adult females when compared to males. In conclusion, these results may identify a mechanism with which women in adult hood are protected from cerebrovascular incidences compared to males due to their greater vasodilator capacity that is associated with higher BK channel function. Supported by AHA SDG (13SDG14000006) to Mallikarjuna R. Pabbidi.

3.35

CEREBROVASCULAR DYSFUNCTION IN THE DAHL S RAT MODEL OF SUPERIMPOSED PREECLAMPSIA

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Preeclampsia is a multi-faceted pregnancy disorder characterized by increases in blood pressure after 20 weeks of gestation coupled with either an increase in proteinuria (renal insufficiency), or other major organ involvement (liver, lung, brain). In regards to neurological complications, common symptoms include nausea, drowsiness, blurred vision, scotomata, and persistent headaches, with more severe cases leading to seizures (eclampsia), cerebral edema and intracranial hemorrhage. Our lab has recently characterized the Dahl salt sensitive rat (Dahl S) as a model of spontaneous superimposed preeclampsia, displaying characteristic symptoms of increased blood pressure, urinary protein excretion, as well as fetal demise and intrauterine growth restriction. Groups of Dahl S rats were used in different experiments during late pregnancy to test the overarching hypothesis that pregnant Dahl S rats exhibit cerebrovascular dysfunction as commonly observed in preeclamptic women. Group 1 (n=4-6): Brains were harvested and weighed on day 20, heated at 60°C for 72 hours, and re-weighed to determine brain water content (edema). Pregnant Dahl S rats have increased posterior brain dry:wet weight ratios compared to virgin littermates (78.0±0.1 vs. 77.4±0.2%, $p<0.05$) with no significant difference in the anterior brain (79.2±0.2 vs. 78.8±0.3%, $p=0.33$). Group 2 (n=4-6): Evan's Blue extravasation into the brain was measured to assess blood-brain barrier function. Evan's Blue concentration was increased in both posterior (0.040±0.012 vs. 0.018±0.001 ng/g tissue/plasma concentration, $p=0.07$) and anterior (0.021±0.001 vs. 0.110±0.068 ng/g tissue/plasma concentration, $p=0.06$) brain in pregnant Dahl S rats compared to virgin littermates, respectively, indicating BBB dysfunction. Group 3 (n=5): Cannulated middle cerebral artery (MCA) segments were used to assess vascular permeability by measuring the fluid flux of water through the vascular wall of pressurized arteries. MCA's from pregnant Dahl S rats showed a greater drop in intravascular pressure compared to virgin littermates (Δ=50.2±3.7 vs. 24.4±5.0mmHg, respectively, $p<0.01$). Together, these results suggest that the pregnant Dahl S rat, a model of superimposed preeclampsia, exhibits increases in cerebrovascular permeability which could play a role in increased cerebral edema and blood-brain barrier permeability. Therefore, pregnant Dahl S rats may be a useful model to

investigate mechanisms leading to cerebrovascular abnormalities that occur during preeclampsia.

4: SESSION 2: PHYSIOLOGY AND GENDER: AGING AND SENESCENCE

4.1

SEX DIFFERENCES, AGING AND VASCULAR FUNCTION

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The incidence of coronary heart disease (CHD) in women lags behind men by 10 years for total CHD and by 20 years for more serious clinical events such as myocardial infarction and sudden death. Under age 75 years, there is a higher proportion of cardiovascular disease events due to CHD in men than in women. Indeed, between the ages of 45 and 65 years, the incidence of myocardial infarction and stroke is higher in men compared with women, in spite of no differences in the prevalence of traditional risk factors. The mechanisms responsible for age- and sex-related differences in vascular endothelial function are complex and not completely understood. Vascular endothelial cell function is critical to cardiovascular health and vitality. However, due to its unique anatomical position the endothelium is susceptible to blood-borne injury, mechanical forces and cardiovascular risk factors that can impair cell function resulting in a proatherogenic endothelial phenotype that is key in the initiation, progression and clinical complications of vascular disease. Many of the cardiovascular complications associated with age are due, at least in part, to endothelial dysfunction, particularly vasomotor dysregulation and fibrinolytic impairment. It is now recognized that the vascular endothelium is significantly influenced by circulating microparticles. The initial notion that microparticles in the circulation were merely “inert cellular debris” rapidly changed when it became apparent that circulating microparticles can trigger a proatherogenic endothelial cell phenotype by disrupting endothelial nitric oxide production, inducing endothelial inflammation, oxidative stress and senescence as well as altering apoptosis. In addition, microparticles have been shown to contribute to plaque development, thrombogenicity and instability. Microparticles are small (100 to 1000 nm diameter) anucleoid phospholipid vesicles released into the circulation by various cell types (e.g. endothelial cells, platelets, leukocytes, monocytes) in response to a myriad of triggers including: high shear stress, cellular activation stemming from proinflammatory, prothrombotic and/or proapoptotic stimuli, cellular differentiation, cell senescence and apoptosis. The potential of circulating microparticles to be vascular-protective or -destructive underscore their biological importance in cardiovascular health and disease. This seminar will review the influence of aging and sex on endothelial cell function and introduce circulating microparticles as novel mediators of age- and sex-related differences in endothelial dysfunction.

4.2

SEX DIFFERENCES IN THE EFFECTS OF PREDIABETES ON VASCULAR CONTRIBUTIONS TO DEMENTIA

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Background: Diabetes causes endothelial dysfunction and is a major risk factor for vascular contributions to cognitive impairment and dementia (VCID). Diabetic women have increased risk of VCID compared to diabetic men. Data on the effects of prediabetes, which is 3 times more common than type 2 diabetes, are lacking. Prediabetes can be modeled in mice via long-term administration of a high fat (HF) diet, which we have shown causes cognitive deficits. Young females are protected from the metabolic effects of the diet. We hypothesized the middle-aged females would lose their protection, relative to males, from the metabolic and cognitive effects of a HF diet in a mouse model of VCID.

Methods: Middle-aged (8.5 month old) male and female C57BL/6J mice were placed on a HF or control diet for 6.5 months (15 months old at the end of the study). After 3 months on the diet, they received either VCID surgery (unilateral carotid artery occlusion) or sham surgery. Body weight and glucose tolerance were monitored. After 6 months on the diet, blood flow through the left carotid was measured via ultrasound and then cognitive function was assessed in the Morris water maze. Finally, blood flow was measured via laser speckle contrast imaging. Brain were also collected and we will be performing histology to examine neuroinflammation, white matter damage, and blood brain barrier damage.

Results: HF diet caused greater increases in body weight and glucose intolerance in females than males. Blood flow through the left carotid was reduced in HF-fed mice of both sexes. Cerebral blood flow was reduced in the right hemisphere following VCID surgery, with reductions being more severe in the HF female VCID mice. Spatial memory was impaired in VCID males, regardless of diet; however, in females, either HF diet or VCID (or a combination of the two) impaired spatial memory.

Conclusions: The data show that HF-fed/prediabetic middle-aged-females have more severe metabolic and cognitive deficits than prediabetic males. This is in line with the increased risk of VCID in diabetic women compared to men. Our data suggests that prediabetes might also be a greater risk factor for VCID in women.

4.3

SEX DIFFERENCES IN THE ROLE OF THE SMOOTH MUSCLE CELL MINERALOCORTICOID RECEPTOR IN CARDIOVASCULAR AGING

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The process of vascular aging involves the development of vasomotor dysfunction and vessel stiffening, which leads to cardiovascular disease (CVD). Clinical data supports that the time course of CVD development with aging occurs differentially in males vs. females, suggesting distinct sex-specific mechanisms of vascular aging. The mineralocorticoid receptor (MR) is a steroid hormone receptor known to regulate renal sodium handling. We previously demonstrated that male mice with the MR specifically deleted from smooth muscle cells (SMC-MR-KO), have lower blood pressure, vascular tone, and vasoconstriction as they age. The purpose of this study was to determine whether there are sex differences in the role of SMC-MR in vascular aging. All evaluations of the mice took place at 3 ages: 3, 12, and 18 months, chosen as these roughly correspond to adult (3 mo.), middle age or "peri-menopause" (12 mo.) and elderly or "post-menopause" in females (18 mo.). First, we show that vascular MR increases with age differentially in male and female mice; with vascular MR increasing at 12 months of age in males but not until 18 months of age in females. This change in MR expression is accompanied by a concomitant down-regulation of vascular expression of miR-155 at 12 months in males vs. 18 months in females, which is prevented in SMC-MR-KO mice. In vitro reporter assays showed that MR transcriptionally represses miR-155 promoter activity, whereas estrogen receptor (ER) upregulates miR-155 promoter activity, and is further enhanced with estrogen treatment. These studies suggest that MR and ER regulate miR-155 in an opposite manner, and that the decline in miR-155 in females may be driven by combination of the increase in MR and a loss of estrogen. We previously identified miR-155 as a regulator of vascular target genes including the angiotensin II type 1 receptor (AT1R) and the pore-forming subunit of the L-type calcium channel (LTCC), Cav1.2, both mediators of vasoconstriction and enhanced vascular tone. Indeed, we find that AT1R- and LTCC-mediated vasoconstriction is increased at 12 months in males and 18 months in females and these vasomotor alterations are prevented in SMC-MR-KO mice of both sexes. We next examined alterations in vascular stiffness, a hallmark of vascular aging. In vivo aortic stiffness studies, as measured by pulse wave velocity (PWV), demonstrate the same temporal difference with aging with increased vascular stiffness at 12 months in males vs. 18 months in females, that is prevented by SMC-MR-KO. We explored the contribution of SMC to stiffness via atomic force microscopy of freshly dispersed SMC from ~18 month old mice. These studies revealed that females exhibit more SMC stiffness vs. males, and that this is partially prevented by SMC-MR-KO in females only. To assess the role of fibrosis in the aging-induced alterations in stiffness, we measured carotid artery fibrosis histologically. Carotid fibrosis is increased at 12 months in males vs. 18 months in females, and partially prevented by SMC-MR-KO in males only. Together, these data suggest that the temporal difference in vascular stiffening is associated with distinct sex-specific mechanisms driving vascular stiffness with aging and that SMC-MR plays a differential role in males and females. Next, we examined the effect of aging on cardiac function in males and females by in vivo echocardiography. Cardiac function declines at 18 months in both males and females, but is attenuated by SMC-MR-KO in females only. Further characterization of overall cardiovascular function via exercise capacity testing revealed that exercise capacity declines in males at 12 months vs. 18 months in females, and is partially restored by SMC-MR-KO in males only. In summary, the time course and mechanisms of cardiovascular aging are distinct between males and females with SMC-MR playing a differential role in males versus females. Furthermore, these studies suggest that sex-specific therapies may be essential to improve CVD outcomes in the aging population.

4.4

MATERNAL AGING AND CARDIOVASCULAR DYSFUNCTION

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Pregnancy has an important influence on both short- and long-term cardiovascular outcomes for women. The age at which women experience their first pregnancy has increased, however, normal pregnancy-induced vascular adaptations may be impaired with aging. Aging is associated with cardiovascular impairments, hence the increased demands of pregnancy may exceed the aging maternal cardiovascular capacity.

Our data shows that advanced maternal age results in impaired vascular function and reduced uteroplacental perfusion, which may underlie adverse pregnancy outcomes. We used aged female rats (9.5 months; equivalent to ~35 yr old human), to investigate the impact of maternal age on pregnancy outcomes and vascular function.[1] Delayed pregnancy reduced fertility

(46%), reduced litter size (36%), caused fetal growth restriction, increased placental weight, and increased maternal systolic blood pressure (16 mmHg). Both uterine and mesenteric arteries from aged dams had greater active myogenic responses (228% and 151% respectively). Moreover, both aging and complicated pregnancies, which may include advanced maternal age, are known risk factors for later-life maternal cardiovascular disease.

Using the aged rat model, we further investigated vascular function in never pregnant (virgin), previously pregnant (postpartum) and previously mated but never delivered (nulliparous) rats at ~13.5 months of age (3 months postpartum or equivalent).[2] Nulliparous rats had significantly reduced vasodilation responses (methylcholine [MCh] E_{max}: 54±13%) vs. virgin and postpartum rats (85±4% and 85±3% respectively); suggesting a worsened vascular pathology associated with pregnancy loss. Further, in mesenteric arteries from postpartum rats, endothelium-derived hyperpolarization (EDH) mediated vasodilation was reduced and a constrictive prostaglandin effect was apparent. These data demonstrate mechanisms which may lead to worsened outcomes at an advanced maternal age; including early pregnancy termination and later life cardiovascular dysfunction.

We recently tested whether being born from a dam of advanced maternal age may constitute a prenatal stress with cardiovascular consequences for the offspring.[3] In 4-month-old offspring (young adult), we observed impaired endothelium-dependent relaxation in males ($P<0.05$), but not females born from aged dams. Interestingly, there was a significant increase in nitric oxide-induced relaxation in females, but not males, born from aged dams (delta E_{max}: young dam -25±12 vs. aged dam -69±8, $P<0.05$). Cardiac susceptibility to an ischemia-reperfusion insult was also increased in male, but not female, offspring born from aged dams ($P<0.001$).

These data illustrate that offspring born from aged dams have an altered cardiovascular risk profile that is sex-specific. Given the increasing trend toward delaying pregnancy, our findings have significant population and health care implications and further illustrate pregnancy as a window of opportunity to assess cardiovascular health.

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4.5

CENTRAL LEPTIN RECEPTOR ANTAGONISM ATTENUATES THE DEVELOPMENT OF MENOPAUSAL-INDUCED HYPERTENSION IN THE RAT

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The fact that estrogen plays a protective role against hypertension in pre-menopausal women is well established. Additionally, the rapid increase in the rate of hypertension among post-menopausal female provides additional support for beneficial effects of estrogen. However, the events that occur in the absence of estrogen to augment the development of hypertension in post-menopausal women have not been fully elucidated. One event that occurs in menopausal women that may contribute to the development of hypertension is the significant increase in body weight in the form of adipose tissue. Increased adiposity is associated with an increase in the levels of circulating adipokines, substances released from adipose cells. One such adipokine is leptin, which is known to increase with increasing adiposity and play a role in the regulation of cardiovascular function, energy homeostasis and autonomic tone. Therefore, in the current study, using a post-menopausal rat model, we hypothesize that leptin, acting through central leptin receptors, augments menopausal-induced hypertension. Bilateral ovariectomized (OVX) female rats were used in these experiments. On the day the ovaries were removed, rats were instrumented with a telemetry probe for continuous monitoring of blood pressure and heart rate and an osmotic pump to deliver the leptin antagonist (LAN-6) (3µg/day, n=6) or vehicle (saline, n=6) into the lateral ventricle for four weeks. Sham animals were subjected to the same surgeries as OVX animals; however, the ovaries were not excised after opening the abdominal cavity. These animals received only vehicle infusion (SHAM-saline, n=4). Four weeks after the surgery, OVX-saline and OVX-antagonist daily food intake (58 grams) and average weight gain after 4 weeks (74 grams) were significantly greater than SHAM-saline (23 and 21 grams respectively; $p<0.05$). Four (4) weeks following the OVX surgery, the blood pressure of OVX-

saline rats was significantly higher than SHAM-saline (106±2 mmHg vs. 96±2 mmHg respectively, P=0.0017), confirming the menopausal-induced hypertension model. The blood pressure of OVX-antagonist rats was similar to SHAM-saline (102±1 mmHg, P>0.05). To determine if the change in blood pressure in this model was due to differential activation of the sympathetic nervous system, we tested the blood pressure response to an acute injection of hexamethonium (30ug/kg, IP). The drop in blood pressure was significantly greater in OVX-saline animals, as compared to OVX-antagonist (-41±2 mmHg vs. -30±3 mmHg, P=0.021), which suggests an enhanced basal sympathetic tone in menopausal-induced hypertension due to leptin receptor activation. Taken together, these data suggest leptin, acting on its receptors in the central nervous system, modulates sympathetic nervous system activity and augments blood pressure in menopausal-induced hypertension.

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4.6

BIOENERGETIC AND METABOLIC CONSEQUENCES OF THE LOSS OF OVARIAN FUNCTION IN WOMEN

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Biological systems age at different rates and the consequences of aging in one system can influence other systems. For example, reproductive aging, which results in decreased systemic sex hormone levels, is known to increase osteoporosis risk. This is of particular concern in women, because the loss of gonadal function occurs in mid-life. The skeletal consequences of the loss of estrogen have been well studied, but potential consequences on other systems have not. There is strong preclinical evidence that loss of ovarian function triggers a disruption in energy balance that causes excess fat gain, particularly in central body regions. Some of the consequences of gonadal ablation in female rodents include insulin resistance and dyslipidemia, which can be prevented by estrogen treatment or by programmed exercise. Preclinical research has demonstrated that this disruption of energy balance involves both increased energy intake (in some species) and decreased energy expenditure. The latter reflects both the suppression of resting metabolic rate and a dramatic reduction in spontaneous physical activity; both are reversed by estradiol. To advance the translation of such findings to humans, we utilize a pharmacologic model of gonadal ablation to experimentally isolate the effects of sex hormones. Using this approach, we have demonstrated that suppressing ovarian function results in a decrease in resting metabolic rate (-50 kcal/d), which is prevented by estradiol therapy. Total energy expenditure is reduced even more dramatically (-130 kcal/d). Ovarian suppression also results in an increase in abdominal adiposity and a decrease in muscle mass, both of which are prevented by estradiol. A key question is whether regular exercise can mitigate these consequences of the loss of ovarian function. Our findings suggest that exercise can attenuate some (e.g., loss of bone), but not all (e.g., decline in resting metabolic rate), of the consequences of ovarian hormone suppression.

5: SESSION 3: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE HEART

5.1

SEX DIFFERENCES IN BLOOD PRESSURE HEMODYNAMICS DURING SEXUAL MATURATION AND AGING: RESULTS FROM THE SAGUENAY YOUTH STUDY

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Blood pressure (BP) and its underlying biology show marked sex differences. These may be particularly pronounced during reproductive age when male and female sex hormones are most active. They may present as sex-specific patterns of BP hemodynamics. Here we investigated this possibility in the Saguenay Youth Study (SYS).

The SYS is a community-based study of 1000 adolescents (12-18 years, 52% females) and 650 of their middle-aged parents (36-65 years, 56% females). In each participant, we measured, beat-by-beat, BP and underlying hemodynamic factors, i.e., stroke volume (SV), total peripheral resistance (TPR) and heart rate (HR) during a 52-minute protocol mimicking daily-life activities. We assessed the relative contributions of SV, TPR and HR to BP separately in male and female adolescents and adults. We found that SV was the main determinant of BP in all but male adults in whom it was TPR ($p=0.03$ to $p<0.001$). Consistently, in individuals with 'high' vs. 'low' BP (by median split), SV was higher in all but male adults in whom TPR was higher ($p<0.001$ for all). In further subset analyses, male adolescents with 'high' (vs. 'low') genetic androgenicity, and female adults in menopause (vs. pre-menopause) showed greater contribution of TPR to BP that, in both cases, this contribution was not different from that in male adults.

In conclusion, the results from the SYS suggest that marked sex differences in BP hemodynamics exist during adult reproductive age, with TPR being the main contributor of higher BP in males, whereas SV being the main contributor of higher BP in females. These sex differences emerge with male pubertal development and diminish with female menopause.

5.2

SEX DIFFERENCES IN HEALTHY HUMAN HEART REVEALED BY CAP ANALYSIS GENE EXPRESSION (CAGE)

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Background: Cardiovascular diseases remain the primary cause of death worldwide. Several epidemiologic and investigative studies have shown the evidence of sex hormones affect in cardiac electrophysiology through genomic effects (ion-channels expression) and non-genomic effects (ion-channels function modulation). However, very little is known about the molecular basis for gender-related discrepancies in cardiac electrophysiology. Due to physiologically distinct functions of atria (electrical impulse initiation) and ventricles (blood pumping), there is a difference in ion-channel expression within a heart that causes different disease susceptibilities between both sexes.

Objective: Compare ion channel gene expression associated with sex differences using CAGE analysis on left atrial (LA) and left ventricular (LV) human donor hearts.

Materials and Methods: Total RNA was extracted from left atria (LA) and left ventricle (LV) of human donor hearts n=4 males (mean age = 53.75) and n=3 females (mean age = 58.7), the cause of death was determined to be non-cardiogenic. Samples were analyzed with CAGE, which is high throughput method for transcriptome analysis that utilizes 'cap-trapping'. The number of tags gives a frequency of usage that provides information about transcription start sites as well as transcript expression levels. Normalization of raw CAGE tag count was performed as counts per million. The two-sample t-test was used to determine statistical significance. **Results and Discussion:** Our results confirmed higher expression of SCN5A in ventricles compared to atria. SCN5A gene encodes Nav1.5 channel α-subunit (I_{Na}) which is more abundant in working myocardium compared to nodal cells. Loss of sodium channel function is associated with Brugada phenotype that is more predominant in males. Indeed, males had a lower expression of the gene that might indicate on their higher predisposition to the disease. KCND3 that encodes for outward current potassium voltage-gated channel (I_{to1}) that is the main contributing current to repolarizing phase 1 of the cardiac action potential had a significantly higher expression in female atria. KCNIP2, voltage-gated potassium channel ($I_{to,f}$) maintains early repolarization. During heart failure it augments $Ca_v1.2$ (CACNA1C) and $K_v4.3$ (KCND3). Since the hearts were healthy, high expression of KCNIP2 in atria did not affect the expression of KCND3 and CACNA1C. Estrogen is known to up-regulate CACNA1C which was indeed found in female hearts although not statistically significant for our postmenopausal female group. Mutations in potassium-channel genes, KCNH2 (I_{Kr}) and KCNQ1 (I_{Ks}), have been associated with Long QT syndrome type 2 and 1 respectively. Our results for these two ions channels did not show a statistically significant difference in expression across sexes. Interestingly, gene for inward rectifier potassium channel (I_{K1}), KCNJ3, that plays an important role in heartbeat generation is up-regulated in atria, the region with pacemaker cells. Another inward-rectifier potassium channel (I_{KACh}), KCNJ2 is up-regulated in ventricles. That demonstrates ion channel specificity to the anatomical areas of the heart and might carry a unique electrophysiological function.

Conclusion: In the past decade there has been a push towards sex-specific drug development since it has been established that medications affect males and females differently. Our study reveals that there are sex-dependent gene expression differences in cardiac ion channels and that CAGE approach allows high-throughput gene expression profiling which can be beneficial for gender-specific drug development and personalized medicine overall. Supported by NIH 5R01HL114395.

5.3

HEART FAILURE WITH PRESERVED EJECTION FRACTION (HFPEF) IS AUGMENTED IN OBESE MICE WITH AN XX SEX CHROMOSOME COMPLEMENT

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Objective: Heart failure with preserved ejection fraction (HFpEF) accounts for at least 50% of cardiomyopathies associated with left ventricular dysfunction. HFpEF is associated with diastolic hypertension, atrial fibrillation, obesity, age, and female sex. Alarmingly, given its high prevalence, there are no effective therapies for HFpEF and few experimental models that exhibit features of the human disease. Since female sex is a risk factor for HFpEF, we used a novel murine model (the four core genotypes) that enables dissection of the relative contributions of sex hormones and sex chromosomes. We also incorporated diet-induced obesity and advancing age as risk factors for HFpEF into the experimental design.

Methods and **Results**: Male (M, XY and XX) and female (F, XX and XY) mice (6 months of age) were fed a 60% high-fat (HF) diet for 6 months. Carotid artery catheters and radiotelemeters were implanted at week 22, mice were allowed to recover for 1 week, and then blood pressure was recorded for 7 days. Echocardiography was performed by ultrasound under anesthesia at study endpoint. There was a significant effect of sex (M>F) and

genotype (XX>XY) on systolic (SBP) and diastolic blood pressures (DBP) of HF-fed mice. Within a sex, SBP and DBP were higher in XY than XX males, but a surprising opposite effect was observed in females (XX>XY SBP and DBP). Notably, left ventricular diastolic diameter (XX: M, 3.9 ± 0.1; F, 3.9 ± 0.1 mm; XY: M, 4.1 ± 0.08; F, 4.2 ± 0.06 mm; P<0.05) and volume were significantly decreased in XX compared to XY obese mice regardless of gonadal sex (P<.05). Consistent with indices of HFpEF, ejection fraction and fractional shortening were not different between groups. However, stroke volume was decreased in XX compared to XY obese mice, regardless of gonadal sex (XX: M, 35.3 ± 2; F, 35.9 ± 3 µl; XY: M, 44.7 ± 4.4; F, 39.6 ± 2.8 µl; P<0.05). Assessment of baroreceptor activity from blood pressure records indicated that baroreceptor slope (gain) and activity were impaired in XX mice, regardless of gonadal sex. Measurements of potential HFpEF biomarkers demonstrated increased serum total TGF-β in female mice with an XX sex chromosome complement (XX, 166 ± 20; XY, 116 ± 11 ng/ml; P<.05). Studies evaluating cardiomyocyte size and assessing cardiac collagen are underway in hearts from XX versus XY mice.

Conclusions: Using mice with differing sex chromosome complement, we have generated a murine model of HFpEF that exhibits features of the human disease, including obesity, female (XX) sex, advancing age, hypertension, diastolic stiffness, an impaired baroreceptor response, and higher serum TGF-β concentrations. Future studies will use this model to define potential therapeutic targets for HFpEF.

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5.4

ESTROGEN MODULATION OF THE CARDIAC RAS IN DIASTOLIC DYSFUNCTION

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Heart failure with preserved ejection fraction predominates in postmenopausal women. Emerging epidemiologic evidence proposes that early menopause is positively associated with incident heart failure, which suggests potential protective roles for estrogens in left ventricular diastolic dysfunction (LVDD) development and disease progression. To date, there are no proven pharmacologic therapies to delay or reverse age – and female sex hormone-related LVDD; in fact, data from large trials imply that ACE-inhibitors and angiotensin II (Ang II) receptor blockers (ARBs) effectiveness may be less pronounced in women than in men receiving treatment for hypertension and heart failure. One reason for the low efficacy of ACE inhibitors and ARBs is that these agents might not directly prevent or limit Ang II intracellular formation. Additionally, chymase rather than ACE may be a primary Ang II-forming enzyme in humans. Local tissue-dependent actions of Ang II have a critical role in the progression of adverse cardiac remodeling to subsequent heart failure. Inappropriate activation or suppression of various components of the cardiac RAS have been linked to diastolic dysfunction. LV hypertrophy and increased interstitial fibrosis, induced by Ang II, impairs diastolic calcium handling and LV chamber compliance via its actions on Ang II receptors (AT1R). Loss of the counter-regulatory axis of the RAS, specifically ACE2/Ang-(1-7)/Mas receptor, further enhances the progression of diastolic dysfunction and susceptibility to heart failure by limiting its anti-hypertrophic, anti-fibrotic, and anti-oxidant effects. To this end, the objectives of the presentation will be to: 1) provide an overview of the cardiac tissue pathways to Ang II formation; 2) document the effects of ovariectomy (OVX) on the expression and activity of the cardiac RAS in female hypertensive and normotensive rodents; and 3) describe a critical RAS-related role of G protein-coupled membrane estrogen receptor (GPER) activation in the maintenance of cardiac structure and function after estrogen loss. Specifically, we found GPER cardioprotective effects include anti-proliferation of cardiac mast cells and associated attenuations of cardiac chymase/Ang II along with reductions in cardiac collagen deposition and myocyte hypertrophy. Taken together, our findings support the presence of an intracellular chymase-mediated Ang II-forming system that is responsive to estrogens. The observation that cardiac tissue chymase activity correlates with worsening of diastolic function buttresses the importance of chymase rather than ACE as a critical tissue Ang II forming enzyme and its role in modulating cardiac function. These findings provide weight to an urgent need to address how sex-related impact on cellular physiology may drastically affect the effectiveness of current therapies.

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5.5

PRENATAL DIABETES AND HIGH-FAT DIET EXPOSURE IMPAIR MITOCHONDRIAL FUNCTION IN ADULT RAT CARDIOMYOCYTES

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Background: Both diabetes and obesity increase the risk of heart disease in adults, and mounting evidence shows that infants born to diabetic or obese mothers also have a higher risk of heart disease in adulthood. We have shown that newborn rats prenatally exposed to maternal diabetes and high-fat (HF) diet have diastolic and systolic dysfunction, myocardial lipid accumulation, decreased respiratory capacity, oxidative injury and mitochondrial

dysfunction that mimics that of adult diabetic cardiomyopathy. However, do these adverse cardiac effects carry into adulthood? If so, is either sex more severely affected?

Objective: Determine whether prenatal exposure to maternal diabetes or HF diet impairs bioenergetics or mitochondrial function in primary isolated adult rat cardiomyocytes (ARCM).

Methods: Sprague-Dawley rats received control or HF diet 28 days before and throughout pregnancy. On gestational day 14, citrate buffer or streptozotocin was given to induce diabetes, which was then treated twice daily with sliding-scale insulin. Dams delivered controls, diabetes-exposed, HF diet-exposed and combination-exposed offspring which, on postnatal day 1, were cross-fostered and raised by healthy dams. At 12-13 months, ARCM were isolated for extracellular flux analysis (glycolytic, mitochondrial, and palmitate stress tests and permeabilized assays), live-cell confocal imaging of stress responses, mitochondrial copy number, and lipid peroxidation assays. Groups were compared using two-way ANOVA for diet, diabetes, and interaction with significance set at $p \leq 0.05$.

Results: Results to date show that male ARCM have a greater ability to oxidize fatty acids and lower glycolytic capacity than female ARCM. In both sexes, diet- and diabetes-exposed ARCM had decreased proton leak, reduced respiratory spare capacity, and impaired tolerance for uncoupler-induced stress. Confocal imaging further demonstrated these effects by showing 15-50% faster membrane potential loss in both sexes and 30-50% faster cell death in female ARCM prenatally exposed to diet and diabetes. Poor spare capacity is likely related to impaired fuel flexibility. All ARCM demonstrated poor glycolytic capacity. Diet-exposure reduced exogenous and endogenous fatty acid oxidation in males while diabetes-exposure reduced endogenous fatty acid oxidation in females. In males, diet-exposed ARCM had reduced oxidation of the complex I fuel glutamine, complex II fuel succinate, and complex IV fuel TMPD/ascorbate. In females, combination-exposed ARCM had lower oxidation of complex II succinate and complex III duroquinol. Results for mitochondrial copy number and lipid peroxidation are pending.

Conclusions: Prenatal exposure to maternal HF diet and diabetes causes sex-specific changes in mitochondrial function and fuel flexibility in ARCM. Findings highlight the role of mitochondria in fetal origins of adult cardiovascular disease.

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7: SESSION 4: PHYSIOLOGY AND GENDER: OBESITY AND METABOLISM

7.2

PROGESTERONE UPREGULATES ENDOTHELIAL MINERALOCORTICOID RECEPTOR EXPRESSION WHICH PREDISPOSES FEMALE MICE TO OBESITY-INDUCED ENDOTHELIAL DYSFUNCTION

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Clinical data indicates that obesity ablates the protection of premenopausal women from cardiovascular disease. Compelling evidence indicates a higher efficacy of mineralocorticoid receptor (MR) blockade for the treatment of cardiovascular disease in obese and diabetic females than in males, however, the origin of this sex-specific effect is unknown. We have shown that the adipocyte-derived hormone leptin mediates endothelial dysfunction in obese female mouse models via aldosterone-dependent activation of MR and, further, that female mice are more sensitive to aldosterone-induced endothelial dysfunction. Therefore, we hypothesized that females express higher endothelial MR (ECMR) expression than males which predisposes females to obesity-associated endothelial dysfunction. RT-PCR analysis in isolated aortic endothelial cells (magnetic cell sorting technique) of Balb/C mice revealed a higher NR3C2 (MR gene) expression in females compared to males (2.9±0.5-fold from male, $P<0.05$), however, no such difference was observed in non-endothelial cells (0.1±0.3-fold from male). Endothelial NR3C2 expression was further elevated in female obese mice (5.8±1.1-fold from male obese, $P<0.05$). Similarly, human aortic (5.4-fold from male) and adipose tissue (2.1±0.1 fold from male, $P=0.07$) endothelial cells derived from female patients exhibited higher NR3C2 mRNA expression than those of males. Western blotting analysis confirmed that ECMR protein expression is correspondingly elevated in aortic endothelial cells from female patients compared to those of males (0.5±0.1 male vs 1.4±0.5 female ratio/β actin). Female sex-hormones suppression (ovariectomy) decreased ECMR expression in female mice (-0.8±0.2-fold from sham, $P<0.05$), which was restored by progesterone supplementation (-0.1±0.1-fold from sham). Increases in progesterone levels with the diestrous phase of the menstrual cycle and pregnancy were associated with a gradual increase in NR3C2 mRNA (diestrous: 1.6±0.1-fold

from estrous, pregnancy day 16: 9.2±0.2-fold from estrous, P<0.05). In parallel, progesterone dose-dependently increased ECMR protein expression in human endothelial cells *in vitro* (P<0.05). Endothelial function was determined in male and female mice by wire myography via aorta relaxation responses to acetylcholine (10^{-9}-10^{-5} M concentration range). Increases in ECMR associated with higher progesterone levels in pregnant females were associated with an increased sensitivity to leptin-induced endothelial dysfunction in mice. In parallel, while leptin induced endothelial dysfunction in intact ECMR female mice (P<0.05), specific deletion of MR in endothelial cells protected female mice from leptin-induced endothelial dysfunction. No differences were observed in leptin-mediated endothelial function in male mice regardless of intact ECMR expression. Further, no changes in endothelium-independent relaxation responses (relaxation to sodium nitroprusside) in any groups were observed. These data indicate that progesterone drives the sex-difference in the levels of ECMR expression and predisposes female mice to leptin-induced endothelial dysfunction. In addition, these data provide a rational for the higher efficacy of MR blockade in obese and diabetic women suffering from cardiovascular disease. Supported by: NIH 1R01HL130301-01, AHA 16IRG27770047, AHA 17POST33660678 and NIH 5F32HL136191-02.

7.3

IDENTIFICATION OF SEX-SPECIFIC MIRNA AND MRNA EXPRESSION PATTERNS IN TYPE 1 DIABETES

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Type 1 Diabetes (T1D) is a chronic autoimmune disease characterized by progressive loss of insulin producing β cells. Sex biased differences exist in the incidence rate, mortality rate and severity of T1D. While T1D is more common in males, the mortality rate and severity is higher in females. The sex-specific difference may also extend into the molecular mechanisms contributing to the development of T1D and has not yet been studied for T1D.

We hypothesized that these sex-specific differences may also extend to molecular pathways activated within the β cell during the evolution of Type 1 diabetes. To address this, human islets from 5 male and 5 female donors were treated with or without cytokines (50 U/ml IL-1β and 1000 U/ml IFN-γ) for 24 hrs, to mimic the pro-inflammatory conditions of T1D. Stratified analysis based on sex was performed. mRNA and miRNA sequencing were performed using Illumina NextSeq and Ion Proton System, respectively. Partek flow, R statistical program (DESeq2 package) and Ingenuity Pathway analysis tools were utilized for analyzing the datasets. mRNAs with a fold change ≥ 2.0 and false discovery rate < 0.05 and miRNAs with a fold change ≥ 1.5 and p < 0.05 in cytokine-treated islets were considered as differentially expressed (DE). miRNA targets were predicted using TargetScan, which were then overlapped with DE mRNAs identified from islets.

Our data revealed striking sex differences in miRNA expression profiles in cytokine-treated islets. Notably, only 4 DE miRNAs were common between the two sexes, while 16 miRNAs and 25 miRNAs were unique in males and females, respectively. Classically these variations have been attributed to differences in sex chromosomes and the action of sex hormones. However, we found that > 95% of these miRNAs originated from autosomes and none of these miRNAs are known to be under the influence of sex hormones. Whereas, 1061 mRNAs were common from a total of 1667 and 1186 DE mRNAs from male and female islets, respectively. Among the significant canonical pathways, 12 pathways were common between males (total = 21; unique = 9) and females (total = 26; unique = 14) and each of these common pathways (Eg: Sphingosine-1-phosphate) were predicted to be regulated by unique miRNAs for males and females. Similarly, common gene ontology terms were observed to be regulated by predominantly unique miRNAs in males and females. For example, apoptosis of pancreatic cells was observed to be regulated by miR-146a-5p, miR-338-3p, miR-155-5p, miR-6891-5p, miR-296-3p, miR-181a-2-3p, miR-675-5p in males, but the same function was predicted to be regulated by miR-146a-5p, miR-194-3p, miR-22-3p, miR-320b, miR-30e-3p, miR-124-3p, miR-338-5p in females.

Taken together, our data suggests that sex-biased expression patterns among mRNAs and their upstream regulatory miRNAs may drive sex-specific activation or inhibition of molecular pathways involved in T1D pathogenesis. Understanding these differences are critical to consider in our efforts to develop biomarkers for T1D risk and heterogeneity and when designing trials of novel disease-modifying therapies.

7.4

SEX DIFFERENCES IN THE DEVELOPMENT OF RENAL INJURY ASSOCIATED WITH OBESITY

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Obesity has grown at an alarming rate within the last decade and has been associated with the development of chronic kidney disease (CKD) and end stage renal disease (ESRD). In the US, half of the ESRD population are nondiabetic and are morbidly obese. Obesity contributes to sex differences in the risk for CKD in which

obese males tend to develop CKD earlier in life than obese females. To study sex differences during the development of renal disease associated with obesity our laboratory uses obese leptin receptor mutant Dahl salt-sensitive (SS^{LepR}mutant) rats which were derived from Zinc-finger nucleases. We observed an increase in body weight in both female and male SS^{LepR}mutant rats when compared to lean SS rats at 18 weeks of age. The SS^{LepR}mutant strain also developed hyperinsulinemia in comparison to their lean SS counterparts. However, blood glucose in the SS^{LepR}mutant strain remained within normal range throughout the course of the study regardless of sex. Female and male SS^{LepR}mutant rats developed severe systolic hypertension by 18 weeks of age when compared to SS rats. Yet, the rise in arterial pressure occurred earlier in female SS^{LepR}mutant rats than males. Protein excretion was significantly higher in the SS^{LepR}mutant strain versus SS rats at 18 weeks of age regardless of sex. The kidneys from the SS^{LepR}mutant strain displayed increased glomerulosclerosis and interstitial fibrosis compared to SS rats. Female and male SS^{LepR}mutant rats had a significant increase in plasma creatinine levels compared to the SS strain suggesting the presence of severe CKD. While conducting these first set of experiments, we observed a significantly higher mortality rate in female SS^{LepR}mutants. Therefore, in the second set of experiments, we determined the survival rate of female and male SS and SS^{LepR}mutant rats through 18 weeks of age. The survival rate of female SS^{LepR}mutant rats was markedly reduced compared to their male counterparts. Recently, we reported that male rats display proteinuria and podocyte injury by 6 weeks of age independent of hyperglycemia and elevations in arterial pressure. However, female SS^{LepR}mutant rats were not examined during this same time period. Thus, we examine whether there were sex differences during the development of renal injury between 4 to 8 weeks of age. Proteinuria was significantly higher in female and male SS^{LepR}mutant rats compared to lean SS rats at 4 weeks of age and remained elevated throughout the course of the study. When examining renal function during this time period by the clearance of FITC-sinistrin, we did not detect any strain or sex differences. The kidneys from the SS^{LepR}mutant rats displayed significant glomerular injury and marked renal fibrosis when compared to lean SS rats during this time period. While we observed sex differences in metabolic and cardiorenal disease in older obese SS^{LepR}mutant rats, we did not detect any sex differences in younger SS^{LepR}mutant rats. Overall, these data indicate that the SS^{LepR}mutant strain may be a useful model to study sex differences during the development of CKD associated with obesity. Further studies are needed to determine the cause of the sex difference in mortality in obese SS^{LepR}mutant rats. This research was supported by GM104357 and DK109133.

7.5

FEMALE RATS OFFERED FREE ACCESS TO LARD, SUCROSE, AND CHOW DEVELOPED FEATURES OF METABOLIC SYNDROME AND PERIUTERINE ADIPOSE TISSUE EXPANSION

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Objective: Free access to lard, sucrose solution, and chow results in the development of obesity and the metabolic syndrome within 3 weeks in male rats. The impact of obesity on adipose tissue expansion differs with each adipose depot throughout the body, suggesting that obesity affects adipose tissue in a regional manner. We determined if free access to lard, sucrose, and chow would result in development of the metabolic syndrome and expansion (i.e. hypertrophy and hyperplasia) of periuterine adipose tissue in female rats.

Methods: Two cohorts of virgin Sprague-Dawley female rats were divided into 2 weight-matched groups: 1) choice group: *ad* libitum access to chow, 30% sucrose solution, and lard (choice diet) and 2) chow group: *ad* libitum access to standard rodent chow for 3 weeks. Food intakes and body weights were recorded daily. Glucose clearance was assessed with a glucose tolerance test and insulin sensitivity was assessed with an insulin tolerance test. Fat depots and trunk blood were collected to measure visceral adiposity and triglyceride concentrations, respectively. Adipocyte morphology (cell size and count) was assessed in hematoxylin and eosin-stained periuterine adipose tissue sections using NIS Elements software.

Results: Total energy intake was greater in choice rats than chow rats (1590 ± 41 vs. 1036 ± 19 kcal, $p < 0.0001$). Choice rats had higher percent carcass fat (10.3 ± 1.0 % vs. 4.1 ± 0.2 %, $p = 0.0005$) compared to chow rats, but body weight did not differ between groups ($p = 0.51$). Glucose tolerance and insulin sensitivity were not different in choice rats than chow rats, but choice rats had higher fasting glucose (122 ± 4 v. 110 ± 2 mg/dL, $p = 0.03$) compared to chow rats. Choice rats had greater visceral adiposity, serum concentrations of triglycerides (45.2 ± 3.8 vs. 31.0 ± 2.5 mg/dL, $p = 0.003$), and higher mean arterial pressure (95.79 ± 1.82 v. 88.55 ± 1.25 mm Hg, $p = 0.01$) compared to chow rats. Cross-sectional area/cell was greater in periuterine adipose tissue from choice compared to chow rats (779.6 ± 47.6 vs. 492.0 ± 27.8 μm^2/cell, $p = 0.0001$), indicating a hypertrophic response. Number of cells/unit area was fewer in periuterine adipose tissue from choice rats (1.24×10^{-3} ±

4.66 x 10^{-5} vs. 8.36 x 10^{-4} ± 6.60 x 10^{-5} cells/μm^2, p = 0.0002), indicating a hypoplastic response.

Conclusion: Free access to lard, sucrose, and chow induced some features of the metabolic syndrome. However, we did not observe glucose intolerance, as previously observed in male rats of the same strain, indicating potential sex differences in sensitivity to high-fat, high-carbohydrate diets. Choice diet-induced morphological changes in periuterine adipose tissue may affect reproductive capacity (i.e. fertility) and pregnancy outcomes in females with preconceptional obesity.

Funding**:** UNT HSC Pilot Seed Grant

7.6

X MARKS THE SPOT: SEX CHROMOSOMES REGULATE HYPERCHOLESTEROLEMIA AND ATHEROSCLEROSIS

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It is generally accepted that males have a more proatherogenic lipid profile and more coronary artery disease than females prior to menopause, when a proatherogenic lipid profile emerges in females and may exceed that of males. The majority of studies investigating sex differences in lipid profiles and atherosclerosis have focused on sex hormones. However, sex chromosome complement is the other primary biologic determinant of sex, and the X chromosome contains as much as 5% of the human genome. We used the 4 core genotype model to examine effects of a Western (WD) high fat with added cholesterol diet on hypercholesterolemia, obesity, and atherosclerosis in XX and XY male and female low density lipoprotein receptor deficient mice (Ldlr-/-). Initial studies defined basal or short-term effects of the WD on whole body metabolism in male (XY and XX) and female (XX and XY) Ldlr-/- mice. XX mice, regardless of gonadal sex, exhibited increased food intake compared to XY mice. Moreover, gastrointestinal fat absorption was increased in XX compared to XY mice, regardless of gonadal sex. However, liver lipoprotein secretion was not different between XX and XY mice (male or female) fed standard murine diet. When fed the WD for 3 months, XX mice (male and female) had increased body weight, and markedly higher serum cholesterol concentrations, and these effects were present even in castrated XX compared to XY mice, suggesting a prominent role for sex chromosomes. The extent of atherosclerosis was markedly increased in XX (male and female) compared to XY mice. Alterations in expression levels of key genes implicated in absorption of fat by the small intestine of XX mice, coupled with increased fat absorption and food intake, augment hypercholesterolemia and the development of atherosclerosis. These results suggest that an XX sex chromosome complement favors lipid absorption, which may contribute to proatherogenic lipid profiles and increased atherosclerosis in post-menopausal females.

8: POSTER SESSION 2

8.1

LONG TERM CONSEQUENCES OF FOOD RESTRICTION ON BODY COMPOSITION AND ANGIOTENSIN SYSTEM

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Introduction: The women population is more predispose to do severe diets and people who does severe food restriction (**FR**) are at risk for developing cardiovascular disease during the FR period and also long term well after the FR period has ended. Abnormalities in the heart, vascular system and kidneys are common problems in individuals who have experienced FR. Unfortunately, the long-term risk for cardiovascular system due to earlier exposure to FR is an underappreciated and understudied problem and there are no known therapeutic strategies designed to prevent the increased risk after recovery from the FR period.

Methods*:* Female Fischer rats with 4 month old were divided in two groups, control (**CT**) with ad libitum diet and FR with 60% reduction in the daily food intake for 14 days. After 14 days of FR the rats received chow ad libitum for 3 months. On FR day 1 to 4 and day 14 of FR until day 4 of recovery period the rats were placed in a metabolic cage.

Aim: Determine the FR short and long-term consequences on body composition and angiotensin system.

Results*:* After 14 of FR the body weight (BW) was reduced by 18%, the water intake by 50%, the rats also showed a reduction in plasma volume (p=0.03), blood pressure (p=0.03), heart rate (p=0.03), kidney weight, kidney protein, high plasma Angiotensin (**Ang**) II (p<0.05) and angiotensinogen (p<0.001). No difference was observed in plasma aldosterone, sodium and and potassium. The rats with FR were in balance for sodium during the first 4 days of FR although for the first day the rats showed a negative balance for potassium (p<0.05) returning to the balance on the 2ndday. 3 days of FR also stopped the estrous cycle on diestrus returning to the ovulatory phase only after 7 days on *ad libitum* diet. The rats recovered the BW only 21 days in the reseeding protocol, although they had a higher deposition of abdominal fat after 40 days of receding and no difference on the lean tissue. After 3 months of ad libitum diet, the rats normalized the water and food intake, however the

Ang II infusion increased more the blood pressure ($p<0.05$), even though the blood pressure was normal.

Conclusion*:* The FR causes a short-term change on body composition and a high Ang system activation with a possible miscommunication between Ang-Aldo-Sodium-potassium. After 3 months of refeeding, the FR rats recovered the body weight and the blood, but had more fat deposition and pressor responsiveness to Ang II.

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8.2

PROGESTERONE UPREGULATES ENDOTHELIAL MINERALOCORTICOID RECEPTOR EXPRESSION WHICH PREDISPOSES FEMALE MICE TO OBESITY-INDUCED ENDOTHELIAL DYSFUNCTION

Jessica Faulkner[1], Simone Kennard[1], Galina Antonova[1], Zsolt Bagi[2], Iris Jaffe[3], Vijay Patel[4], Eric Belin de Chantemele[1]

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Clinical data indicates that obesity ablates the protection of premenopausal women from cardiovascular disease. Compelling evidence indicates a higher efficacy of mineralocorticoid receptor (MR) blockade for the treatment of cardiovascular disease in obese and diabetic females than in males, however, the origin of this sex-specific effect is unknown. We have shown that the adipocyte-derived hormone leptin mediates endothelial dysfunction in obese female mouse models via aldosterone-dependent activation of MR and, further, that female mice are more sensitive to aldosterone-induced endothelial dysfunction. Therefore, we hypothesized that females express higher endothelial MR (ECMR) expression than males which predisposes females to obesity-associated endothelial dysfunction. RT-PCR analysis in isolated aortic endothelial cells (magnetic cell sorting technique) of Balb/C mice revealed a higher NR3C2 (MR gene) expression in females compared to males (2.9±0.5-fold from male, $P<0.05$), however, no such difference was observed in non-endothelial cells (0.1±0.3-fold from male). Endothelial NR3C2 expression was further elevated in female obese mice (5.8±1.1-fold from male obese, $P<0.05$). Similarly, human aortic (5.4-fold from male) and adipose tissue (2.1±0.1 fold from male, $P=0.07$) endothelial cells derived from female patients exhibited higher NR3C2 mRNA expression than those of males. Western blotting analysis confirmed that ECMR protein expression is correspondingly elevated in aortic endothelial cells from female patients compared to those of males (0.5±0.1 male vs 1.4±0.5 female ratio/β actin). Female sex-hormones suppression (ovariectomy) decreased ECMR expression in female mice (-0.8±0.2-fold from sham, $P<0.05$), which was restored by progesterone supplementation (-0.1±0.1-fold from sham). Increases in progesterone levels with the diestrous phase of the menstrual cycle and pregnancy were associated with a gradual increase in NR3C2 mRNA (diestrous: 1.6±0.1-fold from estrous, pregnancy day 16: 9.2±0.2-fold from estrous, $P<0.05$). In parallel, progesterone dose-dependently increased ECMR protein expression in human endothelial cells *in vitro* ($P<0.05$). Endothelial function was determined in male and female mice by wire myography via aorta relaxation responses to acetylcholine (10^{-9}-10^{-5} M concentration range). Increases in ECMR associated with higher progesterone levels in pregnant females were associated with an increased sensitivity to leptin-induced endothelial dysfunction in mice. In parallel, while leptin induced endothelial dysfunction in intact ECMR female mice ($P<0.05$), specific deletion of MR in endothelial cells protected female mice from leptin-induced endothelial dysfunction. No differences were observed in leptin-mediated endothelial function in male mice regardless of intact ECMR expression. Further, no changes in endothelium-independent relaxation responses (relaxation to sodium nitroprusside) in any groups were observed. These data indicate that progesterone drives the sex-difference in the levels of ECMR expression and predisposes female mice to leptin-induced endothelial dysfunction. In addition, these data provide a rational for the higher efficacy of MR blockade in obese and diabetic women suffering from cardiovascular disease.

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8.3

CD8 T-CELLS ISOLATED FROM FEMALE MICE HAVE INCREASED *IN VITRO* CELL ACTIVATION IN RESPONSE TO NUTRIENT DEPRIVATION

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Nutritional status is critically important for immune cell function. T-cells from animal models of obesity have an early and critical role in inducing inflammation. Conversely, in the setting of malnutrition, T-cells have decreased effector function and proliferative capacity. We hypothesized that CD8+ T-cells isolated from females would show increased activation in response to nutrient

deprivation leading to enhanced secretion of inflammatory proteins. CD8+ T-cells were isolated from the spleens of C57BL/6J wild-type mice (male=3.8±0.1 month old and female=3.6±0.1 month old; n=5/sex) and cultured in RPMI 1640 (10% FBS; 1% antibiotic/antimycotic) with T-cell activation beads (CD3ε and CD28) in 5% CO_2 at 37°C. After serum starvation in media with 0.1% FBS for 24 h, the secretome was collected and analyzed by mass spectroscopy. Proteomic analysis identified 3991 unique peptides from 3161 proteins. Of these proteins, 279 were significantly different ($p<0.05$ for all) and at least 2-fold different in expression between sexes (31 proteins downregulated and 248 upregulated in females compared to males). E3 ubiquitin-protein ligase, B-cell lymphoma 11b, tissue growth factor β, macrophage colony stimulating factor receptor, insulin-like growth factor binding protein, and Trp4 associated protein were the top regulators of sex differences based on fold change and p-value. Ingenuity pathway analysis indicated more than one-third (104 of the 279 proteins) mapped to networks involved in regulating cell death (↓), cell viability (↑), leukocyte trafficking (↑), angiogenesis (↑), or fibrosis (↓; arrows indicated direction of female response vs male response; $p<0.05$ for all). In conclusion, CD8+ T-cells isolated from females have an exacerbated response to metabolic stress induced by serum starvation. Key biological pathways were identified as regulators of sex differences in T-cell metabolic adaptation during nutrient stress.

8.4

ARGINASE 2 IS INVOLVED IN DIET-INDUCED OBESITY AND METABOLIC DYSREGULATION IN MALE AND FEMALE MICE

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Arginase is a ubiquitous enzyme, present in 2 isoforms, the cytosolic A1 or mitochondrial A2. Arginase competes with nitric oxide synthase for their common substrate L-arginine and can limit synthesis of nitric oxide (NO), which is needed to maintain normal adipocyte (AC) physiology and metabolic function. In our studies, male WT mice and mice globally lacking A2(-/-) were fed either normal chow diet (ND) or high fat, high sucrose (HFHS) diet for 16 weeks. A2 deletion blunted the HFHS-induced increases in body weight and visceral adipose tissue (VAT) weight in WT mice by 22% and 57%, respectively ($p<0.05$). In line with these observations, metabolic chamber studies showed that A2-/- mice feed HFHS diet also exhibited a higher respiratory exchange ratio (RER) compared to WT HFHS mice ($p<0.05$). A2-/- mice on HFHS diet showed a trend to an increase in energy expenditure compared to WT-HFHS ($p=0.06$). HFHS mice lacking A2 also exhibited a lower fasting blood glucose than WT-HFHS ($p<0.05$). A2 expression in ACs isolated from VAT of WT HFHS mice was elevated 4-fold compared to WT on ND. In differentiated AC (3T3-L1) exposed to high levels of palmitate (250 μM) and glucose (25 mM), A2 levels were 3-fold higher than cells in normal control media. WT-HFHS mice also exhibited reduced mRNA levels of PPAR-ɣ, as well as, the anti-inflammatory adipokine - adiponectin, insulin receptor substrate-1 (IRS-1) and glucose transporter (GLUT)-4 ($p<0.05$) vs ND controls. However, A2-/- HFHS mice showed preserved expression levels of these factors at almost control levels. A2 deletion also blunted the reduction in mitochondrial density in VAT seen in WT HFHS mice as well as the reduced AC mRNA expression of PPAR-ɣ coactivator-1α (PGC-1α), a regulator of mitochondrial biogenesis as well as the mRNA expression of the thermogenic markers - UCP1 and CIDE-A.

In female mice, lack of A2 produced a similar phenotype as in HFHS males, but with a more pronounced decrease in body weight (32%) and VAT weight (74%) compared to WT-HFHS ($p<0.05$). RER was not different between WT and A2-/- HFHS fed groups ($p>0.05$). However, A2 deletion significantly blunted the decrease in energy expenditure exhibited by WT group fed HFHS diet ($p<0.05$). In addition, fasting blood glucose was significantly lower in HFHS A2-/- mice compared to WT HFHS diet ($p<0.05$).

In conclusion, our study shows that A2 is critically involved in HFHS-induced obesity and metabolic abnormalities in both males and females with a more prominent role in the females.

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8.5

IDENTIFICATION OF SEX-SPECIFIC MIRNA AND MRNA EXPRESSION PATTERNS IN TYPE 1 DIABETES

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Type 1 Diabetes (T1D) is a chronic autoimmune disease characterized by progressive loss of insulin producing β cells. Sex biased differences exist in the incidence rate, mortality rate and severity of T1D. While T1D is more common in males, the mortality rate and severity is higher in females. The sex-specific difference may also extend into the molecular mechanisms contributing to the development of T1D and has not yet been studied for T1D.

We hypothesized that these sex-specific differences may also extend to molecular pathways activated within the β cell during the evolution of Type 1 diabetes. To address this, human islets from 5 male and 5 female donors were treated with or without cytokines (50 U/ml IL-1β and 1000 U/ml IFN-γ) for 24 hrs, to mimic the pro-inflammatory conditions of T1D. Stratified analysis based on sex was performed. mRNA and miRNA sequencing were performed using Illumina NextSeq and Ion Proton System, respectively. Partek flow, R statistical program (DESeq2 package) and Ingenuity Pathway analysis tools were utilized for analyzing the datasets. mRNAs with a fold change ≥ 2.0 and false discovery rate < 0.05 and miRNAs with a fold change ≥ 1.5 and $p < 0.05$ in cytokine-treated islets were considered as differentially expressed (DE). miRNA targets were predicted using TargetScan, which were then overlapped with DE mRNAs identified from islets.

Our data revealed striking sex differences in miRNA expression profiles in cytokine-treated islets. Notably, only 4 DE miRNAs were common between the two sexes, while 16 miRNAs and 25 miRNAs were unique in males and females, respectively. Classically these variations have been attributed to differences in sex chromosomes and the action of sex hormones. However, we found that > 95% of these miRNAs originated from autosomes and none of these miRNAs are known to be under the influence of sex hormones. Whereas, 1061 mRNAs were common from a total of 1667 and 1186 DE mRNAs from male and female islets, respectively. Among the significant canonical pathways, 12 pathways were common between males (total = 21; unique = 9) and females (total = 26; unique = 14) and each of these common pathways (Eg: Sphingosine-1-phosphate) were predicted to be regulated by unique miRNAs for males and females. Similarly, common gene ontology terms were observed to be regulated by predominantly unique miRNAs in males and females. For example, apoptosis of pancreatic cells was observed to be regulated by miR-146a-5p, miR-338-3p, miR-155-5p, miR-6891-5p, miR-296-3p, miR-181a-2-3p, miR-675-5p in males, but the same function was predicted to be regulated by miR-146a-5p, miR-194-3p, miR-22-3p, miR-320b, miR-30e-3p, miR-124-3p, miR-338-5p in females.

Taken together, our data suggests that sex-biased expression patterns among mRNAs and their upstream regulatory miRNAs may drive sex-specific activation or inhibition of molecular pathways involved in T1D pathogenesis. Understanding these differences are critical to consider in our efforts to develop biomarkers for T1D risk and heterogeneity and when designing trials of novel disease-modifying therapies.

8.6

SEX SPECIFIC UPREGULATION OF HEPATIC FGF21 EXPRESSION IN METABOLIC STATES CONTRAST BY NUTRIENT AVAILABILITY

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Many aspects of metabolic homeostasis are known to be regulated differently in males and females. Fibroblast growth factor 21 (FGF21) is a hormonal regulator of carbohydrate and lipid metabolism, which is induced by both catabolic and anabolic signals (1). Diet induced obesity (anabolic state) was demonstrated to cause sex specific upregulation of FGF21 in liver tissue (2). No previous studies have addressed the possible sexual differences in FGF21 response to fasting (catabolic state). The aim of this study was to investigate whether there is sexual dimorphism in the effects of dietary obesity and fasting on the hepatic FGF21 expression and its signaling.

Male and female C57Bl mice were subjected to 24h-fasting or to sweet-fat diet (10 weeks). Circulating FGF21 levels and hepatic expression of *Fgf21* and genes involved in FGF21 signaling (*Ppar-α, Pgc1, Klb*) were measured.

FGF21 plasma concentrations and hepatic *Fgf21* expression were increased by the fasting and the degree of upregulation was significantly higher in the livers of female mice. PPARα gene expression, which has been reported as upstream of FGF21, also was upregulated more potently in the liver of fasted female than male mice. Reversed sexual dimorphism in upregulation of FGF21 gene expression was observed in obese mice. Obesity induced male-specific upregulation of FGF21 in liver tissue. PPARα expressions were also upregulated more potently in the liver of obese male than female mice. Consistently, higher levels of FGF21 were detected in serum obtained from obese male mice compared to that of female mice. In both fasted and obese mice, hepatic expression of *Fgf21* gene did not correlate to expressions of genes involved in FGF21 signaling: PPARγ coactivator protein-1α (*Pgc-1α*), a key transcriptional regulator of energy homeostasis and β-klotho *(Klb)* – co-receptor of FGF21 receptor.

Thus, sex specific upregulation of hepatic PPARα-FGF21 endocrine signaling pathway was observed in metabolic situations contrast by nutrient availability. Upregulation of these genes was biased toward females under catabolic condition (fasting) and toward males under anabolic condition (diet induced obesity). The data suggest important role of hepatic FGF21 in the sexually dimorphic regulation of metabolic homeostasis.

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8.7

FEMALE RATS OFFERED FREE ACCESS TO LARD, SUCROSE, AND CHOW DEVELOPED FEATURES OF METABOLIC SYNDROME AND PERIUTERINE ADIPOSE TISSUE EXPANSION

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Objective: Free access to lard, sucrose solution, and chow results in the development of obesity and the metabolic syndrome within 3 weeks in male rats. The impact of obesity on adipose tissue expansion differs with each adipose depot throughout the body, suggesting that obesity affects adipose tissue in a regional manner. We determined if free access to lard, sucrose, and chow would result in development of the metabolic syndrome and expansion (i.e. hypertrophy and hyperplasia) of periuterine adipose tissue in female rats.

Methods: Two cohorts of virgin Sprague-Dawley female rats were divided into 2 weight-matched groups: 1) choice group: *ad* libitum access to chow, 30% sucrose solution, and lard (choice diet) and 2) chow group: *ad* libitum access to standard rodent chow for 3 weeks. Food intakes and body weights were recorded daily. Glucose clearance was assessed with a glucose tolerance test and insulin sensitivity was assessed with an insulin tolerance test. Fat depots and trunk blood were collected to measure visceral adiposity and triglyceride concentrations, respectively. Adipocyte morphology (cell size and count) was assessed in hematoxylin and eosin-stained periuterine adipose tissue sections using NIS Elements software.

Results: Total energy intake was greater in choice rats than chow rats (1590 ± 41 vs. 1036 ± 19 kcal, $p < 0.0001$). Choice rats had higher percent carcass fat (10.3 ± 1.0 % vs. 4.1 ± 0.2 %, $p = 0.0005$) compared to chow rats, but body weight did not differ between groups ($p = 0.51$). Glucose tolerance and insulin sensitivity were not different in choice rats than chow rats, but choice rats had higher fasting glucose (122 ± 4 v. 110 ± 2 mg/dL, $p = 0.03$) compared to chow rats. Choice rats had greater visceral adiposity, serum concentrations of triglycerides (45.2 ± 3.8 vs. 31.0 ± 2.5 mg/dL, $p = 0.003$), and higher mean arterial pressure (95.79 ± 1.82 v. 88.55 ± 1.25 mm Hg, $p = 0.01$) compared to chow rats. Cross-sectional area/cell was greater in periuterine adipose tissue from choice compared to chow rats (779.6 ± 47.6 vs. 492.0 ± 27.8 μm^2/cell, $p = 0.0001$), indicating a hypertrophic response. Number of cells/unit area was fewer in periuterine adipose tissue from choice rats ($1.24 \times 10^{-3} \pm 4.66 \times 10^{-5}$ vs. $8.36 \times 10^{-4} \pm 6.60 \times 10^{-5}$ cells/μm^2, $p = 0.0002$), indicating a hypoplastic response.

Conclusion: Free access to lard, sucrose, and chow induced some features of the metabolic syndrome. However, we did not observe glucose intolerance, as previously observed in male rats of the same strain, indicating potential sex differences in sensitivity to high-fat, high-carbohydrate diets. Choice diet-induced morphological changes in periuterine adipose tissue may affect reproductive capacity (i.e. fertility) and pregnancy outcomes in females with preconceptional obesity.

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8.8

PRENATAL DIABETES AND HIGH-FAT DIET EXPOSURE IMPAIR MITOCHONDRIAL FUNCTION IN ADULT RAT CARDIOMYOCYTES

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Background: Both diabetes and obesity increase the risk of heart disease in adults, and mounting evidence shows that infants born to diabetic or obese mothers also have a higher risk of heart disease in adulthood. We have shown that newborn rats prenatally exposed to maternal diabetes and high-fat (HF) diet have diastolic and systolic dysfunction, myocardial lipid accumulation, decreased respiratory capacity, oxidative injury and mitochondrial dysfunction that mimics that of adult diabetic cardiomyopathy. However, do these adverse cardiac effects carry into adulthood? If so, is either sex more severely affected?

Objective: Determine whether prenatal exposure to maternal diabetes or HF diet impairs bioenergetics or mitochondrial function in primary isolated adult rat cardiomyocytes (ARCM).

Methods: Sprague-Dawley rats received control or HF diet 28 days before and throughout pregnancy. On gestational day 14, citrate buffer or streptozotocin was given to induce diabetes, which was then treated twice daily with sliding-scale insulin. Dams delivered controls,

diabetes-exposed, HF diet-exposed and combination-exposed offspring which, on postnatal day 1, were cross-fostered and raised by healthy dams. At 12-13 months, ARCM were isolated for extracellular flux analysis (glycolytic, mitochondrial, and palmitate stress tests and permeabilized assays), live-cell confocal imaging of stress responses, mitochondrial copy number, and lipid peroxidation assays. Groups were compared using two-way ANOVA for diet, diabetes, and interaction with significance set at $p \leq 0.05$.

Results: Results to date show that male ARCM have a greater ability to oxidize fatty acids and lower glycolytic capacity than female ARCM. In both sexes, diet- and diabetes-exposed ARCM had decreased proton leak, reduced respiratory spare capacity, and impaired tolerance for uncoupler-induced stress. Confocal imaging further demonstrated these effects by showing 15-50% faster membrane potential loss in both sexes and 30-50% faster cell death in female ARCM prenatally exposed to diet and diabetes. Poor spare capacity is likely related to impaired fuel flexibility. All ARCM demonstrated poor glycolytic capacity. Diet-exposure reduced exogenous and endogenous fatty acid oxidation in males while diabetes-exposure reduced endogenous fatty acid oxidation in females. In males, diet-exposed ARCM had reduced oxidation of the complex I fuel glutamine, complex II fuel succinate, and complex IV fuel TMPD/ascorbate. In females, combination-exposed ARCM had lower oxidation of complex II succinate and complex III duroquinol. Results for mitochondrial copy number and lipid peroxidation are pending.

Conclusions: Prenatal exposure to maternal HF diet and diabetes causes sex-specific changes in mitochondrial function and fuel flexibility in ARCM. Findings highlight the role of mitochondria in fetal origins of adult cardiovascular disease.

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8.9

WITHDRAWN

8.10

HIS AND HER COMPUTATIONAL MODELS OF LONG-TERM BLOOD PRESSURE REGULATION

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Hypertension is a global health challenge. Hypertensive men and women are typically treated with the same approach, with less effective outcome in women. To address the critical need to better understand the mechanism of blood pressure control in both men and women, we have developed sex-specific computational models of long-term blood pressure control.

The model represents sex differences in the kidney's pressure natriuresis response, whereby increases in renal perfusion pressure lead to increases in Na+ excretion; that in turns lowers salt and water retention and reduces effective circulating volume.

Females tend to exhibit a leftward shift in the pressure-natriuresis relation relative to males. The model also includes detailed representation of the renin-angiotensin aldosterone system (RAAS), a non-sex hormonal system critical for maintaining blood pressure and effective circulating volume. Major sex differences in the RAAS have been identified, including how substrate is produced, how angiotensin interacts with receptors, and baseline aldosterone levels. Those sex differences are included in the model. Differences in renal sympathetic nervous activity (RSNA) stimulation are also included.

Using the developed model, we conclude that increased afferent arteriole resistance causes a larger increase in mean arterial pressure than other causes of hypertension. We also conclude that females are protected against hypertension caused by increased afferent arteriole resistance (a 17 mmHg increase in females vs a 22 mmHg increase in males) due to differences in RSNA sensitivity.

We also conclude that observed sex differences in anti-hypertensive drug efficacy, (i.e. that angiotensin converting enzyme inhibitors (ACEI) work better than angiotensin receptor blockers (ARB) in males while the opposite is true in females) is due to the effects of angiotensin II on renal vascular resistance when bound to the angiotensin type 2 receptor (AT2R-bound Ang II). Abolishing this effect from our model also abolishes the sex difference in drug treatment efficacy.

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8.11

SEX DIFFERENCES IN THE ROLE OF THE SMOOTH MUSCLE CELL MINERALOCORTICOID RECEPTOR IN CARDIOVASCULAR AGING

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The process of vascular aging involves the development of vasomotor dysfunction and vessel stiffening, which leads to cardiovascular disease (CVD). Clinical data supports that the time course of CVD development with aging occurs differentially in males vs. females, suggesting distinct sex-specific mechanisms of vascular aging. The mineralocorticoid receptor (MR) is a steroid hormone receptor known to regulate renal sodium handling. We previously demonstrated that male mice with the MR specifically deleted from smooth muscle cells (SMC-MR-KO), have lower blood pressure, vascular tone, and vasoconstriction as they age. The purpose of this study was to determine whether there are sex differences in the role of SMC-MR in vascular aging. All evaluations of the mice took place at 3 ages: 3, 12, and 18 months, chosen as these roughly correspond to adult (3 mo.), middle age or "peri-menopause" (12 mo.) and elderly or "post-menopause" in females (18 mo.). First, we show that vascular MR increases with age differentially in male and female mice; with vascular MR increasing at 12 months of age in males but not until 18 months of age in females. This change in MR expression is accompanied by a concomitant down-regulation of vascular expression of miR-155 at 12 months in males vs. 18 months in females, which is prevented in SMC-MR-KO mice. In vitro reporter assays showed that MR transcriptionally represses miR-155 promoter activity, whereas estrogen receptor (ER) upregulates miR-155 promoter activity, and is further enhanced with estrogen treatment. These studies suggest that MR and ER regulate miR-155 in an opposite manner, and that the decline in miR-155 in females may be driven by combination of the increase in MR and a loss of estrogen. We previously identified miR-155 as a regulator of vascular target genes including the angiotensin II type 1 receptor (AT1R) and the pore-forming subunit of the L-type calcium channel (LTCC), Cav1.2, both mediators of vasoconstriction and enhanced vascular tone. Indeed, we find that AT1R- and LTCC-mediated vasoconstriction is increased at 12 months in males and 18 months in females and these vasomotor alterations are prevented in SMC-MR-KO mice of both sexes. We next examined alterations in vascular stiffness, a hallmark of vascular aging. In vivo aortic stiffness studies, as measured by pulse wave velocity (PWV), demonstrate the same temporal difference with aging with increased vascular stiffness at 12 months in males vs. 18 months in females, that is prevented by SMC-MR-KO. We explored the contribution of SMC to stiffness via atomic force microscopy of freshly dispersed SMC from ~18 month old mice. These studies revealed that females exhibit more SMC stiffness vs. males, and that this is partially prevented by SMC-MR-KO in females only. To assess the role of fibrosis in the aging-induced alterations in stiffness, we measured carotid artery fibrosis histologically. Carotid fibrosis is increased at 12 months in males vs. 18 months in females, and partially prevented by SMC-MR-KO in males only. Together, these data suggest that the temporal difference in vascular stiffening is associated with distinct sex-specific mechanisms driving vascular stiffness with aging and that SMC-MR plays a differential role in males and females. Next, we examined the effect of aging on cardiac function in males and females by in vivo echocardiography. Cardiac function declines at 18 months in both males and females, but is attenuated by SMC-MR-KO in females only. Further characterization of overall cardiovascular function via exercise capacity testing revealed that exercise capacity declines in males at 12 months vs. 18 months in females, and is partially restored by SMC-MR-KO in males only. In summary, the time course and mechanisms of cardiovascular aging are distinct between males and females with SMC-MR playing a differential role in males versus females. Furthermore, these studies suggest that sex-specific therapies may be essential to improve CVD outcomes in the aging population.

8.12

GENDER AND AGEING INFLUENCE ON VASCULAR RESPONSES IN A PULMONARY HYPERTENSIVE RAT ANIMAL MODEL

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Female gender has been shown to be a risk factor in the development of pulmonary arterial hypertension (PAH). This life-threatening condition involves an elevation of pulmonary arterial pressure (PAP), accompanied with remodelling of pulmonary arteries (PA), inflammation, thrombosis, and finally death from right heart failure. Despite the protective role of estrogens in female individuals, the fact that they are at a higher risk of developing PAH gives way to the so-called "estrogens paradox".

We studied if there is a sexual dimorphism pattern in the vascular responses in PAH, using a chronic hypoxia (CH)

rat animal model, where male and female rats were exposed to 10%O_2 for 2 weeks. Ageing also plays a key role in the estrogens levels, as they decrease markedly in females. Therefore this prompted us to further study the estrogens role in PAH development using both genders aged rats (>20 months old).

Young female rats showed a faster progression of PAH after 2 weeks of exposure to 10% O_2, with a high increase of PAP (almost doubled; determined by right heart catheterization) and right ventricular hypertrophy (Fulton index >30%) respect to their male counterparts. Ageing didn't affect the values of PAP or Fulton index in both genders when compared with young animals, with no differences between sexes. CH in the old animals didn't have as much effect as it was observed in the young animals in PAP, although changes in the Fulton index were in the same direction than in the young animals, but no differences were observed between old male and female animals.

Opposite to these hemodynamic and cardiac results, the dose response carbachol-induced relaxation on preconstricted PA as indicative of vascular dysfunction showed that CH generated endothelial damage in young male rats ($p<0.001$), with no damage in the females ($p>0.05$). As for only ageing, the endothelial dysfunction appeared just in female animals ($p<0.01$), with no further damage after CH exposure. Ageing in males didn't cause any significant damage before or after CH exposure ($p>0.05$ in both cases).

All data is suggesting a PAH sexual dimorphism in rat, with females developing PAH more rapidly than males at young ages. Conversely their PA are more prone to endothelial damage in CH. As for ageing, where estrogens levels should be decreased, effects of CH are blunted, and differences between male and female animals absent.

In conclusion we postulate that the connection between sex and pulmonary circulation rests on different vascular contractility properties.

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8.13

THE ROLE OF SEX IN OXIDATIVE STRESS AND IMPLICATIONS ON VASCULAR FUNCTION AND BLOOD PRESSURES

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The abrupt loss of estrogen leads to a substantial burden on female vasculature and blood pressures (BP) and is a reason why females display a considerable increase in cardiovascular disease development and progression following menopause. It is unknown how estrogen maintains arterial compliance and BP, but may be due to the role of estrogen in increased nitric oxide bioavailability and subsequent reduction in oxidative stress (OS) levels. This link between OS and estrogen is unknown and not well studied. Reducing OS through acute dosages of antioxidants has been previously shown to improve vascular function in males with elevated OS, such as older adults and patients with coronary artery disease. It is unknown if individuals with high estrogen (young females) versus individuals with low estrogen (young males) might respond differently to decreases in OS mediated through antioxidant supplementation and increases in OS mediated through a stimulated oxidative stressor, such as hyperoxia. Therefore, the purpose of this study was to determine if estrogen plays a role in the response to elevations (oxygen supplementation) and decrements (antioxidant supplementation) in OS. Twenty males and females (10 males, 10 females; mean age = 22 years) underwent a baseline visit and then received both ascorbic acid (AOX, 2000 mg) and 100% oxygen supplementation (OXY) in a randomized, cross-over design. Peripheral and central blood pressures (brachial, aortic, and carotid) were measured using an automated sphygmomanometer and applanation tonometry (SphygmoCor) and arterial function measures were assessed using ultrasonography (Arietta 70, Aloka). Conditions were compared using a 3-way ANOVA (baseline x AOX x OXY) and followed up with independent and dependent t-tests when significant. There was an effect of sex with males displaying higher brachial pulse pressure (PP), carotid systolic BP, and carotid PP with all conditions. Males had significantly higher aortic PP at baseline and AOX (values shown as baseline; AOX; OXY [34 mmHg; 33 mmHg; 34 mmHg]) and females had significant decreases in aortic PP with AOX and significant increases in aortic PP with OXY (30 mmHg; 29 mmHg; 32 mmHg). There were no significant differences in measures of carotid beta stiffness or arterial compliance. These results demonstrate that although there were no sex differences in response to changes in OS in arterial function measures, blood pressures were altered differently between sexes, with males consistently displaying elevated pressures. Female aortic PP was significantly affected by both AOX and OS conditions.

8.14

ESTROGEN DETERMINES THE SEX-DIFFERENCES IN ADRENERGIC VESSEL TONE REGULATION

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Introduction: Sex-specific differences in adrenergic vasoconstriction and vasorelaxation have been demonstrated in rats and humans. Although we have previously shown that differences in rats rely on endothelial β-adrenoceptors, neither translational relevance in humans nor the role of sex-hormones in endothelial β-adrenoceptor-related vessel tone regulation has been shown.

Aims: We investigated the role of endothelium in sex-specific differences of adrenergic vasoconstriction and vasorelaxation in human vessels, as well as the role of female and male sex-hormones on adrenergic vessel tone regulation in a rat model.

Methods: In human mammary arteries, obtained from the Heart Center Dresden (patient age: 50 to 70 years), vasoconstriction (norepinephrine) and vasorelaxation (isoprenaline and β_3 agonist BRL) with and without endothelium were assessed using Mulvany myography. Five weeks old female and male wistar rats were respectively ovariectomized and orchiectomized. As controls, a sham-operated, hormone substituted (2 mg/kg, twice a week) and a vehicle group of rats were examined. At age of 12 weeks, aortas were isolated for assessment of vasoconstriction and vasorelaxation. Additionally, a qRT-PCR for quantification of β-adrenoceptor mRNA levels in aorta was performed.

Results: Mammary arteries of women constricted less (P<0.05) in response of norepinephrine than arteries of men. Removal of endothelium eliminated this sex-specific difference by significantly (P<0.05) increasing vasoconstriction in arteries of women, without affecting vasoconstriction in arteries of men. Vasorelaxation caused by isoprenaline was greater (P<0.05) in mammary arteries of women compared to arteries of men. This sex-specific difference in vasorelaxation was abolished after removal of endothelium. Similar to human arteries there were sex-specific differences in vasoconstriction and relaxation in rat aorta, which was eliminated after ovariectomy in female rats. Compared to sham operated females, ovariectomy increased aortic vasoconstriction in response to norepinephrine more than 2-fold. Vasorelaxation by isoprenaline and β_3-agonist was significantly (P<0.01) reduced after ovariectomy. Compared to vehicle, estrogen substitution largely (P<0.05) restored sex-specific differences in vasoconstriction and vasorelaxation in ovariectomized rats. Differences in vasoconstriction and vasorelaxation between sexes were diminished in presence of selective β_1- and β_3-adrenoceptor antagonists and L-NMMA. Consistently, mRNA levels of β_1- and β_3-, but not β_2-adrenoreceptors were significantly (P<0.05) higher in aortas of sham operated females than in aortas of sham operated males. Ovariectomy abolished this difference by decreasing β_1- and β_3- adrenoreceptor expression in female rats. Consequently, estrogen substitution in ovariectomized females largely (P<0.05) restored β_1- and β_3-adrenoreceptor expression. Orchiectomy and testosterone treatment did not change aortic vasoconstriction and vasorelaxation nor ß-adrenoceptor expression in aortas of male rats.

Conclusion: We reveal that sex-specific differences in vasoconstriction and vasorelaxation in human mammary artery are endothelium-dependent. We also demonstrate that sex-differences in a rat model are estrogen, but not testosterone-dependent. Estrogen determines these differences via regulation of vascular endothelial β_1- and β_3-adrenoreceptor expression.

8.15

INHIBITION OF NEPRILYSIN ATTENUATES ANGII-INDUCED ABDOMINAL AORTIC ANEURYSMS (AAAS) AND ATHEROSCLEROSIS IN HYPERCHOLESTEROLEMIC MALE MICE

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Objective*:* Abdominal aortic aneurysm (AAA) is a symptomatic deadly vascular disease of elderly men. Plasma levels of brain natriuretic peptide (BNP), which is degraded by the metalloendpeptidase, neprilysin, have been suggested as biomarkers of incident AAA. However, it is unclear if neprilysin plays a role in AAA development. Entresto®, a neprilysin inhibitor in combination with an angiotensin receptor blocker, has demonstrated efficacy in human heart failure and is under investigation for treatment of other cardiovascular diseases. In this study, we examined the effect of the neprilysin inhibitor, sacubitril, on AngII-induced AAAs in male *LDLr*$^{-/-}$ mice.

Methods and Results*:* Male (8-12 weeks of age) *LDLr* $^{-/-}$ mice were fed a Western diet (Teklad TD88137) for the duration of the study. Vehicle or sacubitril (S, 1, 6 or 20 mg/kg/day) were administered by osmotic minipump for one week, and then minipumps containing vehicle or S (at respective doses) in combination with AngII (1,000 ng/kg/min) were implanted for 28 day delivery. Body weights were similar in all groups. Sacubitril decreased systolic blood pressure (measured by tail cuff during week 3 of AngII infusions) in a dose-dependent manner, with maximal effects S6 mg/kg/day (vehicle, 150 ± 5; S1 mg/kg/day, 142 ± 7; S6 mg/kg/day, 118 ±5; S20 mg/kg/day, 122 ± 5 mmHg). Sacubitril dose-dependently reduced suprarenal aortic lumen diameters (day

28: vehicle, 1.8 ± 0.02; S1, 1.9 ± 0.2; S6, 1.6 ± 0.2; S20, 1.2 ± 0.1 mm; P<0.05) and maximal AAA diameters at study endpoint (vehicle, 2.3 ± 0.2; S20, 1.0 ± 0.1 mm; P<0.05). AAA incidence (89% in vehicle-infused mice) was significantly reduced by S20 mg/kg/day (20%). Similarly, sacubitril reduced atherosclerosis in a dose-dependent manner (vehicle, 8 ± 1.3; S1, 9.2 ±1.1; S6, 5.6 ±1.4; S20, 2.7 ± 0.4 % lesion surface area; P<0.05). Interestingly, AngII-induced reductions in plasma renin concentrations were reversed by sacubitril (vehicle, 3.5 ±0.1; S20, 22.3 ± 3.2 ng/ml; P<0.05).

Conclusions: These results demonstrate that inhibition of neprilysin protects against AngII-induced atherosclerosis and AAAs in male *LDLr*$^{-/-}$ mice. Future studies will determine mechanism of action in neprilysin inhibition, and whether combination with an angiotensin receptor blocker is an effective therapeutic in the prevention and treatment of atherosclerosis and AAA progression.

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8.16

WITHDRAWN

8.17

WITHDRAWN

8.18

SEX DIFFERENCES IN VASCULAR REACTIVITY AND BIOMARKERS OF INFLAMMATION IN OFFSPRING OF DAMS EXPOSED TO PERINATAL HIGH SALT DIET.

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Hypertension is an important risk factor for cardiovascular diseases and excess of dietary salt is the most common environmental factor that contributes to the development of hypertension (Meneton *et al.,* 2005). However, *in utero* factors have been implicated in the pathogenesis of hypertension (Alexander, 2006). To assess the effect of perinatal high salt diet (HSD) on the offspring, pregnant Sprague-Dawley rats were exposed to perinatal normal (0.3%) or high (8%) salt diet from day 1 of pregnancy till term. The following experiments were conducted in the male and female offspring at 12 weeks of age: Blood pressure (BP) was measured via arterial cannulation under urethane and α-chloralose anesthesia (5ml/kg body weight i.p) using ADinstrument powerlab. Isolated abdominal aorta reactivity to noradrenaline (NA) and acetylcholine (ACh) in the presence or absence of endothelial nitric oxide synthase (eNOS) inhibitor ; L-nitro arginine (L-NA) was determined. Serum concentrations of C-reactive proteins (CRP), TNF-α and IL-6 was also measured using commercially available kits. Perinatal HSD elevated BP ($p< 0.05$) in both sexes but to a greater magnitude in male when compared with female offspring. Basal vascular tone was higher in the male offspring from dams fed a perinatal HSD when compared with male from dams fed a normal salt diet and female from dams fed a HSD. Female offspring from dams fed a HSD has a higher contractile response to NA before and after eNOS inhibition by L-NA when compared to male. However, male offspring from dams fed a perinatal HSD exhibit impaired vasorelaxation response to ACh both in the presence or absence of L-NA when compared with females. High salt diet increased CRP, TNF-α and IL-6 in both the male and female offspring, but the magnitude is higher in males when compared with females. Findings from this study suggest that perinatal exposure of dams to a high salt diet causes hypertension via mechanisms involving vascular function impairment as well as systemic and vascular inflammation. However, this effect of perinatal HSD is more pronounced in male offspring suggesting sexual disparity in the effect on offspring, of perinatal exposure of dams to HSD.

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8.19

NITRIC OXIDE HELPS MAINTAIN THE BUFFERING CAPACITY OF PERIVASCULAR ADIPOSE TISSUE IN FEMALE DAHL SS IN RESPONSE TO A HIGH FAT DIET DESPITE INCREASES IN BLOOD PRESSURE AND VASCULAR INFLAMMATION

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Local inflammation in perivascular adipose tissue (PVAT) is linked to high fat diet (HFD)-induced increases in BP and vascular dysfunction in males. There is limited data on the impact of HFD on BP or vascular function in females.Thus the goal of this study was to test the hypotheses that HFD will 1) increase BP and vascular inflammation and 2) PVAT will exacerbate HF diet-induced vascular dysfunction in female DSS. 6-wko female DSS were fed a normal-fat diet (NFD; 7.2% fat) or HFD (35% fat) diet for 10 weeks, and BP was measured by telemetry. At 16 wko, aortic rings (+/- PVAT) were mounted for isometric myography and cumulative concentration response curves to phenylephrine (PE) or acetylcholine (Ach) were generated in the absence or presence of the nonselective nitric oxide synthase (NOS)

inhibitor L-N^G-Nitroarginine methyl ester (LNAME) or polyethylene glycol (PEG)-catalase. In separate rats, aortic T cells were measured by flow cytometry. HFD increased BP (mmHg: 176 ± 8 HFD vs 130 ± 4 NFD, P<0.001) and led to greater numbers of total aortic T cells (P=0.05), T cell activation (P=0.002), and pro-inflammatory Th17 cells (P=0.002) compared to NFD. There was no change in anti-inflammatory T regulatory cells (P=0.67). HFD alone had no effect on vascular function. Although the presence of PVAT did not increase relaxation to Ach, it did attenuate PE-induced constriction [Area Under Curve (AUC): effect of PVAT P<0.01] regardless of diet. HFD is known to promote oxidative stress via increased production of reactive oxygen species, including H_2O_2. Interestingly, Peg-catalase uncovered a PVAT-mediated vasoconstrictor with HFD (AUC: effect of diet: P=0.015; effect of PegCat: P=0.17; interaction: P=0.0091) while LNAME increased force generation to PE in the presence of PVAT regardless of diet (effect of LNAME: P=0.047; effect of diet: P-0.17). Thus, in contrast to what has been shown in male DSS following a HFD in other studies,vascular function is maintained in female DSS; and PVAT enhanced the vasodilatory capacity of the aorta regardless of diet. Further, our data suggests that overproduction of NO rather than H_2O_2 plays a role in maintaining the anti-contractile effect of PVAT in response to a HFD. Future studies will determine 1) which NOS isoform contributes to the enhanced buffering capacity of PVAT in response to a HFD and 2) whether or not this capacity is mediated by the endothelium using endothelium intact and denuded vessels.

8.20

AFFERENT ARTERIOLAR RESPONSIVENESS TO ENDOTHELIN RECEPTOR ACTIVATION: DOES SEX MATTER?

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The pathogenesis of hypertension is distinct between men and women. Endothelin-1 (ET-1) is a potential contributor to sex-differences in the pathophysiology of hypertension. ET-1 is known to participate in blood pressure regulation through activation of endothelin A (ET_A) and endothelin B (ET_B) receptors in both the renal tubule and vasculature. However, little is known about sex-differences in ET-1 dependent renal microvascular reactivity. Our lab previously reported that renal medullary ET-1 reduces medullary blood flow in male, but not female rats. Orchiectomy (ORX) eliminated ET-1 dependent decreases in medullary blood flow, but ovariectomy (OVX) had no apparent effect on this sex-difference. Thus, we hypothesized that sex and sex steroids regulate the afferent arteriolar responses to ET receptor activation. To test that, we used 15-17 week old male and female Sprague Dawley rats subjected to gonadectomy or sham surgery. Three weeks later, kidneys from those rats were prepared for assessment of renal microvascular responses to ET-1 (ET_A and ET_B agonist, $10^{-12}$to 10^{-8}M) and sarafotoxin 6c (S6c, ET_B agonist, $10^{-12}$to 10^{-8}M) using the blood-perfused juxtamedullary nephron preparation. Baseline afferent arteriolar diameter at 100 mmHg averaged 15.3±0.3 and 14.6±0.3 µm for sham male and female rats, respectively (n=12, each). Gonadectomy had no significant effect on baseline arteriolar diameter. In sham males, ET-1 produced significant concentration-dependent decreases in afferent arteriolar diameter, with 10^{-8}M ET-1 decreasing diameter by 84±1 % (n=6). Similarly, ET-1 induced concentration-dependent vasoconstrictor responses in sham female rats, with 10^{-8}M ET-1 decreasing the diameter by 82±1 % (n=6). The vasoconstrictor responses to ET-1 within the afferent arteriole were unchanged by ORX or OVX. In addition, ET_B receptor activation by S6c induced a concentration dependent decline in the afferent arteriolar diameter, with 10^{-8}M S6c decreasing diameter by 77±3 and 76±3 % in sham male and female rats, respectively (n=6, each). These data do not support our original hypothesis and suggest that sex or sex hormones do not significantly influence afferent arteriolar reactivity to ET receptor activation. They further suggest that reported sex differences of the renal ET-1 system on blood pressure are most likely mediated through renal tubular activity of the ET_A and ET_B receptors as we have previously reported.

8.21

SEX-SPECIFIC DIFFERENCES IN PRIMARY NEONATAL LUNG FIBROBLASTS AND MICROVASCULAR ENDOTHELIAL CELLS EXPOSED TO HYPEROXIA *IN VITRO*: IMPLICATIONS FOR BRONCHOPULMONARY DYSPLASIA (BPD)

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Background: Despite the well-established sex-specific differences in the incidence of bronchopulmonary dysplasia (BPD), the molecular mechanism(s) behind these are not completely understood. Human pulmonary microvascular endothelial cells (HPMECs) provide a robust *in vitro* model for the study of endothelial cell physiology and function. Alterations in fibroblast phenotype may underlie some of the changes observed in babies with BPD, which is characterized by impaired

alveolarization and vascular growth. Notch pathway activation leads to fibroblast activation and proliferation in response to changes in oxygen and is also to modulate angiogenesis.

Objective: To elucidate the sex-specific differences in male and female human neonatal pulmonary microvascular endothelial cells at normoxic and hyperoxic conditions and delineate the differences in Notch pathway activation and test the hypothesis that male fibroblasts would display a greater pro-fibrotic phenotype and greater Notch activation upon exposure to hyperoxia.

Methods: The HPMEC cells (18-22 weeks gestation; 3 male and 3 female) were obtained from ScienCell (Carlsbad, California). They were cultured in endothelial cell media per protocol. Cells from passage from 3 to 6 were used for experiments. Murine lung fibroblasts were isolated from 7-day old male and female mice. Cells (passage 2 and 3) from three different isolations were used. For hyperoxic exposure, confluent murine fibroblasts were incubated in 95% O_2 and 5% CO_2 at 37 °C for upto 72 hours. Normoxia was air and 5% CO_2. Cell proliferation (thymidine incorporation), viability (trypan blue exclusion), cell migration, angiogenesis and expression of fibrosis related genes (PCR array) and Notch pathway mediators (RT-PCR and immunocytochemistry and western blot) were analyzed. Data were analyzed using 2-way ANOVA.

Results: Hyperoxia exposure decreased cell viability and proliferation markedly in male HPMECs at 48 and 72 h ($P<0.01$). HPMECs had significantly higher cell migration when assessed by the scratch assay. Even at baseline normoxic conditions, female HPMECs formed detailed 3D plexus structures and showed greater sprouting compared to similarly maintained male endothelial cells. Upon exposure to hyperoxia, there was decreased expression of Dll4 (delta like ligand 4) in female endothelial cells both at the mRNA and protein level. In normoxic conditions, proliferation was significantly higher in female lung fibroblasts ($P<0.01$). Upon exposure to hyperoxia, cell proliferation was arrested both in male and female fibroblasts ($P<0.01$), but cell viability was preserved.PCA analysis demonstrates that male and female are very different in their fibrosis phenotype at baseline in room air. In response to hyperoxia, male and female fibroblasts demonstrate distinct changes in fibrosis related genes. Female fibroblasts from normoxia and hyperoxia are clustered closer in the PCA analysis as compared to the males. Many pro-fibrotic genes related to the TGF-beta pathway were downregulated in females, while pro-fibrotic genes such as IL-13 and EGF were exclusively upregulated in males. Notch pathway activation was noted in male fibroblasts with greater expression and nuclear localization of NICD and increased expression of activated Notch in lung fibroblasts by western blot.

Conclusion: The results indicate that sex differences exist between male and female neonatal pulmonary fibroblasts and endothelial cells *in vitro* at baseline and after hyperoxia exposure. Differential Notch pathway activation upon hyperoxia exposure may modulate these sex-specific diferences. These differences could explain in part the mechanisms behind sex-specific differences in BPD.

8.22

SERELAXIN INFUSION DOES NOT ATTENUATE THE DEVELOPMENT OF HYPERTENSION IN A MOUSE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic lupus erythematosus (SLE) is an autoimmune disease that most commonly affects women of reproductive age. Sex steroid hormones have long been thought to contribute to disease susceptibility; however, little is known about other sex-specific factors, including the peptide hormone relaxin, in the progression of SLE. While the role of relaxin in SLE is unclear, there is indirect evidence suggesting that reduced relaxin levels may contribute to some complications of the disease. Because relaxin has known vasodilatory and cardiovascular protective effects, we tested the hypothesis that administration of relaxin would attenuate the development of hypertension and renal injury in a murine model of SLE. Serelaxin (human recombinant relaxin-2, 0.5 mg/kg/day, Novartis) or vehicle (citrate buffer) was administered via osmotic mini-pumps (Alzet 1002) in female NZBWF1 (SLE) mice (aged 30 weeks) and NZW (control) mice (aged 31 weeks) for a total of 24 days (pumps were replaced after 14 days). NZBWF1 mice are an established mouse model of SLE that shares many of the characteristics of SLE as seen in humans, including a female sex bias, increased circulating autoantibodies, hypertension and renal injury. Uterine weights were collected at the termination of the study as *in vivo* confirmation of bioactivity of the serelaxin in NZBWF1 and NZW mice. Serelaxin-treated NZW mice had significantly increased uterine weights ($p < 0.05$) compared to vehicle-treated NZW mice, but there was no difference in uterine weights between vehicle- and serelaxin-treated NZBWF1 mice, suggesting the control NZW mice were more responsive to serelaxin than the SLE mice. Mean arterial pressure was measured over two consecutive days at the conclusion of the study in conscious mice via carotid artery catheters. Preliminary data suggest that a four-week administration period of serelaxin was not sufficient to attenuate the development of hypertension in this mouse model of SLE. Mean arterial pressure was

significantly increased ($p < 0.05$) in both vehicle- (135 ± 6 mmHg, n=7) and serelaxin-treated NZBWF1 mice (138 ± 5 mmHg, n=5) compared to vehicle or serelaxin treated NZW mice (109 ± 3, n=7, and 113 ± 4 mmHg, n=7). Urinary albumin, a marker of renal injury, was determined by dipstick assay at the termination of the study, and the prevalence of albuminuria was similar between vehicle- and serelaxin- treated NZBWF1 mice with 55.5% (n=9) of serelaxin-treated NZBWF1 mice and 45.5% (n=11) of vehicle-treated NZBWF1 mice with albumin > 100 mg/dL. Although serelaxin infusion does not attenuate the development of hypertension or urinary albumin excretion under these experimental conditions, the effects of serelaxin on other cardiovascular risk factors including endothelial dysfunction during SLE have not been tested. Therefore, future experiments will determine if serelaxin will improve the endothelial dysfunction observed in NZBWF1 mice and will ultimately provide further insight into potential cardiovascular protective effects of serelaxin.

8.23

PURINOCEPTOR-DEPENDENT REGULATION OF SODIUM EXCRETION IS SEXUALLY DIMORPHIC

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Premenopausal women have a lower risk of hypertension and renal disease compared to age-matched men. Recently, prominent roles have been assigned to $P2Y_2$ and $P2Y_4$ purinoceptor subtypes in promoting sodium excretion, implicating dysfunction of these receptors as potential contributors to hypertension. We recently reported that activation of $P2Y_2$ and $P2Y_4$ receptors in the renal medulla by UTP promotes sodium excretion in male rats. In intact females, UTP did not stimulate sodium excretion while ovariectomy unmasked UTP-induced natriuresis. These observations led us to hypothesize that intact females have higher basal renal medullary activity of $P2Y_2$ and $P2Y_4$ receptors in regulating sodium excretion compared to male and ovariectomized (OVX) rats. To test that, we determined (i) $P2Y_2$ and $P2Y_4$ mRNA and protein expression in the inner medulla from male, intact female and OVX Sprague Dawley rats and (ii) the effect of inhibiting medullary purinoceptors (P2 receptors) on sodium excretion in those rats. We found that $P2Y_2$ and $P2Y_4$ mRNA expression was higher in the inner medulla from females compared to males (1.00±0.09 vs. 0.70±0.05 and 1.00±0.22 vs. 0.29±0.05, respectively, $P<0.5$, n=5-10). These sex differences in $P2Y_2$ and $P2Y_4$ mRNA expression were eliminated by ovariectomy (0.60±0.06 and 0.29±0.04, respectively, $p<0.5$,n=5,8). Consistently, Western blots on inner medullary lysates showed that intact females have higher expression of $P2Y_2$ receptor, compared to males. In anesthetized rats, medullary P2 receptor inhibition by suramin (P2 receptor antagonist, 750 µg/kg/min) significantly attenuated sodium excretion in intact females (0.4±0.1 vs. 0.9±0.2 µmol/min, $P<0.5$, n=7), but not in male or OVX rats. To test whether estradiol (E_2) increases the expression of $P2Y_2$ and $P2Y_4$ receptors, we subjected cultured mouse inner medullary collecting duct cells (mIMCD3) to different concentrations of E_2 (0, 10, 100 and 1000 nM). We found that E_2 dose-dependently increased the expression of $P2Y_2$ and $P2Y_4$ mRNA in mIMCD3. These data suggest that females have enhanced $P2Y_2$ and $P2Y_4$-dependent regulation of sodium excretion in the renal medulla, compared to male and OVX rats, at least partially via an E_2-dependent mechanism. This pathway may contribute to facilitated renal sodium handling in premenopausal females.

8.24

DIFFERENTIAL PROTEIN EXPRESSION OF RENAL DOPAMINE RECEPTORS BUT SIMILAR AT_1R ACTIVITY IN SALT-SENSITIVE MALE AND FEMALE C57BL/6J MICE

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Male C57Bl/6J mice are salt-sensitive that is related, in part, to the functional state of the renal dopamine D_1 receptor (D_1R). However, sex-related difference on salt sensitivity in mice is not well documented. Therefore, we studied the effect of sodium intake on blood pressure (BP), renal dopamine receptors, angiotensin type 1 receptor (AT_1R), and sodium transporters in male and female C57Bl/6J mice. Similar to previous reports in male mice, the BP (telemetry) of female mice (n=4) was also increased by 4% NaCl diet (1 wk) relative to 0.4% NaCl diet which was associated with a decrease in serum renin but an increase in renal AT_1R protein. The salt-induced increase in BP was ameliorated by 18-20 mm Hg with the AT_1R antagonist, candesartan (1 mg/kg/day, subcutaneously administered with osmotic mini-pumps, 1 wk), in both sexes on high salt intake (6% NaCl) (4 groups, n=5/group). Except for the lower body weight of female mice, relative to male mice, there were no sex differences in food/water intake, urinary excretions of water, Na^+, K^+, and Cl^-, and serum concentrations of creatinine, Na^+, K^+, and Cl^-. Relative to vehicle, candesartan caused a similar decrease in renal protein expressions of sodium-hydrogen exchanger isoform 3, sodium-potassium-2 chloride cotransporter, sodium-chloride cotransporter, and a, b and g epithelial sodium channel but not type 2 sodium phosphate cotransporter and a1Na^+K^+ATPase in male and female mice on high salt

diet. However, candesartan increased renal D_1R (144±9, % of vehicle group) and D_5R (151±6%), but not D_2R, D_3R, or D_4R, protein expression in female mice but increased renal D_5R protein expression (240±44%) only in male mice. We conclude that the salt sensitivity of BP and AT_1R activity in C57BL6/J mice is not sex-related. However, the amelioration of high salt induced-hypertension by AT_1R blockade may be mediated by a differential increase in renal dopamine receptor subtype expression but a similar reduction in renal apical sodium transporters in male and female C57Bl/6 mice.

8.25

RENAL ISCHEMIA REPERFUSION INJURY IN A PIG MODEL REVEALS GENDER SPECIFIC EXPRESSED GENES AS POTENTIAL NEW BIOMARKERS OF RENAL INJURY/REGENERATION PROCESSES DRIVING TO CHRONIC KIDNEY DISEASE.

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Kidney diseases are a global public health problem, that is reaching epidemic proportions. Renal ischemia/reperfusion injury (IRI) is a major cause of acute kidney injury (AKI) leading to injury of proximal tubule epithelial cells (PTEC). After injury, the kidney can either regenerate or be engaged in remodeling processes driving to fibrosis and chronic kidney disease (CKD). Men are more prone to AKI and CKD than women and it is accepted that androgens participate on that. The molecular mechanisms involved in regeneration as well as in gender related outcomes upon injury remain to be elucidated. We postulate that the identification of differentially expressed genes in male and female kidney pigs, both in basal and in IRI conditions might unravels genes and pathways useful to understand the different outcomes observed in men and women. We aim to provide new candidate repair regulators able to promote restoration of kidney function. Renal IRI was performed in female and male pigs and mice to identify genes of translational relevance for humans that could be also studied in mouse models. Pre-ischemic, ischemic and post-ischemic kidney tissues from male and female pigs were collected for microarray assays. Moreover, systems biology-based mathematical models for the analysis of microarray data were conducted. The most promising targets that exhibit sexual dimorphism along the injury/regeneration process have been selected and characterized. The mRNA levels, the protein expression and localization in the kidney have been assessed. The early results strongly suggest that the selected targets are potentially androgen regulated. In order to further study the molecular mechanisms of these targets, *in vitro* IRI models of pig and human PTEC cultured cells are currently under development.

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8.26

FEMALE RATS WITH PREEXISTING CKD EXHIBIT IMPAIRED RECOVERY FROM AKI AND THE SUBSEQUENT DEVELOPMENT OF PROTEINURIA

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Interactions between acute kidney injury (AKI) and chronic kidney disease (CKD) predispose to the development of end-stage renal disease (ESRD). A previous study from our group showed that such deleterious AKI-CKD interactions include impaired recovery from AKI and the development of *de novo* mechanisms of CKD progression in male rats with preexisting CKD. The goal of this study was to determine if impaired recovery from AKI is also observed in female rats with preexisting CKD. We induced two levels of CKD in 10-12 week old male and female Sprague-Dawley rats by performing either 50% renal mass reduction via a right uninephrectomy (UNX, n=14, 6 females) or 75% renal mass reduction via a right UNX + surgical excision of 1/2 of the left kidney (3/4 NX, n=12, 6 females). Rats recovered for two weeks to allow for completion of compensatory adaptations in renal size and function. Rats were then subjected to 35 minute ischemia-reperfusion (IR)-induced AKI under isoflurane anesthesia with core body temperature maintained at 37° C. Blood samples were obtained prior to IR and at 48 hours, 7 days, 14 days and 28 days post IR to assess plasma creatinine (P_{Cr}). A 24-hour urine collection was performed in a subset of rats prior to IR and at 28 days post IR to assess proteinuria. At the end of the study, kidneys were fixed in paraformaldehyde and paraffin embedded sections were stained with H&E to assess renal pathology and tubular vimentin expression was assessed using immunohistochemistry. Tubular vimentin expression 28 days post IR identifies sublethally injured tubules that have failed to redifferentiate, which is a robust index of impaired recovery. Vimentin expression was semiquantitated on a scale from 0-4 with 0 representing no tubular vimentin staining and 4 representing tubular vimentin staining in >75% of

tubules. Vimentin scoring was conducted in a blinded fashion. The severity of AKI, based on P_{Cr} levels 48 hours post AKI, was similar between UNX vs. 3/4 NX groups within both males and females. The severity of AKI was lower (P<0.05) in females vs. males with 3/4 NX (1.9±0.3 vs. 3.3±0.3 mg/dl) but not significantly different between females vs. males with UNX (1.7±0.6 vs. 2.7±0.4 mg/dl). While minimal injury and vimentin expression (0.7±0.2) was observed in female rats with UNX, females with 3/4 NX exhibited greater (P<0.05) tubular vimentin staining (1.3±0.3), tubular injury and fibrosis 28 days post IR. Moreover, recovery of P_{Cr} over 28 days post IR was delayed in females with 3/4 NX vs. UNX, which is also indicative of impaired recovery from AKI. Finally, female rats with 3/4 NX developed substantial (P<0.05) increases in proteinuria 28 days post IR as compared to pre-IR levels (195±57 vs. 62±20 mg/day) while proteinuria was similar at 28 days post IR vs. pre-IR in female rats with UNX (21±4 vs. 23±5 mg/day). Similar to our previous study, males with 3/4 NX exhibited impaired recovery from AKI and the development of substantial proteinuria 28 days post AKI as compared to males with UNX. In conclusion, these data support previous studies documenting resistance to IR-induced AKI in female vs. male rats. However, our data indicate that preexisting CKD of greater than 50% renal mass reduction predisposes female rats to impaired recovery from AKI and the subsequent development of mechanisms of CKD progression, similar to male rats.

8.27

ORAL L-ARGININE TREATMENT SIGNIFICANTLY INCREASES RENAL TREGS IN FEMALE DOCA SALT HYPERTENSIVE RATS

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The immune system plays a critical role in the development of hypertension. Our lab has previously reported that there is a sex difference in the renal T cell profile in DOCA salt hypertension, with males having more pro-inflammatory Th17 cells, whereas females have more anti-inflammatory regulatory T cells (Tregs). DOCA treated males also have a greater blood pressure (BP) response to DOCA than females, and we propose this is due to the relative abundance of Tregs in the female. We have also previously reported that an intact nitric oxide (NO) system is required for Treg maintenance in hypertension, and the DOCA model has been characterized by impaired NOS. In the current study, we hypothesized that treatment with L-arginine, a substrate for NO production, would increase renal Tregs and attenuate the sex difference in hypertension in male and female DOCA treated rats. Briefly, male and female Sprague Dawley rats (n=5-15, 10 wks of age) were uni-nephrectomized and subcutaneously implanted with a DOCA pellet (200 mg/rat, 60-d time release) and given 0.9% NaCl to drink ad libitum. A subset of rats were treated with L-arginine (L-arg, 350 mg/kg/d, via drinking water). BP was measured by tail cuff plethysmography at baseline and after 3 wks of DOCA treatment. After 3 wks of treatment, kidneys were processed for flow cytometric analysis of T cells ($CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD4^+FOXp3^+$ Tregs, and IL-10^+CD3^+ T cells). BP was comparable between treatment groups within each sex at baseline, although males had a higher BP than females (Table, effect of sex, p<0.001, 2-Way ANOVA). DOCA treatment increased BP in all groups compared to baseline values (Table, effect of DOCA, p=0.002). L-arginine treatment attenuated DOCA-induced increases in BP in both sexes, although this effect was greater in females (Table, effect of L-arginine, p=0.02, interaction of sex and L-arginine, p=0.03). Females had a more Tregs than males, and L-arginine treatment increased Tregs in females alone (Table, effect of sex, p<0.01, effect of L-arg, p<0.01, interaction, p<0.01, 2-Way ANOVA). Interestingly, despite the Treg numbers remaining unchanged with L-arg, IL-10 increased in both sexes to a similar extent (Table, effect of sex, p=0.29, effect of L-arg, p=0.03, interaction, p=0.93, 2-Way ANOVA). Future studies will examine additional mechanisms by which L-arginine supplementation increases Tregs in females.

	BP Baseline (mmHg)	BP Wk 3 (mmHg)	CD3	CD4	Tregs	IL-10
M DOCA	153±2	225±2	0.9±0.1	65±1.2	3.4±0.3	6.8±0.5
M DOCA + L-Arg	153±1	208±1	1.2±0.2	72±2.0	3.7±0.4	8.5±0.6
F DOCA	142±2	216±2	0.8±0.1	61±1.2	3.9±0.2	7.7±0.5
F DOCA + L-Arg	140±3	180±3	0.6±0.1	64±1.7	6.6±0.8	9.2±1.1

8.28

NECROX-5 ABOLISHED MATURATION INDUCED SEX DIFFERENCES IN BLOOD PRESSURE (BP) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

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It is well established that hypertension is accompanied by cell death, however, less is known regarding the role of cell death in blood pressure (BP) control. Necrosis is an uncontrolled pathologic form of cell death that is associated with inflammation. Therefore, the goal of the current study was to test the hypothesis that necrosis contributes to the development of hypertension in SHR. Initial studies measured renal necrosis in 13 wk old male and female SHR (n=9) using flow cytometry. Male SHR had greater renal necrosis compared to female SHR (3.7±0.4% vs. 0.4±0.2% of total renal cells, respectively; p<0.0001). Additional male and female SHR were then randomized to receive vehicle or Necrox-5 from 6-12 weeks of age (1 mg/kg via IP injection twice weekly; n=4); Necrox-5 is a cell permeable inhibitor of necrosis that blocks oxidative stress induced necrotic cell death. BP was measured weekly via tail-cuff and via telemetry from 10-13 wks of age. Following 6 wks of treatment, kidneys were isolated and necrosis was measured by flow cytometry. Treatment with Necrox-5 beginning at 6 wks of age attenuated maturation-induced increases in BP in male SHR (Necrox-5: 152±11 vs Control: 177±4 mmHg; p=0.07). BP in female SHR was not altered by chronic Necrox-5 treatment (Necrox-5: 152±14 vs. Control: 146±11 mmHg; p=0.72). In addition, the sex difference in BP apparent in control rats was abolished by Necrox-5 treatment. Consistent with these results, Necrox-5 treatment for 6 wks decreased renal necrosis (Necrox-5: 1.5±0.5% vs Control: 3±0% of total renal cells), only in males. Taken together, our data suggest that greater cell death in male SHR compared to female SHR contributes to sex differences in BP by exacerbating age-related increases in BP in males.

8.29

ANDROGEN INFLUENCE ON RENAL FIBROSIS ASSOCIATED WITH PYELONEPHRITIS

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Renal scarring after pyelonephritis is linked to long-term health risks for hypertension and chronic kidney disease. Testosterone signaling through the androgen receptor increases susceptibility to, and severity of, uropathogenic *Escherichia coli* (UPEC) pyelonephritis in both male and female mice (1), while anti-androgen therapy is protective against severe UTI (2). Mice with severe pyelonephritis develop renal fibrosis and scarring (3). This work elucidates the molecular mechanisms of renal fibrosis in androgenized female C3H/HeN and C57BL/6 mouse backgrounds, and determines how these pathways are altered by the presence of testosterone. C3H/HeN mice feature vesicoureteral reflux (VUR), which allows for severe pyelonephritis and widespread renal fibrosis. C57BL/6 mice do not have VUR, but still exhibit alterations in renal fibrosis markers and display scarring following upper-tract UTI. We demonstrate that renal fibrosis after pyelonephritis involves both the TGF-β/Activin A and Hedgehog pathways, with altered local expression of proteins in the Smad family, TGFβ1, Activin A, and Gli1. Elevated circulating testosterone levels drive Ly6C+ monocyte recruitment to the kidney in the uninfected state and upon urinary tract inoculation with UPEC. Our results are consistent with a model in which testosterone increases recruitment of Ly6C+ monocytes, and that these cells are activated in the presence of UPEC during renal infection, driving local expression of pro-inflammatory and pro-fibrotic markers and thereby promoting fibrosis and renal scar formation.

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8.30

COLLECTING DUCT NOS1, SPECIFICALLY NOS1β, IS CRITICAL FOR MAINTAINING FLUID-ELECTROLYTE BALANCE IN BOTH MALES AND FEMALES

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Over the past few years, we have reported that collecting duct (CD) nitric oxide synthase-1 (NOS1) is necessary for maintaining fluid-electrolyte balance during high sodium intake. Genetic deletion of NOS1 (specifically the splice variant NOS1β) in the CD, results in inappropriate sodium handling, and a salt-sensitive increase in blood pressure in male mice. The purpose of this study was to determine if CD NOS1β is also a critical mediator in maintaining fluid-electrolyte balance in female mice. Similar to male mice, NOS1β is specifically expressed in CD. Female CD NOS1 knockout (CDNOS1KO) mice were

randomly assigned to receive either low sodium (LS, <0.01%), normal sodium (NS, 0.16%), or high sodium (HS, 1.6%) diet for 1 week. Food and water intake was similar between littermate control and CDNOS1KO mice. When switched from a LS to HS diet, female CDNOS1KO mice produced significantly less urine (3.4 ± 0.4 vs 4.9 ± 0.1 ml/day, p = 0.04) and excreted less sodium on day 1 of HS compared to controls (1.7 ± 0.2 vs 2.5 ±0.1 mmol/day, p = 0.02). However, by day 2 of HS, urine production and sodium excretion were similar between the genotypes. In male mice, we found a similar sodium handling defect, but with a delay of 4 days to come into sodium balance after the HS challenge. Mean arterial blood pressure was also significantly higher in CDNOS1KO females than controls while on HS (113.5±1.3 vs 107.6±1.6 mmHg, p = 0.046), and very similar to what we observed in male CDNOS1KO. Glomerular filtration rate (GFR) was determined by FITC-sinistrin clearance in conscious mice. In male and female, CDNOS1KO and controls, GFR was similar on LS (male control 260.9 ± 9.8 vs CDNOS1KO 242.7 ± 6.3 µl/min; female control 238.9 ± 7.0 vs CDNOS1KO 280.7 ± 12.4 µl/min) and NS diets (male control 224.2 ± 6.4 vs CDNOS1KO 218.0 ± 10.4 µl/min; female control 258.0 ± 16.6 vs CDNOS1KO 325.1 ± 42.1 µl/min) . However, on day 1 of HS diet, GFR significantly increased in controls compared to CDNOS1KO mice (males: 329.8 ± 12.4 vs 255.1 ± 22.8 µl/min, p = 0.03; females: 296.7 ± 14.1 vs 244.0 ± 10.8 µl/min, p = 0.001). Male control and CDNOS1KO had similar GFR on day 7 of HS compared to GFR on LS and NS (251.3 ± 16.0 vs 290.6±26.7µl/min). However, female control and CDNOS1KO had significantly higher GFR (323.2 ± 29.7 and 358.0 ± 32.4 µl/min) compared to LS and NS. In conclusion, CD NOS1β is critical for maintaining fluid-electrolyte balance and blood pressure control during high salt feeding. However, female mice return to sodium balance much quicker than males, although blood pressure remains high. Interestingly in both sexes, acute (within 24 hr) changes in GFR are dependent on CD NOS1β activation, but chronic (7 day) changes in GFR are independent of CD NOS1β. Moreover, salt-dependent increases in GFR are maintained in female mice but not male mice. Future experiments will determine the mechanisms behind the sex and salt dependent changes in GFR. Funding K01DK105038 to KAH and HL136267, HL69999, AHA24450002 to JSP.

8.31

TISSUE-SPECIFIC ESTROGEN RECEPTOR PROFILING USING DROPLET DIGITAL PCR

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Estrogen signals via th ree identified receptors ERα, ERß, and the G Protein-Coupled Estrogen Receptor (GPER). The relative contribution of each receptor to estrogenic signaling may elucidate the disparate effects of this sex hormone across tissues. Moreover, a novel PCR technology termed droplet digital PCR (ddPCR) now allows direct comparison of multiple target sequences due to absolute transcript quantification. Therefore, we hypothesized that utilization of this new technology would reveal tissue- and sex-specific differences in mRNA for the three estrogen receptors and aromatase. The following tissues were collected from Sprague-Dawley rats (6 female, 6 male) at 13 weeks of age: reproductive (gonads, uterus, mammary gland), cardiovascular (heart, aorta, kidney, adrenal gland), and brain (somatosensory cortex, hippocampus, and prefrontal cortex). GPER expression was relatively stable across all tissues in both sexes, ranging from 14-113 copies/ng RNA, an approximate 8-fold difference. ERα and ERß were more variable although relatively stable within each organ system. ERα displayed a range of 4.5-614 copies/ng RNA, a fold change of ~136 while ERß ranged from 0.2-83 copies/ng RNA or 415-fold. Both ERß and aromatase were highly expressed only in the ovary, with slightly higher levels detected in the brain. Significant sex differences were broadly absent except for renal ERα (female 206 vs. male 614 copies/ng RNA, p<0.001), somatosensory ERα (8.5 vs. 4.5 copies/ng RNA, p<0.001), gonadal ERß (83 vs. 0.30 copies/ng RNA, p<0.01), and gonadal GPER (5.5 vs 48 copies/ng RNA, p<0.001). Cardiovascular tissues showed a predominance of ERα followed by GPER, while ERß was nearly undetectable. In contrast, GPER was the predominant transcript in the three selected brain regions, with similarly low levels of ERα and ERß. While the data revealed surprisingly few sex differences, significant differences were found in the range of receptor mRNA across tissues as well as the estrogen receptor profile between organ systems. These data provide an overview of estrogen receptor expression at the organ level, but future studies are needed on cell-specific receptor profiles. In conclusion, estrogen receptor profiling will enhance understanding on the mechanisms by which estrogen elicits tissue-specific effects. Funding: NIH HL133619 (SHL), COBRE P30GM103337.

8.32

A STUDY ON THE INCIDENCE AND TYPES OF TWINNING IN THE SOUTH INDIAN POPULATION DR.R.SHAKTHI KUMARAN, CRRI FOR ABSTRACT-BASED TRAINEE TRAVEL AWARDS

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The study was undertaken to know the true incidence and types of twinning or other multiple births in Vijayawada under the following protocol- incidence of twinning, multiple births, frequency of twinning and maternal aging with gravidity of mother, sex incidence of twin and the zygosity of twins. In the present study, there is 0.6% incidence of twinning

8.33

T CELL SPECIFIC KNOCKDOWN OF ESTROGEN RECEPTOR-A DOES NOT ELIMINATE PREMENOPAUSAL PROTECTION FROM ANGIOTENSIN II-INDUCED HYPERTENSION, BUT DOES IMPACT RENAL T CELL EXPRESSION OF CD28 AND CTLA-4

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There is extensive evidence that the immune system is required for the development of angiotensin II (Ang II) induced hypertension in males. In contrast, we have shown that premenopausal females are protected from T cell-mediated Ang II hypertension. However, following menopause, female protection from T cell-mediated Ang II hypertension is lost. Adoptive transfer of $CD3^+$ T cells into postmenopausal Rag-$1^{-/-}$mice significantly increased Ang II-induced SBP (SBP Δ28 ± 3mmHg; $p<0.05$), whereas in the absence of T cells, the Ang II-induced systolic blood pressure (SBP) response in postmenopausal Rag-$1^{-/-}$ mice was similar to premenopausal mice (SBP Δ12 ± 2mmHg). Thus, we hypothesize that the loss of estrogen in postmenopausal mice eliminates female protection against T cell-mediated hypertension. Further, we examined if T cell-specific estrogen receptor alpha (ERα) signaling is required for premenopausal protection from T cell-mediated hypertension. $CD3^+$ T cells were purified from ERα null mice ($CD3^+$ERαKO) or wild-type mice ($CD3^+$WT) and adoptively transferred into premenopausal female Rag-$1^{-/-}$ mice (no T/B cells). Ang II was infused via osmotic mini-pump for 14 days (490 ng/kg/min). Similar to our previous studies, premenopausal female Rag-$1^{-/-}$ mice were resistant to T cell-mediated Ang II hypertension following T cell transfer from wild type mice (SBP: $CD3^+$WT/Ang Δ6 ± 5 mmHg). Additionally, there was no significant difference in SBP when ERαKO T cells were adoptively transferred (SBP: $CD3^+$ERαKO/Ang Δ5 ± 4 mmHg). Flow cytometric analysis of renal $CD4^+$, $CD8^+$, and regulatory $Foxp3^+$ T cells identified that the absence of ERα increases expression of the costimulatory receptor CD28 in all three T cell subtypes ($CD3^+$ERαKO/Ang vs. $CD3^+$WT/Ang: $CD4^+$ 138%, $CD8^+$ 144%, $Foxp3^+$ 141%; $p<0.05$). Further, the absence of ERα significantly reduced Ang II-induced expression of the anti-inflammatory CTLA-4 receptor on renal regulatory $Foxp3^+$ T cells ($CD3^+$ERαKO/Ang vs. $CD3^+$WT/Ang: $Foxp3^+$ 67%; $p<0.05$). Our studies show that menopause (loss of estrogen signaling) increases female susceptibility to T cell-mediated hypertension. However, T cell-specific ERα signaling does not seem to play a key role in premenopausal protection from T cell-mediated Ang II hypertension, but may impact anti-inflammatory pathways that regulate T cells.

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8.34

SEX SHAPES CANCER CACHEXIA AND THE RESPONSE TO THERAPEUTIC BLOCKING OF ACVR2B LIGANDS IN THE GENETICALLY ENGINEERED KPC MOUSE MODEL OF PANCREATIC DUCTAL ADENOCARCINOMA

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Background: Women and men are genetically different and this difference influences other levels of biological organization (cell, organ, organ system and organism). Sex has been recognized to impact clinical manifestation and therapeutic effects in diseases including heartdisease; yet little information is available for sex differences in cachexia, a metabolic syndrome characterized by progressive fat/muscle loss and associated fatigue and dysmobility. PDAC is among the most lethal of malignancies in part because patients with PDAC have the highest rates and greatest severity of cachexia. Pre-clinical studies indicate that blocking cachexia improves quality of life and survival in PDAC. Activin is elevated in the circulation of patients with PDAC-associated cachexia; thus inhibition of Activin receptor signaling has been trialed for cachexia in patients with pancreatic cancer, with unclear benefit.

Hypothesis: Biological differences between males and females affects the manifestation of PDAC cachexia and the response to therapies.

Objective: Characterize sex-specific phenotypes in PDAC cachexia and in response to anti-cachexia therapy.

Strategy: We characterized cachexia in the genetically engineered mouse model of PDAC, KPC (LSL-**K**rasG12D;LSL-Tr**p**53R172H;Pdx1-**C**re). We monitored spontaneous tumor initiation and changes in body weight and body composition over the course of tumor

growth up to 33 weeks of age. We used the ACVR2B/Fc, a soluble receptor-chimera trap for Activins and related proteins, to block the ACVR2B-mediated signaling and assessed the response of male vs. female mice to the soluble receptor. Collected tumors and organs were analyzed for sex differences in RNA and protein expression.

Results: Male and female KPC mice had a same median PDAC tumor latency of 17 weeks of age, similar tumor growth dynamics, and same median survival of 25 weeks. ACVR2B/Fc did not significantly inhibit tumor growth in either sex. However, sex-specific differences in body weight loss, organ wasting, and response to ACVR2B/Fc were observed. Male KPC ceased gaining weight and fat mass by 17 weeks of age. Organs, including skeletal and cardiac muscle, fat, and kidney started to waste from early stages of tumor growth and wasting became more severe at late stage. Organ wasting was mostly prevented by ACVR2B/Fc treatment. In contrast, female KPC ceased fat mass gain by 20 weeks but did not lose overall body weight despite identical tumor growth/latency. Organ wasting was less severe in females at early stages, but progressed to severe cachexia quickly at late stage, similar to males. However, prominent differences in the heart and kidney wasting persisted, indicating that some factors in females may specifically protect these two organs from wasting throughout tumor progression. Unlike males, female KPC mice essentially did not respond to ACVR2B/Fc, and organ mass was not preserved. Consistent with these differences at the level of organ mass, differential activation of known catabolic signals and their downstream markers was observed in male vs. female. As well, RNA-sequencing of the skeletal muscle from male and female KPC euthanized at early or late cachexia, with or without ACVR1B/Fc-treatment, revealed many differentially activated pathways. Further analysis is underway.

Conclusions*:* We demonstrate that sex impacts the manifestations of PDAC-associated cachexia, including onset and severity, and the response to anti-cachexia therapy. The KPC model recapitulates many of the phenotypic and genotypic features of human cancer cachexia and could be useful for testing sex differences in cachexia therapies.

8.35

EFFECT OF OLEANOLIC ACID ON LIPID METABOLISM IN NEONATAL RATS WITH METABOLIC SYNDROME

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For the past two decades, emergence of the metabolic syndrome, which is a modern epidemic with frightful consequences to the health of humans worldwide [1]. Diet is an important integral part of human health and livelihood. For instance, fructose intake has increased at an alarming rate and its intake is linked to the epidemic of obesity and diabetes [2, 3]. Lipid synthesis and oxidation are two processes major processes of metabolism. [4]. Lipid accumulation may add to the development of metabolic disorders such as obesity, type 2 diabetes, insulin resistance and cardiovascular related diseases [5]. The use of medicinal plants to curb human ailments has been in use for some time in African and Asian countries. Oleanolic acid is pentacyclic triterpenoid complex which possesses many promising pharmacological activities, such as hepatoprotective, anti-inflammatory, antioxidant, and anticancer activities. However, there is limited information about direct influence of oleanolic acid on mechanism of antidiabetic activity of oleanolic acid, hence it the reason to undertake this research to determine the effect of oleanolic acid on lipid metabolism in fructose induced Sprague dawley rats.

Materials and Methods: This study was conducted using 40 male pups of Sprague Dawley rats. Rats were randomly divided into five groups namely the Control (CON), Oleanolic acid (OA), High fructose (HF), Oleanolic acid and High fructose (OA+HFD) and Metformin and High fructose (MET+HFD). The experiment was conducted in accordance with protocols approved by the Animal Ethics Screening Committee (AESC) of the University of Witwatersrand, Johannesburg, South Africa (AESC approval number 2014/47/D).

Gas Chromatography Mass Spectrometry (GS-MS) was used to determine expression of polyunsaturated fatty acid carried out according to modified method of Association of Official Analytical Chemists AOAC (2005). Fatty acids are identified by comparing their retention times to the retention times of the suitable standard using Aligent 7890A Gas Chromatograph. Real Time Polymerase Chain Reaction (qRT-PCR) was performed using the PowerUp SYBR Green master mix (Applied Biosystems, Life Technologies) according to the manufacturer's protocol.

Results: These results indicate that expression of Oleanolic acid was supressed in HFD group however this was reversed when HFD was accompanied by oleanolic acid administration, indicating promising influence of OA in ameliorating metabolic syndrome. It was interesting to see EPA increasing, the increases observed suggests, activation of the desaturation/elongation pathway. In high fructose diet increased levels of arachidonic acid higher than in treated samples, metformin showed a threefold decrease compared to HFD group.

Conclusions: This study demonstrates that dietary modification may delay or prevent the transition from

developing metabolic syndrome or its related complications. The use of oleanolic acid has showed great significant increase in upregulating the expression of eicosapentaenoic acid (EPA) while being downregulated in arachidonic acid. Oleanolic acid has increased the expression lipid oxidation genes (CPT-1) and decreased the expression of lipid synthesis (FAS). Therefore, oleanolic acid can be used to ameliorate development of metabolic disorders and its related complications.

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8.36

EFFECTS OF TENDER COCONUT WATER ON THE CORONARY ARTERY OF MALE DIABETIC WISTAR RATS

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Diabetes is a major risk factor for cardiovascular disease (CVD). Tender coconut water (TCW) is the liquid endosperm of coconut fruit and it is rich in minerals (Potassium, calcium and magnesium), Vitamins (B and C) which are known to reduce the risk of developing Coronary Heart Disease (Anurag and Rajamohan 2003). It is also a major source of the amino acid L-arginine (Boges and Bode-Boger, 2001). TCW have been reported to have antioxidant and cardio-protective effect (Nnodim *et. al.,* 2013). However, there are very few studies on its cardio-protective effect in diabetes. Therefore the aim of this study was to investigate the effects of Tender coconut water on the coronary artery of male diabetic Wistar rats. Twenty (20) male Wistar rats were selected for this study. The rats in each group were allowed access to feed and water *ad libitum*. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Diabetes mellitus was induced in rats by a single intraperitoneal injection of streptozotocin (STZ) (60mg/kg body weight) dissolved in 0.1M sodium citrate buffer (pH 4.5). Rats in group A "control" were injected with corresponding volumes of the citrate buffer equivalent to the volume of STZ administered intraperitoneally. Rats found to have blood glucose levels between ≥ 250 mg/dl were considered diabetic and were randomly assigned into groups B, C and D. Diabetes was allowed to develop and stabilize in these STZ-treated rats over a period of 72 hours. Before the induction of diabetes, all the rats were fasted for 16-h (overnight), but still allowed free access to water throughout. At the end of the 16-h fasting period – taken as 0 time (i.e., 0h) – blood glucose levels (initial glycemia, G_0) were determined and recorded. Same procedure was repeated weekly after induction of diabetes and Fasting blood glucose levels (G_1, G_2, G_3 and G_4) of the fasted normal (control), and other experimental rats were recorded. Rats in group A served as control; group B served as untreated diabetic group; diabetic rats in group C received 1IU/kg/day of Humulin subcutaneously and group D diabetic rats received 1ml/100g/day of TCW from freshly harvested Tender coconuts. The experimental period lasted for four weeks after initial glyceamia. The results obtained showed that there was a significant reduction ($P<0.05$) in the Fasting Blood glucose concentration of diabetic rats treated with Humulin and TCW compared with untreated diabetic rats. There was a significant ($P<0.05$) increase in serum TG, TC, LDL-C and VLDL-C of untreated diabetic rats compared with diabetic rats treated with Humulin and TCW and control while HDL-C and AAI of untreated diabetic rats were significantly ($P<0.05$) decreased compared with diabetic rats treated with Humulin and TCW and control. Histological studies of the heart of untreated diabetic rats showed coronary artery with focal intimal ulceration, lumen obstruction and hypertrophy of the muscular wall. The wall of the coronary artery of Diabetic rats treated with TCW and Humulin appear normal compared with the control. Histochemistry of elastin fibers showed that there was a strong positive stain for elastin in the wall of the coronary artery of rats in control and those of diabetic rats treated with Humulin and TCW respectively. The wall of the coronary artery of untreated diabetic rats appear negative to elastin stain. The coronary artery of Wistar rats induced with diabetic and treated with humulin and TCW showed normal vascular wall as compared with control. The implication of the result is that Tender coconut water has both glucose lowering and cardio-protective properties in diabetic rats.

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8.37

THERAPEUTIC ROLE OF INTRAPARTUM PDE-5 INHIBITION ON BLOOD PRESSURE AND RENAL INJURY IN OFFSPRING OF PREECLAMPTIC RATS

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Up to 10% of pregnancies are complicated by preeclampsia. As a result, up to 15 million US citizens today are offspring of preeclamptic pregnancies. Offspring of preeclamptic pregnancies have increased blood pressure (BP) during childhood and nearly double the risk of stroke later in life. The Barker hypothesis proposes that the adverse intrauterine environment created by preeclampsia programs fetal tissues and organs to develop high BP from early childhood. Animal models of hypertension have demonstrated the ability of various therapies such as nitric oxide (NO) donors to reprogram hypertension. Sildenafil citrate, a phosphodiesterase-5 (PDE-5) inhibitor, prolongs the NO signaling cascade and improves the maternal syndrome of preeclampsia; however, determination of optimal timing, effectiveness, and safety during perinatal use have yet to be reported. This project tests the hypothesis that use of a PDE-5 inhibitor during preeclamptic pregnancy improves the long-term BP and renal injury in the offspring. Female Dahl S rats on a 0.3% salt diet, a previously characterized spontaneous model of superimposed preeclampsia, were mated and treated orally with sildenafil (50 mg/kg/day), labetalol (currently used to manage hypertension in preeclamptic patients, 10 mg/kg/day), or vehicle from gestational day 10 to delivery. Dams were placed on normal rat chow at delivery throughout weaning at four weeks of age. At seven weeks of age, male and female offspring were acclimated to restraints for four days before tail cuff BP measurement on day five. The process was repeated at 11 weeks of age for analysis of the time-dependent change in systolic BP. Systolic BP increased in Dahl S rats of untreated mothers as expected; however, the rise in BP was abolished in offspring from sildenafil treated dams (VEH: +28 mmHg ±7; SLD: -7 mmHg ±5, p=0.007). This protective effect was not elicited by treatment with labetalol (+13 mmHg ±5). Tubulointerstitial scarring was measured in 4 µm kidney sections stained with Masson's trichrome (Nikon 55I microscope with DS-Fl1 5-Meg Color C digital camera and Nis-Elements Image-analysis software version 3.03) from Dahl S offspring of sildenafil and vehicle-treated dams at age three months. Tubulointerstitial scarring is increased in male Dahl S offspring of untreated mothers as compared with offspring of sildenafil treated dams (Area: VEH: 9 ±0.6%; SLD: 5 ±0.6%, p=0.006), but no changes were observed in kidney sections from female rats. Urine was collected via 24-hour metabolic cage for measurement of urinary protein (Bradford assay). No significant differences in urinary protein excretion were observed in either male or female offspring. These data support the hypothesis that use of a PDE-5 inhibitor during preeclamptic pregnancy improves the long-term BP and renal injury in the offspring.

8.38

EFFECTS OF PRENATAL SILDENAFIL TREATMENT ON LONG-TERM CARDIOVASCULAR FUNCTION IN OFFSPRING FROM DAHL SALT-SENSITIVE RATS

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Introduction: Fetal growth restriction (FGR) is associated with increased risk for cardiovascular disorders in later life. Cardiovascular disease is the most common cause of death worldwide. Previous studies report that prenatal sildenafil improves pregnancy outcomes, such as birthweight, in FGR animal models; however, whether sildenafil treatment is protective against long-term cardiovascular disease in these offspring is unknown.

Objective: We hypothesize that prenatal sildenafil reduces blood pressure and endothelial dysfunction in FGR offspring from Dahl salt-sensitive (SS) rats on normal salt intake.

Methods: Sildenafil citrate (60 mg/kg/day) or control gel diet was administered from gestational day 10 until birth. Birthweight and litter size were measured (treated n=10; untreated n=8 dams). Telemetry devices (DSI) were implanted via the femoral artery to measure mean arterial pressure (MAP) from weeks 5-8 in the offspring (treated n=12; untreated n=4). Aortic rings were isolated from 10 week old offspring to assess vascular sensitivity ($logEC_{50}$) to endothelial-dependent (acetylcholine) and -independent (sodium nitroprusside, SNP) vasorelaxation (treated n=10; untreated n=10). Data shown as mean ± S.E.M.

Results: No sex differences were observed in any variables; therefore, data were pooled between males and females. Sildenafil improved birthweight (treated

6.8±0.2; untreated 6.2±0.1 g; p=0.02) without significantly changing viable litter size (treated 9.6±0.9; untreated 7.9±1.0; p=0.23). While MAP at 5 weeks was similar between groups (treated 107±1; untreated 108±2 mmHg; p=0.55), there was a trend towards lower MAP in prenatally treated offspring at 8 weeks (treated 116±1; untreated 120±2 mmHg; p=0.09). Aortas from offspring of treated dams displayed enhanced sensitivity to acetylcholine ($logEC_{50}$: treated -7.4±0.3; untreated -6.6±0.3 mol/L; p=0.03), but not to SNP (treated -8.2±0.3; untreated -7.9±0.2 mol/L; p=0.43).

Discussion: Prenatal sildenafil treatment improves birthweight in a model of FGR. In young adult offspring, there was a trend towards a sex-independent lowering of blood pressure and increased endothelium-dependent relaxation.

8.39

TESTOSTERONE SUPPLEMENTATION IN POSTMENOPAUSAL HYPERTENSIVE RATS

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The indication of testosterone supplements (T) is increasing in the aging population of the US. It is useful in relieving both the physical and psychological symptoms of androgen insufficiency in clinically affected women and men. However, while in aging men T may have increased risk for hypertension and cardiovascular events, in aging women, its effect is not clear.

Aim: In the present study, we determined whether T would affect blood pressure and induce endocrine-metabolic disorders in a model of postmenopausal (PM) hypertension.

Methods: Aging female spontaneously hypertensive rats (PMR), aged 18-22 mos (n=4/grp), were randomized into two groups and treated with testosterone propionate (18 mg/silastic pellet, SC: PMR+T) or placebo (PMR+P) for six weeks, respectively. After four weeks of treatment, we implanted telemetry probes, and after two weeks of recovery, we measure the mean arterial pressure (MAP) and heart rate (HR). Also, to evaluate whether T affects body composition, we analyzed body weight, lean and fat mass by ECHO MRI once a week for three weeks.

Results: After six weeks of treatment, MAP was higher in PMR+T than controls (PMR+T: 190±2 mmHg, n=4 vs. PMR+P: 174±2 mmHg, n=4, p<0.05, respectively). While T has no effect on body weight or lean mass, fat mass was reduced in PMR+T (PMR+P: 24±2 g vs. PMR+T: 17±1 g, n=4, p<0.05). These data suggest that testosterone supplements in postmenopausal women may improve some metabolic syndrome parameters, but measurement and maintenance of blood pressure control is imperative. Supported by NIH-R01HL66072, PO1HL51971 (JFR), 14POST18640015 (ROM), P20GM121334 (to JFR and ROM).

8.40

HYPOGONADAL HYPERTENSION IN MALE SPRAGUE-DAWLEY RATS IS REVERSED BY TESTOSTERONE REPLACEMENT THERAPY, WHICH DOWN-REGULATES RENIN-ANGIOTENSIN SYSTEM MESSAGE EXPRESSION

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Acutely, testosterone (TES) and other androgens are efficacious vasodilators, both *in vitro* and *in vivo*; however, their long-term effects on arterial blood pressure (BP) are unclear. We previously reported that castration (CsX) produced hypertension in male rats, and that TES replacement therapy (TRT) was antihypertensive and normalized BP. Thus, long-term effects of endogenous TES and exogenous TRT on BP and renin-angiotensin system (RAS) function were studied in intact (InT) and castrated (CsX) male Sprague-Dawley (SD) and Testicular-feminized male (Tfm, androgen receptor defective) rats (12-13 wk old). Weekly measurements of systolic BP (tail cuff plethysmography) revealed a progressive rise in BP over 10 wks in CsX (108 ± 0.9 *vs.* 139 ± 1.2 mmHg), while BP remained stable in InT (109 ± 3.1 *vs.* 115 ± 0.5). During the next 5 weeks, half of CsX received TRT (CsX+TES-enanthate-replaced; 1.75 mg/kg, 2X/wk). BP gradually declined to normal in CsX+TES replaced rats (115 ± 1.2), while BP remained elevated in CsX (141 ± 1.2) and normal in InT (113 ± 0.3). In separate CsX-SD rats, treatment with Losartan (LST; 250 mg/L drinking water) prevented development of hypertension at 10 wks (95 ± 0.8 CsX+LST *vs.* 139 ± 1.2 in CsX). During the next 5 weeks with TRT, BP declined in CsX+TES (113 ± 1.3) and remained lower in CsX+LST (99 ± 0.4). In Tfm, CsX resulted in a similar rise in BP (108 ± 0.6 *vs.* 139 ± 0.4 mmHg), and TRT reduced BP to a similar extent. Real-time PCR (rt-PCR) of kidney from InT, CsX, and CsX+TES rats revealed that CsX increased expression of renin (45.5%), AT_1 receptor (AT_1R; 38%), and angiotensin converting enzyme (ACE; 239%) mRNA, while TRT normalized expression of renin, AT_1R, and ACE mRNA to levels of InT rats. In contrast, CsX reduced expression of angiotensinogen (Angt) mRNA (59%) while TRT restored Angt mRNA to 78% of InT rats. 24 hr urine output (UO) in InT-SD was 48.3 ml/kg at baseline, 34.3 at 10 wks, 38.4 at 12 wks, and 34.8 at 15 wks. UO in CsX-SD was 47.6, 30.3, 25.2, and 27.7; UO in CsX+TRT was 46.1, 29.2 36.2, and 29.5. These data suggest that: 1) endogenous androgens (TES) exert antihypertensive effects in male SD and Tfm rats; 2) the antihypertensive effect of TES appear to involve a diuretic effect on the kidney, which is

non-genomically mediated; and 3) these antihypertensive effects may involve TES-induced reductions in RAS expression in the kidney. (State of Texas)

9: SESSION 5: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE KIDNEY

9.1

SEX DIFFERENCES IN RENAL FUNCTION: LESSONS FROM THE ET-1 SYSTEM

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The incidence of hypertension and chronic kidney disease in premenopausal women is less frequent compared to age-matched men. Since we know that high salt diets contribute to these major health problems, it is important to understand sex differences in renal control of salt and water balance. Our lab has a long-standing interest in the renal endothelin (ET-1) system that includes a major role in renal excretion of high salt diets. Focusing primarily on rat models, we observed some basic sex differences in the renal medulla, the location with very high ET-1 expression and activity. Urinary ET-1 excretion, a reflection of intrarenal production, is enhanced in response to a high salt diet and is consistently higher in females compare to males. Genetic or pharmacological blockade of the ET_B receptor results in salt-dependent hypertension. The ratio of ET_A to ET_B receptors is much lower in female compared to male rats. Exogenous administration of ET-1 produces a natriuretic response in female, but not male rats. At the same time, ET-1 reduces medullary blood flow in males, but not females. This could be explained by lower ET_A-dependent vasoconstriction in females that would allow for a more robust natriuresis. One of the more unexpected findings from our lab in recent years has been the observation that the adaptation of a high salt diet is more rapid in female compared to male rats. As classically explained in textbooks, it takes several days for male rats to come into balance when transferred from a low to a high salt diet. In contrast, female rats come into balance within the very first day of the high salt diet despite eating an identical amount of salt as males. This fits with more recent findings that the response to an acute high salt load in a model of salt-dependent hypertension – ET_B receptor deficiency – female rats excrete the salt load faster than males, which is dependent upon the time of day that the salt load is given. We have continued to explore how other natriuretic pathways, that is, the purinergic system, contribute to salt handling via activation of the ET-1 system. We observed that activation of $P2Y_2$ and $P2Y_4$ receptors in the renal medulla by UTP promotes Na^+ excretion in male rats. In intact females, UTP did not stimulate Na^+ excretion while ovariectomy unmasked UTP-induced natriuresis. An ET_B antagonist inhibited purinergic dependent natriuresis. Since the purinergic and ET_B systems work via inhibition of the epithelial Na channel (ENaC), it is interesting to note that expression of the alpha subunit of ENaC is higher in female compared to male animals. However, we have observed that the natriuretic response to ENaC inhibition is greater in male compared to female rats. Collectively, these observations demonstrate clear differences in sodium handling by renal medullary structures in the kidney. Our lab continues to explore potential mechanisms that can explain sex differences in handling of sodium. We posit that a better understanding of these sex-specific pathways can provide one of the most fundamental aspects of personalized medicine, sex-based therapy in hypertension and renal disease.

9.2

SEX-SPECIFIC REGULATION OF SIRTUIN-3 MEDIATES DIFFERENCES IN ISCHEMIA-REPERFUSION KIDNEY INJURY

Background: While the pathogenesis of ischemic acute kidney injury (AKI) is better defined, the therapeutic options remain limited. Sex influences susceptibility to kidney ischemia-reperfusion injury (IRI), and sex hormones play a crucial role. We have previously shown that a pathway from stanniocalcin-1 (STC1) mediated activation of AMPK to induction of mitochondrial sirtuin-3 (SIRT3) suppresses ROS generation and confers resistance to kidney IRI. Our observations reveal increased baseline kidney expression of STC1, activated AMPK, and SIRT3 in female mice vs. males, and we hypothesize that SIRT3 protects from IRI and mediates the observed sexual dimorphism in response to injury.

Methods: We subjected wild-type (WT) and SIRT3 transgenic (Tg) male or female mice to bilateral kidney IRI (clamping of renal pedicles for 30 minutes). A group of male or female WT mice were treated with testosterone by subcutaneous implantation of a 200 mg (21-day release) testosterone pellet for 2 weeks. Cultured HEK 293T cells were treated with 17β-estradiol, testosterone or vehicle.

Results: We observed higher kidney expression of STC1, mitochondrial SIRT3 and activity of AMPK in WT female mice compared with males. While there was age-dependent decline in kidney SIRT3 and AMPK activity, differential expression in males and females persisted. Aged 6 months-old SIRT3 Tg male mice display less tubular vacuolization vs. similarly aged WT male mice. Compared with WT male mice, SIRT3 Tg male mice demonstrated resistance to 30-minutes of kidney IRI characterized by: improved survival; preserved creatinine clearance (CrCl); decreased morphological damage and

ROS production. SIRT3 Tg male mice tolerated IRI with survival and kidney function impairment similar to WT females. WT or SIRT3 Tg female mice display no measurable change in kidney function with 30-minutes of kidney IRI. In WT female mice, kidney mitochondrial SIRT3 expression correlates with both plasma estradiol and testosterone levels; in WT male mice, kidney mitochondrial SIRT3 expression correlates with only plasma testosterone level. Testosterone administration to aged 6 months-old WT male mice increased plasma testosterone ~4-fold, caused kidney injury (decreased CrCl and increased tubular vacuolization), and decreased kidney mitochondrial SIRT3 expression (with no effect on whole cell SIRT3). Testosterone treatment to WT female mice caused no measurable kidney injury, but increased whole cell and mitochondrial SIRT3 expression; possibly due to an associated increase in plasma estradiol level. In cultured HEK cells, estradiol increased whole cell and mitochondrial SIRT3 protein expression, and SIRT3 mRNA in a dose-dependent manner. Testosterone decreased mitochondrial SIRT3 protein expression in a dose-dependent manner, but had no effect on whole cell SIRT3 protein expression and SIRT3 mRNA. Estradiol also increased estrogen receptor-β and estrogen related receptor-α mRNA expression.

Conclusion: The data suggest that: 1) SIRT3 ameliorates kidney IRI, and decreased SIRT3 expression in males mediates the increased susceptibility to ischemic injury; 2) sex steroids regulate mitochondrial SIRT3 expression; estrogen via transcriptional regulation and testosterone via inhibition of mitochondrial targeting.

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9.3

SEX DIFFERENCES IN RENAL SODIUM TRANSPORTERS

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Pre-menopause, females have lower blood pressure, blunted hypertensive response to angiotensin II infusion (AngII-HTN), and a leftward shift in pressure natriuresis compared to males. Veiras *et al* (1) examined whether the female advantage was associated with sex dependent pattern of transporters' abundance and/or activation along the nephron, using carefully validated quantitative immunoblots and physiological assays of renal function in rats. Female proximal tubule (PT) exhibited differences in transporters, claudin-2 and water channels (AQP1) indicative of less PT reabsorption in females versus males. Supporting these differences physiologically, females excreted a saline load more rapidly and exhibited both lower bicarbonate reabsorption (determined by micropuncture) and higher volume flow from the PT (determined by lithium clearance) than males. Along the distal nephron and collecting duct, sodium transporters, channels and claudin-7 were more abundant and activated in females associated with lower baseline plasma [K+]. A K+ rich meal increased urinary K+ and decreased Na-Cl cotransporter (NCC) phosphorylation in females indicating acute regulation of the lower K+ set point. The findings suggest lower PT reabsorption in female rats expedites excretion of a saline load and provokes higher NCC and ENaC abundance and activation which may increase K+ secretion and re-set plasma K+ at a lower level. Similar sex dependent profiles were evident in female versus male C57BL/6 mice except for lower PT AQP1 in females and no sex dependence of the response to saline challenge.

The Layton lab (2) generated a sex-specific computational model of solute and transport in the rat PT that accounts for the sex differences in abundance levels of the apical and basolateral transporters, in single-nephron glomerular filtration rate, and in tubular dimensions. Model simulations predict more reabsorption of the filtered volume and Na+ by male PT (71%) than female PT (39%). The lower fractional volume reabsorption in female can be attributed to their smaller transport area and lower AQP1 expression level. The latter also results in a larger contribution of the paracellular pathway to water transport. The lower fractional Na+ reabsorption in female is due primarily to their smaller transport area and lower Na+/H+-exchanger (NHE3) and claudin-2 expression levels. Notably, Na+/glucose cotransporter 2 expression levels are 2.5-fold higher in female which the simulations suggest may compensate for lower PT tubular transport area to achieve a similar hyperglycemic tolerance as male.

Studies on the effects of Angiotensin II-hypertension (AngII-HTN) and high salt diet (HSD) in females vs. males are nearing completion. In males, AngII-HTN depresses PT NHE3 and raises DT NCC-P while in females AngII-HTN increases PT NHE3 and does not raise NCC-P. HSD in males raises NHE3-P and depresses NCC, NCC-P. In females, HSD elicits a more robust natriuresis associated with lower PT NHE3 at baseline coupled to reduced distal transporter NCC, NKCC activation. The studies in AngII and HSD indicate sex specific differences in regulatory patterns that indicate careful attention should be paid to therapeutic effects of diuretics and RAS inhibitors in females vs males.

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9.4

ORAL L-ARGININE TREATMENT SIGNIFICANTLY INCREASES RENAL TREGS IN FEMALE DOCA SALT HYPERTENSIVE RATS

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The immune system plays a critical role in the development of hypertension. Our lab has previously reported that there is a sex difference in the renal T cell profile in DOCA salt hypertension, with males having more pro-inflammatory Th17 cells, whereas females have more anti-inflammatory regulatory T cells (Tregs). DOCA treated males also have a greater blood pressure (BP) response to DOCA than females, and we propose this is due to the relative abundance of Tregs in the female. We have also previously reported that an intact nitric oxide (NO) system is required for Treg maintenance in hypertension, and the DOCA model has been characterized by impaired NOS. In the current study, we hypothesized that treatment with L-arginine, a substrate for NO production, would increase renal Tregs and attenuate the sex difference in hypertension in male and female DOCA treated rats. Briefly, male and female Sprague Dawley rats (n=5-15, 10 wks of age) were uni-nephrectomized and subcutaneously implanted with a DOCA pellet (200 mg/rat, 60-d time release) and given 0.9% NaCl to drink ad libitum. A subset of rats were treated with L-arginine (L-arg, 350 mg/kg/d, via drinking water). BP was measured by tail cuff plethysmography at baseline and after 3 wks of DOCA treatment. After 3 wks of treatment, kidneys were processed for flow cytometric analysis of T cells ($CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD4^+FOXp3^+$ Tregs, and $IL\text{-}10^+CD3^+$ T cells). BP was comparable between treatment groups within each sex at baseline, although males had a higher BP than females (Table, effect of sex, $p<0.001$, 2-Way ANOVA). DOCA treatment increased BP in all groups compared to baseline values (Table, effect of DOCA, $p=0.002$). L-arginine treatment attenuated DOCA-induced increases in BP in both sexes, although this effect was greater in females (Table, effect of L-arginine, $p=0.02$, interaction of sex and L-arginine, $p=0.03$). Females had a more Tregs than males, and L-arginine treatment increased Tregs in females alone (Table, effect of sex, $p<0.01$, effect of L-arg, $p<0.01$, interaction, $p<0.01$, 2-Way ANOVA). Interestingly, despite the Treg numbers remaining unchanged with L-arg, IL-10 increased in both sexes to a similar extent (Table, effect of sex, $p=0.29$, effect of L-arg, $p=0.03$, interaction, $p=0.93$, 2-Way ANOVA). Future studies will examine additional mechanisms by which L-arginine supplementation increases Tregs in females.

	BP Baseline (mmHg)	BP Wk 3 (mmHg)	CD3	CD4	Tregs	IL-10
M DOCA	153±2	225±2	0.9±0.1	65±1.2	3.4±0.3	6.8±0.5
M DOCA + L-Arg	153±1	208±1	1.2±0.2	72±2.0	3.7±0.4	8.5±0.6
F DOCA	142±2	216±2	0.8±0.1	61±1.2	3.9±0.2	7.7±0.5
F DOCA + L-Arg	140±3	180±3	0.6±0.1	64±1.7	6.6±0.8	9.2±1.1

9.5

SEX DIFFERENCES IN RENAL ISCHEMIA-REPERFUSION INJURY

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Postischemic organ dysfunction is influenced by gender and sexual steroids in several organs. Renal ischemia-reperfusion injury leading to acute renal failure continues to be an important clinical problem, especially in situations where the kidney is subjected to periods of warm ischemia, such as suprarenal aortic surgery and renal transplantation. The initial ischemic injury of the kidney is one of the most important risk factor for acute and chronic transplant dysfunction.

What is the impact of gender and sexual steroids on the development of renal damage following ischemic injury? We have first reported that sex and sex hormones influence the susceptibility to renal ischemia/reperfusion injury, since female rats have better survival rates and

improved renal recovery following ischaemic insult. Since the first report of gender related differences in renal ischemia/reperfusion injury several study was performed to investigate the background and pathophysiology of this difference. While these studies explored many different pathways and described several different answers to ischemia/reperfusion injury the exact pathophysiology is still not known. The importance to answer this question is not only to know the pathophysiology but to develop therapeutic possibilities in human settings.

In this review we summarize the recent knowledge about renal ischemia/reperfusion injury and treatment possibilities.

9.6

SEX DIFFERENCES IN RENAL AMMONIA METABOLISM

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Renal ammonia excretion is a critical component in maintaining acid-base homeostasis. Sex differences are well recognized as an important biological variable in many aspects of renal function. However, sex differences in renal ammonia metabolism have not been previously reported. This study's objective was to investigate sex differences in renal ammonia metabolism. We compared 4-month-old C57/BL6 male (M) and female (F) mice fed a normal diet, with measurement of plasma electrolytes, urinary ammonia excretion, morphometric analysis of renal structure, and evaluation of changes in key proteins involved in ammonia metabolism using immunoblot analysis and immunohistochemistry. Despite similar level of food intake (F, 8.9±0.9; M, 9.6±1.2 gram/day; P=NS), and thus protein intake, which is the primary determinant of endogenous acid production, female mice excreted significantly more ammonia (F, 72±23; M, 46±19 µmol/day; P<0.01) than did male mice. This difference in ammonia excretion was not due to differences in urine acidification, as urine pH did not differ significantly (F, 6.40±0.18; M, 6.36±0.14; P=NS). Titratable acid excretion (F, 53±26; M, 74±20 µmol/day; P=NS) another component of net acid excretion, did not differ significantly. Serum Na^+, K^+, and HCO^-_3 did not differ significantly. There are fundamental structural differences between the female and male kidney. In the female kidney, proximal tubules account for a lower percentage of the renal cortical parenchyma than the male kidney (F, 42±3; M, 60±3%; P<0.01), whereas the collecting ducts account for a greater percentage of the renal parenchyma (F, 15.4±2.0; M, 9.6±1.6%; P<0.001). Phospho*enol*pyruvate, a major proximal tubule (PT) ammonia generating protein, was significantly greater in female mice than male mice. Expression of glutamine synthetase, which recycles ammonia, was significantly greater in the PT of female mice. Expression of NBCe1, a basolateral PT transporter, recently shown to regulate PT ammonia metabolism did not differ significantly between the sexes. Expression of NHE3, which is believed to be the major mechanism of PT ammonia secretion, did not differ significantly between the sexes. Expression of NKCC2, which mediates thick ascending limb ammonia reabsorption, was significantly greater in the female kidney than the male kidney. The collecting duct secretes the majority of urinary ammonia and the Rhesus glycoproteins, Rhbg and Rhcg are the primary collecting duct ammonia transporting proteins. Rhbg was significantly greater in connecting segment cells and intercalated and principal cells in the collecting duct in the cortex and inner stripe of the outer medulla (ISOM) in female mice. Expression of Rhcg was significantly greater in female mice in connecting segment cells and in the basolateral membrane of intercalated and principal cells in the collecting of the ISOM. Thus, there are sex differences in basal ammonia metabolism that involves both renal structural differences and differences in expression of critical proteins involved in ammonia metabolism. These studies were supported by funding from the National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-045788 (IDW), RO1-DK-107798 (IDW and JWV) and 5T32-DK-10472.

9.7

SEX DIFFERENCES IN THE REGULATION OF BLOOD PRESSURE BY THE CIRCADIAN CLOCK PROTEINS PER1 AND BMAL1 IN C57BL/6J MICE

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Blood pressure (BP) exhibits a circadian rhythm in healthy individuals in which there is a peak during the active period and a decrease during the rest phase. The core transcriptional mechanism of the circadian clock consists of four proteins: BMAL1, CLOCK, CRYTOCHROME (CRY), and PERIOD (PER). BMAL1 and CLOCK interact with E-box response elements in the promoters of clock-controlled genes, including those for PER and CRY, to regulate expression. PER and CRY act in a negative feedback loop to inhibit the actions of BMAL1 and CLOCK. To date, every clock gene mutant mouse that has been tested has exhibited some type of BP phenotype, yet these studies have exclusively been performed in male mice. We have previously demonstrated that male global PER1 knockout mice exhibit non-dipping hypertension in response to a high salt (HS) diet plus mineralocorticoid (DOCP) treatment (Solocinski et al. Acta Physiol. 2017). Curtis et al. demonstrated that

male global BMAL1 knockout mice exhibited a BP that was ~100 mm Hg less than control mice and did not have a detectable rhythm. The goal of the present study was to test whether female global PER1 KO mice and female kidney-specific BMAL1 KO mice exhibit a BP phenotype relative to littermate controls. We also tested the sensitivity of the female mice to the HS/DOCP treatment. The results demonstrate that both PER1 and BMAL1 contribute to the regulation of BP in a sex-specific manner that may be independent of dietary salt and mineralocorticoid.

11: SESSION 6: FEMALE-SPECIFIC CARDIOVASCULAR, RENAL AND METABOLIC COMPLICATIONS

11.2

BETA-CAROTENE METABOLISM IN THE MATERNAL HEART DURING PREGNANCY

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Pregnancy-related deaths associated with cardiovascular diseases have recently increased in the U.S. with a higher occurrence among African American and Hispanic women. Low intake of fruits and vegetables, the main source of vitamin A, is a hallmark of poor nutrition that is manifested in these populations that are mainly affected by gestational cardiac complications. Many signaling pathways have been associated with the physiological hypertrophy (remodeling) of the heart that occurs during pregnancy [1]. However, how these pathways are activated and influenced during pregnancy has yet to be fully understood. Retinoic acid, the active form of vitamin A that functions as a transcriptional regulator, has been implicated in cardiac remodeling in the adult [2], but whether or not retinoids (vitamin A and its derivatives) are essential during the cardiac hypertrophy of pregnancy is still unknown. Preliminary data from our laboratory revealed a small but significant decrease in *Dhrs3* (*Dehydrogenase reductase 3*) and *Lrat* (Lecithin:retinol acyltransferase) in the heart of pregnant wild-type mice at 14.5 dpc, suggesting that during gestation, heart retinoid metabolism may be shunted towards retinoic acid formation rather than storage. Thus, if cardiac retinoid acid synthesis is favored in wild-type mice during pregnancy, the active vitamin A metabolite may play a potential role in sustaining maternal cardiac hypertrophy (remodeling).

Beta-carotene, the most abundant dietary precursor of vitamin A, can be cleaved asymmetrically by the mitochondrial beta-carotene 9',10'-oxygenase (BCO2) to generate beta-apo-10'carotenal, which can serve as a precursor of retinoids, but may also antagonize retinoid acid action *per se* [3]. Preliminary observations in our lab indicate that cardiac mRNA levels of *Bco2,* which is the only carotenoid cleavage enzyme expressed in the adult mammalian heart [4], were elevated in wild-type pregnant mice at mid-gestation. Moreover, in the absence of BCO2 (*Bco2-/-* mice) the maternal heart failed to enlarge during pregnancy. Notably during pregnancy, the heart of *Bco2-/-* mice showed significantly reduced retinyl ester levels compared to wild-type pregnant mice. Based on these premises, we hypothesized that BCO2 may contribute to the physiological hypertrophy of the maternal heart during pregnancy. Current studies aim at further understanding the effects of the lack of murine BCO2 on retinoid metabolism and cardiac function in the maternal heart. Understanding the role of carotenoid and retinoid metabolism in this process will allows us to ultimately design dietary preventative measures to potentially decrease adverse cardiac function during pregnancy

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11.3

VASCULAR CHANGES IN THE POSTMENOPAUSAL FEMALE

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The number of years women live past menopause has drastically increased over the last century. A decline in estrogen induces hypertension and arterial stiffness, accelerating end organ damage and heart failure. In contrast, adverse cardiovascular outcomes have been reported in response to currently available menopausal hormone therapy, indicating that new treatment strategies are needed. Our goal is to elucidate estrogen receptor pharmacology to allow the development of more selective pharmaceuticals that provide cardiovascular protection. We focus our efforts on the

vascular effects of the G protein-coupled estrogen receptor (GPER). GPER is membrane-bound and activates acute intracellular signaling pathways, distinguishing it from the nuclear estrogen receptors ERα and ERβ. Moreover, this receptor is unique in that it does not impact traditional estrogenic actions in reproductive tissues but induces protective cardiovascular effects, indicating a potential drug target. Activation of GPER attenuates aortic remodeling, and GPER deletion increases pulse wave velocity, an *in vivo* indicator of arterial stiffness. GPER also decreases vascular oxidative stress via regulation of NOX4. Moreover, we find that vascular GPER expression decreases during aging, which may further promote vascular disease and worsen the response to menopausal hormones. Current studies in the lab are assessing the impact of sex and aging on not only GPER but expression profiles of all estrogen receptors in order to give a clearer picture of the contribution of each signaling pathway in different tissues. Our overall goal is to promote the improvement of menopausal hormonal therapy and therefore quality of life in aging women.

11.4

FEMALE RATS WITH PREEXISTING CKD EXHIBIT IMPAIRED RECOVERY FROM AKI AND THE SUBSEQUENT DEVELOPMENT OF PROTEINURIA

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Interactions between acute kidney injury (AKI) and chronic kidney disease (CKD) predispose to the development of end-stage renal disease (ESRD). A previous study from our group showed that such deleterious AKI-CKD interactions include impaired recovery from AKI and the development of *de novo* mechanisms of CKD progression in male rats with preexisting CKD. The goal of this study was to determine if impaired recovery from AKI is also observed in female rats with preexisting CKD. We induced two levels of CKD in 10-12 week old male and female Sprague-Dawley rats by performing either 50% renal mass reduction via a right uninephrectomy (UNX, n=14, 6 females) or 75% renal mass reduction via a right UNX + surgical excision of 1/2 of the left kidney (3/4 NX, n=12, 6 females). Rats recovered for two weeks to allow for completion of compensatory adaptations in renal size and function. Rats were then subjected to 35 minute ischemia-reperfusion (IR)-induced AKI under isoflurane anesthesia with core body temperature maintained at 37° C. Blood samples were obtained prior to IR and at 48 hours, 7 days, 14 days and 28 days post IR to assess plasma creatinine (P_{Cr}). A 24-hour urine collection was performed in a subset of rats prior to IR and at 28 days post IR to assess proteinuria. At the end of the study, kidneys were fixed in paraformaldehyde and paraffin embedded sections were stained with H&E to assess renal pathology and tubular vimentin expression was assessed using immunohistochemistry. Tubular vimentin expression 28 days post IR identifies sublethally injured tubules that have failed to redifferentiate, which is a robust index of impaired recovery. Vimentin expression was semiquantitated on a scale from 0-4 with 0 representing no tubular vimentin staining and 4 representing tubular vimentin staining in >75% of tubules. Vimentin scoring was conducted in a blinded fashion. The severity of AKI, based on P_{Cr} levels 48 hours post AKI, was similar between UNX vs. 3/4 NX groups within both males and females. The severity of AKI was lower ($P<0.05$) in females vs. males with 3/4 NX (1.9±0.3 vs. 3.3±0.3 mg/dl) but not significantly different between females vs. males with UNX (1.7±0.6 vs. 2.7±0.4 mg/dl). While minimal injury and vimentin expression (0.7±0.2) was observed in female rats with UNX, females with 3/4 NX exhibited greater ($P<0.05$) tubular vimentin staining (1.3±0.3), tubular injury and fibrosis 28 days post IR. Moreover, recovery of P_{Cr} over 28 days post IR was delayed in females with 3/4 NX vs. UNX, which is also indicative of impaired recovery from AKI. Finally, female rats with 3/4 NX developed substantial ($P<0.05$) increases in proteinuria 28 days post IR as compared to pre-IR levels (195±57 vs. 62±20 mg/day) while proteinuria was similar at 28 days post IR vs. pre-IR in female rats with UNX (21±4 vs. 23±5 mg/day). Similar to our previous study, males with 3/4 NX exhibited impaired recovery from AKI and the development of substantial proteinuria 28 days post AKI as compared to males with UNX. In conclusion, these data support previous studies documenting resistance to IR-induced AKI in female vs. male rats. However, our data indicate that preexisting CKD of greater than 50% renal mass reduction predisposes female rats to impaired recovery from AKI and the subsequent development of mechanisms of CKD progression, similar to male rats.

11.5

LONG-TERM SEQUELAE OF PREECLAMPSIA: A CLINICAL PERSPECTIVE

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Preeclampsia complicates 5-7 % of pregnancies and remains a leading cause of fetal growth restriction, premature birth, as well as infant and maternal morbidity and mortality. It is primarily a disease of the vascular endothelium. Accordingly, women with a history of preeclampsia have now been documented to have an increased risk for the development of hypertension, stroke, coronary artery disease as well as end stage renal disease later in life. This increased vascular risk is likely mediated by abnormalities in vascular physiology,

including significant impairments in flow-mediated vasodilatation as well as increased arterial stiffness that remain present into the postpartum period, and are most marked in women with a history of early onset, severe preeclampsia accompanied by fetal growth restriction, suggesting that vascular dysfunction may, in fact, be the predisposing factor for both abnormal placentation as well as the development of vascular disease in later life. It is critical that clinicians recognize the relationship between placental disease and future vascular disease to ensure those women most at risk are appropriately counselled and targeted with risk reduction strategies.

11.6

ANGIOTENSIN II INDUCES A PRO-INFLAMMATORY SHIFT IN THE SPLENIC CD4$^+$ T CELL PROTEOME IN MENOPAUSAL MICE

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Premenopausal female mice are protected against Angiotensin II-induced hypertension, however this protection against Ang II-induced hypertension is lost following the onset of menopause. T cells are required for the development of Ang II hypertension in male mice and we have shown that premenopausal females are protected from T cell-mediated hypertension. This protection is also lost in menopausal mice. The purpose of this study was to utilize a bottom-up shotgun proteomics-based approach to examine how CD4$^+$ T cell activation and pro-inflammatory signaling is modified in Ang II-infused menopausal mice compared to premenopausal counterparts. 10-week-old C57BL/6J female mice received intraperitoneal injections of 4-vinylcyclohexene diepoxide (VCD; sesame oil vehicle) for 20 consecutive days to induce menopause. Cyclicity was monitored daily to determine the day of onset of menopause. Ang II (800ng/kg/min) was infused for 14 days via osmotic minipump into VCD-treated menopausal (Meno/Ang II) or vehicle-treated premenopausal (Ang II) mice. After 14 days of Ang II infusion, splenic CD4$^+$ T cells were isolated and purified via negative immunomagnetic selection. CD4$^+$ purity was measured via flow cytometry and protein was obtained from these cells. Splenic CD4$^+$ T cell protein samples were fractioned via SDS PAGE prior to trypsin digestion and Zip Cleaning on C18 columns. These peptide samples were analyzed via label-free MS/MS tandem mass spectrometry and were subsequently identified and quantified using Mascot and Progenesis software. 7,123 proteins were identified from the peptide samples. From this protein list, 5,857 proteins were identified by more than one unique peptide sequence and were used for subsequent analysis. 964 proteins were differentially expressed between control, Ang II and Meno/Ang II groups ($p < 0.05$). Of the 964 differentially expressed proteins, 350 were significantly different between Ang II and Control, while 639 proteins were differentially expressed between Meno/Ang II and Control, and 248 between Meno/Ang II and Ang II. Gene Ontology (GO) enrichment of the 964 differentially expressed proteins was assessed using Perseus software and the DAVID database. Ang II infusion resulted in the overexpression of 220 GO biological pathways ($p < 0.05$), including positive regulation of cell adhesion (5.1-fold enrichment), negative regulation of interleukin-6 production pathway (4.5-fold enrichment), and negative regulation of cell cycle arrest (4.1-fold enrichment). Overall, expression of proteins positively regulating cell adhesion and negatively regulating interleukin 6 were decreased in the Ang II group versus control and were further decreased in the Meno/Ang II group (see attached table). Proteins associated with negative regulation of cell cycle arrest were equally increased by Ang II in premenopausal and menopausal female mice, suggesting an increase in CD4$^+$T cell proliferation. These results demonstrate that Ang II induces a significantly greater shift in the splenic CD4$^+$ T cell proteome in female mice after menopause. This shift results in a proteomic profile favoring the proliferation and migration of pro-inflammatory T cells in postmenopausal females.

Protein Name	Control	Ang II	Meno+Ang II
Positive Regulation of Cell Adhesion			
Intercellular adhesion molecule 1	1.00 ± 0.04	0.79 ± 0.06*	0.73 ± 0.08*
Intagrin subunit alpha-2	1.00 ± 0.10	0.80 ± 0.08	0.63 ± 0.03*
Tyrosine-protein kinase SRC-1	1.00 ± 0.13	0.80 ± 0.11	0.51 ± 0.03*#
Platelet 12-LOX	1.00 ± 0.06	0.80 ± 0.07	0.54 ± 0.07*#
Negative Regulation of Interleukin 6 Production Pathway			
Beta-arrestin 1	1.00 ± 0.10	0.81 ± 0.07*	0.69 ± 0.05*
Interleukin-1 receptor-associated kinase	1.00 ± 0.34	0.55 ± 0.08*	0.30 ± 0.08*#
Toll-like receptor 9	1.00 ± 0.15	0.71 ± 0.10	0.58 ± 0.08*
Zinc finger CCCH-type containing 12A	1.00 ± 0.08	0.91 ± 0.14	0.52 ± 0.08*#
Negative Regulation of Cell Cycle Arrest			
Cyclin dependent kinase 4	1.00 ± 0.10	1.87 ± 0.20*	1.55 ± 0.20
JunD	1.00 ± 0.04	1.33 ± 0.12*	1.36 ± 0.19
Ras associated domain-containing protein 1	1.00 ± 0.04	1.24 ± 0.06*	1.28 ± 0.13
Nucleosomal binding protein 1	1.00 ± 0.01	1.17 ± 0.10	1.38 ± 0.08*

*p<0.05 vs Control; #p<0.05 vs Ang II

11.7

LEPTIN ADMINISTRATION LOWERS REGULATORY T CELLS AND ACCELERATES THE DEVELOPMENT OF HYPERTENSION IN AN EXPERIMENTAL MODEL OF AUTOIMMUNE DISEASE

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Systemic lupus erythematosus (SLE) is a prototypic multisystem autoimmune disorder that predominately

affects women during childbearing years. SLE is characterized by a loss of immunological tolerance and the expansion of autoreactive T and B lymphocytes, leading to the production of autoantibodies. The autoantibody production leads to downstream chronic inflammation resulting in high rates of hypertension, renal injury, and cardiovascular disease in SLE patients. The immunomodulatory adipocytokine leptin plays a key role in the maintenance and development of inflammation, in part by promoting the expansion of proinflammatory T helper 1 (T_H1) cells and inhibiting the differentiation of regulatory T cells (T_{REG}). Although women have higher circulating leptin levels than men, and the levels are even higher in women with SLE, it is unclear whether leptin plays a direct role in the pathogenesis of SLE. In the present study, we hypothesized that administration of leptin would lower circulating T_{REG} and accelerate the development of hypertension in a female mouse model of SLE. To test this hypothesis, 30 week old female SLE (NZBWF1, n=21) and control (NZW, n=25) mice were implanted with microosmotic pumps to continuously deliver recombinant mouse leptin at a rate of 0.5 mg/kg/day or vehicle (0.9% NaCl) for four weeks. Body composition was assessed using Echo MRI and fat mass, as a percentage of body mass, was not changed in control mice (20.9±1.1% Control-vehicle vs. 19.6±1.2% Control-leptin p=0.99), but was significantly lower in SLE-leptin treated mice (26.5±1.6% SLE-vehicle vs. 16.2±3.1% SLE-leptin, p<0.05). Circulating levels of anti-dsDNA IgG autoantibodies, a marker of SLE disease activity, were higher in SLE mice compared to controls (0.45±0.1 Control-vehicle vs. 1.3±0.3 SLE-vehicle OD450, p<0.01), but the administration of leptin did not significantly increase anti-dsDNA IgG production (0.98±0.2 SLE-vehicle vs. 1.3±0.1 SLE-leptin OD450, p=0.58). Plasma levels of IgG isotypes were also analyzed, and circulating levels of IgG2a were higher in SLE mice as compared to control (0.25±0.006 mg/mL Control-vehicle vs. 0.79±0.2 mg/mL SLE-vehicle, p<0.01), and were further increased in SLE mice administered leptin (0.79±0.2 SLE-vehicle vs. 1.2±0.05 SLE-leptin, p<0.05), suggesting increased T_H1 activity. Circulating $CD4^+FoxP3^+$ T_{REG}, as assessed by flow cytometry, were lower in SLE mice than in control mice (3.6±0.5% Control-vehicle vs. 1.1±0.2% SLE-vehicle, p<0.05), as previously reported by our laboratory. Leptin administration further decreased the levels of circulating T_{REG} in SLE mice (1.1±0.2% SLE-vehicle vs. 0.52±0.1% SLE-leptin, p<0.05). Mean arterial pressure (MAP; mmHg), measured in conscious mice by carotid catheter, was higher in SLE mice than in control mice (113±3 Control-vehicle vs. 128±3, p=0.06), and leptin further increased blood pressure in SLE mice (SLE-leptin: 134±3; p<0.01 vs. Control-vehicle and Control-leptin). Taken together, these data suggest that SLE mice may have enhanced sensitivity to leptin and that increased leptin enhances T_H1 responses and decreases T_{REG}. In addition, therapeutics aimed at modulating leptin activity could have potential benefit for patients with SLE.

12: SESSION 7: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE VASCULATURE

12.1

IMPACT OF SEX ON VASCULAR FUNCTION IN CARDIOVASCULAR AND METABOLIC DISORDERS

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Obesity is a major risk factor for cardiovascular disease in males and females. Whether obesity triggers cardiovascular disease via similar mechanisms in both the sexes is, however, unknown. In males, the adipokine leptin highly contributes to obesity-related cardiovascular disease by increasing sympathetic activity. Females secrete 3× to 4× more leptin than males, but do not exhibit high sympathetic tone with obesity. Nevertheless, females show inappropriately high aldosterone levels that positively correlate with adiposity and blood pressure (BP). We hypothesized that leptin induces hypertension and endothelial dysfunction via aldosterone-dependent mechanisms in females. Leptin control of the cardiovascular function was analyzed in female mice sensitized to leptin via the deletion of protein tyrosine phosphatase 1b (knockout) and in agouti yellow obese hyperleptinemic mice (Ay). Hypersensitivity to leptin (wild-type, 115 ± 2; protein tyrosine phosphatase 1b knockout, 124 ± 2 mm Hg; P<0.05) and obesity elevated BP (a/a, 113 ± 1; Ay, 128 ± 7 mm Hg; P<0.05) and impaired endothelial function. Chronic leptin receptor antagonism restored BP and endothelial function in protein tyrosine phosphatase 1b knockout and Ay mice. Hypersensitivity to leptin and obesity reduced BP response to ganglionic blockade in both strains and plasma catecholamine levels in protein tyrosine phosphatase 1b knockout mice. Hypersensitivity to leptin and obesity significantly increased plasma aldosterone levels and adrenal CYP11B2 expression. Chronic leptin receptor antagonism reduced aldosterone levels. Furthermore, chronic leptin and mineralocorticoid receptor blockade reduced BP and improved endothelial function in both leptin-sensitized and obese hyperleptinemic female mice. Together, these data demonstrate that leptin induces hypertension and endothelial dysfunction via aldosterone-dependent mechanisms in female mice and suggest that obesity leads to cardiovascular disease via sex-specific mechanisms.

12.2

ESTROGEN DETERMINES THE SEX-DIFFERENCES IN ADRENERGIC VESSEL TONE REGULATION

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Introduction: Sex-specific differences in adrenergic vasoconstriction and vasorelaxation have been demonstrated in rats and humans. Although we have previously shown that differences in rats rely on endothelial β-adrenoceptors, neither translational relevance in humans nor the role of sex-hormones in endothelial β-adrenoceptor-related vessel tone regulation has been shown.

Aims: We investigated the role of endothelium in sex-specific differences of adrenergic vasoconstriction and vasorelaxation in human vessels, as well as the role of female and male sex-hormones on adrenergic vessel tone regulation in a rat model.

Methods: In human mammary arteries, obtained from the Heart Center Dresden (patient age: 50 to 70 years), vasoconstriction (norepinephrine) and vasorelaxation (isoprenaline and β_3 agonist BRL) with and without endothelium were assessed using Mulvany myography. Five weeks old female and male wistar rats were respectively ovariectomized and orchiectomized. As controls, a sham-operated, hormone substituted (2 mg/kg, twice a week) and a vehicle group of rats were examined. At age of 12 weeks, aortas were isolated for assessment of vasoconstriction and vasorelaxation. Additionally, a qRT-PCR for quantification of β-adrenoceptor mRNA levels in aorta was performed.

Results: Mammary arteries of women constricted less (P<0.05) in response of norepinephrine than arteries of men. Removal of endothelium eliminated this sex-specific difference by significantly (P<0.05) increasing vasoconstriction in arteries of women, without affecting vasoconstriction in arteries of men. Vasorelaxation caused by isoprenaline was greater (P<0.05) in mammary arteries of women compared to arteries of men. This sex-specific difference in vasorelaxation was abolished after removal of endothelium. Similar to human arteries there were sex-specific differences in vasoconstriction and relaxation in rat aorta, which was eliminated after ovariectomy in female rats. Compared to sham operated females, ovariectomy increased aortic vasoconstriction in response to norepinephrine more than 2-fold. Vasorelaxation by isoprenaline and β_3-agonist was significantly (P<0.01) reduced after ovariectomy. Compared to vehicle, estrogen substitution largely (P<0.05) restored sex-specific differences in vasoconstriction and vasorelaxation in ovariectomized rats. Differences in vasoconstriction and vasorelaxation between sexes were diminished in presence of selective β_1- and β_3-adrenoceptor antagonists and L-NMMA. Consistently, mRNA levels of β_1- and β_3-, but not β_2-adrenoreceptors were significantly (P<0.05) higher in aortas of sham operated females than in aortas of sham operated males. Ovariectomy abolished this difference by decreasing β_1- and β_3- adrenoreceptor expression in female rats. Consequently, estrogen substitution in ovariectomized females largely (P<0.05) restored β_1- and β_3-adrenoreceptor expression. Orchiectomy and testosterone treatment did not change aortic vasoconstriction and vasorelaxation nor ß-adrenoceptor expression in aortas of male rats.

Conclusion: We reveal that sex-specific differences in vasoconstriction and vasorelaxation in human mammary artery are endothelium-dependent. We also demonstrate that sex-differences in a rat model are estrogen, but not testosterone-dependent. Estrogen determines these differences via regulation of vascular endothelial β_1- and β_3-adrenoreceptor expression.

12.3

GENOTYPE CONTRIBUTES TO SEX DIFFERENCES IN MICRO- AND MACROVASCULAR ENDOTHELIAL PHENOTYPE

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Among humans, at the whole-body level, while both sexes may be in homeostasis, how they achieve that state appears to differ by sex. It is well known that in females the state of pregnancy results in profound changes in fluid distribution with resulting changes in hemodynamics (Duvekot *et al.*, 1993; Mudrovcic *et al.*, 2017). One supposition is that because females must possess the mechanisms to reversibly alter fluid distributions to accommodate pregnancy, the multiple factors regulating fluid and solute flux between the vasculature and metabolizing tissue will likewise differ by sex. In animal studies we and other have demonstrated profound differences in the basal levels of protein and/or fluid flux in some portions of the microvasculature and not others (Huxley *et al.*, 2004, 2007, 2010, Sasaki *et al.*, 2007, Wang *et al.*, 2010). These same studies also demonstrated varied sex-dimorphism in permeability responses to a variety of vasoactive agents under conditions where the reproductive hormone levels were known or not varying suggesting a role for genomic control of barrier function. To assess the role of genomic sex a comprehensive study of endothelial cell (EC) phenotype was undertaken of passage 4.4 EC derived from age-matched sexually mature rat aorta (macrovascular) and skeletal muscle (microvessel) maintained under identical conditions of culture and low, unvarying levels of reproductive hormones. The data

demonstrated that both genomic sex and position in the vasculature played distinct roles in EC morphology, growth, wound healing, lactate production, messenger RNA, and expression of key proteins (including sex hormone receptors for estrogen (ERα and ERβ) and androgen; barrier proteins PECAM-1 and VE-CAD; αvβ3 and N-Cadherin influencing matrix interactions; ICAM-1 and VCAM-1 mediating EC/white cell adhesion). A hierarchy of variable importance was unveiled when the EC growth data were analyzed as precision improved assuming EC homogeneity < Sex < Vessel Origin < Sex and Vessel Origin. Many identified sex differences are subtle and easily ignored. In the aggregate, though, they can profoundly alter phenotype, especially under conditions of pregnancy, exercise, and disease states ranging from diabetes to heart failure. Overall, ignoring either sex (and/or age) is inappropriate and will prevent the design and implementation of appropriate interventions to present, ameliorate, or correct vascular dysfunction. Supported by NIH NIDDK R01 DK095501

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12.4

NITRIC OXIDE HELPS MAINTAIN THE BUFFERING CAPACITY OF PERIVASCULAR ADIPOSE TISSUE IN FEMALE DAHL SS IN RESPONSE TO A HIGH FAT DIET DESPITE INCREASES IN BLOOD PRESSURE AND VASCULAR INFLAMMATION

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Local inflammation in perivascular adipose tissue (PVAT) is linked to high fat diet (HFD)-induced increases in BP and vascular dysfunction in males. There is limited data on the impact of HFD on BP or vascular function in females.Thus the goal of this study was to test the hypotheses that HFD will 1) increase BP and vascular inflammation and 2) PVAT will exacerbate HF diet-induced vascular dysfunction in female DSS. 6-wko female DSS were fed a normal-fat diet (NFD; 7.2% fat) or HFD (35% fat) diet for 10 weeks, and BP was measured by telemetry. At 16 wko, aortic rings (+/- PVAT) were mounted for isometric myography and cumulative concentration response curves to phenylephrine (PE) or acetylcholine (Ach) were generated in the absence or presence of the nonselective nitric oxide synthase (NOS) inhibitor L-N^G-Nitroarginine methyl ester (LNAME) or polyethylene glycol (PEG)-catalase. In separate rats, aortic T cells were measured by flow cytometry. HFD increased BP (mmHg: 176 ± 8 HFD vs 130 ± 4 NFD, $P<0.001$) and led to greater numbers of total aortic T cells ($P=0.05$), T cell activation ($P=0.002$), and pro-inflammatory Th17 cells ($P=0.002$) compared to NFD. There was no change in anti-inflammatory T regulatory cells ($P=0.67$). HFD alone had no effect on vascular function. Although the presence of PVAT did not increase relaxation to Ach, it did attenuate PE-induced constriction [Area Under Curve (AUC): effect of PVAT $P<0.01$] regardless of diet. HFD is known to promote oxidative stress via increased production of reactive oxygen species, including H_2O_2. Interestingly, Peg-catalase uncovered a PVAT-mediated vasoconstrictor with HFD (AUC: effect of diet: $P=0.015$; effect of PegCat: $P=0.17$; interaction: $P=0.0091$) while LNAME increased force generation to PE in the presence of PVAT regardless of diet (effect of LNAME: $P=0.047$; effect of diet: P-0.17). Thus, in contrast to what has been shown in male DSS following a HFD in other studies,vascular function is maintained in female DSS; and PVAT enhanced the vasodilatory capacity of the aorta regardless of diet. Further, our data suggests that overproduction of NO rather than H_2O_2 plays a role in maintaining the anti-contractile effect of PVAT in response to a HFD. Future studies will determine 1) which NOS isoform contributes to the enhanced buffering capacity of PVAT in response to a HFD and 2) whether or not this capacity is mediated by the

endothelium using endothelium intact and denuded vessels.

12.5

FUNCTIONAL IMPLICATIONS OF SEXUAL DIMORPHISM OF TRANSPORTER PATTERNS ALONG THE RAT NEPHRON

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The goal of this study is to investigate the functional implications of the sexual dimorphism in transporter patterns along the nephron. To do so, we began by developing sex-specific computational models of solute and transport in the proximal convoluted tubule of the rat kidney. The models account for the sex differences in expression levels of the apical and basolateral transporters, in single-nephron glomerular filtration rate, and in tubular dimensions. Model simulations predict that 70.6 and 38.7% of the filtered volume is reabsorbed by the proximal tubule of the male and female rat kidneys, respectively. The lower fractional volume reabsorption in female can be attributed to their smaller transport area and lower aquaporin-1 expression level. The latter also results in a larger contribution of the paracellular pathway to water transport. Similar fractions (70.9 and 39.2%) of the filtered Na^+ are reabsorbed by the male and female proximal tubule models, respectively. The lower fractional Na^+ reabsorption in female is due primarily to their smaller transport area and lower Na^+/H^+-exchanger (NHE3) and claudin-2 expression levels. Notably, unlike most Na^+ transporters, whose expression levels are lower in female, Na^+/glucose cotransporter 2 (SGLT2) expression levels are 2.5-fold higher in female. Model simulations suggest that the higher SGLT2 expression in female may compensate for its lower tubular transport area to achieve a similar hyperglycemic tolerance as male.

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12.6

AFFERENT ARTERIOLAR RESPONSIVENESS TO ENDOTHELIN RECEPTOR ACTIVATION: DOES SEX MATTER?

Eman Y. Gohar[1], Anthony K. Cook[1], Edward W. Inscho[1], David M. Pollock[1]

[1]Division of Nephrology, Department of Medicine, University of Alabama at Birmingham

The pathogenesis of hypertension is distinct between men and women. Endothelin-1 (ET-1) is a potential contributor to sex-differences in the pathophysiology of hypertension. ET-1 is known to participate in blood pressure regulation through activation of endothelin A (ET_A) and endothelin B (ET_B) receptors in both the renal tubule and vasculature. However, little is known about sex-differences in ET-1 dependent renal microvascular reactivity. Our lab previously reported that renal medullary ET-1 reduces medullary blood flow in male, but not female rats. Orchiectomy (ORX) eliminated ET-1 dependent decreases in medullary blood flow, but ovariectomy (OVX) had no apparent effect on this sex-difference. Thus, we hypothesized that sex and sex steroids regulate the afferent arteriolar responses to ET receptor activation. To test that, we used 15-17 week old male and female Sprague Dawley rats subjected to gonadectomy or sham surgery. Three weeks later, kidneys from those rats were prepared for assessment of renal microvascular responses to ET-1 (ET_A and ET_B agonist, $10^{-12}$to 10^{-8}M) and sarafotoxin 6c (S6c, ET_B agonist, $10^{-12}$to 10^{-8}M) using the blood-perfused juxtamedullary nephron preparation. Baseline afferent arteriolar diameter at 100 mmHg averaged 15.3±0.3 and 14.6±0.3 µm for sham male and female rats, respectively (n=12, each). Gonadectomy had no significant effect on baseline arteriolar diameter. In sham males, ET-1 produced significant concentration-dependent decreases in afferent arteriolar diameter, with 10^{-8}M ET-1 decreasing diameter by 84±1 % (n=6). Similarly, ET-1 induced concentration-dependent vasoconstrictor responses in sham female rats, with 10^{-8}M ET-1 decreasing the diameter by 82±1 % (n=6). The vasoconstrictor responses to ET-1 within the afferent arteriole were unchanged by ORX or OVX. In addition, ET_B receptor activation by S6c induced a concentration dependent decline in the afferent arteriolar diameter, with 10^{-8}M S6c decreasing diameter by 77±3 and 76±3 % in sham male and female rats, respectively (n=6, each). These data do not support our original hypothesis and suggest that sex or sex hormones do not significantly influence afferent arteriolar reactivity to ET receptor activation. They further suggest that reported sex differences of the renal ET-1 system on blood pressure are most likely mediated through renal tubular activity of the ET_A and ET_B receptors as we have previously reported.

12.7

THE ROLE OF THE CEREBROVASCULATURE IN ISCHEMIC STROKE INJURY AND RECOVERY: SEX AND DIABETES INTERACTIONS

Adviye Ergul[1]

[1]Physiology, Augusta University

Over 30 million Americans who have diabetes are not only at a 2 to 6-fold higher risk of having an acute ischemic stroke but also suffer from poor recovery and greater physical and cognitive disability. Furthermore,

diabetic patients are more likely to bleed into the brain (hemorrhagic transformation, HT), especially with the use of tissue plasminogen activator (tPA), which remains to be the only pharmacological treatment for ischemic stroke. The lack of understanding on how increased bleeding occurs and influences the restorative and regenerative processes within the neurovascular networks hindered the development of new therapeutic strategies for stroke. While clinically it is known that women have poorer functional outcomes, the inadequate inclusion of female animals coupled with the limited use of diabetic models in preclinical stroke research has further deepened this gap.

For neurorestorative strategies to be effective and facilitate functional recovery, a supportive microenvironment is needed. Undoubtedly, cerebral microvasculature is a key component of this reparative & restorative niche. Our early studies in male diabetic animals showed that a) there is robust pathological neovascularization of the brain in a lean and moderate model of type 2 diabetes, b) a reperfusion injury superimposed on this pathology amplifies HT and worsens neurological deficits without increasing infarct size, and c) in the presence of HT, toll like receptor TLR4, a major mediator of innate inflammatory response, is upregulated in the ischemic region and especially in cerebral microvasculature. While testing the hypotheses that any form of bleeding into the brain is detrimental for stroke recovery and excess iron attenuates neurovascular restoration via the activation of TLR-4, an administrative supplement enabled us to incorporate female animals to our ongoing studies in male rats. Our exciting results suggest that 1) young diabetic female rats lose the neuroprotection typically seen in control female animals and develop greater HT than in controls and even diabetic male rats; 2) matrix metalloprotease (MMP)-3, an enzyme known to cause HT and to be regulated by TLR4, is increased to a greater degree in cerebral microvessels of female diabetic rats; 3) while male diabetic animals show significant loss of cerebrovasculature by activation of multiple cell death pathways in the recovery period, female diabetic animals do not, but rather undergo phenotypic changes in endothelial cells resembling endothelial-mesenchymal transition, EndMT, a process associated with scarring and impaired healing, and 4) in the long-term, diabetes worsens sensorimotor and cognitive recovery in both sexes.

Thus, our current knowledge of the impact of diabetes on cerebrovascularization in both sexes will be reviewed with a focus on stroke injury and recovery.

13: SESSION 8: MALE-SPECIFIC CARDIOVASCULAR, RENAL AND METABOLIC COMPLICATIONS

13.1

ANDROGENS IN CARDIOVASCULAR HEALTH AND DISEASE

Jane F. Reckelhoff[1]

[1]**Department of Cell and Molecular Biology, University of Mississippi Medical Center, Jackson**

Androgens have differential effects whether in men or women. For example, most chronic diseases, such as heart disease, obesity, chronic kidney disease, cancer, cause reductions in androgen levels in men. What is not clear is whether the reduction in androgens in men leads to chronic diseases or whether men with chronic diseases experience reduced androgen levels since there have been no serial longitudinal studies in men to address this question. In obese male animals, for example, androgen supplements reduce body weight, increase activity, improve insulin resistance with reductions in plasma insulin and leptin, decrease hyperlipidemia, reduce inflammation with reductions in cytokine levels. But androgen supplements increase blood pressure in the males. In female animals, androgen supplements increase food intake and body weight, increase leptin, cause insulin resistance and hyperlipidemia, and inflammation. Androgen supplements in females also cause elevated blood pressure. Elevated levels of androgens in women are symptomatic of polycystic ovary syndrome. In both women and female with increased androgen levels, reproductive issues are prevalent. If female animals become pregnant, their male offspring have reductions in plasma testosterone, but female offspring do not develop symptoms of polycystic ovary syndrome or hyperandrogenemia. The mechanisms by which androgens cause increases in blood pressure in males and females are likely different and are the focus for future research. These studies are supported by NIH R01HL66072, R01HL69194, P01HL55971, P20GM121334, and R01HL135089.

13.2

GROUP IV CYTOSOLIC PHOSPHOLIPASE A_2A IS REQUIRED FOR 6B-HYDROXYTESTOSTERONE MEDIATED ANGIOTENSIN II INDUCED HYPERTENSION IN MALE MICE

Ajeeth Pingili[1], Purnima Singh[2], Chi Young Song[2], Ji Soo Shin[2], Joseph Bonventre[3], Kafiat Malik[2]

[1]Medicine, University of Tennessee HSC, [2]Pharmacology, University of Tennessee HSC, [3]Renal Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School

Previously we showed that cytochrome P450 (CYP) 1B1-testosterone derived metabolite 6β-hydroxytestosterone (6β-OHT), by acting as a permissive factor, contributes to the development of angiotensin II (Ang II)-induced hypertension in male mice. Also, we reported that Ang II-induced hypertension is mediated by group IV cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$) activation, resulting in arachidonic acid (AA) release, and generation predominantly of eicosanoids with pro-hypertensive effects. This study was performed to investigate the interaction of CYP1B1 and cPLA2α/AA system by testing the hypothesis that 6β-OHT contributes to Ang II-induced hypertension by promoting $cPLA_2\alpha$ activation and generation of eicosanoids with pro-hypertensive effects. Male intact or castrated (Cas) *Cyp1b1*$^{+/+}$/$cPLA_2\alpha^{+/+}$, *Cyp1b1*$^{-/-}$, and $cPLA_2\alpha^{-/-}$ mice (8 weeks old, n=4-5) were infused with Ang II (700 ng/kg/min) and injected with 6β-OHT (15 μg/g, i.p. every 3rd day), for 2 weeks, and systolic blood pressure (SBP) was measured by tail cuff. In *Cyp1b1*$^{+/+}$/$cPLA_2\alpha^{+/+}$ mice, castration or CYP1B1 gene disruption minimized the Ang II-induced increase in SBP (127±3 and 148±3 vs. 188±3 mmHg, respectively, P<0.05). Ang II infusion in 6β-OHT, but not its vehicle (DMSO, 50ml) treated *Cyp1b1*$^{-/-}$mice increased SBP (189±5 vs.148±3 mmHg, P<0.05); this increase was minimized by the AA metabolism inhibitor, 5,8,11,14-eicosatetraynoic acid (25 mg/kg, i.p. every 3rd day) (140±4 mmHg, P<0.05). Ang II infusion with 6β-OHT treatment increased SBP in Cas $cPLA_2\alpha^{+/+}$mice, but not in Cas $cPLA_2\alpha^{-/-}$mice (176±7 vs. 122±2 mmHg, P<0.05). Treatment with antagonists of prostaglandin (PG) E2 receptors EP1 (SC19220) and EP3 (L-798106) (28 μg/g, s.c. every 2nd day) attenuated the Ang II-induced increase in SBP in 6β-OHT treated Cas $cPLA_2\alpha^{+/+}$ mice (123±4, 123±6 vs. 189±5 mmHg, respectively, P<0.05). These data suggest that 6β-OHT contributes to Ang II-induced increase in SBP via $cPLA_2\alpha$ activation, the release of AA and generation of eicosanoids, most likely PGE2 that exerts pro-hypertensive effects by stimulating EP1 and EP3 receptors. Therefore, the development of agents that selectively inhibit the $cPLA_2\alpha$ activity or block EP1 and EP3 receptors could be useful in treating hypertension and its pathogenesis.

13.3

PRETREATMENT WITH LOW DOSE LIPOPOLYSACCHARIDE ATTENUATES MEDULLARY CONGESTION IN MALE WKY FOLLOWING ACUTE KIDNEY INJURY.

Sarah Ray[1], G. Ryan Crislip[1], Riyaz Mohamed[1], Bansari Patel[1], Katie Wilson[1], Jinging Sun[1], Paul O'Connor[1]

[1]Physiology, Augusta University

Acute kidney Injury (AKI) is a sudden loss of renal function which can result in future complications or mortality, and prevalence of AKI continues to increase, specifically in hospitalized patients. Ischemia-reperfusion is a well-accepted experimental technique of AKI in rodents. Renal medullary congestion has been demonstrated to augment ischemia reperfusion (IR) in rodent models (1) and we have recently reported a sex difference in recovery from AKI, in which medullary congestion and indices of kidney function are worse in males than females at 7 days post-IR. As low grade inflammation can promote rouleaux, we hypothesized that prior exposure to LPS would worsen medullary congestion and augment AKI following IR. To test this hypothesis, we examined the effect of pretreatment with incrementing doses of lipopolysaccharide (LPS) on renal congestion following ischemia reperfusion. Male Wistar-kyoto rats (WKY, 9wks) were treated with 10 (n=3), 100 (n=3), 1000 (n=3) μg/kg LPS or control (saline, n=3) for 7 days (i.p), and then subjected to a 30 minute warm, bilateral ischemia reperfusion. Rats were allowed 24h to recover then anesthetized and humanely sacrificed. The right kidney was taken for histology and stained using Gomori's Trichrome Stain (Thermo 87020). Blood was collected by tail vein at baseline, at days 2 and 7, and at sacrifice. C-reactive protein (CRP, Thermo ERCRP), a marker of inflammation, increased for all groups from baseline to post-IR (p_{time} <0.0001), with control treatment having the greatest increase and highest levels of CRP, and 1000ug/kg LPS demonstrating the lowest levels CRP ($p_{interaction}$=0.09), at sacrifice. In difference to our hypothesis, outer Medullary peritubular congestion (blinded scoring) showed LPS pre- treatment reduced congestion when compared to saline treated controls (% congestion: control=80%, 10 and 100ug/kg LPS=40%, 1000ug/kg LPS= 20%). We conclude that, despite promoting inflammation, paradoxically, prior low dose LPS exposure prevents red blood cell congestion in the outer-medulla following IR. As we have recently reported that peristaltic contractions of vasa recta pericytes may prevent RBC congestion in the renal outer-medulla (2), we speculate that LPS activation of toll-like receptors of vasa recta pericytes may prime pericytes to contract preventing congestion. Further investigation of these mechanisms may lead to novel therapeutic approaches to prevent AKI.

1. Hellburg PO, Bayati A, Kallskog O, Wolgast M. Red cell trapping after ischemia and long-term kidney damage.

Influence of Hematocrit. *Kidney Int* 37: 1240-1247. 1990. Doi:10.1038/ki.1991.107

2. Crislip CR, O'Connor PM, Wei Q, Sullivan JC. Vasa recta pericyte density is negatively associates with vascular congestion in the renal medulla following ischemia reperfusion in rats. *Am J Physiol Renal Physiol* 313: F1097-F1105. 2017.

13.4

ANDROGEN INFLUENCE ON RENAL FIBROSIS ASSOCIATED WITH PYELONEPHRITIS

Teri Hreha[1], Christina Collins[1], Allyssa Daugherty[1], David Hunstad[1,2]

[1]Pediatrics Infectious Disease, Washington University in St. Louis, [2]Molecular Microbiology, Washington University in St. Louis

Renal scarring after pyelonephritis is linked to long-term health risks for hypertension and chronic kidney disease. Testosterone signaling through the androgen receptor increases susceptibility to, and severity of, uropathogenic *Escherichia coli* (UPEC) pyelonephritis in both male and female mice (1), while anti-androgen therapy is protective against severe UTI (2). Mice with severe pyelonephritis develop renal fibrosis and scarring (3). This work elucidates the molecular mechanisms of renal fibrosis in androgenized female C3H/HeN and C57BL/6 mouse backgrounds, and determines how these pathways are altered by the presence of testosterone. C3H/HeN mice feature vesicoureteral reflux (VUR), which allows for severe pyelonephritis and widespread renal fibrosis. C57BL/6 mice do not have VUR, but still exhibit alterations in renal fibrosis markers and display scarring following upper-tract UTI. We demonstrate that renal fibrosis after pyelonephritis involves both the TGF-β/Activin A and Hedgehog pathways, with altered local expression of proteins in the Smad family, TGFβ1, Activin A, and Gli1. Elevated circulating testosterone levels drive Ly6C+ monocyte recruitment to the kidney in the uninfected state and upon urinary tract inoculation with UPEC. Our results are consistent with a model in which testosterone increases recruitment of Ly6C+ monocytes, and that these cells are activated in the presence of UPEC during renal infection, driving local expression of pro-inflammatory and pro-fibrotic markers and thereby promoting fibrosis and renal scar formation.

1. Olson PD, Hruska KA, Hunstad DA. Androgens enhance male urinary tract infection severity in a new model. J Am Soc Nephrol 2016; 27: 1625-1634.PMC4884108.

2. Olson PD, McLellan LK, Liu A, Briden KL, Tiemann KM, Daugherty AL, Hruska KA, Hunstad DA. Renal scar formation and kidney function following antibiotic-treated murine pyelonephritis. Dis Model Mech 2017; 10: 1371-1379.PMC5719254.

3. Olson PD, McLellan LK, Hreha TN, Liu A, Briden KE, Hruska KA, Hunstad DA. Androgen exposure potentiates formation of intratubular communities and renal abscesses by *Escherichia coli*. Kidney Int 2018; in press.

13.5

HYPOGONADISM IN MALES: ONE SIZE DOES NOT FIT ALL

Licy Yanes Cardozo[1]

[1]Cell and Molecular Biology and Medicine, University of Mississippi Medical Center

In recent years, we have evaluated the effect of androgens on cardiometabolic risks factors in diverse male rodent models. We reported that long-term testosterone supplementation in obese male Zucker rats causes an increase in blood pressure; despite improvement of other cardiovascular risk factors, such as dyslipidemia, insulin resistance and inflammation. Also, we reported that testosterone upregulates intrarenal angiotensin system in an animal experimental model of salt sensitive hypertension. More recently, we observed a differential effect on blood pressure of testosterone supplementation in young versus old male spontaneously hypertensive rats. The negative effect of endogenous androgens in the cardiovascular system had been reported in other animal experimental models of hypertension. However, in clinical studies performed with hypogonadal males, the role of endogenous and exogenous testosterone upon cardiovascular system remains still unclear. The recent 2018 Clinical Guideline for "Testosterone Therapy in Men With Hypogonadism" from the Endocrine Society states that testosterone replacement is contraindicated in hypoganadal men with uncontrolled heart failure, myocardial infarction or stroke within the last 6 months. In specific populations such as in subjects with Klinefelter syndrome or Diabetes, testosterone have clear beneficial effects on cardimetabolic risk factors. More research is needed to elucidate the complex effects of testosterone upon cardiometabolic risk factors across different populations

*Indicates Invited Speaker

*Indicates Invited Speaker

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Special Abstract Issue

THURSDAY, OCTOBER 25TH, 2018

Plenary Lecture

1.0 **PLENARY LECTURE**
SPONSORED BY THE JOURNAL OF EXPERIMENTAL BIOLOGY

Thur., 5:00 -6:00 PM, Astor Ballroom

5:00 PM **1.1** Tesla Valves and Other Fluidic Devices in Reptile Lungs
CG Farmer. *Univ. of Utah*

6:00 PM **OPENING RECEPTION**

Thur., 6:00 -8:00 PM, Grand Gallery

FRIDAY, OCTOBER 26TH, 2018

Oral

2.0 **HIBERNATION AND DAILY TORPOR: ABSTRACT DRIVEN SESSION – 1**

Fri., 9:00 - 10:30 AM, Astor Ballroom I & II

Chair: **Frank van Breukelen.** *Univ. of Nevada*

9:00 AM **2.1** Extreme Physiological Plasticity in a Hibernating Basoendothermic Mammal, *Tenrec Ecaudatus*
Frank van Breukelen. *Univ. of Nevada*

9:30 AM **2.2** Hibernation and Daily Torpor in Neotamias Cinereicollis
Ana Fabio Braga. *Northern Arizona Univ.*

9:45 AM **2.3** Biologging and Endocrinology: Tools to Understand the Physiological Limits of Free-Living Arctic Ground Squirrels
Victor Zhang. *Northern Arizona Univ.*

10:00 AM **2.4** Anoxia-Reoxygenation Does Not Alter Mitochondrial Function in Ground Squirrels During Hibernation
Leah Hayward. *Univ. of Western Ontario*

10:15 AM **2.5** Effects of PH and Temperature on Blood Oxygen Transport in Hibernating and Non-Hibernating Rodents
Anne B. Kim. *Univ. of British Columbia*

Oral

3.0 **CARDIOVASCULAR: ABSTRACT DRIVEN SESSION – 1**

Fri., 9:00 - 10:30 AM, Astor Ballroom III

Chair: **Todd Gillis.** *Univ. of Guelph*

9:00 AM **3.1** Powering a Zombie Heart: Metabolic Fuel Utilization in the Excised Hagfish Heart During Anoxia Exposure
Todd Gillis. *Univ. of Guelph*

9:30 AM **3.2** The Functional Significance of Plasma-Accessible Carbonic Anhydrase for Cardiovascular Oxygen Transport in Teleosts
T. S. Harter. *Univ. of British Columbia*

9:45 AM **3.3** Convergent Evolution of Reduced Temperature Dependent Hemoglobin-Oxygen Affinity in Regionally Endothermic Fishes
Phillip R. Morrison. *Univ. of British Columbia*

10:00 AM **3.4** Hemoglobin Adaptations to High Altitude Augment Arterial O_2 Saturation in Hypoxia but not Aerobic Capacity in Deer Mice (*Peromyscus Maniculatus*)
Oliver Wearing. *McMaster Univ.*

10:15 AM **3.5** Cardiac Performance of Juvenile Red Drum (Sciaenops Ocellatus) During Acute Hypoxia and the Effect Following Crude Oil Exposure.
Derek Nelson. *Univ. of North Texas*

Oral
4.0 **OSMOREGULATION: ABSTRACT DRIVEN SESSION – 1**

Fri., 9:00 - 10:30 AM, Toulouse A & B

Chair: **Ana Lyons.** *Univ. of California, Berkeley*

9:00 AM **4.1** Role of the Aquaporin Gene Family in Conferring Tolerance to Multiple Environmental Stressors in Tardigrades
Ana Lyons. *Univ. of California, Berkeley*

9:15 AM **4.2** The Septate Junction Protein Mesh Is Required for the Form and Function of Drosophila Malpighian Tubule
Sima Jonusaite. *Univ. of Utah*

9:30 AM **4.3** The Impact of Salt Contaminated Freshwater on the Physiology of the Rectum and Malpighian Tubules of Mayfly (*Hexagenia Rigida*) Nymphs
Fargol Nowghani. *York Univ.*

9:45 AM **4.4** A Novel Technique for Measuring Hindgut Reabsorption in Drosophila Reveals Adaptive Differences Between Species with Different Thermal Tolerance
Mads Kuhlmann Andersen. *Aarhus Univ.*

Oral
5.0 **DEVELOPMENTAL PHYSIOLOGY: ABSTRACT DRIVEN SESSION**

Fri., 9:00 - 10:30 AM, St. Charles Ballroom

Chair: **Jason Breves.** *Skidmore College*

9:00 AM **5.1** Cortisol and Estrogenic Compounds Modulate Insulin-Like Growth-Factor Binding Protein Gene Expression During Vulnerable Life Stages of Atlantic Salmon
Jason Breves. *Skidmore College*

9:30 AM **5.2** Intestinal Hydrolase Transcriptional Responses During Rapid Diet Adjustment in Nestling House Sparrows (*Passer Domesticus*)
William Karasov. *Univ. of Wisconsin-Madison*

9:45 AM **5.3** Developmental Variation in Embryos of Two Killifish Populations
Lindsey Daniel. *Univ. of North Texas*

10:00 AM **5.4** Transgenerational Epigenetic Inheritance Induced by the Combined Exposure to Crude Oil and Hypoxia in the Zebrafish
Naim Martinez. *Univ. of North Texas*

10:15 AM **5.5** Hypoxic Incubation Has No Effect on Permeabilized Cardiac Muscle Mitochondrial Oxygen Flux or ROS Production in the American Alligator
Edward Dzialowski. *Univ. of North Texas*

10:30 AM **COFFEE BREAK**

Oral
6.0 **CONNECTING GENOMES TO PHENOMES TO POPULATIONS**
COSPONSORED BY THE AMERICAN PHYSIOLOGICAL SOCIETY AND THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

Fri., 11:00 AM -1:00 PM, Astor Ballroom I & II

Chair: **Allyson Hindle.** *Massachusetts General Hospital*

11:00 AM **6.1** Sex Dependent Phenological Plasticity in an Arctic Hibernator
C. Loren Buck. *Northern Arizona Univ.*

11:30 AM **6.2** Insights Into Mutational Pathways of Biochemical Adaptation Using Ancestral Protein Resurrection
Jay Storz. *Univ. of Nebraska*

12:00 PM **6.3** The Genomic and Physiological Basis of Rapid Adaptation to Temperature in a Globally Invasive Crab
Carolyn Tepolt. *Woods Hole Oceanographic Institution*

12:30 PM **6.4** Modeling Photoperiodism in Subterranean Rodents
Gisele Oda. *Universidade de São Paulo, Instituto de Biociências*

Oral
7.0 **VERTEBRATE ENERGETICS: ABSTRACT DRIVEN SESSION**

Fri., 11:00 AM-12:30 PM, St. Charles Ballroom

Chair: **Matthew Pamenter.** *Univ. of Ottawa*

11:00 AM **7.1** Digging Up the Evolutionary Origins of Hypoxia-Tolerance: Physiological Adaptations to Acute Hypoxia in 9 Species of African Mole Rats
Matthew Pamenter. *Univ. of Ottawa*

11:30 AM **7.2** Hummingbird Daily Energy Expenditure Allometry: Is Bigger Better?
Anusha Shankar. *Stony Brook Univ.*

12:00 PM **7.3** The Cost of Good Parenting: Altered Maternal Care in High Altitude Deer Mice, *Peromyscus Maniculatus*
Cayleih Robertson. *McMaster Univerity*

12:15 PM **7.4** Colonizing High Altitude Hypoxic Environments: Strategies to Deal with Metabolic Needs
Christian Arias-Reyes. *Institut universitaire de cardiologie et de pneumologie de Québec, Centre Hospitalier Universitaire de Québec (CHUQ), Faculty of Medicine, Université Laval*

Oral
8.0 **VENTILATORY FUNCTION: ABSTRACT DRIVEN SESSION**

Fri., 11:00 AM-1:00 PM, Toulouse A & B

Chair: **Mark Bayley.** *Aarhus Univ.*

11:00 AM **8.1** Learning to Air Breathe; the First Steps
Mark Bayley. *Aarhus Univ.*

11:30 AM **8.2** Characterizing the Branchial Hypercarbia Recovery Mechanisms Following Extreme Hypercapnia in the Highly CO_2 Tolerant Hagfish
Greg Goss. *Univ. of Alberta*

12:00 PM **8.3** Developmental Changes in the Ventilatory Response to CO_2 in Semi-Fossorial Mammals
Ryan Sprenger. *Univ. of British Columbia*

12:15 PM **8.4** Haemoglobin Adaptations to High Altitude Alter Breathing Pattern in Deer Mice (*Peromyscus Maniculatus*)
Catherine Ivy. *McMaster Univ.*

12:30 PM **8.5** The Effect of Chronic Hypercapnic Incubation on Breathing Patterns in American Alligator (*Alligator Mississippiensis*)
Justin Conner. *Univ. of North Texas*

12:45 PM **8.6** Effects of Gravidity on Grasshopper Oxygen Delivery
Scott Kirkton. *Union College*

Oral
9.0 **THERMAL BIOLOGY: ABSTRACT DRIVEN SESSION -1**

Fri., 11:00 AM-12:45 PM, Astor Ballroom III

Chair: **John VandenBrooks.** *Midwestern Univ.*

11:00 AM **9.1** Oxygen Limitation of Thermal Tolerance Varies Depending on the Life Stage and Behavior of Terrestrial Organisms
John VandenBrooks. *Midwestern Univ.*

11:3 0AM **9.2** Ultra-Violet B Radiation, the Often Neglected Ubiquitous Environmental Stressor in Aquatic Environments
Craig Franklin. *The Univ. of Queensland*

12:00 PM **9.3** Understanding the Effects of Food Availability, Thermal Tolerance, and Sirtuin Activity on the Feeding Physiology of Mytilus Californianus
Melissa May. *California Polytechnic State Univ.*

12:15 PM **9.4** A Distal Bat Wing Muscle Operates at Low Temperature in Vivo, and Has Low Thermal Sensitivity of Contractile Properties
Andrea Rummel. *Brown Univ.*

12:30 PM **9.5** Assessing Summertime Thermoregulatory Properties Across the Pelage Molt in a Polar Pinniped: The Weddell Seal
Skyla Walcott. *Univ. of Alaska, Anchorage*

1:00 PM **LUNCH ON YOUR OWN**

10.0 CANCELED

Concurrent
11.0 COMPARATIVE INSIGHTS INTO ANIMAL RESPONSES TO HYPOXIA AND ANOXIA

Fri., 2:00 – 4:00 PM, Astor Ballroom I & II

Chair: **Jon Harrison.** *Arizona State Univ.*

2:00 PM **11.1** Role of the Mitochondrion in Low Oxygen Signalling in the Painted Turtle.
Leslie Buck. *Univ. of Toronto*

2:30 PM **11.2** Re-Oxygenation Resilience - The Other Aspect of the Crucian Carp's Anoxia Tolerance
Sjannie Lefevre. *Univ. of Oslo*

3:00 PM **11.3** Learning from the Experts: How Marine and Freshwater Bivalves Cope with Anoxic Transgression
Doris Abele. *Alfred Wegener Institute for Polar and Marine Research*

3:30 PM **11.4** Flies Are Not Turtles or Carp: Non-Conventional Anoxia Tolerance
Jacob Campbell. *USDA-ARS*

Concurrent
12.0 MICRORNAS IN COMPARATIVE AND EVOLUTIONARY PHYSIOLOGY

Fri., 2:00 – 4:00 PM, St. Charles Ballroom

Chair: **Julie Reynolds.** *Ohio State Univ.*

2:00 PM **12.1** For Everything There Is a Season: MicroRNA Regulation of Insect Diapause
Julie Reynolds. *Ohio State Univ.*

2:15 PM **12.2** The Role of MiRNA Regulation on Phenotypic Responses to Environmental Stressors in Fish
Paul Craig. *Univ. of Waterloo*

2:30 PM **12.3** A Cool Story: Non-Coding RNAs in Natural Models of Cold Adaptation
Pier Jr Morin. *Université de Moncton*

3:00 PM **12.4** Regulation of MicroRNA Activity to Promote Multipotent Cell Fate During Dauer Diapause
Xantha Karp. *Central Michigan Univ.*

3:30 PM **12.5** A Network of MicroRNAs and RNA Binding Proteins Acts Maternally to Regulate Sex Determination in the C. *Elegans* Embryo
Katherine McJunkin. *National Institutes of Health*

Concurrent
13.0 THE ROLE OF GASOTRANSMITTERS IN HYPOXIC AND CHALLENGING ENVIRONMENTS

Fri., 2:00 – 4:00 PM, Toulouse A & B

Chair: **Michael Tift.** *Univ. of California, San Diego*

2:00 PM **13.1** Introduction to Gasotransmitters and the Role of Carbon Monoxide (CO) in Hypoxia-Tolerant Species
Anthony Signore. *Univ. of Nebraska, Lincoln*

2:30 PM **13.2** Roles of NO and H_2S Signaling in Hibernators
Angela Fago. *Aarhus Univ.*

3:00 PM **13.3** Carbon Monoxide Signaling in the Control of Breathing and Impacts for High-Altitude Adaptation
Erica Heinrich. *Univ. of California, San Diego*

3:30 PM **13.4** Hydrogen Sulfide and Oxygen Sensing: From Evolution to Function
Kenneth Olson. *Indiana U Sch Medicine South Bend*

Concurrent
14.0 **THE ROLE OF THERMAL PERFORMANCE CURVES IN PHYSIOLOGY, ECOLOGY AND CONSERVATION**
SPONSORED BY THE SOCIETY OF EXPERIMENTAL BIOLOGY

Fri., 2:00 – 4:00 PM, Astor Ballroom III

Chair: **Johannes Overgaard.** *Aarhus Univ.*

2:00 PM **14.1** The Thermal Performance Curve: Mechanisms, Applications, and Pitfalls for a Concept that Just Turned 40 (Happy Birthday!)
Michael Angilletta. *Arizona State Univ.*

2:30 PM **14.2** Translating Physiology to Fitness Using Thermal Performance Curves
Timothy Clark. *Deakin Univ.*

3:00 PM **14.3** Assessing the Role of Acclimation and Adaptation in Thermal Performance Curves
Johannes Overgaard. *Aarhus Univ.*

3:30 PM **14.4** Comparing Thermal Performance Curves Across Traits: How Consistent Are They?
Vanessa Kellermann. *Monash Univ.*

Poster
15.0 **POSTER SESSION 1: ODD NUMBERED POSTER PRESENTATIONS**

Fri., 4:00 – 6:00 PM, Grand Ballroom

Board #

1 **15.1** Mapping Key-Words in Our Journal to Predict the Future of Comparative Physiology
Tobias Wang, Jens Peter Andersen, Flemming Skov. *Aarhus Univ.*

3 **15.2** Mechanisms Underlying Forelimb Vs. Hindlimb Function During Terrestrial Locomotion in Juvenile Alligators
Adrien A. Arias, Emanuel Azizi. *Univ. of California, Irvine*

5 **15.3** Effect of PIT Tagging on Aerobic Metabolism and Growth of the Gulf Killifish, Fundulus Grandis
Jessica Reemeyer, Brennalyn LeMaire, Bernard Rees. *Univ. of New Orleans*

7 **15.4** The Effects of Body Mass on Immune Cell Concentrations of Terrestrial Mammals
Cynthia Downs, Ned Dochtermann, Ray Ball, Kirk Klasing, Lynn Martin. *Hamilton College; North Dakota State Univ.; Zoo Tampa; Univ. of California, Davis; Univ. of South Florida*

9 **15.5** Sequence Analysis, Expression, and Preliminary Functional Characterization of Aedes Aegypti Sodium-Dependent Cation-Chloride Cotransporters
Christopher M. Gillen, Grace F. Riley, John C. Crow, Adrienne C. DeBrosse, Mary E. Sawyer, Megha Kalsi, Peter M. Piermarini. *Kenyon College; The Ohio State Univ.*

11 **15.6** Hypoxia Avoidance Behavior in Two Air-Breathing Fishes
Corey Jew. *Univ. of California Irvine*

13 **15.7** Parasitic Infection-Associated Resemblance Between Locomotor Muscles of Dragonflies and Obese Vertebrates
Rudolf Schilder. *Penn State Univ.*

15 **15.8** Beneficial Effects of Fluctuating Thermal Regimes in the Alfalfa Leafcutting Bee, Megachile Rotundata
Kendra Greenlee, George Yocum, Joseph Rinehart, Julia Bowsher. *North Dakota State Univ.; USDA-ARS Red River Valley Agricultural Research Center*

17 **15.9** Intraspecific Variation in Thermal, Hypoxia and Acute High PH Tolerance in Rainbow Trout
Nicholas Strowbridge, Patricia Schulte. *Univ. of British Columbia*

19 **15.10** Adenosine A_1 Receptor Agonist-Induced Hibernation: Effects of Agonist and Seasons on Neuronal Pathways
Carla Frare, Mackenzie Jenkins, Kelly Drew. *Univ. of Alaska, Fairbanks*

21 **15.11** Characterization of the HIF-1 Pathway in Response to an Acute Heat Stress in Antarctic Notothenioid Fishes
Anna Rix, Kristin O'Brien. *Univ. of Alaska, Fairbanks*

23 **15.12** Variation in Thermoregulation and Linking Whole Organism Behavior to Thermosensory Neurophysiology in the Porcelain Crab, Petrolisthes Cinctipes
Emily Lam, Alex Gunderson, Brian Tsukimura, Jonathon Stillman. *Univ. of California, Berkeley; Tulane Univ.; California State Univ., Fresno; San Francisco State Univ.*

25 **15.13** Bone Composition of an Elite Mammalian Diver, the Weddell Seal: Implications for the Use of Bone as a Buffer
Katrina Theiss, Allyson Hindle, Daniel Warren. *St. Louis Univ.; Massachusetts General Hospital, Harvard Medical School*

27 **15.14** Identification and Characterization of a Sodium/Hydrogen Exchanger in Coral: A Potential Role in Biomineralization
Mikayla Ortega, Angus Thies, Martin Tresguerres. *Scripps Institution of Oceanography, Univ. of California, San Diego*

29 **15.15** Cortisol Mediates Claudin-28b Abundance and Its Contribution to Model Gill Epithelium Barrier Properties Via the Mineralocorticoid Receptor
Dennis Kolosov, Scott Kelly. *York Univ.*

31 **15.16** Osmotic Activation of Motility and Expression of Aquaporin Proteins in Sperm from the Gray Treefrog Dryophytes Chrysoscelis
Deja Miller, David Goldstein, James Frisbie. *Wright State Univ.*

33 **15.17** Does the Capacity for Seasonal Plasticity Differ Between Aquatic and Terrestrial Life-History Stages in the Eastern Newt (*Notophthalmus Viridescens*)?
Patrick Mineo, Roxanne Siuda. *Elmhurst College*

35 **15.18** Plastic Plasticity: Phenotypic Plasticity at One Time Scale Changes Plasticity at Another Time Scale in Tigriopus Californicus
Timothy Healy, Ronald Burton. *Univ. of California San Diego*

37 **15.19** Diversification of Characteristics Related to Endothermy in Thunnus Tunas
Barbara Block, Adam Ciezarek, Owen Osborne, Oliver N. Shipley, Edward J. Brooks, Sean Tracey, Jaime McAllister, Luke Gardner, Michael J.E. Sternberg, Vincent Savolainen. *Stanford Univ.; Imperial College London; The Cape Eleuthera Institute; Univ. of Tasmania*

39 **15.20** Hypoxia-Induced Oxidative Stress in Fundulid Killifish
Ryan Hoffman, Brittney Borowiec, Chelsea Hess, Graham Scott, Fernando Galvez. *Louisiana State Univ.; McMaster Univ.*

41 **15.21** Integration of Endocrinology, Behavior and Body Temperature of the South-American Tegu Lizard Salvator Merianae
Lucas Zena, Danielle Dillon, Kathleen Hunt, Carlos Navas, Kênia Bícego, C. Loren Buck. *Northern Arizona Univ.; Univ. of São Paulo*

43 **15.22** Hot and Dry: Effects of Heat Waves and Water Limitation on Metabolic and Evaporative Water Loss Rates
Jordan Glass, Sugjit Singh, Zachary Stahlschmidt. *Univ. of the Pacific*

45 **15.23** Skeletal Muscle Thermoregulation and Metabolic Control in Hibernating Arctic Ground Squirrels
Moriah Hunstiger, Jishnu Krishnan, Jace Rogers, S. Ryan Oliver. *Univ. of Alaska, Fairbanks*

47 **15.24** Fat to the High-Altitude Fire: Thermoregulation in Deer Mice
Sulayman Lyons, Grant McClelland. *McMaster Univ.*

49 **15.25** Modeling Energy Use of Overwintering Hatchling Turtles Using Over a Decade of Nest Temperatures
Tim Muir, Dat Tran, Lawrence Catalan, Marguerite Bednarek, Andrew Sward. *Augustana College*

51 **15.26** Regulation of Muscle Pyruvate Dehydrogenase in High Altitude Deer Mice
Soren Coulson, Grant McClelland. *McMaster Univ.*

53 **15.27** d-Amphetamine Exposure to Early Embryonic Zebrafish Reveal Neural and Developmental Consequences
Lisa Ganser, Brad Serpa, Jenn Bullard. *Kennesaw State Univ.*

55 **15.28** Developmental Oxygen Preconditions Cardiovascular Response to Acute Hypoxic Exposure and Maximal B-Adrenergic Stimulation of Anesthetized Juvenile American Alligators (*Alligator Mississippiensis*)
Brandt Smith, Janna Crossley, Ruth Elsey, James Hicks, Dane Crossley. *Univ. of North Texas; Louisiana Wildlife & Fisheries; Univ. of California, Irvine*

57 **15.29** Chronic Crude Oil Exposure Affects Physiology and Sexual Differentiation to Zebrafish (*Danio Rerio*)
Karem Vazquez Roman, Naim Bautista, Amelie Crespel, Warren Burggren. *Univ. of North Texas; Univ. of Glasgow*

59 **15.30** Development and Characterization of a Primary Cultured Model of the Larval Sea Lamprey (*Petromyzon Marinus*) Gills
Chun Chih Chen, Mike P. Wilkie, Scott P. Kelly. *York Univ.; Wilfrid Laurier Univ.*

63 **15.32** Participation of Orexin Receptor-1 in the Modulation of Respiratory Motor Activity in the Bullfrog (*Lithobates Catebeianus*)
Elisa Fonseca, Tara Janes, Stéphanie Fournier, Luciane Gargaglioni, Richard Kinkead. *Laval Univ. - Centre de Recherche de l'Institut Universitaire De Cardiologie et Pneumologie de Québec; Univ. of São Paulo State*

65 **15.33** Does the Spotted Gar, Lepisosteus Oculatus, Express a Functional Endothelial Nitric Oxide Synthase?
Melissa Cameron, Shigehiro Karaku, Susumu Hyodo, John Donald. *The Univ. of Sydney; RIKEN Center for Biosystems Dynamics Research (BDR); The Univ. of Tokyo; Deakin Univ.*

67 **15.34** Toxicity of Crude Oil Extracts in Chicken Embryos
Lara Amaral-Silva, Maria Rojas-Antich, Benjamin Dubanski, Hiroshi Tazawa, Warren Burggren. *Univ. of North Texas*

69 **15.35** Cardiac Proteome Changes in the Western Painted Turtle in Response to Cold Acclimation and Anoxia
Claire Riggs, Daniel Warren. *Saint Louis Univ.*

71 **15.36** Biotinylation of Elephant Seal Blood to Determine RBC Lifespan and Total Blood Volume
Robby Boparai, Christina Blaul, Daniel Crocker, Judy St. Leger, Todd Schmitt, Scott Johns, Mark Fuster, Tatum Simonson, Michael Tift. *Univ. of California, San Diego; Sonoma State Univ.; Seaworld*

73 **15.37** Linking Genotypes to Phenotypes Reveals the Underlying Mechanisms of Intestinal Brushborder Remodeling in Snakes
Stephen Secor, Blair Perry, Todd Castoe. *Univ. of Alabama; Univ. of Texas, Arlington*

75 **15.38** Rapid Evolution of Starvation Resistance in Drosophila: Physiological and Molecular Mechanisms
Austin J. McKenna, Alaric Smith, Allen G. Gibbs. *Univ. of Nevada, Las Vegas*

77 **15.39** Testing the Functional Consequences of Genetic Variation in Insulin-Like Growth Factor 1 (IGF1) in Lizards Via Primary Culture Experiments
Amanda Clark, Abby Beatty, Tonia Schwartz. *Auburn Univ.*

79 **15.40** Modelling Human APOL 1 Variant Related Kidney Dysfunction in Guinea Pigs
Kolawole Ajiboye, William Nabofa. *Babcock Univ.*

81 **15.41** Metabolomic Profiles Reveal That Upregulation of Protein Degradation and Nicotinamide Pathways Are Linked with Successful Pregnancy in Weddell Seals
Michelle Shero, Amy Kirkham, Gregg Adams, Robert McCorkell, Jennifer Burns. *Woods Hole Oceanographic Institution; Univ. of Alaska Anchorage; Univ. of Saskatchewan; Univ. of Calgary*

83 **15.42** Evolution of Thermal Tolerance in Pumpkinseed Sunfish (*Lepomis Gibbosus*)
Brittney Borowiec, Reem Hashem, Derek Campos, Anna Rooke, Michael Fox, Vera Almeida-Val, Graham Scott. *McMaster Univ.; National Institute for Research of the Amazon; Trent Univ.*

85 **15.43** Calcium Transport Across the Placenta in a Placentotrophic Lizard: New Insights About Gestation
Yurany Nathaly Hernández Díaz, Francisca Leal, Martha Patricia Ramírez-Pinila. *Universidad Industrial de Santander; Univ. of Florida*

87 **15.44** A Role for Kisspeptin Receptor in the Pituitary Gonadotroph in Male Mice
Olubusayo Awe, Yaping Ma, Sheng Wu, Andrew Wolfe. *Johns Hopkins School of Medicine*

SATURDAY, OCTOBER 27TH, 2018

Oral
16.0 **HIBERNATION AND DAILY TORPOR: ABSTRACT DRIVEN SESSION – 2**

Sat., 9:00-10:30 AM, Astor Ballroom I & II

Chair: **Sylvain Giroud.** *Univ. of Veterinary Medicine Vienna*

9:00 AM **16.1** The Effect of Lipids on Hibernation and Cardiac Function
Sylvain Giroud. *Univ. of Veterinary Medicine Vienna*

9:30 AM **16.2** A Systems Level Approach Reveals Incomplete Caspase Cascade Function During Mammalian Hibernation
Michael Treat. *Univ. of Nevada*

9:45 AM **16.3** Changes in Protein Phosphorylation and Acetylation Correspond with Suppression of Mitochondrial Metabolism During Mammalian Hibernation
Katherine Mathers. *Univ. of Western Ontario*

10:00 AM **16.4** Perineuronal Nets Cover Parvalbumin-Positive Neurons in Ground Squirrel Cerebral Cortex
Christine Schwartz. *Univ. of Wisconsin-La Crosse*

10:15 AM **16.5** The Impacts of Snow Cover Variation Across Elevation on Overwintering Montane Insects
Kevin T. Roberts. *Univ. of California, Berkeley*

Oral
17.0 **MITOCHONDRIAL BIOLOGY: ABSTRACT DRIVEN SESSION**

Sat., 9:00-10:30 AM, Toulouse A & B

Chair: **Daniel Munro.** *Univ. of Ottawa*

9:00 AM **17.1** Digging Up the Mitochondrial Origins of Hypoxia-Tolerance in African Mole Rats
Daniel Munro. *Univ. of Ottawa*

9:30 AM **17.2** A Comparative Analysis of Mitochondrial Supercomplexes in Vertebrates
Amanda Bundgaard. *Aarhus Univ.*

9:45 AM **17.3** The Influence of Thyroid Hormone Manipulation on Cardiac Muscle Mitochondrial Function in Developing Chickens
Jessica Rippamonti. *Univ. of North Texas*

10:00 AM **17.4** Metabolic Underpinnings of Life History Allocations: Mitochondrial Function Is Fine-Tuned to Meet Divergent Energetic Demands in Two Species of Wing-Polymorphic Crickets
Lisa A. Treidel. *Univ. of California, Berkeley*

10:15 AM **17.5** Cellular Metabolism and Oxidative Stress as a Possible Determinant for Longevity in Small Breed and Large Breed Dogs
Ana Jimenez. *Colgate Univ.*

Oral
18.0 **OMIC RESPONSES TO STRESS: ABSTRACT DRIVEN SESSION**

Sat., 9:00-10:30 AM, St. Charles Ballroom

Chair: **Wes Dowd.** *Washington State Univ.*

9:00 AM **18.1** Physiological Mean-Variance Relationships Among Intertidal Mussels Depend on Environmental Context
Wes Dowd. *Washington State Univ.*

9:30 AM **18.2** Transcriptomic Responses to Low Salinity Among Locally Adapted Populations of Olympia Oyster, an Estuarine Foundation Species
Tyler Evans. *California State Univ. East Bay*

9:45 AM **18.3** Unexpected Natural Modification of Mt-DNA Alters Centenarian Bivalve Physiology and Ecology
Doris Abele. *Alfred Wegener Institute for Polar and Marine Research*

10:00 AM **18.4** Integrating the Effects of Food Availability and Sirtuins on Stress Tolerance to Multiple Levels of Biological Organization
Lars Tomanek. *California Polytechnic State Univ.*

10:15 AM **18.5** Evolution of Higher Rate of Living Leads to Enhanced Inducibility of Gene Expression: Evidence from Heat Shock Genes in Drosophila
Josh Alpern. *Queens Univ.*

Oral
19.0 **ENERGETICS: ABSTRACT DRIVEN SESSION**

Sat., 9:00-10:30 AM, Astor Ballroom III

Chair: **Timothy Healy.** *Univ. of California San Diego*

9:00 AM **19.1** Polygenic Mapping Reveals Genetic Associations with Variation in Routine Metabolic Rate in Fundulus Heteroclitus
Timothy Healy. *Univ. of California San Diego*

9:30 AM **19.2** Evolutionary Variation in Hypoxia Tolerance in Fundulidae Killifishes
Brittney Borowiec. *McMaster Univ.*

9:45 AM **19.3** Protein Turnover: A Biochemical Basis for Endogenous Variation in Growth and Energy Metabolism
Scott L. Applebaum. *Univ. of Southern California*

10:00 AM **19.4** Bioenergetics of Protein Metabolism Under Experimental Environmental Change
Francis Pan *Univ. of Southern California*

10:15 AM **19.5** Dietary Antioxidants and Flight Exercise Affect the Extent to Which Antioxidants are Delivered to the Mitochondria and How Female Birds Allocate Nutrients to Eggs
Scott McWilliams. *Univ. of Rhode Island*

10:30 AM **COFFEE BREAK**

Concurrent
20.0 **EVOLUTION OF PHENOTYPIC PLASTICITY IN PHYSIOLOGICAL SYSTEMS**
SPONSORED BY THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

Sat., 11:00 AM-1:00 PM, Astor Ballroom I & II

Chairs: **Graham Scott.** *McMaster Univ.*
Anne Dalziel. *Université Laval*

11:00 AM **20.1** Mechanisms Underlying Thermal Acclimation and Their Evolution
Frank Seebacher. *Univ. of Sydney*

11:30 AM **20.2** Evolution of the Acclimation Responses to Hypoxia and Cold in Deer Mice Native to High Altitudes
Graham Scott. *McMaster Univ.*

12:00 PM **20.3** Cold Adaptation Drives Evolution of Metabolic Plasticity in Drosophila Melanogaster
Caroline Williams. *Univ. of California, Berkeley*

12:30 PM **20.4** Evolution of Osmoregulatory Flexibility During Transitions Between Marine and Freshwater Habitats in Fishes
Andrew Whitehead. *Univ. of California Davis*

Oral
21.0 CARDIOVASCULAR: ABSTRACT DRIVEN SESSION – 2

Sat., 11:00 AM-1:00 PM, Astor Ballroom III

Chair: **Hans Malte.** *Aarhus Univ.*

11:00 AM **21.1** Assessing the Full Significance of the Bohr/Haldane Effect for Gas Exchange in the Tissues
Hans Malte. *Aarhus Univ.*

11:30 AM **21.2** Weddell Seals Selectively Limit Guanylyl Cyclase-Mediated Vasodilation: Implications for Perfusion of the Brain During Diving
Allyson Hindle. *Massachusetts General Hospital*

12:00 PM **21.3** Developmental Differences in Anoxia-Induced Gene Expression in the Heart of the Painted Turtle
Cornelia Fanter. *Saint Louis Univ.*

12:15 PM **21.4** The Influence of Cellular Stretch on Extracellular Connective Tissue Deposition in Cultured Trout Cardiac Fibroblasts
Elizabeth Johnston. *Univ. of Guelph*

12:30 PM **21.5** Re-Assessment of the Biochemistry of Metabolic Acidosis Using Metabolite and Reaction H^+ Coefficients Computed from Multiple Competitive Cation Binding
Robert Robergs. *Queensland Univ. of Technology*

Oral
22.0 MAMMALIAN MOLECULAR PHYSIOLOGY: ABSTRACT DRIVEN SESSION

Sat., 11:00 AM-12:30 PM, Toulouse A & B

Chair: **Jane Khudyakov.** *Univ. of the Pacific*

11:00 AM **22.1** Obesity-Related Gene Expression During Fasting in a Naturally Obese Marine Mammal
Jane Khudyakov. *Univ. of the Pacific*

11:30 AM **22.2** The Weddell Seal Skin Transcriptome Reflects Local Mechanisms in Endocrine Regulation of Molt
Amy Kirkham. *Univ. of Alaska, Fairbanks*

12:00 PM **22.3** Development of a Biomarker Panel of Chronic Stress in Free-Ranging Marine Mammals
Laura Pujade Busqueta. *Univ. of the Pacific*

12:15 PM **22.4** Insights Into the Structure-Function Relationships of I-Band Titin and Its Evolution Across Mammals
Jenna Monroy. *Claremont Colleges*

Oral
23.0 THERMAL BIOLOGY: ABSTRACT DRIVEN SESSION – 2

Sat., 11:00 AM-1:00 PM, St. Charles Ballroom

Chair: **Suzanne Currie.** *Acadia Univ.*

11:00 AM **23.1** Social Cues Can Push Amphibious Fish to Their Thermal Limits
Suzanne Currie. *Acadia Univ.*

11:30 AM **23.2** Developmental Changes in Oxygen Consumption and Hypoxia Tolerance in the Heat- and Hypoxia-Adapted Tabasco Line of the Nile Tilapia (*Oreochromis Niloticus*)
Warren Burggren. *Univ. of North Texas*

12:15 PM **23.4** Acute Thermal Tolerance, Not Hypoxia Tolerance, Affects the Temperature Sensitivity of Hypoxia Tolerance in Marine Fishes
Derek Somo. *The Univ. of British Columbia*

12:30 PM **23.5** Critical Windows in Rainbow Trout Embryos: Effects of Thermal Shifts on Survival, Growth and Oxygen Consumption
Christopher Melendez. *California State Univ. San Marcos*

1:00 PM **LUNCH ON YOUR OWN**

Concurrent
24.0 COMPARATIVE ASPECTS OF ACID-BASE REGULATION

Sat., 2:00 – 4:00 PM, St. Charles Ballroom

Chair: **Colin Brauner.** *Univ. of British Columbia*

2:00 PM **24.1** Evolutionary Patterns of Acid-Base Regulation in Vertebrates
Colin Brauner. *Univ. of British Columbia*

2:30 PM **24.2** Evolutionarily Conserved Mechanisms for Acid-Base Sensing
Martin Tresguerres. *Univ. of California San Diego*

3:00 PM **24.3** Bicarbonate-Sensing Soluble Adenylyl Cyclase in Fishes
Jinae Roa. *Univ. of British Columbia*

3:30 PM **24.4** The Alkaline Tide: Acid-Base Regulation During Digestion
Tobias Wang. *Aarhus Univ.*

Concurrent
25.0 EVOLUTION OF METABOLIC PROTEINS

Sat., 2:00 – 4:00 PM, Astor Ballroom III

Chairs: **Chris Moyes.** *Queen's Univ.*
Jeffrey Richards. *Univ. of British Columbia*

2:00 PM **25.1** Molecular Evolution of Cytochrome C Oxidase in Hypoxia Tolerant Fish
Gigi Lau. *Univ. of Oslo*

2:30 PM **25.2** Function and Evolution of Cellulase and Hemicellulase Enzymes Within Invertebrates That Do Not Consume Significant Amounts of Plant Cellulose
Stuart Linton. *Deakin Univ.*

3:00 PM **25.3** Evolutionary Phylogenomics of UCP1 and Sarcolipin: Key Players Underlying Adaptive Thermogenesis Across Eutheria?
Kevin L. Campbell. *Univ. of Manitoba*

3:30 PM **25.4** Evolution of Metabolic Proteins: Pyruvate Dehydrogenase in Anaerobiosis
Michael Berenbrink. *Liverpool Univ.*

Concurrent
26.0 **HARNESSING NATURALLY EVOLVED TORPOR TO BENEFIT HUMAN SPACEFLIGHT**
CO-SPONSORED BY THE SOCIETY OF EXPERIMENTAL BIOLOGY

Sat., 2:00 – 4:00 PM, Astor Ballroom I & II

Chairs: **Hannah Carey.** *Univ. of Wisconsin-Madison*
Matthew Regan. *Univ. of Wisconsin-Madison*

2:00 PM **26.1** Enhancing Metabolic Flexibility in Humans: Insights from Hibernation to Benefit Spaceflight
Hannah V. Carey. *Univ. of Wisconsin-Madison*

2:30 PM **26.2** Central Mechanisms of Torpor Induction
Matteo Cerri. *Univ. of Bologna*

3:00 PM **26.3** The Relationship Between Sleep and Torpor
Vladyslav Vyazovskiy. *Univ. of Oxford*

3:30 PM **26.4** The Spaceflight Environment
Jessica Meir. *NASA*

Concurrent
27.0 **PHYSIOLOGY FROM THE NEOTROPICS: RHYTHMS, TEMPERATURE AND SEASON**

Sat., 2:00 – 4:00 PM, Toulouse A & B

Chairs: **Kenia Cardoso-Bicego.** *Sao Paulo State Univ.*
Luciane Gargaglioni. *Sao Paulo State Univ.*

2:00 PM **27.1** Seasonal Physiology of a Hibernating and Facultative Endothermic Lizard
Kenia Bicego. *Sao Paulo State Univ.*

2:30 PM **27.2** Orexin in Ectotherms: Modulatory Role on Ventilation
Luciane H Gargaglioni. *Sao Paulo State Univ.*

3:00 PM **27.3** Temperature Effects on Cardiorespiratory Function in Amphibians and the Aplication of a Non-Invasive Methodology
Lucas Zena. *Univ. of São Paulo*

3:30 PM **27.4** Plasticity of 24h Body Temperature Rhythms in a South American Subterranean Rodent
Patricia Tachinardi. *Univ. of São Paulo*

Poster
28.0 **POSTER SESSION 2: EVEN NUMBERED POSTER PRESENTATIONS**

Sat., 4:00 – 6:00 PM, Grand Ballroom

Board #		
2	**28.1**	On the Dynamics of Actomyosin Binding **Dean Culver.** *Army Research Laboratory*
4	**28.2**	Effect of Substrate Compliance on Coordinated Landing in Rhinella Marina **Alex Duman, Emanuel Azizi.** *Univ. of California, Irvine*
6	**28.3**	The Interaction of Incompressible Fluid and Extracellular Connective Tissues in Lobster Muscle **David Sleboda, Caroline Wolek, Thomas Roberts.** *Brown Univ.*
8	**28.4**	Effects of Passive Integrated Transponder Tagging on Cortisol Release by the Gulf Killifish Fundulus Grandis **Jasmine Harris, Ariel Hernandez, Bernard Rees.** *Univ. of New Orleans*
10	**28.5**	On the Role of the Visual and Vestibular Systems in Stabilising Perching in Zebra Finches **Natalia Perez-Campanero, David Perkel, Graham Taylor.** *Univ. of Oxford; Univ. of Washington*
12	**28.6**	Research of Development of Adaptive Processes to Psycho-Emotinal Stress in Medical Students **Larysa Chernobay, Oksana Vasylieva.** *Kharkiv National Medical Univ.*
14	**28.7**	A Comparision of Thermal Performance Among Latitudinally Separated Populations of the Intertidal Barnacle Balanus Glandula **Sarah Gilman, Gordon Ober, Rhianon Rognstad, Maddy Bunnenberg-Ross, Juanita Man.** *Scripps College; Claremont McKenna College; Pitzer College*
16	**28.8**	Active and Passive Energetics of Thermoregulation from Thermoconformity to Partial Thermoregulation **Adam Parlin, Asgeir Bjarnason, Paul Schaeffer.** *Miami Univ.; Star Oddi*
18	**28.9**	Clocks and Meals Keep Mice from Being Cool **Steven Swoap, Vincent Van der Vinne, Mark Bingaman, David Weaver.** *Williams College; Univ. of Massachusetts Medical School*
20	**28.10**	Expression of TRP Channels in Notothenioid Fish **Julia York, Harold Zakon.** *Univ. of Texas at Austin*
22	**28.11**	Investigating Changes in Thermal Physiology in Response to a Gut Infection in the Dragonfly, Libellula Pulchella **Stanley Stupski, Rudolf Schilder.** *Pennsylvania State Univ.*
24	**28.12**	Stage-Specific Oxygen Limitation of Thermal Tolerance in Schistocerca Cancellata **Jacob P. Youngblood, John M. VandenBrooks, Michael J. Angilletta Jr.** *Arizona State Univ.; Midwestern Univ*
26	**28.13**	The Effect of Salinity on Expression of Aquaporins 1 and 5 in the Gastric Caecae of Aedes Aegypti Mosquito Larvae **Elia Grieco, Lidiya Misyura, Andrew Donini.** *York Univ.*

28 **28.14** Comparative and Functional Analysis of Na^{+}/Ca^{2+} Exchangers Across Nematodes
Damien O'Halloran, Vishal Sharma. *George Washington Univ.*

30 **28.15** Identification of the First Member of the Gap Junction Protein Family in the Protozoa Trypanosoma Cruzi, the Etiological Agent of Chagas Disease
Juan Güiza, Iván Barría, Francisco Solis, Valeska Molina, Pedro Zamorano, Jorge González, Jonathan Canan, Romina Sepulveda, Fernando González-Nil, Juan Carlos Sáez, José Luis Vega. *Antofagasta Univ.; Universidad Andrés Bello; Pontifica Universidad Católica de Chile*

32 **28.16** Trade-Offs in Reproduction and Regeneration in Anolis Lizards
Abby Beatty, David Mote, Tonia Schwartz. *Auburn Univ.*

34 **28.17** The Effect of Food Availability, Temperature and Sirtuin Inhibition on the Metabolic Rate of California Mussel Gill Tissue
Chessie Cooley-Rieders, Amanda Frazier, Tinh Ton That, Sarah Nancollas, Melissa May, Maria Christina Vasquez, Erin Flynn, Lars Tomanek, Anne Todgham. *Univ. of California, Davis; Cal Poly San Luis Obispo; Cal Poly San Luis Obispo.*

36 **28.18** The Importance of Tidal Acclimation in Assessing the Physiological Responses of the Intertidal Crab Carcinus Maenas to Emersion
Sarah Nancollas, Iain McGaw. *Univ. of California, Davis; Memorial Univ. of Newfoundland*

38 **28.19** Hypoxia and the Metabolic Phenotype in Daphnia
Kurtis Westbury, William Nelson, Christopher Moyes. *Queen's Univ.*

40 **28.20** Establishing an Index of Habitat Quality and Reproductive Success for the Northern Fur Seal
Gregory Merrill, Ward Testa, Jennifer Burns. *Univ. of Alaska Anchorage; NMFS Alaska Fisheries Science Center*

42 **28.21** Hypoxia Induces Differential Changes in Thermoregulation and Metabolic Rate Base on Body Size in the Bumblebee Bombus Impatiens
Sara Wilmsen, Edward Dzialowski. *Univ. of North Texas*

44 **28.22** Does Individual Variation in Heat Loss Influence Thyroid and Metabolic Responses to Cold?
François Vézina, Theunis Piersma, Olivier Chastel. *Université du Québec à Rimouski; NIOZ Royal Netherlands Institute for Sea Research; Centre National de la Recherche Scientifique*

46 **28.23** Does the Risk of Overheating Limit Maximum Rates of Energy Expenditure in Breeding Birds?
Gary Burness, Simon Tapper, Joe Nocera. *Trent Univ.; Univ. of New Brunswick*

48 **28.24** Evidence for the Influence of Triiodothyronin on Maximal Heat Production in Birds.
Emily Cornelius Ruhs, Theunis Piersma, Olivier Chastel, François Vézina. *Université du Québec à Rimouski; NIOZ Royal Netherlands Institute for Sea Research; Centre National de la Recherche Scientifique*

50 **28.25** Withdrawn

52 **28.26** Depressing Mitochondrial Function During Paradoxical Anaerobism Leads to an Alcoholic Fish
Stanley Hillyard, Martin Jastroch , Frank van Breukelen. *Univ. of Nevada Las Vegas; Stockholm Univ.; Univ. of Nevada*

54 **28.27** The Impact of Developmental Hypoxia on the Cardiovascular Chemoreflex in Embryonic Snapping Turtles (*Chelydra Serpentina*)
Kevin Tate, John Eme, Dane Crossley. *Texas Lutheran Univ. ; California State Univ. San Marcos; Univ. of North Texas*

56 **28.28** Scaling of Major Organs in Hatchling Female American Alligators (*Alligator Mississippiensis*)
John Eme, Cassidy Cooper, Andrew Alvo, Juan Vasquez, Sara Muhtaseb, Thomas Schmoyer, Susan Rayman, Ruth Elsey. *California State Univ. San Marcos; Louisiana Department of Wildlife and Fisheries*

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60 **28.30** Evolution of the Development of Respiratory Physiology in Deer Mice Native to High Altitude
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88 **28.44** Whole Genome De Novo Sequencing of the Atlantic and Pacific Bluefin Tuna Genomes
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SUNDAY, OCTOBER 28TH, 2018

Oral
29.0 **CLINICAL PHYSIOLOGY: ABSTRACT DRIVEN SESSION**

Sun., 9:00-10:30 AM, Astor Ballroom III

Chair: **Mirit Eynan.** *Israel Naval Medical Institute*

9:00 AM **29.1** Blood Glucose Levels and Hyperbaric Pressure in SOD2 Enzyme Knockdown Mice
Mirit Eynan. *Israel Naval Medical Institute*

9:30 AM **29.2** A Proposed Role for the Mammalian Dive Response in Sudden Unexpected Death in Epilepsy
Jose Vega. *Novant Health, Forsyth Medical Center*

9:45 AM **29.3** A Copmaritive Study of Pulmonary Slowly Adapting Receptors Between Rabbits and Rats
Ping Liu. *Univ. of Louisville*

10:00 AM **29.4** Contribution of Group II Metabotropic Glutamate Receptors in the Dorsal Medullary Neuronal Groups During Hypertension Development
Julia Chu-Ning Hsu. *Graduate School of Agricultural and Life Sciences, The Univ. of Tokyo*

10:15 AM **29.5** The Evolving Cholecystokinin 1 Receptor as a Unique G Protein-Coupled Receptor Permanently Activated by Singlet Oxygen (GPCR-PABSO)
Zong Jie Cui. *Beijing Normal Univ.*

Oral
30.0 **OSMOREGULATION ION REGULATION: ABSTRACT DRIVEN SESSION – 1**

Sun., 9:00-10:30 AM, St. Charles Ballroom

Chair: **Alexander Clifford.** *Univ. of Alberta*

9:00 AM **30.1** Going Against the Gradient: Active NH_4^{+} Excretion by the Ammonia Tolerant Hagfish (*Eptatretus Stoutii*)
Alexander Clifford. *Univ. of Alberta*

9:30 AM **30.2** Ammonia Transporter Expression and Distribution in Organs of Caribbean Subpopulations of the Mosquito, Aedes Aegypti, Collected from Freshwater and High Ammonia Habitats
Andrea Durant. *York Univ.*

9:45 AM **30.3** Potential Role of a Rh Channel in Delivery of Ammonium from Coral Host Cells to Their Endosymbiotic Algae
Angus Thies. *Scripps Institution of Oceanography*

10:00 AM **30.4** The Dual-Purpose Saltwater Mitochondria Rich (MR) Cell of Sea Lampreys (*Petromyzon Marinus*): An Organ of Osmoregulation and Ammonia Homeostasis
Michael Wilkie. *Wilfrid Laurier Univ.*

10:15 AM **30.5** Impact of Sugar Beet De-Icing Liquid on Salt and Water Balance in Mayfly Nymph, Hexagenia Limbata
Laura Ana Cuciureanu. *York Univ.*

Oral
31.0 DIGESTIVE PHYSIOLOGY: ABSTRACT DRIVEN SESSION

Sun., 9:00-10:30 AM, Astor Ballroom I & II

Chair: **Matthew Regan,** *Univ. of Wisconsin-Madison*

9:00 AM **31.1** Exploring How a Shifting Gut Microbiome May Influence the Hibernation Phenotype
Matthew Regan. *Univ. of Wisconsin-Madison*

9:15 AM **31.2** Unraveling the Complexity of Seasonal Phenotypic Flexibility in Small Birds Via Omics Integration
Bernard W.M. Wone. *Univ. of South Dakota*

9:30 AM **31.3** Withdrawn

9:45 AM **31.4** The Role of Microbial Symbionts in Bonnethead Shark Seagrass Digestion
Samantha Leigh. *Univ. of California-Irvine*

10:00 AM **31.5** Will Abalone Survive Climate Change? Comparative Digestive Physiology and the Effect of Temperature Stress on Abalone Across the Pacific Ocean
Alyssa Frederick. *UC Irvine*

10:15 AM **31.6** Quickly Becoming an Omnivorous Lizard: Interactions of Diet, Physiology, and Ecology Lead to Dynamic Changes in a Rapidly Evolving System
Beck Wehrle. *Univ. of California, Irvine*

10:30 AM **COFFEE BREAK**

Concurrent
32.0 CONDUCTING MECHANISTIC INVESTIGATIONS IN COMPARATIVE PHYSIOLOGY USING IN VITRO AND EX VIVO SYSTEMS

Sun., 11:00 AM-1:00 PM, Astor Ballroom I & II

Chair: **Jose Vazquez-Medina.** *Univ. of California, Berkeley*

11:00 AM **32.1** Induced Pluripotent Stem Cells from 13-Lined Ground Squirrels: To Learn “hibernation” in a Dish?
Jingxing Ou. *National Institutes of Health*

11:30 AM **32.2** Molecular Manipulations: The Power of Cell Culture for Defining Mechanisms of Anoxia Tolerance
Sarah Milton. *Florida Atlantic Univ.*

12:00 PM **32.3** Identifying Anti-Inflammatory Properties of Serum That Could Protect the Lungs of Deep- Diving Seals
Allyson Hindle. *Massachusetts General Hospital*

12:30 PM **32.4** Studying Natural Tolerance to Ischemia/Reperfusion Using Endothelial Cells Derived from Seals
Kaitlin Allen. *Univ. of California Berkeley*

Concurrent
33.0 INTEGRATING PHENOTYPES AND FUNCTIONAL GENOMICS TO UNDERSTAND MECHANISMS OF REMODELING AND GROWTH

Sun., 11:00 AM-1:00 PM, Toulouse A & B

Chairs: **Todd Castoe.** *Univ. of Texas Arlington*
Stephen Secor. *Univ. of Alabama*

11:00 AM **33.1** A New Perspective from Snakes on Conserved Vertebrate Stress and Growth Pathways Underlying Intestinal Regeneration
Todd Castoe. *Univ. of Texas Arlington*

11:30 AM **33.2** Using Natural Genomic Variation and Experimental Approaches to Understand the Function and Evolution of the Insulin and Insulin-Like Signaling Network in Reptiles
Tonia Schwartz. *Auburn Univ.*

12:00 PM **33.3** Transcriptome Dynamics in Hibernation: Cause or Consequence of Physiology?
Sandra L. Martin. *Univ. of Colorado School of Medicine*

12:30 PM **33.4** Cardiomyocyte Polyploidization Creates a Barrier to Heart Regeneration in Zebrafish
Juan Manuel González-Rosa. *Harvard Univ.*

Oral
34.0 THERMAL BIOLOGY: ABSTRACT DRIVEN SESSION – 3

Sun., 11:00 AM-1:00 PM, Astor Ballroom III

Chair: **Michael Dillon.** *Univ. of Wyoming*

11:00 AM **34.1** Geographic Variation in Bumblebee Thermal Tolerance: Implications for Past and Future Range Shifts
Michael Dillon. *Univ. of Wyoming*

11:30 AM **34.2** Defying the Temperature Size Rule in Flight: Bigger Bees Perform Better at Higher Temperatures
Meghan E. Duell. *Arizona State Univ.*

11:45 AM **34.3** One for All or All for One: Emergent Thermal Physiology of Ant Colonies Along Tropical Mountain Ranges
Kaitlin Baudier. *Arizona State Univ.*

12:00 PM **34.4** Simultaneous Stress: Effects of Hypoxia-Temperature Interactions on Mortality, Thermal Tolerance, and Transcriptome of Drosophila Melanogaster
Leigh Boardman. *Univ. of Florida*

12:15 PM **34.5** Why Insects Die at Low Temperature: Depolarization Mediated Ca^{2+} Overload Causes Cell Death in Locusta Migratoria
Jeppe Bayley. *Aarhus Univ.*

12:30 PM **34.6** How to Assess Drosophila Heat Tolerance: Unifying Static and Dynamic Tolerance Assays to Predict Heat Distribution Limits
Lisa Bjerregaard Jørgensen. *Aarhus Univ.*

12:45 PM **34.7** Evolution of Body Size Toward Temperature-Dependent Oxygen Conditions in 188 Rotifer Species
Aleksandra Walczynska. *Jagiellonian Univ.*

Oral
35.0 OSMOREGULATION ION REGULATION: ABSTRACT DRIVEN SESSION – 2

Sun., 11:00 AM-12:30 PM, St. Charles Ballroom

Chair: **Carol Bucking.** *York Univ.*

11:00 AM **35.1** The Role of the Pyloric Ceca in Ion Balance in Rainbow Trout: Integrating Across Techniques to Understand Active Calcium Transport
Carol Bucking. *York Univ.*

11:30 AM **35.2** Cellular Mechanism for Teleost Otolith Calcification, and Their Responses to Acid-Base Disturbances
Garfield Kwan. *Scripps Institution of Oceanography*

11:45 AM **35.3** No Water, No Problem: A Metabolomics Analysis of Desiccated Annual Killifish Embryos
Daniel Zajic. *Portland State Univ.*

12:00 PM **35.4** Distinct Ion Transport Properties in Airways of the Marsh Rice Rat (*Oryzomys Palustris*)
Leah Reznikov. *Univ. of Florida*

1:00 PM **LUNCH ON YOUR OWN**

Concurrent
36.0 ANIMAL INTESTINAL MICROBIOMES: COMMUNITY DIVERSITY AND SERVICES PROVIDED TO THE HOST

Sun., 2:00 – 4:00 PM, Toulouse A & B

Chairs: **Beck Wehrle.** *Univ. of California, Irvine*
Brian Trevelline. *Univ. of Pittsburgh*

2:00 PM **36.1** It's Not Easy Eating Green: The Importance of the Gut Microbiome in Facilitating Herbivory
Kevin Kohl. *Univ. of Pittsburgh*

2:30 PM **36.2** The Enteric Microbial Communities of Sharks, Fishes, Island-Dwelling Lizards, and Abalone: Dietary and Phylogenetic Considerations
Donovan German. *Univ. of California, Irvine*

3:00 PM **36.3** Host Genetic Background Contributes to Resistance to Microbiota Disruption and Host Development in an Evolution Model Organism
Kathryn Milligan-Myhre. *Univ. of Alaska, Anchorage*

3:30 PM **36.4** Gut Microbial Community Dynamics in Arctic Ground Squirrels: Microbially-Liberated Urea-Nitrogen Use Across the Annual Cycle of Hibernation and Activity
Khrys Duddleston. *Univ. of Alaska, Anchorage*

Concurrent

37.0 **COMPARATIVE PHYSIOMICS: SYSTEMS-LEVEL APPROACHES TO COMPARATIVE PHYSIOLOGY**
SPONSORED BY THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

Sun., 2:00 – 4:00 PM, Astor Ballroom I & II

Chair: **Jane Khudyakov.** *Univ. of the Pacific*

2:00 PM **37.1** Species-Specific Responses of Juvenile Rockfish to Elevated PCO_2 and Hypoxia
Cheryl Logan. *California State Univ. Monterey Bay*

2:30 PM **37.2** The Role of Small Noncoding RNAs in the Regulation of Metabolic Dormancy and Extreme Stress Tolerance
Jason Podrabsky. *Portland State Univ.*

3:00 PM **37.3** Using Proteomics to Investigate Regulation of Stress Tolerance by Sirtuins in Mytilus Mussel Congeners
M. Christina Vasquez. *Loyola Marymount Univ.*

3:15 PM **37.4** Metabolic Response to Stress in Marine Mammals
Cory Champagne. *National Marine Mammal Foundation*

3:30 PM **37.5** From Genome to Phenome: Exploiting 13-Lined Ground Squirrel "Omics" to Achieve a Deeper Understanding of Hibernation
Katharine Grabek. *Stanford Univ.*

Concurrent

38.0 **MANAGING FUEL METABOLISM UNDER LIMITED OXYGEN AND ENERGY SUPPLY**
SPONSORED BY THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

Sun., 2:00 – 4:00 PM, Astor Ballroom III

Chair: **Jean-Michel Weber.** *Univ. of Ottawa*

2:00 PM **38.1** Metabolic Suppression Mechanisms for Fasting and Hypoxia
Jean-Michel Weber. *Univ. of Ottawa*

2:30 PM **38.2** Now or Later: Differential Fates for Glucose and Fructose in a Nectarivore
Morag Dick. *Univ. of Toronto*

3:00 PM **38.3** Feeding the Machine at the Top of the Food Chain: A Carnivore Conundrum
Terrie Williams. *Univ. of California - Santa Cruz*

3:30 PM **38.4** Fuelling Locomotion and Thermogenesis in High Altitude Native Deer Mice
Grant McClelland. *McMaster Univ.*

Concurrent

39.0 **MECHANISMS OF CHANGE, PHYSIOLOGICAL RESPONSE TO ENVIRONMENTAL STRESSORS**
SPONSORED BY THE SOCIETY OF EXPERIMENTAL BIOLOGY

Sun., 2:00 – 4:00 PM, St. Charles Ballroom

Chairs: **Sarah Alderman.** *Univ. of Guelph*
Todd Gillis. *Univ. of Guelph*

2:00 PM **39.1** Sublethal Effects and Biomarkers of Crude Oil Exposure in Anadromous Fish
Sarah Alderman. *Univ. of Guelph*

2:30 PM **39.2** Physiological Responses to Social Stressors
Kathleen Gilmour. *Univ. of Ottawa*

3:00 PM **39.3** Exploring Thermal Physiology: Effects of Environmental Temperature in Embryonic to Larval Frogs and Juvenile to Adult Copepods
Casey A. Mueller. *California State Univ. San Marcos*

3:15 PM **39.4** Effects of Acute and Chronic Thermal Exposure on the Swimming Performance and Aerobic Scope of Sheepshead Minnows (*Cyprindon Variegatus*).
Amanda Reynolds Kirby. *Univ. of North Texas*

3:30 PM **39.5** Hypoxia and Ammonia Exposures Have Differential, Developmental-Stage Specific, and Long-Term Consequences on the Stress Response in Zebrafish
Nicholas Bernier. *Univ. of Guelph*

Oral
40.0 SCHOLANDER AWARD ORAL PRESENTATIONS

Sun., 4:00 – 6:00 PM, St. Charles Ballroom

Chair: **Lynn Hartzler.** *Wright State Univ.*

4:00 PM The Functional Significance of Plasma-Accessible Carbonic Anhydrase for Cardiovascular Oxygen Transport in Teleosts
T. S. Harter. *Univ. of British Columbia (**3.2**)*

4:15 PM The Septate Junction Protein Mesh is Required for the Form and Function of Malpighian Tubule
Sima Jonusaite. *Univ. of Utah (**4.2**)*

4:30 PM Polygenic Mapping Reveals Genetic Associations with Variation in Routine Metabolic Rate in *Fundulus Heteroclitus*
Timothy Healy. *Univ. of California San Diego (**19.1**)*

4:45 PM Metabolic Underpinnings of Life History Allocations: Mitochondrial Function is Fine-Tuned to Meet Divergent Energetic Demands in Two Species of Wing-Polymorphic Crickets
Lisa A Treidel. *Univ. of California, Berkeley (**17.4**)*

5:00 PM Going Against the Gradient: Active NH4 + Excretion by the Ammonia Tolerant Hagfish (*Eptatretus Stoutii*)
Alexander Clifford. *Univ. of Alberta (**30.1**)*

5:15 PM Evolutionary Variation in Hypoxia Tolerance in *Fundulidae* Killifishes
Brittney Borowiec. *Univ. McMaster (**19.2**)*

5:30 PM Convergent Evolution of Reduced Temperature Dependent Hemoglobin-Oxygen Affinity in Regionally Endothermic Fishes
Phillip R. Morrison. *Univ. of British Columbia (**3.3**)*

5:45 PM The Influence of Cellular Stretch on Extracellular Connective Tissue Deposition in Cultured Trout Cardiac Fibroblasts
Elizabeth Johnston. *Univ. of Guelph (**21.4**)*

AWARDS BANQUET
Sun., 7:00 – 9:00 PM, Astor Ballroom

Plenary Lecture

41.0 PLENARY LECTURE

Sun., 8:00 – 9:00 PM, Astor Ballroom

8:00 PM **41.1** Ecophysiology: Physiology Can Inform Ecology, and Ecology Can Inform Physiology
Raymond Huey. *Univ. of Washington, Seattle*

2018 APS INTERSOCIETY MEETING
COMPARATIVE PHYSIOLOGY: COMPLEXITY AND INTEGRATION
OCTOBER 25 – 28, 2018
NEW ORLEANS, LOUISIANA

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1: PLENARY LECTURE SPONSORED BY THE JOURNAL OF EXPERIMENTAL BIOLOGY

1.1

Tesla Valves and other Fluidic Devices in Reptile Lungs

CG Farmer[1,2]

[1]Biology, Univ. of Utah, [2]Zoology, Trinity College Dublin

The vertebrate respiratory system is one of the most diverse organ systems, but the reasons for this diversity are poorly understood. Among the most striking features of the respiratory system is the variation that exists in the conducting airways and patterns of airflow within the amniotes, especially among reptiles. The Classes Reptilia and Mammalia form a monophyletic group, the Amniota. The vast majority (79%) of the diversity of amniotes is found within the Reptilia, with birds comprising about 10,600 species and non-avian reptiles about 9,600. In mammals, the broncho-alveolar lung appears to be quite similar across all species, with a conducting airway that arborizes and tidal airflow. In contrast, the conducting airways and the path of gases within the lungs of birds and non-avian reptiles shows much greater variation due to the presence of aerodynamic valves. The lungs of crocodilians and a wide range of species of lizards have now been found to contain aerodynamic valves and one-way airflow. A preliminary study of a turtle, the red-eared slider, has revealed a diode that operates much like a Tesla valve. Given how rich in species and enormously diverse reptiles are in body form and life history, it is quite possible their lungs contain other fluid dynamic devices waiting to be discovered.

NSF IOS 1055080

2: HIBERNATION AND DAILY TORPOR: ABSTRACT DRIVEN SESSION - 1

2.1

Extreme physiological plasticity in a hibernating basoendothermic mammal, *Tenrec ecaudatus*

Frank van Breukelen[1], Michael Treat[1]

[1]School of Life Sciences, Univ. of Nevada

Malagasy common tenrecs, *Tenrec ecaudatus*, have many plesiomorphic traits and may represent a basal placental mammal. We established a laboratory population of *T. ecaudatus*. Tenrecs exhibit extreme plasticity in thermoregulation and metabolism, a novel hibernation form, variable annual timing, rapid growth independent of age, and remarkable reproductive biology. For instance, tenrec body temperature (T_b) may approximate ambient temperature to as low as 12°C even when tenrecs are fully active. Conversely, hibernating tenrecs may have T_bs of 28°C. During the active season, oxygen consumption may vary 25-fold with little or no changes in T_b. During the Austral winter, tenrecs are consistently torpid but the depth of torpor may be variable. A righting assay revealed that T_b contributes to but does not dictate activity status. Homeostatic processes are not always linked e.g. a hibernating tenrec experienced a ~34% decrease in heart rate while maintaining constant body temperature and oxygen consumption rates. This species may have as many as 32 young in a litter. Tenrecs may possess indeterminate growth and growth rates vary but young may grow ~40-fold in the 5 weeks until weaning. Despite all of this profound plasticity, tenrecs are surprisingly intolerant to extremes in ambient temperature (<8 or >34°C). We contend that while plasticity may confer numerous energetic advantages in consistently moderate environments, environmental extremes may have limited the success and distribution of plastic mammals.

2.2

Hibernation and daily torpor in *Neotamias cinereicollis*

Ana Fabio Braga[1], Loren Buck[1]

[1]Biology, Northern Arizona Univ.

Animals living in natural environments face seasonal variation of temperature, humidity, light, and other environmental variables. Endothermic animals are capable of producing sufficient endogenous heat which allows for constant body temperature (Tb), allowing certain degree of independence from fluctuation in environmental temperature. Nevertheless, some endotherms regulate their Tb for several hours or even weeks at a time significantly below euthermic levels. Decreased Tb lasting for less than 24h is classified as daily torpor whereas episodes lasting for more than 24h are classified as multiday torpor or hibernation. Western chipmunks (Neotamias) are represented by 23 species that inhabit very specific niches. *N. cinereicollis* occurs in coniferous forests at elevations between 1,950 and 3,440 meters of central and eastern Arizona, and central and southwest New Mexico. Little is known of activity patterns for this species, and it is not clear whether they display hibernation or even torpor. The aim of this study was to investigate torpor and hibernation patterns displayed by wild caught *N. cinereicollis* (n=6) in semi-natural conditions and relate these patterns with ambient temperature. Animals were implanted with temperature loggers (iButtons DS1922; Maxim; Dallas-TX) and kept in outdoor enclosures with water and food *ad libitum* from November 2017 to May 2018. During this period several environmental variables were recorded, including ambient temperature (HOBO U30; Onset; Bourne-MA). While not torpid, Tb=36.7±1.52ºC; during hibernation minimum Tb=1.55ºC. Half of the animals displayed bouts of hibernation and 33% displayed only daily torpor. A regression between Tb and ambient temperature was created for each individual. The slopes were analyzed in a t-test to access their deviation from 0. The obtained slopes for Tb and ambient temperature differed from 0

(p<0.001), indicating that ambient temperature can predict some of the variation in Tb. The next step of this study is to include other environmental variables (*i.e.*, rain, snow cover, and wind speed) to better understand the abiotic drivers of the observed patterns of hibernation and torpor. It is critical to understand the interaction of biological rhythms and the environment; changes in climate and resource availability are expected to be major stressors for organisms, potentially leading to species decline. Understanding the interaction between abiotic drivers and organismal physiology allows for development of predictions of species resilience and vulnerability to climate change.

Financial support: Center for Bioengineering Innovation, Northern Arizona Univ.

2.3

Biologging and endocrinology: tools to understand the physiological limits of free-living arctic ground squirrels

Victor Zhang[1], Cory Williams[2], Rupert Palme[3], Loren Buck[4]

[1]Biological Sciences, Northern Arizona Univ., [2]Biology & Wildlife, Institute of Arctic Biology, Univ. of Alaska Fairbanks, [3]Department of Biomedical Sciences & Biochemistry, Veterinary Medical Univ. Vienna, [4]Biological Department of Biological Sciences & Center for Bioengineering Innovation, Northern Arizona Univ.

All species have evolved mechanisms to anticipate and/or respond to predictable seasonal changes such as temperature, photoperiod and precipitation. However, beyond seasonal changes, animals also encounter daily unpredictable environmental stressors, e.g., predation, competition, and inclement weather. The HPA axis is a primary physiological mechanism that enables animals to cope with unpredictable environmental stressors and its action is manifest through acute elevations in "stress hormones", such as cortisol. Cortisol is a signaling molecule involved in the tradeoff between survival and reproduction, allowing an animal to navigate normal life-history processes despite changes to its environment (1). Currently, extremely little is known of the relationship between cortisol and specific behavioral outcomes in free-living mammals. This is likely due to the challenges associated with mammals in the wild that must be frequently trapped for study, as well as the fact that the magnitude of an adrenocortical response to a stressor varies depending on the time of year in seasonal animals (2). However, recent advances in biologging technologies now allow researchers to noninvasively sample the physiology and behavior of organisms at fine temporal resolution across their annual and daily cycles.

In this study, we used biologgers and field endocrine methods to detect and quantify seasonal patterns of activity and stress in free-living of arctic ground squirrels (*Urocitellus parryii*), providing a more comprehensive understanding of how cortisol plays a role modulating animal behavior in the context of seasonal environmental changes. Seven male and six female arctic ground squirrels were fastened with collars affixed with accelerometers and light loggers, which quantified above-ground overall dynamic body acceleration (ODBA), an index of activity-specific energy expenditure. In addition, we measured fecal cortisol metabolites (FCM) to non-invasively assess adrenocortical activity of squirrels across their active season. Female FCM concentrations were highest during the mid-lactation interval and lowest during post-lactation, while male FCM did not vary across the active season. Overall, males had higher baseline FCM levels than females across the year (male LS-mean = 6.18, SE = 0.01; female LS-mean=5.73, SE=0.09). Levels of above-ground activity were consistent with expectations of reproductive demand; activity levels of males were higher than females during the mating period and lower than in females during the mid-lactation period. Cold and wet weather, which is known to adversely affect conditions of thermal exchange, were associated with higher FCM and lower above-ground activity levels in both sexes. Furthermore, when temperatures were lowest in the early season, weather had the greatest effect on FCM concentrations, possibly due to reduced forage availability associated with early springtime in the Arctic. Lastly, we show that increases in cortisol secretion is associated with decreased above-ground activity and time spent above-ground in female, but not male, arctic ground squirrels. Our results suggest that female squirrels may respond to unpredictable environmental conditions by employing a "sit-and-wait" strategy, whereas males may have required more intense and/or prolonged environmental stressors than those observed for specific behavioral responses to become apparent. Collectively, this study furthers our understanding of how sex, reproductive state and environmental conditions may interact to influence behavioral choices, stress and physiology in free-living mammals.

Funding: This research was supported by Dr. Loren Buck's Northern Arizona Univ. start-up package.

References:

1. McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. Horm. Behav. 43: 2–15, 2003.

2. Romero LM. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocrinol. 128: 1–24, 2002.

2.4

Anoxia-reoxygenation does not alter mitochondrial function in ground squirrels during hibernation

Leah Hayward[1], Katherine Mathers[2], James Staples[1]

[1]Biology, Univ. of Western Ontario, [2]Physiology and Pharmacology, Univ. of Western Ontario

The hibernation phenotype is associated with tolerance to models of ischemia-reperfusion in tissues (specifically

liver[1] and brain[2]). Interestingly, hibernators such as the thirteen-lined ground squirrel exhibit reversible mitochondrial metabolic suppression during torpor-arousal cycling, which is characteristic of anoxia-tolerant animals[3]. We hypothesized that these mechanisms that allow for metabolic flexibility during hibernation also confer mitochondrial anoxia tolerance, thereby providing a mechanism for tissue ischemia-reperfusion tolerance. We isolated liver mitochondria from adult ground squirrels during the hibernation season (in torpor or interbout euthermia; IBE) and summer, as well as rats (non-hibernators). Using high-resolution respirometry and TPP^+ electrodes, we compared three performance metrics (membrane potential and state 3 and 4 respiration rates using both complex I and II-linked substrates) of mitochondria before and after 5 minutes of anoxic exposure. Anoxia decreased absolute state 3 respiration rates by ~30-50% in all groups following 5 minutes of anoxia. Decreases in state 3 are paralleled by decreases in V_{max} of complex I in summer squirrels and rats, and complex II in IBE squirrels and rats, but no such decreases were observed for torpid mitochondria. Absolute state 4 respiration rates increased in all groups following anoxic exposure; however, for any given initial state 4 respiration rate, final state 4 respiration was significantly higher in summer squirrels than in winter squirrels. Membrane potential in state 4 was maintained in winter squirrels, and depolarized in summer squirrels and rats following anoxic exposure. These data suggest greater anoxia tolerance in winter squirrels, especially in torpor. We will measure mitochondrial antioxidant capacity to elucidate mechanisms underlying these differences.

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2.5

Effects of pH and temperature on blood oxygen transport in hibernating and non-hibernating rodents

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Hibernators typically lower their body temperature to 1-2 °C above ambient temperatures during the winter. This greatly increases solubility of CO_2 in the blood. Furthermore, there is a mismatch between ventilation and metabolic rate suppression during entrance into torpor that leads to retention of CO_2 (hypoventilation). Together these changes result in an altered acid-base state in steady-state torpor. These changes in acid-base state and T_b influence the oxygen binding affinity of hemoglobin due to thermal sensitivity and the Bohr effect. To quantify the effects of temperature, pH change and hibernation state on blood oxygen transport, we constructed oxygen equilibrium curves (OECs) across the physiologically relevant temperature and pH ranges experienced by three species with different thermal strategies. We constructed OECs from whole blood of an obligate hibernator (13-lined ground squirrels; *Ictidomys tridecemlineatus*) during euthermia and hibernation, facultative hibernator (Golden Syrian hamsters; *Mesocricetus auratus*) during euthermia and multi-day torpor bouts and a non-hibernating rodent (Sprague Dawley rats; *Rattus norvegicus*) within their thermoneutral zone. Preliminary data suggest that the oxygen binding affinity of hemoglobin is inherently less temperature sensitive in species capable of torpor, in both euthermia and torpor. On-going experiments will allow us to elucidate how the two opposing factors of lowered temperature and lowered pH on hemoglobin's oxygen binding affinity contribute to metabolic rate suppression during torpor by reducing oxygen supply to the tissues. This research was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

3: CARDIOVASCULAR: ABSTRACT DRIVEN SESSION - 1

3.1

Powering a zombie heart: metabolic fuel utilization in the excised hagfish heart during anoxia exposure

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Pacific hagfish, *Eptatretus stoutii,* can recover from 36 hours of anoxia and the systemic hearts of these animals continue to work throughout the exposure. Recent work demonstrates that glycogen stores are utilized in the *E. stoutii* heart during anoxia but that these are not sufficient to support the measured rate of ATP production. One metabolic fuel that could supplement glycogen during anoxia is glycerol. This is because glycerol can be derived from lipid stores, stored in the

heart, or delivered via the blood. The purpose of this study was to determine if glycerol could be used to fuel the heart during anoxia exposure. When excised *E. stoutii* hearts, perfused with metabolite free saline (mf-saline), were exposed to anoxia for 12 hours, there was no difference in heart rate, pressure generation (max-dP), rate of contraction (max-dP/dt_{sys}), or rate of relaxation (max-dP/dt_{dia}) compared to hearts perfused with mf-saline in normoxia. However, hearts perfused with saline containing glycerol (gly-saline) in anoxia had higher max-dP, max-dP/dt_{sys}, and max-dP/dt_{dia} than hearts perfused with mf-saline in anoxia. When glycerol was added to the perfusate in normoxia, there were higher levels of tissue glycerol, however, there was no such increase when glycerol was added to the perfusate in anoxia. Anoxia exposure did not cause the release of fatty acids into the perfusate or affect the activities of triglyceride lipase, glycerol kinase, or glycerol-3-phosphate dehydrogenase. Together, these results suggest that glycerol is utilized by the hagfish heart during anoxia but it is not derived from stored lipids. This work was supported by a Discovery Grant, and a Discovery Accelerator Supplement, from the Natural Sciences and Engineering Research Council of Canada to T.E. Gillis.

3.2

The functional significance of plasma-accessible carbonic anhydrase for cardiovascular oxygen transport in teleosts

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A novel mechanism has recently been discovered in rainbow trout that enables an elevated partial pressure of oxygen (PO_2) in the red muscle of this species (4). Here we show the importance of the mechanism for cardiovascular O_2 transport *in vivo*, in salmonids and perhaps all teleosts. Most teleost species can actively regulate the intracellular pH (pH_i) of their red blood cells (RBC) by adrenergically stimulated sodium-proton exchangers (β-NHE) that create H^+ gradients across the RBC membrane. These H^+ gradients are short-circuited in the presence of plasma-accessible carbonic anhydrase (paCA) at the tissues, creating a large arterial-venous pH shift that greatly enhances O_2 unloading from pH-sensitive hemoglobin (Hb). Thus, we tested the hypothesis that teleosts increase the O_2 capacitance of their blood (β_b) by a metabolon of RBC pH_i regulation and a heterogeneous distribution of paCA, which has functional significance for O_2 transport *in vivo*.

After β-NHE short-circuiting at the tissues, RBC pH_i must recover during venous transit (30-90 s) to enable renewed O_2-loading at the gills, and only then can the system enhance β_b on a systemic level. Therefore, the halftimes ($t_{1/2}$) and magnitudes of β-NHE stimulation, short-circuiting with paCA and recovery of RBC pH_i, were assessed *in vitro*, on rainbow trout whole blood. Results indicate that: i) the $t_{1/2}$ of β-NHE short-circuiting is likely within the time of RBC capillary transit; ii) the $t_{1/2}$ of RBC pH_i recovery is 17 s and within the time of RBC venous transit; and iii) after short-circuiting RBCs re-establish the initial H^+ gradient across the membrane. These findings are in line with a system that can sustainably enhance β_b, with every pass through the circulation.

To validate the role of paCA in cardiovascular O_2 transport *in vivo*, Atlantic salmon swimming at a moderate speed (50% of maximal swimming speed) were injected with C18 (a membrane-impermeable CA inhibitor) while cardiac output ($\dot{Q}$) and the rate of O_2 consumption ($\dot{M}O_2$) were recorded. The inhibition of paCA in resting or swimming fish required a compensatory increase in $\dot{Q}$ of ~30% to maintain $\dot{M}O_2$ and swimming speed. At faster swimming speeds (~75% of maximal) fish collapsed after the inhibition of paCA. Therefore, the exercise performances typically observed in Atlantic salmon are not, even remotely, possible without the enhancement of β_b that is facilitated by paCA. Furthermore, the discovery of paCA in the heart lumen of coho salmon may indicate that β-NHE short-circuiting also facilitates cardiac O_2 supply in salmonids; a critical finding in species where reproductive success depends on the ability to increase $\dot{Q}$ during spawning migrations (2). We show, for the first time, a functional link between β-NHE and paCA activities, and the resulting increase in β_b that reduces the requirements on the salmonid heart by about a third; the system is recruited over a wide range of conditions, including rest, exercise, recovery from exercise, and was further enhanced following acclimation to hypoxia.

It appears that all salmonids and most teleosts meet the mechanistic requirements to enhance cardiovascular O_2 transport by β-NHE short-circuiting, namely: i) pH sensitive Hbs, ii) β-NHE activity, and iii) a heterogeneous distribution of paCA (1, 3, 5). Thus, actively creating and eliminating H^+ gradients across the RBC membrane may be an integral part of the teleost mode of cardiovascular O_2 transport, with important implications for future work on the physiology, the conservation and the evolutionary history, of nearly half of all vertebrates.

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3.3

Convergent evolution of reduced temperature dependent hemoglobin-oxygen affinity in regionally endothermic fishes

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Several lineages of pelagic predatory fishes have independently evolved the capacity for regional endothermy. Whereas most fishes lose metabolic heat to the environment when blood thermally equilibrates with water at the gills, endothermic fishes have evolved heat exchanging retia that cool venous blood before it reaches the gills, enabling localized retention of metabolic heat. This remarkable evolutionary convergence enables tuna, billfishes, Pacific smalleye opah (*Lampris incognitus*), lamnid sharks, and common thresher shark (*Alopias vulpinus*) to maintain select tissues warmer than ambient water, favouring physiological functions that may give these predators a competitive edge over their prey. Having warm tissues also causes blood temperature to rapidly change in regionally endothermic fishes, as much as 10°C or more between the gills and cranium of the swordfish (*Xiphias gladius*), which requires hemoglobin (Hb) to maintain function over a steep temperature gradient. Adaptive changes in Hb-O_2 affinity and its rapid change between the respiratory surface and the metabolizing tissues ensure matching between O_2 supply and demand. Because heme-oxygenation is exothermic, increasing temperature decreases Hb-O_2 affinity in most vertebrates, which could potentially disrupt O_2 transport and be maladaptive in regionally endothermic fishes. However, for endothermic tunas, lamnid sharks, and marlins, the effect of temperature on Hb-O_2 affinity is greatly reduced, which reduces premature Hb-O_2 offloading and may be associated with the evolution of heat exchanging retia. If this is correct, we propose that all regionally endothermic fishes should posses Hbs with reduced temperature sensitivities, but this has not been thoroughly investigated in swordfish, opah, or common thresher shark. To investigate this, we examined the effect of temperature on whole blood Hb-O_2 affinity in these species. Opah and common thresher shark Hb-O_2 affinities were independent of temperature, but the temperature dependence of swordfish Hb-O_2 affinity was pH dependent. At low blood pH (< 7.4) temperature had a negligible effect on swordfish Hb-O_2 affinity, whereas at higher blood pH there was no effect of temperature below 50% saturation but increasing temperature decreased Hb-O_2 affinity above 50% saturation. A reduced effect of temperature on Hb-O_2 affinity is mechanistically linked to oxygenation dependent release of allosteric effectors (i.e., hydrogen ions and organic phosphates), but the relative contributions of these effectors, and potentially the evolution of novel binding sites, differs among species. We have confirmed reduced effects of temperature on the Hb-O_2 affinities of three additional regionally endothermic fishes. Thus, the repeated evolution of endothermy in fishes seems to be associated with a remarkable functional convergence for a reduced effect of temperature on Hb-O_2 affinity, which may facilitate a heat exchanging function for retia.

3.4

Hemoglobin Adaptations to High Altitude Augment Arterial O_2 Saturation in Hypoxia But Not Aerobic Capacity in Deer Mice (*Peromyscus maniculatus*)

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Deer mice native to high altitude have evolved higher aerobic capacity ($\dot{V}O_2$max) in hypoxia than mice from low altitude, a trait that appears to be selectively advantageous in the cold hypoxic environment at high altitude. High-altitude deer mice also possess hemoglobin (Hb) adaptations that increase O_2 affinity, which could foreseeably contribute to these adaptive increases in $\dot{V}O_2$max by supporting higher arterial O_2 saturation (SaO_2). However, this relationship has never been tested in a way that disentangles the benefits of Hb adaptations from evolved differences in other cardiorespiratory traits. We examined this issue by hybridizing wild deer mice from high and low altitudes to produce an F_2 inter-crossed population, such that the effects of distinct globin haplotypes could be assessed on an admixed genetic background. Thermogenic $\dot{V}O_2$max and several traits that underlie it (breathing, pulmonary O_2extraction, SaO_2, and heart rate at $\dot{V}O_2$max) were measured before and after acclimation to hypobaric hypoxia (12 kPa O_2) for 6 weeks. As predicted, mice with highland globin haplotypes had higher blood-O_2 affinity and higher SaO_2 in hypoxia than those with lowland haplotypes, when comparing mice that were acclimated to normoxia. However, hypoxia acclimation reduced blood-O_2 affinity in mice with highland globin haplotypes, such that SaO_2 was then similar between haplotypes. Surprisingly, variation in SaO_2 had no effect on $\dot{V}O_2$max, and $\dot{V}O_2$max was similar across haplotypes. This suggests that Hb adaptations to high altitude do not underlie adaptive increases in $\dot{V}O_2$max, or that their adaptive benefit is contingent upon the evolution of other cardiorespiratory traits. Supported by NSERC of Canada.

3.5

Cardiac Performance of Juvenile Red Drum (*Sciaenops ocellatus*) During Acute hypoxia and the Effect Following Crude Oil Exposure.

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Red drum (*Sciaenops ocellatus*) are a near shore fish species found in the Gulf of Mexico that encounter both environmental and anthropogenic stressors. Prior studies of crude oil exposure have shown detrimental effects on multiple organisms at the level of the cardiovascular system. However, questions remain regarding the combine action of oil exposure and environmental stressors such as hypoxia. We investigate how convective oxygen transport is impacted following crude oil exposure by measuring cardiac output indices in juvenile red drum during a stepwise acute hypoxia challenge, six steps from normoxia (19.5 kPa O_2) to severe hypoxia (4 kPa O_2). Our working hypothesis was crude oil and hypoxia will result in greater negative effects of cardiovascular function that would be predicted due to a sum of these stressors alone. Baseline (n=7) heart rates during normoxia was 62 ± 2 bpm with a stroke volume of 0.97 ± 0.06 ml·kg^{-1} resulting in a cardiac output of 57.2 ± 8.3 ml·min^{-1}·kg^{-1}. Bradycardia (56 ± 1 bpm) was pronounced at moderate hypoxia (15.5 kPa O_2) with reduced cardiac output (47.1 ± 7.2 ml·min^{-1}·kg^{-1}) as both, heart rate and cardiac output, proceeded to decrease throughout the challenge until severe hypoxia (50 ± 2 bpm, 36.9 ± 5.7 ml·min^{-1}·kg^{-1}, respectfully). However, stroke volume remained constant until severe hypoxia (0.89 ± 0.06 ml·kg^{-1}). This study is ongoing and supported by the GoMRI RECOVER Consortium to A.R and D.C.

4: OSMOREGULATION: ABSTRACT DRIVEN SESSION - 1

4.1

Role of the Aquaporin gene family in conferring tolerance to multiple environmental stressors in tardigrades

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Members of the phylum *Tardigrada* are well known for their ability to survive a wide range of environmental extremes, yet the evolutionary history, diversity, and functional roles of tardigrade aquaporins—especially in relationship to various environmental stressors—remain unknown. Previous literature computationally identified ~10-11 putative aquaporins in three Eutardigrade species, yet no experimental work confirmed these proteins' function *in vitro* or *in vivo*. Here, we utilize additional computational and experimental methods to characterize an expanded number of tardigrade aquaporins and aquaporin-like proteins in *Hypsibius dujardini, Ramazzottius varieornatus* and *Milnesium tardigradum*. Tardigrade proteins with E-value of 1.0e-1 were aligned via MUSCLE and manually inspected for the presence of motifs important for known aquaporin function. Our computational analysis—alongside analysis of *de novo* modeling of predicted candidate aquaporin structures—suggests that there may be numerous additional unannotated and uncharacterized aquaporin-like proteins in these tardigrade species, spanning a variety of functional classes. Swelling assays of heterologously expressed tardigrade candidate aquaporins, conducted in *Xenopus* embryos, show patterns of permeability of these proteins. *In vivo* inhibition of candidate tardigrade aquaporins explores the importance of these proteins in conferring tolerance to various environmental stressors (desiccation, chill coma, osmotic stress), via a suite of stress-specific phenotypic assays. This study is among the first to examine a connection between a gene family (aquaporins) present in multiple species of Eutardigrades, alongside its functional role in promoting tolerance to multiple environmental stressors.

4.2

The septate junction protein mesh is required for the form and function of *Drosophila* Malpighian tubule

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Background/objective: Septate junctions (SJs) are specialized occluding cell-cell junctions that form paracellular diffusion barriers in the epithelia of invertebrates. In arthropods, there are two types of SJs: pleated SJs (pSJs) and smooth SJs (sSJs), found in ectodermally and endodermally derived epithelia, respectively. SJs are composed of transmembrane and cytoplasmic scaffolding proteins. In *Drosophila*, a number of pSJ-associated proteins have been identified, characterized and shown to be involved in epithelial barrier function. However, proteins of sSJs have received considerably less attention and many questions about their role in maintaining epithelial integrity and regulating paracellular solute transport remain open. Recently, an integral protein, mesh, has been identified within the sSJs of *Drosophila* midgut and Malpighian tubule epithelia, and shown to be required for the barrier function of the midgut. Here, we studied mesh in adult fly Malpighian tubules, which rely on regulated transepithelial ion and water transport to maintain internal homeostasis.

Methods: The GAL4-UAS system and a temperature sensitive GAL4 repressor, *tub-GAL80^{ts20}*, were used to achieve *mesh* knockdown throughout development or

during adulthood. Expression and localization of mesh and a scaffolding SJ protein, discs large (Dlg), in control and mutant tubules were determined using immunohistochemistry. Tubule ultrastructure was examined using electron microscopy. Transepithelial fluid and K^+ transport in the main segment of control and *mesh* knockdown tubules were measured using the Ramsay assay and K^+-specific electrode.

Results: The Malpighian tubules of an adult fly have four major morphologically and functionally distinct segments and are made up of the larger principal cells and smaller intercalated stellate cells. We found that developmental *mesh* knockdown in the tubule principal cells was associated with early lethality in adult flies. The tubules of these flies revealed defects in epithelial architecture, SJ organization and reduced junctional expression of Dlg. Furthermore, main segment transepithelial fluid secretion and K^+ flux was completely abolished in developmental principal cell *mesh* knockdown tubules and significantly reduced in tubules subjected to principal cell *mesh* knockdown in adulthood as compared to the control groups.

Conclusions and significance: The sSJ protein mesh is essential for the development and maintenance of a functional *Drosophila* Malpighian tubule epithelium. Loss of mesh has profound effects on both the tubule epithelial cell and SJ integrity and transepithelial ion and water transport. Although future studies will be required to unravel the functional role of mesh in regulating the paracellular permeability of *Drosophila* Malpighian tubules, our data support the notion that integral SJ proteins play an important role in insect ionoregulatory epithelia and homeostasis. Funding: NIH DK106350.

4.3

The impact of salt contaminated freshwater on the physiology of the rectum and malpighian tubules of mayfly (*hexagenia rigida*) nymphs

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Salt is a significant environmental contaminant of freshwater ecosystems and in northern temperate climates sodium chloride from winter road salting is a major contributor to this problem. This puts many freshwater (FW) animals at risk because they are normally osmoregulators that actively maintain solute levels in body fluids far in excess of their surrounding habitat. Our previous work has demonstrated functional changes at the gills of mayfly nymphs (*Hexagenia rigida*) in response to salt contaminated water (SCW). In this study the physiology and biochemistry of two other important osmoregulatory organs, the rectum and Malpighian tubules (MTs), were examined in nymphs exposed to SCW. Exposure to SCW elevated hemolymph ion levels and increased body water content, suggesting that nymphs are able to maintain ionoregulatory processes in SCW at a new steady state. In response to SCW, the rectum displayed a significant decline in K^+ absorption relative to FW control nymphs. Na^+-K^+-ATPase (NKA) and V-type H^+-ATPase (VA) activity in this region tended to decline in SCW but not significantly. Localization of basolateral NKA and apical VA along the MTs depicted a complex multicellular composition. MTs also exhibited a decline in K^+ absorption in response to SCW as well as a significant decrease in NKA and VA activity. Data provide insight into how FW mayfly nymphs regulate salt and water balance in response to the environmental problem of FW salination.

4.4

A novel technique for measuring hindgut reabsorption in *Drosophila* reveals adaptive differences between species with different thermal tolerance

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It has become increasingly clear that the ability to maintain osmotic and ionic balance at low temperature dictates cold tolerance in many insects, *Drosophila* included. In insects, maintenance of osmotic and ionic balance is primarily achieved through the secretory actions of the Malpighian tubules and the reabsorptive nature of the hindgut which act in synchrony to regulate ion and fluid balance in the hemolymph. Previous studies have used Ramsay assays to investigate inter- and intraspecific differences in the capacity of Malpighian tubules to regulate secretion at low temperature in *Drosophila*. These studies have revealed adaptive differences that support variation in cold tolerance among and within species but little focus has been directed towards the capacity of the hindgut. This shortage is mainly due to the lack of methods to study fluid and ion transport in small insects. Here, we present and discuss the use of a novel assay that allows for simultaneous measurements of net ion (Na^+ and K^+) and fluid reabsorption of the drosophilid hindgut and employ it in a comparative study on the underlying mechanism of *Drosophila* cold tolerance. Specifically, we study fluid and ion transport at high and low temperature in three *Drosophila* species with marked differences in cold tolerance. Preliminary results indicate that fluid reabsorption is similar across three *Drosophila* species at benign temperature and that the main ion reabsorbed is Na^+. In response to cold, we observed marked decreases in fluid reabsorption of all three species, but a larger degree of suppression in the least cold tolerant species. Interestingly, Na^+ reabsorption was supressed more in cold sensitive species and actually reversed (net Na^+ secretion) whereas cold tolerant species were able to better maintain Na^+ reabsorption while reducing K^+ reabsorption. These results indicate that our newly

developed assay is capable of detecting small differences in fluid and ion flux across the *Drosophila* hindgut and our result help to explain previous observations showing superior homeostatic capacity of cold adapted species.
From a methodological point of view this study introduces a novel assay that can deliver repeated measures of fluid and ion transport in the hindgut of small *Drosophila*. Further work is needed to optimize the protocol, but we are optimistic that this assay holds the potential to study reabsorptive processes in a similar manner as the Ramsay assay has been used in hundreds of studies to investigate the mechanisms of ion and fluid secretion in insects.

5: DEVELOPMENTAL PHYSIOLOGY: ABSTRACT DRIVEN SESSION

5.1

Cortisol and estrogenic compounds modulate *insulin-like growth-factor binding protein* gene expression during vulnerable life stages of Atlantic salmon

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The growth hormone (Gh)/insulin-like growth-factor (Igf) system is a major controller of growth and development in vertebrates, including teleost fishes. The biological activities of Igfs are modified via their interactions with Igf binding proteins (Igfbps). Anadromous Atlantic salmon (*Salmo salar*) express a broad suite of *igfbp* transcripts in key metabolic tissues throughout their early life history. The manner in which Igfbps are modulated by extrinsic and intrinsic factors during their vulnerable early life-stages remains unresolved. In a series of experiments, we examined the effects of cortisol, 17α-ethinylestradiol (EE_2), 17β-estradiol (E_2) and 4-nonylphenol (NP) on various freshwater life-stages of juvenile salmon. Parr implanted with cortisol for 14 days exhibited a dose dependent reduction in growth rate. Cortisol lowered hepatic *igf1* mRNA levels in accord with diminishing the anabolic actions of Igf1. Cortisol simultaneously stimulated hepatic *igfbp1b1* and *-1b2* mRNA levels as a means for their translated products to further attenuate the effects of Igf1. With respect to estrogenic compounds, exposures to EE_2 and NP for 21 days reduced *igf1* and *-2* mRNA levels in fry. EE_2 and NP reduced hepatic *igfbp1b1*, *-2a*, *-2b1*, *-4*, *-5b2* and *-6b1*, and stimulated *igfbp5a* mRNA levels. In smolts, a 4-day exposure to EE_2 diminished plasma Gh and Igf1 levels in parallel with a reduction in hepatic *igf1* mRNA. Consistent with patterns observed in fry, EE_2 and E_2 diminished hepatic *igfbp1b1*, *-4* and *-6b1*, and stimulated *igfbp5a* mRNA levels in smolts. Interestingly, while the *igfbp* system operated in a fashion to pause somatic growth when cortisol was elevated, the *igfbp* system seemingly acted in a fashion to counterbalance the catabolic effects of estrogenic compounds via their effects on Igf1/*igf1*. Collectively, our experiments reveal that hepatic *igfbps* are key modulators of the Gh/Igf system during the freshwater life-stages of Atlantic salmon.

5.2

Intestinal hydrolase transcriptional responses during rapid diet adjustment in nestling house sparrows (*Passer domesticus*)

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Many bird species increase intestinal hydrolase activity when exposed to an increase of the specific substrate in the diet. To increase understanding of the underlying mechanism(s), we studied nestling house sparrows (*Passer domesticus*) switched between high protein diet (63% protein/5% starch) and high starch diet (30% protein/38% starch). In nature, the nestlings undergo a natural switch from high protein insects to starch-containing seed diet during development. Both intestinal α-glucosidase (AG; maltase and sucrase) and peptidase (APN; amino peptidase-N) activity, and their respective mRNA, were induced by their respective substrates within 24 h of a diet shift - consistent with the hypothesis that altered gene expression occurs in mature enterocytes on intestinal villi. For AG, evidence indicated that induction of transcription began $\geq$18 h after diet switch, and incorporation of new protein and activity in the apical brush border membrane occurred in $\leq$6 h after that. For APN, evidence indicated that induction of transcription began as fast or faster than for AG. For both AG and APN, downward modulation of mRNA and activity appeared to begin within 18 h of a large reduction in their respective inducing agent. Overall, changes in intestinal hydrolase activity are so fast ($\leq$1 d) that nestlings could potentially track daily changes in resource availability with fine-tuned changes in digestive performance. Supported by NSF IOS-1354893.

5.3

Developmental Variation in Embryos of Two Killifish Populations

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The first 4 hours post-fertilization represent a critical time point in the developmental trajectory of teleosts. It is an overlooked source of phenotypic variation seen later in development. Such critical developmental events may be unique to a species, with variation in early development even occurring among populations within a species. Critical developmental time points drive the development in embryos of the killifish *Fundulus grandis*, as for other teleosts. Cell division of the blastocyst of *Fundulus* is temperature sensitive, so temperature-induced variation in cleavage rate, cell morphology, and Q_{10} may vary between fish populations.

This experiment targets cell stages in early embryos (fertilization to ~4 hours) from two distinct killifish populations. Patrick's Bayou (TX) killifish reside in a polluted area within the Houston Ship Channel and are resistant to dioxin-like compounds. Populations outside the Houston Ship Channel are non-resistant – i.e. are affected during development by DLC's (dioxin-like compounds). Accompanying these differences are a suite of biochemical and physiological differences, as well. To test the hypothesis that temperature influences critical time points of early killifish embryonic development and that the responses are population-dependent, fertilized killifish embryos from resistant and non-resistant populations were maintained in one of three different temperatures (22°C, 25°C, and 28°C). Repeated measures of each blastocyst cleavage per embryo tracked individual variation to the 64 cell stage.

Resistant populations of Gulf killifish at 22°C in non-polluted water showed a significantly ($p<0.01$) higher development rate of 13.5 cleavages/hour compared to the non-resistant population's cleavage rate of 13.2 cleavages/hour in non-polluted water, indicating intrinsic differences in cleavage rate associated with each population. Further, cleavage rate of the resistant population to 64 cells was significantly ($p<0.01$) less temperature sensitive (Q_{10} = 1.8) then that in the non-resistant population (Q_{10} = 2.8). The difference in Q_{10} values indicates significant differences in the developmental trajectory of the resistant vs. non-resistant population, as expressed by cleavage time to 64 cells.

Alterations in physiological and morphological phenotypes associated with exposure to different temperatures indicate that temperature alters initial developmental trajectory, as expected. Unexpected was that this fundamental biological process would vary between different killifish populations. Additional experiments are underway to determine the mechanism(s) for these differences.

5.4

Transgenerational Epigenetic Inheritance Induced by the Combined Exposure to Crude Oil and Hypoxia in the Zebrafish

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Organisms face constant selection pressures imposed by the interaction of natural and anthropogenic factors, and respond with phenotypes that influence offspring survival and fitness. However, the majority of previous experimental designs have considered only a single factor at a time, and their analysis and interpretation embrace only the generation that has been directly exposed. Usually not investigated are the potential effects on offspring generations. In fish, especially in the Gulf of Mexico, hypoxic conditions and exposure to crude oil may co-occur, However, how the all-important interactions between these environmental stressors affects parental and offspring populations are still poorly understood. Consequently, a multi-scale approach employing the zebrafish (*Danio rerio*) was used to test the chronic responses over 5 weeks of exposure in four parental experimental groups: 1) Control (normoxia, control diet); 2) Hypoxia (~60% DO, control diet); 3) Oil (normoxia, oil-loaded diet) and; 4) Hypoxia-Oil (~60% DO, oil-loaded diet). Offspring from each parental group was exposed to conditions similar to the parental exposures and their survival and performance were determined. Survival (~95%), growth rate, hypoxia resistance (LOE), and heart rate (~240bpm) did not significantly differ among parental groups, indicating that the adult populations were not excessively stressed despite combinations of hypoxia and oil exposure. However, global DNA methylation in adults was significantly decreased in heart tissue ($P<0.05$) but not in gonads. F_1 offspring obtained from parents exposed to hypoxia and oil conditions exhibited higher survival (up to 50%) compared with the control group when raised in hypoxia combined with crude oil conditions. However, regardless of parental exposure, F_1 exposed to oil and/or hypoxic conditions exhibited significant bradycardia in comparison with non-exposed larvae (~110 and ~180 bpm, respectively). Percentage of whole larvae Global DNA methylation was significantly decreased ($P<0.05$) in larvae from hypoxic-oil exposed parents in comparison with control and any of the two one-factor exposed parents. Although chronic exposure to environmental stressors in parental populations might not elicit phenotypic modifications, they do prompt signals to offspring populations via transgenerational epigenetic inheritance, likely helping offspring populations to survive while facing persistent environmental conditions. Epigenetic inheritance plays an important role in shaping phenotypic responses in offspring populations facing

changing environments. However, responses to specific factors may compromise individual performance when more than one stressor is present. Furthermore, considering the parental exposure-experience and also more than one factor in experimental designs will offer a more holistic approach to understand how organisms cope with environmental stressors.

5.5

Hypoxic incubation has no effect on permeabilized cardiac muscle mitochondrial oxygen flux or ROS production in the American alligator

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Alligator eggs developing in nest mounds have the potential to be exposed to hypoxic conditions during incubation. Here we investigated the effects of hypoxic incubation on development and cardiac muscle mitochondrial respiration and emission of reactive oxygen species in developing American alligator (*Alligator mississippiensis*). Alligator eggs were incubated in normoxia or 10% hypoxia at 30^{o}C. One group of animals was examined at 90% of incubation and another group of animals was allowed to hatch and develop for a year in normoxia before measurements were made. Mitochondrial respiration and ROS production under leak and oxidative phosphorylation states were measured in permeabilized cardiac muscle with high-resolution respirometry coupled with fluorometry. To examine the response of mitochondria to anoxia and reoxygenation, permeabilized cardiac muscles were exposed to 25 min of anoxia, followed by reoxygenation during measurement of mitochondria respiration and ROS production. Hypoxic incubation blunted growth of the embryos and these mass differences were maintained through the first year of life. In contrast, hypoxic incubation had no effect on mitochondria respiration or ROS production at either 90% of incubation or 1-year post hatching. After exposure to anoxia for 25 min, the rate of oxidative phosphorylation of permeabilized cardiac muscle returned to pre-anoxia levels. There was no change in ROS production observed upon reoxygenation of the permeabilized cardiac muscle. Our results suggest that hypoxic incubation has little influence on cardiac myocyte mitochondrial physiology in the developing alligator. This study was funded in part by NSF grant IOS 1146758 to EMD.

6: CONNECTING GENOMES TO PHENOMES TO POPULATIONS
COSPONSORED BY THE AMERICAN PHYSIOLOGICAL SOCIETY AND THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

6.1

Sex dependent phenological plasticity in an arctic hibernator

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Hibernation allows animals to conserve energy during predictable seasonal intervals of low resource availability. However, many organisms are now facing rapid changes in the temporal distribution of resources and their capacity to plastically adjust their timing of key seasonal life-cycle events, including hibernation and reproduction, is likely a critical aspect of individual and population resilience to climate change. For vertebrates, one of the most commonly reported consequence of climate change involves warmer spring temperatures leading to earlier timing, although climate-driven changes in precipitation patterns can also alter the temperature dependency of phenology. For example, in the Arctic, higher spring temperatures are associated with increased intensity and frequency of late season snow events and have resulted delayed environmental phenology. The degree to which mammals can prolong the expression of seasonal dormancy is unclear. We have shown that free-living arctic ground squirrels exhibit an endogenously programmed and sex specific chronology of annual cycle events with chronology and phenology of expression apparently independent of environmental cues (Buck and Barnes, 1999, Williams et al. 2016). Aided by biologging devices, here we show that arctic ground squirrels exhibit sex-dependent plasticity in the physiology and phenology of hibernation in response to late spring snowstorms (Williams et al., 2017). Females and non-reproductive males responded to a >1 month delay in snow melt by extending heterothermy or re-entering hibernation after several days of euthermy, leading to a 2-3 weeklong delays in reproduction. Extended hibernation consisted of repeated 2-3 day torpor bouts with a slightly elevated minimum body temperature as compared to minimum body temperatures measured during torpor in mid-hibernation. In contrast, reproductive males did not extend or re-enter hibernation. It is likely that reproductively mature males are unable to re-enter a torpid state because androgen secretion associated with seasonal gonadal recrudescence inhibits expression of torpor. Our findings reveal intriguing differences in how

males and females respond to climatic stressors, which may lead to phenological mismatches between the sexes. Funding was provided by the National Science Foundation, Integrative Organismal Systems under grant numbers 1558056 and 1147187 to CLB and CTW.

6.2

Insights into mutational pathways of biochemical adaptation using ancestral protein resurrection

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The step-by-step evolution of novel phenotypes is central to several fundamental questions in biology. In studies of novel protein functions, the problem becomes experimentally tractable if it is possible to identify and functionally characterize the complete set of causative mutations. With such a system, it is possible to address key questions: Do novel functions evolve via the successive fixation of beneficial mutations that each produce an adaptive change in phenotype when they first arise? Alternatively, are evolutionary transitions in protein function facilitated by neutral mutations that produce no adaptive benefit when they first arise, but which potentiate the function-altering effects of subsequent mutations? By reconstructing all possible mutational pathways that connect ancestral and descendant proteins it is also possible to address fundamental questions about the roles of contingency and determinism in protein evolution. For example: Can novel functions evolve from any possible ancestral starting point, or are specific evolutionary outcomes contingent on prior history? We address these questions by experimentally dissecting the molecular basis of biochemical adaptation. Using ancestral protein resurrection in conjunction with a combinatorial protein engineering approach based on site-directed mutagenesis, we examine the effects of sequential mutational steps in the evolution of novel functional properties in avian and crocodilian hemoglobins.

6.3

The genomic and physiological basis of rapid adaptation to temperature in a globally invasive crab

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Marine invasive species are characterized by broad dispersal and an ability to thrive in a variety of novel environments over very short time scales. I hypothesize that this success is due both to phenotypic plasticity and to rapid genotypic selection, despite the limited adaptive potential suggested by high gene flow and population bottlenecks. Using the globally invasive European green crab (*Carcinus maenas*), I compared thermal adaptation in populations spanning thermal gradients in the species' native and invasive ranges. Heat and cold tolerance (measured via cardiac photoplethysmography) differed significantly among populations even after one month of acclimation under common conditions. In addition, within a population, there were significant plastic differences in tolerance observed after acclimation to 5°C versus 25°C. Transcriptome sequencing revealed ten putatively-selected genomic regions closely associated with both temperature and population-level thermal tolerance. These associations were observed in the species' native range and were recapitulated in under 200 years after introduction to North America. One such region represents a likely genomic island of divergence, and contains at least 18 distinct genes in very strong linkage disequilibrium. This region is significantly enriched for non-synonymous SNPs relative to the transcriptomic data as a whole. Four genomic regions, including the potential island of divergence, also showed a strong and significant association with winter temperature in an independent test of five invasive populations spanning >1,500 km, all of which were descended from a single, highly bottlenecked introduction in 1990. Overall, these results strongly indicate local adaptation in the species' native range, and suggest a role for ongoing, rapid selection to temperature in the success of this widespread marine invader.

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6.4

Modeling photoperiodism in subterranean rodents

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Photoperiodism plays an important role in the synchronization of populational phenomena such as seasonal reproduction. Organisms that inhabit the extreme photic environment of the subterranean provide an opportunity to verify persistence of daily and seasonal biological rhythms, as well as the minimal photic input for 24h entrainment and photoperiodism. The genus *Ctenomys* of South American subterranean rodents, commonly known as tuco-tucos, has circa 60 described species, and can be found south of 12oS Latitude. We obtained automated recordings of daily light exposure and activity patterns of individual tuco-tucos in the field, as well as preliminary data on their seasonal reproduction times. Laboratory experiments have shown that their circadian oscillators display "splitting" of locomotor activity under constant light conditions, which is a hallmark of a 2 coupled clock structure in epigeous

organisms. These 2 neuronal populations that comprise the circadian oscillator is associated to photoperiod decoding through mathematical modeling. We have developed modeling studies to approach photoperiodic time measurement in tuco-tucos, based on field data and laboratory experiments, aiming at integrating multilevel neuronal population phenomena to individual and populational, seasonal biological rhythms (FAPESP, CONICET, FONCyT).

7: VERTEBRATE ENERGETICS: ABSTRACT DRIVEN SESSION

7.1

Digging up the evolutionary origins of hypoxia-tolerance: physiological adaptations to acute hypoxia in 9 species of African mole rats

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Mammals rely on continuous O_2 delivery for energy production but O_2 availability is often limited by environmental factors. Some mammals have evolved to survive hypoxic environments; studying these animals may provide key insights into the evolution of adaptive mechanisms that support life in hypoxia. Of particular interest are African mole rats, which experience periods of hypoxia while sleeping and digging in poorly ventilated underground burrows. During a recent field trip to South Africa, we measured metabolic, ventilatory, and thermal responses to stepwise exposure to acute hypoxia (18 to 2% O_2) in 9 species of African mole rats. These species spanned a range of magnitude in physical size and included animals that live solitarily versus in complex social groups, and under varying soil particulate size and density. All species except *Bathyergus suillus* tolerated 3% O_2, whereas *Heliophobius argenteocinereus* was the only species to tolerate 2% O_2. All species exhibited robust and progressive reductions in metabolic rate with progressive hypoxia, small to moderate reductions in body temperature (in most cases to nearly ambient temperature), and a hypoxic ventilatory response that was characterized by increasing ventilation with concomitantly decreasing O_2 levels, mediated primarily by increased tidal volume and a lesser contribution from breathing frequency. Hypoxia-tolerance did not correlate with body mass (*B. suillus* was the largest species, but *H. argenteocinereus* was the 2nd largest), degree of sociality, or soil density. We conclude that all African mole rat species are tolerant to environmental hypoxia and exhibit broadly similar physiological responses to acute hypoxic exposure.

7.2

Hummingbird daily energy expenditure allometry: Is bigger better?

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Allometric scaling relationships examine how the scaling of one trait (e.g. body size) correlates with the scaling of another trait (e.g. field metabolic rate, brain size). These scaling relationships have intrigued scientists for decades because there seem to be rules that govern how metabolic processes scale up with body size. In birds, field metabolic rate (FMR) scales with body mass (M) in the form FMR = $aM^{0.68}$ (Anderson and Jetz 2005; Nagy 2005), where a is a constant. However, localized taxon-level patterns could be masked by looking only at the scaling exponent of all birds. Hummingbirds have among the highest mass-specific rates of all vertebrates, as well as a much higher wing aspect ratio than predicted for their size. Previous studies estimate the FMR-body mass slope for hummingbirds to be 1.21 (Nagy et al. 1999a), without accounting for the phylogeny. This would indicate that bigger hummingbirds are more metabolically inefficient than smaller birds. We collected FMR and mass data, which combined with published data, spanned 17 hummingbird species over a 6-fold size range (2.7 - 17.5 g), and eight of the nine hummingbird clades. After accounting for phylogenetic relatedness, field metabolic rate scales with body mass as FMR = $2.19*Mass^{0.89}$. This is a much lower slope than what has been predicted for hummingbirds previously. Further, since temperate hummingbirds tend to feed on densely clumped resources more, and are closer to their thermoneutral zone than tropical hummingbirds are, we expected tropical birds to have a higher FMR to body size exponent than temperate birds to support increased foraging effort. Contrary to the usual trend among birds and mammals, we find, both with and without adjusting for phylogenetic relatedness, that tropical hummingbirds have a *higher* FMR to mass slope on average than temperate birds.

7.3

The cost of good parenting: Altered maternal care in high altitude deer mice, *Peromyscus maniculatus.*

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Low oxygen and temperatures at high altitude (HA) are particularly challenging for small mammals due to the high energetic costs of aerobic thermogenesis. Altricial rodents are born without the capacity to independently

thermoregulate and instead rely exclusively on maternal care early in postnatal development. The onset of independent endothermy occurs in the first month of life, a period of high mortality and a potential selective window. Previously, we found that the development of thermo-effector organs and the onset of endothermy is delayed in HA compared to low altitude (LA) native deer mouse pups. HA pups likely rely more on their mothers during this sensitive window. Thus, we tested the hypothesis that high altitude adaptation includes enhanced maternal care, using lab-reared descendants of deer mice native to LA (400 m a.s.l) and HA (4300 m a.s.l.). Mothers reared their litters postnatally under common garden warm + normoxia and under combined cold + hypoxia (5^{o}C: 430mmHg). We characterized maternal energetic demands and quality of care. The metabolic cost of provisioning offspring in a cold/hypoxic environment is exceptionally high. We found that the resting metabolic rate of lactating HA females was 70% of their VO_2 max while the cost for LA females was even higher (85%, VO_2 max). Surprisingly, despite rearing /provisioning larger litters than their LA con-specifics, HA mothers spent considerably less time caring for their pups under any condition. Both populations altered their maternal behavior in response to cold/hypoxic conditions, however, LA pups were significantly developmentally delayed whereas HA pups developed normally. Our data suggest a conflict between offspring and maternal energetic demands at high altitude that manifests as a trade-off in the quality/efficiency of maternal care.

7.4

Colonizing high altitude hypoxic environments: strategies to deal with metabolic needs

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The aptitude of mammals to colonize high altitude (HA) environments is limited by their ability to tolerate decreased oxygen availability. While rats are not found under natural conditions above 2500 meters of altitude, mice are commonly found in such habitats. Previous research in our lab showed that rats and mice display divergent physiological and molecular responses after acute (short-term) exposure to hypoxia. It is well known that the response to hypoxia is linked with the regulation of metabolism, in consequence, we aimed to identify the strategies underlying the metabolic response of rats and mice to short- and long-term exposure to hypoxia.

Sprague – Dawley rats and FVB mice (all males) were exposed to hypoxia (12% O_2) for short (6 hours and 24 hours) and long (7 days and 21 days) periods of time. During the last hour of the exposure, the metabolic rate (O_2 consumption (V_{O2}) and CO_2 production (V_{CO2})) and minute ventilation (V_E) were measured by indirect calorimetry and whole-body plethysmography methods. The hematological response was evaluated by the quantification of hematocrit and hemoglobin concentration. As the brain is particularly vulnerable to hypoxic conditions due to its high energy requirements, we assessed the mitochondrial respiration in permeabilized brain cortex samples using the high-resolution respirometer Oxygraph-2k (Oroboros Inc.).

In comparison to normoxic controls, mice showed a higher metabolic rate (increased V_{O2} and V_{CO2}) after 7 days of hypoxia. No change in rat's metabolism was observed. In line with the increased V_{O2}, mice showed a continuous augmentation in V_E with a peak at 7 days of exposure. Rats showed a weaker rise in the V_E at 6h with no further increase. At brain level, mice showed an increased mitochondrial respiration after 24 hours of hypoxia, while those exposed to 7 and 21 days were similar to the controls. Though in rats only weak changes in the ventilation and no response at brain-mitochondrial respiration were observed, they showed a strong hematological response. The hemoglobin concentration and hematocrit increased in a sustained way starting at 24 hours, reaching a plateau at 21 days of exposure to hypoxia. Hematological adjustments occurred in mice only after 21 days.

Altogether, our results suggest that mice privilege an increase of their ventilatory activity to cope with hypoxia resulting in an enhanced metabolic rate and preserved mitochondrial activity in brain cortex. In rats, whereas the ventilatory response is weak, they favour a rapid and sustained hematological response. We propose that the ability to tolerate hypoxia ultimately relies on the strategy to capture, distribute and use the available oxygen to cope with the metabolic needs during the process of acclimatization.

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8: VENTILATORY FUNCTION: ABSTRACT DRIVEN SESSION

8.1

Learning to air breathe; the first steps

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Air-breathing in vertebrates has evolved many times amongst the bony fish whilst in water. Its appearance fundamentally impacted the regulation of respiratory gas exchange and acid base status with terrestrial vertebrates combining ventilatory control of both; something that is not available to the water-breathers. We review the physico-chemical constraints imposed by water and air on gas exchange, place the extant air-breathing fish into this framework and show how the advantages of combining control of ventilation and acid base status become clearly available in the most obligate of air-breathing fish. Presenting new data on acid base control across temperature in several teleosts, we argue that ventilatory control linked to internal (though not necessarily central) CO_2/H^+sensing is probably widespread across the Actinopterygii, but normally hidden by the hypoxic ventilatory drive.

8.2

Characterizing the branchial hypercarbia recovery mechanisms following extreme hypercapnia in the highly CO_2 tolerant hagfish

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As scavengers that feed on decaying carrion (*e.g.* fish, large marine mammals), hagfish (*Eptatretus stoutii*) can be subjected to noxious environmental stresses such as high ammonia, anoxia and hypercapnia. While the physiological impacts and the hagfishes' tolerance of these stressors have been well characterized, little data exists on the mechanisms and strategies for recovery from these stressors that would be mandatory in the natural environment. Indeed, hagfish can tolerate extreme hypercapnia (>30 Torr; 72h) by building up plasma $[HCO_3^-]$ over 24 – 48 h of exposure, attaining the highest plasma $[HCO_3^-]$ levels ever observed in any organism (>70 mmol $HCO_3^-L^{-1}$). The goal of this study was to characterize the hypercarbia recovery strategies of the highly CO_2 tolerant following hypercapnia exposure at the whole animal and tissue (gill) levels. We exposed hagfish to hypercapnia (4% CO_2) for 48 h to induce severe hypercarbia before being allowed to recover in normocapnic seawater. During this recovery period, measurements of blood acid/base status, plasma $[Cl^-]$ and net H^+/HCO_3^- flux were made to elucidate the recovery strategies of the hagfish. Upon reintroduction into normocapnic conditions, hagfish rapidly (<8h) offloaded the compensatory base load (65.8 ± 2.1 mmol $HCO_3^-L^{-1}$) while sustaining an incredible blood alkalosis of ~0.8 pH units compared to post-exposure conditions, to a blood pH 8.67 ± 0.03 within 4h of recovery. During recovery, rates of HCO_3^- offloading paralleled rates of Cl^- uptake. While increases in both whole-animal HCO_3^- excretion and glomerular filtration were observed throughout recovery (2-8 h), neither can account for the majority of the observed rates of whole-animal HCO_3^- loss, which peaked at ~3.5 mmol kg^{-1} h^{-1}. Inhibition of all carbonic anhydrase activity via infusion of acetazolamide revealed that restoration of plasma $[HCO_3^-]$ from hypercapnia-induced hypercarbia is likely facilitated in a dualistic manner, initially relying on both carbonic anhydrase mediated CO_2 offloading and secondarily, by increasing Cl^-/HCO_3^- exchange processes, both of which are likely either upregulated or further activated as recovery progresses. Using isolated gills from hypercapnia-exposed hagfish allowed to recover for various amounts time (informed by whole-animal studies), we utilized a recently developed *in situ* hagfish dual gill perfusion/perifusion (extracellular aspect/water duct) technique along with a panel of permeant (Acetazolamide) and impermeant (C-18) CA pharmacological inhibitors, in an attempt to further characterize whether this CA-dependent HCO_3^--equivalent mechanism is driven by intracellular and/or plasma-accessible carbonic anhydrase. Similarly, using both pharmacological profiling and Cl^--free artificial seawater/salines, we also evaluated the roles of Na^+/HCO_3^-cotransporter and Cl^-/HCO_3^-exchanger in gill in base-recovery in this highly CO^-_2-tolerant organism.

8.3

Developmental Changes in the Ventilatory Response to CO_2 in Semi-Fossorial Mammals.

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Adult semi-fossorial rodents such as the Golden-Syrian hamster (facultative hibernator) and the 13-Lined ground squirrel (seasonal hibernator) have a blunted ventilatory response to CO_2 in comparison to adult non-fossorial rodents. Rats display a "sensitive period" during respiratory development where ventilatory responses to hypercapnia become blunted before rising to typical adult levels (a triphasic response). This study used pneumotachography and whole body plethysmography to examine the metabolic and ventilatory responses to CO_2 of Golden-Syrian hamsters, 13-Lined ground squirrels, and Sprague-Dawley rats through neonatal development (P0-30) to determine whether the blunted adult response seen in semi-fossorial rodents is a retention of the transient blunted response seen in rats. Hypercarbia (1, 5, and 7% CO_2) did not affect oxygen consumption ($V_{O2,}$ mL/min/kg) in any species but initially

elevated ventilation and the air convection requirement (mL/mLO_2) in all three species. Rats in our study exhibited the triphasic ventilatory response (when expressed as %Δ) to CO_2 previously described. Hamsters had an early (P0-2) robust response that progressively waned to a blunted adult response. Squirrels also exhibited an early, robust ventilatory response to CO_2 that decreased within days and remained attenuated through development to adulthood. Our study shows three distinct developmental patterns that appear to be shaped by lifestyle despite all animals being raised in normocarbic (non-fossorial) conditions. This research was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

8.4

Haemoglobin Adaptations to High Altitude Alter Breathing Pattern in Deer Mice (*Peromyscus maniculatus*)

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Hypoxia at high altitudes constrains O_2 supply to support activity and thermoregulation. Many highland taxa have adapted to these challenges by optimizing O_2 uptake, through changes in the pattern and/or control of breathing, enhancements in gas-exchange capacity of the lungs, and/or genetically based increases in haemoglobin-O_2 affinity. These evolved changes are believed to safeguard arterial O_2 saturation (SaO_2) in hypoxia to maintain O_2 supply to tissues. For example, deer mice native to high altitude possess haemoglobin (Hb) adaptations that augment blood-O_2 affinity, arising from genetic variants in the HbA and HbB gene cluster. Despite the common belief that haemoglobin plays no direct role in the control of breathing, we show that allelic variation in Hb function also contributes to changes in breathing pattern in high-altitude deer mice. We created an F2 inter-crossed population by hybridizing wild mice from high and low altitudes, which allowed us to compare mice with highland and lowland Hb genotypes on an admixed genetic background. The hypoxic ventilatory response was measured before and after hypoxia acclimation (12 kPa O_2 for 6-8 weeks). Hypoxia acclimation led to the expected increases in total ventilation, SaO_2 in hypoxia, and haematocrit. HbA genotype had a significant influence on breathing pattern, with mice possessing the highland genotype exhibiting deeper but less frequent breaths across a range of inspired O_2. This was not clearly associated with effects of HbA genotype on SaO_2, because differences in breathing persisted when mice breathed hyperoxic air. These findings suggest that the evolution of Hb genes may have pervasive effects across the O_2 transport cascade, and that Hb may contribute to environmental adaptation via physiological mechanisms that are not commonly ascribed to this protein.

8.5

The Effect of Chronic Hypercapnic Incubation on Breathing Patterns in American alligator (*Alligator mississippiensis*).

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During ontogeny phenotypic plasticity is a mechanism responsible for trait differences caused by the environment. This epigenetic regulation of phenotype may be particularly influential in embryonic reptiles that naturally experience fluctuations in environmental variables such as carbon dioxide, which could result in chronic acidosis (16-35 torr). However, the phenotypic outcomes of exposure to changes in CO_2 are relatively unknown in reptiles. Terrestrial vertebrates have two mechanisms for maintaining acid-base homeostasis: metabolic compensation and respiratory compensation. A prior study on America alligators demonstrated that hypercapnic incubation causes kidney enlargement suggesting a lasting impact of the incubation environment on the post hatched organism. Based on this we hypothesized that embryonic hypercapnia would decrease the sensitivity to acute hypercapnic exposures in juvenile American alligators (*Alligator mississippiensis*). To test this hypothesis, we monitored ventilation parameters and metabolism of juvenile alligators in response to hypoxic and hypercapnic gas PO_2=15,10,5,3 and PCO_2= 4,6,8,10 kPa. We found resting tidal volumes for animals incubated in hypercapnia were significantly lower (8.8mL/kg) when compared to control animals (14.7mL/kg). During hypoxic exposure oxygen consumption and the ratio of CO_2 produced relative to O_2 consumed or respiratory quotient (RQ) were constant for the control animals while hypercapnic incubated animals decreased oxygen consumption and increased RQ. Hypercapnic exposure did not affect oxygen consumption in either experimental group, while carbon dioxide production increased at 4 kPa for both groups. Carbon dioxide production was significantly lower in hypercapnic animals at 8 and 10 kPa when compared to normocapnic animals. Our initial findings support our hypothesis that developmental hypercapnia alters ventilatory function in juvenile American alligators.

8.6

Effects of Gravidity on Grasshopper Oxygen Delivery

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Although the insect tracheal system is extremely efficient, life history changes can reduce oxygen delivery. For example, intermolt growth results in compressed tracheae, lower tracheal volumes, and reduced femoral air sac ventilation rates. During gravidity, the egg mass of *Schistocerca americana* grasshoppers can reach up to 40% of their body mass. We investigated whether this increase in egg mass compressed tracheae, lowered tracheal volumes, and reduced femoral air sac ventilation rates in the abdomen and femur. Using micro-dissections and live video analysis, we examined the tracheal system of thirty-six female grasshoppers. We found that abdominal compression rates correlated with the inflation of proximal and distal femoral air sacs. In addition, abdominal compressibility decreased with gravidity, suggesting lower tidal volumes. However, there was a positive correlation between gravidity and tracheal compression rate. Current work is using micro-computed tomography to examine whether gravid females have reduced tracheal volumes. If so, gravid females may compensate for reduced tidal and tracheal volumes by increasing tracheal ventilation rates to improve oxygen delivery. This research was funded in part by NSF award 1531850 to SDK.

9: THERMAL BIOLOGY: ABSTRACT DRIVEN SESSION -1

9.1

Oxygen limitation of thermal tolerance varies depending on the life stage and behavior of terrestrial organisms

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Oxygen availability and temperature are two of the most important environmental factors affecting all of animal life. However, the two are not independent of each other and may exert similar selective pressures on animals. Animals should be most susceptible to high temperatures and oxygen variation during times of high performance and during early developmental stages. Through a series of experiments on lizards, birds, and insects, we have begun to examine the interactive effect of oxygen and temperature on terrestrial animals exposed to hypoxia, high temperatures, or a combination of both during various life stages and levels of activity.

The results of these experiments have been mixed in their support for the concept of an effect of oxygen during periods of thermal stress. Oxygen did limit thermal tolerance during embryonic development in lizards. However, while extreme hypoxia limits thermal tolerance in adult lizards, we have shown that ecologically relevant oxygen levels have no effect on voluntary maximum temperature. In adult flies from a variety of DGRP lines, there was a genetic correlation between flight performance during hypoxia and flight performance during high temperatures. However, while both hypoxia and high temperature have a detrimental effect on flight in adult Oregon-R flies, there was no interactive effect between temperature and oxygen during development. Lastly, we have been begun to look at the effect of metabolic rate on thermal tolerance in quail eggs through the use of thyroid hormone manipulation. Based on these experiments, the effect of oxygen on thermal tolerance varies depending on the life stage and activity level of these organisms. However, more experiments under ecologically relevant conditions and behaviorally relevant activities need to be carried out to further test this hypothesis.

9.2

Ultra-violet B radiation, the often neglected ubiquitous environmental stressor in aquatic environments

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High energy, short wave ultra-violet B radiation (UVBR) is a powerful natural stressor emitted by the sun. Although the majority of solar UVBR reaching the outer atmosphere is absorbed by stratospheric ozone, a small amount does reach the Earth's surface and can penetrate aquatic environments, especially in shallow water bodies or near the surface where fish and amphibian larvae often live. UVBR can interact with a range of biological molecules, and is capable of causing extensive cellular and molecular (DNA and proteins) damage as well as generating ROS. At the organismal level, UVBR exposure can adversely impact survival, immune function, growth rates, developmental trajectories and locomotion. UVBR can also interact with other environmental drivers within aquatic environments, such as temperature, hypoxia, and pH. Multi-factorial studies have been identified as a key area that is needed to disentangle the underlying mechanisms behind global amphibian declines. This presentation will examine the interaction of UVBR and temperature where the effects of UVBR are more pronounced at low temperatures. New data provide insight into the increased susceptibility of amphibians, both larvae and adults (via carry-over effects through metamorphosis) to UVBR at low temperatures which in turn may help to explain the predominance of amphibian declines in montane regions.

9.3

Understanding the effects of food availability, thermal tolerance, and sirtuin activity on the feeding physiology of *Mytilus californianus*

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The California mussel, *Mytilus californianus,* is a dominant member of coastal marine communities where they have a profound impact on intertidal and subtidal community structure. Mussels are regularly exposed to temperature extremes during periods of emersion and persistence of mussels along the coast depends, in part, on the ability of adult mussels to withstand environmental stress. Thermal tolerance in mussels is influenced by diet (i.e. phytoplankton composition) and mussels are known to modulate their feeding physiology in response to both environmental conditions and food availability. Previous studies in Mytilids suggest that sirtuins (a class of deacylases that regulate numerous cellular processes through posttranslational modification) are activated during food limitation and act as a cellular energy sensor linking food availability and thermal tolerance in mussels. To investigate the mechanistic link between diet and temperature sensitivity, we conducted a large-scale experiment with high temporal resolution to evaluate the dynamics of the stress response in *M. californianus* across multiple levels of biological organization (i.e. cellular, organ, and organismal). As part of this study, we evaluated how acclimation to a combination of feeding regimes (low or high algal abundance) and thermal history (exposure to 20°C or 30°C maximum aerial temperature during each daytime low tide period) altered the feeding physiology of mussels, measured as variation in clearance rate, ciliary activity, and siphon opening during the last 48 h of the three-week acclimation period. Following acclimation, we chemically inhibited sirtuin activity in half of the mussels, exposed all mussels to acute heat shock (6 h heat ramp to 33°C), and measured how sirtuin inhibition and acute heat shock altered the feeding physiology over the course of 48 h in each of the treatment groups. Preliminary analysis of the data suggest that diet plays an important role in the feeding behavior of mussels and that both acute heat stress and sirtuin inhibition decrease ciliary activity, clearance rate, and siphon opening. However, the response of feeding physiology to heat stress and sirtuin inhibition is complex and depends on acclimation history and time of day. For instance, clearance rates were reduced in mussels exposed to acute heat stress and in those exposed to both sirtuin inhibitors and acute heat stress relative to our baseline measurements, but these effects were exacerbated during the day and in those fed the low algal food ration. This research is funded by the National Science Foundation (IOS- IOS-1557500 & 1557496).

9.4

A distal bat wing muscle operates at low temperature *in vivo*, and has low thermal sensitivity of contractile properties

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Flight in bats requires fast and coordinated movement of the forelimbs to maintain wing cycling frequencies of approximately 12 Hz or more, and involves contributions from muscles along the trunk, arm, and forearm. As nocturnal fliers, small bats likely incur substantial thermoregulatory cost from forced convection and radiative heat loss to the night sky; however bat wings are poorly-insulated, and muscles in the wing may operate at relatively low temperatures. Since rate-related processes in muscle slow at cooler temperatures, temperature effects in the wing muscles may impair flight performance. Here, we ask: (1) are bat wing muscles cold during flight, and (2) if so, how do bats compensate for effects of temperature on muscle contractile properties? We investigated these questions by measuring the temperature of flight muscles during wind tunnel flights in *Carollia perspicillata*, a small neotropical fruit bat, at an environmental temperature of 22°C; then by determining the temperature dependence of contractile properties in the extensor carpi radialis longus muscle (ECRL), a forearm extensor muscle. We compared bat ECRL properties to a distal but well-insulated limb muscle in the mouse, the extensor digitorum longus (EDL), at 22, 27, 32, 37, and 42°C. Our continuous measurements of muscle temperature during flight suggest a steep proximal-to-distal temperature gradient in the wings. Core body temperature (T_b) was maintained at approximately 39°C for all individuals. After commencement of flight in the wind tunnel, biceps and ECRL temperatures dropped substantially over the course of minutes-long flights to approximately 5°C and 12°C cooler than T_b, respectively. Instantaneous measurements immediately post-flight indicate that the deep pectoralis may operate at or several degrees above T_b during flight. The thermal dependence of the bat ECRL is low relative to the mouse EDL for all isotonic and isometric properties we studied, including shortening velocity ($Q_{10,\ ECRL}$= 1.1 *vs* $Q_{10,\ EDL}$ = 1.5 from 32–37°C) and half-relaxation times for twitch and tetanus; the mouse EDL was stable *in vitro* to a higher maximal temperature (42°C). When we evaluate Q_{10}s for each muscle relative to maximum experimental temperature, the thermal performance curves are of the same shape, suggesting a shift in temperature optimum rather than an increase in performance breadth. Preliminary data for contractile properties of the pectoralis muscle in *C. perspicillata* indicates that it is more temperature sensitive than other studied skeletal muscles, including the bat ECRL and the mouse EDL. The high temperature

sensitivity of the pectoralis may relate to its proximal location and maintenance at or above T_b during rest and activity. This work was approved by the Brown Univ. IACUC and funded by NSF and AFOSR.

9.5

Assessing summertime thermoregulatory properties across the pelage molt in a polar pinniped: the Weddell seal

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Antarctic pinnipeds face complex thermoregulatory challenges, as they must conserve heat in both air and water. Primary adaptations to reduce heat loss, or flux, include a thick blubber layer and the ability to limit the amount of blood flow to the peripheries. These adaptations reduce heat flux by decreasing the temperature gradient between the animal and the environment. However, during the annual pelage molt, hair follicles may require more constant blood perfusion which would compromise heat conservation and result in higher rates of heat loss and energy expenditure. Thus, polar species such as the Antarctic Weddell seal (*Leptonychotes weddellii*), may only have a short temporal window during which molting can be accomplished without incurring large energetic costs.

To determine how ambient conditions influence heat loss and energy expenditure, surface temperature (ST) and heat flux (HF) were directly measured in 77 adult female Weddell seals prior to, during, and following the molt. Seal ST was mean±SE 6.64°C ± 0.34, and 73% of ST variation could be explained by intrinsic (mass, surface area, blubber thickness and molt status) and extrinsic (ambient temperature, wind speed, relative humidity and solar radiation) factors measured at the time of animal handling (General Linear Model). Surface temperature increased most significantly with increased ambient temperature and decreased wind speed, which explained 47.8% and 46.0% of the variation respectively. Surface temperature was not related to molt status. In contrast, HF was most impacted by molt status, with molting seals having higher HF than those handled prior to the molt start. As HF is a direct proxy for energy expenditure, this suggests that perfusion is increased during molt to support hair growth despite the increased thermoregulatory costs.

Finally, to determine if seals offset the increased HF during the molt by reducing time in water (due to higher conductivity of water), we determined the time-activity budgets of 55 seals using time-depth recorders, and paired their haul-out behavior with information on ambient conditions. On average, seals spent 9.98 ± 0.16 SE hours per day hauled out, and the amount of time seals spent hauled out was independent molt status. Quantifying the contributions of behavior and intrinsic and extrinsic conditions will allow us to understand how energy requirements might be altered by changing environmental conditions.

11: COMPARATIVE INSIGHTS INTO ANIMAL RESPONSES TO HYPOXIA AND ANOXIA

11.1

Role of the Mitochondrion in Low Oxygen Signalling in the Painted Turtle.

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The western painted turtle is an anoxia-tolerant vertebrate that provides a useful model to study the natural cellular mechanisms that are protective of low oxygen stress. It wasn't long ago that mitochondria were not considered capable of being low oxygen stress sensors; however, more recently that opinion has changed. We know in anoxic turtle brain that pyramidal neurons undergo: an increase in GABA receptor currents, a decrease in glutamate receptor currents, an increase in whole-cell conductance, a movement of membrane potential towards the Cl^- reversal potential, decrease in reactive oxygen species, and a depolarization of mitochondria membrane potential (MMP), and calcium release through mitochondrial K_{ATP} channels (mK_{ATP}). We know little about the role of mitochondria in non-excitable tissues such liver; therefore, we used electrophysiological and fluorescent imaging techniques to examine membrane potential, whole-cell conductance, mitochondrial Ca^{2+} release and MMP in hepatocytes during a normoxic to anoxic transition. Unlike brain, hepatocyte membrane potential remains constant at -28 mV and conductance decreases. There was no anoxia-mediated increase in cytosolic Ca^{2+}levels but similar to brain MMP did decrease to a new steady state, and the addition of an uncoupler further decreased the MMP. An inhibitor of the mK_{ATP} channel partially reversed the decrease in MMP during anoxia and an activator decreased MMP during normoxia. While mitochondria in both tissues undergo depolarization with anoxia, this only leads to increases in Ca^{2+} levels in brain tissue. Therefore, in pyramidal neurons mitochondria can act as a low oxygen sensor but in hepatocytes it remains unclear.

11.2

Re-oxygenation resilience - the other aspect of the crucian carp's anoxia tolerance

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The crucian carp *Carassius carassius* is capable of surviving without oxygen for several months, by carefully matching ATP supply and demand. It is aided by its unique ability to produce ethanol as an anaerobic end-product and undergo partial metabolic rate suppression, shutting down non-vital functions such as vision. In this presentation, however, I will focus more on another aspect of anoxia, namely the associated re-oxygenation, which in hypoxia- and anoxia-insensitive species leads to severe tissue damage and even death, but which the crucian carp tolerates when its habitat is re-oxgenated every spring. In addition to investigating the effect of anoxia and re-oxygenation on brain cell death, oxidative damage, and memory and learning ability, we have used next-generation sequencing followed by de-novo transcriptome assembly to identify mRNA sequences differentially regulated between normoxic, anoxic and re-oxygenated crucian carp, in order to find processes and pathways that may respond differently in crucian carp as compared to non-tolerant species.

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11.3

Learning from the experts: how marine and freshwater bivalves cope with anoxic transgression

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Maximum performance, such as highest respiration and heart rates, or pumping activities are often interpreted as marking the range of "optimal physiological performance" in marine species. Mollusks and especially some bivalves display, however, a hypoxia-adapted phenotype to the point where they maintain extremely low oxygen partial pressure (<4 kPa pO_2) in shell water and hemolymph *against* normoxic (21 kPa) "outside" pO_2 over periods that can last many hours. As their respiration is usually "oxyconforming" in that low pO_2 range, "underperformance" is not necessarily a sign of physiological strain in these organisms, but essentially self-induced. When exposed to environmental oxygen shortage, these specialists withstand prolonged hypoxia for many days and up to weeks by reducing metabolism to extremely low rates of sometime less than 10% of normoxic performance. I will present new insight into the complex strategies employed by hypoxia tolerant marine and freshwater bivalves, combining different organizational levels from the whole animal and its microenvironment to subcellular reorganization.

With help of their microbial biofilms and gut microbiome, bivalves create their own chemical microenvironment inside the closed shell. Within minutes of shell closure, the shell water pO_2 becomes hypoxic and eventually anoxic. Using fluorescent dyes that react with reactive oxygen and nitrogen species, we showed how NO accumulates in the endothelial cells surrounding the blood vessel in *Mytilus edulis* gills as pO_2 was lowered to 7kPa (30 % oxygen saturation), conferring relaxation of the endothelium and improving hemolymphatic flow to counteract the oxygen shortage. As pO_2 declines further, facultative anaerobic nitrate respiring bacterial biofilms produce nitrite which accumulates and eventually disproportionates to nitrous oxide (N_2O) and nitric oxide (NO) (Stief 2013). NO accumulating in shell water competitively inhibits cytochrome-c oxidase (CCO) activity in peripheral tissues (gills), slowing down gill respiration and supporting oxygen distribution to the central body.

Many bivalves have preserved special anaerobic mitochondrial pathways, inheritance from bacterial endosymbionts (Tielens et al. 2002), which support reduction of short-chained organic acids (fumarate) to succinate and from there to propionate for slightly better ATP yield compared to anaerobic glycolysis. Mitochondria that switch between aerobic and anaerobic electron transport are often also endowed with an *a*lternative end-*ox*idases (AOX) that receives electrons directly from the ubquinone pool, bypassing complexes III and CCO (complex IV). AOX has recently been sequenced in several sediment dwelling bivalves and we find it is strongly upregulated in hypoxia and anoxia exposed freshwater mussels (Yusseppone et al. 2018). As AOX is resistant to sulfide inhibition, it can stabilize mitochondrial electron flow and membrane potential under low oxygen and potentially under sulfidic conditions. Maintenance of low mitochondrial membrane potential lowers the risk of reactive oxygen species (ROS) production upon reoxygenation. Therefore, significant induction of antioxidants during hypoxic exposure is often not detected, and presumably not necessary in these bivalves. Furthermore, lower shell water pO_2 enables constitutive protein levels of hypoxia-inducible transcription factor (HIF-α) in bivalve and gastropod tissues which reinforces expression of genes supporting anoxic survival. I will show bivalve examples in support of these low oxygen strategies in evolutionary early non-model organisms, which can be so different from all we perceive as "normal".

Stief, P. (2013) *Biogeosciences, 10*(12), 7829.

Tielens et al. (2002) *TRENDS in Biochemical Sciences, 27*(11), 564-572.

Yusseppone, et al. (2018) *Frontiers in Physiology*, 9:100.

11.4

Flies are not turtles or carp: non-conventional anoxia tolerance

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The ability to tolerate bouts of oxygen deprivation varies tremendously across the animal kingdom. Animal species and individuals from different regions show large variation in tolerance to hypoxia; additionally, it is widely known that neonatal mammals are much more tolerant to anoxia than their adult counterparts, including in humans. Furthermore, oxygen limitation plays a key role in many pathologies, and yet we still lack a fundamental understanding of the mechanisms responsible for intra- and interspecific variation in hypoxia/anoxia tolerance. Here, we use a variety of experiments designed to test hypotheses for how adult *Drosophila melanogaster* survive longer than larvae (LT50: ~8 vs. 1 h). First, we tested the most common mechanism for anoxia tolerance in vertebrates: are more tolerant individuals better able to maintain ATP and thereby prevent ionic disruption during anoxia? During the first two hours, larval ATP fell to <1% of normal and hemolymph [K^+] rose by 50%; survival decreased with time, in strong correlation with the fall in ATP and rise in [K^+]. During the same time period in adults, ATP also fell strongly, while hemolymph [K^+] rose even more strongly than in larvae, but survival was 100%. During the next six hours, adults maintained high survival, while ATP was maintained at 2% of normal levels, and hemolymph [K] continued to rise to 5x normal. After 8 h of anoxia, adult ATP levels decreased further and [K^+] continued to rise; and over this time period, both of these variables correlated with decreased survival. The superior anoxia tolerance of adult *Drosophila* appears to be due to the capacity to maintain and tolerate very low ATP levels, and to the ability to tolerate high extracellular [K^+]. This suggests that protective mechanisms downstream of ATP depletion and an ionic disruption may be important for surviving anoxic bouts. Next, we used a targeted H-NMR metabolomics approach to investigate three questions. Are developmental differences in anoxia tolerance associated with metabolic rate, anaerobic capacity, and/or protective metabolites? Despite the fact that metabolic rates were not different after 30 min of anoxia, adults did suppress metabolic rates to levels 40% lower than larvae during the first 30 min of anoxia, likely due to the paralysis of adults vs the escape locomotion of the larvae. Secondly, adults utilize alternate anaerobic end products (alanine, succinate and acetate) more so than larvae, likely attaining a better ATP/H^+ratio and redox balance. Lastly, adults have higher levels or increased concentrations of several putatively protective metabolites (i.e. polyols, β-alanine, taurine) that likely reduce cellular damage associated with osmotic or antioxidant stress during or after anoxia. In a third set of experiments, we use the *Drosophila* Genetic Reference Panel to assess genetic variation in anoxia tolerance. We show that anoxia tolerance is a highly variable trait, and that much of this variation is determined genetically. Male and female anoxia tolerance is tightly correlated, yet there is still a substantial amount of variation within some lines. Genome-wide association analyses for adult anoxia tolerance identified many genes with functions closely related to immune/inflammatory response, consistent with the strong up-regulation of immune genes after anoxic/hypoxic exposure. These data strongly suggest that genetically-based differences in immune function are key differentiators of anoxia-tolerance, but the mechanisms responsible remain elusive. GWA also identified multiple ion transport function genes whose allelic variation affected anoxia-tolerance in adults; examination of how these alleles affect ionic disruption or tolerance of ionic disruption may provide important insights into the maintenance of genetic variation in these ion transporters. Altogether, this study suggests that a new focus of research in anoxia-tolerance should be the mechanisms by which animals can survive and quickly recover from such hypo-energetic and ionically-disrupted conditions. Supported by NSF IOS1256745.

12: MICRORNAS IN COMPARATIVE AND EVOLUTIONARY PHYSIOLOGY

12.1

For Everything There is a Season: microRNA Regulation of Insect Diapause

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Diapause is an endogenously regulated dormancy that provides insects, and other animals, a means to “escape” seasons of inimical conditions and to synchronize periods of growth and reproduction with seasons of abundant resources. Diapause is a complex, alternative phenotype, characterized by changes in developmental timing, metabolism, and stress tolerance, that is coordinated by molecular mechanisms that are not completely understood. MicroRNAs, small (18-25 nucleotide) non-coding RNAs) are emerging as components of a conserved “toolkit” of processes that regulate gene expression before, during, and after diapause in evolutionarily diverse insect species including flesh flies, mosquitoes, and moths. Combined RNA-seq and qRT-PCR studies have been used to identify differences in the abundance of miRNAs in diapausing insects relative to their nondiapause counterparts. Pupal diapause in the flesh fly, *Sarcophaga bullata* is characterized by changes in the abundance of at least ten conserved microRNAs[1]. MiR-289-5p and miR-1-3p are increased by as much as 2-fold in diapausing pupae, while miR-9c-5p, miR-13b-3p,

miR-31a-5p, miR-92b-3p, miR-275-3p, miR-276a-3p, miR-277-3p, and miR-305-5p are underexpressed in diapausing pupae compared to their nondiapausing counterparts. Many of these (e.g., miR-13b-3p, miR-275-3p, miR-277-3p, and miR-305-5p) are also underexpressed in adult females of *Culex pipiens* mosquitoes that are programmed to enter diapause compared to females not programmed to enter diapause[2]. In the moth, *Helicoverpa zea*, downregulation of miR-289-5p and miR-277-3p following termination of pupal diapause[3] provide evidence these miRNAs regulate at least some some diapause-relevant changes in Lepidoptera as well as in Diptera. The precise targets of miRNAs differentially regulated in *S. bullata*, *Cx. pipiens*, and *H. zea* have yet to be identified in these species, but their functions, inferred from studies on *Drosophila melanogaster* and *Aedes aegypti*, suggest they regulate diapause-relevant processes including cell cycle progression, developmental timing, suppression of metabolism, and stress responses. Future challenges include integrating these control molecules into the "big picture" of previously identified signaling pathways and gene networks that mediate the diapause entry, maintenance, and termination.This work was funded by National Science Foundation Grants NSF_IOS-1354377 and NSF_IOS- 1755318, and USDA/NIFA Grant 2015-67013-23416.

1. Reynolds et al., 2017. IBMB, 84:1-14.
2. Meuti et al., 2018. Submitted
3. Reynolds et al., 2018. Submitted

12.2

The role of miRNA regulation on phenotypic responses to environmental stressors in fish.

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MicroRNA (miRNA) are small (~21-22 nucleotides in length), non-coding RNA that can bind to multiple target transcripts, effectively reducing translation. This can ultimately result in decreased functionality and altered phenotype of a given protein or pathway. MicroRNA have been an influential tool in studying pathologies of numerous human related diseases, such as cancer and heart disease. However, less than 3% of comparative physiology approaches examine the functional consequences of miRNA, which is instrumental in the regulation of phenotypic plasticity. This presentation will provide an overview of the recent advancements in understanding microRNA regulation of phenotype from individual pathways all the way to whole animal impacts in fish. Emphasis will be placed on how the environment plays an essential role in mediating phenotypic response in teleosts, through changes in microRNA. Unique facets of microRNA will also be discussed, including the conservation of binding sites in key transcripts across millions of years of evolution, secretion and circulation of miRNAs following a stressful event, and miRNA regulation of energetics. A final discussion will focus on the use of miRNA shed into the water as a non-invasive marker for the identification of aquatic communities that are under stress. At the conclusion of this presentation, it is hoped that there is a greater appreciation and understanding of microRNA regulation of phenotypic responses related to environmental challenges in teleosts. This work is funded through NSERC Discovery.

12.3

A cool story: Non-coding RNAs in natural models of cold adaptation

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Characterization of the underlying molecular changes associated with cold adaptation has revealed insightful clues on how various models cope with low temperatures. Nevertheless, much remains to be accomplished in order to clarify the complete molecular picture linked with life at low temperatures and to translate this knowledge into practical applications. Several natural models of cold adaptation exist, such as mammalian hibernators and cold-hardy insects, and hold keys to our understanding of processes at play during this phenomenon. Decreased metabolic rate and down-regulation of energy-consuming processes including protein translation are notably observed. Our work is aligned with the latter and aims at better understanding the roles played by non-coding RNAs, including microRNAs and long non-coding RNAs, by amplifying and quantifying these molecules in select models exposed to low temperatures. Whether via the quantification of cold-associated microRNA footprints using high-throughput sequencing approaches or through the assessment of the functional consequences that result from such changes using bioinformatics- or RNAi-based methods, this talk will primarily highlight results collected on cold-responsive non-coding RNAs in the hibernating ground squirrel *Ictidomys tridecemlineatus* and the Colorado potato beetle *Leptinotarsa decemlineata*. Overall, characterization of signatures of non-coding RNAs holds the potential to yield valuable information on how natural models of cold adaptation deal with temperature stress and how this strategy could have applications in fields as diverse as pest management and organ preservation. This work is notably supported by the Natural Sciences and Engineering Research Council of Canada and Agriculture and Agri-Food Canada.

12.4

Regulation of microRNA activity to promote multipotent cell fate during dauer diapause

Xantha Karp[1], Allison Cale[1], Megan Wood[1], Isaac Smith[1], Amelia Alessi[2], Mallory Freeberg[2], John Kim[2], Liberta Nika[1], Kyal Lalk[1], Mikayla Schmidt[1], Anna Zinovyeva[3], Alexis Santos[1], Payton Salomon[1]

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Animal developmental programs are robust to the diverse environmental and physiological conditions experienced in nature. One mechanism used by multiple species to withstand adverse environmental conditions is entry into diapause, or developmental arrest. When conditions improve, development resumes and is completed normally. During diapause, multipotent stem and progenitor cells must retain the capacity to give rise to all appropriate cell types. The mechanisms that enable development to proceed normally after diapause are incompletely understood, and this question forms the basis of our work. In *C. elegans,* adverse conditions drive entry into dauer diapause midway through larval development [1]. If environmental conditions improve, dauer larvae recover and complete development normally. However, there are differences in the genetic network required to specify development after dauer [2]. One of the best understood examples of this phenomenon is the cell fate specification of lateral hypodermal seam cells, a *C. elegans* stem cell model. During larval development seam cells are multipotent and undergo self-renewing divisions to produce additional seam cells as well as differentiated cell types. Seam cell fate is specified by a network of "heterochronic" genes [3]. During early development, transcription factors and RNA-binding proteins specify early seam cell fates. Progression to later cell fates depends on the expression of microRNAs that downregulate the early-promoting genes [3]. Intriguingly, many heterochronic genes that are normally necessary for development are completely dispensable after dauer. Furthermore, seam cell fate appears to be re-set during dauer [2]. Using a combination of genetic mutants and gene expression data, we find that this dauer-specific developmental program involves modulation of microRNA activity. Mutants with compromised microRNA-induced silencing activity display penetrant phenotypes during non-dauer development but appear completely healthy after dauer [4]. During dauer, expression of heterochronic microRNAs is low. We find that a subset of the genes that control the decision to enter dauer diapause are also required to prevent inappropriate expression of heterochronic microRNAs during dauer, and thereby maintain multipotent cell fate. Surprisingly, these heterochronic microRNAs act through a novel pathway, distinct from the canonical pathway in which they act to promote developmental progression during non-dauer development. Thus, we have identified a new, microRNA-dependent mechanism that coordinates diapause with multipotent cell fate in *C. elegans.* Because the genes involved are highly conserved in animals, this mechanism is potentially relevant to the maintenance of multipotent stem cell fate outside of nematodes.

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12.5

A network of microRNAs and RNA binding proteins acts maternally to regulate sex determination in the *C. elegans* embryo.

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[1]NIDDK Intramural Research Program, National Institutes of Health

Although many roles of microRNAs in differentiated tissues have been described, relatively few microRNAs are known to function in early embryonic development. In particular, little is understood about the function of microRNAs expressed prior to the maternal to zygotic transition, when post-transcriptional control of gene expression is widespread. Seeking to elucidate the function of microRNAs in early development, I have focused on the *mir-35-41* microRNA cluster in *C. elegans*, which is expressed maternally and in embryos, and is essential for embryonic development and fecundity.

Here I show that the *mir-35-41* microRNA cluster regulates sex determination, preventing aberrant activation of male-specific gene expression in hermaphrodite embryos. Two predicted *mir-35-41* target genes are required for the sex determination phenotypes of *mir-35-41* mutant embryos, suggesting that they act downstream of *mir-35-41.* These target genes, *sup-26* and *nhl-2*, both encode RNA binding proteins, thus delineating multiple new layers of post-transcriptional regulation of the sex determination pathway.

Most players in the sex determination pathway are regulated zygotically, after the inheritance of both sex chromosomes. In contrast, the maternal load of *mir-35-41* is largely responsible for regulating sex determination. Because of this maternal contribution to an inherently zygotic process, I propose that *mirs-35-41* act as a developmental timer, ensuring a period of naïveté in early embryos, and preventing premature decision-making in sex determination and possibly other developmental processes.

Using CRISPR/Cas-9 to manipulate the endogenous *mir-35-41* seed match in the *nhl-2* 3' UTR, I observe that

repression of *nhl-2* by *mir-35-41* is not only required for proper sex determination but also for viability, showing that a single microRNA target site can be essential. Our work thus also makes progress towards understanding the essentiality of this maternally-contributed microRNA family.

13: THE ROLE OF GASOTRANSMITTERS IN HYPOXIC AND CHALLENGING ENVIRONMENTS

13.1

Introduction to gasotransmitters and the role of carbon monoxide (CO) in hypoxia-tolerant species

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Certain gases [carbon monoxide (CO), nitric oxide (NO) and hydrogen sulfide (H2S)] that were previously thought to be strictly pollutants, are now known to be endogenously generated and act as critical signaling molecules in many physiological processes. Further, these gasotransmitters have recently shown to elicit protective or therapeutic effects, making the study of these gases an emerging field in medicine, biochemistry and physiology. Carbon monoxide (CO) is naturally produced in the body from the regular turnover of heme (a major component of many heme-proteins) by heme oxygenase enzymes. Ironically, CO also binds tightly to the same site on hemoglobin as oxygen (creating carboxyhemoglobin), leading to an allosteric increase in hemoglobin-oxygen affinity. Too much carboxyhemoglobin can therefore limit oxygen delivery to tissues. However, a moderate increase in hemoglobin-oxygen affinity is thought to confer tissue protection during severe hypoxia. Increased hemoglobin oxygen affinity is consistently seen in species adapted to tolerate chronic hypoxia, yet this phenotype is not thought to be present in diving mammals. Our research highlights that some deep-diving mammals exhibit elevated levels of carboxyhemoglobin that correlate well with their increased heme-protein stores. This results in an increase in hemoglobin-oxygen affinity, which can improve tissue oxygen delivery during the severe hypoxia these animals face while diving. This is the first direct evidence of a hypoxia-tolerant animal utilizing CO as a mechanism to achieve increased hemoglobin-oxygen affinity.

13.2

Roles of NO and H_2S signaling in hibernators

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Surviving winter at nearly subzero temperatures without food is an extreme physiological challenge common to several vertebrates, including the brown bear, crucian carp and freshwater turtles. Common to these hibernating species is the ability to undergo metabolic suppression and to tolerate end products of energy metabolism and oxidative stress at reoxygenation. These responses are potentially controlled at least in part via nitric oxide (NO) and hydrogen sulfide (H_2S) signaling pathways. Analyses of type and distribution of NO and H_2S metabolites in the blood of brown bears in the wild reveal significant changes in the sulfide distribution during hibernation, suggesting recycling of sulfide oxidative products into H_2S, but no apparent major changes in NO metabolites [1]. These results suggest that H_2S may contribute to controlling aerobic metabolism suppression in hibernating bears. Conversely, in anoxic and cold-acclimated turtle and crucian carp, NO metabolites of most tissues increase markedly during anoxia, particularly in the heart, suggesting a key role of NO in the protection against oxygen deprivation [2]. Surprisingly, anoxic turtle hearts retain similar levels S-nitrosation of mitochondrial complex I, a major NO-dependent modification preventing reactive oxygen species (ROS) generation at reoxygenation [3]. In addition, changes in H_2S metabolites measured in cold-acclimated turtles during anoxia are less pronounced than for NO and limited to selected organs. In conclusion, these results suggest distinct biological roles of NO and H_2S signaling in the aerobic and anaerobic suppression of hibernating endotherms and ectotherms, respectively.

(Grants: Danish Council for Independent Research, Aarhus Univ. Research Foundation, Medical Research Council UK, Wellcome Trust, National Institute of Health USA)

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13.3

Carbon monoxide signaling in the control of breathing and impacts for high-altitude adaptation

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Animals living at high altitude are subject to strong selective pressure to improve oxygen delivery and utilization efficiency. Tibetan and Andean human populations developed distinct physiological adaptations to high altitude, including different ventilatory sensitivity to oxygen. This may be explained by variation in heme oxygenase 2 (HO-2; *HMOX2*), which is implicated in carotid body oxygen sensing. HO-2 produces carbon monoxide in the presence of oxygen which inhibits hydrogen sulfide production by inhibiting cystathionine γ-lyase activity. Furthermore, heme oxygenase activity may contribute to differences in hemoglobin concentration across these groups since it is a key player in heme catabolism. We conducted whole genome sequencing and genotyped *HMOX2* variants in Tibetan and Andean populations to determine if *HMOX2*, previous demonstrating signals of evolutionary selection in Tibetans, is also under selection in Andeans and if the putatively adaptive *HMOX2* variants differ across these groups. We also collected hypoxic ventilatory response measurements in Andean highlanders to determine if *HMOX2* variants are associated with ventilatory sensitivity to oxygen. We found that while *HMOX2* demonstrates signals of selection in Tibetans, the gene encoding the inducible heme oxygenase isoform, *HMOX1*, is under selection in Andeans. Whether the adaptive significance of *HMOX1* is related to oxygen sensing in the carotid body, hemoglobin concentration, alterations in inflammatory profiles, and/or cytoprotective effects remains to be determined.

13.4

Hydrogen Sulfide and Oxygen Sensing: From Evolution to Function

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Life appeared nearly 4 billion years ago (BYA) in anoxic and sulfidic environments and nearly seven-eights of evolution occurred in these conditions. When oxygen appeared 0.6 BYA most organisms readily became dependent on this efficient O_2 acceptor[1]. This necessitated development of elaborate O_2 sensing mechanisms to monitor availability of environmental O_2, ensure adequate O_2 delivery to tissues and to regulate cellular metabolism. Although low Po_2 is directly coupled to physiological effectors in these cells, the identity of this O_2 "sensor" has remained enigmatic despite over 50 years of intensive research. In 2006 we proposed a novel O_2 sensing mechanism based on the balance between constitutive cellular production of biologically active hydrogen sulfide (H_2S) and its inactivation through oxidation, the latter directly coupled to O_2 availability[2]. Since then numerous studies on a variety of animals and tissues have shown that 1) H_2S production is O_2 independent, whereas there are numerous mechanisms of O_2-dependent H_2S metabolism, 2) tissue H_2S concentration is intimately coupled to O_2 concentration and this is regulated at physiologically relevant Po_2s, 3) physiological responses to exogenous H_2S are identical to hypoxic responses, most notably as the paradoxical hypoxic pulmonary vasoconstriction or dilation in terrestrial vs diving mammals, 4) compounds that inhibit or augment H_2S production inhibit or augment hypoxic responses, respectively, and 5) H_2S acts upon effector mechanisms known to mediate hypoxic responses[3]. Recent studies have shown that H_2S mediated O_2 sensing has both a rapid on/off component but can maintain its effectiveness for extended periods. These studies have employed "Kroghian" physiology and Occam's razor to demonstrate that H_2S mediated O_2 sensing is a simple, yet elegant remnant of an anoxic past where sulfide-based metabolism became adapted to detect the molecule that eventually replaced it as the energetic center of redox metabolism. Support, NSF IBN 0235223, IOS 0641436, IOS 1051627, IOS 1443610.

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14: THE ROLE OF THERMAL PERFORMANCE CURVES IN PHYSIOLOGY, ECOLOGY AND CONSERVATION
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14.1

The thermal performance curve: mechanisms, applications, and pitfalls for a concept that just turned 40 (happy birthday!)

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In the autumn of 1978, Huey and Stevenson penned an influential paper to introduce the concept of the thermal performance curve (1979, *American Zoologist* 19: 357-366). This concept catalyzed a theory of thermal adaptation and helped biologists to understand why animals thermoregulate. In recent years, thermal performance curves have been used to explain everything from global patterns of biodiversity to extinction during global warming. Given how widely biologists have applied thermal performance curves, we should periodically question the implicit assumptions and consider ways to improve the concept. In this talk, I will focus on three assumptions taken for granted in most applications of thermal performance curves: 1) a curve reflects the thermal sensitivity of performance; 2) the thermal sensitivity of performance reflects the thermal niche of a genotype; and 3) thermal sensitivity does not depend on factors other than temperature. Because these assumptions are usually false, I will demonstrate ways to relax these assumptions when modeling thermal adaptation or thermal ecology in a changing environment.

14.2

Translating physiology to fitness using thermal performance curves

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Lab-derived thermal performance curves (TPCs) provide a tangible means for predicting the responses and fitness of wild ectothermic animals in the face of spatial and temporal thermal variability. The use of TPCs has become even more important in the current era of human-induced climate warming, whereby forecasts of the performance of wild animals can be made using characteristics of TPCs including their slope, magnitude and breadth. Given that a TPC for fitness generally follows a bell-shaped pattern across the naturally occurring temperature range of a species, many lab studies attempt to isolate physiological processes that follow the same pattern and position along the (horizontal) temperature axis, thus opening the door to a cause-and-effect understanding of the physiological mechanisms underlying temperature-dependent fitness.
In this regard, the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis is based on the TPC for aerobic scope (maximum minus minimum aerobic metabolism) and has been touted (including in an IPCC report) as a universal mechanism underlying temperature-dependent patterns in fitness of ectothermic animals. Controversy surrounds the OCLTT hypothesis, with mounting evidence demonstrating that the hypothesis lacks relevance and predictive power for the majority of ectothermic vertebrates. In this talk, I will outline some of the controversy related to the OCLTT hypothesis, including the negative consequences of marketing a hypothesis as universal while ignoring studies that contradict the foundational basis and predictions of the hypothesis. Moving beyond OCLTT, I will highlight promising avenues that are deserving of our scientific attention. I hope to promote healthy skepticism to inspire broader, multi-disciplinary research programs investigating the use of TPCs in predicting the lifetime fitness of wild animals.

14.3

Assessing the role of acclimation and adaptation in thermal performance curves

Johannes Overgaard[1], **Torsten N. Kristensen**[2], **Jesper G. Sørensen**[3], **Vanessa Kellermann**[4], **Kristian Beedholm**[1], **Volker Loeschcke**[3], **Heidi J. Maclean**[1]

[1]**Zoophysiology, Aarhus Univ.,** [2]**Department of Chemistry and Bioscience, Aalborg Univ.,** [3]**Genetics and Ecology, Aarhus Univ.,** [4]**Sch of Biological Sciences, Monash Univ.**

Environmental temperature is arguably one of the most important factors dictating the distribution of animals. All animals, including insects, must experience periods with temperatures that allow for growth and reproduction. It is, therefore assumed that thermal performance curves (TPC) for growth and reproduction have evolved to match the species thermal environment. Likewise, species can only exist in climates where they avoid lethal thermal stress. It is, therefore also assumed that tolerance to thermal extremes has evolved to match the occurrence in their environment. Nevertheless, there are few studies that have systematically investigated how capacity traits (growth and reproduction) and thermal tolerance traits (CT_{min} and CT_{max}) vary among and within species and how such variations relate to their natural distribution.
Capacity traits of ectotherms are often depicted using TPCs and textbook examples often highlight that temperate species have broader TPCs and lower optimal temperatures compared to tropical species. Furthermore, theory predicts that acclimation to low or high temperatures could shift the TPC and again it is

assumed that species originating from variable temperate environments are more plastic.

Here we measure TPCs of three fitness components (fecundity, egg to adult viability and developmental rate) of flies developed at 20°C and tested at seven test temperatures using 22 species of *Drosophila* originating from a wide range of temperate and tropical environments. For 10 of these species, we also measured how thermal capacity changed following developmental acclimation to three additional temperatures (15, 23 and 27°C). In parallel with these studies we also measured thermal tolerance traits (CT_{min} and CT_{max}) and investigated how these traits varied among and within species. Using these data, we test if *Drosophila* TPC's conform to the fundamental assumptions about the evolution and plasticity of TPCs.

We found that cold tolerance varied strongly between the species with higher tolerance in temperate species whereas heat tolerance was similar among species. Similarly, we found a positive and predictable response to both heat and cold acclimation. These results suggest that adaptation to temperate environments have involved the evolution of increased cold tolerance while the level of plasticity was similar among temperate and tropical species. Contrary to our expectation for fitness traits we find that the breadth and optimum of TPCs is similar in temperate and tropical species and we also find that the plasticity of TPCs is very limited. This suggests that TPC's in *Drosophila* species are stable within and among species irrespective of origin or acclimation treatments. Together our data suggest that thermal tolerance limits have evolved to overcome extreme environmental conditions whereas evolution of the temperature range for optimal population growth is more likely to be governed by temperatures that prevail during the thermally benign growing season. These results therefore support previous findings suggesting that species distribution of Drosophila can be modeled reliably using the tolerance traits CT_{min} and CT_{max}, while models using thermal capacity traits are unable to make reliable predictions of current distributions.

14.4

Comparing thermal performance curves across traits: how consistent are they?

Vanessa Kellermann[1]

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Thermal performance curves (TPCs), describing the relationship between temperature and fitness, are commonly integrated into species distributional models for understanding species responses to climate change. However, the accuracy of these predictions rests on a number of underlying assumptions, primarily that measured TPCs are static in time and space. TPCs however are unlikely to be static, which will have implications for the use of TPC's in climate change models. A few reasons TPCs may vary are: because different traits have different thermal sensitivities, traits vary with the timing and duration of the temperature exposure (short exposures: mins – hrs, long exposures: days – months), or because trait variation is sensitive to other factors rarely measured such as biotic interactions. Here I explore the extent to which TPCs in *D. melanogaster* vary across traits (fecundity, viability, activity and metabolic rate) and how the timing of the temperature exposure may influence the shape and descriptors of the TPC. Moving beyond intra-specific variation I also examine the role of species interactions in shaping TPC's for metabolic rate.

15: POSTER SESSION 1: ODD NUMBERED POSTER PRESENTATIONS

15.1

Mapping key-words in our journal to predict the future of comparative physiology

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Life sciences, including comparative physiology, develop as increased and novel insight provoke new academic questions and as emerging techniques enable mechanisms to be studied at more levels of biological organization. As part of this development, some research split, while others combine as new connections arise. Neurobiology, for example, started as a classic physiological discipline, but is now dominated by molecular biology. In an attempt to characterize the development of comparative physiology, we developed a scientific reference map based on key-words in papers published our leading journals (Journal of Experimental Biology, Journal of Comparative Physiology, Physiological and Biochemical Zoology and Comparative Biochemistry and Physiology) over the past three decades. This reference map is constructed from basic bibliographic information in Web of Science, and co-occurring keywords appear close to another and vice versa. The analysis allows a chronological tracking of the (dis-)appearance of certain key-words (i.e. scientific sub-disciplines) and reveal a clear emerging trend of recent publications in comparative physiology being focused more on ecology and conservation physiology. It is our hope that this type of quantitative and visual analysis can provide a foundation for informed discussion on the future of comparative physiology. A central emerging question, for example could be whether we are currently losing a firm grip on mechanisms whilst making the transition to ecophysiology? This type of analysis can also be used by editorial boards to identify emerging areas of research to be included in their journal and can in

general serve as a good foundation to discuss future directions. Finally, it is easy to map the position of individual researchers and can therefore be instrumental when evaluating job applicants or when identifying missing research fields in a given group of researchers.

15.2

Mechanisms underlying forelimb *vs.* hindlimb function during terrestrial locomotion in juvenile alligators

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Most biomechanical models of quadrupedal locomotion rely on the assumption that the animal has a center-of-mass (CoM) located equidistant from the pectoral and pelvic girdles. Previous work based on the whole-body mechanics of crocodilians suggest their caudally-displaced CoM imparts a braking role on their forelimbs (FLs) and a propulsive role on their hindlimbs (HLs). However, it remains unclear how crocodilians distribute these roles across the various limb joints involved in terrestrial locomotion. Here we use high-speed videography and force-plate ergometry to develop an inverse dynamics model for the limb joints in the American alligator (*Alligator mississippiensis*). We test how mechanical energy production (propulsion) and dissipation (braking) are distributed amongst FL and HL joints during steady-state walking. Our preliminary results show the wrist and elbow spend larger percentages of stance phase flexing, whereas the ankle and knee spend more time extending. We also find a greater braking impulse in the FLs and greater propulsive impulse in the HLs, and no significant difference in mediolateral or vertical impulses between limb pairs. Furthermore, the transition from braking to propulsion occurs later in the FLs when compared to the HLs. These results highlight the disparities in limb joint mechanics between corresponding distal joints in the fore- and hindlimb of alligators and will serve as a first step in quantifying joint moments during walking. Joint moments will help determine which limb joints, and therefore which limb muscles, contribute most significantly to the FL's net braking impulse and the HL's net propulsive impulse in crocodilians. This work will improve our overall understanding of skeletal muscle function in the context of the whole-organism and allow us to effectively characterize functional muscle specializations during terrestrial locomotion.

15.3

Effect of PIT tagging on aerobic metabolism and growth of the Gulf killifish, *Fundulus grandis*

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[1]Biological Sciences, Univ. of New Orleans

Passive integrated transponder (PIT) tags allow unambiguous identification of individual animal subjects in field and laboratory studies. Typically, PIT tags are implanted into the animal, raising the question of whether the tagging procedure could alter subsequently collected physiological data. Here, we evaluated the effects of PIT tagging on the standard metabolic rate (SMR) and specific growth rate (SGR) of the Gulf killifish, *Fundulus grandis*, a small estuarine fish native to the Gulf of Mexico. SMR, determined by intermittent-flow respirometry, and SGR, determined as percent change in body mass per day, were measured prior to PIT tagging and once per week after tagging for one month. Our results demonstrate that PIT tagging has no effects on SMR or SGR for the duration of the study. SMR was positively related to body mass, as expected, with the slope of a log-log relationship of 0.81. In addition, the SMR of male fish significantly decreased over the course of the experiment, independent of PIT tagging. We also found that male fish had higher SGR than female fish, again independent of tagging. We conclude that body mass, sex of fish, and duration of the experiment may affect SMR or SGR, but that neither variable was influenced by PIT tagging between 1 and 4 weeks after tagging. We suggest that PIT tagging can be used in *F. grandis,* and probably in other small fishes, with minimal or no impact on subsequent physiological measurements. Funding for this work was provided by the Greater New Orleans Foundation and the Audubon Nature Institute.

15.4

The effects of body mass on immune cell concentrations of terrestrial mammals

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Body mass is likely to affect the way organisms evolve, develop, and use immune defenses. Risk of exposure to parasites, physical constraints affecting transport and metabolism of defensive cells and their secreted molecules, and tradeoff options among alternative life histories all impinge on how hosts of different body sizes cope with infections. Here, we investigated whether and how body mass and blood neutrophil and lymphocyte concentrations were related among 300+ terrestrial mammalian species. First, we tested whether predictions derived from existing theories (Protecton Theory, basal metabolic rate constraints, or risk of parasite exposure) best-predicted slope coefficients. We then evaluated the predictive power of body mass for these leukocyte concentrations compared to sociality, diet, life history, and phylogenetic relatedness. Phylogeny was the most important predictor of

leukocyte concentrations; taxon explained 61% of variation in lymphocytes and 67% of variation in neutrophils. Body mass explained only a small portion of the variation (3% in lymphocyte and 9% in neutrophils), and other factors combined show similar importance. Our data for lymphocytes revealed a scaling coefficient close to, but not overlapping the slope predicted by the Protecton theory, whereas no hypothesis was supported for neutrophils. Indeed, the strong positive effect of body mass on neutrophils was unexpected: we found that, extrapolated to total cell numbers, a 3800 kg elephant circulates 13,300,000 times the neutrophils of a 15 g mouse, whereas their masses differ by only 250k-fold. We hypothesize that such high neutrophil numbers might offset i) higher parasite exposure that large animals face as they traverse more risk space per unit movement and/or ii) provide broad protection against the relatively higher cellular replication capacities of pathogens relative to that of large mammals.

15.5

Sequence analysis, expression, and preliminary functional characterization of *Aedes aegypti* sodium-dependent cation-chloride cotransporters

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The genome of the yellow fever mosquito *Aedes aegypti* contains three genes with sequence similarity to vertebrate Na-K-Cl cotransporters (NKCCs). One gene, aeNKCC1, codes for an ortholog to *Drosophila melanogaster* ncc69, which is a bumetanide-sensitive NKCC (1,2). Two genes, aeCCC2 and aeCCC3, group within an insect-specific clade of transporters that have not been functionally characterized. These paralogs are consecutive in the genome, have similar exon structures, and have orthologs in all mosquito genomes that we have evaluated. Thus, they probably arose from a tandem gene duplication at the base of the mosquito lineage. In prior qPCR experiments (3), we found aeCCC3 to be 100-fold more abundant in larvae than in adults. In larval tissues, aeCCC2 was 2-fold more abundant in Malpighian tubules compared to anal papillae. In contrast, aeCCC3 was nearly 100-fold more highly expressed in larval anal papillae compared to Malpighian tubules, suggesting a role in absorption. Consensus phosphorylation sites for Ste20-related proline alanine-rich kinase (SPAK) are present on aeCCC3 and the aeCCC2-X1 splice variant but not aeCCC2-X2 splice variant. Additionally, aeCCC2-X1 has several consensus protein kinase A sites that are not found on aeCCC3 or aeCCC2-X2. Both aeCCC2 and aeCCC3 lack a dileucine motif that targets human NKCC1 to the basolateral membrane (4) and is present in the aeNKCC1 sequence. Quantitative PCR with splice variant specific primers indicated that when both variants are measured, aeCCC2 was approximately 200 fold more highly expressed in adult female hindgut tissue than in Malpighian tubule, whereas the aeCCC2-X1 variant was equally expressed in the two tissues. An antibody produced against an aeCCC2-specific peptide detected bands at and above 120 kDa in adult and larval tissues and stains the basolateral membrane of larval Malpighian tubules. To evaluate aeCCC2 function, we measured uptake of lithium, a tracer for sodium, into oocytes injected with cRNA encoding aeCCC2. Following exposure to buffers containing lithium, oocytes were lysed in distilled water and lithium concentration was assessed by cation chromatography. Oocytes expressing aeCCC2 transported lithium at greater rates than water-injected controls. These findings suggest potential roles for aeCCC2 and aeCCC3 in mosquito transepithelial Na^+ secretion and absorption. Funding: NSF-IOS-1557230, Kenyon College, and State and Federal funds appropriated to the OARDC of the Ohio State Univ.

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15.6

Hypoxia Avoidance Behavior in two Air-breathing Fishes

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Aquatic animals are known to be sensitive to low O_2 environments. Aquatic hypoxia can limit an animal's capacity for aerobic metabolism, reducing its ability for locomotion, growth, reproduction, and survival. It is thus important for a fish to be able to detect and avoid hypoxic environments or develop an auxiliary method for obtaining O_2, such as breathing air. Air breathing allows a fish to access environments and maintain aerobic metabolism independent of environmental O_2 levels. In this study, we test two air-breathing fishes, *Polypterus* and *Pangasianodon*, for the presence of a hypoxia avoidance behavior both when allowed to freely breathe air and when air access is denied. Fish were placed in a shuttle box consisting of two circular arenas attached to allow free movement between the two. The position of the fish was monitored and O_2 was reduced on one arena and then returned to normal while the other side was reduced. Separate trials were run with a subsurface net to deny air breathing. Polypterus did not show a hypoxia avoidance behavior when allowed air access, however it

did when denied air access, similar to a solely water-breathing fish. *Pangasianodon* did not show a hypoxia avoidance behavior with or without air access even at O_2 levels below its air-breathing threshold and Pcrit. The lack of hypoxia avoidance behavior may correspond with the physiologically ecology of these two fishes. Hypoxia avoidance behavior may be important to *Polypterus*, being a demersal predatory fish, in the wild when surfacing to air breath maybe be costly, (e.g., when predation risk is high, while stalking prey, or in deep water). In contrast, a blunted response to external hypoxia may be advantageous for *Pangasianodon*, usually found in surface water with convenient air-access. Internal PO2 would be the more important to detect than external PO_2 to maintain O_2 delivery if frequently inhabiting hypoxic water but air-breathing is not naturally inhibited.

15.7

Parasitic infection-associated resemblance between locomotor muscles of dragonflies and obese vertebrates

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Metabolic disease with high similarity to vertebrate obesity and type 2 diabetes exists in natural populations of *Libellula pulchella* male dragonflies, and is caused by a protozoan gut parasite. Previous work examined how these parasites affect male *L. pulchella* flight behavior and flight muscle metabolism, and here I have extended those studies by examining infection effects on *in situ* muscle performance- and sarcomere traits relevant to *L. pulchella* flight performance, but that are also known to be negatively affected by vertebrate metabolic disease. I will show that infection impairs normal relationships between body mass, and flight muscle power output, and molecular composition. Moreover, infection affects *in situ* flight muscle endurance and causes a left-shift of the optima of flight muscle power-cycle frequency curves. Interestingly, power-frequency curves of infected *L. pulchella* resemble those of non-infected, teneral (i.e., physiologically immature) *L. pulchella* males. These findings show that effects of metabolic disease on skeletal muscle physiology and function in natural insect systems can be very similar to those observed in vertebrates maintained in laboratory settings.

15.8

Beneficial effects of fluctuating thermal regimes in the alfalfa leafcutting bee, *Megachile rotundata*

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Exposure to low temperature during metamorphosis often results in high mortality or sublethal effects. A growing number of studies have shown that exposing insects to recurrent brief warm pulses during low temperature stress (fluctuating thermal regimes, FTR) improves survival and ameliorates sublethal effects observed during exposure to constant low temperatures. However, FTR can fluctuate over many parameters. To determine which parameters are the most important, we exposed groups of developing *Megachile rotundata* pupae and emergence-ready adults to one of eight thermal profiles. All thermal profiles had base temperatures of 6°C, upper temperatures of 12°C or 18°C (peak temperature), different durations of exposure to the upper temperature (pulse length), and either a square or wave-based thermoprofile (shape). An additional group of insects were exposed to a constant low temperature of 6°C. Generalized linear models that treated components of the thermal profiles separately were better at explaining the variation in survival than average degree day models or other models that accounted for total temperature exposure. Within each life stage, bees exposed to the higher peak temperature (18°C) as a group had improved survival compared to the 6°C control and 12°C pulse thermal profiles. Within the 18°C peak temperature, bees exposed to the square profile had greater survival than bees under a wave-based profile, but that survival benefit did not hold for profiles with a peak temperature of 12°C. Survival of the eye-pigmented pupae and of emergence-ready adults appears to be not directly related to the total degrees accumulated, but to specific aspects of a thermal profile.

15.9

Intraspecific Variation in Thermal, Hypoxia and Acute High pH Tolerance in Rainbow Trout

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Increased temperatures due to climate change are predicted to reduce available habitat for coldwater fishes such as Rainbow Trout (*Oncorhynchus mykiss*) by up to 50% in North America. In addition, the indirect consequences of rising temperatures, such as decreased dissolved oxygen and altered pH can also have negative effects. The resilience of a species to altered environmental conditions is, at least in part, determined by a combination of intrinsic tolerance, the degree of intraspecific variation in tolerance, and the extent of phenotypic plasticity. In addition, when multiple environmental factors are changing simultaneously, phenotypic or genetic correlations among tolerance traits may also be important. Here, we examined intraspecific variation and plasticity in tolerance to elevated temperature, decreased oxygen, and elevated pH, environmental challenges which are facing Rainbow Trout populations in British Columbia. We determined Critical thermal maxima (CT_{max}), incipient lethal oxygen saturation (ILOS), and acute high (9.5) pH tolerance for

multiple strains of Rainbow Trout that are used in stocking programs that support the recreational fishery for this species. We detected significant differences in upper thermal tolerance, measured as CT_{max} among strains at the fry life stage (Blackwater River strain, mean = 28.2 ± 0.05 °C vs Carp Lake strain fry, mean = 28.4 ± 0.04 °C, $p < 0.05$) and ILOS (Blackwater River strain, mean = 12.1 ± 0.12 % sat. vs Carp Lake strain fry, mean = 11.3 ± 0.11 % sat. $p < 0.05$) but there was little difference in acute high (9.5) pH tolerance for the same strains. At the yearling stage, these trials were completed on individuals as repeated measures to determine if there was a correlation within individuals from particular strains with respect to tolerance of these traits. CT_{max} and ILOS were significantly correlated ($F(1,896) = 1369$, $p < 2.2e\text{-}16$; R^2 of .604), indicating that more thermally tolerant fish tend to also be more hypoxia tolerant. We also detected significant plasticity in these traits with differences in CT_{max} and ILOS across multiple acclimation temperatures (12, 18 and 24 °C, $p < 0.05$) within individual strains. We are currently examining the physiological and biochemical mechanisms that underlie these effects. Taken together, our findings will aid in the preservation of recreational fisheries and Rainbow Trout as a species by informing stocking programs as to which strains appear most resilient in the face of rapid climate change and hence most appropriate for stocking.

15.10

Adenosine A_1 receptor agonist-induced hibernation: effects of agonist and seasons on neuronal pathways

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Hibernation is an adaptive strategy characterized by metabolic suppression and a decrease in body temperature (T_b). Previous study in the Arctic Ground Squirrel (AGS) reported the role of A_1 adenosine receptor (A_1AR) in inducing hibernation. Treatments with N^6-cyclohexyladenosine (CHA), an A_1AR agonist, promotes the onset of hibernation, but showed a seasonal difference of A_1AR sensitivity to its agonist. However, what regulates the seasonal control of the agonist response is still unknown. We test the hypothesis that thermoregulatory pathways are differentially activated by CHA depending on season.

CHA or vehicle (0.5mg/kg) was administered intraperitoneally and after 3 hours AGS were perfused with 4% paraformaldehyde and brains removed for immunohistochemical analysis. Free-floating immunohistochemistry was used to localize active nuclei as indicated by cFos-immunoreactivity (mouse anti-cFos 1:20,000, Millipore). Data analysis was performed in R.

In our results, CHA-induced hibernation correlates with higher activation in the Nucelus Tractus Solitarius (NTS). The NTS has been previously identified as a site of action of CHA in the rat. The Median Preoptic Nucleus (MnPO) and the Raphe Pallidus (rPA) are thermoregulatory nuclei; MnPO and rPA show a lower cFos activation in winter compared to summer AGS following CHA treatment ($p<0.05$ treatment by season ANOVA); thus CHA response in winter may be mediated by these nuclei, decreasing thermogenesis leading to hibernation onset.

Other brain regions show difference in neuronal activation as the Tuberomamillary Nucleus (TMN), a region regulating wakefulness. The TMN shows a decrease in cFos activation after CHA treatment. This suggests that CHA inhibits wakefulness to promote hibernation. The rPA shows a lower activation in winter compared to summer in non-treated AGS. This result correlates with the lower euthermic body temperature of AGS in winter compared to summer.

In conclusion, hibernation is characterized by a seasonal decreased in thermogenesis; and CHA-induced hibernation correlates with a further suppression of thermogenesis and decrease in wakefulness.

15.11

Characterization of the HIF-1 pathway in response to an acute heat stress in Antarctic notothenioid fishes

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The long evolution of Antarctic fishes in the cold, oxygen-rich waters of the Southern Ocean may have reduced their capacity to respond to hypoxia, thereby contributing to their low thermal tolerance. The transcription factor, hypoxia-inducible factor-1 (HIF-1), a heterodimer of HIF-1α and HIF-1β subunits, is the master regulator of oxygen homeostasis in all metazoans. In Antarctic notothenioid fishes, HIF-1α has a polyglutamine (Q) and glutamic acid (E) repeat that is longer in the more oxygen sensitive and hemoglobinless icefishes (16-34 amino acids) than in red-blooded species (4-16 amino acids). The functional effect of the polyQ/E repeat in HIF-1α of notothenioids is unknown, but in humans, the expansion of polyQ repeats causes protein aggregation and is associated with several diseases, while shorter polyQ repeats (~ <35-40 residues) are prevalent in transcription factors and enhance transcriptional activity and facilitate protein-protein interactions. We sought to characterize the activity of the HIF-1 pathway in Antarctic notothenioids during an acute heat stress induced by exposure to their critical thermal maximum (CT_{MAX}). Studies were focused on the heart ventricles of the red-blooded notothenioid, *Notothenia coriiceps* (polyQ/E repeat = 9 amino acids), and the icefish, *Chaenocephalus aceratus* (polyQ/E repeat = 34 amino acids), held at ambient temperature or exposed to their CT_{MAX}. HIF-1α was quantified in nuclear extracts using western blotting and HIF-1 DNA binding was measured using an

electrophoretic mobility shift assay. Transcript levels of lactate dehydrogenase A (LDH-A), a glycolytic enzyme regulated by HIF-1, were quantified using quantitative real-time PCR. Levels of HIF-1α and HIF-1 DNA binding were significantly lower in heart ventricles of *C. aceratus* than *N. coriiceps* and did not increase in response to exposure to CT_{MAX}, nor did transcript levels of LDH-A change in response to exposure to CT_{MAX}. The significantly lower level of nuclear HIF-1α and HIF-1 DNA binding activity in hearts of *C. aceratus* may be due to the polyQ/E repeat preventing gene transcription and/or nuclear localization of the protein. Alternatively, the polyQ/E repeat may enhance HIF-1 transcriptional activity, requiring less protein to drive gene expression. Future studies will determine if the lack of a HIF-1-mediated response following exposure to CT_{MAX} is caused by the short duration of the CT_{MAX} experiment (3-4 hours), the loss of the ability of these fishes to respond to hypoxia, or CT_{MAX} not leading to hypoxic conditions in the heart. Funding was provided by grants from the National Science Foundation (ANT 1341663 to KOB) and Alaska INBRE through an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103395.

15.12

Variation in thermoregulation and linking whole organism behavior to thermosensory neurophysiology in the porcelain crab, *Petrolisthes cinctipes*

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Small-scale shifts in species distributions are expected to occur under future climate scenarios for many species. These shifts can have consequences for population dynamics, and therefore it is important to understand when and why they occur. The intertidal crab *Petrolisthes cinctipes* currently experiences temperatures near lethal levels. However, the extent to which crabs move in response to temperature and the thermal thresholds that trigger migration to cooler microhabitats remain unknown. We tested for effects of body size and reproductive state on escape temperature (Tesc). In addition, we tested for the relationship between Tesc and the temperature of peak action potential firing frequency in sensory afferent neurons. We found that both size and reproductive state influence behavioral sensitivity to temperature. Small crabs tolerate significantly higher temperatures before they move to cool refuges (a higher Tesc) compared to large crabs. In addition, non-gravid crabs have significantly higher Tesc than gravid females. We also found that Tesc is positively correlated with peak neural performance of spontaneous action potentials (R^2=0.26). We find that behavioral sensitivity to temperature varies consistently with size and reproductive state. These findings have implications for species persistence, rates of dispersal and community dynamics. The vulnerability of marine organisms to global change is predicated on their ability to utilize and integrate these physiological and behavioral strategies to promote survival and reproductive fitness; understanding these strategies will allow predictions of species distributions under warming and the potential for extirpation.

15.13

Bone composition of an elite mammalian diver, the Weddell seal: Implications for the use of bone as a buffer.

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The Western painted turtle, *Chrysemys picta bellii*, can survive exceptionally long periods of anoxia, up to 177 days at 3°C, in part, because they utilize their skeletal system, including their shells, to buffer lactic acidosis that results from reliance on anaerobic respiration. Buffering is achieved, in part, using carbonates from their bones to maintain plasma pH at survivable levels. Studies of reptilian and amphibian bone have demonstrated this ability to be a generalized property of vertebrate mineralized tissues that correlates with its carbonate content. To determine whether this property might extend to mammalian deep-divers, we analyzed the composition of dermal bone from the Weddell seal (*Leptonochytes wedellii*), a species which voluntarily dives beyond its calculated aerobic dive limit, resulting in the accumulation of lactate in plasma. Bone from 2 adult and 5 pup seals, 3 adult painted turtles, and 3 adult dogs was dried, powdered, and total carbonates were measured using the nitric acid/CO_2 evolution technique. Levels of CO_2 measured in dermal bone from Weddell seal adults and pups (mean ± SEM, 774.3 ± 17.4 mmol/kg dry bone) were lower than those present in painted turtles (mean=1343.2 ± 159.1 mmol/kg dry bone), and similar to those in canine bone (mean=863.7 ± 9.5 mmol/kg dry bone). There were no differences in bone CO_2 between adult and seal pups. ICP-OES analysis of bone ash indicated that calcium/phosphorous ratios in Weddell seal (mean=1.58 ± 0.01) are similar to those in painted turtles (mean=1.63 ± 0.02), but less than in dogs (mean=1.86 ± 0.03). There were no qualitative differences between the pup and the adult seals for any of these measurements. Based on these compositional analyses, we conclude that Weddell seal dermal bone does not possess exceptional levels of carbonate, unlike the acidosis-tolerant painted turtle bone, suggesting Weddell seals do not likely utilize bone as a buffer in the

same way. Further studies are needed to determine the relative importance of mineralized tissue in buffering lactic acidosis in elite mammalian divers.

15.14

Identification and Characterization of a Sodium/Hydrogen Exchanger in Coral: a potential role in biomineralization.

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Although coral skeleton biomineralization is responsible for creating the massive reef ecosystems found throughout the world, the cellular mechanisms are mostly unknown. Biomineralization requires the transport of $Ca^{2+}$and dissolved inorganic carbon to the subcalicoblastic medium (SCM), the microenvironment located underneath coral tissues where the skeleton is built. Additionally, H^+ are constantly produced as a byproduct of calcium carbonate precipitation and must be removed from the SCM to prevent acidification that would impair biomineralization. We investigated the potential role of Na^+/H^+ Exchangers (NHEs) in this process. Although NHEs are essential for H^+ secretion and pH regulation across the animal kingdom, they have not been characterized in corals. BLAST searches identified several genes encoding for NHE-like proteins in *Acropora, Stylophora,* and *Orbicella* corals. We focused on an *Acropora* gene that shares the strongest homology with mammalian NHE2. Bioinformatics analyses predicted *Acropora*NHE2 is ~93 kDa in size, has 10 transmembrane domains typical of NHEs, a long extracellular C-terminus tail, and abundant potential glycosylation sites. To further characterize *Acropora*NHE2, we generated specific antibodies and studied its protein abundance and cellular localization in *A. yongei*. Western blotting on membrane-enriched fractions detected a specific protein of ~114 kDa. The discrepancy with the smaller predicted size is most likely due to glycosylation, a possibility we are currently investigating. Immunohistochemical analysis revealed *Acropora*NHE2 was highly abundant in the calcifying cells. *Acropora*NHE2 was also present in desmocytes that anchor coral tissue to the skeleton, in intracellular structures resembling the Golgi apparatus in symbiocytes, and in the apical membrane of oral ecotdermal cells. The high abundance of *Acropora*NHE2 in calcifying cells suggests it is important for H^+ removal from the SCM, therefore promoting biomineralization, as well as in intracellular pH regulation. However, those roles must be confirmed by functional experiments. This type of basic information about the cellular mechanisms behind coral biomineralization is essential to be able to predict responses to environmental change, as well as to identify potential species-specific mechanisms that determine differential vulnerability and resilience. This

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15.15

Cortisol mediates claudin-28b abundance and its contribution to model gill epithelium barrier properties via the mineralocorticoid receptor

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Cortisol-induced tightening of a primary cultured gill epithelium model derived from the gill of freshwater (FW) rainbow trout (*Oncorynchus mykiss*) occurs in conjunction with a significant reduction in the permeability of the paracellular pathway. With this, the molecular physiology of the epithelium tight junction (TJ) complex reorganizes so that select barrier-forming TJ proteins become more abundant. Using pharmacological blockers of corticosteroid receptors (CRs), it has been suggested that the molecular composition of the TJ complex is rearranged by cortisol through binding to the epithelium mineralocorticoid receptor (MR) as well as glucocorticoid receptors (GRs). However, because pharmacological blockers of CRs in fishes can sometimes produce ambiguous results, this study considered how cortisol influenced gill epithelium permeability and TJ properties by transcriptional knockdown (KD) of the gene encoding MR. Following *mr*-KD a significant reduction in MR protein abundance was observed in the gill epithelium. In the absence of cortisol, *mr*-KD reduced epithelium transepithelial resistance (TER) and increased the paracellular flux of [^{3}H] polyethylene glycol (MW 400 kDa, PEG 400). Cortisol treatment significantly increased TER and reduced PEG 400 permeability, and this was further enhanced in *mr*-KD preparations, indicating that a reduction in MR abundance allowed the epithelium to become tighter in the presence of cortisol. Cortisol treatment significantly increased the transcript and protein abundance of TJ proteins such as claudin (*cldn*/Cldn) -8d, and -28b. However, in *mr*-KD preparations, Cldn-28b protein abundance did not significantly alter in response to cortisol treatment, while Cldn-8d was significantly elevated as observed in control preparations. These data suggest that while the barrier protein Cldn-8d is responsive to cortisol through both the MR and GR, Cldn-28b protein abundance may be modulated by cortisol via the MR only. In this regard, there appears to be a distinction between MR and GR mediated pathways in the regulation of gill epithelium paracellular permeability.

15.16

Osmotic activation of motility and expression of aquaporin proteins in sperm from the gray treefrog *Dryophytes chrysoscelis*

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Gametes of gray treefrogs, *Dryophytes chrysoscelis*, are deposited into freshwater ponds. We tested the hypothesis that exposure to a hypotonic environment activates sperm motility. We also hypothesized that osmotic water uptake is facilitated by expression of water channel proteins from the aquaporin family. To test these hypotheses, we collected sperm from captive treefrogs maintained with food and water at 22C and assessed motility of sperm immersed in solutions of varying osmolarity. We also assessed expression of mRNA and protein for two aquaporins, HC-1, a homolog of the water channel AQP1, and HC-7, a homolog of the glyceroporin (glycerol/water channel) AQP7, in sperm and testes from those warm-acclimated animals and from animals that were cold-acclimated during the autumn and winter. Sperm from gray treefrogs could be rendered immotile by immersion in isotonic (280 mosmol/l) PBS. Motility was increasingly activated at more hypotonic concentrations, to maximum motility at 50 mosmol/l, with diminished motility in more dilute solutions. The specific type of motility (e.g., forward moving sperm vs. "wobblers") also varied with osmolarity. We detected mRNA for both the aquaporin HC-1 and the glyceroporin HC-7 in testes from warm-acclimated, cold-acclimated, and post-freezing thawed animals, but not in emitted sperm. Western blotting indicated both HC-1 and HC-7 were expressed in testes from animals in all three thermal conditions. Immunolocalization using confocal microscopy of cross sections of testes from warm-acclimated treefrogs indicated HC-1 protein expression in the mesentery surrounding each testis, in the epididymis, and in interstitial cells. No expression was evident in the immature spermatogonia or in mature spermatozoa within the tubular lumen. HC-7 in warm testes was expressed in the interstitial tissues of the testes and, at low levels, in primary spermatocytes within the seminiferous tubules. No HC-7 expression was detected in mature sperm. We conclude that treefrog sperm require osmotic activation to acquire motility. That water uptake likely is achieved via water channel proteins, but the specific aquaporins expressed in mature spermatozoa remains to be confirmed.

15.17

Does the capacity for seasonal plasticity differ between aquatic and terrestrial life-history stages in the eastern newt (*Notophthalmus viridescens*)?

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Climate change is impacting biodiversity worldwide, and the survival of some species may depend on their capacity for phenotypic plasticity to buffer the effects of environmental change on organismal function. Specifically, seasonal plasticity (via thermal acclimatization) compensates for the effects of temperature on performance, allowing some ectotherms that live in temperate climates to maintain physiological function despite seasonal variation in body temperature. Studies comparing different populations of the same species suggest that ectotherms from diverse thermal environments have evolved different degrees of plasticity. However, few studies have examined how animals that alternate between aquatic and terrestrial habitats at various life-history stages differ in their capacity for seasonal plasticity. Therefore, the goal of this study was to determine if differences in the stability of the thermal environment has resulted in differences in the seasonal plasticity of metabolism between terrestrial and aquatic life-history stages in the eastern newt (*Notophthalmus viridescens*). The life-history of eastern newts is unique among amphibians—aquatic larvae metamorphose into terrestrial efts, and after 2 to 7 years the efts metamorphose into aquatic adults. As adults, eastern newts remain active in winter by upregulating the activities of oxidative enzymes in skeletal muscle to compensate for the negative effects of temperature on metabolism, but little is known about their capacity to acclimatize during the terrestrial eft stage. Because seasonal plasticity requires a predictable thermal cue in order to benefit the organism, we predict that the plasticity of metabolism will differ between aquatic adults and terrestrial efts. To test this hypothesis, we measured whole-animal rates of oxygen consumption and the activities of metabolic enzymes in skeletal muscle of both adult and juvenile eastern newts that were acclimated to either winter (8˚C, 10L:14D) or summer (26˚C, 14L:10D) conditions for eight weeks. Preliminary results indicate that rates of oxygen consumption are higher in winter compared to summer-acclimated adults, but do not differ between winter and summer-acclimated efts.

15.18

Plastic plasticity: phenotypic plasticity at one time scale changes plasticity at another time scale in *Tigriopus californicus*

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Phenotypic plasticity, or the expression environmentally mediated alternative phenotypes, is a key process through which organisms respond to changes in their environment. For instance, plasticity in response to changes in temperature may play a major role in mitigating the effects of climate change on organisms

and ecosystems. Even considering only within generation plasticity in response temperature variation, plasticity occurs over a range of time scales. Differences in temperature experienced during development can result in irreversible phenotypic differences that persist throughout an organism's life, whereas changes in temperature experienced later in life often result in reversible modifications of phenotypes. Despite the fact that both developmental and reversible plasticity often affect the same important physiological traits, such as thermal tolerance, little is known about the capacity for developmental plasticity to influence the expression of reversible plasticity later in life. In the current study, we examine the combined effects of developmental and reversible plasticity on the thermal tolerance of the intertidal copepod *Tigriopus californicus*. Populations of *T. californicus* are found along the west coast of North America from Baja, Mexico to Alaska, USA, and are known to demonstrate local adaptation of upper thermal tolerance across this latitudinal range. Additionally, temperatures experienced during development result in plasticity of upper thermal tolerance in *T. californicus*, as in essentially all populations 25 °C-developed copepods tolerate higher temperatures than 20 °C-developed copepods. Our data reveal that variation in developmental temperature not only results in plasticity of adult thermal tolerance, but also changes the reversible plasticity of thermal tolerance in adults. Copepods developed at 25 °C demonstrated an increase in upper thermal tolerance when adult acclimation temperature was increased from 20 to 25 °C, whereas copepods developed at 20 °C had no change in tolerance in response to the same change in adult acclimation temperature. These results suggest that the extent to which phenotypic plasticity is able to mitigate the organismal consequences of climate change will depend on the interactive effects of plasticity across a range of time scales.

15.19

Diversification of Characteristics Related to Endothermy in *Thunnus* Tunas

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Birds, mammals, and certain fishes, including tunas, opahs and lamnid sharks, are endothermic, conserving internally generated, metabolic heat to maintain body or tissue temperatures above that of the environment. Bluefin tunas, among the most threatened, but commercially important, fishes worldwide are renowned for their endothermic physiology, maintaining elevated temperatures of the oxidative locomotor muscle, viscera, brain and eyes, and occupying cold, productive high-latitude waters. Less cold-tolerant tuna, such as yellowfin tuna, by contrast, remain in warm-temperate to tropical waters year-round, reproducing more rapidly than temperate bluefin tuna. Little is known of the genetic processes underlying the diversification of tuna. In collecting and analyzing sequence data across 29,556 genes, we found that parallel selection on standing genetic variation has driven the evolution of endothermy in bluefin tunas. This includes two shared substitutions in genes encoding glycerol-3 phosphate dehydrogenase, an enzyme which underlies thermogenesis in bumblebees and mammals, as well as four genes involved in the Krebs cycle, oxidative phosphorylation, β-oxidation and superoxide removal. Using phylogenetic techniques, we further illustrate that the eight *Thunnus* species are genetically distinct, but found evidence of mitochondrial genome introgression across two species. Phylogeny-based metrics highlight conservation needs for some of these species.

15.20

Hypoxia-Induced Oxidative Stress in Fundulid Killifish

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Aquatic hypoxia is a naturally occurring environmental stressor that has the potential to promote adaptation in fish populations. Additionally, aquatic hypoxia and fluctuations in environmental oxygen availability have been linked to an increase in the production of reactive oxygen species and oxidative stress. We investigated the relationship between hypoxia tolerance and resistance to hypoxia-induced oxidative stress in species of Fundulid killifish that differ in their tolerance of oxygen deprivation based on their time to loss of equilibrium at 3% O_2 saturation. These fish species were exposed to acute hypoxic challenges normalized to the species' critical pressure of oxygen (P_{crit}) for 12 hours with and without subsequent re-oxygenation. Lipid peroxidation, which is a marker of oxidative stress, was measured in the brain, muscle, liver, and gill using the xylenol orange assay. These data will be used to assess the relationship between hypoxia tolerance and oxidative stress during and following hypoxia exposure in closely-related species of Fundulid killifish.

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15.21

Integration of endocrinology, behavior and body temperature of the South-American tegu lizard *Salvator merianae*

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The tegu lizard *Salvator merianae* exhibits annual cycles of high activity during the spring and summer, and hibernation during winter, a seasonal rhythm that is independent of ambient temperature. *S. merianae* exhibits a unique combination of traits among lizards — thermogenic capacity and seasonal metabolic adjustments, with a regularly occurring hibernation phase — that makes this species an interesting subject for study of comparative aspects of endocrine regulation of seasonal changes in physiology and behavior. We investigated seasonal changes in body temperature (Tb), activity (overall dynamic body acceleration; ODBA) and plasma concentrations of hormones involved in regulation of energy metabolism (thyroid hormones T_4 and T_3; corticosterone) and reproduction (testosterone in males and estrogen/progesterone in females) across the annual cycle of a captive population of *S. merianae*. Following emergence from hibernation in August (late winter in southeastern Brazil), males maintained a higher average Tb than females (25.4±0.3 vs 24.3±0.1°C; $P<0.001$, respectively). The transition from low (June-hibernation) to peak (September-reproduction) levels of plasma testosterone in males (0.65± 0.13 *vs* 4.82±0.83 $ng.mL^{-1}$; $P<0.001$, respectively) was positively related to ODBA (August: $r=0.72$; $P=0.017$) and Tb (August: $r=0.93$; $P<0.001$), suggesting that testosterone may trigger the end of dormancy like seen in some mammalian hibernators. Estradiol in females also peaked in spring coincident with reproductive behaviors. Progesterone in females gradually increased from low (June) to high levels in October (0.28± 0.04 *vs* 0.85±0.08 $ng.mL^{-1}$; $P<0.001$) when putative ovulation occurs and gravid females build nests. Nest building behavior contributes to a higher ODBA in females relative to males (0.025± 0.002 *vs* 0.020± 0.001g; $P=0.011$; respectively). The thyroid hormones, known for influencing energy metabolism, varied seasonally with some sex-dependent differences. T_4 gradually increased from an annual nadir during prehibernation and hibernation to high concentrations during spring in both sexes. In contrast, T_3 was not seasonally modulated in males, but females showed a two-fold increase in T_3 during the spring reproductive season. This sex-dependent seasonal change in T_3 secretion may underlie reproductive physiology and behavior of female *S. merianae* by supporting increased metabolism and modulating energy allocation during annual cycles of folliculogenesis, nest building and oviposition. Corticosterone was significantly elevated during the active season in both sexes, suggesting its involvement in mobilization of energy stores and modulation of behavior and physiology. This is the first study to characterize the integrated seasonal profile of reproductive, adrenal and thyroid hormones, as well as Tb and activity in this endemic and physiologically unique South American lizard. Our findings provide greater understanding of tegu physiology and behavior, insights needed for management of free-living tegus in South America, as well as management of invasive populations in other parts of the world.

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15.22

Hot and Dry: Effects of heat waves and water limitation on metabolic and evaporative water loss rates

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Environmental temperature is important because it influences a range of animal processes, including behavior, energy use, locomotion, and reproduction. In addition to warming (increasing mean temperature), environments are expected to continue to exhibit an increased frequency of extreme temperature events, such as heat waves. Some animals can respond to heat waves by reducing their metabolic rates (after controlling for test temperature) to conserve energy. Heat waves often coincide with reduced water availability (e.g., drought), and water is critical to homeostasis. Therefore, a combined heat wave and drought may reduce rates of both energy and water use. To investigate, we employed a 2 x 2 factorial manipulation of temperature (field-parameterized heat wave vs. control diel regime) and water availability (*ad libitum* vs. absent) in fasted variable field crickets (*Gryllus lineaticeps*). After 4 days of treatment, we used flow-through respirometry to estimate metabolic rate (VCO_2) and evaporative water loss rates at 28°C. We will discuss whether temperature regime and water availability exhibit additive, synergistic, or antagonistic effects on rates of metabolism and water loss. Together, our results will provide new insight into the effects of shifts in co-varying environmental factors (e.g., combined heat wave and drought) on animals' water and energy budgets.

15.23

Skeletal Muscle Thermoregulation and Metabolic Control in Hibernating Arctic Ground Squirrels

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Skeletal muscle shivering, brown adipose tissue (BAT), and metabolic rate are the identified major sources of heat production in mammals to maintain homeostasis. Recently, skeletal muscle non-shivering thermogenesis has been highlighted as a significant thermogenic source, which occurs via sarcolipin (SLN) uncoupling of sarcoendoplasmic reticulum (SR) calcium ATPase (SERCA)-mediated ATP hydrolysis and ryanodine (RyR) mediated calcium leakage from the SR (1,2,3). The SLN/SERCA pathway may play a significant role in thermoregulation of hibernating animals due to the wide temperature range experienced and periodic rapid metabolic increase every 3-4 weeks, which briefly return squirrels to euthermic temperatures (inter-bout arousals). We hypothesized that skeletal muscle uncoupling is a significant source of thermogenesis and inhibiting this pathway can alter metabolic rates during hibernation in Arctic Ground Squirrels (AGS). This work aims to elucidate skeletal muscle SLN uncoupling and its connection with BAT heat production in thermoregulation. Initial studies sampled tissues from AGS housed at 4 °C across the hibernation season, and compared to AGS exposed to extreme cold (-10 °C) for 2 weeks during the hibernation cycle, to determine if increased metabolic stress would cause modulation of thermogenic pathways. The following season, AGS were treated with inhibitors of BAT-uncoupling or skeletal muscle-uncoupling during hibernation (4 °C). Rate of temperature increase and change in VO_2 were compared to internal controls during inter-bout arousals to quantify the effect of suppressing BAT or skeletal muscle uncoupling throughout hibernation. Preliminary results show that SERCA/SLN expression is increased during periods of cold temperature stress and fluctuate throughout the hibernation cycle. Protein expression of SERCA, RYR, and SLN are seen to increase during the later portions of hibernation bouts and post hibernation season. Proteins were also seen to increase in squirrels exposed to extreme cold. This indicates that increased metabolic stressors and increasing body temperature during periods of rewarming could be achieved by recruitment of the SLN/ SERCA pathway. Additional results show that suppressing skeletal muscle uncoupling by SLN, using dantrolene (RyR inhibitor that decreases cytosolic calcium) caused a dose dependent reduction in metabolic rate during AGS rewarming. Inhibition of BAT thermogenesis did not reduce metabolic rate highlighting the importance of SLN in thermoregulation and metabolism. The SLN/ SERCA pathway appears to act in conjunction and independently of the BAT uncoupling pathway to increase and control thermogenesis and metabolism during periods of inter-bout and complete arousal at the end of the hibernation season. Thermogenesis control is directly linked to metabolic rate and energy loss regulation, as seen in hibernating AGS, and thus has the potential to innovate treatments for obesity and metabolic syndromes.

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15.24

Fat to the High-Altitude Fire: Thermoregulation in Deer Mice

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It has been shown that the ability to effectively thermoregulate is under a strong directional selection in deer mice (*Peromyscus maniculatus*) native to high altitude. These small endotherms are constantly exposed to a cold and hypoxic (CH) stress all-year round. Therefore, thermogenesis is a demanding metabolic process and is fuelled mainly by lipids. Recently, our lab has determined that the maximal rates of lipid oxidation during maximal bouts of thermogenesis are three-four times higher compared to the maximal lipid oxidation rates observed during exercise. We have also shown that highland native deer mice have a greater capacity to oxidize lipids in muscle compared to their lowland conspecifics. How these mice are able to oxidize lipids at higher rates for heat production than exercise, and how highlanders maintain elevated lipid oxidation rates compared to lowlanders, remains unclear. To address this issue, I used lowland and highland native deer mice born and raised in common laboratory conditions to examine potential population differences in lipid storage, mobilization, circulatory transport, muscle uptake and mitochondrial oxidation. I also examined the role of phenotypic plasticity on lipid oxidization pathways at

high altitude by acclimating mice to combined CH. Results suggest that unacclimated highlanders maintain increased capacity to uptake fatty acids from the blood into the gastrocnemius, increased capacity for mitochondrial oxidation, and increased intramuscular triglyceride stores compared to lowlanders. Both populations increased thermogenic capacity with CH exposure, but showed distinct phenotypic plasticity changes along the lipid oxidation pathway. Whether the differences and/or changes along the lipid oxidation pathway are responsible for the higher rates of whole animal lipid oxidation in highlanders is unclear. My project and future research will be the first step in understanding the mechanistic underpinnings responsible for the highest observed lipid oxidation rates seen in any mammal.

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15.25

Modeling energy use of overwintering hatchling turtles using over a decade of nest temperatures

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Many animals endure extended bouts of dormancy during which they are aphagic and thus, must rely on endogenous energy stores to survive and to fuel post-arousal activities. Because temperature directly influences metabolic rate in ectothermic animals, the thermal environment during dormancy profoundly affects energy use and conservation by dormant ectotherms. Moreover, temperature varies among microhabitats both spatially and temporally such that dormant animals inhabiting different microhabitats may incur very different energy costs. We investigated the potential variation in energy costs among hatchling painted turtles (*Chrysemys picta*) that overwinter in their natal nests. First, we measured rates of oxygen consumption (VO_2) for individual turtles at varying temperatures (-1.5 – 25°C) during a multi-month acclimation to winter. Hatchling turtle VO_2 was very sensitive to temperature (range of Q_{10}: 3.3 – 7.4), and was characteristic of reverse metabolic compensation as the turtles were acclimated to lower temperatures. We then used those data to generate a predictive model of VO_2 as a function of temperature and fit that model to the thermal profiles (recorded hourly from 15 September to 21 March) of 138 successful natural nests from 2001 to 2013. Mean nest temperature varied significantly ($P = 0.004$) across years with 2004-05 having the highest nest temperatures. However, predicted rates of energy consumption did not vary significantly among years ($P = 0.116$). The marked variation among all nests, regardless of year, and the ~4-fold difference between the lowest (7.7 ml O_2 g^{-1}) and highest (33.4 ml O_2 g^{-1}) predicted energy consumptions suggest that the microhabitat of the nest site has profound energetic implications for hatchling turtles. Indeed, the literature suggests that hatchling *C. picta* are in poor condition after having consumed 24.9 – 56.7 ml O_2 g^{-1} during dormancy which means that turtles in some of the natural nests may have exhausted their energy reserves. A multiple regression analysis of all nests showed that predicted energy consumption was separately and positively correlated to both mean nest temperature (coefficient = 0.43, $P < 0.001$) and the thermal variability of a nest (coefficient = 0.80, $P < 0.001$). Not only does this suggest that hatchling turtles in warm and thermally variable nests incur greater energy costs than those in cool and thermally stable nests, but the coefficients suggest that thermal variability has an even greater impact on energy use than does mean nest temperature. By using this simple energetic model, we were able to take advantage of more than a decade of thermal profiles from natural *C. picta* nests to better understand the energy budget of hatchling turtles. We suggest that a similar approach could be used on any dormant ectotherm.

15.26

Regulation of muscle pyruvate dehydrogenase in high altitude deer mice

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Low O_2 availability at high altitude (HA) can impair the ability for animals to engage in aerobic submaximal exercise. However, deer mice (*Peromyscus maniculatus*) are surprisingly abundant in the high alpine. HA deer mice have higher aerobic capacities than low altitude (LA) mice and a greater reliance on carbohydrate oxidation (CHO) at equivalent submaximal intensities as an O_2-saving strategy (1). It is unclear if differences in muscle metabolic phenotypes are responsible for population differences in fuel use. However, activities of most glycolytic enzymes of gastrocnemius muscles are equivalent in HA and LA deer mice and resistant to hypoxia acclimation (1). We hypothesized that altered metabolic regulation of the CHO pathway in muscle allows higher rates of CHO oxidation during submaximal exercise at HA. To test this we used first generation lab born and raised LA and HA deer mice to determine activation of pyruvate dehydrogenase (PDHa) in muscle using electrical stimulation of varying intensity relative to maximal force production *in situ*. Results show an induction of PDH activity with contraction and a positive PDHa to intensity relationship in both populations. While maximal PDH activity values in normoxic HA mice were slightly higher than LA mice, the degree of PDHa activation was ~20% higher than LA mice. PDHa activation in hypoxia-acclimated HA mice were ~50% higher than normoxic HA mice at similar muscle

workloads. However, kinetics of the PDH reaction might be different under acute hypoxic condition, where muscle O_2 availability is decreased. By identifying trends in PDH activation with exercise, we can provide a mechanistic explanation for whole-animal fuel selection strategies during exercise *in vivo*.

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15.27

d-Amphetamine Exposure to Early Embryonic Zebrafish Reveal Neural and Developmental Consequences

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By 2016, more than 9.4% of US children aged 2-17 had been diagnosed with attention deficit disorder, and of those 6.1 million adolescents, 62% were medicated with prescribed amphetamines. Not only are rates of diagnosis of behavioral attention disorders and amphetamine prescription to treat symptoms growing, but also illicit use is more prevalent than ever among college aged and working adults who seek easy ways to improve productivity and concentration, and even decreasing appetite. With both prescriptive and illicit amphetamine consistently increasing, risks for exposure to nervous systems are more and more likely, especially during critical embryonic, childhood, or adolescent neurodevelopmental stages. To understand specifically how amphetamines affect somatic and neural growth, we exposed early stage zebrafish embryos (0-1 hour post fertilization, hpf, through 72 hpf) to four amphetamine treatment concentrations (0, 10, 20, 30 mg/ml). We assayed growth and survival parameters (average length, developmental stage, natural chorion emergence, mortality, and morphological anomalies), as well as locomotor behaviors to measure the effects of amphetamines on the development and function of escape circuits. At 24hpf, amphetamine-exposed zebrafish embryos yielded mortality rates ranging from 19-20% of total, while mortality among controls was only 12%. Likewise, general development parameters were significantly delayed in amphetamine-exposed embryos compared to controls. Average natural chorion emergence for controls was 49.2 hpf, while hatching rates were slowed in amphetamine-exposed embryos (61 hpf for 10 µg/ml, 57.8 hpf for 20 µg/ml, and 58.8 hpf for 30 µg/ml). Body lengths in amphetamine-exposed embryos were significantly shorter than controls: 10 µg/ml ($p = 0.000039$), 20 µg/ml ($p = 0.00007$), and 30 µg/ml ($p = 0.000321$), and showed more common morphological anomalies, including blunted tails, spinal curvature, and pericardial and yolk sac edema. Amphetamine-exposed fish also showed more prevalent spastic episodes during elicited escape behaviors. Common spastic phenotypes included a hyper-wound, “corkscrew” C-bend coil, and stuttered, choppy, or hyper-paralytic coils and bends, resulting in circular movement or active paralysis, significantly lengthening the time to complete the C-bend portion of escape behavior when compared to controls ($p < 0.0001$). Finally, in an attempt to understand the underlying causes of spastic behaviors in amphetamine-treated embryos, we measured differences in neurotransmitter receptor populations in the spinal cord that underlie the excitatory (NMDA)/inhibitory (glra1) signaling balance necessary for patterned locomotor behaviors like the escape response. Significant differences in inhibitory glycine receptor protein (glycine receptor alpha-1 protein, glra1) and excitatory glutamate receptor proteins (NMDAR) were measured in Florescence Arbitrary Units (FAU). Only in the 30ug AMP group did we see a significant increase in glra1 expression compared to controls ($p = 0.0074$) and to 20 ug AMP ($p = 0.0085$). There were no significant differences in FAU of NMDAR among treatment groups ($p > 0.2921$).

15.28

Developmental oxygen preconditions cardiovascular response to acute hypoxic exposure and maximal b-adrenergic stimulation of anesthetized Juvenile American alligators (*Alligator mississippiensis*).

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During embryonic development, environmental factors, such as oxygen have been shown to induce characteristic phenotypic changes thereby shaping organismal traits and potentially impacting post hatching animal performance. The embryonic American Alligator (*Alligator mississippiensis*) cardiovascular (CV) system has been extensively investigated in this regard. In response to incubation in 10% oxygen (hypoxia), alligator embryos possess larger hearts, lower heart rates, and maintain metabolic rate during hypoxic exposure. If these cardiovascular traits persist in the hatching and subsequent adults, they may convey a greater capacity to adjust cardiovascular function during periods of elevated oxygen demand or extended dives. We hypothesized that developmental hypoxia would result in a dampened response to acute hypoxia and an increased response to beta adrenergic stimulation. 4-year-old alligators that were previously incubated in control (21% oxygen) and hypoxic conditions were studied under anesthesia.

Animals were instrumented for measurements of intraventricular pressures, systemic and pulmonary blood flow during exposure to acute hypoxia and beta-adrenergic stimulation. At baseline, left ventricle stroke volume was greater while heart rate and dP/dt max were lower in the hypoxic incubated juveniles compared to control animals. In both groups, 5% oxygen exposure decreased heart rate and peak ventricular pressure however the response was dampened in the hypoxic incubated juveniles. Further dP/dt max was unchanged during exposure to 5% oxygen. Beta adrenergic stimulation increased stroke volume, blood flow and heart rate in both groups. Beta adrenergic stimulation during 5% oxygen exposure produced greater increases in total systemic blood flow and left ventricular stroke volume in the hypoxic compared to the control juveniles. Collectively our findings suggest that exposure to 10% oxygen during embryonic development has lasting effects on cardiovascular function in American alligators and these changes may impact animal performance during periods of elevated oxygen demand.

15.29

Chronic crude oil exposure affects physiology and sexual differentiation to zebrafish (*Danio rerio*).

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Crude oil is composed of environmental toxicants, such as Polycyclic Aromatic Hydrocarbons (PAHs), that affect physiological processes (e.g. cardiorespiratory performance), sexual differentiation, as well as morphological traits in aquatic organisms. Nevertheless, there is a little information about the factors controlling zebrafish development during chronic crude oil exposure, especially sexual determination. We hypothesized that chronic exposure to crude oil would disrupt sexual differentiation, as well as affect cardiorespiratory physiology and morphological traits in zebrafish (*Danio rerio*). To corroborate these hypotheses, zebrafish larvae and juveniles were chronically exposed through dietary exposure to standardized oil mixtures call “High-energy water accommodated fraction (HEWAF)” consisting of 2 g of oil blended into 1000 ml water. Four different conditions were used: three treatments using aquarium water and crude oil mixture added to food at different concentrations (10% HEWAF, 50% HEWAF, 100% HEWAF), and a control group fed only with food spiked with aquarium water. HEWAF exposure lasted 16 days, beginning from 20 and finished at day 35 post fertilization (dpf). Oxygen consumption, loss of equilibrium in hypoxic water, heart rate, body mass and length were measured at 25, 30 and 36 days post fertilization (dpf). Preliminary results showed that there were no significant differences in the time to loss of equilibrium, body mass and length. In addition, heart rate during 25 and 30 dpf was not different among groups. These data suggest that the early larvae were not unduly stressed by the crude oil exposure. However, with further development to 36 dpf, significant differences in the heart rate began to develop. In the 100% HEWAF treatment, heart rate was significantly decreased to 208 ±7 beats/minute from 236 ±4 beats/minute in the control group (P= 0.005). The difference between 100% HEWAF and 50% HEWAF treatment (230 ±7 beats/minute) was also significant (P= 0.034) but more modest. Those results suggest that, even in the absence of gross morphological changes in zebrafish, more subtle physiological responses occur due to lengthy periods of exposure to PAHs.

15.30

Development and Characterization of a Primary Cultured Model of the Larval Sea Lamprey (*Petromyzon marinus*) Gills

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The parasitic sea lamprey (*Petromyzon marinus*) is an extant agnathan. As a model that provides insight into chordate evolution, these aquatic vertebrates have generated significant research interest in comparative physiology/endocrinology and functional biology. With regard to the regulation of salt and water balance, the sea lamprey presents an opportunity to study an organism with distinct osmoregulatory strategies in different lifecycle stages. However, to the best of our knowledge there is no simplified surrogate model to study the physiology of gill epithelium function in lamprey. This study reports a primary culture method using larval sea lamprey cells derived from the gill tissue. Cultured gill cells were examined using molecular biology and biochemistry and found to express tight junction-associated proteins. In this regard, the preparation and maintenance of the primary cultured gill cells is discussed along with application of endocrine-mediated physiology studies of the sea lamprey gill cells.

15.31

Withdrawn

15.32

Participation of orexin receptor-1 in the modulation of respiratory motor activity in the bullfrog (*Lithobates catebeianus*)

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Orexin releasing neurons are known to participate in many important physiological processes, such as sleep–wakefulness, feeding behavior, energy homeostasis, metabolism, hormonal secretion, and additionally, it has been shown that the orexinergic system also modulates respiration in rodents. Orexin's effects on respiration likely involve participation in central chemoreflexes. Orexinergic neurons are profoundly affected by changes in CO_2 and pH as demonstrated by *in vitro* and *in vivo* experiments, which suggests that they are intrinsically chemosensitive. Furthermore, focal dialysis of SB-334867 (OX_1R antagonist) in the retrotrapezoid nucleus (RTN) or medullary raphe of conscious rats decreased the hypercapnic ventilatory response. However, there are currently no *in vitro* data demonstrating the role of this important neuropeptide in the modulation of respiratory control in amphibians.

Based on this background and the scarcity of studies on respiratory control in non-mammalian vertebrates, the main objective of this study was to investigate the contributions of orexin receptor-1 (OX_1R) to central respiratory motor activity and its participation in the CO_2 chemoreflex in adult bullfrogs. We hypothesized that orexin, acting through the OX_1R, potentiates central CO_2 chemoreflexes in adult frogs to stimulate ventilation during hypercapnia. Consequently, we predicted that inactivation of OX_1R's by a selective antagonist would attenuate fictive lung ventilation in response to hypercapnia.

To this end, we used *in vitro* brainstem preparations transected rostral to the optic chiasma to keep hypothalamic orexinergic neurons intact. Brainstems were perfused with artificial cerebrospinal fluid (aCSF) containing SB-334867 – 10µM (OX_1R antagonist) during control (98% O_2, 2% CO_2) and high CO_2 conditions (95% O_2, 5% CO_2). All experiments complied with the guidelines of the Canadian Council on Animal Care.

Contrary to our hypothesis, our preliminary results show that OX_1R antagonism potentiated the normal response of fictive lung ventilation to hypercapnia, suggesting that orexin (via OX_1R) attenuates the hypercapnic chemoreflex in adult bullfrogs. Further experiments are underway to explain these results, however, it is possible that the orexinergic system of amphibians is controlled by autoregulatory mechanisms (thus imparting dose-dependence), as shown for mammals. This work will bring new insights to our understanding of the role of the orexinergic system in vertebrate physiology and its evolution.

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15.33

Does the spotted gar, *Lepisosteus oculatus*, express a functional endothelial nitric oxide synthase?

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The evolution of endothelium-derived nitric oxide (NO) in blood vessels, and its associated signalling pathway, remains contentious in comparative cardiovascular physiology. The advancement of comparative genomics has provided insight into the evolution of the NO synthase (NOS) enzymes (NOS1, NOS2 and NOS3), in particular, that of NOS3 that is expressed in the endothelium of mammalian blood vessels. Until recently, the parsimonious view was that NOS3 evolved early in the tetrapods, as all three isoforms are found in amphibians, but only two isoforms (NOS1 and NOS2) are found in the genomes of teleost and chondrichthyan fishes. Recently, all three NOS proteins have been predicted in the genome of the non-teleost actinopterygian, *Lepisosteus oculatus*, which potentially reshapes our understanding of NOS3 evolution. This study aimed to characterise the *nos3* gene and determine if NOS3 is a functional protein within the vasculature of *L. oculatus*. The predicted *nos3* gene did not demonstrate a conserved synteny with the *nos3* genes of mouse and *Xenopus*, respectively, compared to the conserved synteny that is found for the *nos1* and *nos2* genes. Phylogenetic analysis showed that the predicted NOS3 protein grouped with other vertebrate NOS3 proteins; *L. oculatus* NOS1 and NOS2 grouped with their respective isoforms. To confirm the genomic sequence, we cloned the *L. oculatus nos3* mRNA, which was found to be 99% similar to the predicted sequence. We then designed a specific *L. oculatus* NOS3 antibody, and demonstrated NOS3-immunoreactivity in the adventitia of both small and large blood vessels, as well as in chloride cells in the gill filaments. To determine if the presence of NOS3 within the vasculature contributed to vasodilation, myography was performed on the dorsal aorta. Interestingly, addition of the NO

donor, sodium nitroprusside, had no effect on vascular tone indicating that NO generated by endogenous NOS3 is not involved in vasodilation. Taken together, these data suggest that *L. oculatus* does express a NOS3 protein within the vascular wall, but the function of NO does not appear to involve regulation of vascular tone.

15.34

Toxicity of crude oil extracts in chicken embryos

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Crude oil from oil spills can be a toxic contaminant to bird embryos, affected directly by contaminated water or by feathers and feet of contaminated birds during parental care. This study is part of a series of studies examining embryotoxicity of crude oil (CO) water accommodated fraction (WAF). In the current report we studied the embryotoxicty and blood physiology in fertile eggs injected with water accommodated fraction (WAF) and crude oil. We first investigated body mass (BM) of hatchlings, time of hatch and hatchability of eggs injected with 10, 30, 50, 70 or 90 µL of 100% WAF (2g-crude oil stirred in 1L-tap water) or vegetable oil (V-oil, sham) into the air cell on embryonic day 4 (D4) or D10 beside control (intact) eggs. Hatchability and hatchling BM, ratio of BM to egg mass and time of hatch were not different in any volume or day injected, indicating the lack of major toxicity at the employed doses. Thus, we investigated the survival rate of embryos in eggs injected with higher doses: 1, 3 and 5 µL of CO or V-oil into the air cell along with control eggs on D4 and D10 since 1 µL of CO correspond to ~six-fold volume of crude oil contained in 90 µL of WAF in embryotoxicity. No effect on the injection itself was found since sham and control embryos showed the same survival rate. However, the survival rate of embryos injected with CO on D4 was lower than the control 88%, 94% and 100% for 1, 3 and 5 µL injected respectively ($p<0.001$). D10 embryos injected with 3 µL of CO had a 58% decreased survival from control ($p<0.001$) and a further decrease to just 5% of control was observed in embryos injected with 5 µL of CO (<0.001). However, no effect on survival occurred at 1µL of CO injection ($p=0.260$). Based on these survival test results, we hypothesized that physiological variables would be negatively affected in D10 embryos suffering severe toxicity of CO (5 µL-injection), but that in lower doses, embryos would be able to show physiologically plasticity to mitigate toxicity of CO (1 µL-injection). To examine the hypothesis, 1, 3 or 5 µL of CO was injected into the air cell of d10 embryonated eggs and the arterialized blood was analyzed on d15 of incubation. Blood O_2 transport variables as Hct, [RBC], [Hb] were actually increased (4.5%, 3.7% and 3.9% respectively) in response to injection of 1 µL of CO. However, counterintuitively, they were significantly decreased (8.9%, 9.1% and 7.8% respectively) by injection of 5 µL of CO. Blood gas variables (pH, $[HCO_3^-]$, Pco_2, Po_2) remained unchanged in 1 and 3 µL of CO injections, but 5 µL injection caused metabolic acidosis (pH 7.58 to 7.55) and decrease of 3.8% in arterial Po_2. Lactate ($[La^-]$) was increased progressively at all doses injected (1% at 1 µL, 181% at 3 µL and 204% at 5 µL. Water concentration in blood increased 0.9% just in the 5 µL injected. The harmful CO effects in higher CO dose could be generated by a disturbance of chorioallantoic membrane, decreasing the O_2 diffusing capacity, resulting in selective embryo hydration and acidosis because of the large increase in $[La^-]$.Our results suggest that lower doses of CO are mitigated by embryo plasticity while higher doses of CO are harmful to embryo.

15.35

Cardiac proteome changes in the western painted turtle in response to cold acclimation and anoxia

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The western painted turtle *Chrysemys picta bellii* is the most anoxia-tolerant tetrapod known, with survival lasting more than 170 days a 3°C. This ability allows the turtles to overwinter in frozen lakes in the northern reaches of the US, which can become anoxic during the winter. Successful entry into and exit out of metabolic depression is critical to surviving anoxia, and during anoxia the cardiac function of the painted turtle is suppressed, beating only once per minute at 3˚C. Shutting down energy consuming processes, such as protein synthesis, is critical to entering metabolic depression; yet changes in abundance levels of particular proteins may be key to maintaining cardiac function under energy limiting conditions. Therefore, we conducted a study on the effects of cold acclimation, anoxia, and recovery on the cardiac proteome of adult western painted turtle. Turtles were held at 20˚C, acclimated to 3˚C, exposed to anoxia for 20 days, and allowed 6 days of aerobic recovery following anoxia. Ventricle was sampled and 2D fluorescent Difference Gel Electrophoresis (DIGE) was used to compare the proteome of the turtle ventricle at 20˚C, following cold acclimation, exposure to anoxia, and recovery from anoxia. Proteins of interest were identified by MALDI-QIT-ToF mass spectrometry. While work has been conducted on changes in the cardiac transcriptome during anoxia, this is the first study of its kind on the proteome. As such, these data will be particularly instrumental in furthering our understanding of anoxia tolerance in the painted turtle.

15.36

Biotinylation of Elephant Seal Blood to Determine RBC Lifespan and Total Blood Volume

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While carbon monoxide (CO) is typically thought of as toxic, the gas has recently been highlighted as being therapeutic at moderate levels in hypoxic conditions. Furthermore, it was recently discovered that elephant seals are the only animal in nature known to have concentrations of CO in their blood at levels similar to those that have shown cytoprotective properties in other models.

The source of high CO production in elephant seals is currently unknown; however, in other mammals, including humans, endogenous CO is produced from the natural turnover of heme stores (primarily from erythrocyte and hemoglobin turnover). Understanding the source of CO production and the potential cytoprotective effects in the elephant seal model will help inform about the potential use of CO as a therapeutic drug.

Our hypothesis is that the elephant seal will exhibit a reduced red blood cell lifespan, which will increase heme turnover and CO production via heme oxygenase activity. Our novel method uses biotinylated red blood cells (RBCs) to measure total systemic volume of RBCs and lifespan in elephant seals directly, as opposed to the current Evans Blue dye method which estimates the volume of RBCs from plasma volume. Specifically, our method labels the membrane of approximately 1% of RBCs in the animal with biotin. Biotinylated RBCs can then be tracked via serial collection of small blood samples (~50ul) and analyzed with flow cytometry. We have successfully biotinylated 1% of the RBCs in a mouse analyzed these on a flow cytometer. Our next steps will be to 1) biotinylate 1% of RBCs from a vial of elephant seal blood (knowing that the RBCs will be larger), 2) biotinylate 1% of RBCs directly from an elephant seal, and inject the labeled cells back into the animal to measure RBC lifespan and volume in the living organism, and 3) to use a novel method of co-oximetry to check the effects of endogenous CO production on an elephant seal's hemoglobin oxygen affinity curve.

15.37

Linking genotypes to phenotypes reveals the underlying mechanisms of intestinal brushborder remodeling in snakes

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The adaptive interplay between feeding ecology and digestive physiology is exemplified for snakes with the noted dichotomy in the capacity to regulate intestinal performance with feeding and fasting. Frequently-feeding snake species experience narrow spans of intestinal response with feeding and fasting, in contrast to the very broad regulatory shifts in intestinal form and function exhibited by infrequently-feeding species. Driving this dichotomy at the cellular level are the distinct differences in the response of the brushborder membrane. Feeding and fasting induces no change in microvillus length and thus functional surface area for frequently feeding species, whereas infrequently-feeding species experience with each meal a 3- to 5-fold lengthening of the microvilli and matched increases on function. Upon the completion of digestion, microvilli shorten and function is downregulated. This distinct variation in microvillus regulation among snakes provides a unique opportunity to explore and identify the signaling, cellular, and molecular mechanisms that underlie microvillus growth and homeostasis. In our comparative approach we have identified specific pathways, regulatory molecules, and specific genes that are associated with the mobilization and insertion of microvillus cytoskeleton and membrane proteins during the postprandial remodeling of the brushborder membrane for infrequently feeding snakes. Specifically, several pathways related to the nucleation and elongation of actin filaments are uniquely predicted to be activated in infrequently-feeding species. These pathways largely center around the regulatory activity of Rho-family GTPases, which are known regulators of cytoskeleton dynamics and have been implicated as regulators of microvillus formation. Additionally, several known positive regulators of this family are upregulated in infrequently-feeding species only. Our results provide a promising set of candidate pathways, regulators, and specific genes that likely drive microvillus dynamics in infrequently-feeding snakes. Many of these components also intersect with general growth and development pathways previously implicated in regenerative growth of the snake intestine, suggesting potential points of integration between the signaling cascades driving multiple facets of the overall regenerative response. Deciphering the signaling and molecular mechanisms of brushborder remodeling will facilitate the discovery of those mechanisms that underlie the remodeling of other tissues (e.g., liver, kidney, heart) and the development of hypotheses to explain the independent evolution of

signaling and expression programs responsible for the dichotomy in digestive responses. This work was supported by funding from the National Science Foundation (IOS 0466139 to Secor and IOS 1656138 to Secor and Castoe).

15.38

Rapid Evolution of Starvation Resistance in *Drosophila*: Physiological and Molecular Mechanisms

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We subjected five replicate populations of *Drosophila melanogaster* to selection for starvation resistance and compared them to a founding control population that had been maintained at large population sizes to reduce linkage disequilibrium. After only one generation of selection, all five replicate selected populations survived longer without food than the control population, and starvation survival continued to increase by ~5 hr/generation over four subsequent generations. Previous studies have shown that long-term starvation-selected *Drosophila* contain more lipid, have lower metabolic rates and develop more slowly than controls. Lipid contents in our selected populations increased within three generations, and development tended to be slower within five. However, starvation-selected flies did not not have lower metabolic rates than controls. Samples were collected each generation for a genome-wide association study to link changes in SNP allele frequency with evolved phenotypic changes. Preliminary findings of the GWAS will be presented. Supported by NSF award IOS-1355210.

15.39

Testing the Functional Consequences of Genetic Variation in Insulin-like Growth Factor 1 (IGF1) in Lizards via Primary Culture Experiments

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Insulin-like growth factors (IGFs) are key hormone peptides regulating the Insulin and Insulin-like signaling (IIS) pathway, a pathway required for growth, metabolism, and reproduction. IGFs and other key proteins in the IIS pathway are highly conserved across vertebrate lineages including reptiles, but there are still gaps in our knowledge about the function of the IIS pathway and its members in reptiles [1,2]. Previous work has shown that although IGF2 is highly conserved across reptiles, IGF1 has experienced diversifying selection across the reptile clade. This is in contrast to mammals where IGF1 is under purifying selection [2]. Substantial amino acid diversity in IGF1 between green anole (*Anolis carolinensis*) and brown anole (*A. sagrei*) lizards is concentrated in a domain associated with IGF1 Receptor binding affinity [2,3]. In mammalian *in vitro* cell cultures, IGFs are known to be fibrogenic/mitogenic and involved in cellular proliferation [4,5]. Cell culture provides a model for studying physiological and biochemical function and preliminary insight on cellular and possibly organismal response to drugs, growth factors, and/or stressors, but optimization of this method and associated culture assays in non-model organisms is not well documented [6,7]. We describe the establishment of three fibroblast lines from *A. sagrei* (brown anole lizard) tail tips and their use in characterizing the function of reptilian IIS pathway with species-specific recombinant IGFs expressed in and purified from *E. coli*. To optimize and assess prolific response to IGF treatments, cells were seeded and synchronized before serial time- and dose-dependent exposure to recombinant brown anole IGF1 and IGF2 treatments. Cellular proliferation in response to IGF dose and exposure time was assessed via growth curve analysis and BrdU assay. We then test the bioinformatic prediction that the amino acid sequence variation in IGF1 between green and brown anoles has a functional effect on cell proliferation via binding to the IGF1 receptor through the application of brown or green anole IGF1 or IGF2 to culture wells. Cellular proliferation in response to the hormones was directly assessed via growth curve analysis and indirectly via cell metabolism assays, after time- and dose-optimized exposure to peptide treatments. Expression of transforming growth factor-β1 (TGF-β1), cytokine expressed in response to IGF stimulation in human dermal fibroblasts, was quantified via qPCR [5]. Results to be discussed include insights and challenges of primary culture with non-model ectothermic organisms, functional verification and use of lab purified recombinant proteins from non-model organisms, and cellular response to IIS activation by IGF proteins in the context of known genetic sequence variation and conservation patterns.

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15.40

Modelling Human *APOL 1* Variant Related Kidney Dysfunction In Guinea Pigs

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Humans of early West African ancestry are more prone to kidney dysfunction. Earlier studies demonstrate that the reason for this phenomenon is because of the mutation of *APOL 1* gene in most humans of early West African ancestry. Although several *APOL* genes are expressed in mammals, *APOL 1* is only naturally expressed in primates. The restricted expression of *APOL 1* to only primates has limited the exploration of the functional role of *APOL1* variants in kidney disease development. The aim of this preliminary study was to develop a guinea pig model of *APOL1* variants and demonstrate possible biological basis of *APOL 1*-mediated kidney injury as is observed in humans given that both humans and guinea pigs are HDL mammals.

Expression of *APOL1* gene variants in Guinea pigs was done by hydrodynamic gene delivery (HGD). 5 sets of male and female guinea pigs were injected with plasmids containing various *APOL1* gene variants. *APOL1* protein presence in Guinea pig plasma and kidney tissues was determined by WESTERN blotting and immunohistochemistry (IHC) respectively.

Induction with APOL1 gene variant in Guinea pigs resulted in a derangement of renal function as evidenced by creatinine accumulation and distorted renal histoarchitecture. There was a scattered inconsistent IHC staining for APOL1 in the experimental group although the WESTERN blot assay showed a consistently elevated protein presence in the same group compared to control. Subsequent studies will seek to improve on the APOL1 induction method, gather more genetic information in addition to determining the full extent of renal structure and function compromise in the Guinea pig specie.

Keywords: APOL1, Kidney dysfunction, FSGS, End stage renal failure, HDL

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15.41

Metabolomic profiles reveal that upregulation of protein degradation and nicotinamide pathways are linked with successful pregnancy in Weddell seals

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Mechanisms governing reproduction vary widely across and within taxa, yet numerous challenges often prohibit the study of intrinsic physiological factors that influence reproductive outcomes in non-model or wild organisms. Weddell seals (*Leptonychotes weddellii*) in Erebus Bay, Antarctica offer a unique opportunity to elucidate the physiological processes regulating the establishment of successful pregnancy in marine mammals, due to a long-term demographic study and high site-fidelity of females. We determined pregnancy status of 77 adult female Weddell seals during the peri-implantation period (January/February) from 2014-2017, using transrectal ultrasound (embryos detectable ≥3mm diameter). Pregnancy rates were high (78%), and 16% of females that were detected pregnant returned the following year but did not produce a pup (i.e., lost the pregnancy). Hormone quantification and metabolomics approaches were used to determine biochemical pathways that were up- or down-regulated during early gestation for female Weddell seals that had a successful pregnancy (*n*=14) as compared to females that were not detected pregnant (*n*=10) or experienced pregnancy loss (*n*=12).

Hormones used to diagnose pregnancy in domestic animals, such as progesterone and estrogen, did not

differ between non-pregnant and pregnant Weddell seals during the peri-implantation period. However, pregnant individuals and seals that successfully produced a pup had significantly higher serum thyroxine levels than seals that failed to give birth. As thyroid hormones are potent regulators of metabolism, this suggested that there may be differences in the catabolism of tissues and substrate use to meet the demands of a growing fetus. An untargeted metabolomics approach identified 600 biochemicals in Weddell seal serum. Of these, 25 were significantly (p<0.05) and 50 slightly (p<0.10) different between non-pregnant and pregnant Weddell seals. Most of the metabolic shifts associated with pregnancy suggest a decrease in lipolysis and increased protein degradation and amino acid transport. This was evidenced by a decrease in the relative abundance of free fatty acids (long chain; polyunsaturated) and an increase in the metabolic end-products in a diverse set of metabolic pathways (alanine and aspartate; lysine; tryptophan; leucine, isoleucine, and valine; arginine and proline, creatine, and gamma-glutamyl amino acid metabolism pathways) in pregnant animals. Further, females that successfully carried their pregnancies to term had significantly higher abundance of nicotinamide, 1-methylnicotinamide, and N1-methyl-2-pyridone-carboxamide than females that experienced pregnancy loss, suggesting greater flux through the nicotinamide metabolic pathways. Increased splanchnic uptake of amino acids is critical for proper embryonic development, and disruption of nicotinamide synthesis has been implicated in miscarriages in humans and other mammals. This study is the first to pair ultrasonography with the metabolomic-fingerprint of pregnancy in a wild mammal, and can aid in the development of minimally-invasive markers of successful reproduction while monitoring wild populations.

15.42

Evolution of thermal tolerance in pumpkinseed sunfish (*Lepomis gibbosus*)

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Historical transplantations of non-native species to novel warm environments are natural experiments for studying the potential mechanisms of climate change adaptation. We compared the thermal tolerance of warm-adapted pumpkinseed sunfish that were transplanted ~140 years ago from North America to Europe with native pumpkinseed populations from southern Canada. We found that Spanish populations (Ter River and Susqueda Reservoir) have evolved greater heat tolerance than Canadian populations (Rice Lake and Otonabee River), as reflected by a higher critical thermal maximum (CT_{max}). This greater heat tolerance of Spanish populations was not associated with differences in resting metabolism, hypoxia tolerance, haematology, or the maximal activities of anaerobic and aerobic enzymes in muscle or liver, suggesting that other physiological mechanisms may have contributed to the evolution of thermal tolerance. Evolved differences in thermal plasticity were also observed, with fish from the Canadian Rice Lake population showing a much greater increase in CT_{max} with acclimation to warmer temperature. This was associated with greater plasticity in the mitochondrial respiratory capacity of the heart in Rice Lake fish (as reflected by measurements of oxidative phosphorylation in permeabilized cardiomyocytes), suggesting that adjustments in cardiac energy metabolism are important for thermal acclimation. Therefore, pumpkinseed appear capable of increasing heat tolerance via evolutionary adaptation and thermal acclimation, possibly underpinned by changes in heart function, which may help them cope with climate change in their native range. Supported by NSERC of Canada.

15.43

Calcium transport across the placenta in a placentotrophic lizard: New insights about gestation

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Lizards species of the genus *Mabuya* distributed in the Neotropics[1] are characterized by some exclusive and very interesting features in their reproductive and developmental biology. These traits include viviparity with long gestation time (from 9 to 10 months), microlecithal eggs[2], and associated with this type of oocytes, the development of a placenta that is both morphologically and physiologically the most complex and specialized within Squamata reptiles[3]. These traits make them particularly important as they contribute with a system that allows to perform comparative physiological, metabolic, and developmental biology investigations.

Previous studies on Colombian *Mabuya* sp IV[1] have provided clues about the morphology and physiology of their gestation, for example, the placenta fulfills the fundamental functions for gaseous exchange and the absorption, transfer or transport of nutrients[4,5], such as water, glucose, some lipids, proteins and ions. Here we report preliminary data on gene expression of calcium transporters in placental tissues of *Mabuya* sp IV by quantifying the relative expression of mRNA and characterizing its temporal dynamics through its gestation by real-time reverse transcription PCR. To do this we analyzed gene expression of Calcium-binding proteins as calbindin-D9K and D28K, the Plasma

Membrane Ca^{2+} ATPase (PMCA) and the Transient Receptor Potential cation channel subfamily V member 6 (TRPV6) in non-pregnant female's oviducts and oviductal eggs, early, mid and advanced extraembryonic membranes from pregnant females. Individuals were capture by hand, killed by intrathoracic injection of lidocaine 2% v/v and dissected. All work conducted with the animals was consistent with government guidelines on the ethical treatment of animals and all applicable regulations and follows the considerations of The Herpetological Animal Care and Use Committee (HACC).

We found that all of the calcium transporters were present in the placenta and they were highly expressed at mid and late gestation. This gene expression prove calcium transfer to the embryo-fetus must be completely mediated by the placenta, it also highlights that calcium is one of the most important ions required during pregnancy, not only for the development of the skeleton from mid pregnancy, but also for the establishment of gestation, growth and fetal homeostasis.

Our results emphasize *Mabuya* sp as the only reptile species where the presence of calbindin-D9K, a protein previously restricted to mammalian species, has been observed nowadays. Finally, due to the homology of tissues we can compare and associate it with eutherian mammals, demonstrating a conserved and similar biological function in this placentotrophic species.

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15.44

A role for kisspeptin receptor in the pituitary gonadotroph in male mice

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The anterior pituitary secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) regulate gonadal development, gametogenesis and the secretion of the gonadal steroid hormones. The gonadotroph is primarily regulated by hypothalamic secretion of gonadotropin-releasing hormone (GnRH) from neurons of the rostral hypothalamus and is mediated by GnRH receptor signaling. Recently, kisspeptin (KISS1)/kisspeptin receptor (KISS1R) signaling in GnRH neurons has been shown by our group and others to play an essential role in HPG axis function. However, whether kisspeptin signaling via the Kiss1r could also regulate reproductive function at the level of pituitary is not yet established. Using Cre/Lox technology, we knocked out the *Kiss1r* gene in pituitary gonadotropes (PKiRKO). Our results revealed that PKiRKO males have normal external genital development, have normal ages of puberty as assessed by preputial separation and also have comparable body and testes weight to WT male mice. While there were no differences in basal serum LH and FSH levels, we observed a significant attenuation ($P < 0.05$) in GnRH stimulated luteinizing hormone (LH) levels in PKiRKO male mice compared with WT male mice and this was associated with attenuated expression of *Gnrhr*. To directly assess cellular response, calcium (Ca2+) assays were performed on primary pituitary cell cultured *ex vivo*, and demonstrated that cells from WT male pituitaries were more responsive to GnRH (100nm) and kisspeptin (10nm) than were pituitaries from PKiRKO males. Overall, these findings indicate that the pituitary KISS1R may plan an important modulatory role in augmenting pituitary responsiveness.

16: HIBERNATION AND DAILY TORPOR: ABSTRACT DRIVEN SESSION - 2

16.1

The Effect of Lipids on Hibernation and Cardiac Function

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Many small mammals and birds have developed specific mechanisms of energy saving, via active and controlled

reduction of metabolic rate (MR) and body temperature (T_b), *i.e.* torpor and hibernation. Torpor involves tight regulations within the organism of many bodily systems. However, physiological mechanisms that regulate this overwintering strategy are still poorly understood.

Polyunsaturated fatty acids (PUFA) are one of the main factors affecting torpor and hibernation performance. Heterothermic mammals fed diets containing plant oils that are rich in n-6 PUFA, notably linoleic acid (LA, C18:2 n-6), show a higher propensity of torpor use, lengthen torpor bout duration, lower minimum T_b and hence increase energy savings. Conversely, there is evidence for adverse effects of n-3 PUFA on hibernation performance.

The membrane lipid composition is thought to play an important role in regulating hibernation. This is supported by the fact that small hibernators show seasonal changes of n-6 and n-3 PUFA contents in phospholipids (PL) of specific organs. Similar changes in lipid composition also occur in a large hibernator, the brown bear, suggesting that the implication of lipids in regulating hibernation is a conserved phenomenon of the hibernation phenotype.

The heart, which activity is tightly regulated during torpor, plays a pivotal role in maintaining body homeostasis. As previously hypothesized, the influence of PUFA may be mediated via effects of the membrane lipid composition on the sarcoplasmic reticulum (SR) calcium ATPase (SERCA) in the hibernators' heart. This trans-membrane pump is responsible for removing calcium into the SR and hence for continued cardiac function at low T_b in torpor.

We tested the hypotheses that high proportions of n-6 PUFA in general, or specifically of LA, in PL are associated with increased cardiac SERCA activity, and allow animals to reach lower minimum T_b in torpor. SERCA activity and SR-PL fatty acid composition were assessed from hearts of hibernating and non-hibernating Syrian hamsters (*Mesocricetus auratus*), a granivorous, food-storing hibernator, and of hibernating garden dormice (*Eliomys quercinus*), an insectivorous, fat-storing hibernator.

In both species, we found that SERCA activity was strongly up-regulated by increased proportion of LA in SR-PL, but was negatively affected by the content of docosahexaenoic acid (DHA; C22:6 n-3). In hibernating hamsters, high levels of LA and low proportions of DHA were found in SR-PL. As a result, SERCA activity was significantly higher during entrance into torpor and in torpor compared to inter-bout arousal, *i.e.* phase of high MR and euthermic T_b between torpor bouts. A subgroup of hamsters, which remained euthermic throughout winter, displayed a phenotype similar to animals in summer, *i.e.* lower LA levels and increased DHA proportions in SR membranes. Similarly, a group of dormice, which delayed their mean onset of hibernation by almost 4 days (range 0-12 days), showed extremely high DHA levels prior to hibernation. Both hamsters and dormice with increased SERCA activities reached lower T_b during torpor. Interestingly, SERCA activity in torpor was three-times higher in garden dormice than in Syrian hamsters at similar DHA proportions in SR-PL.

We conclude that (1) fatty acid composition of SR membranes modulates cardiac SERCA activity, hence determining the minimum T_b tolerated by hibernators, and (2) high DHA levels prevent hibernators from entering into torpor, but the critical levels differ substantially between species. These specific roles of PUFA in regulating cardiac activity, hence body homeostasis during torpor and hibernation might shed light on the benefit for humans to enter a torpor-like state during long-term spaceflight.

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16.2

A systems level approach reveals incomplete caspase cascade function during mammalian hibernation

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Hibernating golden-mantled ground squirrels, *Spermophilus lateralis*, experience numerous conditions during the winter that are known to be pro-apoptotic in other mammal systems (e.g. extreme hypothermia, ischemia and reperfusion, acidosis, increased reactive oxygen species, bone and muscle disuse). Studies suggest that hibernators may invoke a protective phenotype to limit widespread apoptosis during the hibernation season. Could regulating apoptosis provide protection against the harmful conditions experienced during the hibernation season? To address this, we systematically examined the caspase cascade (caspases 1-12) for evidence of apoptotic signaling during hibernation. Caspases comprise a family of cysteine-aspartate proteases that are central to apoptosis and inflammation where upon proteolytic activation, they participate in a complex signaling cascade. Using ground squirrel liver, we determined the availability and activation status of caspases with western blots, performed caspase-specific proteolysis activity assays, and analyzed multiple caspase-mediated cellular events for indications of caspase signaling during hibernation. Surprisingly, we found the canonical apoptotic caspases 3, 6, and 9, as well as inflammatory caspases 11 and 12, appeared activated during hibernation. Caspase activation typically has dramatic effects on proteolytic activity. For instance, in other systems, when caspase 3, the "key executioner of apoptosis," is processed into the active 17 kDa (p17) fragment, caspase 3 proteolytic activity increases up to ~10,000X vs. the procaspase form (p32). Caspase 3 activation, therefore, is thought to commit a cell to apoptosis. We found caspase 3 p17 increased ~2X during

hibernation which may indicate significant apoptotic commitment. Did these winter-activated caspases display increased activity? We found no indications of dramatically increased caspase activity expected with a seeming caspase activation. To better understand the implications of caspase "activation" during hibernation, we used a systems-level approach to analyze numerous events downstream of caspase activation. We looked for indications of caspase 6 activity (nuclear lamin A cleavage), caspase 3 activity (degradation of the inhibitor of caspase-activated DNase (ICAD), inactivation of DNA repair enzyme poly (ADP-ribose) polymerase (PARP), and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) activity), and inflammatory caspase activity (IL-1b and IL-18 cytokine activation and serum transaminase levels). We found no evidence of downstream caspase activity. Despite the pro-apoptotic conditions of hibernation and the seeming caspase "activations", there was no evidence suggesting widespread apoptosis and inflammation during ground squirrel hibernation. These data demonstrate the importance and utility of the systems-level approach in studying complex cellular signaling pathways like apoptosis in non-steady state physiological contexts like mammalian hibernation.

16.3

Changes in protein phosphorylation and acetylation correspond with suppression of mitochondrial metabolism during mammalian hibernation

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Small hibernators such as the 13-lined ground squirrel (*Ictidomys tridecemlineatus*) cycle between distinct metabolic states from late autumn through early spring. Periods of torpor last approximately 12 days during which body temperature (T_b) is maintained near 5°C and metabolic rate (MR) is very low. Torpor is spontaneously interrupted by arousals when MR increases approximately 100-fold and T_b rises to near 37°C within a few hours. During interbout euthermia (IBE) these high levels of T_b and MR are maintained for ~10 hours before animals enter another torpor bout. The whole-animal suppression of MR during torpor is mirrored at the mitochondrial level; state 3 respiration rates of liver mitochondria isolated from torpid ground squirrels can be 70% lower than those from IBE animals. This suppression of organelle function corresponds with decreases in maximal activities of electron transport system (ETS) complexes I and II during torpor, compared with IBE. We hypothesized that changes in post-translational modifications to mitochondrial enzymes were responsible for the rapid and reversible changes in mitochondrial metabolism between torpor and IBE. Differential 2D gel electrophoresis (DiGE) and Blue-Native PAGE of proteins from purified liver mitochondria revealed that the isoelectric point or molecular weight of several proteins changed between torpor and IBE. MALDI mass spectrometry revealed that these proteins are involved in β-oxidation, the Krebs cycle, ROS detoxification and the ETS. Immunoblots for total acetylated protein of 2D gels showed that subunit 1 of ETS complex IV had 2.4-fold higher acetylation in torpor compared with IBE. Phosphoprotein staining revealed that the phosphorylation of the 75 kDa subunit of ETS complex I increased 1.5-fold during torpor, whereas phosphorylation of flavoprotein subunit of complex II decreased 4.6-fold. Given that differences in phosphorylation state of complexes I and II corresponds with differences in maximal enzymatic activity of these proteins between torpor and IBE, we attempted to manipulate enzyme activity by inducing dephosphorylation in liver mitochondria from torpid and IBE animals. *In vitro* treatment with alkaline phosphatase increased maximal activities of complex I in liver mitochondria isolated from animals in torpor, but had no effect on complex I from IBE animals. By contrast, exogenous phosphatase treatment decreased complex II activity in IBE liver mitochondria, but not torpor. These findings suggest that the rapid changes in mitochondrial and, perhaps, whole animal metabolism between torpor and IBE are mediated by post-translational modifications of key metabolic enzymes. Our future work will focus on understanding how changes in the cellular environment between torpor and IBE affect the activities of intramitochondrial kinases, phosphatases and deacetylases, which ultimately catalyze the changes that we observe.

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16.4

Perineuronal nets cover parvalbumin-positive neurons in ground squirrel cerebral cortex

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Thirteen-lined ground squirrels go through seasonal hibernation, which is characterized by extreme changes in physiology. These animals enter periods of torpor, where they exhibit near freezing body temperature, reduced blood flow, and low metabolism. Bouts of torpor are regularly interspersed with short returns to the physiological parameters typical of a summer, non-hibernating ground squirrel, called interbout arousals (IBAs). The brain is known to go through extreme changes during the transitions between torpor and IBA, specifically related to connectivity and synapse structure. The extracellular matrix could play a key role in this dynamic nature of the brain, particularly

perineuronal nets (PNNs), which are protective and stabilizing structures known to regulate plasticity. Previous work investigating PNNs in the thirteen-lined ground squirrel brain showed that expression of PNNs was not global throughout the brain, but in specific regions, including areas of the hypothalamus, cerebral cortex, amygdala, and septum. Additionally, the overall presence of PNNs did not differ seasonally, as PNNs were found in all brain regions of both hibernating (Torpor and IBA) and non-hibernating (summer) animals. Here, we further characterize PNNs in the cerebral cortex using fluorescent *Wisteria floribunda* lectin histochemistry, focusing analysis on three areas: the anterior cingulate cortex, and two areas of the dorsal and lateral cerebral cortex just cranial to the hippocampus. Fluorescent Nissl staining paired with the lectin histochemistry revealed that PNNs surround neuron cell bodies and extend out along the projections, some extending more than 25 um from the cell body. The PNNs were found specifically in cortical layers III and V. Within these layers, the nets do not cover all neurons, but are restricted to specific, multipolar neurons. Double labeling with the *Wisteria* lectin and a parvalbumin antibody revealed that the PNNs specifically surround parvalbumin-positive interneurons. The average neuron area covered by the PNN was 207.29 ± 8.49 um^2 in layer III and 221.54 ± 6.69 um^2 in layer V (n=11). PNN wrapped neurons were found at a frequency of 5.69 ± 0.62 cells/100 um^2 in layer III and 6.99 ± 0.35 cells/100 um^2 in layer V (n=11). There was no significant difference in count or average size among three time points analyzed (torpor, IBA, or summer/non-hibernating) in any of the cerebral cortex areas. Importantly, the three cerebral cortex areas quantified and analyzed here serve as representative areas. The entire cerebral cortex exhibited the same general PNN expression. Overall, this work supports previous PNN expression analyzed in other species, where these structures are known to regulate neuronal plasticity and promote protection, but further work is needed to support the idea that these extracellular matrix structures could serving a similar role during hibernation to allow for the dynamic changes occurring in brain.

16.5

The impacts of snow cover variation across elevation on overwintering montane insects

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During winter, organisms are faced with multiple physiological challenges, including cold exposure and limited access to nutritional resources. To cope with these difficult environmental conditions organisms often enter dormancy. During dormancy individuals rely on energy reserves accumulated during resource-abundant summer months. Surviving a lengthy dormancy requires large energy stores and physiological modification. Metabolic rate dictates rate of consumption of energy stores, and is strongly influenced by environmental temperature. For organisms that overwinter beneath snow, temperature is modulated by snow cover, with snowy years being relatively warm and stable, while dry years are cold and variable. Impact of winter snow cover varies across elevation, winter snow fall increases with elevation while mean winter temperature decreases. We expect more reliable buffering at high elevations, but higher risk of cold damage when exposed to air temperature. The willow beetle *Chrysomela aeneicollis* lives in high elevation habitats in the Sierra Nevada Mountains, where it overwinters in leaf litter below its host plant for eight months each year. In these habitats there is considerable variation in snow fall across elevation and between winters, exposing populations to variable winter cold stress. To test the hypothesis that snow cover modulates winter energy use and protects from cold stress, in October 2015 we placed field-caught individuals under soil in plots exposed to natural snow cover or sheltered from snow. After seven months, survival was assessed and whole body lipid profile was measured using thin layer chromatography (TLC-FID). The experiment was repeated in October 2017, which was a dry winter with no significant snowfall until February and a minimum air temperature that was 5°C higher than 2015. During winter 2015 beetle survival did not differ between snow and no snow conditions, lipid stores decreased over the course of winter in both conditions, and beetles under snow had significantly lower lipids at the end of winter compared to beetles exposed to environmental temperature. During winter 2017, overall survival was higher than 2015, and survival of beetles that were not under snow was significantly higher than beetles that were under snow. This field experiment was coupled with metabolic rate measurements that were used to calculate expected winter energy use across elevation during differing winter conditions. Low elevation sites had higher expected overwintering energy costs, and were more sensitive to changes in precipitation than high elevation sites. Beetles at low elevation sites also spent more time below a freezing threshold than higher elevations. Together this suggests that variation in snow cover timing and duration will impact overwintering energetics of dormant insects, but the extent is dependent on the overall severity of winter, and not all elevations will be impacted similarly.

17: MITOCHONDRIAL BIOLOGY: ABSTRACT DRIVEN SESSION

17.1

Digging up the mitochondrial origins of hypoxia-tolerance in African mole rats

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African mole-rats live in densely populated burrows and putatively experience periods of limited oxygen availability throughout their lives. This has likely driven the evolution of mechanisms to cope with hypoxia at multiple levels of their physiology. For hypoxia-intolerant species, such as rats, reoxygenation following hypoxia causes well-characterized tissue injury, particularly in heart. It has been suggested that succinate accumulation during hypoxia and subsequently elevated mitochondrial reactive oxygen species (ROS) production during reoxygenation underlies this injury [1]. We hypothesized that African mole rats would exhibit adaptations at the mitochondrial level that limit ROS damage during hypoxia/reoxygenation. To examine this hypothesis, we compared oxygen consumption and the capacities for H_2O_2 production and H_2O_2 elimination of heart mitochondria from five species of African mole rat and the rat. Consistent with their generally lower metabolic rate, African mole rats had lower rates of H_2O_2 production and elimination (although not significant with our sample size). However, African mole rats all exhibited significantly lower rates of oxygen consumption under respiratory substrate conditions that provide electrons to complex II (succinate/rotenone + ADP and succinate/rotenone + ADP + CytC), whereas oxygen consumption was comparable to the rat using substrates of complex I. In hypoxia-tolerant species, lower complex II activity may reduce both the rate of succinate accumulation during short term hypoxia and the rate of succinate oxidation during reoxygenation. This finding is consistent with the reverse electron flow ROS production hypothesis of hypoxia/reoxygenation tissue injury [1], and may represent a constitutive mitochondrial adaptation to hypoxia in hypoxia-tolerant species. This research was funded by an NSERC Discovery grant, an NSERC Tier 2 research chair, and a National Geographic Explorer grant to M. Pamenter, and a FRQS postdoctoral fellowship to D. Munro.

Reference:

1. Chouchani, E.T., Pell, V.R., Gaude, E., Aksentijević, D., Sundier, S.Y., Robb, E.L., Logan, A., Nadtochiy, S.M., Ord, E.N.J., Smith, A.C., *et al.* (2014). Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature *515*, 431–435.

17.2

A comparative analysis of mitochondrial supercomplexes in vertebrates

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Mitochondrial electron transport chain enzyme complexes have recently been found to be arranged in supercomplexes (SCs), and it has been suggested that SCs may either improve electron transfer between individual complexes or stabilise individual complexes. In mammals, SCs are only observed when using very gentle detergents like digitonin and consist of CI and CIII with or without CIV in varying stoichiometries. Using selected species for vertebrate classes, we show here that heart mitochondrial SCs are much more stable in ectotherms, where they can be observed even in the presence of the much harsher detergent dodecyl maltoside (DDM). Reptilian SCs are the most stable, with fish and amphibians having a SC stability in between that of mammals and reptiles. Using mass spectrometry complexomics of isolated mitochondria with DDM, we found that turtle heart SCs consists of CI and CIII, and that all CI is found in the SC state. We observed no SCs containing CIV. We found that the content of SCs correlates with the content of cardiolipin in the mitochondrial membrane, but found no correlation between respiration rate, ROS production and SC content of isolated mitochondria. This suggests that SC formation depends on the phospholipid composition of the inner mitochondrial membrane and may thus depend on the body temperature of the species. Whether the mitochondrial membrane phospholipid composition has evolved to maintain SC integrity or if SC stability is a consequence of a given phospholipid composition is an interesting question to address next. Our present data does not support the view that SCs enhance electron transfer efficiency.

17.3

The Influence of Thyroid Hormone Manipulation on Cardiac Muscle Mitochondrial Function in Developing Chickens

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As chickens hatch, their thermoregulatory ability changes from an ectothermic to an endothermic phenotype. Associated with attaining endothermy at hatching is an increase in mitochondria function within different tissues correlated with endothermic capacity. Thyroid hormones (TH), key regulators of avian metabolism, are thought to influence development of endothermy. In birds, TH regulates skeletal muscle

growth, which directly impacts a chick's ability to thermoregulate via shivering thermogenesis. To better understand the role of TH in the timing of hatching, development of thermogenic capacity, and metabolic rate, we manipulated plasma TH levels in chicken embryos beginning at 85% development (day 17 of a 21 day incubation) with either the thyroperoxidase inhibitor methimazole (MMI) or supplemental triiodothyronine (T3). After TH manipulation, we characterized mitochondrial function and reactive oxygen species (ROS) production in cardiac muscle from embryos and neonates using high-resolution respirometry coupled with fluoromery (Oxygraph-O2k). Additionally, we measured citrate synthase activity of cardiac and skeletal muscle. Thyroid hormone manipulation had a significant effect on cardiac mitochondria respiration with no influence on ROS production in externally pipped embryos. These results allow for a comprehensive view of the role of TH on the development of metabolic capacity of skeletal and cardiac muscle in the developing chicken. The development of increased metabolic capacity is essential for maturation of endothermic capacity at hatching in these animals.

17.4

Metabolic underpinnings of life history allocations: Mitochondrial function is fine-tuned to meet divergent energetic demands in two species of wing-polymorphic crickets

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Organismal fitness depends on having sufficient energy available to invest in life history traits such as activity, growth, maintenance, and reproduction. Through development, energetic demands fluctuate as organisms differentially allocate acquired resources amongst life history traits. Energetic constraints limit life history investments, and in turn can shape evolutionary patterns of biodiversity. To avoid constraints, variable life history demands are met via differential flux of ingested nutrients through catabolic and anabolic metabolic pathways. These pathways are linked by mitochondrial ATP production and consumption. Thus, changing energetic requirements may elicit concurrent shifts in mitochondrial function. We hypothesized that alternative life-history allocations are underpinned by differences in mitochondrial function, and predicted that ontogenetic shifts in energy demand are met by changes in mitochondrial bioenergetics. Many species of *Gryllus* field crickets have two morphs within populations, a flight-capable dispersal morph and a flightless reproductive morph. The polymorphism is maintained by a resource-based flight-oogenesis trade-off. Metabolic demands are highly divergent between morphs and change during ontogeny, making this an ideal system to test our hypothesis. Energy demands of the reproductive morph are high during early adulthood while investing in ovary synthesis but decline within a week, once reproductive maturity is reached. In contrast, high metabolic requirements of flight in dispersal morphs extend past the first week of adulthood. Here, we determined the extent to which mitochondrial function differs between adult wing morphs of two cricket species (*Gryllus firmus* and *Gryllus lineaticeps*) across ontogeny (1, 3 or 5 day-old). Mitochondrial function was assessed using high-resolution respirometry to measure respiration rates of isolated mitochondria from fat body when fueled by NADH (malate, glutamate, pyruvate) or $FADH_2$-generating (succinate) substrates. In early adulthood, when energy demands are elevated for both morphs, mitochondrial respiratory function is similar, but diverged gradually through ontogeny in both species. By day five, mitochondria from dispersal morphs exhibited a higher oxidative phosphorylation capacity compared to reproductive morphs. Cytochrome *c* oxidase (Complex IV) expression levels and specific activity were also elevated in dispersal morphs. Thus, increases in mitochondrial content and electron transport chain activity may serve to enhance ATP production capacity to meet the high energetic demands of flight. Overall, our results demonstrate a divergence of mitochondrial activity through development, which suggests that adjustments to the bioenergetic machinery of this organelle play an integral role in maintaining energetic homeostasis and supporting alternative life-history allocations. Additionally, these findings indicate that life history evolution has played a prominent role in shaping mitochondrial function.

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17.5

Cellular metabolism and oxidative stress as a possible determinant for longevity in small breed and large breed dogs.

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Among species, larger animals tend to live longer than smaller ones, however, the opposite seems to be true for dogs - smaller dogs tend to live significantly longer than larger dogs across all breeds. We were interested in the mechanism that may allow for small breeds to age more slowly compared with large breeds in the context of cellular metabolism and oxidative stress. Primary dermal fibroblasts from small and large breed dogs were grown in culture. We measured basal oxygen consumption (OCR), proton leak, and glycolysis using a Seahorse XF96 oxygen flux analyzer. Additionally, we measured rates of

reactive species (RS) production, reduced glutathione (GSH) content, mitochondrial content, lipid peroxidation (LPO) damage and DNA (8-OHdg) damage. Our data suggests that as dogs of both size classes age, proton leak is significantly higher in older dogs, regardless of size class. We found that all aspects of glycolysis were significantly higher in larger breeds compared with smaller breeds. We found significant differences between age classes in GSH concentration, and a negative correlation between DNA damage in puppies and mean breed lifespan. Interestingly, RS production showed no differences across size and age class. Thus, large breed dogs may have higher glycolytic rates, and DNA damage, suggesting a potential mechanism for their decreased lifespan compared with small breed dogs.

18: OMIC RESPONSES TO STRESS: ABSTRACT DRIVEN SESSION

18.1

Physiological mean-variance relationships among intertidal mussels depend on environmental context

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The ability of environmental variation to mask or, alternatively, to reveal physiological variation among individuals will almost certainly play a role in how populations respond to changing environmental conditions. Yet, the contributions of temporal and spatial environmental variation to inter-individual physiological differences remain poorly resolved. Each individual's physiological profile depends on how its unique microhabitat conditions and past experience interact with the biochemical/physiological and behavioral mechanisms at its disposal. Stressful conditions promoted the expression of inter-individual variation in antioxidant capacities within a population of rocky intertidal zone mussels (*Mytilus californianus*). In a separate study, hotter individuals expressed higher levels of some antioxidants in the field. However, preliminary measures of inter-individual variation in oxidative damage and global protein expression patterns in the same manipulations appear to tell a surprisingly different story, with greater magnitudes of inter-individual variation observed under relatively benign conditions. There are several possible explanations for this contradiction between measures of variation in "defense" and "damage". Using transcriptomics and proteomics approaches, we are exploring which underlying biochemical networks contribute the most to these patterns of inter-individual variation. Highly variable networks, or key regulators thereof, represent possible avenues of selection under novel environmental conditions.

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18.2

Transcriptomic responses to low salinity among locally adapted populations of Olympia oyster, an estuarine foundation species

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The Olympia oyster (*Ostrea lurida*) is a foundation species inhabiting estuaries along the North American west coast. In California estuaries, *O. lurida* is adapted to local salinity regimes and populations differ in low salinity tolerance. In this study, oysters from three California populations were reared for two generations in a laboratory common garden and subsequently exposed to low salinity seawater. Comparative transcriptomics was then used to understand species-level responses to hyposmotic stress and population-level mechanisms underlying divergent salinity tolerances. Gene expression patterns indicate Olympia oysters are sensitive to hyposmotic stress: all populations respond to low salinity by up-regulating transcripts indicative of protein unfolding, DNA damage, and cell cycle arrest several days before mortality. Among *O. lurida* populations, transcriptomic profiles differed constitutively and in response to low salinity. Despite two generations in a common garden, transcripts encoding apoptosis modulators were constitutively expressed at significantly different levels in the most tolerant population. Expression of cell death regulators may facilitate cell fate decisions when salinity declines. Following low salinity exposure, oysters from the more tolerant population expressed a small number of mRNAs at significantly higher levels than less tolerant populations. Proteins encoded by these transcripts regulate ciliary activity within the mantle cavity and may function to prolong valve closure and reduce mortality in low salinity seawater. Collectively, gene expression patterns suggest sub-lethal impacts of hyposmotic stress in Olympia oysters are considerable and that even oysters having garnered greater low salinity tolerance via natural selection will be vulnerable to future freshwater flooding events.

18.3

Unexpected natural modification of mt-DNA alters centenarian bivalve physiology and ecology

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Mitochondrial DNA (mtDNA) is normally maternally inherited and encodes for subunits of respiratory chain complexes and ATP synthase, among many other processes. The integrity of mtDNA is crucial for all eukaryote species function, from sub cellular processes to species ecology and evolution. Mitochondrial DNA mutations have been associated with cellular energetic and redox homeostasis dysfunction, and can affect individual well-being and longevity. Bivalves are the only zoological group in which Doubly Uniparental Inheritance (DUI), characterized by the presence of 2 divergent mtDNAs within different tissues of male individuals, is frequently observed. The F-mtDNA, maternally inherited, is found in somatic tissues of both sexes and female gonads whereas the M-mtDNA is inherited exclusively in male gonads (Dégletagne et al 2015). Our recent investigations highlighted the existence of this particular mtDNA inheritance system in long-lived populations of the ocean quahog, Arctica islandica. This clam is distributed throughout the North Atlantic shelf regions. Due to different environmental regimes (salinity, temperature, oxygen), the maximum lifespan of its populations varies between >500 years around Iceland and 35 years in the Baltic Sea.

Our new analyses of mitochondrial marker gene sequences in somatic tissues indicate North Atlantic populations to be genetically homogenous, but reveal the existence of a “divergent” mtDNA haplotype with a genetic difference ~6% in all somatic tissues (gill, mantle, foot) of 10% of Iceland animals, male and female. This “divergent” mtDNA is similar to the M-mtDNA which is present in 100% of *A. islandica* male gonads. We sequenced the “divergent” mtDNA and showed that the sequence of all genes is modified but that gene order is conserved. Using transcriptomic and biochemical approaches, we highlighted that this “divergent” mtDNA is exclusively expressed and reduces the maximum capacities of respiratory chain complexes (complex I, III and cytochrome-c oxidase) by approximately 30% compared to the normal F-mtDNA type. These results combined with our analyses of mitochondrial respiratory capacities, ROS production, and data on individual growth and longevity suggest that these animals carrying this “divergent” mtDNA in their somatic tissues, and among those especially the female specimens, could be the ones with an extremely long lifespan prognosis.

Altogether, these new findings will allow us to enlarge the discussion about the link between natural mitochondrial genome modifications and animals’ phenotype and ecology.

Dégletagne, C., Abele, D. Held, C. (2015). A distinct mitochondrial genome with DUI-like inheritance in the ocean quahog *Arctica islandica*. Molecular Biology and Evolution, 33(2), pp.375-383.

18.4

Integrating the effects of food availability and sirtuins on stress tolerance to multiple levels of biological organization

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Sirtuins remove acyl groups, which represent several metabolites, from proteins and thereby regulate enzyme activity through modifying post-translational modifications (PTMs). Levels of sirtuins are generally upregulated in response to caloric restriction and affect a number of cellular processes in mammals. We investigated the effect of sirtuin (SIRT) and food on the stress tolerance of several closely related mussel species. We discovered that overall levels of the mitochondrial sirtuin 5 (SIRT5) are three times higher in gill of the heat-sensitive mussel *Mytilus trossulus* than the more heat-tolerant Mediterranean *M. galloprovincialis*, which replaced the former from the warmest part of its range in California over the last century. Despite the higher constitutive levels of SIRT5, the heat-sensitive species only showed an increase with heat stress. Further, inhibiting SIRT activity during heat stress increased SIRT5 levels in the heat-sensitive but not the -tolerant species, suggesting an active signaling feedback pathway. Using proteomics, inhibitor studies showed that sirtuins affect molecular chaperones, oxidative stress proteins, metabolic enzymes and signaling proteins during heat stress, suggesting a general influence on the cellular stress response (CSR). We extended the studies to test for the effect of food availability and frequent heat stress during acclimation on levels of sirtuins and the effect of sirtuin inhibition on the CSR, several measures of gill performance (particle velocity, ciliary beat frequency and clearance rate) and respiration in *M. californianus*. Our initial results suggest that the changes initiated by food availability and SIRT inhibition integrate up to affect gill respiration and interact to affect clearance rate at the level of the whole organism. Finally, these results are dependent on the circadian rhythm of mussels, in part because SIRT5 levels undergo circadian changes in

abundance. Our studies suggest that integration across levels of organization requires a high temporal resolution and several types of performance measures at the level of the organ and the organism.

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18.5

Evolution of higher rate of living leads to enhanced inducibility of gene expression: evidence from heat shock genes in *Drosophila*

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For most organisms, the ability to respond to stressful and changing environments is essential for survival and Darwinian fitness. In response to stress, individuals undergo shifts in both behaviour and physiology. Species/populations with early reproduction and short lifespan may be under pressure to respond to changing conditions rapidly because reproductive success is determined in a narrow window of time. Additionally, a higher metabolic rate may predispose faster activation of underlying molecular responses to a stressor. Either way, one might expect species with a high "rate of living" – typically smaller and faster growing species – to also be quicker in up-regulating stress-mitigating pathways. In this study, we address whether there is such a relationship between rate of living (ROL) and rate of cellular response to stress using populations of *Drosophila melanogaster* which have been selected for highly divergent ages of reproduction for over 1000 generations. One selection treatment, here designated "Fast" for simplicity, goes through a complete generation every 9 days, while it's "Slow" counterpart reproduces every four weeks; each treatment has five-fold replication at the population level and each population is kept in large cohorts. In response to selection, Fast and Slow flies now differ markedly in many traits. Fast flies develop in seven days, compared to 9 – 10 days for Slow. Fast flies are also smaller-bodied at reproductive maturity. These lines also differ in sperm production, food requirements, desiccation and starvation tolerance and lifetime fecundity. Most important for this study, Fast and Slow flies have dramatic differences in longevity, with Slow flies living 2-3X longer. To investigate the relationship between stress response and ROL, flies from both selection treatments were exposed to temperatures known to up-regulate heat shock proteins (Hsps). We predicted that, due to their higher ROL, Fast flies would mount a stress response by up-regulating Hsps more rapidly in response to heat shock than Slow flies would. To measure the rate of heat shock response, we set up a time-course of heat exposure for flies from both selection treatments. We then measured gene expression of Hsps using qRT-PCR. We found that, in line with our predictions, Fast flies increase expression of Hsps quicker than Slow flies, consistent with a link between ROL and kinetics of the stress response. Unexpectedly, Fast flies not only ramped up HSP expression faster but also displayed increased overall expression than Slow flies. We are following up this work by looking at other stressors to determine if there is an innate difference in sensitivity to stress between the two selection treatments. *Supported through NSERC Discovery Grants to AC and CDM.*

19: ENERGETICS: ABSTRACT DRIVEN SESSION

19.1

Polygenic mapping reveals genetic associations with variation in routine metabolic rate in *Fundulus heteroclitus*

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Variation in metabolic rate is thought to be a key factor influencing the ecology of individuals, populations and species. Consequently, identifying the genetic mechanisms that underlie variation in this trait is critical for understanding not only the potential for evolution of metabolic rate, but also the processes through which evolution of metabolic rate is likely to occur. Despite this clear need to link genetic variation with phenotypic variation in metabolism, there are few empirical datasets available that address this important issue. In the current study, we assess variation in routine metabolic rate in three populations of Atlantic killifish, *Fundulus heteroclitus*. There are two subspecies of this species along the east coast of North America: *F. h. heteroclitus* from Florida, USA to New Jersey, USA, and *F. h. macrolepidotus* from New Jersey to Nova Scotia, Canada. The northern subspecies has previously been shown to have a higher routine metabolic rate than the southern subspecies. However, this pattern could be to the result of either genetic differences between the subspecies or the result of developmental plasticity caused by the different environmental conditions at the northern and southern end of the species range. Here, we take advantage of a New Jersey population located in a region of genetic admixture between the subspecies to assess the relationship between genetic variation and variation in metabolic rate in a relatively large sample of fish (286) collected from a single location. Through the use of random forest models, we identified 62 single-nucleotide polymorphisms (SNPs) that explained up to 43% of the variation in routine metabolic rate among individuals. This suggests that metabolic rate is polygenic to a large

extent, and that a substantial amount of variation in this key physiological and ecological trait is genetically determined. Additionally, several of our identified SNPs demonstrated allele frequency differences among populations that were consistent with population-level variation in metabolic rate. Thus, it is possible that variation at a subset of the 62 SNPs we identified may contribute to variation in metabolism among populations as well as among individuals. Many of these SNPs were located within or nearby genes that have plausible functional connections to differences in metabolism, including several upstream regulatory proteins of central cellular kinases such as phosphatidylinositol 3-kinases, mitogen-activated protein kinase and the mechanistic target of rapamycin, as well as other genes involved in metabolic pathways such as monocarboxylate transporter 7.

19.2

Evolutionary variation in hypoxia tolerance in *Fundulidae* killifishes

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Hypoxia is a pervasive stressor in the aquatic environment, and fish exhibit a variety of mechanisms for coping with hypoxia that arise from phenotypic flexibility and/or evolutionary adaptation. We investigated the evolved variation in hypoxia tolerance and the hypoxia acclimation response in killifish from the family *Fundulidae*, a widely distributed group that naturally experiences daily and seasonal cycles of hypoxia. We compared resting O_2 consumption rate (MO_2) and three indices of hypoxia tolerance – critical O_2 tension (P_{crit}), O_2 tension (PO_2) at loss of equilibrium (LOE), and time to LOE in severe hypoxia – across 8 species (*F. heteroclitus, F. confluentus, F. rathbuni, F. grandis, F. diaphanus, F. similis, Lucania goodei,* and *L. parva)*. We also examined the effects of acclimation to constant hypoxia (2 kPa O_2) or to intermittent cycles of nocturnal hypoxia (12h normoxia during the day, and 12h of 2 kPa O_2 at night) in a subset of 5 species. Larger fish generally had lower mass-specific MO_2 and were more hypoxia tolerant (P_{crit}, and PO_2 at LOE) after accounting for evolutionary relatedness using phylogenetically independent contrasts. Acclimation to constant hypoxia increased hypoxia tolerance in all species, but there was interspecific variation in the magnitude of the response. Acclimation responses to intermittent hypoxia were idiosyncratic, with changes occurring in only some species. This variation in hypoxia acclimation responses was not generally associated with variation in MO_2 or with the relative severity of the O_2 stress to each species (as reflected by acclimation PO_2 relative to P_{crit}). Our results suggest that there is appreciable evolutionary variation in hypoxia tolerance and the hypoxia acclimation response across the *Fundulidae*. Supported by the Natural Sciences and Engineering Research Council of Canada, the National Science Foundation, the National Institute of Environmental Health Sciences, the American Physiological Society, the Society for Experimental Biology, and the Louisiana Sea Grant program.

19.3

Protein turnover: A biochemical basis for endogenous variation in growth and energy metabolism

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Protein turnover is regulated by complex, counteracting dynamics of synthesis and degradation. When the balance of these process favors synthesis over degradation, protein growth occurs. Protein turnover is energy intensive, with protein synthesis often being the major consumer of metabolic energy in animals, accounting for ~50% of total ATP consumption. Variation in protein turnover will therefore have important implications for metabolic energy expenditure and growth. While the regulation of protein turnover by exogenous (environmental) factors has been of long-standing interest in the field of comparative biology, less effort has been directed towards understanding endogenous (genetic) regulation of protein turnover and its role in genetically determined growth variation. We used factorial crosses of inbred lines of the Pacific oyster (*Crassostrea gigas*) to produce larval families that showed classical hybrid vigor (i.e., the superior growth of hybrids over both parental lines). We then tested for variation in protein turnover among families of larvae with contrasting growth phenotypes. Despite a lower growth rate than hybrid larvae, inbred larvae had higher fractional rates of protein synthesis (synthesis rate as a percent of whole-body protein content). Consequently, protein depositional efficiency (the ratio of protein accretion to total protein synthesized) was much lower for inbred families – reflecting higher protein degradation rates in these slow-growing larvae. We next assessed energy use and costs for protein turnover. During growth, respiration was dependent on larval size and did not differ among families (i.e., was not genetically determined). Differences in protein turnover between inbred and hybrid families did, however, have major effects on energy expenditure. Inbred larvae used ~2-fold more ATP to support protein synthesis, relative to hybrid larvae. Our analysis provides a mechanistic explanation for hybrid vigor and suggests that genotypes with increased energy demand for protein synthesis, as well as high degradation rates, may be limited in their ability to allocate ATP to other essential processes. This

work was supported by funding from the U.S. National Science Foundation (Emerging Frontiers No. 121220587).

19.4

Bioenergetics of protein metabolism under experimental environmental change

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The identification of the integrative processes that regulate physiological traits remains a key goal in biology. Furthermore, in multicellular organisms it remains challenging to define how processes at the cellular level can be used to predict responses at the whole-organism level under changing environmental conditions. In general terms, such responses are regulated by complex genotype-environment-phenotype interactions; for most organisms, however, the details of these interactions are not understood. In this study, we present findings based on a combination of physiological and genetic analyses focused on the study of biological variation in growth and metabolic energy (ATP) allocation. Crosses of pedigreed lines were used to produce contrasting phenotypes in developmental stages of a highly fecund marine invertebrate mollusc (*Crassostrea gigas*). On average, during growth 82% of the allocation of cellular ATP was accounted for by only three processes: protein synthesis and degradation (48%), sodium-potassium regulation (20%), and calcification (14%). Significant variation on this average hierarchy of ATP allocation was observed across different larval families, with noteworthy physiological consequences. For instance, variation in ATP allocation to protein synthesis predicted 72% of growth variation. Analysis of genetic, environmental, and physiological components of variation revealed a tradeoff between ATP allocation for fast growth and susceptibility to environmental stress. This bioenergetic framework suggests that a biochemical 'tipping point' can be identified as a predictive index of sublethal-to-lethal stress responses.

19.5

Dietary antioxidants and flight exercise affect the extent to which antioxidants are delivered to the mitochondria and how female birds allocate nutrients to eggs.

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Physiological challenges during one part of the annual cycle can carry over and affect performance at a subsequent phase, and antioxidants could be one mediator of trade-offs between phases. We performed a controlled experiment with zebra finches to examine how songbirds use nutrition to manage trade-offs in antioxidant allocation between endurance flight and subsequent reproduction. Our four treatment groups included a factorial combination of exercise/sedentary groups and diet supplemented/non-supplemented groups with the supplement including water- and/or lipid-soluble antioxidants. Dietary Vitamin E was delivered to mitochondria within 22 hrs of ingestion but only in exercised and not sedentary birds. After flight training, birds were paired within treatment groups for breeding. We analyzed eggs for lutein and vitamin E concentrations and the plasma of parents throughout the experiment for non-enzymatic antioxidant capacity and oxidative damage. Exercised birds had higher oxidative damage levels than non-exercised birds after flight training, despite supplementation with dietary antioxidants. Supplementation with water-soluble antioxidants decreased the deposition of lipid-soluble antioxidants into eggs and decreased yolk size. Flight exercise also lowered deposition of lutein, but not vitamin E, to eggs. These findings have important implications for future studies of wild birds during migration and other oxidative challenges.Supported by NSF (IOS-0748349 & IOS-135417 to S.R.M.), USDA (RIAES-538748 to S.R.M.), and AOU and Sigma Xi student research awards to M.M.S.

20: EVOLUTION OF PHENOTYPIC PLASTICITY IN PHYSIOLOGICAL SYSTEMS SPONSORED BY THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

20.1

Mechanisms underlying thermal acclimation and their evolution

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Thermal acclimation manifests as reversible shifts in reaction norms within organisms, which can confer resilience to environmental change on individuals. Acclimation is thought to have evolved in response to recurring environmental variation within generations. I suggest that thermal acclimation emerges from regulatory systems that evolved to maintain energy homeostasis within cells. For example, the principal regulator of energy homeostasis in cells, the AMP-stimulated protein kinase (AMPK), has an ancient prokaryotic origin and switches between anabolic and catabolic processes in response to environmental energy availability. AMPK is a principal node in a complex regulatory network that also includes the ancient histone deacetylases and the more derived thyroid hormones. The interaction between regulatory mechanisms permitted an increasingly sophisticated fine-tuning of phenotypes, and regulatory complexity increased over evolutionary time. It is constructive to interpret thermal

plasticity in the context of explicit regulatory mechanisms, which can replace hypothetical "genes for plasticity" in evolutionary models.

20.2

Evolution of the Acclimation Responses to Hypoxia and Cold in Deer Mice Native to High Altitudes

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High-altitude environments provide fertile ground for investigating the evolution of phenotypic plasticity in the physiological systems underlying animal performance. The cold and oxygen-depleted ('hypoxic') environment at high altitudes requires that endothermic animals sustain high rates of O_2 consumption for thermogenesis and locomotion while facing a diminished O_2 supply. This appears to result in strong directional selection for high aerobic capacity (VO_2max) in hypoxia in deer mice (*Peromyscus maniculatus*) native to high altitude. I will present our work on the evolution of phenotypic plasticity of VO_2max in high-altitude deer mice. Hypoxia acclimation augments hypoxic VO_2max much more in highlanders than in low-altitude populations of deer mice and white-footed mice (*P. leucopus*). This is associated with higher pulmonary O_2 extraction, arterial O_2 saturation, cardiac output, and tissue O_2 extraction. The evolution of hypoxia-induced plasticity in systems-level function involves coordinated changes at tissue, cellular, and transcriptomic levels of organization. In contrast, cold acclimation has a similar effect on VO_2max in highlanders and lowlanders, and the effects of cold acclimation arise primarily from increases in cardiac output and tissue O_2 extraction. Therefore, high-altitude adaptation has augmented plasticity in response to chronic hypoxia, but not chronic cold, and involves a series of integrated changes across the O_2 pathway.

20.3

Cold adaptation drives evolution of metabolic plasticity in *Drosophila melanogaster*

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Metabolic pathways are strongly affected by temperature, and the thermal sensitivity of metabolism frequently evolves in response to variable thermal environments leading to differences in metabolic plasticity. Metabolic plasticity can occur across the levels of the biological hierarchy, from transcript or metabolite networks, pathway flux, and organismal performance. In some cases, plasticity at one level of the hierarchy can canalize important responses at another level. We use data from an integrative study of metabolic plasticity in response to cold adaptation in *Drosophila melanogaster* to illustrate this principle, and show that evolution of metabolic plasticity can preserve organismal function in response to thermal stress.

We show that adaptation of *Drosophila melanogaster* to acute low temperature exposure increases rates of growth, activity, respiration, catabolism and anabolism in flies that have not received a cold exposure. During cold exposure, cold-hardy flies shut down catabolism to a greater degree than do cold-susceptible flies, leading to greater plasticity in nutrient catabolism and greater metabolic flexibility. At the metabolite level, this flexibility in catabolism allows them to maintain metabolic and energetic homeostasis more effectively. Thus, plasticity in metabolic flux can respond to selection imposed by acute low temperature exposure, and can be key to the maintenance of homeostasis in variable environments.

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20.4

Evolution of osmoregulatory flexibility during transitions between marine and freshwater habitats in fishes

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Adaptive divergence between marine and freshwater environments is important in generating phyletic diversity within fishes, but the mechanistic basis of adaptation to fresh water remains poorly understood. We examine divergence in osmoregulatory abilities between populations of *Fundulus heteroclitus* that are native to brackish (BW-native) or freshwater (FW-native) environments along a salinity gradient. We find that BW-native fish showed a reduced ability to regulate plasma Cl− in fresh water, while FW-native fish showed a reduced ability to regulate plasma Na+ in brackish water, suggesting that cell–cell junctions are involved in the divergence of physiological abilities. We used population genomics to identify genomic regions that affect fitness between BW and FW environments, and genome wide association to reveal the genetic variation that is associated with variation in salinity tolerance. Our analyses implicate candidate genes likely involved in evolved physiological capabilities, some of which support hypotheses about the importance of cell-cell junctions.

21: CARDIOVASCULAR: ABSTRACT DRIVEN SESSION - 2

21.1

Assessing the full significance of the Bohr/Haldane effect for gas exchange in the tissues

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Despite the fact that the Bohr and Haldane effects are of equal size at the molecular level due to their thermodynamic linkage, the influence of the Bohr effect

on the utilization of blood borne oxygen has been deemed secondary to the influence of the Haldane effect on the uptake of carbon dioxide by the blood. Here we show that the opposite is the case. Using a simple two-ligand, two-state formulation we modelled the simultaneous oxygen and proton binding to hemoglobin as well as the resulting acid-base changes of the surrounding solution. When the Bohr effect is blocked in this model system, we see a dramatic increase in the oxygen affinity, with a fall in oxygen half saturation pressure (P_{50}) from 27 to 6 mmHg. We also show that the P_{50} and the Bohr factor are not independent but directly related. Thus, everything else being equal, varying the number of Bohr groups from 0 to 8 per tetramer results in an increase in the Bohr factor from 0 to -0.9 and an increase in P_{50} from 6 to 46 mmHg at a constant Pco_2 of 40 mmHg. Therefore, changes in hemoglobin structure that lead to changes in the Bohr factor will inevitably also change hemoglobin oxygen affinity. The full extent of the impact of the Bohr effect on oxygen unloading cannot be assessed by comparing oxygen equilibrium curves measured in the lab at different constant Pco_2 or pH because each of these curves are already shaped by the Bohr/Haldane effect.

21.2

Weddell seals selectively limit guanylyl cyclase-mediated vasodilation: Implications for perfusion of the brain during diving

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In terrestrial animals, the nitric oxide-cGMP (NO–cGMP) signal transduction pathway plays a critical role in inducing vasodilation, including the systemic response to hypoxia. Diving mammals experience profound hypoxia during submergence, while they maintain selective vasoconstriction in peripheral tissues, suggesting that hypoxic vasodilation may modulate cardiovascular responses to diving. The objective of this study was to investigate NO–cGMP signaling in Weddell seals, using tissues including arteries collected at necropsy. We focused our investigation on soluble guanylyl cyclase (GC), which produces the vasodilator cGMP upon stimulation by NO. GC protein abundance, baseline activity, and NO-responsiveness were consistently lower in Weddell seals compared to terrestrial mammals. In seal lung homogenate, GC produced less cGMP (pmol•mg protein^{-1}•min^{-1}) than the lungs of dogs (-80±144), sheep (-472±96), rats (-664±104) or mice (-1160±104; P<0.004). GC activity was also lower in seal brain, heart, skeletal muscle, kidney, and artery homogenates compared to the same tissues in sheep, indicating that seals produce less vasodilatory cGMP at baseline and in the presence of NO. Amino acid sequences of the GC enzyme alpha subunits differed between seals and terrestrial mammals, impacting hydrophobicity and polarity of the seal proteins and potentially affecting their structure and function. GC abundance, activity, and NO-responsiveness declined with maturation in some seal tissues: NO-stimulated GC activity declined 15-fold in lung, 25-fold in muscle and 39-fold in heart of adults versus pups. Reduced cGMP production via low GC activity in selected adult seal tissues could support the dive response by potentiating sympathetically-mediated vasoconstriction. Yet, peripheral vasoconstriction in diving Weddell seals is not homogenous, with arterial blood perfusion distributed to favor the brain and heart. To better understand tissue-specific vasoregulation during diving, we compared NO-cGMP signal transduction in the brain versus the kidney (which experiences vasoconstriction, even during short dives). Seal GC was more responsive to stimulation by a NO-donor in the brain (58-fold activity increase) than in the kidney (24-fold increase), consistent with the prioritization of cerebral perfusion during diving. *Nos3* expression was also high in the seal brain relative to the carotid artery and the renal circulation (renal artery and kidney tissue; P=0.01), which could improve NO production and vasodilatory potential in the hypoxic brain. Conversely, *Pde5a* expression was highest in the seal renal artery (P=0.04), identifying this as a site of cGMP scavenging and supporting vasoconstriction in the kidney. Taken together, these results suggest that Weddell seal-specific tissue alterations in the expression and function of proteins in the NO-cGMP signal transduction pathway may be critical features of the diving response. Funded by NSF Office of Polar Programs 1443554 and NIH U54 HG003067-08.

21.3

Developmental differences in anoxia-induced gene expression in the heart of the painted turtle

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The Western painted turtle, *Chrysemys picta bellii*, is the most anoxia-tolerant tetrapod known, capable of surviving 170 days of anoxia at 3˚C. During anoxia, the turtle suppresses its cardiac metabolic rate in order to decrease energy consumption to levels that can be met by glycolysis. Although many of the functional changes that occur during anoxia have been described, the

transcriptomic changes have received less attention. Turtles also exhibit developmental differences in anoxia tolerance; hatchling turtles survive for only 40 days at 3˚C. Our study compared the transcriptomic changes that occur in the turtle ventricle during 20 days of anoxia followed by 6 days of recovery at 3˚C in both adult and hatchling painted turtles. The anoxia-tolerant adult turtles exhibited several unique changes in gene expression after anoxia and recovery, including a significant decrease in mRNA expression levels of 58 ribosomal proteins after anoxia, ($p = 0.001$), all of which increased in hatchlings ($p = 0.001$). After reoxygenation, ribosomal protein expression levels returned to control levels for both development stages. These results suggest that downregulation of genes encoding ribosomal proteins, and of the process they regulate, may be essential for cardiac survival during anoxia in adult painted turtles.

21.4

The influence of cellular stretch on extracellular connective tissue deposition in cultured trout cardiac fibroblasts

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Rainbow trout undergo cardiac hypertrophy and extracellular collagen deposition in response to cold (4 °C) acclimation. During cold acclimation, whole blood viscosity initially increases, which in turn leads to increased vascular resistance and ultimately places more workload on the heart. At the cellular level, myocytes, fibroblasts, and endothelial cells within the myocardium are thought to undergo increased deformation as a result. In mammals, cellular deformation stimulates mechanosensory cellular components and initiates a signaling cascade through mitogen activated protein kinase (MAPK) pathways, influencing the gene and protein expression of collagen, angiotensin and transforming growth factor-beta 1 (TGF-β1). Previously, we have demonstrated that exogenous human TGF-β1 is able to increase collagen synthesis in trout cardiac fibroblasts, as well as differentiate these cells into myofibroblasts. Therefore, it is expected that many of the major remodeling pathways are conserved throughout vertebrates, such as mechanotransduction through extracellular physical cues. We hypothesized that trout cardiac fibroblasts are deformed in response to vascular resistance, and predicted that stretching these cells would result in the activation of MAPK pathways and ultimately collagen remodeling. Fibroblast cultures from rainbow trout ventricles were subjected to 10% stretch on a rubber membrane for 10 min, 20min, 24h and 3d and then their MAPK pathways were compared to that of control cells that were cultured on the same type of membrane, but not stretched. p38 MAPK and extracellular-regulated kinase (ERK) phosphorylation was quantified using Western blotting. No changes were detected after 10min of stretch, however, after 20min, p38 MAPK phosphorylation increased by 4.2-fold compared to cells that were not stretched ($P<0.05$). While ERK was not significantly phosphorylated, an interesting finding was noted. The total amount of unphosphorylated ERK was increased by 1.7-fold in stretched cells after 20min, without a change in total protein. After 24h of stretch at 10% deformation, p38 continued to be phosphorylated by 4.7-fold more than control cells ($P<0.05$). At this time, collagen type I was reduced by 70% in stretched cells. Collagen types I and III are currently being quantified after 3d of stretch. The results of this study provide support for the hypothesis that cardiac remodeling in trout is initially triggered by vascular resistance, leading to cellular deformation and mechanotransduction.

21.5

Re-Assessment of the Biochemistry of Metabolic Acidosis Using Metabolite and Reaction H^+ Coefficients Computed From Multiple Competitive Cation Binding

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Background*:* The purpose of this research was to complete pH dependent calculations of H^+ balance during intense exercise corrected for pH and competitive cation binding. **Methods*:*** Metabolite accumulation data were acquired from prior published research of intense exercise conditions causing muscle anoxia, and where volitional exhaustion occurred within 3-4 min (1-3,5,9-11). Such data, adjusted for muscle water, pathway flux and carbon numbers where appropriate, were used to quantify the capacities of each reaction of non-mitochondrial ATP turnover in contracting skeletal muscle as well as profile each metabolite production curve over time. Muscle ATP turnover during intense exercise was modeled based on the data obtained from Spriet et al. (9,10). Data for H^+ dissociation constants for metabolites were obtained from the NIST database (6), Robergs (7), Kushmerick (4), and Vinnakota et al. (12). Custom software was completed (LabVIEW, Austin, Texas, USA) to allow computations of pH dependent H^+ consumption and release for skeletal muscle non-mitochondrial energy catabolism for this exercise condition. **Results and Conclusions:** Computed data results revealed that the total H^+ load of this intense exercise condition, where negative numbers indicate H^+ release, was -174.6 mmol/L. The net H^+ balance for the sum of the CK, AK, AMPD, myosin ATPase and amino acid deamination reactions was 32.87 mmol/L. The combined glycogenolytic and glycolytic H^+ balance, including lactate production, was -33.54 mmol/L. The overall H^+ balance of ATP hydrolysis equated to -44.57 mmol/L. Lactate production had a H^+ balance of 44.14 mmol/L (assuming

a total lactate accumulation of 45 mmol/L). Total metabolic H^+ buffering was 129.33 mmol/L. Structural buffering was 49.53 mmol/L, with an estimated total muscle buffer capacity for this pH change (7.0 to 6.1) of 55.03 Slykes. The H^+ release to lactate ratio was close to 4.0 (174.6/45=3.88). When correcting past errors of a lactic acid origin of metabolic acidosis, muscle H^+ release during intense exercise is up to 4-fold larger than previously assumed based on the now disproven lactic acid construct. Lactate production removes approximately 25% of the intracellular metabolic H^+ release of intense muscle contractions. The data have important implications to the teaching of metabolic biochemistry and interpretations of research data concerning systemic and cellular acid-base balance.

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22: MAMMALIAN MOLECULAR PHYSIOLOGY: ABSTRACT DRIVEN SESSION

22.1

Obesity-related gene expression during fasting in a naturally obese marine mammal

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Adipose tissue is a complex energy storage and endocrine organ that regulates metabolic homeostasis via adipocyte-derived hormones (adipokines). Excess adiposity in humans is correlated with increased adipokine levels, insulin resistance, and inflammation, and is a major risk factor for metabolic pathologies. With some of the largest subcutaneous adipose stores in the animal kingdom, marine mammals may provide valuable insights into the role of adipose tissue in health and disease. Capital breeding marine mammals rely on lipid stores in blubber to sustain fasting periods associated with terrestrial breeding and migration. Northern elephant seals (Mirounga angustirostris) undergo two annual fasts during breeding and molting haul-outs, losing up to half of their fat mass over a period of 1-3 months while maintaining high circulating fatty acid and glucose levels and insulin resistance, similarly to obese humans. We examined changes in expression of genes associated with obesity in humans in blubber of fasting adult female elephant seals using quantitative PCR. Target genes included the adipokines leptin, adiponectin, and retinol-binding protein 4 (Rbp4), fat mass and obesity-associated gene (Fto), and angiopoietin-like 4 (Angptl4), among others. Normalized gene expression values (dCt) were compared between early breeding, late breeding, early molting, and late molting groups using linear mixed models. Expression values were significantly different between groups and the majority of adipokine genes were upregulated in late fasting compared with early fasting seals, despite a concomitant decline in fat mass. This suggests a mechanism by which elephant seals maintain insulin resistance and lipid oxidation during fasting periods characterized by high energy expenditure and provides insights into rapid weight loss in mammals.

22.2

The Weddell seal skin transcriptome reflects local mechanisms in endocrine regulation of molt

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Pinnipeds replace their fur each year in an annual pelage molt, the timing of which is tightly regulated to conserve energy. While various hormones are known to influence hair growth, the role of local gene expression in endocrine control of molt is not well understood. To characterize how serum hormones and skin gene expression dynamics impact molt, we examined hair follicle cycling in prime-age female Weddell seals (*Leptonychotes weddellii*) during the Antarctic summer (November-February) (n=121, each seal handled once or twice). Hair cycle phases of seals were determined histologically from skin biopsies, and associated hormone profiles were assessed by radioimmunoassay. Skin transcriptomes were profiled with Illumina RNA-Seq during three of the observed hair cycle phases: early anagen (initiation of hair growth), late anagen (rapid hair growth), and telogen (quiescence) (n=3 per phase).

Weddell seal hair follicles entered early anagen in November/December, at least a month prior to the onset of visible shedding, and did not enter telogen until late January. Animals with higher serum concentrations of

prolactin, cortisol, and estrogens and lower concentrations of thyroxine completed molt later, suggesting these hormone levels slow hair growth. The Weddell seal skin transcriptome changed drastically across the molt, with >4000 genes differentially expressed between hair cycle phases. The late anagen transcriptome was most distinct, and featured enrichment of genes in a number of signaling pathways likely involved in regulating the molt. For example, during late anagen, expression levels of receptors for prolactin (PRLR) and thyroid hormone (THRB) were upregulated, while those for estrogen (GPER1) and cortisol (NR3C1) were downregulated. Thyroid hormone deiodinases were also differentially expressed across molt stages, with upregulation of DIO3 expression during late anagen suggesting that thyroxine is more rapidly inactivated in skin at this phase. Local changes in both hormone receptors and modifiers thus may alter the biological signal of circulating hormones during molt. Our study is the first to examine how skin transcriptomics reflect the mechanisms of molt in pinnipeds, and our findings offer key context for interpreting the roles of hormones in regulating this life history event.

22.3

Development of a biomarker panel of chronic stress in free-ranging marine mammals

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Increasing anthropogenic disturbances in marine ecosystems such as fishing, oil-drilling, and noise pollution associated with human activity can have detrimental effects on the reproduction and survival of apex predators such as marine mammals. Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, resulting in increased circulating glucocorticoid hormones, which alter expression of target genes encoding metabolic enzymes and other mediators of stress. Prolonged or repeated HPA axis stimulation may increase catabolism of nutrient stores and suppress the immune and reproductive functions, impacting the fitness of marine mammals. Our objective was to develop and validate a biomarker panel of stress that can discriminate between acute and chronic stress states in marine mammals. We previously characterized endocrine and metabolic profiles and identified genes that were differentially expressed in response to single and repeated adrenocorticotropic hormone (ACTH) administration in juvenile northern elephant seals (*Mirounga angustirostris*). We found that blubber genes upregulated during the response to repeated ACTH administration include those encoding lipid particle proteins (perilipins PLIN1 and PLIN4, cell death activator CIDE-A), oxidative stress enzymes (GPX3 and MGST1), adipokines (ADIPOQ and LEP), lipid transporters and lipid metabolism enzymes (ABCA6, ABCA10, AZGP1, and ACSL1), and an adipogenesis promoting transcription factor (DKK1). Downregulated genes include an adipogenesis inhibitor (TGFB1) and an adipokine that inhibits gluconeogenesis and inflammation (C1TQNF3). To validate our biomarker panel, we collected blood and blubber samples from 30 juvenile northern elephant seals of varying body condition and baseline stress states. Endocrine (cortisol, aldosterone, total triiodothyronine (tT3) and reverse T3) and metabolic (triglyceride) markers were measured in blood using immunoassays and colorimetric assays. Expression of candidate genes in blubber was measured using real-time polymerase chain reaction. Gene marker expression levels were significantly correlated with elevated stress hormones, decreased triglycerides, and body condition (standard length/axillary girth) in elephant seals. These markers provide insights into molecular mediators of the stress response and its physiological consequences, such as depletion of lipid stores in blubber, and can be used as a potential diagnostic panel for differentiating stress states in free-ranging marine mammals.

22.4

Insights into the structure-function relationships of I-band titin and its evolution across mammals

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The giant elastic protein, titin plays a key role in vertebrate striated muscle, where it acts as a molecular spring responsible for passive and active muscle elasticity. Titin spans an entire half sarcomere from z-disk to m-line and its elastic I-band region is composed of three domains: the proximal tandem Ig segment; the unique N2A (skeletal muscle), N2B (cardiac muscle), or N2BA (cardiac muscle) sequence; and the PEVK region. Through alternative splicing, titin can be expressed as isoforms of varying lengths which correlate with the passive properties of different muscle types. Muscles with long titin isoforms have low passive stiffness whereas muscles with shorter isoforms have higher passive stiffness. However, little is known about how the variability of titin contributes to differences in muscle performance across a wide range of muscle types and species. A more extensive description of I-band titin could reveal evolutionary trends across vertebrates. In this study, we developed an annotation tool to characterize the PEVK region of the titin gene across 41 mammalian species with a range of muscle physiologies. Our results reveal contrasting patterns of constraint and divergence across the PEVK region, suggesting two distinct sub-regions with distinct evolutionary dynamics. The PEVK-N region shows a relatively

conserved length and exon structure over evolutionary time, but evidence of diversifying selection and more variable amino acid content. In contrast, the PEVK-C region varies dramatically in length and exon number across mammals, but these exons tend to be more similar. These data suggest that "essential" PEVK-N exons play key roles in vertebrate muscle function but the total length of the PEVK-C region, rather than selection on any particular exon, dominates the evolution of the PEVK-C. It is possible PEVK-N exons are necessary for increasing titin stiffness in active muscle whereas alternative splicing of the PEVK-C region determines the stiffness of the titin "spring". Future work can focus on disentangling the effects of natural selection acting on specific codons, and how both regions contribute to evolutionary adaptation of titin.

23: THERMAL BIOLOGY: ABSTRACT DRIVEN SESSION - 2

23.1

Social cues can push amphibious fish to their thermal limits

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Social context can impact how animals respond to changes in their physical environment. We used an aggressive, amphibious fish, the mangrove rivulus (*Kryptolebias marmoratus*) with environmentally-determined sociality to test the hypothesis that social interactions would push fish to their thermal limits. We capitalized on the propensity of rivulus to emerse from warming water and demonstrated that social stimuli, produced by their reflection, increased emersion threshold without changing critical thermal maximum, effectively diminishing thermal safety margins. When rivulus were denied air access, surface behaviours dramatically increased, supplanting social interactions. This suggests that assessing the terrestrial environment is crucially important. We conclude that social stimulation narrows the scope for survival in naturally stressful conditions.

23.2

Developmental changes in oxygen consumption and hypoxia tolerance in the heat- and hypoxia-adapted tabasco line of the nile tilapia (*oreochromis niloticus*)

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The genus *Oreochromis* is among the most popular of the tilapiine cichlid tribe for aquaculture. However, their temperature and hypoxia tolerance, if tested at all, is usually tested at temperatures of 20-25°C, rather than at the considerably higher temperatures of 30-35ºC typical of tropical aquaculture. We hypothesized that both larvae and adults of the heat- and hypoxia-adapted Tabasco line of the Nile tilapia, *Oreochromis niloticus*, would be relatively hypoxia-tolerant. Oxygen consumption rate (MO_2), Q_{10}, and aquatic surface respiration (ASR) was measured using closed respirometry at 2 (~0.2g), 30 (~2-5g), 105 ~(10-15 g) and 240 (~250 g) days of development, at 25ºC, 30ºC and 35ºC. MO_2at 30ºC was inversely related to body mass: ~90 uM O_2/g/h in larvae down to ~1 µM O_2/g/h in young adults. Q_{10} for MO_2 was typical for fish over the range 25-35 ºC of 1.5-2.0. ASR was exhibited by 50% of the fish at PO_2s of 15-50 mmHg in a temperature-dependent fashion. However, the largest adults showed notable ASR only when PO_2 fell to below 10 mmHg. Remarkably, P_{Crit} for MO_2 was 12-17 mmHg at 25ºC-30ºC and still only 20-25 mmHg across development 35ºC. These values are among the lowest measured for teleost fishes. Noteworthy is that all fish maintain equilibrium, ventilated their gills, and showed routine locomotor action for 10-20 minutes after MO_2 ceased at near anoxia, and when then returned to oxygenated waters, all fish survived, further indicating a remarkable hypoxic tolerance. Remarkably, data assembled for MO_2 from >30 studies showed a >2000X difference, which we attribute to calculation/conversion errors. Nonetheless, P_{Crit} was very low for all *Oreochromis* sp., and lowest in the heat- and hypoxia-adapted Tabasco line.

23.4

Acute thermal tolerance, not hypoxia tolerance, affects the temperature sensitivity of hypoxia tolerance in marine fishes

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There is considerable interest in understanding the combined effects of ocean warming and deoxygenation on species distribution and abundance. Recent studies have used the temperature sensitivity of the critical oxygen tension for standard oxygen uptake rate (P_{crit}) as an index of hypoxia tolerance to model changes in species-specific habitat suitability and availability with projected changes in ocean temperature and oxygen content. Though this approach is theoretically well-founded, data to validate the models are limited to a handful of disparate taxa. Many questions remain regarding the effects of variability in hypoxia tolerance and thermal tolerance on species' responses to changes in environmental temperature and oxygen availability. In this study we asked whether species that are more tolerant of hypoxia and high temperature show reduced temperature sensitivity of hypoxia tolerance. We measured the effect of acute temperature increase on standard oxygen uptake rate ($\dot{M}o_{2,Standard}$) and P_{crit} in 9

species of marine cottid fishes with native habitats spanning the inter-to-subtidal environment. We then compared the temperature sensitivity of P_{crit} (β_{Pcrit}) in these species to P_{crit} at 12°C, a common environmental temperature at which previous investigations have characterized the mechanisms underlying variation in hypoxia tolerance of these species. To determine whether β_{Pcrit} varied with acute thermal tolerance we also measured critical thermal maximum (CT_{max}) in 8 of these species and compared CT_{max} with β_{Pcrit}. The effects of acute temperature increase on P_{crit} were directly correlated with temperature effects on $\dot{M}O_{2,Standard}$. There was no relationship between β_{Pcrit} and P_{crit} at 12°C and a marginally significant relationship between β_{Pcrit} and CT_{max}. Species exhibiting tidepool occupancy, a proxy for variability in habitat temperature and oxygen availability, generally had lower β_{Pcrit}, though this was not unique to tidepool occupants. Together these data suggest: (1) variation in whole-animal hypoxia tolerance is not related to the temperature sensitivity of hypoxia tolerance and (2) acute thermal tolerance may be an important predictor of the temperature sensitivity of hypoxia tolerance, potentially by constraining thermal performance breadth of aerobic metabolism.

23.5

Critical windows in rainbow trout embryos: Effects of thermal shifts on survival, growth and oxygen consumption

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Rainbow trout (*Oncorhynchus mykiss*) are a native anadromous species that inhabit a variety of fresh water rivers and lakes throughout the western portion of the United States. Currently, populations of rainbow trout in California, Oregon, Washington, and Idaho have been designated as threatened or endangered. Environmental temperature is a crucial abiotic factor that influences many physiological functions in fish, and global climate change is contributing to species declines. Understanding temperature effects during development is likely to provide insight into the overall thermal biology of a species and its persistence in a changing climate. To assess the effect of temperature on development of rainbow trout, we examined phenotypes of hatchery embryos reared in various incubation temperatures (5°C, 10°C, 15°C and 17.5°C). To identify the presence of embryonic developmental plasticity within critical developmental time points embryos incubated in 5°C were exposed to either 10°C, 15°C or 17.5°C during gastrulation, organogenesis, system integration or growth windows. A 3-dimensional critical window model was then applied. An increase in constant incubation temperature increased oxygen consumption rate (VO_2), and decreased hatchling survival, mass, and time to 50% hatch. Thermally shifting embryos into increased temperature during distinct windows of development also reduced survival at hatch, and this was most evident following exposure during organogenesis. Likewise, increased temperature reduced hatchling mass, specifically when exposure occurred during system integration. Thermally shifted embryos exhibited a decrease in VO_2 when measured at hatch at 5°C compared to 5°C constant embryos. This decrease in VO_2 was most evident during later developmental windows. Collectively, these results suggest rainbow trout embryos are most sensitive to increased temperature during organogenesis and system integration. Critical window studies such as this one performed across species will eventually allow for the identification of commonalities in plasticity between different species, and allow us to understand when during development particular phenotypic traits are most influenced by temperature.

24: COMPARATIVE ASPECTS OF ACID-BASE REGULATION

24.1

Evolutionary patterns of acid-base regulation in vertebrates.

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Acid-base regulation is a tightly regulated process and pH disturbances in vertebrates are rapidly corrected to restore homeostasis. In most vertebrates investigated to date, acute (<96 h) exposure to elevated environmental CO_2 (hypercarbia) results in a rapid reduction in both blood pH (pHe) and intracellular pH (pHi); recovery of pHi occurs in conjunction with pHe compensation (coupled pH regulation). However, coupled pH regulation may be limited at PCO_2 tensions far below levels that some fishes naturally encounter. Previously, three hypercarbia tolerant fishes had been shown to not compensate pH_e but completely and rapidly regulate pH_i during acute exposure to >3 kPa PCO_2 (preferential pH_i regulation). Here we test the hypothesis that preferential pHi regulation confers improved hypercarbia tolerance, by measuring CO_2 tolerance as well as intra- and extracellular pH regulation in a broad range of phylogenetically separated fishes. Contrary to previous views, we show that preferential pHi regulation is the most common strategy for acid/base regulation within adult fishes and that this strategy is associated with improved hypercarbia tolerance. While preferential pHi regulation has not previously been observed in adult amniotes, we have recently found that embryonic snapping turtles and alligators exhibit preferential pHi regulation but that this trait is lost later in development. We hypothesize that preferential pHi regulation is an embryonic pattern of acid-base regulation in vertebrates

that is either retained or lost during development, depending on the severity of acid-base challenges experienced during adaptation to their environment. By conferring hypercarbia tolerance, preferential pH_i regulation may have been a key exaptation that facilitated evolutionary transitions in vertebrate evolution, such as the evolution of air breathing.

24.2

Evolutionarily conserved mechanisms for acid-base sensing

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The levels of carbon dioxide, pH, and bicarbonate collectively determine the acid-base status of an organism. Because acid-base disturbances can compromise cellular function, maintaining acid-base homeostasis is essential for all organisms. To be able to modulate compensatory responses, organisms must be able to sense the acid-base disturbance in the first place. The bicarbonate-stimulated enzyme soluble adenylyl cyclase (sAC) is an evolutionarily conserved acid-base sensor that modulates diverse physiological processes *via* the cAMP signal transduction pathway. In coral cells, sAC senses acid-base disturbances from both internal and external origin and regulates intracellular pH, a function likely to apply to many (all?) cells from other organisms. In acid-base regulatory organs such as gill and kidney, sAC senses blood acid-base disturbances and modulates compensatory acid and base secretion and absorption. In response to elevated bicarbonate, sAC stimulates intestinal NaCl absorption across the intestine of marine bony fish. sAC is abundantly present in coral calcifying cells, mollusk mantle, fish otolith organ, and shark rectal gland, suggesting universal acid-base related regulatory control of epithelial ion transport. In hagfish heart, sAC regulates heart beat rate. The presence of sAC in the nucleus of some cells and in hemocytes, erythorcytes, and myocytes suggest regulatory control over diverse other physiological processes. However, sAC is by no means the only acid-base sensor. For example, research in mammals has identified a subset of G-protein coupled receptors (GPCRs) that stimulate transmembrane adenylyl cyclases (tmACs) in response to extracellular acidosis. Because sAC is stimulated by bicarbonate and the GPCR-tmAC complex is stimulated by protons, coordination of both sensing mechanisms might allow discriminating between metabolic and respiratory acid-base stress. Additionally, tmAC and sAC activities can have opposite effects, implying exquisite regulatory mechanisms that allow differential physiological regulation within cells by the same messenger molecule, cAMP. Funded by NSF grants IOS # 1354181 and 1754994, and UCSD CRES.

24.3

Bicarbonate-sensing soluble adenylyl cyclase in fishes

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The evolutionarily conserved acid-base sensor soluble adenylyl cyclase (sAC, adcy10) is important for regulation of acid-base status (i.e. $[CO_2]$, pH, and $[HCO_3^-]$) in cells, organs, and whole-animals. sAC is highly expressed in the base-secreting V-H^+-ATPase (VHA)-rich gill cells of both leopard shark (*Triakis semifasciata*) and round ray (*Urolophus halleri*), where it locally senses alkalosis and triggers VHA translocation to the cell membrane and subsequent base secretion. sAC is also highly expressed in acid-secreting Na^+/K^+-ATPase (NKA)-rich gill cells, where it likely regulates acid secretion. Both acid- and base-secreting cells are mitochondrion-rich and have large glycogen stores that serve as an energy source for each ATPase. Glycogen abundance diminished in both NKA-rich cells from sharks experiencing acidosis, and in VHA-rich cells from sharks experiencing alkalosis, demonstrating differential energy utilization during acid and base secretion, respectively. Furthermore, glycogen intracellular localization in isolated gill cells was consistent with that of NKA and VHA: in NKA-rich cells, glycogen was always present near the cell membrane; in VHA-rich cells, glycogen was normally present throughout the cytoplasm but was at the cell membrane during alkalosis. Intriguingly, the co-localization of VHA and glycogen at the cell membrane during alkalosis was blocked by the sAC-selective inhibitor KH7, implicating sAC as a regulator of energy metabolism in base-secreting cells. sAC is also present in other fish species; rainbow trout (*Oncorhynchus mykiss*) have >20 sAC splice variants with potential differential intracellular and tissue localization and functions. We are currently investigating the role of sAC in sensing and regulating acid-base stress in fish using rainbow trout (very CO_2 intolerant) and white sturgeon (*Acipenser transmontanus*; very CO_2 tolerant) as model species because they preferentially regulate extracellular and intracellular pH, respectively. Thus far we have found sAC is present in the liver and gills of both species, and that exposure to elevated CO_2 induced significant changes in carbonic anhydrase, VHA, and cAMP abundance in a tissue- and species-specific manner. Overall, we propose that sAC may be an essential acid-base sensor within fishes (elasmobranchs, teleosts, and basal actinopterygians), and that sAC activity has differential regulatory effects depending on acid-base status, species, tissue type, and even cell subtype; all of which are currently being investigated. Funded by NSF Postdoctoral Fellowship in Biology and APS Porter Physiology Development Fellowship to JNR, NSERC Discovery grant to CJB, and NSF IOS#1354181 to MT.

24.4

The alkaline tide: acid-base regulation during digestion

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Gastric acid secretion during digestion leads to a rise in the bicarbonate concentration in the bodily fluids, whilst pancreatic base secretion will tend to decrease bicarbonate levels. Because gastric functions precede the intestinal secretions, many vertebrates experience an initial rise in plasma bicarbonate upon ingestion of food, and this "alkaline tide" may last for hours or even days in some animals that ingest very large meals. The alkaline tide probably represents the most common acid-base disturbance in the life of any vertebrate. Yet, surprisingly little is know about the attending compensatory mechanisms. In air-breathers (amphibians, reptiles and mammals), arterial pH remain rather unaffected due to a concomitant reduction in air-convection requirement vertebrates that provides for a rise in PCO_2. Air-breathers therefore provide for a respiratory compensation of the metabolic alkalosis, which is consistent with ventilation being primarily regulated to govern acid-base balance. In fish where ventilation is more committed to oxygen, the ventilatory compensation is more benign. I will discuss the alkaline tide in connection with the large metabolic and phenotypic changes that occur during digestion, and will emphasize studies based on animals that ingest large meals upon extended periods of fasting. I will review how the use of specific proton pump inhibitors demonstrate that the ventilatory responses are indeed indicative of pH regulation and I will discuss unpublished data demonstrating the pancreatic secretions are of paramount importance in dampening the rise in plasma bicarbonate concentration that results from gastric acid secretion.

25: EVOLUTION OF METABOLIC PROTEINS

25.1

Molecular evolution of cytochrome *c* oxidase in hypoxia tolerant fish

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Cytochrome *c* oxidase (COX) is an ancient, multi-subunit enzyme that is considered to be older than the rise in atmospheric oxygen (O_2) that occurred 2.4–2.1 billion years ago that led to the expansion of eukaryotic biodiversity [1] Though its original function is unclear, the modern COX is the final step of the O_2 transport cascade in mitochondria and essential for aerobic metabolism. It catalyzes the final transfer of electrons from the electron transport system (ETS) to O_2, reducing it to water while simultaneously pumping protons to generate a proton electrochemical gradient for ATP synthesis *via* the F_1F_O ATP-synthase. Given its functional importance, COX was thought to be highly conserved. In fact, variation in COX function has been observed in organisms that encounter naturally occurring hypoxia in which adaptive modifications of the O_2 transport cascade have been well described. For example, in species of intertidal sculpins (Family Cottidae) that inhabit the nearshore environment and vary in whole animal hypoxia tolerance, there was a relationship between COX apparent O_2 binding affinity and the critical oxygen tension (P_{crit}), where more hypoxia tolerant species showed lower COX apparent O_2 binding affinity compared to less tolerant species. *In silico* analysis revealed interspecific sequence differences on the catalytic COX3 subunit that could affect interaction with membrane phospholipid cardiolipin and subsequently the path of O_2 travel [2]. In mammalian models, hypoxia induces an isoform switch of COX4-1 to COX4-2 which renders the COX protein insensitive to ATP inhibition [3]. Although it was previously shown that the teleost COX4-2 gene was not responsive to hypoxic exposure [4], recent data suggests that the COX4-2 transcript is upregulated and that the isoform switch may occur in the anoxia-exposed crucian carp *Carassius carassius* [5]. In this talk, I will present what is currently known of modifications to COX function and regulation in teleosts adapted to environmental O_2 limitation, and what is still unknown about this complex enzyme.

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25.2

Function and evolution of cellulase and hemicellulase enzymes within invertebrates that do not consume significant amounts of plant cellulose.

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Classic cellulase, endo-β-1,4-glucanase and the hemicellulases, β-1,3-glucanase or laminarinase and endo-β-1,4-mannase are expressed widely amongst the invertebrate animal groups. It is now widely recognized that these enzymes are endogenously produced and are not solely synthesized by symbiotic microorganisms such as bacteria and protozoans. Their presence is commonly used to indicate the ability of an animal to digest cellulose and hemicellulose. The implication is that animals consume and digest plant cellulose and hemicellulose. This may be true for primarily herbivorous or wood feeding invertebrates. However, in animals such as algal and deposit feeding invertebrates, such as crustaceans, molluscs and echinoderms, that do not consume substantial amounts of plant cellulose and hemicellulose, the function is not clear. Thus, the function of the enzyme in these species needs a

reappraisal. This will be attempted here by 1. describing the reaction catalyzed and structure of the enzyme to infer the preferred substrates, and 2. correlating if the substrates are present in the diet and hence ascribe a function. The endo-β-1,4-glucanase and β-1,3-glucanase may also be involved in non-digestive functions such as innate immunity given these enzymes are expressed in non-digestive tissues. They may act as β-glucan binding proteins to stimulate the phenol oxidase system. The cellulase and hemicellulase enzymes are distributed in a gradient of aquatic to terrestrial species and within different feeding specializations ranging from algal feeders, mixed deposit and leaf litter feeding species, those which are primarily herbivorous and omnivores. Hence there is the potential to examine the evolution of the enzymes and this will be attempted in this presentation. This will be done with respect to the colonization of land and the adoption of an herbivorous leaf litter diet. The function of the cellulase and hemicellulase enzymes within omnivorous species that do not consume significant amounts of low grade plant material will also be reviewed.

25.3

Evolutionary phylogenomics of UCP1 and sarcolipin: key players underlying adaptive thermogenesis across Eutheria?

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Two proteins—uncoupling protein 1 (UCP1) and sarcolipin (SLN)—have been implicated to underlie non-shivering thermogenesis (NST) in brown adipose tissue (BAT) and skeletal muscle, respectively, of eutherian mammals. BAT has long been recognized to play a key thermoregulatory role in small-bodied and hibernating species, and is considered to be especially important for neonates of cold-tolerant forms. However, given that body size and energetic considerations are expected to alter the selective advantage of BAT-mediated NST, we posited that evolution of large body mass or reduced thermogenic capacity would be accompanied by relaxed selection and/or inactivation of the *ucp1* locus. More recently, SLN—a 31 residue helical peptide bound to SERCA pumps in the sarcoplasmic reticulum—has been shown to increase heat production *in vitro* by partially uncoupling Ca^{2+} re-sequestration from ATP hydrolysis, leading to the hypothesis that SLN may contribute to muscle NST *in vivo*. To date, however, little is known on the regulation of SLN or its potential role in facultative muscle NST outside of a few small-bodied model systems (rodents and rabbits) that reside within the same mammalian clade. To address these questions, we first mapped *ucp1* sequences from 133 mammals onto a robust species tree and show that inactivating mutations occurred in at least 8 of the 18 traditional placental orders. Selection and timetree analyses further reveal that *ucp1* inactivations temporally correspond with strong secondary reductions in metabolic intensity (xenarthrans and pangolins), or coincided with a ~30 million year episode of global cooling that promoted sharp increases in body mass evident in the fossil record. These findings shed light on the potential energetic costs of BAT and challenge the thermogenic importance of UCP1 in nearly half of all eutherian lineages. Furthermore, while a number of studies conducted on lab mice contend that loss of SLN hinders muscle-based NST or that cold exposure increases SLN expression, our calculations suggest that this mechanism should only contribute ~2% of the heat produced by a single contraction-relaxation cycle. Additionally, as SLN is nearly undetectable in fast-twitch glycolytic fibers that form the bulk of the limb musculature, any NST linked to SLN may be expected to be imperceptible in these small mammals. Intriguingly, SLN mRNA and protein levels are several magnitudes higher in skeletal muscles of pigs, dogs, and humans than lab rodents, raising the possibility that SLN-NST may be of greater thermogenic importance in larger mammals. However, like *ucp1*, this locus may also be expected to have evolved under relaxed selection or even be pseudogenized in very large or tropically distributed species. In contrast to this expectation, not only is SLN functionally intact in all mammals (except possibly sloths), but its primary structure has remained exceptionally well conserved despite highly divergent body sizes and thermoregulatory capacities. This remarkable degree of sequence conservation—in species for which NST should have no apparent adaptive benefit—is not readily compatible with a thermogenic function, though in line with a role in regulating intracellular Ca^{2+} homeostasis by modulating the (apparent) Ca^{2+} affinity of SERCA. Thus, unequivocal support for an adaptive thermogenic role by SLN *in vivo* is lacking. Suggestions that SLN plays a meaningful thermogenic role in larger bodied mammals (or birds) is also without empirical evidence and remains purely speculative. This work was supported by Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery Grants to KLC (RGPIN/238838-2011 and RGPIN/6562-2016).

25.4

Evolution of Metabolic Proteins: Pyruvate dehydrogenase in anaerobiosis

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Without oxygen, most vertebrates die within minutes as they cannot meet cellular energy demands with anaerobic metabolism. However, fish of the genus *Carassius* (crucian carp and goldfish) have evolved a

specialized metabolic system that allows them to survive prolonged periods without oxygen by producing ethanol as their metabolic end-product. Here we show that this has been made possible by the evolution of a pyruvate decarboxylase, analogous to that in brewer's yeast and the first described in vertebrates, in addition to a specialized alcohol dehydrogenase. Whole-genome duplication events have provided additional gene copies of the pyruvate dehydrogenase multienzyme complex that have evolved into a pyruvate decarboxylase, while other copies retained the essential function of the parent enzymes. We reveal the key molecular substitution in duplicated pyruvate dehydrogenase genes that underpins one of the most extreme hypoxic survival strategies among vertebrates and that is highly deleterious in humans.

26: HARNESSING NATURALLY EVOLVED TORPOR TO BENEFIT HUMAN SPACEFLIGHT COSPONSORED BY THE SOCIETY OF EXPERIMENTAL BIOLOGY

26.1

Enhancing Metabolic Flexibility in Humans: Insights from Hibernation to Benefit Spaceflight

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Many mammals from diverse phylogenetic lineages can safely and reversibly lower their metabolic rate and enter torpor, a depressed metabolic state that enables substantial energy savings. Some species undergo daily torpor and continue to forage during active periods. Others hibernate, characterized by multi-day torpor bouts interspersed with periodic arousals to euthermia. Most hibernators cease feeding completely and obtain energy from oxidation of endogenous fuel (primarily lipid). Multiple lines of evidence suggest that the ability to induce natural torpor or hibernation is accompanied by resistance to a variety of pathologic stressors including ischemia-reperfusion, hemorrhagic shock and radiation injury. Thus, engineering the ability to induce a state of "synthetic torpor" in species that are unable to do so naturally would be advantageous for the treatment of multiple pathologic states, routine surgery, and long duration spaceflight missions - when food resources are limited and cosmic radiation impairs survival. What constrains humans from adopting this shifting phenotype for energy savings and cellular protection? This talk will discuss (1) why mimicking mechanisms of natural hibernation/torpor holds more promise for engineering synthetic torpor in humans than do current medical practices that rely on direct body cooling to reduce energy demands; (2) why size matters in translating natural hibernation to humans; and (3) what key discoveries in the field are needed to engineer synthetic torpor for beneficial use in non-hibernating species.

26.2

Central mechanisms of torpor induction

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In mammals, torpor is a state that is characterized by an active reduction in metabolic rate with a progressive decrease in body temperature (1). At the moment, the neural pathways responsible for the metabolic suppression that characterizes torpor are not known. Mice are facultative heterotherms, and torpor in these rodents can be reliably triggered by changing environmental conditions (2). This characteristic consents therefore to trigger torpor almost on command and the marker of neuronal activation cfos can be used to identify groups of neurons activated at torpor onset.

The metabolic suppression that characterizes torpor requires a reduction in the activity of metabolically active organs. Most of these organs, such as the brown adipose tissue, are controlled by the putative sympathetic premotor neurons located within the Raphe Pallidus (RPa) (3). To enter torpor, these neurons have to be necessary inhibited. The inhibition of these neurons in rats induces indeed a state the resembles torpor under many aspects (4). To pinpoint the neural circuits actively inducing the metabolic suppression, a retrograde tracer was injected within the Rpa region. The main aim was to identify populations of neurons projecting to the RPa neurons with torpor-related activity.

Here preliminary results showing the neural regions with torpor-related activity and projections to the neurons within the RPa will be presented.

The experiment was funded by the Univ. of Bologna

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26.3

The relationship between sleep and torpor

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Torpor is a strictly regulated process, associated with pronounced changes in activity, sensory functions, physiology and metabolism. However, many of the mechanisms that regulate this dramatic physiological state remain poorly understood. The nature of the relationship between torpor and sleep has been a controversial topic for several decades. In mammals, sleep and energy metabolism are intimately linked, as evidenced by the numerous bidirectional connections between the neural centres/circuits that govern these processes (1, 2). The maintenance of waking and sleep is regulated by several subcortical structures, which provide neuromodulatory action on the forebrain (3). In addition, wakefulness and sleep are shaped by the interaction of two processes: the homeostatic process, and the circadian process, which provides a temporal framework for specific waking behaviours, sleep and metabolism (4). Several studies implicated the role of hypothalamic centres of thermoregulation and energy homeostasis in the occurrence of torpor both in hibernators and non-spontaneously hibernating species (5, 6). Mice are facultative heterothermic species, and readily display torpor bouts in response to food deprivation (7, 8). It has been proposed that fasting can elicit a torpor response by decreasing energy expenditure and body temperature, while blocking normal cold-induced thermogenesis responses. However, less is known about the specific fasting-related signals which initiate entry into torpor. Behaviourally, torpor in mice resembles sleep, but our study suggests that brain activity measured by EEG is significantly depressed in torpid animals. However, early studies in ground squirrels and our observations in mice suggest that animals during torpor are able to respond to sensory stimuli and may exhibit spontaneous or provoked behaviours. While it appears that torpor is a state neurophysiologically distinct from both waking and sleep, evidence suggests that torpor and sleep are closely related. Specifically, torpor bouts induced by fasting are initiated from deep sleep, while daily torpor in Djungarian hamsters and pharmacologically induced torpor in mice are followed by sleep with high EEG slow-wave activity. The functional role of deep sleep after torpor is debated, but it has been suggested that sleep is important for recovery processes, which could be related to energy homeostasis, synaptic structure and function, and for a renormalisation of learning and waking performance after torpor. We conclude that further studies are necessary to investigate the effects of torpor on brain function and the relationship between torpor and sleep.

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26.4

The spaceflight environment

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Animals that thrive in extreme environments host an array of physiological adaptations that defy the limits of humans. For example, consummate divers like emperor penguins and elephant seals tolerate extremely low levels of oxygen even to critical organs like the brain and heart, as well as a reduction and subsequent reperfusion of blood. Birds that migrate at high altitudes like the bar-headed goose are able to maintain an adequate oxygen supply to support the metabolically costly act of flapping flight even when ambient oxygen levels are as low as 1/3 those of sea-level. Somehow these animals do so without facing the detrimental outcomes associated with humans under similar conditions of oxygen limitation (heart attack, stroke, reperfusion injury, etc.). The benefit that understanding the mechanisms of such impressive adaptation may have to the human medical field continues to motivate comparative physiologists. A natural connection may be made to humans functioning in the extreme environment of space, with particular relevance to the development of long duration missions that will begin to take us away from the proving grounds of low Earth orbit, to destinations like Mars. Among the critical limiting factors of human spaceflight missions is the amount of consumables (food, water, life support

systems) that can reasonably be launched and carried on-board. As mission lengths increase, load limits will quickly be reached. Although developing regenerative life support systems will be critical for future exploration, an added strategy could be to induce some level of metabolic depression or induced torpor in astronaut crewmembers to prolong the supply of such consumables. Astronauts in states of induced torpor may also be able to remain better shielded from the harmful effects of radiation during the journey, a medical risk factor that remains to be resolved before such missions can be realistically undertaken. In addition to the safety and practicality of the astronauts on-board, logistical considerations must also be taken into account according to the given mission architecture, for example the activities involved on the space journey and level of autonomy of the spacecraft itself. The practical application of such states will be discussed from the perspective of the astronaut crewmember.

27: PHYSIOLOGY FROM THE NEOTROPICS: RHYTHMS, TEMPERATURE AND SEASON

27.1

Seasonal physiology of a hibernating and facultative endothermic lizard

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In southeastern Brazil, seasons are characterized by warm and wet spring/summers and mild and dry winters, with small changes in photoperiod. In this scenario, the tegu lizard *Salvator merianae* shows a robust annual cycle of intense daily activity/foraging during spring/summer and hibernation during winter, which starts as early as in the first year of post-hatch life. During autumn, lizards gradually decrease feed intake until eventually hibernate, maintaining a reduced metabolic rate without food ingestion for about 3 months. Important to highlight is that this seasonal metabolic cycle is a temperature-independent phenomenon. In addition, when adults, they show a capacity for increasing the temperature difference between core body and ambient during the reproductive season, in spring, just after hibernation. This presentation will be focused on seasonal variation of metabolism, behavior, cardiorespiratory parameters and hormones involved in energy metabolism and reproduction in this unique species of lizard inhabiting tropical and subtropical biomes in South America. Financial support: FAPESP and CNPq (Brazil) and Northern Arizona Univ. (NAU-USA).

27.2

Orexin in ectotherms: modulatory role on ventilation

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Orexins (or hypocretins) are neuropeptides that are involved in regulating many physiological functions such as sleep, feeding, temperature, energy balance, and breathing. Interestingly, the amino acid sequences of orexins A and B are highly conserved across different groups of vertebrates and the distribution of these neuropeptides has been described in all classes of vertebrates. In most groups, orexinergic neurons are located in the hypothalamus, but instead of being found exclusively within a single nucleus, they are located in various hypothalamic nuclei. Despite being located in a restricted spot, these neurons project widely to the brain including areas involved in respiratory regulation and this modulation is dependent on the phase (light or dark) and, in mammals, is also dependent on the state of activity of the animal (sleep and wakefulness). Additionally, these neurons are considered to be intrinsically CO_2/pH sensitive. This presentation will discuss the orexinergic modulation on breathing in vertebrates, giving emphasis in our recent findings on neotropic amphibian and reptilian species. Financial support: FAPESP and CNPq

27.3

Temperature effects on cardiorespiratory function in amphibians and the aplication of a non-invasive methodology

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Brazil has the highest biodiversity of amphibians in the world with 1,080 species in three orders, including 1,039 anurans, 36 caecilians and 5 species of salamanders. Such diversity of amphibian species is mainly attributed to Brazil's large land mass, in addition to the number of tropical and subtropical ecosystems with distinct climate features. Temperature has profound effects on physiology of ectothermic animals. The respiratory and cardiovascular systems are tightly coupled to maintain adequate oxygen delivery to metabolically active tissues, and cardio-respiratory adjustments are always required whenever metabolic demands change. It is well established that acute exposure to increasing temperature elevates resting metabolic rate and heart rate exponentially in adult anurans (10-35°C, Q_{10} ~ 2.0). In contrast, studies on metabolic needs and cardiovascular performance of larval stages of amphibians, especially against a temperature challenge are very scarce. Cardiorespiratory studies in tadpoles are complicated by methodological issues such as implantation and maintenance of electrodes, cannulae, *etc*. especially in the case of long-term measurements.

We introduce a non-invasive methodology that can contribute to long-term studies of cardiorespiratory performance, and its respective temperature influences, in *in vivo* larval stages of aquatic anuran amphibians. These studies are essential to our understanding of the effects of temperature on cardio-respiratory physiology during a particularly vulnerable life stage.

27.4

Plasticity of 24h body temperature rhythms in a South American subterranean rodent

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Plasticity in the expression of 24h rhythms has been a subject of increasing interest. This plasticity can involve a change in the time of day a certain behavioral or physiological process occurs (such as a change from nocturnal to diurnal) or a change in the amplitude of rhythms, such as in the daily body temperature rhythm. The subterranean rodent tuco-tuco (*Ctenomys* aff. *knighti*) is among the species which display rhythmic plasticity. This animal is nocturnal under standard lab conditions but mostly diurnal in the field. It also shows drastic changes in amplitude of the body temperature rhythm when in the field, with changes in maximum, minimum and mean body temperature values. This plasticity was revealed by several years of data collection on body temperature rhythms of tuco-tucos in the laboratory and in outdoor enclosures. The differences between the rhythms observed in the laboratory and in the field suggest an interaction of the circadian system with a wide set of biotic and abiotic factors that differ between these two conditions. A lot of these variables are related to daily energetic challenges, which are much greater in the field than in the lab. This herbivorous rodent forage by intense digging, in a semi-arid habitat where vegetation is sparse and there are great daily changes in environmental temperature. Noteworthy, food availability and temperature have been changing in the last years, in their natural habitat in Argentina, especially with the increase in rainfall. Increase in the plasticity of rhythmic patterns have been registered along these years and investigation of this phenomenon may provide insights into the role of environmental changes on timing of activity and physiological functions.

28: POSTER SESSION 2: EVEN NUMBERED POSTER PRESENTATIONS

28.1

On the Dynamics of Actomyosin Binding

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Animal skeletal muscle exhibits very interesting behavior at near-stall forces (when the muscle is loaded so strongly that it can barely contract). Near this physical limit, the actin-myosin cross bridges do more work than their energy releasing molecules, Adenosine TriPhosphate (ATP) suggest they can. It has been shown that the advantageous utilization of thermal agitation is a likely source for this increased capacity. Here, we propose a spatial-temporal mechanical model to illustrate how this may occur. The physical interpretation of the system energy also informs how Brownian motion and the probability of actomyosin binding are related, granting a greater physical understanding for binding rate functions that are prevalent in muscle contractile theories.

28.2

Effect of Substrate Compliance on Coordinated Landing in *Rhinella marina*.

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The musculoskeletal system must dissipate mechanical energy to decelerate the body during common modes of terrestrial locomotion like running downhill, decelerating or landing from a jump. The ability to safely dissipate energy upon impact is influenced by the mechanical properties of the environment which can vary greatly in nature. Substrate compliance is one property that may affect the performance of animals as they attempt to safely decelerate their body upon impact. *Rhinella marina* offers an ideal model to understand how substrate stiffness may modulate controlled deceleration behavior as hopping is their main form of locomotion and well-coordinated landings have been well documented. In this study we use high-speed videography and force-plate ergometry to compare landing performance of *R. marina* (n = 5) across four compliance treatments relative to body weight (BW); 0, 2.5, 5 and 10 mm BW^{-1}. Landing performance was normalized and assessed by determining the ratio of energy dissipated by the forelimbs relative to the total energy of the system – animal's center of mass and substrate. We also use inverse dynamics analysis to determine how the energy dissipated by the forelimb is distributed across different joints and how such patterns change with substrate dynamics. Our findings suggest the increased compliance decreases the relative amount of

energy that has to dissipated by the forelimbs during landing. This work will provide fundamental insight into the relationship between the properties of the substrate and the energetics and control rapid deceleration during terrestrial locomotion.

28.3

The interaction of incompressible fluid and extracellular connective tissues in lobster muscle

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Vertebrate skeletal muscle is reinforced by a complex network of fibrous collagen, comprising the endomysial, perimysial, and epimysial layers of intramuscular connective tissue. The physical interaction of these connective tissues with the incompressible muscle fibers they surround has been shown to influence passive tension generated by stretched muscle (1,2), and may also influence active force generated during contraction. Though this interaction is mechanically important in the muscles of vertebrates, its relevance in the muscles of non-vertebrate animals has received relatively little study.

Using a combination of scanning electron microscopy (SEM) and mechanical testing of muscle, we investigated the interaction of connective tissues and incompressible muscle fibers in the claw closer muscle of the American lobster (*Homarus americanus*). Cross sections of claw closer muscle were visualized using SEM and compared to skeletal muscles of three vertebrates: bullfrogs, mice, and alligators. In separate experiments, passive tension was measured from lobster claw closer muscles before and after a perturbation of intramuscular fluid volume. In vertebrates, perturbations of fluid volume have been shown to influence passive tension by altering the interaction of muscle fibers and connective tissues (2).

Micrographs of lobster claw closer muscle differed from those of vertebrates in that fibrous connections between adjacent muscle fibers were relatively scarce. Distinctions between endomysial, perimysial, and epimysial layers of connective tissue were not apparent. Contrary to its effect on vertebrate muscle, altering the fluid volume of claw closer muscle did not alter passive tension generated in response to stretch.

Together, these results suggest that interactions between incompressible muscle fibers and intramuscular connective tissues may not be mechanically relevant in the claw closer muscles of lobsters, and that connective tissue may play a lesser mechanical role in the claw closer muscles than in typical vertebrate skeletal muscles. These results suggest that the mechanics of the extracellular matrix are not uniform across all skeletal muscle, and raises questions about the function and evolutionary history of connective tissue in vertebrate muscle.

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28.4

Effects of passive integrated transponder tagging on cortisol release by the Gulf killifish *Fundulus grandis*

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Passive integrated transponder (PIT) tagging has many advantages as a method for individual identification of animals in field and laboratory studies. One potential drawback, however, is the stress that results from implantation of PIT tags, especially in smaller animals, which could have adverse physiological effects. This study assessed the effects of PIT tagging on levels of the stress hormone cortisol in the Gulf Killifish, *Fundulus grandis*, an estuarine fish of the Gulf of Mexico. First, we optimized a non-invasive procedure for measuring cortisol excreted by individual fish into their aquarium water. Water samples were acidified to release bound cortisol, which was then concentrated by solid phase extraction prior to quantification by enzyme-linked immunoassay. The optimized procedure allowed high rates of recovery of known amounts of cortisol added to aquarium water. Then, using this procedure, cortisol was measured in water samples from fish one week before, immediately after and weekly for four weeks following PIT tagging. Within the first 2 h of tagging, cortisol release rates were dramatically elevated compared to values measured prior to tagging. This immediate cortisol release was significantly higher than that of fish handled identically except not implanted with PIT tags. By one week after PIT tagging, however, cortisol release returned to control levels. The results suggest that PIT tagging causes an immediate stress response, which subsides within one week. Therefore, individuals should be allowed to recover one week after PIT tagging prior to other experimental manipulations. Funding for this work was provided by the Greater New Orleans Foundation and the Audubon Nature Institute.

28.5

On the role of the visual and vestibular systems in stabilising perching in zebra finches

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Birds have outstanding abilities to balance on two legs and maintain upright posture on both rigid and highly flexible moving perches. However, to our knowledge, there has been no formal study investigating which sensory modalities are used to control upright posture in birds and, more importantly, how these are combined. From observation, we hypothesized that birds use a combination of visual, vestibular and proprioceptive information to balance on a perch. We used a perch torque sensor to measure the torques and forces exerted on the perch under different circumstances to explore this question.

To test the degree to which vision contributes to balancing ability, we compared perching ability in the light and dark. Zebra finches (*Taeniopygia guttata*) were assessed for their ability to perch using high speed infrared videography and perch torque measurements in light and dark conditions over several weeks. In the dark perch torque root-mean-squared (RMS) deviation from 0 was higher in light conditions ($p=0.003$), suggesting that perching ability was impaired in the dark. We then assessed the role of the vestibular system in maintaining upright posture while perched by damaging vestibular hair cells with amino glycoside antibiotics, and measuring perching ability in the light and dark over a period of 8 weeks, over which loss of vestibular function and consequent recovery as inner ear hair cells regenerated was recorded. Again, we found root-mean-squared deviation from 0 was higher in light conditions than in the dark, but not significantly so ($p=0.06$). This was attributed to birds being overall less active after treatment, as reflected both in the videography and RMS data ($p=0.01$), which was especially evident in light conditions.

28.6

Research of development of adaptive processes to psycho-emotinal stress in medical students

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Psycho-emotional stress that develops in dynamics of studying in Univ. is a common factor of development of the vast group of neurogenic diseases.

The objective of the research was to study the intersystem relations (integration) which are the base of development of organism`s resistance to the effects of stress factors, i.e. the development of adaptive syndrome.

The object of the research was a group of medical students of 1-3 years which were examined in conditions of educational process. The peculiarities of intersystem integration of cardiorespiratory system based on coupling index (CI), and integrational brain activity based on intellectual workability in conditions of correction test (2 minutes of test, total percentage of completed work, number of mistakes) were studied.

The research of complex of psychophysiological indicators in the dynamics of studying revealed the formation of chronic emotional stress in medical students, the severity of which depends on the individual characteristics of the psychophysiological status of students.

Formation of adaptation to the educational stress has the stage character (3stages) – the initial period is accompanied by activation of non-specific adaptive mechanisms in almost all students (1^{st} stage). From the 2nd year, only in 40% of the students an adequate adaptation to the training load was formed – intellectual performance increases, with the background of rationalization of its vegetative support. Sustainable intersystem relationship that reveals increasing the body's integrity (2^{nd} stage) is formed.

In second group of students (43%) non-specific adaptive mechanisms (1^{st} stage) on the 2nd year of study goes into a stage of specific adaptation (2^{nd} stage), but the severity of changes in psychophysiological indexes is strongly pronounced. On the 3rd year of study it leads to the depletion of adaptive capabilities (3^{rd} stage).

In 17% of students it was seen that the stage of activation of non-specific adaptive mechanisms rapidly changed by their depletion, that is accompanied by decrease of intersystem integration and intellectual workability. This is the evidence of prevalence of disintegrational processes in the organism.

Based on results of the research, a conclusion can be made that the formation of adaptive optimum to psycho-emotional stress occurs only in 40% of students. The majority (60%) of students show either the initially insufficient adaptive capabilities, or their excessive intensity, that naturally leads to psychological and vegetative disorders in the organism.

28.7

A comparision of thermal performance among latitudinally separated populations of the intertidal barnacle *Balanus glandula*

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Intertidal species must adapt to life in a biphasic environment, which alternates daily between marine and terrestrial conditions. These two environments present

very different thermal regimes, with water temperatures generally being more stable and less variable than what animals experience in air. Few studies have measured the energetic cost of exposure to thermal extremes during emersion. Moreover, it is not known how the water temperatures experienced during immersion influence thermal tolerance in air. We measured the energetic costs of low tide exposure in the intertidal barnacle *Balanus glandula*, collected from three populations along the west coast of the United States with distinct thermal environments. Barnacles were collected from southern California (a site with both warm air and water temperatures), northern California (cold air and water temperatures), and northern Washington (warm air and cold water temperatures). All animals were acclimated to a laboratory tidal cycle with native water temperatures and a common low tide temperature of 18 °C. To measure performance, we exposed barnacles from each population to a 5-hour low tide at 10, 15, 20, 25, 30, 35, or 38 °C, followed by a 6-hr high tide. We monitored oxygen consumption throughout the full tidal cycle using a fluorometric O2 sensor system. Overall, aerial respiration was greater than aquatic respiration. This is somewhat surprising for a species that lacks lungs, but it likely reflects the greater concentration of oxygen in air. However we also found evidence for oxygen debts at temperatures over 20 °C, in the form of a spike on oxygen consumption upon immersion. The pattern of total oxygen consumption over the 11 hours generally matched a thermal performance curve with a different thermal optimum for each population. Our results suggest that immersion, rather than emersion conditions, are the driving factor in thermal evolution.

28.8

Active and passive energetics of thermoregulation from thermoconformity to partial thermoregulation.

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Physiological and behavioral mechanisms controlling thermoregulation in ectotherms are not just limited by trade-offs such as missed opportunities and mortality, but by the energy allocated to regulating temperature through processes like cardiovascular adjustments or locomotion. Thermoregulation is a spectrum with varying degrees of regulation ranging from strict thermoconformity to precise thermoregulation. For instance, some species will use cardiovascular adjustments to increase or decrease the rate of heat exchange with the environment for various reasons from survival to performance and to lower daily energy expenditure. Many studies focus on the temperature difference between the animal and the environment as incurring implied energetic costs, but with advanced biologging technology we are able to monitor the fine-scale physiological processes of ectotherms under natural conditions, independent of patterns and processes observed in the laboratory. The goal of our study was to compare the energetics of two contrasting thermoregulatory strategies: thermoconformity and an intermediate, partial thermoregulation, using ECG and temperature biologging devices. We then focused on the energetics related to active and passive heat transfer using an a priori framework. We were interested in changes in body temperature, oxygen pulse in the lab and field, and daily energetics of each species' thermoregulatory capability relative to active and passive heat exchange. We monitored Midland-painted turtles (Chrysemys picta) as our partial thermoregulator in 2017 and Eastern box turtles (Terrapene carolina) as our strict thermoconformer in 2014 and 2015 using similar equipment and protocols in Southwest Ohio. Metabolic rates were calibrated under laboratory conditions using body temperature, heart rate, and body mass and proxies for oxygen consumption. We found that thermoconformers used approximately 25% of the total daily energy than partial thermoregulators. Although we found no significant difference within species throughout their active season, we found a significant difference in the daily energy expenditure between box turtles and painted turtles and time of day within each species. Oxygen pulse was similar to previously reported turtle species, and in the field the average oxygen pulse decreased from the beginning to the middle of the active season for painted turtles but increased for box turtles. Our study shows the energetics of thermoconformity and partial thermoregulation in two turtle species and quantifies a significant difference in the daily energy expenditure using heart rate and body temperature as more accurate proxies for oxygen consumption, and begins to disentangle the relationship between cardiovascular function and active or passive heat exchange.

28.9

Clocks and meals keep mice from being cool

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Daily torpor is used by small mammals to reduce daily energy expenditure in response to energetic challenges. Optimizing the timing of daily torpor allows mammals to maximize its energetic benefits and, accordingly, torpor typically occurs in the late night and early morning in most species. The regulatory mechanisms underlying such temporal regulation have however not been elucidated. Direct control by the circadian clock and indirect control through the timing of food intake have both been suggested as possible mechanisms. Here,

feeding cycles outside of the circadian range and brain-specific mutations of circadian clock genes (*Vgat-Cre*$^{+}$ *CK1δ*$^{fl/fl}$ *ε*$^{fl/+}$*; Vgat-Cre*$^{+}$ *Bmal1*$^{fl/fl}$) were used to separate the roles of the circadian clock and food timing in controlling the timing of daily torpor in mice. These experiments revealed that the timing of daily torpor is transiently inhibited by feeding, while the circadian clock is the major determinant of the timing of torpor. Torpor never occurred during the early part of the circadian active phase, but is preferentially initiated late in the subjective night. Food intake disrupted torpor in the first 4-6 h after feeding by preventing or interrupting torpor bouts. Following interruption, re-initiation of torpor was unlikely until after the next circadian active phase. Overall, these results demonstrate that feeding transiently inhibits torpor while the central circadian clock gates the timing of daily torpor in response to energetic challenges by restricting the initiation of torpor to a specific circadian phase.

28.10

Expression of TRP channels in notothenioid fish

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The Southern Ocean connects all the world's oceans and yet is highly isolated due to the Antarctic Circumpolar Current, the benthic topography, and the thermal environment. This isolation, combined with the strong selective drive of the cold stenothermic environment, has enabled the broad niche diversification of a single fish clade: the notothenioids. While notothenioids are well adapted to typical Antarctic temperature regimes between -2 and +1 degrees Celsius, their metabolic scope is highly limited above 2 degrees C and temperatures above 5 degrees C can be lethal. Given this high thermal sensitivity we wondered about the presence and abundance of molecular thermosensors in these fish. The candidate molecular thermosensors in both vertebrates and invertebrates are a class of cation channels called transient receptor potential (TRP) channels. We investigated the expression of TRP channels in four species of notothenioids: *Harpagifer antarcticus*, *Neopagetopsis ionah*, *Parachaenichthys charcoti,* and *Parachaenichthys georgianus*, and compared them with *Notothenia coriiceps*, a notothenioid for which the complete genome is available. We found the genome contained approximately 15 TRP channels, similar to other teleost fish and approximately half that of mammals. Of those, the notothenioids expressed between five and ten of the channels. All four species with transcriptome data expressed TRPV1, TRPM5, TRPM2, and TRPC1. Channels in the TRPC subclass are not thought to be thermosensitive. This leaves three channels as candidates for determining thermal sensitivity in these fish, and the range of temperatures that might activate these channels remains to be determined. Further study of these channels may help us gain insight into how these fish might cope with or avoid warming waters of the Southern Ocean, which in some places are expected to warm 0.5 degrees C per decade.

28.11

Investigating Changes in Thermal Physiology in Response to a Gut Infection in the Dragonfly, *Libellula pulchella*

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A key goal in thermal physiology is to understand the factors that induce physiological thermal plasticity and their underlying mechanisms. Recently, insulin signaling and disruptions to metabolic homeostasis have been demonstrated to impact thermoregulatory strategies and thermal performance regimes in a number of species. Here we use an endothermic dragonfly, the 12-spotted skimmer, *Libellula pulchella*, as a model to test how a disruption of insulin signaling affects thermal physiology at organismal and molecular levels. *L. pulchella* often harbor a protozoan gut parasite which disrupts insulin signaling, changes flight behavior, and induces a pathology similar to mammalian type 2 diabetes and obesity. One unresolved question regarding this host-parasite interaction is whether this infection produces a thermally plastic phenotype. Here we test if infected *L. pulchella* differentially regulate their thoracic temperature in the field, and whether thermal reaction norms of flight muscle force production differ between infected and healthy individuals. Lastly, we examine the effects of infection on the thermal performance of key carbohydrate and fatty acid enzymes in an attempt to test potential metabolic correlates of thermal plasticity.

28.12

Stage-specific oxygen limitation of thermal tolerance in *Schistocerca cancellata*

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Performance decreases drastically as body temperature increases above a thermal optimum, yet the proximate cause of this heat stress is unknown. One controversial hypothesis, known as oxygen- and capacity- limited thermal tolerance, suggests that metabolic demand during warming outstrips the energy supplied by aerobic respiration, decreasing performance until death. Therefore, reducing oxygen availability should make animals more susceptible to heat stress. This idea has mixed support in adult insects, but younger life-stages have less developed tracheal systems that may make them more susceptible to oxygen limitation. We tested this hypothesis by rearing South American locusts

(*Schistocerca cancellata)* from hatchling to adult in four temperature and oxygen environments: cold-hypoxia (28±7 °C and 13% oxygen), cold-normoxia (28±7 °C and 21% oxygen), hot-hypoxia (35±7 °C and 13% oxygen), and hot-normoxia (35±7 °C and 21% oxygen). Survival to adulthood was only affected by temperature, with locusts reared in hot environments exhibiting decreased survival (21%) compared to those reared in cold (43%). However, early-instar survival was significantly decreased by hypoxia at high temperatures. In the hot-hypoxia treatment, 66% of locusts survived to the 2nd instar, but more than 85% of locusts in all other treatments survived to the same stage. This stage-specific interaction between heat and hypoxia suggests that the mechanism of heat stress changes as organisms grow and develop. These fundamental changes to an organism's physiology should be considered when predicting how species will respond to climate change.

28.13

The effect of salinity on expression of aquaporins 1 and 5 in the gastric caecae of *Aedes aegypti* mosquito larvae

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The *Aedes aegypti* mosquito is a vector that can transmit viral diseases such as Zika, dengue, chikungunya and yellow fever. The larvae of this mosquito can inhabit freshwater (FW), brackish water (BW) and sewage contaminated water. Each of these habitats impose different challenges to osmoregulation and survival. The gastric caecae (GC) are digestive and putative osmoregulatory organs located at the anterior portion of the midgut that have been shown to secrete ions from hemolymph to the GC lumen. External salinity is known to alter the structure of the GC ion-transporting and resorbing/secreting cells; however, the movement of water across the GC has not been studied. Aquaporins (AQPs) are water and/or solute channels which mediate water flux across cell membranes. Here we compare the expression of *A. aegypti* AQP1 and AQP5 in the GC of larvae reared in FW and BW. Water-specific AQP1 was found on the apical membrane of the GC of both FW and BW-reared larvae and did not show changes in protein abundance in response to external salinity. However, localization of AQP1 along the apical membrane became more dispersed in BW-reared larvae compared to FW-reared larvae and coincided with the effect of salinity on ion-motive ATPases in the GC ion-transporting cells. AQP5 transports water and small solutes and was localized to the basal membrane of the GC. Whole mount and cross-sectional immunohistochemistry of the GC showed greater AQP5 immunoreactivity in BW-reared larvae compared to FW-reared larvae, however quantification of AQP5 protein abundance in the GC remained unchanged between the groups. Knock-down of AQP5 resulted in decreased survival of larvae reared in BW. This is the first study to report on the protein expression and localization of aquaporins in the GC of larval mosquitoes and results suggest that aquaporins in the GC play a role in the osmoregulatory strategies mosquitoes employ to cope with alterations in habitat salinity. Funding was provided by NSERC.

28.14

Comparative and functional analysis of Na^+/Ca^{2+} exchangers across Nematodes

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The super-family of Na^+/Ca^{2+} exchangers include transporters that exchange sodium for calcium (NCX), $Na^+/Ca^{2+}/K^+$ exchangers (NCKX) which exchange sodium for potassium and calcium, and Ca^{2+}/Cation exchangers (also called NCLX) which exchange sodium or lithium for calcium. In mammals there are three genes encoding various isoforms of NCX transporters, five NCKX genes and a single NCLX gene. Here, I will give an overview of my lab's data on the comparative genomics of Na^+/Ca^{2+} exchangers across nematode species. We have uncovered a surprising level of diversity within this super-family of exchangers across diverse nematode species. We observed several examples of gene gain and loss, but perhaps most surprisingly was the apparent absence of NCLX-type exchangers from a subset of nematode species that we sampled, these were: *Brugia malayi, Loa loa, Ascaris suum, Trichinella spiralis, Trichuris muris, Trichuris suis*, and *Trichuris trichiura*. This was most unusual considering that this cohort includes some of the most basal nematode species. This diversity suggests that mechanisms regulating calcium homeostasis vary in accordance with physiological demands of individual species. In addition to our comparative physiology data, my lab has also functionally characterized an NCLX-type exchanger in *Caenorhabditis elegans* called NCX-9. During neural circuit formation, migrating axons must interpret secreted guidance cues to facilitate proper path finding and navigation. Defects in the detection or expression of these secreted cues have been shown to perturb guidance, and this system of axon guidance via secreted cues is evolutionarily conserved across invertebrate and vertebrate species. Yet, despite the central and conserved role of secreted guidance cues in the control of neural development, the mechanism of guidance cue secretion is very poorly understood. The mammalian NCLX has been shown to regulate secretion of insulin in pancreatic β-cells, and we have found that the *C. elegans* orthologue of NCLX (called NCX-9) functions with the guidance cue UNC-129/BMP to regulate proper patterning within a motor neuron circuit during development. We also show that NCX-9 functions at the mitochondrion and is expressed in non-neuronal hypodermal cells *in vivo* in which UNC-129/BMP is also expressed. Our findings on NCX-9 are

the first description of a role for NCLX in patterning neural circuits.

28.15

Identification of the first member of the gap junction protein family in the protozoa *Trypanosoma cruzi*, the etiological agent of Chagas disease.

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Introduction: The presence of member of the gap junction protein family has been described in vertebrates (connexin and pannexin) and invertebrates (innexin), but has not been reported in unicellular organisms. The aim of this study was to identify an innexin protein and perform its characterization in *Trypanosoma cruzi* (*T. cruzi*), etiologic agent of Chagas disease.

Methodology: The search of genomic sequences, homologues of gap junction proteins were performed using TritrypBD genomic database. The topology was analyzed with PROTTER software. The innexon structural stability was performed in Modeller 9.10 and dynamic simulation carried out in Amber16 software. The dye uptake assay in epimastigote form of *T. cruzi* was used for innexon functional characterization. Probenecid (400 µM), flufenamic acid (50 µM) and heptanol (2 mM) were used for pharmacological characterization. A FITC-Dextran (~70,000 Da) was used to control for cell membrane damage.

Results: *T. cruzi* presents a homologue of innexin proteins with a length of 257 amino acids and present a highly conserved innexin motif YYQWV. The innexin three-dimensional modeling, showed that the channel model with 8 subunits is more stable than with 6 subunits modeled at 10 ns. While the channel with 6 subunits is closed, the model with 8 subunits presents a pore diameter of 10-12 Å. Moreover, it presents a gradient of electrostatic potential, being electropositive in extracellular regions and electronegative in intracellular regions. Epimastigotes showed an innexon-like activity with permeability to YOPRO-1, DAPI, ethidium bromide and Evans blue, induced by extracellular Ca^{2+}/Mg^{2+} free solution and blocked by 400 µM probenecid, 50 µM flufenamic acid or 2 mM heptanol.

Conclusions: These findings suggest the presence of homologues of connexin proteins in *T.cruzi.* This could be a new molecular target for future studies on the parasite biology.

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28.16

Trade-offs in reproduction and regeneration in *Anolis* lizards.

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Life-history theory has been used as an analytical framework to explain the variation across species in their life history strategy through traits such as birth size, growth, age of maturity, reproductive patterns, and longevity[1]. The energetic investment in regeneration—particularly of large amounts of tissues such as limbs or tails—is predicted to be negatively correlated with investment in other life history traits, such as reproduction[2,3,4]. However, the physiological mechanisms of trade-offs between reproduction and tail regeneration are unclear. Across animals, the Insulin and Insulin-like Signaling (IIS) network regulates cellular processes including pre- and post-natal growth and development, reproduction, longevity and wound healing following tail autotomy[4,5,6,7]. *Anolis* lizards that regenerate tail tissue and produce a single egg every 1-3 weeks provide an opportunity to study the life history patterns associated with tissue regeneration[4], and the role of the IIS network in regulating this trade-off. Female brown anoles (N=40) were split into two treatment groups: Control vs Tail Autotomy. Lizards were kept on a limited diet of 5 crickets weekly. Reproduction was tracked for a period of one month prior to tail autotomy and 3 months post autotomy through measures of egg number, mass, and frequency of oviposition, along with tissue generation post-autotomy. Hatchling size was measured through mass, snout vent length and tail length. Maternal tissues will be assessed for differential expression of IIS hormones and regulators between the treatments. We predict that if tissue regeneration and reproduction are negatively correlated, on a limited resource diet, reproductive females will decrease investment into eggs, slow, or halt reproduction when regenerating tail tissue. Results will be discussed along with future plans for recovery of tradeoffs through manipulation of the IIS network.

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28.17

The effect of food availability, temperature and sirtuin inhibition on the metabolic rate of California mussel gill tissue

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Recent work has demonstrated the importance of food availability in modulating the tolerance of marine organisms to several environmental stressors. Preliminary studies provide evidence that sirtuins (deacylases that respond to caloric intake and maintain cellular homeostasis) mediate the response to differences in food availability and heat stress in intertidal mussels. To investigate the role of sirtuins in mediating physiological performance under conditions of varying food availability and thermal regimes California mussel (*Mytilus californianus*) were acclimated to one of two algae food rations (high or low) and to daytime low tide periods where air temperatures were ramped to 20 or 30°C for three weeks. Post acclimation, half of the mussels in each treatment were exposed to a sirtuin inhibitor during the evening high tide period. During the subsequent low tide, all mussels were exposed to an acute aerial heat shock of 33°C during the daytime low tide period, after which mussels were returned to acclimation conditions. Prior to (baseline) and following sirtuin inhibition (experimental), metabolic rate of gill tissue was assessed by closed respirometry every 3 hours during high tide periods (i.e. 1am, 4am, 1pm, 1pm) for two consecutive tidal cycles (i.e. 4 days total). For the baseline measurements, the availability of food had a significant effect on the metabolic rate, with the high food groups having a higher metabolic rate than low food groups, and metabolic rate was highest at 4pm. Day time temperature acclimations at low tide (20 or 30°C) did not have an effect on metabolic rate. For the experimental measures, food availability significantly increased metabolic rate, similar to what was seen in baseline measurements, and exposure to a sirtuin inhibitor significantly decreased metabolic rate. Time of day and low tide temperature did not have an effect on metabolic rate. Metabolic rate is a measure of aerobic performance, specifically metabolic demand. The baseline data indicate that the degree of temperature change experienced at low tide does not affect metabolic demand during the subsequent high tide period, but that metabolic demands shift on a circatidal rhythm, with highest energy demand in the daytime high tide period 3 hours after low tide. Increased feed availability (i.e. energy supply) is met by increases in metabolic demand in both the baseline and experimental measures, supporting recent findings that food availability plays a vital role in the physiology of the intertidal mussel. Additionally, sirtuin inhibition decreased metabolic demand of mussels, supporting previous preliminary work that sirtuins are an important mediator that links cellular energy state and physiological stress in the California mussel. Taken together, the results provide evidence that sirtuins likely play an important role in modulating the metabolic demand in intertidal mussels but do not influence how mussels are affected by low tide temperature or food availability at the tissue level. This research is funded by the National Science Foundation (IOS-1557496 & IOS-1557500).

28.18

The importance of tidal acclimation in assessing the physiological responses of the intertidal crab *Carcinus maenas* to emersion

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Animals inhabiting the intertidal zone are exposed to abrupt changes in environmental conditions associated with the rise and fall of the tide. The most predictable of these changes is the transition between aquatic and aerial media. For convenience, the majority of laboratory studies on intertidal organisms have acclimated individuals to permanently submerged conditions. However, this is not representative of the daily fluctuations intertidal organisms experience in their natural habitat. We used the green crab *Carcinus maenas* to identify whether individuals acclimated to a simulated tidal regime of continuous emersion-immersion (tidal) exhibited different physiological responses compared with individuals that were held permanently submerged (non-tidal). Oxygen consumption, PaO_2, venous pH, L-lactate and hemocyanin concentration were measured in

individuals from both acclimation groups subjected to 6h-6h-6h cycle of immersion, emersion, and re-immersion. Both tidal and non-tidal crabs exhibited a 50% decline in oxygen consumption during the 6h emersion period but experienced no change in PaO_2. This was associated with a concomitant decline in venous pH and increase in L-lactate in non-tidal crabs, whereas these remained unchanged in tidal crabs. Pre-emersion oxygen consumption rates were rapidly regained in both groups when the crabs were re-immersed, as were pH and L-lactate levels in non-tidal crabs. Tidal crabs maintained higher hemocyanin concentrations than non-tidal crabs throughout the experimental regime. These results suggest acclimation of *C. maenas* to submerged conditions results in a loss of important physiological mechanisms to tolerate emersion. Hemocyanin plays a crucial role in oxygen transport and acid base homeostasis during emersion in *C. maenas* and the elevated levels in tidal crabs are the likely driver behind the physiological differences between the two groups. The results of this study show that caution must be taken when acclimating intertidal organisms to laboratory conditions, as it risks abolishing important physiological responses that play a critical role in the physiological performance of an organism *in situ*. These results further underscore the importance of acclimating organisms to realistic ecological scenarios that incorporate a multifaceted design within the laboratory environment. This study was funded by a NSERC Discovery grant awarded to Iain J. McGaw.

28.19

Hypoxia and the metabolic phenotype in *Daphnia*.

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Low oxygen induces many animals to mount compensatory responses to improve oxygen utilization and/or anaerobic metabolism. In vertebrates, the coordination of these compensatory responses falls to the HIF pathway, which simultaneously induces expression of genes for glycolytic enzymes, glucose transporters, angiogenic factors and erythropoietin. The net result is improved vascular delivery of oxygen and glycolytic capacity. The hypoxic response in invertebrates is less studied, and expected to be different because of the role of the vasculature and the use of extracellular Hb. Daphnia are an intriguing model because they induce Hb genes in response to environmental hypoxia, causing the animal to turn red. This phenotypic plasticity is seen in lakes that undergo summer thermal stratification, with corresponding gradients in oxygen levels. Although the nature of the Hb response is well studied, it remains unknown whether this is sufficient to sustain normoxic metabolism or whether systemic hypoxia results, necessitating induction of glycolytic metabolism and/or glycolytic gene expression. The objective of this study is to explore the extent to which the Hb response is coupled to glycolytic gene induction in relation to the depth and duration of hypoxia, and whether there is any evidence of local (microevolutionary) adaptation in glycolytic gene patterns. *Daphnia* were collected from a series of lakes in the Canadian Shield, differing in the extent to which late summer stratification occurs. Animals were compared before stratification (in June) and after stratification (in September/October). In several lakes, animals that differed in Hb level (pale vs red) showed no difference in glycolytic enzymes (lactate dehydrogenase, pyruvate kinase, enolase). Other lakes showed pronounced increases in levels of both Hb and the suit of glycolytic enzymes, which for the most part increased in parallel with each other. These results suggest that in at least some conditions, Hb induction is sufficient to sustain normoxic metabolism and that there is a flexible coupling of control of Hb and glycolytic genes in response to hypoxia. Current studies integrate metabolic studies with activity patterns of the animal, assessing the potential of glycolytic metabolism and glycogen stores to sustain the animal during diurnal migrations in the water column of stratified lakes. *Supported through NSERC Discovery Grants to WN and CDM.*

28.20

Establishing an index of habitat quality and reproductive success for the Northern Fur Seal

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Northern fur seals (NFS; *Callorhinus ursinus*) support lactation using a central place foraging strategy, alternating foraging trips to sea with shore visits to nurse pups. Prey abundance and availability influence trip duration. The frequency and length of shore-stays influences the rate and amount of milk pups receive. Pup mass, influenced by these factors, is positively correlated with post-weaning survival. Overall, the NFS population on the Pribilof Islands, AK, has declined ~3.5% annually for the past two decades. However, despite continued decline on St Paul Island (SNP), pup production increased ~27% on St George Island (STG) between 2012 – 2016. Maternal females forage in colony-specific areas at sea, each characterized by a distinct oceanographic environment that may influence prey availability and explain the inter-island variation in pup production. We hypothesized that the variability in maternal foraging trip durations (MFTD) could provide an index of offshore habitat quality and explain known differences in pup mass and post-weaning survival; the objective was therefore to identify correlative relationships between colony-averaged MFTD and pup mass between seasons.

Between 2010-2017, the attendance patterns of 239 maternal females were monitored throughout the lactation period using VHF radio transmitters at six Pribilof Island colonies. Additionally, inter-annual variability in the relationship between MFTD and pup mass was assessed for females at Polovina Cliffs, a SNP colony. Likelihood ratio tests identified factors influencing the variability observed in the colony-averaged MFTD (Colony, Year, & Julian Day; P <<< 0.01). Colony average MFTD were longer for SNP females than STG (P <<< 0.01). For Polovina Cliffs, there was a negative correlation between average MFTD and average female pup mass (P = 0.03, r^2 = 0.59), whereby pups lost 0.51kg mass per day mom spent foraging. Regional and temporal variability in colony average MFTD was consistent with trends in oceanographic environment, pup mass, and pup production. Record-breaking warm ocean temperatures, as seen in 2016, corresponded to decreased biomass of important NFS prey species. Subsequently, MFTD increased and pup mass decreased. Longer average SNP MTFD corresponded to the observed decrease in pup production. The interannual and regional correlations between colony-averaged MFTD and pup mass suggests that population-level metrics can be used as a sensitive indicator of habitat quality and reproductive success.

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28.21

Hypoxia induces differential changes in thermoregulation and metabolic rate base on body size in the bumblebee *Bombus impatiens*

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Facultative endothermy is utilized in bumblebees to warm up flight muscles in preparation of flight and also to maintain hive warmth. *Bombus impatiens* is a species of bumblebees that divides labor based on size of the bee. The larger bees are more likely to forage and when in the hive are found at the edges of the colony, while the smaller bees are found in the center of the hive and are more likely to incubate and feed larvae (Jandt & Dornhaus, 2009). In another colonial bee (*Apis mellifera),* oxygen concentrations vary within the hive and have been measure during winter hibernation as low as 10% (Nerum & Buelens, 1997). We Investigated whether bees of differing sizes react differently to several levels of hypoxia using two different regimes of oxygen change. One starting with low oxygen concentration of 2.5% then increasing from 7.5, 10, 15, to 21% and one that started high at 21% and gradually decreased down to 2.5%. We measured thorax temperature using an infrared camera and simultaneously measured metabolic rate using flow-through respirometry. Our results suggest that larger bees when challenged with hypoxia are more likely to decrease thorax temperature and therefore metabolic rate than their smaller counterparts. In addition, larger bees required a higher metabolic rate than smaller bees to maintain the same thorax temperature and overall smaller bees maintained higher thorax temperatures despite having a higher surface area to volume ratio. This indicates that larger bees, those that are more likely to forage, have higher basal metabolic rates and do not thermoregulate as well at low oxygen levels. While the smaller bees increase temperature in lower oxygen environments.

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28.22

Does individual variation in heat loss influence thyroid and metabolic responses to cold?

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Triiodothyronin hormone (T3) is known to play a role in thermoregulation and heat production in birds and is typically higher in cold environments. Additionally, T3 has been shown to correlate positively with metabolic rate; however, these relationships are typically noisy, with a large amount of variation around the mean. One potential cause for this variation is individual-level differences in insulation and consequently heat loss (conductance). For a given temperature, birds losing more heat would perceive their environment as colder and might therefore show a stronger physiological response; leading to the prediction that thermal conductance should correlate positively with T3 levels and metabolic rate. Here, we exposed indoor captive red knots (*Calidris canutus islandica*) to sequential decreases in temperature (15°C to -10°C; by -5°C) while measuring their oxygen consumption to calculate minimal thermal conductance. We also measured T3 blood levels before and after metabolic rate measurements with the expectation that birds with the highest heat loss would show higher T3 levels and higher metabolic responses. Although we found that T3 increased with

the decline in temperature, as expected, our results only provide weak support for the effect of individual heat exchange on T3 and metabolic response. Potential explanations for the correlation between thyroid hormone, metabolic responses and heat conductance will be discussed.

28.23

Does the risk of overheating limit maximum rates of energy expenditure in breeding birds?

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During breeding, adult birds may spend many weeks feeding their chicks, resulting in a sustained energy expenditure of ~4x the parent's resting levels. But why don't parents work even harder, especially if there are presumed fitness benefits to raising more nestlings? The heat dissipation limit theory of Speakman and Król (2010), proposed that maximum sustained rates of energy expenditure are constrained by an individual's capacity to dissipate metabolic heat. To test this, we studied breeding tree swallows (*Tachycineta bicolor*), which forage for up to 15 hours per day gathering insects to feed nestlings. We predicted that if an individual's capacity for energy expenditure is limited by its ability to dissipate body heat, then individuals with an increased capacity to dissipate heat will feed their nestlings at higher rates. To increase the capacity for heat dissipation we experimentally increased the size of the brood patch in females, by trimming the overlying feathers. We also implanted small temperature-sensitive tags, which allowed us to monitor body temperature and feeding rates remotely. Our preliminary analyses suggest that risk of overheating does in fact limit parental performance. Because the risk of overheating may increase with climate warming, such risks may exacerbate the ongoing population declines of many small birds. Funding: Trent Univ.; Natural Sciences and Engineering Research Council (Canada).

28.24

Evidence for the influence of triiodothyronin on maximal heat production in birds

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Triiodothyronin (T3) is considered to be a regulatory hormone of thermoregulation in mammals and birds. Indeed, low ambient temperatures elicit both heat production and higher T3; which has been linked to non-shivering thermogenesis. T3 has also been shown to be correlated with basal heat production (BMR) in birds. However, whether T3 plays a significant role in avian maximal heat production and cold endurance remains unknown (i.e. positively correlates with). Here, we studied indoor captive red knots (*Calidris canutus islandica*) maintained under three thermal treatments over a complete annual cycle. Each month we measured variation in body mass, basal metabolic rate (BMR), maximal thermogenic capacity (M_{sum}), and thyroid hormones: triiodothyronin (T3) and thyroxine (T4); and subsequently investigated correlated variation. T3 levels were positively correlated with BMR across seasons and treatments, confirming previous observations. T3 also correlated positively with M_{sum}, thus suggesting a potential influence of tissue heat production independent from shivering. Our results support recent findings showing improvement of maximal thermogenic capacity independent from muscle size variation in birds.

28.25

Withdrawn

28.26

Depressing mitochondrial function during paradoxical anaerobism leads to an alcoholic fish

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Skeletal muscle mitochondria were isolated from desert pupfish (Cyprinodon spp) that were acclimated to ecologically relevant temperatures of 28 °C (M28) and 33 °C (M33). The respiratory control ratio (RCR) of M33 pupfish was ~2.6X lower than that of M28 pupfish, initially suggesting mitochondria were damaged by the warmer temperature. However, state 4 respiration was below expected values in both M28 and M33 pupfish suggesting little proton leak. Further, membrane potential was lower in the M33 pupfish than M28 pupfish and did not change with assay temperature. These data suggested a reduced proton motive force in M33 pupfish. Analyses of respiration as a function of membrane potential suggest a block in substrate utilization in M33 pupfish. M33 pupfish also experience reduced production of reactive oxygen species (ROS) from the Q site of complex I. Taken all together, the data suggest pupfish may be limiting mitochondrial use to avoid ROS production by restricting access of substrates to the mitochondrion. These results are consistent with our measurements of oxygen consumption in intact fish. Specifically, fish acclimated to 33 °C demonstrate extended periods of paradoxical anaerobism in which

oxygen consumption is absent despite the presence of oxygen. These fish produce ethanol as an alternative end product of metabolism and key indicators such as alpha smooth muscle actin in the liver and cytochrome p4502E1 analogue are consistent with an alcoholic lifestyle.

28.27

The impact of developmental hypoxia on the cardiovascular chemoreflex in embryonic snapping turtles (*Chelydra Serpentina*).

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Developmental hypoxia is a common challenge in developing reptiles which can have lasting effects on the functional phenotype of the embryo and hatchling. Prior studies have shown several aspects of cardiovascular function are altered in reptile embryos by chronic hypoxic incubation. In this study we expanded on our prior studies to investigate the impact of chronic developmental hypoxia on the cardiovascular limb of pharmacologically induced activation of a chemoreflex in developing snapping turtle (*Chelydra serpentina*) embryos. We measured arterial blood pressure and heart rate in embryos incubated in 21%O_2 and 10%O_2 at 70 and 90% of incubation. We hypothesized that hypoxic incubation would blunt the reflexive response to phenylbiguanide (PBG) and sodium cyanide (NaCN) late in incubation due to withdrawal of vagal input on the heart. Chronic hypoxic incubation produced smaller embryos that were hypotensive at 90% of incubation, similar to previous studies. NaCN (0.1, 1, 10mg kg^{-1}) and PBG (800ug kg^{-1}) produced a hypotensive bradycardia, similar to the cardiovascular response produced during acute hypoxia in both incubation conditions and time points previously observed. The cardiovascular response was attenuated with hexamethonium in response to PBG, while atropine completely abolished the response to PBG and reduced the response to NaCN, suggesting that the cardiovascular reflex is mediated through the vagus nerve. This study has confirmed the presence of the cardiovascular limb of a chemoreflex in embryonic snapping turtles and that it is mediated through the vagus nerve acting on cardiac tissue.

28.28

Scaling of Major Organs in Hatchling Female American Alligators (*Alligator mississippiensis*)

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Large non-avian and avian reptile species grow most rapidly during their early life history, with 'clutch' effects for different parentage. The effect of body mass on the relative growth, metabolism, organ size and morphological/anatomical features of animals is an active and long-studied area of biology, ecology and comparative animal physiology. Allometric equations represent relationships between a morphological size or physiological rate and body massas $Y=aM^b$, where Y is a size or rate, a is elevation, b is the exponent describing the shape of the line, and M is animal body mass. We measured visceral organ size in hatchling female alligators up to 6 months-old from 5 clutches at 8-10 timepoints during their first order of magnitude of growth (~45 g to ~500 g wet body mass). We tracked each individual alligator (N=86) from original egg/clutch identity. Clutches differed in initial egg mass, initial hatchling wet mass with yolk, snout-vent length (SVL) and head length (HL). The interaction between initial egg mass and clutch identity was significant for initial hatchling wet mass, but only egg mass, not clutch, had a significant effect on initial SVL and HL. Kidney and liver mass showed biphasic scaling with body mass for both wet and dry values, a 'breakpoint' of ~100 g wet body mass. Kidney and liver wet mass showed slopes b>1.0 as animals increased in wet body mass from ~45 g to ~100 g; kidney and liver wet mass slopes were significantly lower b~0.8-0.9 for larger animals >100g. Lung and heart wet mass did not show biphasic scaling with body mass and b~0.8-0.9. Within kidney and liver mass, below ('small' alligators) and above ('larger' alligators) the breakpoint (~100 g body mass), wet or dry organ mass slopes tended to be similar between clutches. Within lung and heart wet or dry mass between clutches, all clutches had statistically identical slopes. Combined clutch data for wet mass showed distinct regressions with slopes >1.4 for small alligators' kidney and liver mass, compared to larger alligators' kidney and liver mass slopes as well as all alligators' lung and heart mass slopes. The slope for wet heart mass was larger than other slopes, except wet liver mass for larger alligators. Overall, there was variation in size of hatchling alligators due to egg size and clutch effects (genetic effects); however, for a given whole animal size, the relative organ mass of alligators was reasonably similar across clutches through the first order of magnitude of rapid growth. Hatchling alligators appear to undergo a very early period of rapid kidney and liver growth following hatching, with higher rates than lung or heart tissue. Clutch, egg mass, and hatchling size influence organ size, and each factor should be accounted for in future studies exploring reptile morphology and physiology in order to assess environmental *versus* clutch/genetic contributions. This study strongly indicates that given common environmental conditions and diet, alligators dedicate similar energetic resources to visceral organ

growth, regardless of clutch, and that variability in crocodilian organ size may be driven primarily by environmental variation or ecological conditions.

28.29

Effects of Δ9-tetrahydrocannabniol (THC) on Zebrafish Embryo Development

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Δ9-tetrahydrocannabinol is the psychoactive constituent derived from the angiosperm *Cannabis sativa*, and found in the commonly used recreational drug marijuana. Now being examined for its medicinal purposes, *C. sativa* has become a relevant topic for political and social debate due to its ability to trigger the endocannabinoid system, resulting in powerful therapeutic effects like anti-emesis, anti-anxiety and anti-spasticity, but also remaining controversial becauses of its accompanying THC-induced hallucinogenic effects. While research involving the physiological actions of THC have been conducted, very few studies have investigated the deleterious effects on early embryological development. As a preliminary component of my thesis research regarding the reestablishment of balanced excitatory and inhibitory signaling in a muscle spasticity mutant, I examine the developmental effects of THC on the developing zebrafish from 2 hours post fertilization to five days post fertilization. Somatic growth parameters (body length, maturation rates, natural chorion emergence rates and morphologic abnormalities), as well as the achievement and production of smooth, effective and fast-maturing escape behaviors indicate the phenotypic consequences of early embryonic exposure to THC.

28.30

Evolution of the Development of Respiratory Physiology in Deer Mice Native to High Altitude

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High altitude is a challenging environment characterized by unavoidable and unremitting hypoxia (low oxygen), which can constrain O_2 supply to support physiological processes. Based on studies in adults, control of breathing appears to be altered in many highland taxa to optimize O_2 uptake, but few studies have investigated the control of breathing at early developmental stages. We examined the development of the hypoxic ventilatory response (HVR) at post-natal age (P) 7, 14, 21, and 30 in animals that were born and raised in captivity, comparing populations of deer mice (*Peromyscus maniculatus*) native to high altitude to populations of deer mice and white-footed mice *(P. leucopus*) native to low altitude. Breathing, arterial O_2 saturation (SaO_2), and heart rate were measured during step-wise decreases in inspired PO_2. The HVR appeared to develop between P7 and P14, as reflected by robust increases in breathing in response to hypoxia in P14 mice but not P7 mice. This was associated with significant development of the carotid bodies – the peripheral chemoreceptor that initiates the hypoxic chemoreflex – between P7 and P14. Differences in breathing pattern arose in highlanders at P30, who then breathed deeper but less frequently than lowland mice, consistent with differences we have previously observed in adults. Blood-O_2 binding affinity decreased with age, consistent with decreases in SaO_2 during hypoxia, but highlanders had consistently higher binding affinity and SaO_2 than lowlanders. Therefore, high-altitude mice express a high blood-O_2 binding affinity very early after birth, which should help safeguard arterial oxygenation, but evolved changes in the control of breathing do not become apparent until later in post-natal development.

28.31

A legged limitation on insect size? Scaling of tracheal systems in scarab beetles

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Scaling limitations on oxygen supply are often invoked to explain the small size of insects and sometimes other invertebrates. However, body size does not affect the safety margins for oxygen delivery at rest, during flight and during growth of insects, suggesting that oxygen supply matches demand across insect size. In vertebrates, oxygen transporting structures scale isometrically or hypometrically, in the latter case, matching the hypometric scaling of metabolic rate. In insects, there is some evidence for hypermetric scaling of tracheal structures, suggesting that larger insects must invest relatively more in tracheal structures in order to be large, and potentially providing upper limits on insect size. We tested this idea using 15 scarab beetle species ranging in mass from 0.05 to 31 g by using microCT to assess the scaling of the tracheal system, flight muscles, and the brain. Most of the air sacs in the body of scarab beetles form globular, grape-like clusters, appearing morphologically quite different from the air sacs of other insects. However, around the flight muscles, air sacs appear complex in shape similar to those seen in other insects. Total and thoracic air sac scaled isometrically, as did flight muscle volume, consistent with matching convection to tissue oxygen need during flight. The percent of volume occupied by air sacs was greater in the thorax than the head or abdomen, consistent with the high demands of flight. Head and brain volumes scaled hypometrically, and air sacs became an increasing proportion of the head as beetles increased in size,

consistent with the need for greater convection to supply the brain in larger beetles, or alternatively with the use of air sacs to expand head volume for other purposes. Air sac volumes scaled hypometrically in the abdomen. In contrast to all other body segments, the tracheal system of the femur (the segment of the leg containing most leg muscle) scaled strongly hypermetrically. The morphology of the femoral tracheae suggest that they are compressible and used for convective gas exchange in the leg, consistent with the hypothesis that gas exchange to larger legs requires increasingly larger relative investments in tracheal structure and function. These data support the hypothesis that insect maximal size may be partly constrained by gas exchange within the leg. Supported by NSF IOS 1122157.

28.32

The effects of a bacterial endotoxin LPS: neuromuscular junction and cardiac function in fruit fly (*Drosophila melanogaster*) and blowfly (*Phaenicia sericata*) larvae.

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Gram negative bacterial septicemia inflicts humans as well as other animals. The immunological response to bacterial infection activates cascades of defense cytokines and antibody formation. Two common culprits in mammals are *Pseudomonas aeruginosa* (*P.a.*) and *Serratia marcescens* (*S.m.*). The induced cytokines and defense response to the surface antigens on bacteria accounts for some of the immune response but also the secretion of lipopolysaccharides (LPS) is responsible for a large degree of the immune response. The direct action of bacterial LPS endotoxin was shown to enhance synaptic transmission and hyperpolarize the membrane potential at low dosage but block glutamatergic receptors and decrease observable spontaneous events at a high dosage. The dosage effects are LPS type specific. The hyperpolarization is not due to a voltage gated potassium channels or due to activation of nitric oxide synthase (NOS). Comparative effects of LPS on heart rate (HR) were examined in larvae. Acute direct exposure of in situ heart tubes with saline containing at 500 µg/ml LPS from two common bacterial strains (*P.a.* and *S.m*.) showed a dose-dependent effect on HR but different responses for the two fly models. LPS is likely altering ionic balance of the pacemaker potential by inducing a hyperpolarization of the cardiac muscle. Currently, we are investigating lower doses of LPS. The significance of these findings is to gain a better understanding of the direct mechanism of action of LPS on synaptic function and its effects on HR, induced without the effect of an immune response as occurs in intact animals. Knowing the acute and direct actions of LPS exposure on larvae in these species may aid in understanding the underlying mechanisms in other animals during septicemia. We thank Ms. Kameron Roach, Ms. Amanda Paschal, Ms. Alexandra Stanback, Mr. Jaylen Scott, Mr. Mohsin Akhtar, Mr. Jate Bernard and Ms. Nicole Audia for helping in conducting these experiments. Funding was provided by personal funds (R.L.C.), student tuition, and a "Sustaining Excellence-2014" competition grant from the Howard Hughes Medical Institute (Grant #52008116) awarded to the Univ. KY (VM Cassone, PI). The authors confirm that the HHMI funder had no influence over the study design and content.

28.33

Examination of predicted cardiac parameters based on ventricle wall thickness in the Northern bobwhite quail, *Colinus virginianus.*

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Experimental evidence of avian cardiac function for a number of species has been understandably limited due to a number of factors including animal size constraints. However, a number of predictions related to cardiac function have been put forward based on available data for this vertebrate group. In this study we directly measure ventricular pressure parameters of Northern bobwhite quail, *Colinus virginianus*in an effort to test existing models of cardiac parameters in this species. We measured right and left intraventricular pressures under general anesthesia. Heart rate, intraventricular pressures, contractility, change in pressure over time and time of relaxation were measured in adult birds. In an effort to investigate maximal pressure parameters the beta adrenergic agonist dobutamine (100 uglkg) was injected into the right ventricle. At the completion of the study cardiac mass as well as right and left ventricle wall thickness were determined. Birds maintained baseline mean intraventricular pressure of the left and right ventricles at 4.8±0.3 kPa and 1.9±0.4 kPa respectively. Peak systolic pressure was 12.3±0.4 kPa and 3.5±0.4 kPa in the left and right ventricle respectively. Diastolic pressure was 0.5±0.2 kPa and 0.9 ±0.3 kPa in the left and right ventricle respectively. Baseline heart rate was 300±16 beat $min^{-1}$and increased to 360±17 beat min^{-1}following dobutamine injections. All pressure parameters increased following dobutamine injection, with the right ventricle pressure parameters increasing to a greater degree ~40% vs ~20% compared to the left ventricle. Our findings deviate from those values that would be predicted based on an existing predicted values suggesting revision of the model is necessary.

28.34

Heart rate and angiogenesis in chicken embryos exposed to the environmental contaminant TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin)

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TCDD is a persistent pollutant that can be delivered to the mammalian fetus from the mother via placenta and breast milk and via yolk in birds. TCDD is teratogenic and cardiotoxic for chicken embryos, as evident from an enlarged heart and dilated ventricle cavities with thin walls as well as cardiac septal defect. TCDD also causes angiogenic malformations and a reduced number and size of blood vessels in chicken embryos, as well as decrease in vascular endothelial growth factor-A (VEGF-A). However, whether and when TCDD affects early cardiovascular development in the embryo is unclear. Thus, we investigated the effect of TCDD on heart function and vasculature development at the early stages of development in the chicken embryo. We used shell-less cultured embryos that allow full access to embryo and chorioallantoic membrane (CAM) development until embryonic day 5 (E5). The eggs of *Gallus gallus* were incubated until day E2, after which the whole egg content was transferred to a sterilized petri dish covered in plastic wrap. The embryos in the petri dishes were then incubated at 37.5^{o}C. Beside controls, the embryos were exposed to one topical application of 0, 0.002, 2 and 10 ng TCDD/egg on E2. Another population received repeated injections of 0 and 2 ng TCDD/egg daily (E2, E3, E4). Finally, a third population was dosed with 0, 2, 10 and 20 ng TCDD/egg before the incubation (E0) for heart rate analysis and exposed once to 0, 0.002, 2ng for angiogenesis analysis. Videos for heart rate analysis and photographs for angiogenesis analysis were made before the injection and daily at the same time until E5. Heart rate was analyzed by counting the number of heart beats in a 30 sec film and extrapolating to 1 min. Angiogenesis in the CAM was analyzed by a vascular index generated by counting the number of vessels that crossed each of several concentric circles placed 2mm distant for each other until cover all the CAM from the umbilical vessels. Heart rate in control embryos was 128.5±3.4 (E2), 154.6±2.8 (E3), 178.2±1.4 (E4) and 123.3±5.9 beats.min^{-1} (E5). Vessel density index measured on the CAM of control embryos at E5 was 3.2±0.7, 11.2±0.8, 19.4±1.3, 32.8±2.5, 49.4±5.8, 39.2±6.8, 8.6±4.7 and 0.6±0.5 vessels/concentric circle for 2-16 mm in 2 mm distance increments, respectively, from the umbilical vessels. Neither heart rate nor vascular index were significantly changed in any of the described treatments in these early stages. Despite the toxicity and cardiovascular effects of TCDD in late chicken embryos, TCDD does not affect cardiovascular form or function in early stage chick embryos. Past literature shows differences in heart rate and angiogenesis between E5 and E10. Other current experiments from our lab show the sharpest increase of mortality *in ovo* occurs from E9 to E15 when embryos were exposed to dioxins (2-50ng) at E4. This leaves for future investigation the identification of the beginning of the critical window for appearance of TCDD-induced cardiovascular malformation, which now appears to be between E5 and E15.

28.35

Baroreflex changes with body size in the Green iguana

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The baroreflex is a mechanism that continuously regulates acute changes in arterial blood pressure (P_{sys}) at every heartbeat. Mechanoreceptors (*i.e.* baroreceptors) localized at the arterial wall sense variations of P_{sys} by the displacement of the arterial wall during the cardiac cycle. The typical baroreflex response includes rapid changes in heart rate (HR) that regulates blood volume to re-stabilize P_{sys}. The magnitude of this response is denoted baroreflex gain (G_{50}) and indicates the baroreflex sensibility. Increased body size may affect vascular compliance, which is often related to a decreased G_{50}. In reptiles, which may exhibit massive increases in body mass (BM) that can reach 10,000 fold during their life span, bigger BM is often related to decreased HR and increased P_{sys}, possibly affecting G_{50} responses. To test if G_{50} is affected by increased BM, we measured P_{sys} and HR of 7 Green Iguanas (*Iguana iguana*), an arboreal lizard species, with body sizes ranging from 31 to 819g, representing a 27-fold increase in BM. Animals had their femoral artery occlusivelly cannulated and recordings were made 24h after surgery within a climatic chamber set to constant 35^{o}C. Heart rate was derived from blood pressure pulsatile signals and G_{50} was calculated using the sequence method. P_{sys} increased with BM (P_{sys}=50.5BM$^{0.11}$, R^2=0.72), whereas HR and G_{50} decreased with BM (HR=57.6BM$^{0.13}$, R^2=0.56; G_{50}=-125.4BM+188.5, R^2=0.67). Increased P_{sys} suggests perfusion pressure is greater in larger individuals which may facilitate tissue perfusion. The decreased HR suggests a lower specific rate of oxygen consumption of bigger animals, such as observed in mammals and other reptiles. The decreased G_{50} indicates that larger animals possess a decreased vascular compliance or distensibility. This higher sensitivity to regulate acute pressure changes in smaller iguanas suggests that they are better able to circumvent abrupt orthostatic challenges, such as during climbing, when compared to adult individuals, probably increasing their chances to avoid predation.

28.36

Withdrawn

28.37

Changes in the gut microbiota over the course of gestation in oviparous Eastern fence lizards (*Sceloperus undulatus*)

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In humans, pregnancy significantly alters the structure of the gut microbiome. Specifically, gut communities exhibit decreases in alpha diversity and increases in beta diversity (higher inter-individual variability) over the course of pregnancy. Here, we investigated whether similar trends occur in evolutionarily distant hosts. We collected repeated fecal samples from gestating Eastern fence lizards (*Sceloperus undulatus*), and recorded the date of egg laying to determine the time of gestation for each sample. Additionally, fecal samples were collected from non-gestating females. Bacterial inventories were conducted by sequencing the 16S rRNA gene and community profiles were determined using QIIME2. We found that over the course of gestation, lizard gut microbial communities exhibited decreases in alpha diversity (Faith's phylogenetic diversity and number of observed OTUs). Additionally, inter-individual variation was higher towards the end of gestation. The relative abundance of the candidate phylum Melainabacteria was lower in lizards towards late-gestation. The presence of Melainabacteria was detected in 60% of samples from non-gestating individuals, 70% of samples from early-gestation individuals, but less than 40% of samples from late-gestation individuals. Overall, our results are similar to previous results observed in humans, suggesting similar interactions between gestation and the gut microbiome in these disparate lineages. We hypothesize that hormonal, immunological, or metabolic changes associated with gestation may underlie these community shifts. Further studies should investigate the functional effects of these altered communities over gestation in varied animal groups, including humans.

28.38

Comparative analyses of gene expression in snakes yields insight into conserved mechanisms underlying intestinal regeneration

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Some species of snakes possess feeding ecologies that involve extremely long periods of fasting between meals. Rather than maintaining an energetically costly digestive system, these snakes have evolved the capacity to downregulate digestive form and function at the conclusion of digestion and maintain this dormant state throughout the fast. Following feeding, the digestive system is rapidly restored to a fully functional state within just 24 hours through major increases in cell growth and proliferation, metabolism, and overall digestive function. In contrast, frequently feeding snake species do not possess this extreme capacity for regulation, and instead maintain an active digestive system at all times. Here, we compared responses in the intestine of multiple snake species, including those that do and do not regenerate upon feeding, to identify transcriptional responses associated with the intestinal regenerative phenotype in snakes. By comparing and contrasting responses across phenotypes and species, we develop a set of explicit hypotheses about signaling mechanisms underlying regenerative growth in snakes, which includes a surprisingly major role of stress-response signaling pathways.

28.39

Cold-inducible RNA-binding protein as a potential regulator of embryonic gonadogenesis in the red-eared slider turtle

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Temperature-responsive genes, such as those coding for heat shock proteins, play a vital role in embryogenesis and their patterns of spatial expression are shared among vertebrate species. While the hierarchy of expression is not conserved, many of the genes regulating developmental processes, such as gonadal development, are shared between taxa. Vertebrates exhibit multiple forms of sex-determining pathways, including genetic and environmental sex determination. The red-eared slider turtle (*Trachmeys scripta elegans*) exhibits *temperature-dependent sex determination* (TSD), where extrinsic thermal cues trigger gonadal differentiation during the thermosensitive period. During the thermosensitive period, embryos respond to relatively cooler or warmer temperatures to initiate male and female development, respectively, but we do not yet fully understand how temperature affects the molecular mechanisms of sex determination. In this study, we are targeting a candidate protein for driving sex determination in species with TSD, cold-inducible RNA-binding protein (Cirp). Cirp is a heat shock protein present in the gonadal tissues of many vertebrates and it has a potential regulatory role in the sex-determining pathway for *T. s. elegans*. Cirp has sexually dimorphic, temperature-dependent expression in *T. s. elegans* and other TSD species. In addition to Cirp, we are also investigating the potential for intron-retention (IR) to impact sex determination in *T. s. elegans*. IR has been proposed as a regulatory mechanism for sex-specific development in a variety of taxa. RNA-binding proteins can impact gene expression by: 1) stabilizing the bound transcript for transport into the cytoplasm, or 2) triggering degradation of the bound transcript by

recognizing a retained intron that contains a premature stop codon. The objectives of the proposed research are to evaluate the role of Cirp in gonadogenesis in *T. s. elegans* by determining the transcript targets of Cirp and evaluating those targets for intron retention. This will allow us to determine if Cirp binds to ovarian-inducing or testis-inducing transcripts and if the bound transcripts contain retained introns with premature stop codons, thereby implicating Cirp as a regulator of gonadal development in *T. s. elegans*. Turtle eggs were purchased from Concordia Turtle Farm (Concordia, LA) and incubated under fluctuating temperatures within the range of naturally-occurring, sub-surface soil temperatures to mimic an incubating nest. At the start of the thermosensitive period, eggs experienced thermal shifts in the form of heat waves to initiate gonadal differentiation. The duration of the heatwaves were selected to initiate bipotential gonadal development, to allow for testis development, or to trigger ovarian development. Gonads have been harvested from embryos for use in immunoprecipitation and RNA-seq to capture mRNAs bound to Cirp. We will evaluate potential impacts of IR using a *T. s. elegans* de novo transcriptome assembly derived from genes expressed at male-and female-producing temperatures in developing *T. s. elegans* embryos. Transcripts will be aligned to the closely-related painted turtle (*Chrysemys picta*) proteome to identify alignment gaps that correspond to retained introns. We are presently validating the binding efficiency of the Cirp antibody, which has not been specifically developed for *T. s. elegans*. The proposed research represents the first year of a multi-year project investigating the functional role of Cirp in vertebrate sex determination and its impact on the nuclear expression of reproductive genes. This study was approved by ISU IACUC. Funding by: NIH (#1R15ES023995-01), APS Porter Physiology Development Fellowship, ISU Mockford-Thompson Fellowship, and Beta Lambda Phi Sigma Biological Honor Society.

28.40

Determinants of growth in hybrid sunfish: asymmetries in expression of maternal and paternal myogenin genes.

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Sunfish are an intriguing model to study the evolution of metabolism and growth because of inherent differences in their hypoxia tolerance and life history strategies. However, these species can also unidirectionally hybridize when precocious male bluegill (BG, *Lepomis macrochirus*) sneak into pumpkinseed (PS, *L. gibbosus*) nests to fertilize eggs. Interestingly, though the BG fathers are phenotypically small, the hybrids demonstrate larger body size, as is seen in other models of heterosis. Previous studies have shown that BG are much less hypoxia tolerant than PS, but their hybrids have very low tolerance for hypoxia, consistent with a metabolic dysfunction. In this study, we took advantage of the unidirectional hydridization to explore the maternal and paternal patterns of expression of genes associated with body size. We considered that the large body size of hybrids might result from elevated expression of one or more growth factor genes, a dysfunction that might arise if hybrids escaped epigenetic silencing of maternal or paternal alleles that is seen in other models of growth. We surveyed the sequences of a series of growth factors and, where possible, created primers that could discriminate between each parental species. We then examined PS/maternal and BG/paternal expression in hybrids. Though most of the genes examined (myoD1, IGF2, IGFBP5, MEF2, follistatin) showed similar expression of BG and PS genes, myogenin showed marked differences in maternal and paternal gene expression. Specifically, in about one third of the hybrids, BG/paternal myogenin expression was more than an order of magnitude lower than PS/maternal expression. Since myogenin stimulates muscle growth, clearly this depressed myogenin expression was not consistent with the larger body size of hybrids. However, we continued to explore whether this pattern might be a reflection of the genotypes of the small-bodied precocious BG males. We sequenced upstream promoter regions of BG myogenin to see if we could find genetic markers linked to low expression individuals. We identified a series of diagnostic sites for 4 alleles of BG myogenin (A1, A2, A3, A4). We found that 90% of the hybrids displaying very low BG myogenin expression possessed the A4 allele, and 10% the A3 allele. In contrast, the A4 allele was found at about 55% frequency in BG. This appeared to be consistent with a model where enrichment of the A4 allele in hybrids might reflect an overrepresentation of this allele in the genotypes of the small male sneakers and satellites. However, genotyping of males that adopted the sneaker/satellite life history strategy were not found to differ from the population at large in myogenin allele frequency. Though the unexpected allele-specific suppression of myogenin is intriguing, it appears not to be linked to a phenotypic outcome in either BG or hybrids. *Supported through NSERC Discovery Grants to CDM and BDN.*

28.41

Hydrogen sulfide metabolites in tissues of normoxic and anoxic freshwater turtles (*Trachemys scripta*)

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Since the discovery that at low levels hydrogen sulfide (H_2S) acts as a neuromodulator in the brain, H_2S has

gained acceptance as a vital signaling molecule controlling key physiological functions, such as metabolism, cytoprotection, oxygen sensing and vascular tone. Here, we investigate whether and how H_2S metabolism remodeling is involved in the adaptation to anoxia of freshwater turtles (*Trachemys scripta*), one of the very few vertebrates capable of overwintering up to several months in complete absence of oxygen without major tissue damage. As H_2S is inactivated by O_2, we speculate that H_2S signaling could have a prominent impact on tissue metabolic depression during anoxia and cytoprotection.

Turtles were acclimated to low temperature and to normoxia or anoxia. After 9 days of exposure, tissue samples were collected. Sulfide content was analyzed with a fluorescent monobromobimane assay coupled with reverse-phase high-performance liquid chromatography (RP-HPLC) to identify major H_2S storage pools, namely bound sulfane sulfur (mainly persulfides, polysulfides and thiosulfate) and acid-labile sulfide (mainly iron sulfur clusters).

The total pool of sulfide (including bound sulfane sulfur and acid-labile sulfide) in tissues was lowest in the lung, moderate in liver, kidney and brain, and highest in red blood cells, where we found a very high content of bound sulfane sulfur, which may reflect the high protein thiol content of these cells. Overall, there was no major difference in sulfide content between the normoxic and anoxic turtles, except for a decrease of the bound sulfane sulfur pool in the anoxic brain. Whether changes in the free H_2S are linked to anoxia-adaption in turtles remains to be investigated further.

28.42

Effects of hind limb immobilisation and castration on [^{3}H]ouabain binding site content and Na^+, K^+-ATPase isoform abundances in rat soleus muscle

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The Na^+, K^+-ATPase (NKA) protein plays a critical role in skeletal muscle function via maintenance of the [Na^+] and [K^+] gradients across sarcolemmal and t-tubular membranes, and is comprised three α (α_1 - α_3), and three β (β_1 - β_3) isoforms. No study has yet investigated the possible effects of testosterone suppression on skeletal muscle NKA particularly after immobilisation. We therefore investigated the effects of hindlimb immobilisation and testosterone suppression via castration surgery on rat soleus muscle NKA as measured by [^{3}H]ouabain binding site content and NKA isoform abundances.

Methods: Eight week old male Fischer rats underwent sham or castration surgery, and then after 7 days were subjected to 10 days of immobilisation of one hindlimb. For both sham and castration groups, soleus muscles were obtained 7 d after surgery from non-immobilised controls, following 10 days immobilisation and after 14 days of recovery, from both the cast and non-cast leg.

Results: Within the sham group, after immobilisation, the [^{3}H]ouabain binding site content in the cast leg was 26% lower than in the non-cast leg ($p = 0.023$) and 34% lower ($p = 0.001$) than in the non-immobilised control group ($P = 0.012$), but did not differ at 14 d recovery compared to either the non-cast leg or non-immobilised control group. There were no differences in the NKA α_1, α_2, α_3, β_1 or β_2 isoform abundances in the cast leg compared to either the non-cast leg, or the non-immobilised control group, after immobilisation, or at 14 d recovery.

Within the castration group, the [^{3}H]ouabain binding site content in the cast leg after immobilisation was 34% lower ($p = 0.001$) than in the non-immobilised control group and remained depressed by 34% ($p = 0.001$) at 14 d recovery after immobilisation. The α_2 isoform in the cast leg was 60% lower than in both the non-cast leg ($p = 0.004$) and non-immobilised control group ($p = 0.004$) and remained 42% lower than the non-immobilised control group at 14 d recovery ($p = 0.039$). The β_1 isoform in the cast leg after immobilisation was 26 % lower than in the non-cast leg ($p = 0.018$), but did not differ at 14 d recovery compared to either the non-cast leg or non-immobilised control group. The β_2 isoform in the cast leg after immobilisation was 71% lower than the non-cast leg ($p = 0.004$) and 65% lower than non-immobilised control group ($p = 0.012$), but did not differ at 14 d recovery, compared to either the non-cast leg or non-immobilised control group. There were no differences in the abundances of the α_1 and α_3 isoforms between legs or groups .The β_3 isoform abundance could not be detected in either sham or castration groups.

Conclusions: Thus the [^{3}H]ouabain binding site content and α_2 were decreased with immobilisation, and remained depressed at 14 d recovery in the castration group, with the NKA α_2, β_1 and β_2 isoform abundances also decreased with immobilisation compared to sham group. Hence testosterone suppression was associated with impaired restoration of immobilisation-induced lowered NKA α_2 isoform and of the number of functional NKA in rat soleus muscle.

28.43

Preliminary survey of homeodomains in *Lumbriculus variegatus*

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Several animal clades contain members capable of body part regeneration. In particular, many annelids have remarkable regenerative abilities. A recent review of annelid regeneration noted the utility of examining a wide variety of annelid species to help uncover

conserved versus derived regenerative abilities (1). The annelid *L. variegatus* is a superb model organism for regeneration research (2), but is poorly characterized at the molecular level. Since Hox genes code for transcription factors that help establish positional identity during animal development and regeneration (3), we sought to study the expression of Hox genes during *L. variegatus* regeneration. As a first step we cloned Hox genes from *L. variegatus*. Genomic DNA was extracted from *L. variegatus* and conserved homeodomain regions were amplified via PCR with degenerate primers (4). PCR products of the expected size were inserted into the pGEM-T Easy vector and sequences from 18 plasmids were placed into six different groups based on sharing greater than 90% amino acid identity. A member of each group was compared to homeodomain sequences from other organisms and named Lva (*L. variegatus*) followed by the paralog group it is part of (if one could be ascertained). We identified Lva-Scr (95% a.a. identity to earthworm *P. excavatus* (Pex) Scr), Lva-Lox2 (97% a.a. identity to Pex-Lox2), and Lva-Lox5 (95% a.a. identity to Pex-Lox5), all part of the central group Hox gene cluster, and an Xlox paralog (92% identity to Pex-Xlox), part of the ParaHox gene cluster. Two sequences do not show high similarity to any homeodomains (less than 84% a.a. identity to any Pex homeodomains screened). To our knowledge this is the first report of homeodomain sequences from the Lumbriculidae family of annelids. Our study may pave the way for analyzing Hox gene expression along the anterior-posterior axis of adult *L. variegatus* and during regeneration following amputation.

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28.44

Whole Genome *de novo* Sequencing of the Atlantic and Pacific Bluefin Tuna Genomes

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Accurate genome assembly is a critical first step for in depth studies of physiology, population genetics, and evolution. Here, we report the first long-read draft assemblies of the Atlantic (ABFT) and Pacific (PBFT) bluefin tuna genomes. We extracted high quality genomic DNA from one individual from each species, which was sequenced with single-molecule, real-time (SMRT®) sequencing technology. Long read sequence data (average read lengths >15kb) was generated to over 80X genomic coverage for each sample. Diploid assemblies were generated using the FALCON-Unzip assembly algorithm designed for long sequence reads (>10kb). Genome assembly completeness and the degree of heterozygosity of each genome assembly was assessed with the benchmarking universal single-copy orthologs dataset (BUSCO, version 3). The FALCON-Unzip genome assembly algorithm generated a 1.59 Gb assembly for ABFT and a 1.24 Gb assembly for the PBFT. The primary N_{50} was 4.58 Mb for ABFT and 4.96 Mb for PBFT. In both assemblies, the largest contigs were in the 20Mb range approaching theoretical sizes in the chromosomal arm scale. The assembly completeness analysis using the BUSCO gene model set found 95% completeness for ABFT and 93.2% completeness PBFT. The degree of heterozygosity in each assembly found 85.9% duplicated BUSCOs in ABFT and 52.2% duplicated BUSCOs in PBFT, suggesting a higher degree of haplotype merging in the PBFT assembly. Coverage estimates across each assembled base in the two assemblies found ~5% collapse in the ABFT assembly and ~30% collapse in the PBFT assembly. Based on these findings the genome sizes were re-estimated to be 1.67 Gb for the ABFT assembly and 1.61 Gb for the PBFT assembly. These estimated genome sizes are roughly twice the haploid genome sizes published to date for these species suggesting that the assemblies have resolved a majority of the polymorphisms inherent within these individual genomes. Additional data types will be required to finish the annotation of these genomes. The high-quality initial draft assemblies of the Atlantic and Pacific bluefin tuna genomes resulting from long-read technology provide a more complete picture of gene-content and structural variation to help guide future studies of physiology and evolution in tunas.

29: CLINICAL PHYSIOLOGY: ABSTRACT DRIVEN SESSION

29.1

Blood glucose levels and hyperbaric pressure in SOD2 enzyme knockdown mice.

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Introduction: Large numbers of studies have been conducted in the search for the mechanism underlying CNS-oxygen toxicity (CNS-OT), the outcome of which may be fatal when diving with closed-circuit apparatus. In our previous studies in rats, we found a linear correlation between the partial pressure of oxygen at 4–6 atmospheres absolute (ATA), blood glucose levels (BGL), and changes in the membrane potential of the mitochondria. In transgenic mice, knockdown of the antioxidant enzyme Mn-superoxide dismutase (SOD2), which is found only in the mitochondria, resulted in an increase in oxidative stress. In light of this information, it would appear that plasma glucose is influenced by oxidative stress, which in turn depends on the activity of the enzyme SOD2.

Hypothesis: We hypothesized that underexpression of SOD2 would result in greater elevation of BGL than may be seen in the WT as oxygen pressure increases. The purpose of the study was to verify whether BGL might serve as a marker for the development of CNS-OT.

Methods: The study was conducted on 2 groups of mice: 1. Knockdown SOD2; 2. Wild type (WT). Latency to CNS-OT was measured by preliminary exposure of animals to hyperbaric oxygen (HBO) at 5 ATA, and this was used to derive the time for subsequent exposure at the lower pressures. Mice were exposed to HBO from 2–5 ATA in increments of 1 ATA/wk for 60% of their latency to CNS-OT (no convulsions). BGL were measured before and immediately after each exposure. We evaluated the influence of hyperglycemia and hypoglycemia on latency to CNS-OT by injection of 25% glucose solution and 6 U/kg insulin, respectively, prior to HBO exposure.

Results: Glucose levels increased after HBO exposure at 3–5 ATA in the WT mice, whereas in the transgenic mice blood glucose levels increased after HBO exposure at 2–5 ATA. Latency to CNS-OT did not differ between the transgenic mice and the WT on exposure to 5 ATA. However, after the induction of hyperglycemia, latency in the WT mice was prolonged in comparison with the transgenic mice, and compared with the latency observed without hyperglycemia. No change was noted in the transgenic mice. Following the induction of hypoglycemia, latency in the transgenic mice was shorter than it had been without hypoglycemia. No change was noted in the WT mice.

Conclusion: The induction of hyperglycemic and hypoglycemic states showed that transgenic mice with knockdown of the antioxidant SOD2 are more sensitive to oxidative stress. This may be an indication that the mitochondria play a significant role in the development of CNS-OT. Further investigation of mitochondrial activity will be required to assess this theory.

29.2

A proposed role for the mammalian dive response in sudden unexpected death in epilepsy

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Epilepsy patients die suddenly nearly thirty times more frequently than the general population. Victims are often found dead, in bed. Seizures are occasionally witnessed prior to death, but often only a hint of one is discovered (e.g., a tongue bite). This enigmatic phenomenon is known as sudden unexpected death in epilepsy (SUDEP). Clinicopathological reports indicate that SUDEP is most commonly associated with prolonged apnea, severe bradycardia and pulmonary edema. This paper explores important parallels between the physiopathology of SUDEP and that induced by the human form of the mammalian dive response (MDR), as the latter is known to include apnea, hypertension, bradycardia, and the translocation of a large fraction of the total blood volume into the pulmonary vasculature. The author hypothesizes that the MDR is triggered during apneic-generalized-tonic-clonic seizures and, in the worst instances, the drastic cardiovascular adjustments induced by this response result in SUDEP.

29.3

A COPMARITIVE STUDY OF PULMONARY SLOWLY ADAPTING RECEPTORS BETWEEN RABBITS AND RATS

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Pulmonary mechano-sensory receptors provide important inputs to the respiratory center for control of breathing. However, our knowledge regarding the receptor structure-function relationship is still limited. In the current studies, we explored the relationship by comparing the morphology and function of pulmonary slowly adapting receptors (SARs) between rabbits and rats. Sensory units of SARs were recorded from anesthetized, open-chest and artificially ventilated animals and their electrical activities in response to lung inflation were compared at 3 different constant airway pressures (10, 20 and 30 cm H2O). We found that the discharge frequencies were higher in rabbits than in rats. Peak discharge frequencies at pressures of 10, 20 and 30 cmH2O were 83±6, 139±8 and 200±11 impulses/sec for rabbits and were 49±6, 78±7 and 94±9 impulses/sec for rats, respectively ($P<0.001$). For morphological studies,

tracheal or bronchial smooth muscles were histochemically labeled with anti-Na+/K+-ATPase (a3 sub-unit). We found that receptor size is larger in rabbits than rats. Averaged receptor sizes were 368.7±20.5 mm2 (n=78) in rabbits and 222.9±12.9 mm2 (n=73) in rats (P<0.0001). Our results demonstrate that SARs are larger in size and discharge more in rabbits than in rats. It is possible that action potentials are generated from generator potentials, which are, in turn, determined by the local potential on the sensing surface of the receptor. In summation, the larger the surface of a receptor, the greater the generator potential and the discharge frequency, and the lower the activating threshold. Our results support the theory that larger receptors in a sensory unit may result in a low activating threshold and higher discharge frequency.

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29.4

Contribution of Group II Metabotropic Glutamate Receptors in the Dorsal Medullary Neuronal Groups during Hypertension Development

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[Background] It has been known that the nucleus tractus solitarius (NTS) and the area postrema (AP) in the dorsal side of medulla oblongata are crucial sites for regulating blood pressure and controlling baroreflex function via glutamatergic transmission. Although ionotropic glutamate receptors in the NTS and the AP are main receiving docks from baroreceptor sensory information, group II metabotropic glutamate receptors (mGluR2/3) are thought to be modulating baroreflex signal transmission to set the blood pressure properly. Inappropriate baroreflex signalings may cause different set point of blood pressure, e.g. resulting in hypertension, and thus we hypothesized that mGluR2/3 signals are essential to control blood pressure in normal range before developing hypertension.

[Methods] The mGluR2/3 agonist (LY379268; 4, 10, 40 μg/day) was continuously applied onto the dorsal side of the medulla oblongata for 6 weeks, using implantable mini-osmotic pump through a foramen magnum catheter in spontaneously hypertensive rats (SHR) starting at 6 weeks of age. Thereafter, blood pressure was measured twice a week using tail-cuff method more than 9 weeks.

[Results] The systolic blood pressure (SBP) of sham control group increased to 200 mmHg at age of 13 weeks, while SBP in experimental groups showed less than 175 mmHg at 10 μg/day and less than 165 mmHg at 4 μg/day. The 40 μg/day dose of LY379268 application caused low vitality and bradycardia, and thus we considered that this dose was not feasible even though lower SBP was observed. Even after finishing 6 weeks of LY379268 application, SBP in experimental groups was still remaining around 170 mmHg, indicating that blood pressure could be controlled in normal range without mGluR2/3 stimulation in the dorsal medullary neuronal groups.

[Conclusions] Our results suggest that continuous stimulation of mGluR2/3 in the dorsal medullary neuronal groups could prevent hypertension development in SHR and, therefore, the agonist may become a possible prophylaxis medication for hypertension-prone patients.

29.5

The evolving cholecystokinin 1 receptor as a unique G protein-coupled receptor permanently activated by singlet oxygen (GPCR-pABSO)

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The typical class A G protein-coupled receptor cholecystokinin 1 receptor (CCK1R) is unique in that it is permanently activated by singlet oxygen which is normally generated in a Type II photodynamic action. We have previously shown that CCK1R is activated permanently by photodynamic action with the chemical photosensitiser sulphonated aluminium phthalocyanine (SALPC) in freshly isolated rat pancreatic acinar cells. Such permanent photodynamic activation of CCK1R with SALPC could be reproduced in the rat pancreatic acinar tumor cell AR4-2J, and in CHO-K1 cells ectopically expressing the human CCK1R. Further, both CCK1R in AR4-2J cells and ectopically expressed CCK1R in other cell lines (CHO, HEK) could be permanently activated by photodynamic action with genetically encoded protein photosensitisers KillerRed and miniSOG. Ongoing work is examining the potential for CCK1R in Peking duck panctreatic acinar cells and CCK1R analogues from invertebrates to be permanently activated by photodynamic action with SALPC, KillerRed, miniSOG or enhanced variants of KillerRed and miniSOG. Since the CCK-CCK1R system is evolutionally conserved from ancient times the evolution of permanent CCK1R activation by singlet oxygen is actively pursued in our laboratory. The outcomes of such experiments will provide useful tools or toolkits for remote *in vivo* photon-driven manipulations of the CCK-CCK1R system in animal behaviors such as feeding and higher CNS functions from invertebrates to birds, mammals and non-human primates.

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30: OSMOREGULATION ION REGULATION: ABSTRACT DRIVEN SESSION - 1

30.1

Going against the gradient: Active NH_4^+ excretion by the ammonia tolerant hagfish (*Eptatretus stoutii*).

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Hagfishes (*Eptatretus stoutii*) feed on decomposing carrion drops during which time they may be exposed to high concentrations of total ammonia ($[T_{amm}] = [NH_3 + NH_4^+]$). Unlike most fishes, hagfishes are incredibly resilient to high environmental ammonia (HEA) exposure, readily surviving at water $[T_{amm}]$ of 20 mM but limiting plasma T_{amm} accumulation to ~5 mM despite large inwardly directly $NH_3 + NH_4^+$ partial pressure (ΔPNH_3) and electrochemical gradients (ENH_4^+) respectively. To test the hypothesis that hagfish used active transport to excrete ammonia under such conditions, hagfish were exposed to sequentially higher $[T_{amm}]$ (0-20 mM) for 48 h, and plasma $[T_{Amm}]$ was measured after 6, 12, 24 and 48 h. In all cases, plasma $[T_{Amm}]$ were consistently maintained at ~30-70% lower than environmental $[T_{amm}]$, despite sustained inwardly directed ΔPNH_3 (~3800 µTorr) and ENH_4^+ (~40 mV) gradients, supporting our hypothesis. The possibility that NH_4^+ excretion was facilitated by secondary active transport using $Na^+/NH_4^+(H^+)$ exchange *via* apical NHE (Na^+/H^+ Exchanger) antiports was then examined at both the organismal and tissue (gill) level. When hagfish were acclimated to 10 mMHEA for 24h and then transferred to HEA-containing artificial seawater (ASW) or Na^+-free artificial seawater (NFASW), plasma $[T_{amm}]$ increased by ~40%, suggesting that $Na^+$was a necessary substrate for ammonia stabilization. In a separate series, HEA-acclimated were infused with ^{14}C-methylamine (^{14}C-MA), a radiolabeled analogue of NH_4^+ prior to transfer to HEA-ASW or HEA-NFASW for measurement of apparent JNH_4^+ (inferred from J^{14}C-MA). While minimal JNH_4^+ was observed in control hagfish not acclimated to HEA following transfer to HEA-containing ASW, JNH_4^+ increased by ~60-fold in the HEA-acclimated hagfish following transfer to HEA-ASW; yet only a 14-fold increase was observed in animals transferred to HEA-NFASW. These results further support the hypothesis that Na^+ is a required counterion for active NH_4^+excretion. Next, we utilized a newly established *in situ* hagfish dual gill perfusion/perifusion technique to characterize the role of the NHE, using amiloride, and ^{14}C-MA. After the intact hagfish were acclimated to 10 mM HEA for 24 h, the afferent gill pouch arterioles were surgically cannulated and perfused with ^{14}C-MA/4 mM $[T_{amm}]$ hagfish saline while the water ducts draining the gills were cannulated and perifused with 10 mM HEA-ASW spiked with amiloride (500 µM), a putative NHE inhibitor. Amiloride application resulted in a 52% reduction of JNH_4^+, compared to control fluxes suggesting that there was appreciable Na^+/NH_4^+ exchange. We conclude that an active $Na^+/NH_4^+(H^+)$ exchange mechanism, likely mediated *via* NHE, is used by the hagfish to excrete ammonia against large inwardly directed ΔPNH_3 and ENH_4^+gradients which may be encountered when feeding on decomposing animal carcasses on the ocean floor. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

30.2

Ammonia transporter expression and distribution in organs of Caribbean subpopulations of the mosquito, *Aedes aegypti*, collected from freshwater and high ammonia habitats

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Surveys of the presence and abundance of mosquitoes in various urban habitats have demonstrated that *A. aegypti* may select septic tanks as a preferred breeding site, and emerging adults are larger and have significantly higher nutrient reserves. Sewage is typically rich in ammonia (NH_3/NH_4^+) and whilst ammonia serves as an important nutrient for microorganisms and plants, it is toxic to animal cells at relatively low levels. Insects are generally more tolerant to high ammonia in comparison to other animal groups and this may explain the prevalence of *A. aegypti* larvae in high ammonia environments. Important ammonia-excreting organs of these larvae which are in contact with the surrounding environment, the anal papillae, express four ammonia transporters; Rh proteins AeRh50-1 and AeRh50-2, and Amt/Mep proteins, AeAmt1 and AeAmt2. We have previously shown using RNAi that each Rh and Amt protein functions in ammonia excretion at the anal papillae, as well as contributing to hemolymph ammonia homeostasis in mosquitoes from a laboratory colony. The objective of the present study was to investigate ammonia transporter gene expression within Caribbean subpopulations of *A. aegypti* mosquitoes developing and emerging from clean freshwater or high ammonia sewage breeding sites (i.e. septic tanks). We hypothesized that the populations of *A. aegypti* collected from septic tanks would be highly specialized in their ammonia transporting capabilities to tolerate living in these habitats through measurable differences in ammonia transporter expression. We examined the

expression and distribution of Rh and Amt mRNA and protein in the organs of larvae and adults using quantitative PCR, Western blotting, and immunohistochemistry. Results are presented as a comparison between freshwater and sewage collected mosquitoes. We conclude that these populations of *A. aegypti* show vast plasticity in ammonia regulatory and excretory pathways that may contribute to their successful inhabitancy of these high ammonia environments.

30.3

Potential Role of a Rh Channel in Delivery of Ammonium from Coral Host Cells to Their Endosymbiotic Algae

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Intro: Coral reefs are facing mounting threats in the Anthropocene Era, yet basic coral biology remains largely uncharacterized. The purpose of this study is to examine the unexplored physiological mechanisms allowing for the exchange of nitrogenous molecules, such as NH_3/NH_4^+, between corals and their endosymbiotic algae. We identified a Rhesus protein (Rh) in the coral *Acropora yongei* and provide evidence it regulates nitrogen supply to their endosymbiotic algae. **Methods:** Molecular biology and immunohistochemistry techniques were employed to clone *A. yongei* Rh (ayRh) and investigate its physiological role in the coral-algal symbiosis. Custom rabbit-anti-ayRh antibodies were developed by GenScript USA and validated. **Results:** The cloned ayRh cDNA sequence coded for a 52 kDa protein with 12 predicted transmembrane domains typical of Rh proteins. Phylogenetic analysis determined ayRh to be part of the invertebrate Rhp1 subgroup, members of which enhance the diffusion of NH_3 gas. Immunocytochemistry on coral tissues and isolated cells revealed ayRh was colocalized with the vacuolar H^+-ATPase (VHA) in the host-derived symbiosome membrane which surrounds the algae and mediates molecule exchange between host and endosymbiont cells. Localization of ayRh in the symbiosome membrane fluctuated on a diel trend as the percentage of cells exhibiting symbiosome ayRh-localization was significantly greater in the daytime compared to the nighttime (52 ± 5% v. 31 ± 2%; n=3). **Conclusions:** We propose ayRh and VHA in the symbiosome membrane constitute a novel nitrogen concentrating mechanism whereby ayRh facilitates the diffusion of NH_3 and VHA acidifies the symbiosome space resulting in NH_4^+ trapping. The diel variations in symbiosome ayRh localization suggest a mechanism that allows coral host cells to regulate algae metabolism and growth. Enhanced supply of NH_3/NH_4^+ during daytime may provide algae with nitrogen only when required for the maintenance of photosystems and the production of photosynthesis-derived products that are translocated to the coral host. During nighttime, diminished nitrogen supply may limit algal growth and maintain coral control over the symbiosis.

30.4

The dual-purpose saltwater mitochondria rich (MR) cell of sea lampreys (*Petromyzon marinus*): an organ of osmoregulation and ammonia homeostasis

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The life cycle of sea lampreys comprises a prolonged 3-7 year filter-feeding, larval ("ammocoete") phase, followed by a multi-stage metamorphosis which prepares them for the blood-feeding juvenile "parasitic" stage in sea water. The goal of our study was determine how gill structure and function changes following metamorphosis to enable sea lamprey to cope with the dual challenges of osmoregulation and high rates of ammonia production arising from the ingestion of vast quantities of protein-rich blood. Ammocoetes, captured from rivers draining into the Northhumberland Strait, were held in freshwater (T=19°C) for 3-4 months, where approximately 40 % of animals entered metamorphosis. Rates of ammonia excretion averaged 100 nmol/g.h, but decreased by 50-75 % during metamorphosis, before increasing several-fold following metamorphosis. Immunohistochemistry and western blot analyses revealed that changes in J_{Amm} were accompanied by a marked re-distribution of ammonia-transporting, Rhcg-like glycoproteins (Rhcg). In ammocoetes, Rhcg-like proteins and V-ATPase had a punctate distribution on the lamellae. Following metamorphosis, however, Rhcg-like protein abundance massively increased, co-localizing with Na^+/K^+-ATPase (NKA) on the basolateral membrane of saltwater mitochondria rich (MR) cells, within the intra-lamellar spaces. Saltwater acclimation and feeding lead to further increases in Rhcg-like protein abundance in SW MR cells, during which time most ammonia was excreted via the gills. V-ATPase abundance decreased markedly, however. We propose that ammonia excretion across the lamprey gills in saltwater is via Rhcg-like proteins, which transport $NH4^+$ into the cytosol from which is it is excreted to the water via an apical $Na^+(NH4^+)/H^+$ antiporter. We conclude that in addition to osmoregulation, the SW MR cells augment ammonia excretion by juvenile lampreys when they are ingesting large quantities of protein-rich blood during their saltwater parasitic phase.

30.5

Impact of sugar beet de-icing liquid on salt and water balance in mayfly nymph, *Hexagenia limbata*

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With increasing awareness of the negative environmental impact of road salt (NaCl) as a de-icing agent, alternative road de-icers are being developed and marketed as environmentally friendly. This study investigated osmoregulatory changes in freshwater (FW) mayfly nymphs (*Hexagenia limbata*) following chronic exposure to beet juice de-icer (BJD), an alternative to conventional road salt. As benthic macroinvertebrates, mayflies can act as water pollution indicators as they are particularly sensitive to runoff contaminants such as road salts and metals. Knowing that beet juice contains high concentrations of K^+ which is an important ion in maintaining electrochemical gradients; it was hypothesized that excess runoff into FW may disrupt ionoregulatory mechanisms of mayfly nymphs, in part since K^+ settles in the sediment where some nymph species, such as *H. limbata*, burrow. A seven day chronic toxicity test was first carried out to establish LC_{50}, after which mayfly nymphs were exposed to a sub-lethal dose. Following exposure, measurements of hemolymph ions (K^+, Na^+, Cl^-, $NH_4^+$and H^+) as well as Na^+-K^+-ATPase and V-type H^+-ATPase activity in tracheal gills, rectum and Malpighian tubules were used to evaluate any perturbations in systemic salt and water balance. The LC_{50} value for BJD was measured at 40 ppt and the sub-lethal dose of 32 ppt was further used for the seven day exposure. Hemolymph $Na^+$and pHwere significantly higher in BJD nymphs compared to FW nymphs. In contrast, no significant difference was recorded in K^+, Cl^- and NH_4^+hemolymph concentration although the trend showed an increase in $Cl^-$and a decrease in $K^+$and NH_4^+in BJD nymphs compared to FW nymphs. To our knowledge, this is the first study to explore the physiological effects of BJD on FW organisms. This is an important initial step in identifying any potential risk to FW ecosystems that relate to the introduction of BJD products as an environmentally friendly alternative to conventional road salt.

31: DIGESTIVE PHYSIOLOGY: ABSTRACT DRIVEN SESSION

31.1

Exploring how a shifting gut microbiome may influence the hibernation phenotype

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Some animals hibernate during periods of food scarcity, surviving up to nine months without eating. This alters the abundance and composition of the hibernator's gut microbiota, which, because of the microbiota's role in supplying its host with metabolites, may influence the hibernator's own metabolism. To explore this possibility, we orally gavaged 13-lined ground squirrels with ^{13}C-labeled substrates that are degraded only by microbes, and then used stable isotope-based techniques to 1) measure the effects of hibernation on the microbiota's function, and 2) track the movement of microbial-derived metabolites through the squirrel. To measure microbiota function, we used cavity ring-down spectrometry to monitor in real time the $\delta^{13}C$ of the squirrel's exhaled CO_2. Higher values indicate higher rates of substrate metabolism by the microbiota. We found that season significantly influenced these ratios, which peaked in summer and reached a nadir in winter, indicating a diminished microbial metabolic capacity during hibernation. Next, to track microbial-derived metabolites, we used NMR-based metabolomics to identify how ^{13}C-labeled metabolites in gut contents and host tissues varied with season (results pending). This approach will reveal the potential for hibernation-induced microbiota changes to influence the squirrel's metabolic plasticity, the hallmark of the hibernation phenotype.

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31.2

Unraveling the Complexity of Seasonal Phenotypic Flexibility in Small Birds *via* Omics Integration

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Non-migratory small birds in seasonal climates must contend with changes between summer and winter conditions, including alterations in temperature and food availability and type. These birds typically respond by upregulating the capacity for muscular shivering thermogenesis in winter, including changes to either or both muscle masses and cellular metabolic intensity. To better understand the mechanistic basis of seasonal phenotypic flexibility, we conducted a large-scale metabolic profiling of pectoralis muscle in two small North American songbird species, American goldfinches

(*Spinus tristis*) and black-capped chickadees (*Poecile atricapillus*) during summer and winter seasons. These muscle samples were analyzed using non-biased, global metabolomics profiling technology based on UHLC/MS/MS^2 platforms. We integrate these non-targeted metabolomics data with previously published transcriptomics data for the same tissues to gain insight into how combined gene expression and metabolic profiles can provide a more detailed mechanistic understanding of the cellular responses to seasonal changes in organismal metabolic capacity of two small songbirds. This 'omics integration has provided an unprecedented view into the complex regulatory and biochemical mechanisms orchestrating seasonally flexible phenotypes. The metabolomics work was supported by the Department of Biology, Univ. of South Dakota startup funds to BWMW, whereas the transcriptomics work was supported by the National Science Foundation IOS 1021218 to DLS and partially supported by the National Science Foundation IOS 1354934 and IOS 1634219 to ZAC.

31.3

Withdrawn

31.4

The Role of Microbial Symbionts in Bonnethead Shark Seagrass Digestion

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Sharks, uniformly accepted as carnivores, have guts specialized for a high-protein diet. However, the bonnethead shark (*Sphyrna tiburo*) has been shown to consume copious amounts of seagrass (up to 62.1% of gut content mass)[1]. Bonnetheads were found to digest 51.2% of the organic matter in seagrass[2], as well as possess cellulose-component-degrading enzymes (β-glucosidase)[2] in their hindguts. This indicates likely involvement from the gut microbiome as part of the seagrass digestion process. In order to understand the role that the microbiome plays in bonnethead shark seagrass digestion, gut contents and mucosal scrapings were collected along the digestive tract of wild-caught bonnetheads (n=4) and bonnetheads that were fed a 90% seagrass and 10% squid diet in the lab (n=5). Using 16s rDNA sequencing, we determined the taxa of microbes present along the bonnethead shark digestive tract. Using gas chromatography of gut content fluid, we determined the concentrations of short-chain fatty acids, the primary end-products of microbial fermentation, in the different gut regions. Data collection is in progress. Results of this work show that the bonnethead shark is digesting seagrass at higher efficiency than would be expected for a "carnivore," and digestion may be aided by microbial symbionts. These findings have ecological implications because they show that bonnethead sharks are omnivorous and play a different role (including nutrient transport) than assumed within fragile seagrass ecosystems. Funding: UCI Newkirk Center for Science and Society, the UCI Microbiome Initiative, National Geographic, and an NSF GRFP. References: [1]Bethea DM, Hale L, Carlson JK, Cortés E, Manire CA, Gel- sleichter J (2007) Geographic and ontogenetic variation in the diet and daily ration of the bonnethead shark, *Sphyrna tiburo*, from the eastern Gulf of Mexico. *Mar Biol*, 152: 1009–1020. [2]Leigh SC, Papastamatiou YP, and German DP (under review) Seagrass digestion by a notorious 'carnivore.'

31.5

Will abalone survive climate change? Comparative digestive physiology and the effect of temperature stress on abalone across the Pacific Ocean

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Abalone in the northeastern Pacific Ocean have experienced massive population declines over the last century due to historic overfishing, and more recently, disease and stress. Withering syndrome (WS), a bacterial disease, infects the digestive systems of North American abalone and results in starvation and eventual death in affected animals. WS has led to population declines in all species infected (Crosson et al. 2014), is one of the largest threats to the Endangered black abalone, and affects animals more often and more severely during periods of thermal stress (Moore et al. 2009). Despite the severe impact of WS and the ongoing threats of thermal stress from climate change, the understanding of mechanistic impacts of WS and heat stress on abalone digestive function is unknown. This study aims to fill this critical knowledge gap by determining the digestive strategy of abalone and characterize their gut function. To do this, we examined gut function in wild red abalone (*H. rufescens*) and compared their gut function to distantly related New Zealand pāua (*H. iris*), because this species is thought to be basal with respect to the Haliotids and serves as a WS-free control, as WS does not exist in New Zealand. We tested two hypotheses: (1) Abalone use a rate-maximizing digestive strategy; and (2) Abalone digestive function is unable to keep pace with increased metabolic demand at the highest temperatures they experience in the wild, thereby making them more vulnerable to heat stress and WS in the face of climate change. To test hypothesis 1, we collected wild red abalone from northern California and wild pāua from Wellington, New Zealand, and measured enzyme activities, fermentation byproducts, and microbiome communities in distinct gut regions. To test hypothesis 2, we conducted 4-week thermal stress experiments with

both species. Abalone/pāua were divided into two temperature regimes, ambient seawater and 6°C above ambient. We fed animals known diets, and collected fecal material daily. After 4 weeks, we measured organic matter digestibility, as well as digestibility of individual macronutrients (protein, carbohydrate, and lipid) digestibility to determine how thermal stress impacts the animals' ability to extract nutrients and energy from their diet. We also measured metabolic demand at both temperatures to determine whether the changes in digestive function enable animals to extract enough energy to meet their increased metabolic demand at elevated temperatures. Preliminary results show that metabolic demand is significantly elevated during the thermal stress treatment. We predict that ongoing analyses will show that digestive efficiency will not keep pace with increased metabolic demand and abalone will thus have a net energy deficit during thermal stress. This is the first detailed study on abalone digestion, and collaborations with international fishing, aquaculture, and management agencies is enabling this physiological data to be integrated into management.

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31.6

Quickly Becoming an Omnivorous Lizard: interactions of diet, physiology, and ecology lead to dynamic changes in a rapidly evolving system

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After an experimental relocation, a population of Italian Wall Lizards, *Podarcis sicula*, on the island Pod Mrčaru in Croatia became omnivorous and morphologically distinct from its source population on the nearby island of Pod Kopište in <36 years (~30 generations). Additionally, the new omnivores consumed >2x more food than their Pod Kopište counterparts. Observed changes in the Pod Mrčaru lizards, such as the presence of valves in the hindgut, suggest a shift in digestive tract function to accommodate this drastic dietary change.

Determining what an animal eats and how it digests its food requires a multi-faceted approach that considers not only diet, but also digestive tract structure and function. Using Chemical Reactor Theory, a gut can be modeled as a series of chemical and physical reactions, predicting chemical and physical adaptations in the gut to accommodate dietary input. This theoretical framework provides the "ultimate" reasons for why specialization of digestion should arise. Thus, we hypothesized this new population of lizards would have physiological and morphological adaptations for plant eating. We characterized the shifts in form and function of these lizards' digestive tracts in the context of diet and ecology. By comparing the new and source populations, we can test the "proximate" mechanisms through which specialization arises, and the consequences of specialization on organismal performance.

We measured digestive performance of the newly omnivorous lizards of Pod Mrčaru and the Pod Kopište source population on three experimental diets: plant, insect, and mixed. When fed daily, the newly omnivorous Pod Mrčaru lizards had a higher digestibility of plant diets than their source population, attributable to better digestibility of plant-proteins in the Pod Mrčaru lizards. The two populations did not differ in digestibility of the insect diet. However, when fed half as often, a different pattern emerged. Male lizards fed every two days showed no population differences in digestibility of each diet. Surprisingly, female Pod Mrčaru lizards were more efficient at digesting the insect only diet—overall and with respect to carbohydrates and protein—than their Pod Kopište counterparts.

To understand the mechanisms of these performance differences, we investigated the digestive physiology of each of these populations of lizards in their ecological contexts, on a wild diet. We found no differences in gut morphology, apart from the already discovered cecal valves. Digestive enzymes corresponding to major nutrient classes and specific plant- or prey- based food sources did not differ in proximal regions of the gut, where the lizards would have produced these enzymes endogenously. However, in the distal intestine, where we primarily expect microbial digestion to occur, several enzyme activities were higher in the plant eating Pod Mrčaru lizards, although not just enzymes associated with plant digestion This implicates endosymbiotic microbes as the likely source of the functional shift in the gut over this short time span.

The Pod Mrčaru lizards proportionally more plant material in the summer, up to 70% of their diet compared to 30% in spring. The Pod Kopište population, on the other hand, consumed 4-7% plant material year round. While stomach contents revealed no sex differences in diet, stable isotope analyses of the lizards' tissues indicated differences in C and N assimilation by population, sex, season, and even year. Analyses of gut morphology and digestive enzyme activities also reveal interactions of population, sex, and season. Our results suggest that males and females accommodate this dietary shift using different strategies.

Overall, the physiology and ecology of the lizards in this system are dynamic and context dependent, with the hindgut playing a central role in this rapid evolutionary shift.

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32: CONDUCTING MECHANISTIC INVESTIGATIONS IN COMPARATIVE PHYSIOLOGY USING IN VITRO AND EX VIVO SYSTEMS

32.1

Induced pluripotent stem cells from 13-lined ground squirrels: to learn "hibernation" in a dish?

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Hibernatingmammals survive profound hypothermia (<10°C) without injury, a remarkablefeat of cellular preservation that bears significance for potential medicalapplications. However, mechanisms imparting cold-resistance, such ascytoskeleton stability, remain elusive. Using the first iPSC line from ahibernating mammal (13-lined ground squirrel), we uncovered cellular pathwayscritical for cold-tolerance. Comparison between human and ground squirreliPSC-derived neurons revealed differential mitochondrial and protein qualitycontrol responses to cold. In human iPSC-neurons cold triggered mitochondrialstress, resulting in reactive oxygen species overproduction and lysosomalmembrane permeabilization, contributing to microtubule destruction. Manipulationsof these pathways endowed microtubule stability upon human iPSC-neurons and rat(a non-hibernator) retina, preserving its light responsiveness after prolongedcold exposure. Furthermore, these treatments significantly improved microtubuleintegrity in cold-stored kidneys, demonstrating the potential for prolongingshelf-life of organ transplants. Thus, ground squirrel iPSCs offer a uniqueplatform for bringing cold-adaptive strategies from hibernators to humans inclinical applications.

32.2

Molecular manipulations: the power of cell culture for defining mechanisms of anoxia tolerance

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The freshwater turtle *Trachemys scripta* has been studied as a model of anoxia tolerance for nearly 40 years due to its ability to survive prolonged bouts of anoxia and repeated reoxygenation without apparent functional damage. *T. scripta* can survive without oxygen as long as 24h at room temperature to weeks at 3°C during winter hibernation. Originally, research on this model was largely observational, whole animal physiology. More recent studies have focused largely on the brain, since this is the most sensitive organ to oxygen deprivation in vertebrates. However, manipulation of an intact animal to determine molecular mechanisms of anoxia tolerance is difficult. Microdialysis of intact brains or the use of brain slices have proven useful for some studies such as the mechanisms of neurotransmitter balance, but the thickness of the samples can limit utility. Since 2007, our lab has utilized primary cell cultures of brain tissue which allows for direct manipulation at the cellular and molecular level. Neuronal cell cultures are established by digestion of brain tissue in an enzyme cocktail for 4 hours followed by density gradient separation to isolate the neuronal layer. Cell cultures are incubated for 3-4 weeks in Minimum Essential Medium (MEM) with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin at 30ºC in a 5% CO_2 incubator. Our lab has utilized these cell cultures to pharmacologically manipulate molecular pathways including inhibition of the Adenosine A1 Receptor (A1R) and protective kinase pathways as well as knocking down expression of neuroprotective genes such as Neuroglobin, Hsp72 and Bcl-2 utilizing turtle target specific small inhibitory RNAs (siRNAs). Our results indicate that during anoxia/reoxygenation blocking of A1R and decreased expression of Neuroglobin and Hsp72 increased ROS production suggesting that these play an important role in neuroprotection by decreasing oxidative stress damage, while decreasing the expression of Bcl-2 triggers the activation of the apoptotic pathway.

Our current study focuses on the role and regulation of Methionine Sulfoxide Reductase (Msr), an antioxidant and repair mechanism that is highly conserved from prokaryotes to eukaryotes and has been shown to be neuroprotective during anoxia and oxidative stress. We are using both MsrA knock down with target specific siRNA and plasmid transfection to examine the expression, role, and regulation of MsrA in anoxia and under oxidative stress. The induction of FOXO3a protects against cell death when cells are exposed to oxidative stress. Preliminary studies suggest that the induction of FOXO3a also increases MsrA levels. As it may be possible pharmacologically to increase FOXO3a expression, this suggests a way to increase cell survival under conditions that induce oxidative stress. The ability to manipulate cells in culture at the molecular level thus provides an additional tool by which we can define critical protective pathways of survival without oxygen and suggest therapeutic targets for further study. All work is approved by the FAU IACUC.

32.3

Identifying anti-inflammatory properties of serum that could protect the lungs of deep-diving seals

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Seals are accomplished diving mammals, which rely on lung collapse to limit nitrogen absorption and prevent decompression injury. Repeated collapse and re-expansion exposes the lungs to multiple stressors, including ischemia/reperfusion, alveolar shear stress, and inflammation. There is no evidence, however, that diving damages pulmonary function in these species. To investigate potential protective strategies in deep-diving seals, we examined the inflammatory response of seal whole blood exposed to lipopolysaccharide (LPS), a potent endotoxin. IL6 cytokine production elicited by LPS exposure was 50-500× lower in blood of healthy northern elephant seals and Weddell seals compared to that of healthy human blood. In contrast to the ~6× increased production of IL6 protein from LPS-exposed Weddell seal whole blood, isolated Weddell seal peripheral blood mononuclear cells, under standard cell culture conditions using media supplemented with fetal bovine serum (FBS), produced a robust LPS response (~300×). Induction of *Il6* mRNA expression as well as production of IL6, IL8, IL10, KC-like and TNFα were reduced by substituting FBS with an equivalent amount of autologous seal serum. Weddell seal serum, elephant seal serum, and harbor seal serum also attenuated the inflammatory response of RAW 267.4 mouse macrophage cells exposed to LPS. Cortisol level and the addition of serum lipids (free fatty acids) did not impact the cytokine response in cultured cells. These data suggest that seal serum possesses anti-inflammatory properties, which may protect deep divers from naturally occurring inflammatory challenges such as dive-induced hypoxia-reoxygenation and lung collapse. Funded by NSF Office of Polar Programs 1443554. [Reference: Bagchi, A., Batten, A. J., Levin, M., Allen, K. N., Fitzgerald, M. L., Hückstädt, L. A., Costa, D. P., Buys, E. S. and Hindle, A. G. (2018). J. Exp. Biol. 221, 10.1242/jeb.178491]

32.4

Studying Natural Tolerance to Ischemia/Reperfusion Using Endothelial Cells Derived from Seals

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Reperfusion injury is a common complication following ischemia/reperfusion events typical of myocardial infarction, stroke, embolism, and other peripheral vascular diseases. Lack of blood flow to tissues during ischemic episodes activates cellular pathways that upregulate pro-inflammatory signaling and increase oxidant generation. Reperfusion of ischemic tissues recruits inflammatory cells, exacerbating oxidant production and ultimately leading to cell death, tissue injury, and organ dysfunction. Marine mammals exploit the cardiovascular adjustments of the dive response to maximize the use of body oxygen stores while diving. These hemodynamic changes result in selectively reduced perfusion of peripheral tissues, exposing them to potentially damaging cycles of ischemia/reperfusion. Remarkably, seals show no apparent detrimental effects associated with these ischemia/reperfusion events. The cellular mechanisms underlying this natural tolerance to a typically pathological condition, however, remain unknown. Clinical and translational work shows that vascular endothelial cells are particularly susceptible to oxidant- and inflammatory-mediated reperfusion injury. Such injuries could be catastrophic for marine mammals, for whom fine-tuned control of vasoconstriction and dilation is paramount during and after diving. We isolated primary endothelial cells from placental arteries from Weddell seals (*Leptonychotes weddellii*) and northern elephant seals (*Mirounga angustirostris*) to study the mechanisms conferring ischemic tolerance to seal cells. Cells were assayed for dil-acetylated low-density lipoprotein (dil-AcLDL) uptake and immunostained with endothelial markers to confirm the endothelial phenotype of the preparations. Endothelial cells were stimulated with phorbol ester (PMA) and angiotensin II (AngII) to evaluate their capacity to produce oxidants. Live cells were also exposed to acute hypoxia and immunoblotted for hypoxia-inducible factor 1 (HIF-1α). Seal endothelial cell monolayers exhibit the cobblestone-like morphology characteristic of human and murine endothelial cells, and stain positive for platelet endothelial adhesion molecule (CD31) and vascular endothelial cadherin (CD144) by immunofluorescence and flow cytometry. Live seal cells in culture are functionally capable of incorporating dil-AcLDL, generating oxidants when stimulated with PMA or AngII, and upregulating HIF-1α expression when exposed to hypoxia. Together, these results suggest that primary seal endothelial cells in culture are a viable, novel model

to study the cellular mechanisms modulating a natural tolerance to ischemia/reperfusion.

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33: INTEGRATING PHENOTYPES AND FUNCTIONAL GENOMICS TO UNDERSTAND MECHANISMS OF REMODELING AND GROWTH

33.1

A new perspective from snakes on conserved vertebrate stress and growth pathways underlying intestinal regeneration

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Some snake species undergo remarkable feats of physiological remodeling and organ regenerative growth upon feeding. These species tend to substantially downregulate physiological function and atrophy organs while fasting, and must rapidly regenerate tissue and upregulate physiological function upon feeding. This begs the question of what molecular mechanisms do snakes use to direct such regenerative processes. In this study, we focus on regenerative growth in the snake intestine, and use a comparative experimental design that includes snake species that do, and those that do not, undergo regeneration upon feeding. Here we integrate phenotypic data, together with data on gene expression and protein expression, to characterize regenerative growth in snake intestinal tissues. Our results provide surprisingly clear inferences of shared conserved stress and growth pathways that underlie regenerative intestinal growth in snakes, and new evidence for the importance of stress response signaling in tissue regeneration.

33.2

Using natural genomic variation and experimental approaches to understand the function and evolution of the Insulin and Insulin-like signaling network in reptiles

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The Insulin and Insulin-like Signaling (IIS) network integrates signals from the environment and physiological stressors to regulate cellular and organismal growth, reproduction, and aging (i.e. life history traits). Evidence from model organisms indicates the IIS network may regulate trade-offs between these life history traits. Reptiles have considerable diversity in these life history traits, relative to mammals and classical model organisms; however, little is known about the function of the IIS network in this clade. Previous research found evidence of positive (diversifying) selection in key regulators and nodes within the IIS network in reptiles, particularly the interactions between the hormones (IGF1 and IGF2) and their receptors within squamates (snakes and lizards) [1]. Here I summarize what we know and don't know about the interaction of molecular stress responses with the IIS network to regulate life history traits and trade-offs in snakes and lizards [2,3]. I present recent findings from my lab on (1) the genetic variation in the IIS pathway in *Anolis* and *Sceloporus* lizard clades, and (2) the quantitative expression of regulators of the IIS pathway with ontogeny in the brown anole lizard, *Anolis sagrei*. I then describe the tools my lab has been developing to facilitate the study of IIS in reptiles. These tools include genomic sequence data; quantitative assays for IIS proteins and mRNA; the establishment of fibroblast cell culture from lizard tails and organs; production of species-specific recombinant proteins (regulators of the IIS pathway) for experimental manipulations; and organismal dosing of recombinant proteins using slow-release microspheres. Finally, I provide an overview of ongoing projects within my research program that are using these tools to understand the role of IIS in regulating cellular and organismal growth and aging, tissue regeneration, and life history trade-offs.

Funding provided by Auburn Univ..

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33.3

Transcriptome Dynamics in Hibernation: Cause or Consequence of Physiology?

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Small-bodied hibernators including the 13-lined ground squirrel (Ictidomys tridecemlineatus) exploit winter's

environmental cold to achieve extreme metabolic depression. This metabolic depression allows the hibernating mammal to save tremendous amounts of energy, facilitating survival when food is scare. Metabolic rate during the torpor phase of hibernation is as low as 2% of basal for 1-3 weeks at a time while body temperature hovers near freezing, but the animals periodically re-activate metabolism and restore euthermic body temperature for <1 day over a period of several months. Thus these mammals abandon homeothermy to become heterotherms for part of each year. This dynamic hibernating phenotype, like all phenotypes, must be orchestrated via gene expression programs and is thought to include not only mechanisms that give rise to metabolic flexibility, but also mechanisms that confer tissue protection from damage due to cold and ischemia followed by reperfusion. We hypothesize that gene expression patterns control the tissue remodeling needed to orchestrate and survive torpor and that the key genes can be identified by analyzing differential gene expression in carefully timed and collected samples from the distinct phases of the hibernation phenotype. To this end we collected a large tissue bank from telemetered animals. Currently we are using RNA-seq to interrogate various tissues from the bank in order to define both quantitative and qualitative gene expression differences among multiple phenotypic phases of the hibernator's year. Both general patterns and specific gene expression changes of interest that have emerged from these studies will be discussed. For example, in brain, qualitative changes include alternative splicing and 3' end formation as well as RNA editing by deamination of adenosines, the last of which occurs during torpor, and apparently as a consequence of continued cold exposure. In contrast, quantitative changes in the transcriptome are particularly prominent across the brief euthermic arousals of hibernation, and between heterothermy and homeothermy, and may therefore be more directly related to mechanisms underlying the phenotypic switches.

All tissues in this study were collected under the Univ. of Colorado School of Medicine IACUC-approved animal protocol, 44309.

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33.4

Cardiomyocyte polyploidization creates a barrier to heart regeneration in zebrafish

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The adult mammalian heart lacks any appreciable capacity to regenerate ischemia-damaged muscle. By comparison, injured hearts of neonatal mice[2] and some non-mammalian vertebrates, including salamanders and zebrafish, mount an impressive regenerative response driven by myocardial proliferation. Despite these differences in regenerative capacity, the variables that promote or restrict cardiomyocyte proliferation following injury remain incompletely characterized. One often-noted difference between non-regenerative and highly regenerative hearts is the DNA content of their cardiomyocytes. Non-regenerative hearts contain a majority of polyploid cardiomyocytes that form early in life when their diploid predecessors complete one round of DNA replication without cytokinesis. By contrast, highly regenerative hearts contain a majority of cardiomyocytes that are diploid, or assumed to be diploid based on nucleation studies. Although these observations reveal an inverse correlation between myocardial ploidy and cardiac regeneration, a causal relationship has yet to be investigated. Here, we demonstrate that experimental induction of cardiomyocyte polyploidization is sufficient to suppress the high regenerative capacity of the zebrafish heart. We learned that zebrafish cardiomyocytes are diploid, both during homeostasis and regeneration, and become susceptible to polyploidization upon inhibition of the RhoGEF Ect2. To create adult animals with mosaic hearts composed of diploid and polyploid cardiomyocytes, we engineered a transgenic system for transient, cardiomyocyte-specific inhibition of Ect2 during hyperplastic growth of the zebrafish heart. Following cardiac injury, diploid cardiomyocytes outcompeted their polyploid neighbors in producing regenerated muscle, demonstrating that elevated ploidy reduces or abolishes cardiomyocyte proliferation *in vivo*. Moreover, mosaic hearts composed of roughly equivalent percentages of diploid and polyploid cardiomyocytes failed to regenerate, indicating that a critical percentage of diploid cardiomyocytes, one greater than 50%, is required for heart regeneration. Taken together, our data identify cardiomyocyte polyploidization as a significant barrier to heart regeneration and suggest that maximizing the percentage of diploid cardiomyocytes surrounding infarcted myocardium may improve the regenerative capacity of the human heart.

34: THERMAL BIOLOGY: ABSTRACT DRIVEN SESSION - 3

34.1

Geographic variation in bumblebee thermal tolerance: implications for past and future range shifts

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Recent shifts in the geographic ranges of bumblebees (genus *Bombus*) in North America and Europe appear to be tightly linked to changes in climate. Warming temperatures could explain range contraction at the southern edge, but the failure of many bees to move northward in response to warming climates remains enigmatic. As with many other species, we know little about the mechanistic links between changing temperatures and bumblebee physiology. To address this gap, we first measured critical thermal limits of *B. vosnesenkii* workers reared from queens collected across the geographic range of the species (from southern CA to northern OR). We found strong divergence in cold (but not heat) tolerance across latitude and altitude, with CT_{min} closely tracking winter minimum temperatures of the queen collection sites. We then asked whether thermal tolerance could predict past distribution of this species (hindcast) by filtering historic climate data with measured thermal limits. Finally, we used projected climates to predict future range shifts.

34.2

Defying the temperature size rule in flight: Bigger bees perform better at higher temperatures

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The temperature-size "rule" was coined because, both in the field and lab, ectotherms are often smaller in hotter environments. However, neither the evolutionary causes nor the physiological mechanisms for this pattern are clear. As for other insects, bees tend to be larger at higher altitudes and smaller when reared at warmer temperatures in the lab. Larger bees can be endothermic during flight, so it is possible that thermal effects on body size are partially mediated by temperature effects on flight performance. Plausibly, endothermic, larger bees may be more able to fly in cooler temperatures but heat-limited under hotter conditions. If so, such a pattern could provide a partial evolutionary explanation for the temperature-size rule in bees. In the field in Panama, we measured thorax and air temperature to assess the effect of size on thermoregulatory ability. We measured leaf and flower surface temperatures and air temperatures in sun and shade in the tropical forest canopy, to assess the thermal environment of foraging stingless bees. We then measured the flight performance (whether or not bees could fly, flight durations) and flight metabolic rates across a range of air and body temperatures in the lab that reflected thermal conditions in the field using ten species of stingless bees that varied in body size from 2-120mg in body mass. Smaller species flew with body temperatures much closer to air temperature than larger species, which fly at thorax temperatures up to 10°C in excess of air temperature. This is partially explained by the scaling of heat gain and loss as a function of body volume; we found that smaller bees had steeper cooling curves, indicating that they gain and lose heat more rapidly. In the lab, contrary to predictions, the critical thermal maximum temperature at which flight ceased was lower in smaller species. These data suggest that larger bees may have developed capacities to tolerate higher body temperatures because they are routinely exposed to higher temperatures during flight due to their endothermy. Also, the shape of thermal performance curves varied strongly by species, suggesting that other ecological or phylogenetic effects on thermal tolerance are important determinants of flight performance in stingless bees. This research was supported by USAID, the Smithsonian Tropical Research Institute, and NSF IOS 1558127.

34.3

One for all or all for one: Emergent thermal physiology of ant colonies along tropical mountain ranges

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How do individual physiologies relate to group thermal adaptation? Using 16 Neotropical ant species as social models, we compared both individual and group-level thermal adaptations among tropical habitats. Here, we report new patterns of how within-colony thermal tolerance variation and group thermoregulatory tradeoffs scale to biogeographic clines in temperature. Differences in thermal tolerance breadth across habitats were often due to changes in small-caste (weak-link) thermal performance, and these biogeographic patterns appeared to be more actively selected for by thermal variation than by mean environmental temperature. We go on to describe the role synchronous broods play in group thermoregulatory trade-offs across elevation clines of living, self-constructed nests (bivouacs) of the army ant *Eciton burchellii*. Together, these findings suggest that considering social scales of thermal adaptation is key to accurately predicting the effects of climate change on highly social species.

34.4

Simultaneous stress: effects of hypoxia-temperature interactions on mortality, thermal tolerance, and transcriptome of *Drosophila melanogaster*

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Insects live in diverse, multifaceted environments, where several abiotic factors occur concurrently. Despite the relevance, stress responses to multiple, interactive stressors are not well studied. When stressors are combined the results are often unpredictable because we do not know how animals sense and respond to interactive environmental stressors. To understand how oxygen and temperature stressors interact across several organizational hierarchical levels, we studied how combined oxygen partial pressure (PO_2)-temperature stressors affect subsequent mortality, thermal tolerance and the transcriptome. We exposed male *Drosophila melanogaster* to a PO_2-temperature treatment consisting of 21 (normoxia), 10 or 5 kPa O_2 combined with control (23°C), cold (4°C) or hot (31°C) temperature. After 2 h recovery, we measured mortality, chill coma recovery time (CCRT) and heat knock down time (HKDT) as measures of cold and heat tolerance, and ran microarrays to measure the global gene expression profile. Cold treatments resulted in low mortality, regardless of the PO_2 of the treatment. Hot treatments had higher mortality, especially at 5 kPa O_2, and longer CCRT and shorter HKDT in comparison to the cold treatments. We identified genes and pathways associated with the protective effects of combined hypoxia and cold, as well as transcripts correlated with longer CCRT and shorter HKDT. These genes, and their associated pathways, provide targets for future studies on oxygen-temperature stress, and how they may interact to affect insect thermal tolerance and differential survival.

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Reference: Boardman, L., Mitchell, K.A., Terblanche, J.S., and Sørensen, J.G. (2018). A transcriptomics assessment of oxygen-temperature interactions reveals novel candidate genes underlying variation in thermal tolerance and survival. *Journal of Insect Physiology* 106: 179-188.

34.5

Why insects die at low temperature: depolarization mediated Ca^{2+} overload causes cell death in *Locusta migratoria*

Jeppe Bayley[1], Christian Winther[1], Mads Andersen[1], Camilla Grønkjær[1], Ole Nielsen[2], Thomas Pedersen[3], Johannes Overgaard[1]

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Cold tolerance of insects is arguably among the most important traits defining the distribution of this species rich group (Addo-Bediako et al., 2000). Even so, very little is known regarding the causes of cold injury. For many insects it has been observed that cold injury coincides with a cellular depolarization caused by hyperthermia and extracellular hyperkalemia that develops during chronic cold exposure (Andersen et al., 2017). However, prior studies have been unable to determine if cold injury is caused by direct effects of hypothermia, toxic effects of hyperkalemia or by the depolarization that is associated with these perturbations. Here we use a florescent DNA staining method to estimate cell viability of muscle and hindgut tissue from *L. migratoria* and show that the cellular injury is independent of the direct effects of hypothermia or toxic effects of hyperkalemia. Instead, we show that chill injury develops as a consequence of the cellular depolarization caused by these perturbations: 1) Regardless of whether the cells were depolarized by hypothermia or hyperkalemia, they accumulated injury to a similar extent when at similar membrane potentials. 2) The combination of hypothermia and hyperkalemia additively induced injury in both muscle and ileum tissue. 3) Depolarization and cell injury also occured in conjunction when the means of depolarization was TEA. We further hypothesized that the depolarization-induced injury was caused by opening of voltage sensitive Ca^{2+} channels causing a Ca^{2+} overload that triggers apoptotic/necrotic pathways. To test this we pharmacologically manipulated intra- and extracellular Ca^{2+} concentration as well as Ca^{2+}channel permeability and demonstrate that injury is prevented if transmembrane Ca^{2+} flux is prevented by removing extracellular Ca^{2+} or blocking Ca^{2+} influx. Together these findings provide the first demonstration of a causal relation between cold induced hyperkalemia, depolarization and the development of chill injury through Ca^{2+} mediated necrosis/apoptosis.

The work described here was funded by AUFF NOVA

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34.6

How to assess *Drosophila* heat tolerance: Unifying static and dynamic tolerance assays to predict heat distribution limits

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Temperature is a critical determinant of ectotherm distribution, and accordingly, climate change will likely affect future distributions of many species. Prediction of such distributional changes calls for simple and comparable measures of heat tolerance. These measures should ideally correlate closely with the characteristics of the species current thermal environment. The time to heat death is obviously dependent on the intensity of the heat stress and a recent model (thermal tolerance landscapes – TTLs) uses the negative exponential relation between temperature and heat knockdown time to parametrise the interaction between temperature (heat stress intensity) and duration across a range of stressful temperatures. In the present study we established TTLs for 11 *Drosophila* species representing different thermal ecotypes by measuring knockdown time at 9-17 stressful temperatures (at 0.5°C intervals). All species displayed the expected exponential relation between temperature and knockdown time (average R^2=0.98).

Previous analyses of TTLs have described an apparent trade-off between tolerance to acute and chronic heat stress in ectotherms. Here we show that this trade-off is an erroneous artefact that can arise from experimental noise and extrapolations of limited datasets used to feed the TTL model. In accordance with this conclusion we do not find any general trade off between chronic and acute heat tolerance between our 11 species.

Our study also addresses another important debate related to the measurement of heat tolerance in ectotherms. Thus, it has been debated if dynamic heat tolerance assays are better or worse at describing ectothermic heat tolerance. In contrast to the static tolerance assays, dynamic assays gradually increase temperature until knockdown resulting in a knockdown temperature rather than a knockdown time. The comparability of the static and dynamic assays has been questioned, but here we show that static and dynamic assays give comparable information on heat tolerance. Thus, the constants derived from a TTL can be used mathematically model dynamic knockdown temperature and we confirm the validity of this model with empirical measurements of dynamic knockdown temperatures in all 11 species. Finally, we show that both dynamic and static assessment of heat tolerance correlate closely with the environmental gradients that characterise the 11 species studied. Heat tolerance assessed by static and dynamic assays correlations to precipitation of the driest month and maximum temperature of the warmest month combined (R^2=0.68-0.71). These simple laboratory measures of heat tolerance are therefore powerful predictors of the species fundamental thermal niche and both static and dynamic measures of heat tolerance could therefore also be useful to predict the consequences of future climate change.

34.7

Evolution of body size toward temperature-dependent oxygen conditions in 188 rotifer species

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Recent reports suggest the important mediating role of oxygen concentration in body size-to-temperature response, especially in aquatic systems. However, the knowledge of the causal connections is still in a phase of conceptions. We tested the relationship between body size evolution and temperature/oxygen preferences in a large group of rotifer species using a comparative phylogenetic approach. Each species was defined by eight parameters describing its environmental preferences, namely minimum/maximum/optimum/range of temperature/oxygen concentration in the habitat of living. These data were obtained from the published studies. The PCA analysis showed that both range of temperature and of oxygen concentration clustered together, inversely to minimum temperature and minimum oxygen (Factor 1; 38 % of variance explained), while optimal temperature clustered inversely to optimal oxygen concentration (Factor 2; 30 % of variance explained). Factor 3 explained 14 % of variance, but its eigenvalue was still above 1, so we included it in further analyses. This factor differentiated temperature from oxygen and was driven mainly by the inverse connection (a trade-off?) between temperature range and temperature minimum, and between oxygen range and oxygen minimum. We further tested the relationship between species body size and species-specific environmental characteristics using published data on 188 rotifer species and corrected for their phylogeny. A multiple regression analysis for post-PCA scores showed the significant positive relationship of body size with Factor 1 ($p < 0.001$), a tendency of the relationship with Factor 3 ($p = 0.05$), but no relationship with Factor 2 ($p = 0.12$). The interpretation of this pattern is that body size of rotifers increases with wider ranges of preferred temperature and oxygen concentration, but is not related to optimal values of these variables. Instead, body size tends to be affected by minima of temperature and oxygen. Our study points out some interesting patterns in temperature/oxygen preferences in a large group of aquatic organisms. The new findings are that (i) the evolution toward wide thermal preferences was accompanied by the evolution toward wide oxygen preferences, (ii) the evolution toward optimal temperature/oxygen conditions, thus guaranteeing

maximal performance, has evolved separately from temperature/oxygen range, (iii) the interspecific correlation between body size and joint thermal and oxygen conditions discloses that, in the evolutionary scale, body size of aquatic organisms is selected not only by temperature, but by a combination of thermo-oxygenic conditions.

This study was funded by the Polish National Science Centre, Grant Number: 2015/19/B/NZ8/01948.

35: OSMOREGULATION ION REGULATION: ABSTRACT DRIVEN SESSION - 2

35.1

The role of the pyloric ceca in ion balance in rainbow trout: Integrating across techniques to understand active calcium transport

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The gastrointestinal tract (GIT) and dietary ions contribute additional routes and sources to maintaining ion balance in fish. Within the GIT of rainbow trout are vermiform ceca projecting from the anterior intestine ampule. The role of these structures in ion balance has been shown on a broad scale, averaging across all ceca combined using more traditional techniques. Instead, we use the Scanning Ion-selective Electrode Technique (SIET), which offers a powerful tool for studying ion transport, providing rapid and precise measurement of ion transport across GIT tissues. Using SIET we explored active and passive transport in individual ceca isolated from the Anterior, Middle, and Posterior sections of the anterior intestine. The impact of feeding and environmental manipulation exposure to Ion Poor Water (IPW)) revealed a dynamic response in ion transport that was specific for location within the ceca. Furthermore, correlations with enzyme activities within those locations demonstrated an increase in Na^+-K^+-ATPase (NKA) and Citrate Synthase (CS) activity that paralleled changes in ion transport. Overall, feeding and IPW exposure each altered ionoregulation within the caeca in a zone-specific pattern, with the Anterior and proximal portions of the caeca being paramount. Increased carbohydrate and protein metabolism fueled the increased ATP demand of NKA through CS.

35.2

Cellular mechanism for teleost otolith calcification, and their responses to acid-base disturbances

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Otoliths are calcium carbonate structures found in the inner ear of teleost fish, specifically within small epithelial sacs (“otolith epithelium”) and surrounded by an alkaline fluid (“endolymph”). The otolith organ is essential for perceiving gravity, equilibrium, soundwaves, and acceleration. The otolith calcifies in a ring-like pattern similar to a tree that provides valuable information about age, diet, growth rate, and geographical location that is essential for fisheries and ecology research. In addition, recent studies reporting enlarged otolith size in fish exposed to hypercapnia have,raised concerns about potential impacts of future ocean acidification on the balance and hearing of fishes. To promote calcium carbonate precipitation for otolith growth, the surrounding otolith epithelium must transport calcium and bicarbonate into the endolymph, and remove protons from the endolymph. However, the cellular mechanisms behind otolith calcification are poorly understood, especially in marine fish. This information would help identify potential species-specific mechanisms in otolith calcification and regulation that could affect isotope incorporation rate or determine differential responses to ocean acidification. Using immunohistochemical techniques, we identified two ion-transporting cell types in the otolith epithelium of Pacific mackerel (*Scomber japonicus*). The first type of ionocyte expresses Na^+/K^+-ATPase and $Na^+/K^+/Cl^-$-co-transporter, while the second type of ionocyte is enriched in vacuolar H^+pump and a cytosolic carbonic anhydrase. Both types of ionocytes also express the evolutionary conserved acid-base sensing enzyme soluble adenylyl cyclase. To further investigate how otolith calcification may be affected by acid-base disturbances, otolith epithelia were dissected and exposed to elevated [HCO_3^-] levels simulating blood conditions typical of fish exposed to hypercapnia. Immunohistochemistry of whole-tissue, sections, and isolated ionocytes as well as scanning electron microscopy were employed to analyze potential changes in protein localization and ionocyte morphology. Funded by NSF GRFP and GRIP to GTK, and NSF IOS # 1754994 to MT.

35.3

No water, no problem: a metabolomics analysis of desiccated annual killifish embryos

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Water is essential for life and the threat of dehydration puts organisms at risk. However, some organisms have adapted mechanisms for survival when faced with water stress. The annual killifish (*Austrofundulus limnaeus*) survives in ephemeral ponds in the coastal deserts of Venezuela and their embryos have the remarkable ability to tolerate anoxia for months. In addition, *A. limnaeus* must also contend with the seasonal dehydration of their ponds, which they survive through mechanisms that likely highly limit gas exchange. Survival is attributed to the ability of the embryos to enter a state of drastic

metabolic dormancy (diapause) as a part of their normal development. Embryos of *A. limnaeus* exhibit unique resistance to desiccation unseen by aquatic vertebrates. However, dehydration tolerance of these embryos has received much less attention than other aspects of their biology, and many questions remain regarding how long embryos can survive without water and the molecular mechanisms that support survival. To address these unknowns, we assessed the dehydration tolerance of *A. limnaeus* embryos during diapause and across post-diapause embryonic development. We then performed a metabolomics analysis (Metabolon) on embryos exposed to short and long term dehydration stress at two developmentally unique stages (diapause and 4 days post diapause). We sought to identify the metabolic pathways that may provide this species with its remarkable abilities to survive under extreme dehydration stress. Early findings suggest different molecular mechanisms are at play that allow embryos to survive dehydration stress compared to those that support survival of anoxic stress. Further metabolite profiling of desiccated embryos will provide insight into the unique molecular physiology behind embryological survival with no water. (Funding: NSF Grant IOS 1354549 to JEP).

35.4

Distinct ion transport properties in airways of the marsh rice rat (*Oryzomys palustris*)

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The airway epithelium is a critical barrier to the external environment and is protected by a thin layer of fluid known as the airway surface liquid (ASL). ASL volume is driven by osmotic gradients that occur via active ion transport, evaporative water loss and contact with hyper- or hypo- osmotic aerosols. The marsh rice rat (*Oryzomys palustris*)is a medium-sized rodent from the *Cricetidae* family that inhabits salt marshes, which are areas of coastal wetlands marked by periods of flooding, high salinity, and extreme temperatures. In the current study, we examined the ion transport and fluid secretion mechanisms of the marsh rice rat airway. We found limited amiloride-sensitive Na^+absorption and forskolin and IBMX-mediated Cl^-secretion. In contrast, a large carbachol-mediated increase in Cl^-secretion that was sensitive to DIDS, the calcium-activated chloride channel (CaCC) inhibitor CaCCinh-A01, and pretreatment with barium was observed. Consistent with that, marsh rice rat airway cultures exhibited fluid secretion to carbachol. When challenged with 3.5% NaCl apically to mimic exposure to sea water and sea water aerosols, marsh rice rat cultures exhibited a prolonged expansion of the ASL volume. These data suggest that the marsh rice rat airway may have evolved unique ion transport mechanisms to facilitate survival in the dynamic salt marsh environment.

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36: ANIMAL INTESTINAL MICROBIOMES: COMMUNITY DIVERSITY AND SERVICES PROVIDED TO THE HOST

36.1

It's not easy eating green: The importance of the gut microbiome in facilitating herbivory

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Herbivory, or feeding primarily on plant material, is a relatively common feeding strategy for animals. This feeding strategy is advantageous given the high abundance of plants in most ecosystems. However, herbivory is also physiologically challenging because plants often have low protein concentrations, high amounts of indigestible materials such as fiber, and defend themselves with toxic chemicals. In response, herbivorous animals have enlisted the help of microbial partners to cope with these various challenges. I will discuss experimental work in various animal systems examining the physiological contributions of the gut microbiome that allow animals to thrive on herbivorous diets. For example, using a series of experimental manipulations consisting of whole-organism feeding trials, microbiome reduction, and microbial transplants, as well as metagenomic and metabolomic approaches, it was demonstrated that herbivorous woodrats (*Neotoma* spp.) rely on their gut microbiota to consume plant toxins. Additionally, captive feeding trials demonstrated that the gut microbiome of lizards respond to high fiber diets and help lizards cope with these challenging diets. These studies and others across various animal taxa will be important for uncovering universal and novel microbial adaptations to herbivory.

36.2

The enteric microbial communities of sharks, fishes, island-dwelling lizards, and abalone: dietary and phylogenetic considerations

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All animals have microbial communities associated with them in one way or another, and usually, the most diverse community is in the digestive system. These enteric microbes can participate in a number of metabolic pathways that affect digestion, metabolism, and immune function in the host. As a field, we are just scratching the surface of what microbial diversity exists in animal guts, and how these communities provide

services for their hosts. In this talk, I will share the microbial diversity in the guts of four different taxonomic groups that show dietary variation: bonnethead sharks, prickleback fishes and zebrafish, Italian wall lizards, and abalone. Each of these systems has unique characteristics that make their intestinal microbial community of interest. The bonnethead shark (*Sphyrna tiburo*) is the only shark known to consume and digest seagrass. A spike in β-glucosidase activities in their hindguts, as well as elevated (~50%) digestibility of plant fiber—an unexpected result for a "carnivore"—suggest that the microbes matter in this shark's ability to digest plants. Prickleback fishes (family Stichaeidae) feature dietary diversity, sister-taxa with different diets, and convergent evolution of herbivory, all within sympatric species, making this group an excellent model for investigating the role of diet and host evolutionary history in microbial community diversity. Moreover, I will share data on how diet switching experiments affect the microbiome diversity in prickleback fishes and zebrafish (*Danio rerio*), the latter of which is becoming a model for microbiome research. Italian wall lizards (*Podarcis sicula*) showed rapid evolution (~30 generations) of morphological characters relating to a shift to an omnivorous diet, but the shifts in digestive physiology occurred mostly in the hindgut, concomitant with targeted changes in its microbiome. Finally, the abalone (family Haliotidae) are iconic herbivorous molluscan taxa found globally, yet we know little about the digestive process in their guts. Hence, I will share the first efforts to integrate their digestive strategy and enteric microbial diversity with regards to how they make a living and potentially deal with the deadly "Withering Syndrome", which has decimated abalone populations on the west coast of the USA. Overall, by better understanding how intestinal microbiomes correlate with different diets and phylogenetic histories, and coupling these data with functional data on digestive physiology and metabolism of the host, we can move towards understanding the range of services that microbiomes provide to their host organisms.

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36.3

Host genetic background contributes to resistance to microbiota disruption and host development in an evolution model organism

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Background: Communities of gut microbiota play an important role in stimulating development of organs and the immune system of the host, and in maintaining host homeostasis. The host genetic background can influence microbiota composition, in part via the immune response to microbes. In fact, several diseases that are marked by inflammation and gut microbiota disruption, such as Irritable Bowel Syndromes (IBS) and Inflammatory Bowel Diseases (IBD), are known to have high heritability (1,2). However, these are also marked by a strong environmental stressor or microbiota challenge early in development, indicating a gene by environment interaction that drives susceptibility to these diseases.

To determine the extent that the host genetic background contributes to the developmental response to microbiota disruption, we adapted the evolution and biomedical model organism, threespine stickleback fish (*Gasterosteus aculeatus*), for microbiota manipulation experiments. Stickleback are ideal model organisms for these studies due to their large clutch sizes, genetic variation within and between populations that is similar to human genetic variation within and between populations, extensive knowledge about developmental and behavioral variation between and within populations, and the tools available to study host-microbe interactions. I previously found that a lab-adapted anadromous population, which originally spent part of its life in the ocean and part freshwater, had a larger immune response to microbes than a lab-adapted freshwater population (3). These two populations shared ancestors, but have been separated for thousands of years. **Methods:** We developed gnotobiotic protocols to manipulate the microbiota of both lab-raised and wild embryos of stickleback (3). We then raised fish from several different populations in germ free conditions with no detectable microbes (GF), with complex communities added back (conventional, CV), with single microbes (monoassociations, MA), or with mock communities of up to eight members (mock). We also added antibiotics or environmental contaminants to the water with the fish. We compared the somatic development, immune system transcripts, and behavior of the fish to determine how these microbiota manipulations affected fish development. **Results:** We found that the populations varied in their response to these manipulations, indicating that the genetic variation between the populations contributed greater to the relationship between microbes and the host than the variation within the populations. Interestingly, we observed differences in the development of some somatic markers of development but not others very early in development (14 days post fertilization). **Future directions:** We will use these results as a basis for future studies to identify the critical windows in development in which disruptions to gut microbiota result in short- and long-term consequences to host health, and determine the extent to which the host genetic background contributes to the ability of healthy gut microbial communities influence to fitness. We are also working

with collaborators to determine whether changes in the immune responses to microbes occur early in the switch from anadromous to freshwater phenotype.

36.4

Gut microbial community dynamics in arctic ground squirrels: microbially-liberated urea-nitrogen use across the annual cycle of hibernation and activity

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Arctic ground squirrels (AGS) are hibernation extremophiles, spending up to 9 months annually in torpor, subsisting on endogenous body reserves of lipid and protein. We have shown that the gut microbial diversity and metabolic activity in AGS is influenced by the physiological state of the squirrel, and that microbial community composition reflects differences in availability of nutrient substrates across the annual cycle (Stevenson et al., 2014a,b). Urea-nitrogen salvage (UNS)---the diffusion of urea into the gut, its degradation by ureolytic gut microbes to NH_4 and CO_2, and the subsequent incorporation of microbially-liberated urea-N (MLUN) by the host---is posited as an important N-conservation mechanism of hibernators; however, little is known about the extent to which MLUN contributes to host synthetic processes, the identity, number or activity of ureolytic microbes in the gut, or how these aspects change seasonally. Therefore, we injected squirrels with either unlabeled or $^{15}N/^{13}C$-labeled urea periodically across their annual cycle. The magnitude of gut ureolysis was assessed via quantification of $^{13}CO_2$ in breath. To determine host use of MLUN, tissues were collected for analysis of $\delta^{15}N$. Cecal samples were collected to enumerate ureolytic microbes and determine expression of urease genes, and fecal and cecal samples were collected to isolate and characterize ureolytic bacteria. The breath of squirrels was enriched in $^{13}CO_2$, suggesting active ureolytic bacteria in the gut in both hibernation and summer euthermia, and several taxonomically diverse ureolytic bacteria were isolated and identified. Tissues were enriched in ^{15}N to a greater extent in hibernating squirrels compared to summer active squirrels, evidence of the use of MLUN in host protein synthesis across the annual cycle. Enumeration of ureolytic bacteria and analysis of expression of urease genes in the gut is ongoing. Our results indicate that AGS contain a diversity of ureolytic gut bacteria that are active across the annual cycle and confirm the potential importance of UNS in N-conservation in hibernation. All work followed approved UAA-IACUC protocols. FUNDING was provided by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103395 and the National Institute of Diabetes and Digestive and Kidney diseases (IR15DK110784 to KD). The content is solely the responsibility of the authors and does not necessarily reflect the official views of the NIH. Support was provided to KC from the Alaska Native Science and Engineering Program and Sloan Scholar, Alfred P. Sloan Foundation's Indigenous Graduate Partnership (SIGP) Program, awarded in 2015-16.

37: COMPARATIVE PHYSIOMICS: SYSTEMS-LEVEL APPROACHES TO COMPARATIVE PHYSIOLOGY
SPONSORED BY THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

37.1

Species-Specific Responses of Juvenile Rockfish to Elevated pCO_2 and Hypoxia

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Global climate change is predicted to trigger large-scale changes in ocean carbonate chemistry and hypoxia over the next several decades. Projected decreases in mean ocean pH and oxygen levels will be exacerbated in coastal upwelling systems if climate change increases the frequency and magnitude of upwelling events. We compared the effects of exposure to low pH (elevated pCO_2), hypoxia, and a combined stressor treatment on metabolic physiology and transcriptomic profiles of juvenile rockfish (genus *Sebastes*) over acute (<2 weeks) and chronic timescales (>4 months) under both fluctuating and static treatments. To compare high-CO_2 and hypoxia tolerance among species, experiments were conducted simultaneously on congeneric species that inhabit California kelp forests yet differ in early life history traits. Our findings indicate that congeners express different sensitivities to elevated pCO_2 and hypoxia levels, and that hypoxia appears to have a stronger effect than elevated pCO_2 at multiple levels of biological organization. The capacity of long-lived, late to mature, commercially important fish to acclimatize and adapt to a changing ocean over the next 50–100 years is likely dependent on species-specific physiological

tolerances that correspond with the timing and location of recruitment within the kelp forest.

37.2

The role of small noncoding RNAs in the regulation of metabolic dormancy and extreme stress tolerance

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Small noncoding RNAs (ncRNAs) are ubiquitous, abundant, and diverse. These short RNA sequences (15-30 nucleotides) can have a wide range of cellular functions most of which have to do with regulation of gene expression. However, there are a growing number of examples where these sequences may be playing roles in cell and organismal physiology independent of direct gene regulation. While some classes of small ncRNAs, such as miRNAs, are relatively well understood, there is an enormous diversity of sequences that have interesting expression patterns but unknown origins and functions. Exploring the small ncRNA transcriptome promises to shed new light on mechanisms of gene regulation, cellular and organismal physiology. The annual killifish, *Austrofundulus limnaeus* survives in ephemeral ponds by producing stress tolerant embryos that can enter into diapause, a state of developmental and metabolic arrest. Using RNAseq, we have explored the small ncRNA transcriptomes of *A. limnaeus* during transitions into and out of anoxia (1,2), and during entrance into metabolic dormancy (3). This work has identified small ncRNA sequences that may help to mediate survival of anoxia in embryos, including sequences that are derived from the mitochondrial genome. In addition, we have identified a number of interesting sequences that may integrate environmental cues into the developmental program and regulate phenotypic plasticity. Importantly, many of these sequences have unknown functions and may represent new origins and mechanisms of action for small ncRNAs. Funded by NSF IOS-1354549 (JP), NSF DEB-1501414 (JP&CR), NSF GRFP DGE-1057604 (CR).

37.3

Using Proteomics to Investigate Regulation of Stress Tolerance by Sirtuins in *Mytilus* Mussel Congeners

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Understanding physiological tolerances of marine organisms to environmental stressors is key to predicting species adaptability under climate change. Along the Pacific Coast of the U.S.A. intertidal mussel congeners of the genus *Mytilus* vary in their physiological tolerances to stress, with the invasive *M. galloprovincialis* being heat tolerant but vulnerable to hyposalinity while the native *M. trossulus* is vulnerable to heat stress and tolerant of hyposalinity. Our research findings suggest that sirtuins (SIRT), a group of NAD^+-dependent deacylases, influence the environmental stressor tolerances in these two mussel species. Our work uses proteomics to understand the role of sirtuins in regulating the cellular stress response (CSR) in mytilid mussels exposed to a variety of environmental stressors (i.e., submerged or aerial heat, hypoxia, food limitation, and hyposalinity). Mussels are acclimated to laboratory conditions in tidal simulators with a 12 h light:dark cycle and 6 h square wave tide to assess the effect of the circadian and circatidal rhythm. At the end of acclimation, mussels are exposed to chemical sirtuin inhibitors that reduce the activity of SIRT1, 2, 3, and 5. Following inhibition, mussels are then exposed to experimental conditions for 6 h during low or high tide to heat, oxygen and salinity stress. Our studies have found a clear effect on CSR proteins (antioxidants, superoxide dismutase; molecular chaperones, HSP70) key to maintaining cellular homeostasis under stress, proteins related to energy metabolism (isocitrate dehydrogenase), cell signaling (ERK2), and translational regulation (Musashi1). Moreover, through western blot analysis of SIRT5, our studies highlight the stark difference in stress tolerance between the two mussel congeners possibly due to sirtuin regulation. Thus, comparative proteomics reveals sirtuins as regulators of the CSR and stress tolerance and may allow us to make predictions regarding climate change effects on these competing species.

37.4

Metabolic response to stress in marine mammals

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Marine mammals are exposed to a range of stressors, including increased levels of anthropogenic disturbance in the marine environment. The generalized stress response is largely mediated by the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the HPA-axis stimulates glucocorticoid release and results in metabolic alterations that facilitate the response to, and recovery from, proximate stressors. Frequent or chronic stress, however, can result in persistent activation of the stress response with maladaptive consequences on health, survival, and fitness. Far less is known about the metabolic effects of stress responses in wildlife species in comparison to model species commonly used in biomedical studies. We therefore investigated the hormone and metabolic response to stress in two marine mammal species: the bottlenose dolphin (*Tursiops truncatus*) and the northern elephant seal (*Mirounga*

angustirostris). We experimentally induced stress in dolphins under managed care (n = 5) with a single out-of-water stress test, and in free-ranging seals (n = 7) by repeatedly administering adrenocorticotropic hormone (ACTH). Blood samples were collected before, during, and after stress manipulations. To understand the influence of stress on whole-animal metabolism, we characterized the glucocorticoid response and used metabolomics to evaluate changes in the circulating metabolome. Stress had profound influences on metabolism—in both species, over half of the circulating metabolites were significantly altered during the stress response. As anticipated, stress stimulated carbohydrate metabolism, evidenced by increased circulating carbohydrates increased (e.g. glucose, lactate, and pyruvate) in both species, whereas amino acids showed a varied response to stress. The greatest influence of stress was on lipid metabolism, both in the magnitude of change and in the number of compounds affected. Multiple pathways of fat metabolism were altered, including lipolysis, carnitine-mediated transport, β-oxidation, and corresponding tricarboxylic acid cycle activity. The enhanced effect of stress on fat metabolism may be advantageous in marine mammals commonly characterized by high adiposity. Preliminary analysis suggests that there may be a combination of compounds that exhibit differing responses to acute and chronic stress that may help inform stress states in marine mammals.

37.5

From Genome to Phenome: Exploiting 13-Lined Ground Squirrel "Omics" to Achieve a Deeper Understanding of Hibernation.

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Hibernation is a highly dynamic phenotype whose timing, for many mammals, is controlled by a circannual clock and accompanied by rhythms in body mass and food intake [1]. When housed in an animal facility, 13-lined ground squirrels exhibit individual variation in the seasonal onset of hibernation, which is not explained by environmental or biological factors, such as body mass and sex [2]. We hypothesized that underlying genetic factors instead drive variation in this timing. In this project, we first increased the contiguity of the 13-lined ground squirrel draft genome assembly by using a long-range scaffolding technique [3]. We next employed a modified ddRAD sequencing protocol [4] to characterize genetic variation in 153 13-lined ground squirrels. Combining this with datalogger records, we estimated high heritability (61-100%) for the seasonal onset of hibernation. Applying a genome-wide scan with 46,996 variants, we identified 2 loci significantly, and 12 loci suggestively, associated with hibernation onset. These 14 markers alone accounted for 48% of the variance in the phenotype. The most significant marker (SNP 1, $p=3.81x10^{-6}$) was located near *prolactin-releasing hormone receptor* (*PRLHR*), a gene that regulates food intake and energy homeostasis. Other loci were located near genes functionally related to hibernation physiology, including *muscarinic acetylcholine receptor M2* (*CHRM2*), involved in the control of heart rate, *exocyst complex component 4* (*EXOC4*) and *prohormone convertase 2* (*PCSK2*), both of which are involved in insulin signaling and processing. Finally, to further refine genes affected by these loci, we applied an expression quantitative trait loci (eQTL) analysis using existing transcriptome datasets. We identified significant expression associations for 8/14 loci. Our results highlight the power of applying a genetic mapping strategy to hibernation and present new insight into the genetics driving its seasonal onset.

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38: MANAGING FUEL METABOLISM UNDER LIMITED OXYGEN AND ENERGY SUPPLY SPONSORED BY THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

38.1

Metabolic suppression mechanisms for fasting and hypoxia

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Metabolic suppression is a key strategy to cope with prolonged fasting or environmental hypoxia. My talk will address two alternative physiological mechanisms that reduce overall energy expenditure when food or oxygen are in short supply: (1) the inhibition of substrate cycles during long-term fasting, and (2) the inhibition of ion pumps via remodelling of membrane lipids during chronic hypoxia. Rabbits fasting for 6 days decrease their metabolic rate by 28%. After 2 days, *in vivo* tracer kinetics show that the rates of appearance of glycerol (R_a glycerol or lipolysis; +59%) and nonesterified fatty acids (R_a NEFA; +73%) are strongly stimulated, but these responses are abolished after 6 days without food. Simultaneous lipolysis and re-esterification form the triacylglycerol/fatty acid cycle (TAG/FA cycle): a substrate cycle that consumes ATP without net synthesis of any product. By combining tracer kinetics and indirect calorimetry, flux through this cycle can be quantified from R_a glycerol, R_a NEFA, and NEFA oxidation. We have found that TAG/FA cycling is first activated (+47% after fasting 2 days), but that it falls well below baseline after 6 days (-31%). Therefore, when fasting is prolonged, the inhibition of the TAG/FA cycle reduces total energy expenditure. If food deprivation was to have the same effect on other major substrate cycles (glucose, fructose and others), this mechanism alone would play an important role in supporting metabolic suppression.

Fish and mammalian champions of hypoxia tolerance like goldfish and naked mole rats show an outstanding capacity for metabolic suppression. They are able to reduce their rate of oxygen use dramatically by downregulating ion pumps, anabolic pathways, and energy metabolism. Because ion pumping by membrane-bound ATPases is the most energy costly physiological process in resting tissues, we have looked for new mechanisms of ion pump inhibition that could help survival in hypoxic environments. We have recently discovered that goldfish restructure membrane lipids extensively to cope with chronic hypoxia (4 weeks at 10% air saturation). They modulate both the fatty acid composition and the cholesterol content of their membranes in ways that are fully consistent with well known mechanisms of ion pump inhibition. More specifically, they increase membrane cholesterol (in muscle and gills), and decrease membrane unsaturation (in gills and liver) by reducing the relative abundance of polyunsaturated fatty acids, but particularly DHA (docosahexaenoic acid or 22:6 n-3). These large changes in lipid composition are interesting because ion pumps like Na^+/K^+-ATPase and Ca^{2+}-ATPase are inhibited by cholesterol and activated by DHA. Therefore, these observed membrane remodelling responses to low oxygen could be a novel mechanism used by hypoxia tolerant animals to achieve extreme levels of metabolic suppression. We are presently exploring this possibility through direct measurements of ion pump activity during acclimation to hypoxia and by characterizing how mammalian membranes are affected by long-term hypoxia, using the naked mole rat as a model.

38.2

Now or Later: Differential fates for glucose and fructose in a nectarivore

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Hummingbirds fuel their high energy active lifestyle with the fructose and glucose in their nectar diets. These sugars are used to fuel immediate energy needs and build the fat stores to fuel future fasting periods. Remarkably, hummingbirds can quickly shift from fuelling energy-demanding hovering flight from stored fat to either fructose or glucose alone within 20 mins of feeding. However, if and how hummingbirds partition dietary fructose and glucose towards immediate oxidation to fuel foraging behaviour or towards fat storage is unknown. The high fat turnover rates of hummingbirds (50% fat turnover rate in less than 24 h) means that nectar consumed during the day is also required to fuel that night's energy requirements. As such, metabolic partitioning of glucose and fructose to balance the energy needs of now and later may be an important strategy. Theoretically, the distribution and abundance of sugar transporters and metabolic pathways suggests preferential oxidation of glucose and prioritization of fructose for lipogenesis. Using acute and chronic stable isotope tracer studies we are examining how dietary sugars are allocated towards immediate oxidation or fat synthesis in ruby-throated hummingbirds (*Archilochus colubris*). First, we tested for preferential use for *de novo* lipogenesis by feeding hummingbirds diets with either the glucose or fructose enriched with ^{13}C for 5 days. The isotope incorporation into fat was measured via the breath fatty acid signature each morning after an overnight fast. We found higher incorporation of stable isotopes into the fat stores when glucose was enriched compared to fructose suggesting

preference for glucose as a substrate for fatty acid synthesis. Secondly, we are testing for immediate preferences to determine if glucose or fructose is partitioned towards fuelling foraging behaviour. These studies improve our understanding of the adaptations that support the energy needs of hummingbirds during both feeding and fasting periods and the role played by the partitioning of a seemingly simple diet. Funding for this work is provided by the Natural Sciences and Engineering Research Council and Human Frontier Science Program.

38.3

Feeding the Machine at the Top of the Food Chain: A carnivore conundrum

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A defining feature of the 276 species of fish- and meat-eating mammals is a diversity of form and function that enables them to serve as apex predators from mountain tops to ocean depths and from tropical to polar regions. Critical to this success is the integration of physiology, environmental synergy and energetic efficiency that matches how animals are built with the environmental conditions in which they forage and live. Such integration is especially evident in the marked changes in organ morphology and physiology that occurred 30-50 MYA as terrestrial mammals reinvaded the oceans. In this study, we use extant carnivorous mammals to examine the role of metabolic demand and acquisition along the evolutionary pathways leading to modern marine mammals. Maintenance, locomotor and field metabolic rates were measured for fully terrestrial (puma, leopard, grizzly bear), transitional (sea otter, polar bear), and fully marine (bottlenose dolphin, monk seal, Weddell seal) species to characterize the energy demands of each lifestyle. To assess the capacity of the digestive system to process food to meet those demands, we also examined the length of the small intestine of 50 mammalian species specializing in terrestrial, transitional or marine diets. We found that the maintenance metabolism of carnivorous mammals depended on the degree of aquatic specialization, and ranged from 1.3 to >2.2 times predicted basal levels of non-carnivorous mammals of similar body mass. Locomotor costs were as predicted for similarly-sized swimming or running mammals regardless of diet preference. Field metabolic rates of marine mammals, except deep-diving seals, reflected the thermal energetic demands of immersion and averaged 1.8 times that predicted for terrestrial carnivores. An important pre-adaptation for marine living was remodeling of the omnivore and herbivore gut to support these elevated metabolic rates and to fuel seasonal adiposity. Consequently, maintenance energetic demand (kcal/day) was correlated to small intestinal length (m) according to, Energetic demand = 141.9 x small intestinal length$^{1.162}$ (n = 16 species).

Transitional species in this study retained both terrestrial and marine characteristics that included the comparatively high resting metabolic rates of marine-adapted species coupled with relatively short intestinal lengths of their terrestrial ancestors. Thus, the intestinal morphology of the polar bear is similar to that of terrestrial black bears and grizzly bears but must assimilate more calories to meet the higher metabolic demands of marine living. Two different solutions for fueling the metabolic overhead created by this reduced digestive capacity are displayed by these transitional species. Polar bears rely on a diet of calorically-dense, lipid rich prey, while sea otters graze throughout the day and night in areas of abundant protein prey resources. The latter fueling strategy of otters has the added benefit of prolonging the heat increment of food processing to offset thermal metabolic demands. Both fueling strategies enabled ancestral mammalian groups to take advantage of plentiful oceanic prey across evolutionary time. Today, access to critical food resources needed to preserve energy balance within the processing capacities of these transitional carnivorous mammals has been compromised by rapid changes in ecological and environmental conditions. The ensuing energy imbalance is manifested as a reduction in body condition in polar bears and sea otters during periods of high energetic demand with a concomitant increase in uncertainty for long-term survival. (Supported by ONR, NSF-DBI, NSF-Polar.)

38.4

Fuelling locomotion and thermogenesis in high altitude native deer mice

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Alpine regions are challenging for high-altitude animals due to unavoidable low O_2 availability and low temperatures. Reduced aerobic scope in hypoxia may limit the ability to engage in sustained exercise or to avoid hypothermia. However, deer mice (*Peromyscus maniculatus*) have a wide altitudinal distribution, up to ~4300m, and have evolved elevated cold-induced (thermogenic capacity) and exercise-induced maximum oxygen consumption (VO_2max) in hypoxia. Thermogenesis is fuelled by very high rates of lipid oxidation but submaximal exercise relies to a greater extent on carbohydrates than seen in lowland natives. The metabolic phenotype of skeletal muscle and their responses to cold and hypoxia acclimation do not necessarily reflect variation in fuel use. These data suggest a role for metabolic regulation of muscle enzymes or modifications of other parts of substrate pathways in setting fuel use at altitude. Possibilities that

will be discussed include changes in substrate availability, muscle uptake capacities, and /or selective recruitment of fibre metabolic pathways.

39: MECHANISMS OF CHANGE, PHYSIOLOGICAL RESPONSE TO ENVIRONMENTAL STRESSORS SPONSORED BY THE SOCIETY OF EXPERIMENTAL BIOLOGY

39.1

Sublethal effects and biomarkers of crude oil exposure in anadromous fish

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Crude oils are a complex mixture of chemicals and represent a pervasive environmental stressor. Canada sits on the world's third largest crude oil reserve, found as bitumen in the Athabasca oil sands. As plans to bolster the export capacity of this resource intensify, so too do concerns for the added risk of spills and environmental contamination. For example, existing and proposed oil pipeline routes carry bitumen across coastal watersheds, and release of bitumen here could threaten salmon populations that depend on these waters for their anadromous life cycle. My research investigates the physiological and performance impacts of environmentally-relevant bitumen exposure in salmon at different life stages, with an aim to understand the implications of a spill on the capacity of these fish to transition from freshwater to seawater. I have connected molecular and histological changes in the hearts of juvenile salmon with reduced swimming performance, supporting a causal link between crude oil induced cardiotoxicity and impaired aerobic capacity, and warning of reduced migration success in exposed fish. In addition, exposing fish during smoltification blunts the molecular response in the gill required for seawater acclimation, which could further impede the success of their seaward migration and transition to the oceanic life phase. I have shown that exposure during development carries a lasting impact, with high mortality continuing for months even when fish are removed to uncontaminated water, and changes in brain morphology of surviving fish are evident nearly one year later. While the organismal outcomes of these changes in the brain are not known, this finding supports an emerging interest in the neurotoxic effects of crude oil exposure. Finally, analysis of the plasma proteome of exposed fish revealed changes consistent with elevated tissue damage as well as several proteins that may be useful as biomarkers of crude oil exposure. This research is funded by the National Contaminants Advisory Group at Fisheries and Oceans Canada.

39.2

Physiological responses to social stressors

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Many species of fish establish social hierarchies as a consequence of competition for limited resources such as feeding territories or mates. Typically, dominant fish enjoy preferential access to the limited resource and are aggressive towards more subordinate fish. Subordinate fish, by contrast, may exhibit behavioural inhibition including reduced activity and feeding. These behavioural differences are accompanied by distinctive physiologies, particularly with respect to regulation of the stress axis, and metabolism and growth. Subordinate fish often exhibit chronic elevation of the glucocorticoid stress hormone cortisol, and typically do not feed or grow as well as dominant fish. Indeed, the combination of high cortisol levels and low food intake can remodel liver metabolism in subordinate fish to place greater reliance on on-board energy reserves. This presentation will focus on regulation of the stress axis and regulation of growth and metabolism as a result of social interactions in rainbow trout (*Oncorhynchys mykiss*) and the cichlid *Neolamprologus pulcher*. Whereas juvenile rainbow trout form pecking order social hierarchies, *N. pulcher* is a cooperative breeder in which a breeding pair is dominant over a series of subordinate helpers. These contrasting social systems provide a useful framework in which to explore physiological responses to social stressors.

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39.3

Exploring thermal physiology: Effects of environmental temperature in embryonic to larval frogs and juvenile to adult copepods

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The environment is a driving force that has immediate and long-term effects on animal phenotypes. Yet, animals are not passive entities, and phenotypic plasticity is an important avenue by which animals respond to the environment. Plasticity is an important component of thermal responses, particularly when temperature changes occur during development, or fluctuate over short time scales (minutes to hours). One of the main objectives of my lab's research is to understand how temperature shapes phenotypes across different life stages, time scales, and populations. We are exploring the immediate and long-term effects of developmental temperature in the Baja California chorus frog (*Pseudacris hypochondriaca*) and examining how different populations of a supratidal marine copepod (*Tigriopus californicus*) respond to temperature changes across different time scales. Chorus frogs are the most

abundant and successful amphibian on the west coast of North America. Chorus frog embryos demonstrate immediate responses to incubation temperature (10°C-25°C). Warmer temperatures reduce time to hatch, and oxygen consumption rate (Vo_2) is higher and more variable at 20°C. However, there is a limited temperature effect on embryonic growth, with mass similar between temperatures throughout embryonic development and at hatch. The developmental environment may also have long-term effects in shaping phenotypes later in life. Chorus frog larval Vo_2 and critical thermal maximum (CTmax) is influenced by a combination of embryonic temperature and larval acclimation temperature. Timing of thermal changes is also an important consideration in juvenile and adult organisms, particularly those experiencing highly variable environments. *T. californicus* inhabit supratidal 'splash pools' (above the tidal zone) that are exposed to drastic changes in daily temperature and a latitudinal thermal cline from Baja California, Mexico, to British Columbia, Canada. When juvenile and adult copepods are shifted from 20 to 30°C at 2.5°C intervals every 48 h, we observe no temperature effect on Vo_2 measured at each temperature. These results suggest the copepods undertake rapid metabolic compensation. However, when Vo_2 is measured at 0 to 6 h after a temperature increase from 20°C to 25°C, an immediate increase in Vo_2 occurs before rapidly returning to 20°C levels by 6 h. The exact time course of this response varies between copepod populations from Oregon and southern California. Collectively, these studies indicate that a full understanding of a species thermal physiology must include both temporal (changes throughout development, environmental variability) and spatial considerations (geographic population).

39.4

Effects of Acute and Chronic Thermal Exposure on the Swimming Performance and Aerobic Scope of Sheepshead Minnows (*Cyprindon variegatus).*

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Estuarine fish experience daily fluctuations in abiotic factors, which makes them an ideal model organism to study physiological acclimation responses to environmental stressors. One such species is the eurythermal Sheepshead minnow (*Cyprindon variegatus*), which has the capacity to tolerate environmental temperatures from below freezing (-1.9°C) to the point of protein degradation (43°C). It has been suggested that these fish have the largest thermal capacity recorded. However, questions remain about their physiological strategies at these temperatures. To investigate this adult mix-sexed minnows were placed in aquaria where water temperature was changed 1°C daily from 21°C until target temperatures (10 and 32°C) were reached and then held at target temperature for four weeks. For this study, individuals were transferred to a Blazka-style swim respirometer to assess swim performance (U_{crit} and U_{opt}), metabolic rate (SMR, MMR, and aerobic scope) and cost of transport (COT). U_{crit} and U_{opt} in 10°C minnows is decreased by 50% compared to 21 and 32°C minnows. Aerobic scope in 32°C minnows is 5.5 times higher than 10°C minnows but neither were different from 21°C minnows. COT and COT_{min} is not altered by thermal acclimation. To ascertain if observed results were temperature dependent or a product of acclimation, minnows (n=5) were acutely exposed (2°C change per hour) to target temperatures. Preliminary results show Ucrit was unchanged but exhibited a 2.5 fold increase in MMR and aerobic scope. This suggest that swim performance is a temperature dependent response but metabolic rate is not. This research was made possible by a grant from The Gulf of Mexico Research Initiative.

39.5

Hypoxia and ammonia exposures have differential, developmental-stage specific, and long-term consequences on the stress response in zebrafish

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Eutrophication and climate change are increasing the incidence of severe hypoxia and high environmental ammonia (HEA) in freshwater fish nursery habitats yet the short- and long-term consequences of these challenges on stress responsiveness in later life are largely unknown. To identify the potential programming effects of these environmental stressors in fish, we explored the consequences of HEA and hypoxia exposure during early life on the stress response of zebrafish. Larval exposure to HEA stimulated the endocrine stress axis, inhibited neuronal differentiation but also increased ammonia tolerance in later life. While early life HEA had little impact on the cortisol stress response to a repeat HEA exposure, it abolished a later life stress response to a novel vortex stressor in both larval and adult fish, suggesting that early-life HEA exposure can have persistent effects on the stress response. Larval hypoxia exposure also stimulated the endocrine stress axis, inhibited neuronal proliferation and differentiation, and increased hypoxia tolerance in later life. In contrast to the effects of HEA, larval hypoxia exposure inhibited the cortisol stress response to a repeat hypoxia exposure in larval fish, but had no effect on the cortisol response to a novel vortex stressor, and no sustained effects on the stress response to hypoxia in adults. Finally, although anoxia exposure in embryos had no effect on the stress response to hypoxia in later life, adults derived from anoxia-exposed embryos exhibited dominance during dyadic interactions and had lower whole body cortisol

levels. Anoxia-exposed embryos raised to adults were also more aggressive and had higher whole body testosterone levels. These results suggest that acute embryonic anoxia can favor the development of a dominant and aggressive phenotype, and that a disruption in sex steroid production may contribute to the programming effects of environmental hypoxia. Overall, while early life environmental challenges can affect the larval stress response and stress phenotype of adult zebrafish, our results also show that the programming effects of early life environmental stressors are both life stage- and stressor-specific, and dependent on exposure history. Acknowledgements: Supported by an NSERC Discovery Grant to NJB.

41: PLENARY LECTURE

41.1

Ecophysiology: Physiology can Inform Ecology, and Ecology Can Inform Physiology

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My training (late 1960s) was initially in animal ecology and subsequently in evolutionary biology. Not surprisingly, my approach to ecophysiology naturally reflects that ecological and evolutionary perspective. For most of my career, ecologists and evolutionary biologists largely ignored physiological data and insights. That began shifting about 25 years ago when biologists and others started becoming aware of climate warming, but also began to appreciate that the biological impacts of climate warming (and of other environmental perturbations) are inevitably mediated by and transformed by basic physiological processes. I will begin my talk by briefly summarizing my view of the history of ecophysiology in the 20thCentury as well as highlight a few papers that were key to my thinking, and then examine three case studies in ecophysiology. The first two demonstrate that a basic physiology perspective can sometimes be fundamental to understanding certain ecological issues: in fact, a failure to consider physiology can sometimes lead to incorrect answers. The third example demonstrates how an ecological perspective can lead to an awareness of physiological 'unknowns' and thus can motivate novel physiological studies. Overall, my talk develops the theme -- hardly original with me -- that ecophysiology provides synergies to multiple fields. Research supported by NSF grants (1978-2017).

*Indicates Invited Speaker

*Indicates Invited Speaker