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Transitioning from the Bench to the Classroom, an Education-Intensive Career

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The expectation for most PhD students working in a basic science field is to have their own laboratory and become independent scientists. Any deviation from this career path is typically not perceived as success from the perspective of traditional bench scientists, as addressed in "Career Paths Beyond the Ivory Tower" (7). This perception is often coupled with an unsupportive environment, making the transition to a different career path a challenging task.

When I decided to leave the laboratory and become a full-time educator, I met this challenge by seeking help and advice from colleagues, friends, and various resources, such as teaching-focused books and professional society education websites. The transition from the bench to the classroom came with its challenges, many of which I wished I would have known sooner. This article highlights some important lessons learned for making the transition from a position in a basic science laboratory to the classroom as a full-time teaching faculty.

I completed my PhD and postdoctoral training in a basic science field. Soon after my postdoctoral training, I accepted a nontenured position at a research-intensive university as a research assistant professor. Even though I was awarded a federal research grant and had salary support, I wanted to devote more time to teaching.

I thought I knew what teaching entailed because, during my graduate studies, I was asked to lecture and be involved in the laboratory portion

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A Matter of Opinion

Caveat Emptor

This article by Alice Ra'anan and Bill Yates was published October 16, 2015 by *Speaking of Research* (http://speakingofresearch.com/2015/10/16/caveat-emptor/) and is reprinted with permission. As of this writing, Santa Cruz Biotechnology, Inc., has denied USDA's allegations of misconduct.

A current USDA case involving a major antibody producer underscores the need for the research community to demonstrate its commitment to high standards of animal welfare.

On August 18-20, 2015, Santa Cruz Biotechnology, Inc. (SCBT) went before Administrative Law Judge Janice Bullard in Washington to rebut charges of Animal Welfare Act (AWA) violations at its California antibody production site.

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in courses in the Nursing, Nutrition, and Dental School for short periods of time. I really enjoyed these little moments of teaching. Then two separate opportunities to teach an entire course at a community college emerged. The first was when I was a postdoctoral fellow and then again when I was a research assistant professor, and I took them both. However, these part-time teaching opportunities did not fully prepare me for the transition to my next position as a full-time educator.

Teaching Is Not Just Lecturing

It was after I made the transition to teaching full time that I realized teaching was not merely lecturing but also involved engaging the students and so much more, including offering research and service activities to students. My new position was at a professional school, and I had to adapt the classroom material to the students' specific needs. I chose to tackle my new challenge by using the same scientific method approach I had used in the laboratory. My hypothesis became my way of teaching, the aims were my courses, and my test subjects were the students.

During the first years, I went through an immense amount of troubleshooting, including preparing lectures, writing exams, posting grades and lectures using different platforms, analyzing validity of questions, getting involved in shared governance, etc. Although not physically in the laboratory, I had created a laboratory in the classroom. Who would have thought that a PhD experience in a laboratory would have so many similarities with the classroom?

If you think about teaching as monotonous work, think again. Teaching is as diverse as the laboratory. Students are very different within the same course and among different semesters or years; you constantly change your teaching style, maybe even your teaching philosophy, evolving and adapting to the ever-changing world of education. Although similar to the laboratory experience, transferring from test tubes and animal models in the laboratory to human subjects in the classroom requires anticipation, adaptation, and training.

If you are considering becoming a full-time professional educator, then I would highly recommend the following, keeping in mind that all these aspects intertwine with each other.

1) Be Proactive and Get Ready by Getting Information Ahead of Time

It may seem obvious, but you should spend time researching this new world of teaching before making your move. A "teaching only" job involves more than teaching. Research and service are also part of your teaching portfolio, so carefully consider what is involved in an "education-intensive career." Collect feedback from others who have made a similar transition. You are not the first scientist to make this change, so take advantage of the experiences of those who made it. If you do not know anyone personally, ask friends and colleagues and contact scientists who have recently become primarily teachers. They are the best sources to give you thorough and valuable input. Ask detailed questions about technology in teaching, pedagogy, words of advice/mentoring, and start networking in academia.

I would also encourage you to gain your own experience before embarking in the full-time endeavor by looking for teaching, tutoring, or lecturing opportunities. Shadow a teacher, do some informal teaching in a guest lecture, course, laboratory, etc. Start acquiring those experiences early. There are several programs that provide training in college classrooms, such as the NIH Institutional Research and Career Development Award (*http://www.nigms.nih.gov/Training/CareerDev/Pages/TWDInstRes.aspx*).

Teaching is not only presenting materials in a lecture. It is not as sterile as the laboratory. It implies several aspects: *1)* helping to translate knowledge with a personal touch, dealing with students on a daily basis in the classroom, during office hours, through advising, and mentoring; *2)* keeping updated with technology advances that we allow to invade the classroom to keep up with the technology-savvy new generations of students; *3)* dealing with the pressure of exam preparation and grading; *4)* generating scholarship; and *5)* integrating service.

Be aware that research or scholarship are required in addition to service, which translates into more hours in meetings and committee work than you would anticipate, often exceeding lecture or laboratory time (4). Thus do your homework first and get your feet wet. What a better way to start!

2) Network, Network, and Network

Networking is key before, during, and after your transition into full-time educator (3). Before the transition, get input from colleagues outside and inside your institution fostering community. It is less complicated than what it seems. I contacted a few full-time teachers who had left my institution but had continued doing some bench research projects. They shared their experiences and answered my questions.

You do not have to be extroverted to network. You can contact people via e-mail, through social media, or in person. One of my contacts was kind enough to invite me to her lectures and gave me a tour of the institution, while encouraging me to apply for a position that was soon to be posted.

You can also attend conferences where teaching styles and experiences are shared and continue networking.

To succeed as an educator, practice to be a good communicator, network, be proactive, and volunteer for some committees. The more people you interact with, the more you'll know about your new institution and how to succeed in it. Outside of your institution, the APS also offers numerous committee opportunities (5); use them not only for professional development, as we will address later, but also as a networking tool.

3) Seek Professional Development

Basic science doctoral work and courses do not include pedagogy, but a PhD qualifies you for a teaching position without the necessary training in pedagogy. Before your transition, read about teaching techniques, pedagogy, learning styles, etc. It may seem daunting at first, but it will be worthwhile (2).

Once you start your new teaching position, utilize the faculty development courses/workshops/seminars offered by your institution. Make it a priority to attend teaching workshops or seminars. If they are not offered at your institution, find them at conferences. Experimental Biology (*experimentalbiology.org*) offers numerous professional development opportunities for basic scientists in the APS Teaching Section. In 2016, APS will hold the second Institute for Teaching and Learning (*http://www.the-aps.org/itl.aspx*), which is another good opportunity for professional development. The information gathered in these sessions will impact your teaching style and provide networking opportunities.

Do not attend professional development activities only as a part of your portfolio. It is not effective unless you use it to improve your instruction. Remember that effective teaching is the result of study, analyses, practice, and persistent hard work. You never can know enough about how a student learns, what facilitates or impedes learning; your students change, and unless you adapt to them, your teaching will not be effective.

During professional development activities, you will have the opportunity to meet professors at different stages of their career. Use their experience and advice to your benefit, keep in contact with them, and find a mentor. Some institutions provide you with a mentoring system, but others do not. Be proactive and find a mentor in your institution or outside if there is not a suitable mentor for your discipline on site. Finding a mentor is tremendously helpful and valuable for your teaching and for the "promotion and tenure" process inherent to your new career path as an educator.

Professional development will ensure you are up-to-date and will contribute to continuously solidify you as a teacher.

4) Measure, Assess, and Document

Just like in the laboratory, don't wait until the last minute to plan periodic assessments of your teaching style to determine what works and what does not work for you and your students. It is important to measure what and how much the students are learning (1). Is the learned material aligned with the course objectives and the university standards?

Nowadays, you have access to numerous assessment tools, including the ones provided by newer technologies found in many classrooms. Use them if you have them. There are endless assessments and evaluations that will enhance your teaching experience and the learning experience of the students (1).

The skills you bring from the laboratory will be very useful. In the laboratory you learned to document your experimental designs and measure your results. Assessment is vital to continue your research activities. All those years of building up patience, overcoming adversity, trying different alternatives, performing critical analyses, and problem solving will bear their fruit in this alternative path, too. The laboratory is now a classroom with PowerPoint and desks instead of a bench and test tubes.

It constitutes a big time commitment, so set up time apart for assessments, since they are invaluable because you can utilize assessments to build scholarship as you develop your curriculum vitae (see below). Publish your

observations. Invest your time in writing, and publish your teaching scholarship.

5) Protect Your Time

Time management is an important part of achieving your goals. Start with the goal in mind. There are numerous activities in a teaching-focused career that will take your time: scholarship, service, and outreach activities, in addition to class preparation, networking, seeking professional development, and assessing your courses and students, all of which are important aspects of your academic life. You can anticipate and plan for all these activities, dividing your day accordingly. Allow yourself to be flexible, since priorities may fluctuate weekly, but by proactive planning you will reduce the time that you need to work from deadline to deadline.

Administrative tasks and e-mails will also take your precious time away. You will have to decide between being productive vs. being available to students. Set up a time for office hours and include the time you will respond to e-mails in your syllabus. Plan your schedule and be productive in all areas. If you clearly communicate your plan, then students will be more receptive and the process will become more efficient.

Use a similar approach for administrative tasks. Make your calendar available to administration so they can plan around your schedule. Otherwise, they will never be considerate of your time. Ask them to respect your blocks of "busy" time that they can actually see in your shared calendar.

And finally, delegate tasks, especially to the right person, to efficiently protect your time and to allow you to focus on those tasks that need your complete attention.

It may seem daunting and you may not be able to efficiently manage your time during the first year, but by assessing and evaluating your schedule and goals you will eventually get there.

6) Develop Your Curriculum Vitae (CV)

Do not wait to develop your CV until it is time for promotion and tenure. Start on *day 1.* Your curriculum is important for your promotion and tenure, and is mainly evaluated according to three different aspects: teaching, research/scholarship, and service. Use your mentor, networking connections, and professional development opportunities to develop your teaching portfolio.

Know upfront how you will be evaluated; every institution has different processes. The emphasis placed on any of these three aspects in promotion and tenure reviews depends on the mission of the institution and how much weight the institution places on research vs. teaching. Tenure- and nontenure-track expectations differ; however, you should always dedicate time to each of the three aspects.

Service

It may not be in the job description, but service is an important part of being an educator.

You are expected to serve on committees at any institution, but you do not have to be part of every committee. Committee participation can result in very time-consuming tasks. Both meeting preparation and attendance will take time, so be selective but participate. Manage your time wisely. Serving on a committee will introduce you to colleagues and will help you establish connections in your institution that may prove useful when requesting the reference letters for the promotion or tenure process. You will be both networking and developing professionally at the same time while you are getting known by your peers. This is the time to demonstrate your involvement in the institution.

Community service and outreach activities are also part of your service commitment. Select what you can manage efficiently but, remember, do not be over-ambitious. Make sure you have sufficient time to excel in your service, otherwise it may be detrimental for your career.

Be involved with your professional societies, such as APS. For example, APS allows you to work on outreach during Physiology Understanding (PhUn) Week (6); to volunteer to serve as a meeting mentor for one of the APS Minority Travel Fellowship Award recipients, a Porter Physiology Development and Minority Affairs Committee; to contribute to professional development activities in the Teaching Section; and so on. There are numerous Society committees where you can contribute, network, and develop professionally.

Scholarship/Research

Work on scholarship and do not forget to document it. "Publish or perish" still holds true for education-intensive careers in which you are expected to produce scholarly work.

You can get scholarship in areas other than bench research, including education and technology. This refers to research in educational outcomes, learning styles, learning technology, or writing books or chapters that fit the needs of the courses and/or discipline you are teaching.

Alternatively, you may also choose to continue conducting bench research. If you choose bench research as your scholarly activity, you may continue your previous research at your institution or establish connections with other institutions with a greater involvement in bench research.

Regardless of which scholarship activity you choose, you should set a goal, assign time to work on it, and set a time frame to achieve it, and you are on your way. Be realistic – you will not be able to dedicate as much time to scholarship as when you were a bench scientist.

You can also get double credit, since outreach activities, new technologies, and service may be turned into scholarship activities. APS provides outreach opportunities that involve teaching, such as the aforementioned PhUn Week. It is easy to get involved and also very rewarding when you see the impact in the youth. You can design a protocol to perform during the PhUn Week activities and then present it at Experimental Biology, where you will network with a group of professionals all invested in the formation of the scientists of the future. Thus, with one event, you can do service and scholarship without the double time commitment.

Measuring and documenting the impact of service and outreach activities is very valuable when establishing the success of such activities. Earn double or triple credit by implementing new technologies in the classroom that can be utilized as a teaching tool, evaluation tool, and technology tool. Results from technological innovations in the classroom can be showcased in workshops and conferences, and reported as scholarly work. This is double-dipping while growing as a rounded educator.

Teaching

There is a lot of preparation time that goes into developing a course, and do not be fooled by the myth of "it is just the first couple of years." Developing a course is not a static but rather a dynamic process. You want to be up-to-date, finding alternative teaching methods and new technologies to keep up with your class demographics, personalities, and attitudes toward learning.

Teaching and technology are rapidly evolving, and you will have to adapt to them. You will develop your own "teaching philosophy" that will also be part of your "promotion and tenure" portfolio and will evolve with you.

Preparing to Teach

So, how do you get prepared for teaching? First, you need to learn the language of teaching (seek professional development). I highly recommend attending workshops and navigating the internet for articles on teaching theories and techniques to discover which one is yours and which one will be most efficient with your students. Buy a few good books about teaching. Get to know the school's librarian. You will quickly learn how useful such a relationship can be for acquiring books, obtaining loaners, etc.

Experiment with incorporating new teaching technologies, such as using iPads, tablets, Android platforms, hands-on, problem-based learning, and interactive devices. It can be a little time-consuming if you are not tech savvy, so seek help. Do not aim to try every single new theory that arises; some may work in your setting with your type of students and courses you teach and some may not. Also, do not try them all at once: start small and expand; otherwise, it may be a failure and not because of the methodology but due to the lack of proper preparation, time investment, and student perception.

Pearls of Wisdom

While there are more factors that may contribute to a successful transition into a full-time educator position, I included those which I thought were necessary and not so evident when I was planning the change in my career path. Changing career paths is easier said than done, but it helps to be prepared.

If you are considering an education-intensive career path, I offer four pearls of wisdom: *1)* anticipate what to expect by getting information ahead of time and evaluating your new path; *2)* be proactive by engaging in new activities, networking, and professional development activities while managing your time wisely; *3)* effectively use your time by using one activity for multiple outcomes, double credit, addressing any of the levels you will be evaluated on, such as teaching, service, and scholarship; and *4)* document your work by publishing your results and recording your activities, otherwise it will count for you, but it will not count for others in your path to success.

Using these suggestions as your guide will help you make your transition easier from the laboratory to the lecture hall. ●

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To comment on this article or ask a question of the author, see *www.the-aps.org/forum-transition*.

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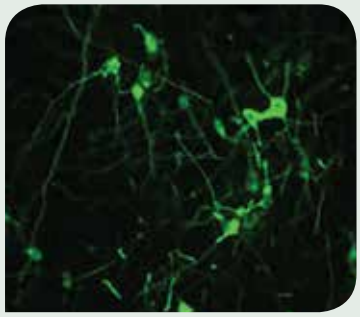
Caveat Emptor

The hearing was supposed to conclude on August 21. However, according to an account of the hearing posted by the Animal Welfare Institute ("Key Hearing in DC from August 18 to August 20" https://awionline.org/archived-action-ealerts/key-hearing-dc-august-18-august-20), the proceedings were suspended on the last day and the parties were given until September 30, 2015 to negotiate a settlement. As of this writing, no settlement agreement has been reached. Therefore, the allegations against SCBT remain just that–allegations: Final judgment must be withheld until the legal proceedings are concluded. Nevertheless, the seriousness of the USDA's charges against SCBT demands attention.

Why Antibodies Matter

Antibodies play an increasingly important role in both clinical medicine and research. The immune system generates antibodies when it detects a foreign protein. Antibodies are proteins that tag these "invaders," enabling other immune cells to find and destroy them. Because each antibody targets a single protein, they also have many useful applications. Antibodies can be used to diagnose and treat diseases, such as cancer (http://www.mayoclinic.org/diseases-conditions/cancer/in-depth/monoclonal-antibody/art-20047808), and autoimmune conditions including rheumatoid arthritis (http://www.webmd.com/rheumatoid-arthritis/guide/biologics) and inflammatory bowel disease (http://www.ccfa.org/resources/biologic-therapies.html). Just this past August, the U.S. Food and Drug Administration approved the antibody-based drug Repatha (evolocumab) (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm460082.htm), the second in a new class of drugs that can lower cholesterol dramatically by targeting a specific protein.



Antibodies "light up" a neurotransmitter in this sample of brain tissue (Yates laboratory, University of Pittsburgh)

Antibodies are also widely used in research to detect specific proteins in blood or tissue (see figure).
Antibody production is a multi-billion dollar industry, and SCBT is a major player.

Making Antibodies

Antibody production starts by injecting animals with the protein to be tagged. One production method involves collecting blood from animals injected with the protein and then extracting the antibodies. This method produces polyclonal antibodies that are comprised of a collection of immune cells.

Another method uses hybridoma technology, which produces monoclonal antibodies that consist of only one type of immune cell. This method also begins by injecting an animal with the protein to be tagged. The next step is to remove an initial batch of antibody-producing cells from the animal's blood and fuse them with a harmless cancer cell to produce a cell line that can generate the desired antibody in the lab. César Milstein and Georges J. F. Köhler shared the 1975 Nobel Prize in Physiology or Medicine for developing this methodology.

When performed properly, the creation of antibodies using either of these methods causes minimal pain or distress to animals.

SCBT produces antibodies with various animals, including goats and rabbits, species regulated under the AWA. The USDA sends inspectors at least once a year to visit all facilities that conduct research, teaching, or testing with regulated animal species to ensure their compliance with the AWA.

In a formal complaint filed August 7, 2015, the USDA accused SCBT of "repeated failures to provide minimally-adequate and expeditious veterinary care and treatment to animals" (https://speakingofresearch.files.wordpress.com/2015/10/usda-3rd-scbt-complaint-7-aug-2015.pdf, *paragraph 5*). USDA said further that the company had "demonstrated bad faith by misleading APHIS personnel about the existence of an undisclosed location" where goats were housed (2015 complaint, *paragraph 6*).

SCBT History of Non-Compliance Citations

This was not the first time SCBT has been cited for AWA compliance issues. According to the August 7, 2015 complaint, in July, 2005, the company paid a $4,600 penalty to resolve allegations of AWA violations from 2002 to 2004 (2015 complaint, *paragraph 7*). Seven years later, on July 19, 2012, USDA filed a complaint against SCBT alleging the following (see https://speakingofresearch.files.wordpress.com/2015/10/usda-1st-scbt-complaint-19-july-2012.pdf):

- SCBT failed to "establish and maintain programs of adequate veterinary care." (2012 complaint, *paragraphs III. B.-C* based on findings from a July 13, 2010 inspection; 2012 complaint, *paragraphs IV. C.-D,* based on findings from a February 8, 2011 inspection; and 2012 complaint, *paragraph VI. B. 5,* based on findings from a March 6, 2012 inspection).
- During the March 6, 2012 inspection, the inspector cited SCBT for not only having "failed to establish and maintain programs of adequate veterinary care under the supervision and assistance of a doctor of veterinary medicine," but also having "failed to provide veterinary care to animals in need of care." (2012 complaint, *paragraph VI. A*).
- On July 13, 2010, the USDA inspector cited SCBT for animal care staff who were not properly trained (2012 complaint, *paragraphs III. A.-B.* and *E.1*).
- On July 24, 2007, the USDA inspector cited SCBT for improper handling of animals (2012 complaint, *paragraph II.D.1.-2*).

The 2012 complaint also noted various shortcomings of SCBT's institutional animal care and use committee or "IACUC." According to the AWA, the IACUC is required to "assess the research facility's animal program, facilities, and procedures," including semi-annual inspections of the facilities that identify and report "significant deficiencies" [9 C.F.R. section 2.31 (c) (1-3)]. A significant deficiency is defined in 9 C.F.R. section 2.31 (c) (3) as a problem that "is or may be a threat to the health or safety of the animals." The IACUC is also required to review and approve animal use protocols before the research commences, to review and approve significant changes to ongoing protocols, and to ensure that animal pain and distress are minimized.

The 2012 complaint alleged these problems with SCBT's IACUC:

- The AWA requires the IACUC to determine that the principal investigator had considered alternatives to potentially painful procedures and had ensured that the animals' pain and distress would be minimized by providing pain-relieving drugs unless there was scientific justification to withhold them [9 C.F.R. 2.31 (d) (1) (ii)]. Alleged failures of the SCBT IACUC to do so were noted in the July 24, 2007 inspection (2012 complaint, *paragraphs II. B.-C*); the February 8, 2011 inspection (2012 complaint, *paragraphs IV.A.-B*); and the March 6, 2012 inspection (2012 complaint, *paragraph VI. B. 2*).

- The AWA requires the IACUC to review and approve significant changes to an ongoing activity [9 C.F.R. 2.31 (c) (7)]. On March 6, 2012, the USDA inspector cited SCBT for an alleged failure of its IACUC to review significant changes. (2012 complaint, *paragraph VI.B.1*).
- The AWA requires the IACUC to determine that animals are housed in conditions appropriate for their species [9 C.F.R. 2.31 (d) (1)]. On March 6, 2012, the USDA inspector cited SCBT for an alleged failure of its IACUC to ensure appropriate housing for animals at the facility. (2012 complaint, *paragraph VI. B. 3*).

2014 Hearing Delayed

The 2012 complaint was to have been adjudicated in 2014, but the hearing was called off 2 weeks before it was scheduled to take place. According to a July 1, 2014 notice issued by Administrative Law Judge Jill S. Clifton, the hearing was cancelled to give SCBT and USDA "ample time to meet to further their attempts to settle the case." However, no resolution to the allegations in the complaint was announced, and, during subsequent visits, USDA inspectors identified more alleged AWA violations at SCBT.

On November 4, 2014, USDA filed a second formal complaint listing alleged violations found during seven inspections between September 26, 2012 and April 22, 2014 (https://speakingofresearch.files.wordpress.com/2015/10/usda-2nd-scbt-complaint-4-nov-2014.pdf). The second complaint charged SCBT with having "failed to allow APHIS officials to inspect" a barn known as Lake Ranch/H7 "from at least March 6, 2012, through October 30, 2012" (2014 complaint, *paragraph III*). This complaint also listed additional instances of failures to provide adequate veterinary care based on findings from inspections of October 31, 2012 (2014 complaint, *paragraph IV. B*), December 18, 2012 (*paragraph V*), and February 20, 2013 (*paragraph VI*).

The 2014 complaint also included these allegations:

- The AWA requires the IACUC to ensure that the proposed activities or significant changes in ongoing activities "will avoid or minimize discomfort, distress, and pain to the animals" [9 C.F.R. 2.31 (d) (i)]. On September 26, 2012, the USDA alleged that SCBT's had failed to execute this requirement (2014 complaint, *paragraph II. A*).
- The AWA requires the IACUC to "review and approve, require modifications in (to secure approval) or withhold approval of proposed significant changes regarding the care and use of animals in ongoing activities" [9 C.F.R. 2.31 (c) (7)]. Alleged failures of the SCBT IACUC to do so were noted during the inspections of October 31, 2012 (2014 complaint, *paragraph IV.A*), May 14, 2013 (*paragraph VII*), and April 22, 2014 (*paragraph IX.A.-B*).
- The 2014 complaint further listed problems with the housing, food, and water provided to animals. These problems were noted in the September 26, 2012 inspection [cited in *paragraph II. C. 1-4* of the 2014 complaint as alleged violations of 9 C.F.R. Sections 3.125 (a), 3.129 (a), 3.131 (a) and (d)], in the October 31, 2012 inspection [cited in *paragraph IV.C.* as alleged violations of 9 C.F.R. Sections 2.26, 2.100 (a), and 3.131 (c)], in the September 10, 2013 inspection [cited in *paragraph VIII.1* as alleged violations of 9 C.F.R. Section 3.127 (a)], and in the April 22, 2014 inspection [cited in *paragraph IX. C.1-3* as alleged violations of 9 C.F.R. Sections 3.56 (a), 3.54 (a), and 3.129 (a)].

USDA'S Latest Complaint

The third USDA complaint was filed August 7, 2015 and reported by the Santa Cruz Sentinel under the headline "Santa Cruz Biotech Faces Third USDA Complaint Alleging Animal Mistreatment" (http://www.santacruzsentinel.com/health/20150819/santa-cruz-biotech-faces-third-usda-complaint-alleging-animal-mistreatment-shooting-goat-in-the-head). As noted above, this complaint asserted that the company had "demonstrated bad faith by misleading APHIS personnel about the existence of an undisclosed location where respondent housed regulated animals" (2015 complaint, *paragraph 6*). It also alleged "repeated failure" by SCBT "to provide minimally-adequate and expeditious veterinary care and treatment to animals" (*paragraph 5*). In support of this allegation, *subparagraphs 8. a.-n.* of the complaint describe 14 instances between 2011 and 2015 where USDA inspectors observed individual goats that appeared to be in poor health and lacking appropriate veterinary care. Several of these goats were thin, appeared anemic, or seemed to be suffering from infections (*subparagraphs 8 a., b., c., d., g., j., k., l., and m.*), whereas others had wounds or other injuries (*subparagraphs 8.e., f., and i.).*

These were two of the most serious cases:

- "Respondent failed to provide adequate veterinary care to a goat (#12267) that sustained a rattlesnake bite on April 28, 2012, and following initial treatment, the goat's condition did not improve, and the goat was not given any further treatment until its death. Specifically, the goat developed a visibly swollen jaw and chest and draining lesion and experienced a 23% weight loss (24 pounds) between April 28 and May 9, 2012. By APHIS's inspection on May 24, 2012,

the goat was observed to be unable or unwilling to close its mouth, which, in conjunction with the goat's other visible conditions, indicated that the goat was unable to eat normally. On June 10, 2012, the goat was observed to have labored breathing, but was not euthanized June 11, 2012." (2015 complaint, *subparagraph 8.f.*).

- "Respondent failed to provide adequate veterinary care to a goat (#21135) that had been diagnosed with urinary calculi [kidney stones] and treated with ace promazine. On July 7, 2015, at approximately 10:30 a.m., APHIS inspectors found the goat in a depressed posture, unwilling to walk, and breathing heavily. Respondent had no veterinarian available to attend to this animal: the respondent's 'on-site' veterinarian was on vacation, and respondent's staff could not contact respondent's attending veterinarian, or any other veterinarian who could provide emergency care. By 3:30 p.m., the goat was agonal [gasping for breath], suffering and in distress. Respondent failed to follow its own 'Standard Operating Procedure' for emergency goat euthanasia, which requires veterinary approval for euthanasia. As no veterinarian was available, respondent's staff used a captive bolt gun alone (without a sedative or secondary euthanasia injection) to effect euthanasia of the goat at approximately 4:15 p.m." (2015 complaint, *subparagraph 8.n.*).

As of this writing, there has been no judicial resolution of the alleged AWA violations by SCBT. That is to say, neither a settlement between USDA and SCBT nor a continuation of the administrative hearing has been announced. (***Update:*** On October 14, 2015, the USDA Administrative Law Judge ordered the suspended hearing to resume on April 5, 2016.)

Animal Welfare Matters

On February 14, 2014, Cat Ferguson wrote in *The New Yorker* about alleged animal welfare problems at SCBT, "Valuable Antibodies at a High Cost" (http://www.newyorker.com/tech/elements/valuable-antibodies-at-a-high-cost). On September 25, 2015, science writer Meredith Wadman published an opinion article in the San Jose *Mercury News* about the 4-day hearing the previous month. In "No Excuse for Cruelty to Goats Raised for Medical Research" (http://www.mercurynews.com/opinion/ci_28879871/meredith-wadman-no-excuse-cruelty-goats-raised-vaccines), Wadman opined that researchers were "the only constituency that Santa Cruz cares about," and urged them to "weigh in" using their purchasing power. According to Wadman, Matt Scott of the Carnegie Institution for Science and Pamela Björkman of the California Institute of Technology have stopped buying antibodies from SCBT. Wadman concluded by asking, "Is it too much to ask other scientists to follow suit?"

Testimony from USDA Veterinary Medical Officer Marcy Rosendale was reported in an account (https://awionline.org/archived-action-ealerts/key-hearing-dc-august-18-august-20#updates) of the August 18-20, 2015 hearing posted by the Animal Welfare Institute. According to this report, Rosendale said she had not observed the same number of animal welfare problems she found at SCBT at other antibody production facilities she had visited.

There is growing recognition that, to ensure the rigor of their work, scientists need more information about the antibodies they use actually, i.e., technical specifications such as what part of the target protein the antibody binds to. Perhaps it is also time to pay more attention to how those antibodies are produced.

USDA inspections are a matter of public record, but meeting the requirements of the AWA should only be the beginning. Antibody producers should be encouraged to take additional steps to affirm their commitment to animal welfare, such as by seeking independent accreditation of their production facilities through AAALAC. The point is that researchers and antibody producers alike must find tangible ways to demonstrate a commitment to high standards of animal care. ●

USDA documents cited:

1. USDA - 1st SCBT complaint 19 July 2012.
2. USDA - 2nd SCBT complaint 4 Nov 2014.
3. USDA - 3rd SCBT complaint 7 Aug 2015.

Speaking of Research invites scientists to advance public understanding of how humanely conducted animal studies contribute to scientific discovery through its "Speaking of Your Research" project. See http://speakingofresearch.com/get-involved/current-campaigns/ for information about how to write and submit a short article about what your research involves; its potential future applications; why animals are essential to the work; and how you consider the welfare needs of animals.

APS News

Novo Nordisk Foundation Continues Support of APS Awards

The Foundation will provide $100,000 over 5 years toward August Krogh Lecture and Bodil Schmidt-Nielsen Award

The American Physiological Society (APS) is pleased to recognize the support of the Novo Nordisk Foundation for the August Krogh Distinguished Lectureship of the American Physiological Society's Comparative and Evolutionary Physiology Section and the Bodil M. Schmidt-Nielsen Distinguished Mentor and Scientist Award. The foundation will provide $50,000 for each award, given in $10,000 increments over 5 years. Both awards will be presented at the Society's annual meeting at Experimental Biology.

"APS is pleased to be able to join with the Novo Nordisk Foundation to recognize the contributions made by the recipients of the August Krogh Distinguished Lecture Award and the Bodil Schmidt-Nielsen Mentor and Scientist Award to physiological understanding. Through the use of comparative approaches to study physiological function, Krogh and Schmidt-Nielsen have contributed significantly to the development of treatments and cures for disease that are celebrated through our partnership with the Novo Nordisk Foundation," says Martin Frank, executive director of APS.

The Novo Nordisk Foundation currently sponsors the August Krogh Distinguished Lectureship and has agreed to additionally support the Bodil Schmidt-Nielsen Award.

About the Awards

The August Krogh Distinguished Lecture of the APS Comparative and Evolutionary Physiology Section is awarded to a distinguished scientist who has made major and meritorious contributions to comparative and evolutionary physiology. The award recipient delivers an honorary award lecture at Experimental Biology, is recognized at the APS Comparative and Evolutionary Physiology Section's annual business meeting, and is invited to submit the lecture for publication in the *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology*. The awardee also receives a $1,000 honorarium and reimbursement of meeting-related travel expenses up to $2,000.

The Bodil M. Schmidt-Nielsen Distinguished Mentor and Scientist Award recognizes an APS member who has made outstanding contributions to physiological research and has demonstrated dedication and commitment to excellence in training and mentoring young physiologists. The award recipient receives a $1,000 honorarium and reimbursement of meeting-related travel expenses up to $1,500.

The Story Behind the Awards

The Comparative and Evolutionary Exercise Physiology Section named its distinguished lectureship after August Krogh because of his contributions to comparative physiology and because his daughter, Bodil Schmidt-Nielsen, a renowned physiologist, was a past APS president and active in the section. Krogh was awarded the 1920 Nobel Prize in Physiology or Medicine for his discovery of the process by which oxygen is supplied to the tissues.

The Novo Nordisk Foundation has a unique connection to Krogh and Schmidt-Nielsen. Krogh is one of its founders. During a lecture tour in the U.S. in 1922, Krogh met with Canadian scientists Frederick Banting, Charles Best, and John Macleod, who had successfully manufactured active insulin the previous year. Krogh received permission to use the methods developed and patented by the scientists to manufacture insulin in his home country of Denmark and the surrounding Scandinavian countries. One condition had to be met, however: The insulin had to be widely available and all profits from sales used for scientific and humanitarian purposes. Krogh formed the non-profit Nordisk Insulin Laboratorium and Nordisk Insulin Foundation, which later became Novo Nordisk A/S and the Novo Nordisk Foundation, to produce insulin on a large scale.

Schmidt-Nielsen was the youngest child of August and his wife, Marie Krogh. Schmidt-Nielsen received the Novo Nordisk Foundations Jacobæus Prize, awarded to internationally recognized researchers who have made significant contributions to medical research, in 1974, the year before becoming the first woman to be elected president of APS.

"By supporting the August Krogh Distinguished Lectureship and the Bodil Schmidt-Nielsen Distinguished Mentor and Scientist Award in collaboration with the American Physiological Society, the foundation wishes to celebrate the outstanding contributions both have made to physiology, as well as recognizing their contributions to the Novo Nordisk Foundation," says Niels-Henrik Holstein-Rathlou, chief scientific officer of the Novo Nordisk Foundation. ●

Chapter News

Nebraska Physiological Society 18th Annual Meeting

The Annual meeting of the Nebraska Physiological Society (NPS) was held on Saturday, October 10 at Sanford School of Medicine on the campus of University of South Dakota, Vermillion. The meeting became the 18th annual meeting of the NPS. The American Physiological Society (APS), Visual Sonics, DSI, VWR, WPI, ADInstruments, and Fisher Scientific, in part, financially supported the meeting.

Seventy-seven registered individuals, including undergraduate and graduate students, postdoctoral associates, and faculty members, participated in the scientific/educational conference. Overall, institutions from Nebraska and South Dakota were represented.

The scientific/educational sessions began with welcome and opening remarks from Doug Martin, President of the NPS from the University of South Dakota. Martin recognized Cindy Norton, Pearl Sorensen, Debra Davis, and Kim Kavan from the Department of Cellular and Integrative Physiology at UNMC and Wendy Pederson and Mike Olson from the University of South Dakota for their help in organizing the meeting.

Following Martin's introductory remarks, Bill Yates from the University of Pittsburgh made the APS-sponsored keynote address. His presentation was entitled "Multisensory Control of Blood Pressure." World Precision Instruments raffle drawing followed the presentation. Alicia Schiller, from University of Nebraska Medical Center, won the WPI Mouse Kit worth $250. Then there was a time for the break in which attendees were able to visit exhibitor booths and view posters. After that, the APS-sponsored Advocacy Address entitled "Research Advocacy: Why Your Voice Matters" was given by Kathryn Meier from Washington State University. The presentation was followed by the VWR Scientific raffle drawing and a coffee break. Jessica Freeling from the University of South Dakota won the VWR $250 gift certificate.

After the break, NPS oral presentations were given. These three oral presentations were selected by an NPS panel of judges before the meeting from the undergraduate, graduate, and postdoctoral categories based on merit. The first presenter was Cleofes Sarmiento, an undergraduate student from Wayne State College. His talk was entitled "Methotrexate Directly Scavenges Superoxide Generated by Xanthine Oxidase." The second presenter was Michael Price, a graduate student from University of Nebraska Medical Center. His talk was entitled "Alcohol-Induced S-Nitrosylation Drives Protein Phosphatase 1-Dependent Motile Airway Cilia Dysfunction." The postdoctoral presenter was Priyanka Prathipati, from University of Nebraska Medical Center. Her talk was entitled "Ablation of MMP9 Alleviates Mitophagy and Mitigates Cardiac Dysfunction in Diabetes."

The afternoon sessions commenced with the poster competition for undergraduate, graduate, and postdoc posters. Overall 21 posters, including 9 faculty posters, were presented during the 2-hour period allocated to poster viewing.

After the poster presentations, Bill Yates, Editor, *Journal of Neurophysiology;* Kathryn Meier, Associate Editor, *Journal of Pharmacology and Experimental Therapeutics;* and Irving Zucker, Editor, *American Journal of Physiology – Heart and Circulatory* coordinated a publication and ask-the-expert workshop. The workshop was very well attended and

NPS Oral Presentation Winners

Cleofes Sarmiento, undergraduate, Wayne State College

Michael Price, graduate, UNMC

Priyanka Prathipathi, postdoctoral, UNMC

created a great deal of discussion and interest among the trainees as well as faculty.

The afternoon session concluded with student awards and recognitions for oral presentations and posters. The award recipients received certificates and monetary awards of $200. The winner of undergraduate poster competition was Sarah Hill from Midland University. The winner of the graduate poster competition was Siguard Hartnett from the University of South Dakota. The postdoctoral winner for poster competition was Bryan Hackfort, from the University of Nebraska Medical Center.

At the closing of the conference, the NPS business meeting was called to order and chaired by NPS President Doug Martin. An update on outreach activities over the past year was reported by Alicia Schiller, from the University of Nebraska Medical Center. NPS Secretary/Treasurer Neeru Sharma, from the University of Nebraska Medical Center, then provided the treasurer's report. Martin presented the Past-President Award to Carol Fassbinder, from the Creighton University, for her service to the NPS. The NPS council members for 2014-2015 were then announced. President Yi-Fan-Li, University of South Dakota; Past-President Doug Martin, University of South Dakota; President-Elect Matthew Zimmerman, University of Nebraska Medical Center; Councilor Evelyn Schlenker, University of South Dakota; Lie Gao, University of Nebraska Medical Center; Adam Case, University of Nebraska Medical Center; Student Councilor Michael Price, University of Nebraska Medical Center; Secretary/Treasurer Neeru Sharma, University of Nebraska Medical Center; Executive Director Cindy Norton, CAP-OM, University of Nebraska Medical Center; CAC Representative Harold Schultz, University of Nebraska Medical Center. Afterward, Yifan Li, discussed new business and vision for the 2016 meeting of NPS. The meeting was then adjourned. ●

Neeru Sharma, PhD
Secretary/Treasurer

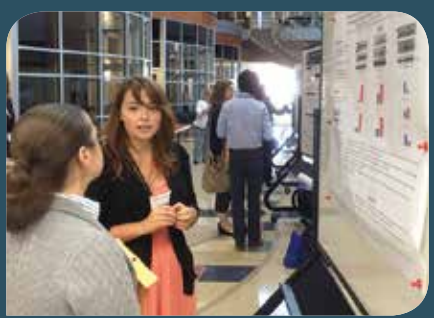
NPS Poster Award Winners

Sarah Hill, undergraduate, Midland University

Sigurd Hartnett, undergraduate, University of South Dakota

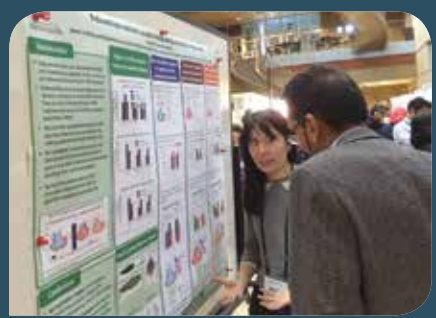
Bryan Hackfort, postdoctoral, University of Nebraska Medical Center

Poster Competition



Erika Boesen, Sabrina Schnack

Peter Pellegrino, Bill Yates



Yanqing Wang, Kaushik Patel

Experimental Biology

EB 2016 Distinguished Lectures

Physiology in Perspective – Walter B. Cannon Memorial Award Lecture

Amira Klip

Hospital for Sick Children, Toronto

Muscle-Immune Cell Crosstalk in the Genesis of Insulin Resistence

Saturday, April 2, 2016, 5:30 PM

Henry Pickering Bowditch Award

Sean D. Stocker

Penn State Coll. of Med.

Sodium-Sensing Central to Salt-Sensitive Hypertension

Sunday, April 3, 2016, 5:45 PM

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

David M. Pollock

Univ. of Alabama at Birmingham

Endothelin as a Master Regulator of Whole Body Sodium Homeostasis

Sunday, April 3, 2016, 4:15 PM

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

Benedito Honorio Machado

Sch. of Med. Ribeirao Preto-USP

Neurogenic Hypertension and the Secrets of Respiration

Monday, April 4, 2016, 8:00 AM

Claude Bernard Distinguished Lecturer of the APS Teaching of Physiology Section

Barbara E. Goodman

Univ. of South Dakota Sanford Sch. of Med.

An Evolution in Student-Centered Teaching

Sunday, April 3, 2016, 10:30 AM

Solomon A. Berson Distinguished Lecturer of the APS Endocrinology and Metabolism Section

Gerald I. Shulman

HHMI, Yale Univ. Sch. of Med.

Cellular Mechanisms of Insulin Resistance: Implications for Obesity, Type 2 Diabetes, and the Metabolic Syndrome

Monday, April 4, 2016, 10:30 AM

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

Paul A. Insel

Univ. of California, San Diego

GPCRomics: Discovering New *Ways Cells Communicate with One Another and the Outside World*

Sunday, April 3, 2016, 2:00 PM

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

Scott K. Powers

Univ. of Florida

Exercise: Teaching Myocytes New Tricks

Monday, April 4, 2016, 2:00 PM

Joseph Erlanger Distinguished Lecturer of the APS Central Nervous System Section

Quentin J. Pittman
Univ. of Calgary
Immune Stress and the Brain: Synaptic Substrates of Sickness
Monday, April 4, 2016, 3:15 PM

Robert M. Berne Distinguished Lecturer of the APS Cardiovascular Section

Stephanie W. Watts
Michigan State Univ.
Oh, the Places You'll Go! My Many Colored Serotonin (Apologies to Dr. Seuss)
Tuesday, April 5, 2016, 2:00 PM

Carl Gottschalk Distintuished Lectureship of the APS Renal Section

David H. Ellison
Oregon Hlth. Sci. Univ.
Aldosterone and Hypertension: What's the DCT Got to Do With It?
Monday, April 4, 2016, 3:15 PM

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

Supported by Novo Nordisk Foundation

Jon F. Harrison
Arizona State Univ.
Physiological and Evolutionary Interactions Among Body Size, Metabolic Rate and Oxygen
Tuesday, April 5, 2016, 3:15 PM

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

Gary C. Sieck
Mayo Clin. Col. of Med.
Mysteries and Maladies of Mitochondrial Dynamics
Tuesday, April 5, 2016, 10:30 AM

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

Bishr Omary
Univ. of Michigan
The Intermediate Filament Cytoskeleton: From Bench to Bedside
Tuesday, April 5, 2016, 3:15 PM

APS Nobel Prize Lecture

Roger Tsien
Howard Hughes Med. Inst., Univ. of California, San Diego
Molecules Against Cancer or for Very Long-Term Memory
Wednesday, April 6, 2016, 4:45 PM

Upcoming EB Symposia

Mark your calendars for professional development symposia at Experimental Biology 2016!

Saturday, April 2: 8:00 AM - 12:00 PM
Keep Your Eye on the Ion – A Refresher Course on Ionic Homeostasis and Systems Physiology (Medical Education Refresher Course)

Get an update on content from leading experts in the field: Regulation of Sodium Homeostasis and Hypertension (**John Osborn,** U Minnesota), Regulation of Potassium Homeostasis and Renal Disease (**Biff Palmer,** UT Southwestern Medical Center), Acid Base Disturbances and Regulation of Potassium (**L. Lee Hamm,** Tulane U School of Medicine), and Cardiac Ischemia: Ionic Currents and the ECG (**Richard Klabunde,** Marian U College of Osteopathic Medicine).

the-aps.org/refresher-ion

San Diego Convention Center, Rm. 24

Tuesday, April 5: 10:30 AM - 12:30 PM
Leadership and Management Skills: What You Might Not See in Your CV (Career Symposium)

A successful career in scientific research is filled with leadership and management opportunities. The challenge is in understanding what kind of leader you are, how you lead/manage, seeing the possibilities, and leading well. Learn how to explore, identify, and apply inherent and learned leadership/management skills.

the-aps.org/leadership

San Diego Convention Center, Rm. 25B

Monday, April 4: 3:15 - 5:15 PM
Negotiating for Success! (Mentoring Symposium)

Negotiation is both a skill and an art. Understanding your strengths and weakness will help you to best promote yourself and succeed in interviews and getting the position you are aiming for. Learn how to use the right tools and the right approach to succeed in any discipline.

the-aps.org/negotiating

San Diego Convention Center, Rm. 25C

Wednesday, April 6: 10:30 AM - 12:30 PM
Now Hiring PhD's: Post Doc Not Required (Trainee Symposium)

It is critical for trainees to become exposed to various career paths available in today's job market for PhD holders and to understand the skills necessary to attain those job opportunities. Learn about *1)* various career options that do not require a postdoc; *2)* how to get the experience and skills needed for those careers; and *3)* creative ways that graduate programs prepare trainees for diverse careers.

the-aps.org/hiring

San Diego Convention Center, Rm. 22

Experimental Biology 2016

April 2–6, 2016, San Diego, CA

PHYSIOLOGY PLATFORM SESSIONS

Saturday, April 2, 2016

Room			
Ballroom 20A			5:30 PM–6:30 PM Cannon Award Lecture **Klip**
Room 22			3:00 PM–5:00 PM *NCAR Section Award Session* Data NCARnation
Room 23	9:30 AM–11:30 AM *MCS Symp* Microcirculation: President's Symposium: Blood Cell-Microvessel Interactions **Rumbaut**	1:00 PM–3:00 PM *MCS Symp* Signal Integration and Microcirculatory Blood Flow Control: Making Parts Whole Using a Network Approach **Jackson**	3:30 PM–5:30 PM *MCS Symp* Advances in Microvascular Permeability/Glycocalyx **Breslin**
Room 24	8:00 AM–12:00 PM *Education Committee Refresher Course* Keep Your Eye on the Ion. Refresher Course on Ionic Homeostasis and Systems Physiology **Rodenbaugh/Scrogin**		2:15 PM–5:15 PM *WEH Section Award Session* WEH Trainee Award Finalists and Data Diuresis
Room 25A		1:00 PM–5:15 PM *PGG Special Session* 3rd Annual APS Physiological Genomics Group Conference	
Room 25B		1:00 PM–3:00 PM *ACE Committee Symp* Having Trouble with Your IACUC? **Henegar**	
Room 25C			3:00 PM–5:00 PM *Communications Committee Symp* Setting the Record Straight for Science: How to Write to Local and National News Outlets **Goodman**
Room 26		1:00 PM–3:00 PM *APS Workshop* Advanced Microscopy Techniques for the Study of Physiology **Kolar/Yosten**	3:15 PM–5:15 PM *APS Workshop* Novel Methods to Perturb Genes for Physiological Examination **Andresen/Joe**

Saturday, April 2, 2016, *cont.*

Room 27			6:00 PM–8:00 PM *MCS Special Session* Microcirculatory Society Reception and Poster Discussion
Room 28AB	9:00 AM–5:00 PM *ETG Conf* Pre-EB Meeting of the Epithelial Transport Group Young Investigators Symposium **Levi**		

Sunday, April 3, 2016

Room	8:00-10:00 AM	10:30 AM-12:30 PM	3:15-5:15 PM
Ballroom 20A		*President's Symp Series – Physiological Mechanisms Responsive to Behavioral and Environmental Challenges* Physiological Processes Underlying Organ Injury in Alcohol Abuse **Murray/Souza**	*Integrative Physiol Symp* Early Life Stress and Sex-Specific Manifestations of Cardio-Respiratory Dysfunction: Insight from Microglial Cells **Baldy/Dasinger** 5:45 PM–6:45 PM Bowditch Award Lecture **Stocker**
Room 22	*NCAR Section FT* NCAR Young Investigator Awards **Ramchandra/Limberg**	*PIC Symp* Metabolic Syndrome and the Pathway of Drug Development: From Bench to Bedside **Zahner/Cornelius**	*Hypoxia Group FT* Chemical Control of Autonomic Function in Health and Disease **Forster**
Room 23	*E&M Section Symp* New Insights into Exercise and Insulin Sensitivity **Richter/McConell**	*CV Section FT* Wiggers Award FT **Meininger**	3:15 PM–4:15 PM MCS Landis Award Lecture **Segal** 4:30 PM–5:30 PM *MCS* Business Meeting and Reception
Room 24	*CV Section Symp* Physiological and Pathological Aspects of Hypertrophic Cardiomyopathy **Steinberg/Sadayappan**	10:30 AM–11:30 AM Teaching Section Bernard Lecture **Goodman**	2:00 PM–3:00 PM CAMP Section Davson Lecture **Insel** 3:15 PM–4:15 PM WEH New Investigator Award Lecture 4:15 PM–5:15 PM WEH Section Starling Lecture **Pollock**

Sunday, April 3, 2016, *cont.*

Room 25A	*WEH Section FT* Neural and Hormonal Modulation of Fluid Balance and Ion Homeostasis in Health and Disease **Banek/Lob**	*CAMP Section FT* Microbiota or Nutrition and Host Cell Signaling **Worrell/Butterworth**	*CAMP Section Symp* Orai/STIM1 Physiology and Pathophysiology **Muallem/Delpire**
Room 25B	*Teaching Section Symp* Standing on the Edge: Transformational Teaching and Learning Beyond the Classroom Walls **Crecelius/Taylor**	*PGG Symp* Omics Applications in Metabolic Physiology **Olfert/Adams**	*CEP Section FT* Comparative and Evolutionary Physiology Trainee Driven FT **Warren/Ivy**
Room 25C	*MBG FT* Muscle Dysfunction in Diabetes: Cause(s) or Effect(s)? **Brozinick**	*Resp Section Symp* Macrophages: A Double-Edged Sword in Inflammatory Tissue Injury **Mehta/D'Alessio**	*Resp Section FT* Intermittent Hypoxia: Respiratory and Cardiovascular Control and Beyond **Solomon/Fields**
Room 26	*CV Section Symp* Microbiome in Cardiopulmonary Diseases: From Association to Causation **Shenoy/Pluznick**	*NCAR Section Symp* Bridging the Gap between Pre-clinical and Clinical Evidence: Treating Cardiovascular Diseases with Autonomic Modulation Therapies **Ruble/Sunagawa**	*NCAR Section Symp* The Brain-Gut Axis: Microbiome in Neural and Metabolic Diseases **Zubcevic/Raizada**
Room 27	*Renal Section FT* Advances in Kidney Physiology **Ortiz**	*Renal Section FT* Renal Section Young Investigator Symp: Novel Signaling and Transport Mechanisms in the Collecting Duct **Prieto-Carrasquero/Peti-Peterdi**	*EEP Section Symp* Emerging Mechanisms of Thermoegulation and Metabolic Control **Clanton/Periasamy**
Room 28A	*PGG Award Session* Physiological Genomics Trainee Highlights	*ETG FT* Epithelial Physiology and Transport I **Bomberger/Bradbury**	*EEP Section FT* What do Both Mitochondrial Protein Turnover and Mitochondrial Function Tell Us About Exercise and Aging? **Miller**
Room 28B	*TIPG FT* Translational Physiology Showcase: Focus on the Effects of Alcohol Abuse, Behavior, Diet, Nutrition, and Extreme Environmental Conditions on Physiology **Young/Bikman**	*CNS Section Symp* The Spinal Control of Motor Output: From Neural Circuits to Mechanics **Frigon/Nichols**	*CV Section FT* Sex Disparities in Cardiovascular Function and Remodeling **Goulopoulou**

Monday, April 4, 2016

Room	8:00-10:00 AM	10:30 AM-12:30 PM	3:15-5:15 PM
Ballroom 20A	*TAC FT* Sex Differences in Health and Disease **Ilatovskaya/Banek**	*APS President's Symp Series. Physiological Mechanisms Responsive to Behavioral and Environmental Challenges* Dietary Influences on Physiological Control Mechanisms – How Much, When and What **Anthony**	*Integrative Physiol Symp* Role of Epithelium in Innate Defence: More than a Barrier **Garnett**
Room 22	*CV Section Symp* Novel Insights in Vascular Disease in Metabolic Syndrome **Weber/Stepp**	*WEH Section Symp* Hydration Physiology: From Cells to Systems and Clinical Health Outcomes **Armstrong**	*E&M Section Symp* The Control of Skeletal Muscle Atrophy in Responses to Disuse: Clinical/Pre-Clinical Contentions and Fallacies of Evidence **Atherton/Lang**
Room 23	*E&M Section FT* Metabolic Consequences of Exercise **Steiner/Yosten**	*EEP Section Symp* Exercise and Cancer: From Cardiovascular Outcomes to the Tumor Microenvironment **Behnke/Jones**	3:15 PM–4:15 PM CNS Section Erlanger Lecture **Pittman** 4:15 PM–5:15 PM *CNS Section* Erlanger Lecture MiniSymp **Pittman/Stocker**
Room 24	8:00 AM–9:00 AM NCAR Ludwig Lecture **Machado**	10:30 AM–11:30 AM E&M Section Berson Lecture **Shulman**	2:00 PM–3:00 PM EEP Section Adolph Lecture **Powers** 3:15 PM–4:15 PM Renal Section Gottschalk Lecture **Ellison**
Room 25A	*GL Section FT* Innate Immune Functions of Epithelial Cells **Frey**	*CAMP Section FT* Cell Signaling: Proteins, Pathways, and Mechanisms **Rao/Rodrigues**	*PGG FT* Environmental Regulators on Microbiome-Mediated Immunity and Inflammation: Genetic and Epigenetic Implications **Claycombe/Meydani**
Room 25B	*Teach Section FT* Innovations in Teaching Physiology **Golden**	*Resp Section FT* Environmental Exposures, Oxidative Stress, and Lung Disease **Waters**	*Teach Section Symp* Scientific Foundation for Clinical Practice: More Than a Pile of Facts **Alarcón Fortepiani/Sanchez-Diaz**
Room 25C	*WEH Section FT* Hypertension: Developing Concepts **O'Connor/Ho**	*CEP Section FT* Avian Osmoregulation: Unique Solutions, Unanswered Questions **Sweazea/Goldstein**	*WIP Committee Symp* Negotiating for Success! **Mathis/Sweazea**
Room 26	*EEP Section Symp* Modulatory Influence of Exercise on Physiological Function with Aging **Seals/Booth**	*GIL Section Symp* Neuro-Immune Crosstalk in the Gut **Gulbransen/Lomax**	*Hypoxia Group Symp* Transcriptional and Epigenetic Regulation of Cardio-Respiratory Homeostasis under Hypoxia **Semenza/Ramirez**

Monday, April 4, 2016, *cont.*

Room 27	*CV Section FT* Cooperation Between Adaptive and Innate Immunity Post-Myocardial Infarction **DeLeon-Pennell/de Castro Bras**	*Renal Section Symp* Novel Mechanisms of Gene Regulation in the Kidney **Gumz/Hoover**	*NCAR Section FT* Vagal-Respiratory Coupling and its Implications in Health and Disease **Dutschmann**
Room 28A	*CV Section FT* Cerebrovascular Dysfunction and Reactive Nitrogen Species **Katakam/Pollock**	*ETG FT* Epithelial Physiology and Transport II **Hamilton/Helms**	*CV Section FT* Cardiopulmonary Effects of Environmental Stressors **Wold**
Room 28B	*Publications Committee Symp* Publishing 101: How to Get Your Work Published and Avoid Ethical Minefields **Sigmund/Scheman**	*CV Section Symp* Thyroid Hormone Modulation of Cardiac Function and Remodeling: Bench to Bedside **Portman/Gerdes**	*Resp Section FT* Inflammation and Its Influence on Lung Function and Respiratory Control **Wilson/Wilson**

Tuesday, April 5, 2016

Room	8:00-10:00 AM	10:30 AM-12:30 PM	3:15-5:15 PM
Ballroom 20A	*PG Journal and ASHG Symp* Beyond GWAS: Attaching Physiology to the Genome **Munroe/Wiltshire**	*President's Symp Series Symp* *Physiological Mechanisms Responsive to Behavioral and Environmental Challenges* Physiological Adaptation to Behavioral, Environmental, and Chronological Stress **Simon/Whitaker**	*Integrative Physiol Symp* Mechanobiology of Fibrosis across Organ Systems **Tschumperlin** 5:45 PM–7:45 PM APS Business Meeting
Room 22	8:00 AM–10:00 AM *GIL Section Special Session* John Forte GIL Section Distinguished Abstract Plenary Session **Uno/Zavros**	*CV Section Symp* Shear Stress-Induced Mechanotransduction in Endothelial Cells: Implications for Vascular Health and Adaptations to Physical Activity **Hellsten/Egginton**	*CV Section FT* Metabolic Regulation of Cardiac Function in Diabetes: Epigenetics and Posttranslational Mechanisms **Wende/Chatham**
Room 23	*CV Section Symp* Redox Mediated Endothelial Responses: Showcasing NOX2 Enzymes in Pathophysiology **Chatterjee/Sampath**	*CV Section Symp* New Insights into the Role of Autophagy in Cardiac Disease **Mellor/Jones**	3:15 PM–4:15 PM CEP Section Krogh Lecture *Supported by Novo Nordisk Foundation* **Harrison**
Room 24	*Resp Section Symp* Opioid-Induced Respiratory Depression: Sites/Mechanisms of Action and Potential Solutions **Forster/Miller**	10:30 AM–11:30 AM Respiration Section Comroe Lecture **Sieck**	2:00 PM–3:00 PM CV Section BerneLecture **Watts** 3:15 PM–4:15 PM GIL Section Davenport Lecture **Omary**
Room 25A	*WEH Section FT* Origins of Adult Cardiovascular and Metabolic Disease **Loria/Gillis**	*CAMP Section FT* Ion Channels and Transporters in Health and Disease **Kravtsov/Thai**	*CAMP Section Symp* Ion, Water, and Gas Movements through the Brain in Health and Disease: Putting it All Together **O'Donnell/Iliff**

Tuesday, April 5, 2016, *cont.*

Room 25B	*MBG FT* Skeletal Muscle Peroxisomal-Mitochondrial Interactions in Health and Disease **Cortright/Noland**	*Careers Committee Symp* Leadership and Management Skills: What You Might Not See in Your CV **Schnackenberg/Richards-Williams**	*E&M Section FT* Hormones and Reproduction **Samson**
Room 25C	*History Group Symp* A Broad History of Temperature Regulation **Leon/Kirkton**	*CEP Section Symp* Context Dependence of Cardiorespiratory Physiology: Temperature Effects, Circadian Cycles, and System Interdependence **Santin/Hartzler**	*MBG Symp* Gene Regulation in Skeletal Muscle **Nader**
Room 26	*CV Section FT* 2016 Gabor Kaley Lecture FT **Nourshargh**	*EEP Section Symp* Mechanisms of Neuromuscular Junction Destabilization and Fragmentation in Aging Skeletal Muscle **Jackson/Hepple**	*WEH Section FT* Cross-Talk Between Salt and Other Factors in Hypertension **Li**
Room 27	*Renal Section FT* Advances in Renal Pathology and Disease **Welling**	*Renal Section Symp* Still Unraveling the Mysteries of the Kidney with Isolated Tubules after All These Years **Sands/Brooks**	*CNS Section FT* Novel Mechanisms at the Level of the Solitary Tract Nucleus (NTS) **McDougall**
Room 28A	*CNS Section Symp* Building Neural Circuits: Wiring and Experience **Van Hooser/Cang**	*NCAR Section FT* The Mind Matters: Psychology as an Overlooked Variable in Autonomic Physiology **Wehrwein/Carter**	*EEP Section Symp* Mechanisms Regulating Skeletal Muscle Mass **Bodine**
Room 28B	*NCAR Section FT* Neural Control of Inflammation-Mediated Hypertension **Lazartigues**	*GIL Section FT* Chronic Liver Diseases Modulated by Transcriptional and Translational Mechanisms **Wang/Machida**	*Resp Section Symp* Neurostimulation to Restore Breathing with Neuromuscular Disorders **Mitchell/Streeter**
Marina Ballroom DE			1:00 PM–2:00 PM *History of Physiology Group Lecture* **Severinghaus**
Room 33ABC			3:00 PM–5:00 PM 2014 Tang Prize in Biopharmaceutical Science **Honjo**

Wednesday, April 6, 2016

Room	8:00-10:00 AM	10:30 AM-12:30 PM	2:30-4:30 PM
Ballroom 20A			4:45 PM–5:45 PM APS Nobel Prize Lecture **Tsien**
Room 22	*CV Section FT* New Approaches for Induction of Arteriogenesis **Rocic**	*TAC Symp* Now Hiring PhD's: Post Doc Not Required **Hernandez-Carretero/Dougherty**	
Room 23	*Pan-American Societies Symp* Cardiac Mitochondria: More than an ATP Powerplant **Antunes/Villa-Abrille**	*AFMR Symp* Emerging New Mechanism in Alcoholic Liver Disease **Liangpunsakul**	
Room 24	*Integrative Physiol Symp* Reprogrammed Cells as Models for Disease **Chilian/Zhang**		
Room 25A	*GIL Section Symp* Mechanisms Underlying Host-Microbial Interactions in Pathophysiology of Diseases, Using Gut Organoids and Animal Models **Dudeja/Sun**	*CAMP Section Symp* Recent Advances in the Structure and Function of Epithelial Tight Junctions **Rao/Vetrano**	*AFMR Symp* Natural Products: Biological Effects and Therapeutic Potential in Human Disease **Prabhakar/Wu**
Room 25B	*Resp Section Symp* Microtubules in Lung Disease and Recovery **Birukova/Stevens**	*Resp Section FT* Redundancy and Plasticity in Respiratory Control **Bavis/Nichols**	
Room 25C	*CNS Section FT* Breathing Disturbances in Neurological Disorders **Moreira**	*CEP Section Symp* Comparative Physiology of Skeletal Muscle – Novel Studies in Plasticity and Structure **Rourke/Horner**	*NDOGS Special Session* ORPHEUS – Developing Best Practices for Graduate Education in Europe **Barnett**
Room 26	*TPIG Symp* Novel Molecular Targets and Therapeutic Approaches in Myocardial Infarction and Heart Failure **Koch/Sharp**	*CV Section FT* Endothelial Dysfunction in Diabetes **Dokken/Meininger**	
Room 27	*Renal Section Symp* Renal Potassium Sensing Mechanisms: A New Paradigm for Potassium Secretion **Ellison/Subramanya**	*ETG Symp* Compartmentalization of Signal Transduction in Epithelial Cell Biology **Fenton/Rieg**	
Room 28A	*EEP Section FT* Hot, Cold, and Old: Aging and the Physiology of Thermal Stress **Schlader/Gagnon**	*EEP Section FT* Recovery from Exercise and Translating Post-Exercise Hypotension **Baynard**	
Room 28B	*E&M Section Symp* Role of Oxytocin in the Control of Energy Homeostasis **Blevins/Samson**	*NCAR Section FT* Actions and Interactions of Baroreflexes, Chemoreflexes and Metaboreflexes in Autonomic Regulation and Heart Disease **Amann/Fadel**	

Education

APS Promotes Physiology to K-12 Teachers at Fall Meetings

The APS highlighted physiology for middle school science teachers and administrators at the annual Association for Middle Level Education (AMLE) Conference held in Columbus, OH from October 14th to 17th. This was the sixth 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 science society's presence, since so few opportunities are available for science-related materials at this meeting. The APS booth was extremely busy and well received with many questions about the APS Life Science Teaching Resource Community, age-appropriate careers materials such as the career trading cards, and the new online Six Star Science Professional Development Fellowship. Next year's conference will be held in Austin, TX from October 9th to 12th.

APS also promoted physiology for K-12 biology teachers at the National Association of Biology Teachers (NABT) Conference in Providence, RI. 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. APS sponsored an exhibit booth and featured speaker as well as presenting two hands-on workshops that highlighted how to transform cookbook labs, the APS Life Science Teaching Resource Community (*www.LifeSciTRC.org*), career materials, and K-12 outreach.

Donald Jackson

This year's sponsored speaker was APS member Donald Jackson, Brown University. Jackson presented "Living Without Oxygen – Lesson from Animal Physiology," an entertaining and informative talk that shared what he learned studying anaerobic metabolic end-products, conserving metabolic substrates, and protecting the heart and brain from irreversible damage in freshwater turtles, such as the painted turtle, and the crucian carp, a close relative of the goldfish.

In a hands-on workshop led by Margaret Shain Stieben (Program Manager, K-12 Education Programs), attendees explored how to transform cookbook labs into ones that *1)* actively engage students in developing hypotheses and methods and exploring concepts; *2)* address a wide variety of learning styles and cultures; and *3)* effectively integrate internet resources. During this standing-room-only workshop, sample labs were rewritten and lessons/labs that are currently available in the *APS Life Science Teaching Resource Community* were discussed.

Patricia Halpin

Patricia Halpin, University of New Hampshire, presented a second workshop, "Physiology: A How-To for K-12 Outreach in an Undergraduate Setting." This session was a primer for undergraduate educators on how to establish K-12 outreach programs for both personal and institutional benefit. Three programs were discussed, followed by a hands-on demo and a 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 provided and discussed with community college attendees. Next year's conference will be held in Denver, CO. For further information, please contact Margaret Shain Stieben, Program Manager, K-12 Education Programs (*mshain@the-aps.org*). ●

Donald Jackson and Patricia Halpin

APS Participates in the 2015 Eastern Regional HAPS Conference

Erin Keen-Rhinehart and Jan Foster
Susquehanna University and North Greenville University


Erin Keen-Rhinehart


Jan Foster

More than 60 Anatomy and Physiology educators from the Eastern United States gathered on October 3rd for the Eastern Regional Human Anatomy and Physiology (HAPS) conference. This year, the conference was hosted by local organizer, Terry Thompson, from Wor-Wic Community College in Salisbury, Maryland.

APS was pleased to sponsor the workshop "Student Centered Learning in Physiology Courses." The workshop was presented by APS members Erin Keen-Rhinehart (Associate Professor, Susquehanna University, Selinsgrove, PA) and Jan Foster (Assistant Professor, North Greenville University, Tigerville, South Carolina).

This workshop introduced various evidence-based student-centered teaching methods. Attendees shared their interests in implementing strategies, such as team-based learning, problem-based learning, and online polling in their physiology classes. Speakers shared information about available APS resources to support excellence in physiology education. For example, speakers informed attendees about the Life Science Teaching Resource Community (www.lifescitrc.org), an online repository of more than 7,000 peer-reviewed teaching resources and community interaction tools. In addition, the workshop included information about the Physiology Education Community of Practice (PECOP; *www.lifescitrc.org/PECOP*), which was formed as an outgrowth of the first bi-annual APS Institute on Teaching and Learning in 2014.

The workshop was well received, garnering participation from approximately one-third of the conference attendees, and it provided an engaging presentation and a lively interactive discussion. Participation in the workshop was supported by NSF RCN-UBE Incubator Grant DBI-1346220. ●

Erin Keen-Rhinehart is an Associate Professor at Susquehanna University in Selinsgrove, PA. Her research program investigates the effects of neonatal energy restriction on the programming of adult appetite regulatory systems and reproductive function. Erin is enthralled by coming up with new ways to teach students challenging concepts interactively. She also enjoys showing students how seemingly complicated scientific information applies to their everyday life. She routinely has undergraduates working in her lab conducting research. She was awarded a Faculty Early Career Development Grant by the National Science Foundation recently. Erin was a 2014 PECOP Fellow.

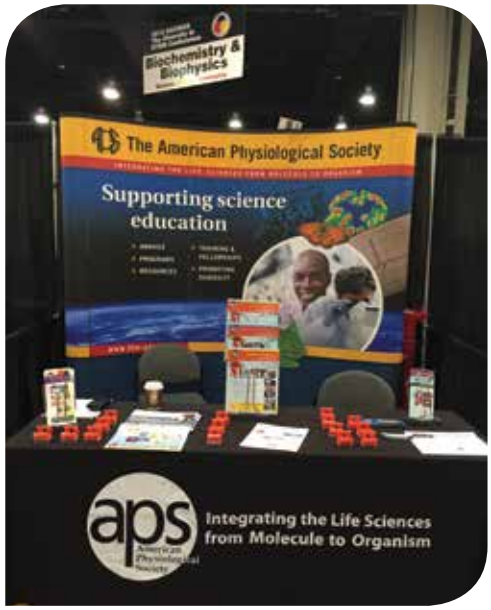
Jan Foster is an Assistant Professor at North Greenville University in Tigerville, SC. She regularly teaches Human Anatomy and Physiology, Histology, and Advanced Physiology. Jan received her PhD in Biomedical Sciences from the Medical College of Georgia in Augusta, GA. She regularly incorporates student-centered learning activities in all of her classes. Additionally, she recently designed a new course, Human Advanced Physiology, to be an entirely primary literature-based student-led discussion course. Jan was a 2014 PECOP Fellow.

APS at SACNAS 2015

The APS was an exhibitor at the 2015 Society for the Advancement of Chicanos and Native Americans in Science (SACNAS) annual meeting at the Gaylord National Resort & Convention Center in National Harbor, MD from October 29 to 31. The theme this year was "Interdisciplinary Collaboration: The Role of Diversity in STEM Innovation."

The APS, represented by Rolando J.J. Ramirez (University of Akron and Porter Physiology Development and Minority Affairs Committee Member), Nicholas Aguirre (University of California, Davis and K-12 Minority Outreach Fellow), and Brooke Bruthers (Senior Program Manager, Diversity Programs), staffed the exhibit booth.

The SACNAS National Conference is designed to motivate, inspire, and engage participants to achieve their highest goals in pursuing education and careers in all disciplines of science, technology, engineering, and mathematics from across the country. Conference programming is specifically tailored to support undergraduate and graduate students, postdoctoral researchers, and career professionals at each transition stage of their career as they move toward positions of science leadership. The conference showcases cutting-edge science and features mentoring and training sessions for students and scientists at all levels. Nearly 4,000 attended the conference, and more than 300 exhibits shared training, research, grad school, and job opportunities. For more information about the SACNAS National Conference, visit *www.sacnas.org*. For more information on APS diversity programs, visit *www.the-aps.org/diversity*. ●



Missed Experimental Biology 2015?
OR
Attended EB2015 but missed APS career/trainee/mentoring/education sessions?

You can still attend them!
Listen to the talks and view the PowerPoint presentations for:

Refresher Course
It's All in Your Head – A Refresher Course on the Brain and Systems Control
the-aps.org/refresher-brain

Mentoring Symposium
Mentoring for Diverse Careers: Mentor and Protégé Perspectives
the-aps.org/mentoringdiversecareers

Career Symposium
Resilience is Power: Dealing with the Ups and Downs of Your Scientific Career
the-aps.org/resilience

Trainee Symposium
Scientists as Supervisors: Hiring, Firing and Beyond
the-aps.org/supervisor

APS Awards and Fellowships

David S. Bruce Awards for Undergraduates in Research

Application Deadline: January 12, 2016

the-aps.org/bruce

See the website for more details and apply online at the-aps.org/awardapps

The David S. Bruce Undergraduate Awards are presented annually to undergraduate students who are first authors on an Experimental Biology (EB) abstract and presenting their research at the EB meeting. There are two types of Bruce Awards that students can apply for through a single application.

David S. Bruce Outstanding Undergraduate Abstract Awards

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

David S. Bruce Excellence in Undergraduate Research Awards

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

Porter Physiology Development Fellowships

Application Deadline: January 15, 2016

the-aps.org/porter

Apply online at the-aps.org/awardapps

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

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

Dale J. Benos Early Career Professional Service Award

Application Deadline: January 24, 2016

the-aps.org/benos

The Dale J. Benos Early Career Professional Service Award honors an early career stage (graduate student, postdoctoral fellow, Assistant Professor, or equivalent position) member of APS. The Award will honor someone who is judged to have made outstanding contributions to the physiology community and demonstrated dedication and commitment to furthering the broader goals of the physiology community. This can be by serving on professional committees, participating in K-12 education outreach, participating in scientific advocacy and outreach programs, or by otherwise strengthening and promoting the physiology community.

See the website for more details and apply online at **the-aps.org/awardapps**

Undergraduate Summer Research Fellowships

Application Deadline: February 1, 2016

the-aps.org/summerresearch

APS is proud to offer four programs that allow undergraduate students to participate in research during the summer (IOSP, STRIDE, UGSRF, UGREF). Recipients spend an average of 10 weeks in the laboratory of an established scientist and APS member. Each program recruits undergraduate students nationwide, two internationally. Some programs are open to students from disadvantaged backgrounds, from underrepresented racial and ethnic groups, and students with disabilities. Each Fellow receives a stipend plus additional funds for travel to present his or her research at a scientific meeting. Research hosts receive funds for student lab supplies.

See the website for more details and apply online at **the-aps.org/awardapps**

APS/NIDDK STEP-UP Undergraduate Summer Research Fellowships

Application Deadline: February 15, 2016

the-aps.org/stepup

STEP-UP Fellows spend an average 8-12 weeks in the laboratory of an established scientist conducting research in the NIDDK mission areas. This program is open to students from disadvantaged backgrounds, from underrepresented racial and ethnic groups, and students with disabilities. Each Fellow receives a stipend plus additional funds for travel to present his or her research at the STEP-UP summer research symposium. Research hosts receive funds for student lab supplies.

See the website for more details and apply online at **a://1.usa.gov/1HyYspl**

Science Policy

APS Members Urge Congress to Raise Budget Caps and Support Research

In September, members of the APS Science Policy Committee (SPC) went to Capitol Hill to meet with their Senators and Representatives. Members of the Animal Care and Experimentation (ACE) held its Capitol Hill Day in October.

Participants in both Hill Day events used the Twitter hashtag #HillDayAPS to reinforce their messages.

SPC Capitol Hill Day: September 21, 2015

The SPC had 27 meetings with 14 Republican and 13 Democratic offices. The SPC highlighted the importance of raising overall budget caps to allow for increased funding for research at the National Institutes of Health (NIH) and the National Science Foundation (NSF). The SPC also discussed pending reauthorization bills for the NIH and NSF.

To learn how you can be an advocate for research, see *the-aps.org/SciencePolicy*.

Giovanna Collu, Katherine Wilkinson, and Amy Davidoff in the Hart Senate Office Building in front of "Mountains and Clouds" by Alexander Calder

Alicia Schiller with Senator Deb Fischer (R-NE)

David Pollock, Jennifer Pollock, and John Chatham in front of the Longworth House Office Building

Chris Westby, Kay Meier, and Allyson Hindle outside the Russell Senate Office Building

Alice Ra'anan, Amy Davidoff, and Giovanna Collu in the office of Congresswoman Chellie Pingree (D-ME 1)

Congressman Gregg Harper (center, R-MS 3), with Linda Yang, Mike Ryan, Karen Uray, and Teresa Luperchio

ACE Committee Hill Day: October 21, 2015

The ACE committee participated in 16 meeting with 11 Republican and 5 Democratic offices. They discussed the importance of establishing regulations that ensure animal welfare and accountability for research funds, but prevent unnecessary administrative burden. The ACE also discussed the need for safe and reliable air and ground transportation for research animals (see the APS position statement on research animal transportation to learn more about this issue: *the-aps.org/AnimalTransport*). ●

Senator Thad Cochran (R-MS) with Mike Ryan

Dan Warren, Lauren Stein, and Barbara Hansen outside the Capitol Building

Congressman David Price (center, D-NC 4) with Richard Auten and Michael Christe

Congressman David Jolly (R-FL 13) with Barbara Hansen

APS Urges U.S. to Oppose International Restrictions on Ketamine

On October 14, 2015, the APS submitted comments providing the Food and Drug Administration (FDA) with information about the importance of ketamine in clinical and research settings. The FDA had requested input on how to represent U.S. concerns at the 36th meeting of the World Health Organization's Expert Committee on Drug Dependence (ECDD), scheduled to take place November 16-20, 2015. WHO asked the ECDD to review a list of psychoactive substances deemed to have significant potential for dependence, abuse, or harm.

Ketamine is a Schedule III controlled substance in the U.S. but is not currently restricted under international treaties such as the Psychotropic Convention or the Single Convention on Narcotic Drugs. Ketamine abuse is a serious problem in China, where criminal gangs have acquired the expertise to synthesize it. The ECCD reviewed the status of ketamine at several recent meetings in response to requests from China to restrict its availability. This resulted in the formal request for comments from member states.

The APS comments focused on the importance of ketamine and included these points:

- Ketamine is currently classified in the U.S. as a Schedule III drug under the Controlled Substances Act. Consequently, it is strictly regulated, and safeguards are in place to prevent its illegal or unauthorized use. A change to this status would have deleterious impacts in clinical and research settings, where ketamine is approved as an anesthetic for both humans and animals.

- Ketamine is used for sedation and analgesia in clinical veterinary practice as well as in animal research. Because ketamine acts rapidly and its effects are short-lived, it is very useful for short surgical procedures in species ranging from rodents to non-human primates. Ketamine promotes animal welfare because the risk of overdose is low, so there are fewer complications.

- For these same reasons – quick onset of effect, rapid recovery, and low risk of complications – ketamine is also used in human surgeries for pediatric patients.

- In longer surgeries in human and animal patients, ketamine is used in combination with other drugs to produce stable anesthesia with fewer side effects.

- In research settings, ketamine is often the anesthetic of choice for short surgical procedures in rats and mice, such as to implant medical devices such as catheters, pressure transducers, and osmotic mini-pumps. These implants enable researchers to measure physiological processes with less handling of the animals. Since ketamine provides rapid recovery with a minimal risk of complications, it further promotes the animal welfare objectives of implants, namely to collect data with a minimal impact on the animals.

- Ketamine has attributes that are essential for certain kinds of research. It is preferable to other anesthetic agents when cortical neuron activity must be measured because it does not silence the spontaneous activity of those neurons. Without ketamine, it would be impossible to conduct many electrophysiological studies and optogenetic studies. Ketamine is also one of the few anesthetics that does not disturb glucose metabolism, making its use critical to studies of diabetes and glucose tolerance.

- Ketamine is incorporated into the research protocols for a large percentage of preclinical research studies. Changing the international regulation of ketamine would have a deleterious impact on this research by severely limiting access to an important anesthetic agent.

- We urge the FDA to safeguard the welfare of humans and animals by strongly opposing any changes to the international regulation of ketamine that would make this drug less accessible to physicians, veterinarians, and research scientists.

Despite China's concerns about ketamine's potential for abuse, it also has been noted that ketamine is widely used in the developing world, where it plays a crucial role by providing safe anesthesia at clinics in remote areas. ●

NIH Simplifies the Vertebrate Animal Section of Proposals

On October 13, 2015, NIH's Office of Extramural Research issued new instructions for completing the Vertebrate Animal Section (VAS) of NIH grant applications, cooperative agreements, and contract proposals. The updates are intended to eliminate redundancy with oversight by the Institutional Animal Care and Use Committee (IACUC) while still meeting the requirements of the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

These requirements apply to all NIH-funded work with live vertebrate animals, including those obtained or euthanized for tissue harvest or to generate custom antibodies. The requirements will go into effect for grant applications starting January 25, 2016. A checklist of items to include and an example of an acceptable VAS may be found at *http://grants.nih.gov/grants/olaw/vertebrate_animal_section.htm*.

Summary of Changes to VAS

According to NIH Notice NOT-OD-16-006, a description of veterinary care and justification for the number of animals used in a study are no longer required. The proposed euthanasia method need only be described if it is not consistent with the American Veterinary Medical Association (AVMA) guidelines.

Criteria for Applicants

All applicants must respond to the first three items. They must respond to the fourth item only if applicable (NIH website on VAS):

1) Describe the proposed use of animals for the study. Identify the species, strains, ages, sex, and total number of animals. If dogs and cats are involved, include their source.
2) Justify that the species is appropriate for the proposed research. Explain why research goals can't be accomplished with an alternative model (e.g., computational, human, invertebrate, in vitro).
3) Describe procedures that will alleviate discomfort, stress, pain, and injury, such as the use of analgesia, anesthesia, sedation, palliative care, and humane end points. If tranquilizers, analgesics, and anesthetics are used, include the name and class of the drug.
4) Indicate whether the proposed method of euthanasia is consistent with the AVMA guidelines. If not, describe the method and provide a scientific justification.

Criteria for Reviewers

Reviewers must determine whether the use of vertebrate animals is appropriate for the proposed scientific work and whether the applicant has addressed all the required items of the Vertebrate Animal Section. ●

Publications

Current Calls for Papers

Physiological Genomics

- Gut Microbiota in Health and Disease
- Systems Biology and Polygenic Traits

Journal of Neurophysiology

- Auditory System Plasticity *(Submission deadline: July 1, 2016)*
- Comparative Approaches in Neurobiology *(Submission deadline: July 1,2016)*
- Glial Cells and Neuronal Signaling *(Submission deadline: July 1, 2016)*

Advances in Physiology Education

- Pre-Professional Education in Transition
- Historical Perspectives and Living Histories

American Journal of Physiology – Gastrointestinal and Liver Physiology

- Microbiome and Host Interactions
- Nutrient Sensing, Nutrition, and Metabolism
- Systems Biology
- Translational Human Pathophysiology

American Journal of Physiology – Heart and Circulatory Physiology

- Cardiovascular Mitochondria and Redox Control in Health and Disease *(NEW deadline: January 15, 2016)*
- Small Vessels – Big Problems: Novel Insights into Microvascular Mechanisms of Diseases *(Submission deadline: January 15, 2016)*
- Cardiovascular Epigenetics: Phenotypes and Mechanisms *(Submission deadline: January 31, 2016)*
- Quantitative Analyses of Coronary Vascular and Cardiac Mechanics in Health and Disease *(Submission deadline: January 31, 2016)*

American Journal of Physiology – Lung Cellular and Molecular Physiology

- Electronic Cigarettes: Not All Good News? *(Submission deadline: October 1, 2017)*
- Ion Channels and Transporters in Lung Function and Disease
- Age-Related Dysfunction in Lung Barrier Function in Health and Disease
- Real-Time Visualization of Lung Function: from Micro to Macro
- Bioengineering the Lung: Molecules, Materials, Matrix, Morphology, and Mechanics
- Biomarkers in Lung Diseases: from Pathogenesis to Prediction to New Therapies
- Sex Differences in the Respiratory System
- Translational Research in Acute Lung Injury and Pulmonary Fibrosis

American Journal of Physiology – Regulatory, Integrative and Comparative Physiology

- Sex and Gender Differences in Cardiovascular, Renal and Metabolic Diseases *(Submission deadline: June 30, 2016)*

American Journal of Physiology – Renal Physiology

- Endothelin in Renal Physiology and Disease *(Submission deadline: June 30, 2016)*
- Imaging Techniques in Renal (Patho)physiology Research *(Submission deadline: June 30, 2016)*
- Inflammation and Inflammatory Mediators in Kidney *(Submission deadline: June 30, 2016)*
- Purinergic Signaling Mechanisms in the Lower Urinary Tract *(Submission deadline: June 30, 2016)*
- Mechanism and Treatment of Renal Fibrosis and Treatment *(Submission deadline: June 30, 2016)*

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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UAH, Huntsville, AL

Njotu Agbor
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Gay R. Holstein
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Rongrong Liu
Fourth Military Med. Univ., Xi'An, China

Yin Liu
Stanford Univ., Stanford, CA

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Washington Univ. Sch. of Med., St. Louis, MO

Claire H. Mitchell
Univ. of Pennsylvania, Philadelphia, PA

Esteban Moya
Univ. of California-San Diego, La Jolla, CA

Shengyu Mu
Univ. of Arkansas for Med. Sci., Little Rock, AR

Fiona Murray
Univ. of Aberdeen, Aberdeen, United Kingdom

Klaus Olgaard
Rigshospitalet, Copenhagen, Denmark

Matthew Orange
Central Connecticut State Univ., New Britain, CT

Per Petersson
Lund Univ., Lund, Sweden

Paulo Wagner Pires
Univ. of Nevada Sch. of Med., Reno, NV

Khaled Qanud
New York Med. Coll., Valhalla, NY

Adam J. Sachs
The Univ. of Ottawa, Ottawa, ON, Canada

Luis Fernando Santana
Univ. of California-Davis, Davis, CA

Brad A. Seibel
Univ. of Rhode Island, Kingston, RI

Jai Shetake
Boston Scientific Neuromodulation, Santa Clarita, CA

Bruno Silva
Federal Univ. of Sao Paulo, Sao Paulo, Brazil

Komal Sodhi
Marshall Univ. Sch. of Med., Huntington, WV

Martin Szinte
Ludwig-Maximilians-Univ. München, Munich, Germany

Tom Taylor-Clark
Univ. of South Florida, Tampa, FL

Guillermo Vazquez
Univ. of Toledo Coll. of Med. and Life, Toledo, OH

Weihua Wang
Univ. of Arizona, Tucson, AZ

T. Cooper Woods
Tulane Univ., New Orleans, LA

Piet Albert Henri Wyffels
Ghent Univ. Hosp., Ghent, Belgium

Masaaki Yoshigi
Univ. of Utah, Salt Lake City, UT

Tianzheng Yu
Uniformed Services Univ., Bethesda, MD

Angelina Zanesco
UNESP, Rio Claro, Brazil

New Graduate Student Members

Balyssa Bell
Univ. of Iowa, Iowa City, IA

Cheavar Blair
Univ. of Kentucky, Lexington, KY

Alex Brownstein
Iowa State Univ., Ames, IA

Sierra Morgan Butcher
Tulane, New Orleans, LA

Songita Choudhury
Univ. of Nebraska Med. Ctr., Omaha, NE

Megan Michelle Clarke
Appalachian State Univ., Boone, NC

Matthew Davies
Univ. of Leeds, Leeds, United Kingdom

Lindsay Ellis
Univ. of British Columbia, Kelowna, BC, Canada

Zeinab Fazlali
Inst. for Res. in Fundamental Sciences (IPM), Tehran, Iran (Islamic Republic Of)

Joshua J. Foster
The Univ. of Chicago, Chicago, IL

Kibrom Gebremedhin
Michigan State Univ., East Lansing, MI

Dayton Joshua Goodell
Univ. of Colorado Anschutz Med. Campus, Denver, CO

Anne Hall
Baylor Coll. of Med., Houston, TX

Jamie Graham Hijmans
Univ. of Colorado Denver, Lakewood, CO

Said Ibeggazene
Univ. of Leeds, Leeds, United Kingdom

Ramakanth Reddy Kankanala
Med. Inst. of Med. Sci., Hyderabad, India

Deepesh Khanna
Texas A&M Univ., College Station, TX

Shawn Kuster
Univ. of Regina, Regina, SK, Canada

Jonathan Lambert
Temple Univ., Philadelphia, PA

Chang Woock Lee
Texas A&M Univ., College Station, TX

Alyssa Lombardi
Temple Univ. Sch. of Med., Philadelphia, PA

Jamie Meegan
Univ. of South Florida, Tampa, FL

Kathleen Byrne Miller
Univ. of Wisconsin-Madison, Madison, WI

Van Kim Ninh
LSUHSC-NO, Slidell, LA

Mark Pepin
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Priya Prasai
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Teryn Roberts
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Caroline Robertson
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Matthew Romero
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Farzana Rouf
Oklahoma State Univ., Stillwater, OK

Frantz Sainvil
Univ. of Science, Arts and Technology, Davie, FL

Aarushi Sharma
Southern Illinois Univ., Springfield, IL

Hannah Spaulding
Iowa State Univ., Ames, IA

Adam Swiercz
The George Washington Univ., Northwest, DC

Laura Tetri
Univ. of Wisconsin-Madison, Madison, WI

Douglas Van Pelt
Univ. of Michigan, Ann Arbor, MI

Matthew Wallen
The Univ. of Queensland, Brisbane, Australia

Lei Wang
Univ. of Florida, Gainesville, FL

Lei Wang
Univ. of Minnesota, Saint Paul, MN

Ashley Ward
Univ. of Kansas Med. Ctr., Kansas City, MO

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Univ. of Nebraska Med. Ctr., Omaha, NE

Meng-Ying Wu
Graduate Inst. of Life Sci. New Taipei City, Taiwan, Taiwan

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Christian Haase
Univ. Kailserslautern, Berlin, Germany

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Emory Univ., Sewickley, PA

Christina Ju Lee
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Affiliate Members

Andreas A. Giannopoulos
Brigham & Women's Hosp., Boston, MA

Joyce Horton
Marian Univ., Indianapolis, IN

Deepak Kadiyala
Univ. of Maryland Med. Ctr., Baltimore, MD

Meetings

2015 APS/ET-14: International Conference on Endothelin: Physiology, Pathophysiology, and Therapeutics

Savannah, Georgia, September 2-5, 2015

The 2015 APS/ET-14: International Conference on Endothelin: Physiology, Pathophysiology and Therapeutics was held in the enchanting historic city of Savannah, Georgia. The conference took place over 4 days at the Hyatt Regency Savannah hotel, located just steps from the Savannah River waterfront, parks that boasted age-old gnarled oak trees that were dripping in Spanish moss, fairytale antebellum houses, and the charming historic district.

The Local Organizing Committee (LOC) was chaired by Adviye Ergul (Georgia Regents University), Anil Gulati (Midwestern University), and David Pollock (University of Alabama at Birmingham) (see Photo 1). The LOC organized a program that would include symposia, oral presentation opportunities for students and postdoctoral fellows, interactive poster sessions, and social networking opportunities to make this conference a valuable experience for those who attended. In addition to the LOC, the Scientific Advisory Committee and the International Advisory Committee assisted the organizers in reviewing and programming all of the abstracts that were submitted to the conference.

The conference was attended by 108 total registrants: 35 (33%) registrants were represented by invited chairs, speakers, and members of the organizing committees, 24 (22%) students, and 12 (11%) postdoctoral fellows. Moreover, 14 (13%) attendees identified themselves as APS members, and the remaining 23 (21%) registered as nonmembers (Photo 2). Table 1 (below) shows the breakdown of the different registration types. The ET-14 conference attracted 51 (47%) registrants from outside the U.S. The international attendees represented countries from Argentina, Austria, Brazil, Canada, Czech Republic, France, India, Italy, Japan, Nigeria, The Netherlands, Saudi Arabia, South Korea, Sweden, Switzerland, and the United Kingdom.

Table 1. Registration statistics

Registration Type	Number of Attendees (%)
APS member	14 (13%)
Nonmember	23 (21%)
Postdoctoral	12 (11%)
Student	24 (22%)
Invited Chairs/Speakers	35 (33%)
Total	**108 (100%)**

Photo 1. Conference organizers *(left to right)*, David Pollock, Adviye Ergul, and Anil Gulati

Photo 2. ET-14 attendees gather for the traditional ET group photograph in front of the Savannah River Queen boat

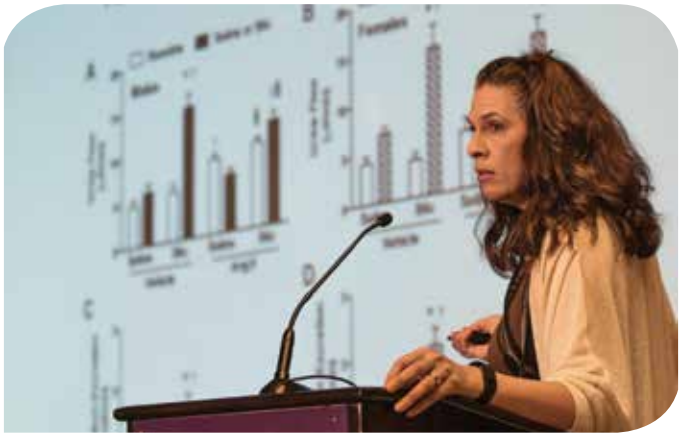


Photo 3. Invited Speaker Jennifer Sullivan presents her research during a session

Photo 4. Attendees interacting with the presenter during the session

The conference program consisted of 11 symposia on a wide variety of topics related to endothelin. The audience was encouraged to share their ideas and thoughts with the speakers at the end of their talks (Photos 3 and 4). Moreover, all of the symposia provided the opportunity for selected oral presentations from the volunteered abstracts that were submitted for the conference. In addition, there was a well attended Trainee Hot Topics Happy Hour session that gave students and postdoctoral fellows the opportunity to show one slide and have a 1-minute presentation (Photo 5). The conference also had several social activities including a Welcome and Opening Reception, which was designed to give attendees a chance to meet with long-time colleagues, create new friendships, and enjoy some hot and cold hor d'oeuves and beverages while listening to live classical music by a local musician. There were also two afternoon poster sessions where scientists presented their work and discussed their findings with other attendees. On the last evening of the conference, a special ticketed event took place at the majestic antebellum Mansion on Forsyth Park. The attendees were treated to traditional Southern hospitality with a host of Southern appetizers, cocktails, and a three-course dinner, which included a Georgia peach dessert.

During the dinner event at the Mansion on Forsyth Park, Ergul, Gulati, and Pollock presented travel awards to 20 postdoctoral fellows and students who were recognized as the recipients of the APS Abstract Travel Award. The following individuals were presented with a certificate and cash prize: Diana Cardero (Florida International University), Louisiane Desbiens (University of Sherbrooke, Canada), Caitlin Dow (University of Colorado, Boulder), Yang Gao (University of Utah), Eman Gohar (University of Alabama at Birmingham), Trevor Hardigan (Georgia Regents University), Jermaine Johnston (University of Alabama at Birmingham), Malgorzata Kasztan (University of Alabama at Birmingham), Philip Kavlich (University of Colorado, Boulder), Alejandro Majali-Martinez (Medical University of Graz, Austria), Yujiro Matsuishi (University of Tsukuba, Japan), Kasi McPherson (University of Mississippi Medical Center), Rebecca Moorhouse (University of Edinburgh, UK), Hary Muliawan (Kobe University Graduate School of Medicine, Japan), Ifeoma Okoli (Imo State University, Nigeria), Javier Pino (Florida International University), Kristen Solocinski (University of Florida, Gainesville), Yoko Suzuki (Kobe University Graduate School of Medicine, Japan), Rebecca Ward (Georgia Regents University), and Haiyan Xiao (Georgia Regents University) (Photo 6).

In addition, Carmen de Miguel and Jermaine Johnston (both from the University of Alabama at Birmingham), Javier Pino (Florida International University), and John Valenzuela (Georgia Regents University) were the recipients of the APS Minority Travel Fellow Travel Award, which is provided to encourage participation of underrepresented minority students in the physiological sciences. With generous support from the APS, the fellowship provides reimbursement of all expenses associated with travel and participation in the conference. The recipients of the award were matched with APS members: Robert Blank (Medical College of Wisconsin), Adviye Ergul (Georgia Regents University), Erika Boesen (University of Nebraska Medical Center), and Rita Tostes (University of São Paulo, Brazil), who

offered guidance and made introductions to the other scientists during the conference.

At the conclusion of the dinner, on behalf of the International Advisory Board on Endothelin, Pollock awarded the 2015 Tomoh Masaki Award to Martine Clozel (Actelion Pharmaceuticals, Switzerland) in recognition of excellence and achievement in the field of endothelin research (Photo 7).

A total of 102 abstracts were submitted for the conference. From the submitted abstracts, 88 were programmed as poster presentations. Out of the 88 poster presentations, 38 were also programmed as oral presentations, allowing for students and postdoctoral fellows to showcase their science. The remaining 14 abstracts were submitted by invited speakers. Of the abstracts submitted for the conference, 37 (36%) were submitted by a female first author; 50 (49%) were submitted from institutions outside of the U.S., including 16 from Japan, 19 from Europe, and 5 from Canada. The remaining abstracts came from Argentina, Brazil, India, Nigeria, and South Korea.

The American Physiological Society and the Organizing Committee gratefully acknowledge the financial support provided though generous educational grants from NIH, National Heart, Lung, and Blood Institute, Actelion Pharmaceuticals, Ltd., Gilead Sciences, Inc., Pharmazz, Elsevier/*Life Sciences*, Data Sciences International, Cell Signaling Technology, BioTek, Thermo Fisher Scientific, British Pharmacological Society, and Retrophin, Inc.

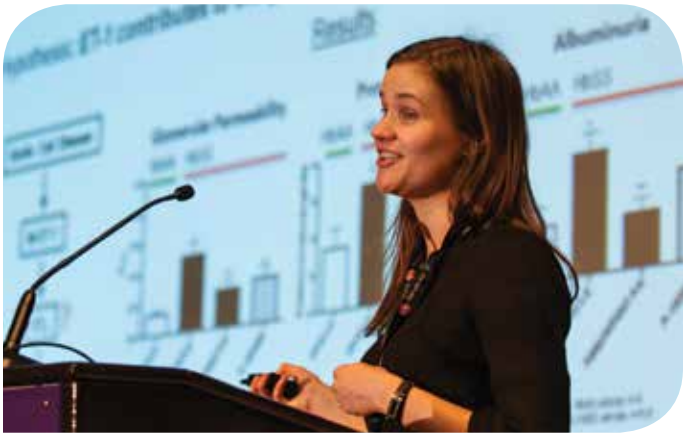


Photo 5. One of the young investigators presenting during the Trainee Hot Topics Happy Hour session

Photo 7. David Pollock congratulates Martine Clozel the 2015 Tomoh Masaki Award at the conference special event

Photo 6. Conference Organizers Pollock, Ergul, and Gulati congratulate the recipients of the APS Abstract Travel Award during the Award Ceremony

Physiological Bioenergetics: From Bench to Bedside

Tampa, Florida, September 9-12, 2015

The 2015 APS Conference: Physiological Bioenergetics: From Bench to Bedside was held in Tampa, Florida over the course of three and a half days. The Organizing Committee included Chair Victor Darley-Usmar (University of Alabama at Birmingham), Co-Chair Sruti Shiva (University of Pittsburgh) (Photo 1), Shannon Bailey (University of Alabama at Birmingham), Andreas Beyer (Medical College of Wisconsin), Paul Brookes (University of Rochester), Janine Santos (NIH), Russell Swerdlow (University of Kansas), and Yisang Yoon (Georgia Regents University). The committee organized a program that included seven symposia, three plenary lectures, and two poster sessions. Moreover, the organizers provided 12 slots during the conference symposia for oral presentations that allowed students and postdoctoral fellows who had submitted abstracts to showcase their work. Finally, a career session was held during the conference that was designed to show attendees how they can move forward in the research field of bioenergetics, whether in the lab or within a company setting.

The conference was attended by 99 total registrants, of whom 35% were represented by young scientists, including 12 postdoctoral fellows and 23 students. Twenty-four (25%) attendees identified themselves as APS members, and 17 (17%) registered as nonmembers; invited chairs and speakers made up the remaining 23 (23%) attendees. Table 1 shows the breakdown of the different registration types. This conference mostly attracted registrants (79%) from inside the U.S. However, there was a small contingent of scientists (21%) who came to the conference from Canada, Columbia, Europe, Israel, Japan, and Nigeria.

Photo 1. Conference Organizers Victor Darley-Usmar and Sruti Shiva

Table 1. Registration Statistics

Registration Type	Number of Attendees (%)
APS member	24 (25%)
Nonmember	17 (17%)
Postdoctoral	12 (12%)
Student	23 (23%)
Invited Chairs/Speakers	23 (23%)
Total	**99 (100%)**

The conference program covered a host of research on bioenergetics and mitochondria. The audience was encouraged to share their ideas and thoughts with the speakers at the end of their talks. The conference also had several social activities, including a Welcome and Opening Reception, and two afternoon poster sessions where scientists presented their work and discussed their findings with other attendees (Photo 2).

A total of 73 abstracts were submitted for the conference. Fifty-one of these abstracts were programmed as poster presentations. The remaining 20 abstracts were submitted by invited speakers. Of the abstracts submitted for the conference, 24 (33%) were submitted by a female first author; a quarter of the submitted abstracts (25%) were submitted from institutions outside of the U.S., including 10 from Europe, 5 from Canada, 2 from Japan, and 1 from Nigeria.

On Friday evening, Darley-Usmar and Shiva hosted the Banquet and Awards Presentation Dinner. Attendees gathered in the hotel ballroom for dinner, wine, and conversation. During the event, 15 postdoctoral fellows and students were recognized as the recipients of the APS Abstract Travel Award. The following individuals

were presented with a certificate and cash prize: Karina Ait Aissa (Medical College of Wisconsin, Milwaukee), Saima Ajaz (King's College London, UK), Christopher Akintayo (Afe Babaloa University, Nigeria), Pawel Albrycht (Warsaw University, Poland), Yang Chen (New Jersey Medical School/Rutgers University), Teague Cole University of Pittsburgh), Anna Czajka (King's College London, UK), Jonathan Gumucio (University of Michigan), Lucas Maddalena (Brock University, Canada), Kiana Mahdaviani (Boston University School of Medicine), Kyle Trudeau (Boston University School of Medicine), Jennifer Valcin (University of Alabama at Birmingham), Yang Wang (Georgia Regents University), Heather Wilkins (University of Kansas Medical Center), and Jimmy Zhang (University of Rochester Medical Center) (Photo 3).

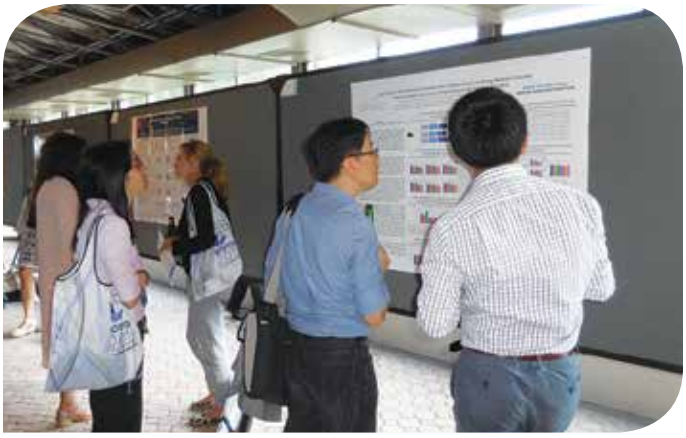


Photo 2. Attendees discuss their work during one of the poster sessions

Photo 3. Sruti Shiva *(far left)* and Victor Darley-Usmar *(third from right)* congratulate the recipients of the APS Abstract Travel Award during the Award Ceremony

In addition, Karina Ait Aissa (Medical College of Wisconsin, Milwaukee), Kennedy Mdaki (Sanford Research), and Jennifer Valcin (University of Alabama at Birmingham), were the recipients of the APS Minority Travel Fellowship Award, which is provided to encourage participation of underrepresented minority students in the physiological sciences. With support from the Society, the fellowship provides reimbursement of all expenses associated with travel and participation in the conference. The recipients of the award were matched with three APS members: Paul Coen (Translational Research Institute/Florida Hospital), William Cade (Washington University in St. Louis), and Jaime Baum (University of Alabama at Birmingham), who attended the conference, offered guidance, and made introductions to other scientists.

The American Physiological Society and the Organizing Committee gratefully acknowledge the financial support provided though generous educational grants from the National Institute of General Medical Sciences, NIH, Seahorse Bioscience, and University of Pittsburgh Center for Metabolism and Mitochondrial Medicine. ●

Cardiovascular, Renal and Metabolic Diseases: Physiology and Gender

Annapolis, Maryland, November 17-20, 2015

The fifth in a series of conferences related to physiology and gender entitled *2015 APS Conference: Cardiovascular, Renal and Metabolic Diseases-Physiology and Gender,* kicked-off in the historic naval city of Annapolis, Maryland. The conference took place over 3 days at the Crowne Plaza Annapolis hotel, which was just a few minutes' drive from the historic downtown area, which boasted the elegant and the oldest continuously used Maryland State House, the quaint harbor front, and the vast selection of some of the best seafood restaurants on the Eastern seaboard.

The Organizing Committee for the conference included Chairs Jane F. Reckelhoff (University of Mississippi Medical Center) and S. Ananth Karumanchi (Harvard University), as well as Heddwen Brooks (University of Arizona, Tucson), Kate M. Denton (Monash University, Australia), Rolando J. Ramirez (University of Akron), Vera Regitz-Zagrosek (Charite University, Germany), Javier Salazar (University of Murcia, Spain), Willis K. Samson (St. Louis University School of Medicine), Kathryn Sandberg (Georgetown University, James R. Sowers (University of Missouri School of Medicine), Jennifer Sullivan (Georgia Regents University), You-Lin Tain (Chang Gung Memorial Hospital, Taiwan), and Rita Tostes (University of São Paulo, Brazil).

Photo 1. Conference Organizer Jane Reckelhoff *(left)* and Jennifer Sullivan *(right)* present the Distinguished Lectureship Award to Chris Baylis *(middle)*

The conference was attended by 159 total registrants: 34 (21%) of registrants were represented by invited chairs, speakers, and members of the organizing committees, 41 (26%) were students, and 21 (13%) were postdoctoral fellows. Moreover, 46 (29%) of attendees identified themselves as APS members, and the remaining 17 (11%) registered as nonmembers. Table 1 (below) shows the breakdown of the different registration types. The conference attracted 29 (18%) registrants from outside the U.S. The international attendees represented countries from Argentina, Australia, Brazil, Canada, Czech Republic, Germany, Israel, Japan, The Netherlands, Saudi Arabia, South Africa, and Spain.

Table 1. Registration Statistics

Registration Type	Number of Attendees (%)
APS member	46 (29%)
Nonmember	17 (11%)
Postdoctoral	21 (13%)
Student	41 (26%)
Invited Chairs/ Speakers	34 (21%)
Total	**159 (100%)**

The conference program consisted of eight symposia on a wide variety of topics related to physiology and gender. The audience was encouraged to share their ideas and thoughts with the speakers at the end of their talks. Moreover, all of the symposia provided the opportunity for young investigators to present their work in a short oral presentation from abstracts that they submitted to the conference. Moreover, the conference had a plenary session presented by Janine Clayton (NIH, Office of Research in Women's Health), as well as the Distinguished Investigator Award Lecture, presented

by Chris Baylis (University of Florida, Gainesville). After the lecture, Jennifer Sullivan and Jane Reckelhoff presented Baylis with an award in appreciation of her work in the gender field (Photo 1). The conference also had several social activities including a Welcome and Opening Reception, which was designed to give attendees a chance to meet with long-time colleagues, create new friendships, and enjoy some hot and cold hor d'oeuves and beverages (Photos 2-3). There were also two afternoon poster sessions where scientists presented their work and discussed their findings with other attendees. On the last evening of the conference, a special banquet and award ceremony was held.

While attendees feasted on traditional Maryland Crab Cakes, organizers Reckelhoff and Karumanchi presented travel awards to 38 postdoctoral fellows and students who were recognized as the recipients of the APS Abstract Travel Award (Photos 4-5). The following individuals were presented with a certificate and cash prize: Brittany Balser (University of Akron), John Bowman (Washington and Lee University), Gene Crislip (Georgia Regents University), John Dasinger (University of Mississippi Medical Center), Kristine DeLeon-Pennell (University of Mississippi Medical Center), Jessica Faulkner (University of Mississippi Medical Center), Michelle Farve (Rutgers University), Ellen Gillis (University of Mississippi Medical Center), Eman Gohar (University of Alabama at Birmingham), Guiomar Gomes (University of São Paulo, Brazil), Rayna Gonzales (University of Arizona, Phoenix), Taben Hale (University of Arizona, Phoenix), Victoria Halperin-Kuhns (Johns Hopkins University School of Medicine), Ronee Harvey (Mayo Clinic), Jaime Hijmans (University of Colorado, Boulder), Edith Hochhauser (Rabin Medical Center, Israel), Shaletha Holmes (Univ. of North Texas Health Science Center), Aline Jarrete (Campinas State University, Brazil), Sofien Laouafa (University of Laval, Canada), Jacqueline Limberg (Mayo Clinic), Rheure Lopes (University of São Paulo, Brazil), Margaret Murphy (University of Kentucky), Iane Novais (São Paulo State University, Brazil), Ajeeth Pingili (University of Tennessee Health Science Center), Dennis Pollow, Jr. (University of Arizona, Tucson), Jonathan Respress (Baylor College of Medicine), Carla B. Rosales (Tulane University), Ibolya Rutkai (Tulane University), Corinna Serviente (University of Massachusetts,

Photo 2. APS Executive Director Martin Frank introduces Conference Organizer and APS President-Elect Jane Reckelhoff at the Welcome and Opening Reception

Photo 3. Longtime APS member Ralph Lydic and members of his lab, Chelsea Angel, Wateen Alami, and Sara Mihalko, enjoying the reception

Photo 4. Conference Organizers S. Ananth Karumanchi and Jane Reckelhoff present the travel awards at the Banquet and Awards Ceremony

Amherst), Sonali Shaligram (University of the Pacific), Kanakadurga Singer (University of Michigan), Lauren Stein (St. Louis University), Jared Tur (University of South Florida), Luciana Veiras (University of Southern California), Martin Vizek (Charles University, Czech Republic), Tracey Weissgerber (Mayo Clinic), Baojian Xue (University of Iowa), and Margaret Zimmerman (Tulane University).

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: Mark Cunningham (University of Mississippi Medical Center), Kristine DeLeon-Pennell (University of Mississippi Medical Center), Katiria Flores (University of Connecticut), Bernard Ogola (Texas Technology University Health Science Center), Ana Palei (University of Mississippi Medical Center), Maria Torres (East Carolina University), Laura Villasana (Oregon Health and Science University), and Junie Warrington (University of Mississippi Medical Center). With generous support from the APS, the fellowship provides reimbursement of all expenses associated with travel and participation in the conference.

Finally, a surprise award was announced to the attendees at the dinner. Courtesy of the *American Journal of Physiology–Regulatory, Integrative, and Comparative Physiology* and Editor-in-Chief Willis K. Samson, an additional eight individuals were announced for providing an outstanding poster presentation at the conference. The recipients of the award were Suzan Al-Gburi (Technical University of Dresden, Germany), Jacqueline Limberg (Mayo Clinic), Colette Miller (Environmental Protection Agency), Dennis Pollow, Jr. (University of Arizona, Tucson), Jessica Santollo (University of Buffalo), Corinna Serviente (University of Massachusetts, Amherst), Lauren Stein (St. Louis University), and Maria Torres (East Carolina University) (Photo 6).

A total of 123 abstracts were submitted for the conference. From the submitted abstracts, 99 were programmed as poster presentations. Out of the 99 poster presentations, 25 were also programmed as

Photo 5. 2015 APS Conference: Cardiovascular, Renal and Metabolic Diseases--Physiology and Gender Abstract Travel Award winners

oral presentations, allowing for students and postdoctoral fellows to showcase their science. The remaining 24 abstracts were submitted by invited speakers. Of 123 abstracts submitted for the conference, over half were submitted by a female first author. Seventy-one women were depicted as the first author on the abstracts submitted (58%) compared with 52 (42%) male first author of abstracts submitted.

The American Physiological Society and the Organizing Committee gratefully acknowledge the financial support provided through generous educational grants from American Heart Association Council, Council on Hypertension, and the University of Mississippi Medical Center, Women's Health Research Center.

Photo 6. American Journal of Physiology–Regulatory, Integrative, and Comparative Physiology Abstract Travel Award Winners pose with the Conference Organizers Karumanchi and Reckelhoff, AJP-RIC Editor-in-Chief Willis R. Samson, and Associate Editor Heddwen Brooks

People and Places

Kibble Presented with Robert J. Glaser Distinguished Teacher Award

Jonathan Kibble

Alpha Omega Alpha Robert J. Glaser Distinguished Teacher Award has been presented to APS Member Jonathan Kibble, Assistant Dean for Undergraduate Medical Education and Professor of Physiology and Medical Education at the University of Central Florida College of Medicine. Kibble is a medical educator who inspires students to learn and whom teachers aspire to emulate in equal measure. ●

Chien Recognized as Franklin Institute Award Laureate

Shu Chien

Shu Chien, APS Past-President and APS Fellow, was recognized as one of the 2016 Franklin Institute Awards Laureates. Chien received the Benjamin Franklin Medal in Mechanical Engineering for contributions to the understanding of the physics of blood flow and for applying this knowledge to better diagnose cardiovascular disease. The Franklin Institute Awards Program dates back to 1824, when the Institute was founded by a group of leading Philadelphians to train artisans and mechanics in the fundamentals of science. Through its awards program, The Franklin Institute seeks to provide public recognition and encouragement of excellence in science and technology, preserving Benjamin Franklin's legacy. ●

Tang Prize Lecture to be Presented at Experimental Biology 2016

Established in 2012, the Tang Prize in Biopharmaceutical Science awards ~$1.3 million in recognition of "original biopharmaceutical or biomedical research that has led to significant advances toward preventing, diagnosing, and/or treating major human diseases to improve human health."

http://bit.ly/TangPrize

Tang Prize Foundation CEO Chern Jenn-chuan with AAA Executive Director Shawn Boynes and APS Executive Director Martin Frank sign a cooperative pact at EB 2015. (Photo Credit: Taiwan Today)

News From Distinguished Physiologists

Letter to Lois Heller

Colin Caro. Photograph courtesy of Nick Watkins

Colin Caro writes: "I must thank you for your recent, unexpected, but most welcome letter, received as I approach nonagenarian status.

"What am I doing now? In many ways, I have been highly fortunate in being able to continue my work, albeit now as Emeritus Professor and Senior Research Investigator in the Department of Bioengineering at Imperial College London.

"I was born in South Africa and read physiology and medicine at the University of the Witwatersrand. Looking back over the years, I have had a continuing interest in mechanics and things mechanical in physiology. In the 1950s, I was a Fellow and later Research Associate in the Department of Physiology and Pharmacology, Graduate School of Medicine of the University of Pennsylvania, first under Julius Comroe and later under Bob Forster and Arthur DuBois. Indeed, I have memories of giving one of my first communications, in the field of respiration mechanics, at the 1958 Fall Meeting of the APS at the University of Western Ontario. I also became a member of the APS at about that time.

"On returning to the UK in 1959, my interest became directed toward circulation mechanics where, following a wish which remains, I could try to link even more closely physiology and physical science. I worked first with Donald McDonald on wave propagation in the pulmonary circulation. I was then able, with the support of the renowned mathematician James (later Sir James) Lighthill to set up at Imperial College the Physiological Flow Studies Unit (PFSU).

"The PFSU, then almost unique in the field, was comprised of engineers, mathematicians, medics, and physiologists coming from many parts of the world. It served as a focus for research and teaching, and many of its members and academic visitors went on to distinguished careers and to form similar structures. I would like to mention some of the early members of the PFSU. They include: Bob Schroter, Tony Seed, Mike Sudlow, Tim Pedley, Kim Parker, Jim FitzGerald, John Lever, Bob Nerem, Shelly Weinbaum, Takami Yamaguchi, and John Tarbell. There were also visits from, among others, Mort Friedman, Lars Walloe, Sy Glagov, Don Giddens, Dick Skalak, Bert Fung, Moto Sugawara, and Alex Silberberg. It may be claimed that the PFSU contributed to the later formation of the Department of Bioengineering at Imperial and that many of the PFSU's members and visitors became lifelong friends.

"In 1969, based on the PFSU's multidisciplinarity, a slender knowledge of fluid mechanics, and pathological observations, I proposed, to account for the patchy distribution of atherosclerosis in arteries, that the process develops preferentially at locations where wall shear is low. It would seem there is still wide acceptance of that proposal, or elaborations of it, and still extensive related fundamental work.

"Skipping a few decades of research, I recognized that the curvature and branching of normal arteries is commonly nonplanar or helical, rather than planar (two-dimensional), and suggested that this observation could have implications for arterial physiology and pathology.

"Work is still needed to discover the significance of the observation. However, one outcome has been the development of a novel stent for the management of arteries obstructed by atherosclerosis. The novel stent, which I devised, has a helical-centerline, in recognition of the helical geometry of arteries, and is produced by a spin-out company (Veryan Medical Limited), of which I was a key founder. Conventional, straight-centerline stents will tend to reduce the physiological curvature of arteries. In contrast, the helical-centerline stent deforms arteries helically, causing swirling and cross-mixing of the flow. In consequence, there is among other changes reduction of extremes of wall shear and hence elevation of wall shear where wall shear is low.

"Pre-clinical porcine studies have demonstrated significantly less intimal hyperplasia in helical-centerline stented than straight-centerline stented arteries; intimal

hyperplasia is the leading cause of stent failure. Recently, a randomized, controlled clinical trial has been undertaken of the helical-centerline stent (BioMimics 3D Nitinol self-expanding stent) in patients undergoing intervention for atherosclerosis affecting the superficial femoral and popliteal arteries. This revealed, among other favorable findings, that the bare-metal helical-centerline stent provided significant improvement in respect of long-term patency, compared with bare-metal straight-centerline control stents. It should be added that, by permitting a stented segment to shorten under compression, the helical-centerline stent reduces the risk of stent fracture.

"It is my hope that I may be able to continue to work with colleagues a little longer, to more fully understand these phenomena, which appear to play roles in the pathogenesis of atherosclerosis and intimal hyperplasia.

"I am asked to provide some 'words of wisdom' for younger colleagues. A primary wish is that, for those so inclined, they should foster the interaction of physiology and physical science." ●

Letter to Lois Heller

Don Frazier writes: "Thank you so much for my birthday card and kind words therein. With respect to my current activities since retirement in 2000, I currently enjoy a post-retirement appointment in the University of Kentucky College of Medicine as an Emeritus Professor in the Department of Physiology. This appointment allows me to continue as the Founder and Director of the UK Outreach Center for Science, which is still going strong. Since its inception in 1992, we have had the opportunity to interact with over 125,000 Kentucky students at all levels, with the primary focus always on the appreciation of the wonderful biological system they possess and the awesome responsibility they have to nurture it. This of course allows us the opportunity to encourage them to acquire the necessary tools of chemistry, math, science, and communication skills. We have slowed down a little but still run about 4,000 students per academic year. I might add, the feedback from the students and teachers brightens my day and helps me maintain my resolve to continue until the time they start to fall asleep during an interactive presentation or, worse, start throwing tomatoes at me. The Center also continues with many summer programs that bring students to our campus for summer science camps and individually funded programs. Although not deserved, as our wonderful staff and volunteers make the difference, I am being honored on September 25, 2015 as the Center is being named after me.

"Other activities include our long-standing NIH-NIGMS-funded grant-writing program. In the mid-90s NIGMS issued a FOA that focused on helping minority faculty or faculty at institutions that served a high percent of minority students get funded. The guidelines stipulated the development of a Web-based internet grant-writing course, which is still up and running. In addition to the course, we continue offering (to eligible faculty) grant-writing workshops on our campus (two per year) and as many as three offsite throughout the country. Our involvement with the participants continues as we provide mentoring and/or proposal reviews for as long as required. We are happy to report that we just received funding for our competing renewal. I still teach one graduate level course per fall semester.

"Marty will be happy to know that I continue to play tennis regularly with wonderful friends at noon rather than eat. It is well known by everyone that our group can make a can of tennis balls last a year, and we never break a string. I continue to be President of the UK Men's and Women's Tennis Programs booster club. In fact, just this year, I was inducted into the Kentucky Tennis Hall of Fame – not for my talent on the court but as a fan and supporter, although I wish they had included skill.

"My passing thought is continue those things that keep you motivated each day and, if at all possible, share your talents, whatever they are, with others." ●

Positions Available

Assistant Professor: The Department of Physiology at Michigan State University (MSU) seeks an engaging teacher with a strong interest in undergraduate education to serve as the course coordinator and instructor of a new upper division laboratory course in physiology. The course is intended to teach a broad range of physiological principles to a diverse group of pre-health and life science majors. Duties will include developing laboratory exercises, teaching multiple sections of the laboratory course throughout the academic year, overseeing course organization and logistics, training and supervising teaching assistants, evaluating student performance, and reporting grades. Additional duties may be assigned as required. This is a fixed-term (non-tenured) faculty appointment, renewable subject to annual review. Qualifications for this position include a PhD or an equivalent advanced degree in a basic biomedical science field that includes formal training in physiology. Prior experience and a strong interest in teaching, familiarity with the logistical demands of an undergraduate teaching laboratory, experience with learning management systems and other contemporary instructional technologies, excellent communication skills, and an ability to work effectively with large numbers of students from diverse educational backgrounds are preferred. The successful candidate is expected to collaborate with current faculty to advance MSU's strong commitment to undergraduate STEM education and to play an active role in the broader mission of excellence in undergraduate education at MSU. *Desired qualifications:* The ideal candidate would have experience developing and teaching student lab experiences at the undergraduate level, working with students as human subjects, using animals or animal tissues to illustrate fundamental principles in physiology, and familiarity with instrumentation and protocols related to digital acquisition of physiological data. Please apply to *www.jobs.msu.edu*; posting no. 2290. Candidates should supply the following: a CV; cover letter; teaching philosophy uploaded as the required document labeled "Learning Philosophy." Review of applications will begin on November 12, 2015 and continue until the position is filled. Thank you for your interest in this position. The screening and selection process is currently underway and will continue until a successful candidate is chosen. Should review of your qualifications result in a decision to pursue your candidacy, you will be contacted.

Assistant Professor: Florida Southern College invites applications for a full-time, tenure-track Assistant Professor of Biology position to begin in August 2016. The successful candidate will be expected to teach undergraduate courses in human anatomy and physiology, developmental biology, histology, and other courses that support students in biology and nursing majors. The position will also require extensive advising for students aspiring to careers in the health professions. Review of applications will begin immediately and continue until the position is filled, but only applications received by November 30, 2015, can be assured full consideration. The position starting date is fall 2016. A complete application consists of a cover letter, curriculum vitae, teaching philosophy (up to 500 words), research statement involving undergraduate students (up to 500 words), contact information for three recommendations, and graduate transcripts. Qualified candidates will have completed a PhD degree in biology, anatomy, physiology, or related fields and have strong credentials teaching undergraduate students. Knowledge of gross human anatomy and organ system physiology is a plus. The successful candidate will demonstrate a commitment to excellence and innovation in undergraduate teaching and be skilled in methods of engaged learning, the use of instructional technology, and directing undergraduate research. In addition, FSC faculty members participate effectively in advising, committee work, and other activities supporting FSC's teaching and learning community. *Application information:* contact Human Resources, Florida Southern College; online app. form: *https://www.flsouthern.edu/human-resources.aspx#hr-working*.

Assistant Professor: EMU's Departments of Biology and Chemistry seek a physiology professor with a terminal degree in biology. Other potential areas of expertise will be considered such as kinesiology or developmental biology. Responsibilities include teaching courses in human physiology and developmental biology, with the potential to teach courses in research methods, ethics, or biomechanics. Course audience can be solely undergraduate or graduate as well as dual-enrollment; thus the successful applicant will demonstrate effective teaching at both introductory and advanced levels. The biology department at EMU emphasizes open-ended, question-driven laboratory experiments, discussion of ethical and religious implications of biomedicine, and research. The biology and chemistry departments

have an excellent record of success for alumni achieving admission to health sciences graduate and professional studies. The ability to work collegially as part of an integrated graduate and undergraduate program and compatibility with the department's mission is essential. Research in a biomedical area that incorporates both undergraduate and graduate students as well as advising both undergraduate and graduate students in the health sciences will be required. *Qualifications:* doctorate in biology (ABD acceptable). Experience teaching is desirable. *Compensation:* Nine-month contract. Salary determined by education and experience. Eastern Mennonite University uses a tenure-with-review contract system. *Appointment date:* Position begins mid-August 2016. Review of applications will begin on November 13, 2015. EMU reserves the right to fill the position at any time or keep the position open. *Inquiries:* Application review begins immediately. Applicants will be asked to respond to questions specific to EMU's mission after the initial inquiry. Send letter of application, curriculum vitae, transcripts (unofficial acceptable), and three letters of reference to Dr. Deirdre Smeltzer, Vice President and Undergraduate Academic Dean, Eastern Mennonite University, 1200 Park Road, Harrisonburg, VA 22802; e-mail, *ugdean@emu.edu*; phone, (540) 432-4141; website, *http://www.emu.edu*. Eastern Mennonite University is an equal-opportunity employer, committed to enhancing diversity across the institution. Eastern Mennonite University does not discriminate on the basis of race, color, national or ethnic origin, sex, disability, age, sexual orientation, or gender identity. EMU conducts criminal background investigations as part of the hiring process. EMU seeks faculty who have demonstrated or show promise of teaching excellence in a Christian liberal arts environment, a commitment to ongoing scholarship, and familiarity with and support for Anabaptist/Mennonite Christian faith practices.

Assistant/Associate Professor: The Department of Kinesiology in the Curry School of Education at the University of Virginia (UVa) seeks applicants for a tenure-track assistant or a tenured associate professor in the area of nutrition/physical activity and health and well-being. Responsibilities include teaching, student advisement, and leadership and service to the program, department, school, university, and profession. The department and UVa offer a highly collaborative, interdisciplinary environment, where the incumbent has the opportunity to design and implement novel nutrition/exercise interventions to control obesity and obesity-related diseases, and to improve health and well-being across the lifespan. Candidates who exhibit a commitment to diversity and equity through their teaching, scholarship, and service are encouraged to apply. A doctoral degree in nutritional science, exercise science, or a related field is required by the start date of the position. Postdoctoral research experience and at least one semester of experience teaching at the university level, as well as prior experience with mentoring students are preferred. A registered dietician nutritionist is preferred. Research expertise in nutritional sciences related to physical activity/exercise and health and well-being is required. Knowledge of culturally responsive pedagogy and the ability to implement a variety of pedagogical techniques appropriate for academically diverse learners is required. Respect for diversity and richness in human difference and the ability to form productive collaborations with faculty across grounds are essential. Finally, a track record of scholarship related to the role of nutrition and physical activity/exercise on health and well-being outcomes related to children and adolescents is required, as is a strong potential for developing an externally funded research program. An extramurally funded research program is required for consideration as an Associate Professor. The University of Virginia is located in beautiful Charlottesville, Virginia, 100 miles south of Washington, DC along the foothills of the Blue Ridge Mountains. The area is widely known for its scenic beauty and historical significance, and is ranked among the most exciting, healthiest, and favorite places to live. The Department of Kinesiology, among the top programs in the U.S., offers a bachelor's degree in kinesiology, and masters and doctoral degrees in kinesiology, with specializations in exercise physiology, athletic training/sports medicine, adapted physical education, and pedagogy. To apply, visit *http://jobs.virginia.edu* and search on Posting Number 0617279. Complete a Candidate Profile online, attach a cover letter, curriculum vitae, statement of teaching philosophy, and contact information for three references. Applicant screening begins October 19, 2015, and the position will remain open to applicants until filled. For questions about the position, please contact Joe Hart, Associate Professor, at *joehart@virginia.edu*. For questions about the application process, please contact Ellen Missana, Curry Director of Human Resources, at *ejm6n@virginia.edu*. The Curry School of Education and the University of Virginia are Equal Opportunity/Affirmative Action employers.

We seek to build a culturally diverse intellectual environment and welcome applications from women, minorities, veterans, and persons with disabilities.

Assistant/Associate Professor: Discover the vision and excitement at Baylor as we seek applications and nominations for the following tenure-track faculty position in the Department of Health, Human Performance and Recreation (HHPR) within the College of Health and Human Sciences. *Position:* Tenure-Track Assistant/Associate Professor of Health, Human Performance and Recreation with a specialization in Exercise Physiology. *Date of appointment:* August 2016. *Background:* Baylor University is a private Christian university and a nationally ranked research institution, consistently listed with highest honors among The Chronicle of Higher Education's "Great Colleges to Work For." Chartered in 1845 by the Republic of Texas through the efforts of Baptist pioneers, Baylor is the oldest continuously operating university in Texas. The university provides a vibrant campus community for over 15,000 students from all 50 states and more than 80 countries by blending interdisciplinary research with an international reputation for educational excellence and a faculty commitment to teaching and scholarship. Baylor is actively recruiting new faculty with a strong commitment to the classroom and an equally strong commitment to discovering new knowledge as we pursue our bold vision, Pro Futuris (*www.baylor.edu/profuturis/*). *Qualifications:* Outstanding scientists are invited to apply for a tenure-track faculty position in the Department of Health, Human Performance and Recreation. We are seeking individuals who have research interests addressing genomics and/or epigenomics in skeletal muscle physiology, muscle and strength changes in aging, immunology, and inflammation, and/or complement existing areas of excellence in genomics, proteomics/metabolomics, and cardiovascular, metabolic, and muscle physiology. To be considered for a tenure-track position at the Assistant/Associate Professor level, applicants must hold a PhD or equivalent degree. Appropriate relevant postdoctoral research experience is preferred. *Responsibilities:* Successful candidates must either currently have or demonstrate potential to obtain significant extramural research funding. Previous university-level teaching experience is highly desirable. Special consideration will be given to candidates who are broadly trained and demonstrate strong backgrounds in one or more of the following areas: *1)* skeletal muscle genomics and/or epigenomics; *2)* skeletal muscle physiology; *3)* influences of aging on skeletal muscle physiology and function; *4)* immunology and/or inflammation as they relate to skeletal muscle, cardiovascular, and metabolic physiology; *5)* transient responses and chronic adaptation to exercise and/or environmental stressors. Research that is conducted largely or partly in human subjects is highly desirable. The successful candidate is expected to establish a vigorous research program supported by extramural funding, engage in collaborative research endeavors with existing faculty members, contribute to the mission of the department in undergraduate and graduate education, teach courses in exercise physiology and/or related areas, and engage in departmental and university service. The successful candidate will also have the ability to work effectively with faculty, staff, and students with diverse backgrounds. *Salary & review date:* Competitive salary support and start-up funds will be provided. Preference will be given to applicants whose philosophy is compatible with the stated mission of the University to be a world-class institution dedicated to Christian principles and ideals. Applications must be complete by October 30, 2015 to guarantee consideration. The review of completed applications will continue until the position is filled. *Application:* The application package must include a formal letter of application (specifically address how you, the applicant, meet qualifications and can fulfill responsibilities described in this position announcement), a list of names and contact information of three references who may be contacted for a letter of recommendation, your full curriculum vitae, copies of degree transcripts, and samples of research publications. Electronic (PDF) copies of all application materials are preferred. Send to Dr. Peter Grandjean, Search Committee Chair, at *Peter_Grandjean@baylor.edu*. Mailing address: One Bear Place # 97313, Waco, TX 76798-7313; phone: 254/7103909; fax: 254/710-3527. To learn more about the above position, visit *www.baylor.edu/hr/facultypositions*; the Robbins College of Health & Human Sciences, *http://www.baylor.edu/chhs/*; the Department of Health, Human Performance and Recreation, *http://www.baylor.edu/hhpr/*. Baylor University is a private not-for-profit university affiliated with the Baptist General Convention of Texas. As an Affirmative Action/Equal Opportunity employer, Baylor is committed to compliance with all applicable antidiscrimination laws, including those regarding age, race, color, sex, national origin, marital status,

pregnancy status, military service, genetic information, and disability. As a religious educational institution, Baylor is lawfully permitted to consider an applicant's religion as a selection criterion. Baylor encourages women, minorities, veterans, and individuals with disabilities to apply.

Assistant/Associate Professor: Founded in 1856, University of Maryland, College Park is the flagship institution in the University System of Maryland. Our 1,250-acre College Park campus is just minutes away from Washington, DC, and the nexus of the nation's legislative, executive, and judicial centers of power. This unique proximity to business and technology leaders, federal departments and agencies, and a myriad of research entities, embassies, think tanks, cultural centers, and non-profit organizations is simply unparalleled. Synergistic opportunities for our faculty and students abound and are virtually limitless in the nation's capital and surrounding areas. The University is committed to attracting and retaining outstanding and diverse faculty and staff who will enhance our stature of preeminence in our three missions of teaching, scholarship, and full engagement in our community, the state of Maryland, and the world. *Position summary:* The Department of Kinesiology, School of Public Health, University of Maryland College Park (*www.sph.umd.edu/KNES/*) invites applications for a 9-month tenure-track position in cardiovascular and/or skeletal muscle exercise physiology. The position can be filled at the Assistant or Associate Professor rank. The successful candidate is expected to conduct research within the broad field of exercise physiology and physical activity and, preferably, to collaborate with ongoing research in the Department, including: paracrine signaling of circulating angiogenic stem cells; calcium signaling in skeletal muscle; effects of exercise on memory, executive function, and overall brain function; mechanical loading and musculoskeletal health; mechanics, energetics, and control of the extremities (hand, arm, lower limb); non-invasive brain biomarkers for cognitive-motor performance and learning; physical activity participation, built environment, and public health. The successful candidate will be expected to develop and maintain a nationally recognized and externally funded program of original research; advise, direct, and teach graduate students; and teach appropriate undergraduate courses. *Minimum qualifications:* Candidates should possess a doctoral degree in kinesiology or a related field. *Preferences:* Postdoctoral research experience is strongly preferred, and a history of extramural funding is desirable. The applicant must demonstrate evidence of a sustainable and focused research program and a strong publication record. Evidence of teaching experience and graduate student mentorship and advising is desirable. The School and University offer opportunities for collaboration across a multidisciplinary faculty. Additional research opportunities are possible with the University of Maryland Baltimore School of Medicine, Johns Hopkins University, NIH, Walter Reed National Military Medical Center, and other universities and organizations in the Baltimore-Washington region. *Application:* Applicants must apply electronically to position no. 106005 listed under faculty positions at *https://ejobs.umd.edu*. Review of applications will begin immediately, and applications will be accepted until the positions are filled. For best consideration, candidates are expected to submit materials by December 1, 2015. Applications should include the following: *1)* cover letter describing qualifications and experience in cardiovascular and/or skeletal muscle exercise physiology, *2)* a curriculum vitae, *3)* a statement of research focus including current and planned research, *4)* a statement of teaching experience and interest, *5)* names of three individuals and contact information who can provide references (to be contacted only with candidate's approval), and *6)* copies of the three most significant publications. Inquiries about the position should be directed to Dr. James Hagberg, Search Committee Chair (*hagberg@umd.edu*). *Salary:* Negotiable, depending on experience; start-up funds competitive. The University of Maryland, College Park, an equal opportunity/affirmative action employer, complies with all applicable federal and state laws and regulations regarding nondiscrimination and affirmative action; all qualified applicants will receive consideration for employment. The University is committed to a policy of equal opportunity for all persons and does not discriminate on the basis of race, color, religion, sex, national origin, physical or mental disability, protected veteran status, age, gender identity or expression, sexual orientation, creed, marital status, political affiliation, personal appearance, or on the basis of rights secured by the First Amendment, in all aspects of employment, educational programs and activities, and admissions.

Assistant/Associate Professor: The Department of Biochemistry and Microbiology in the Joan C.

Edwards School of Medicine and the Marshall Institute for Interdisciplinary Research (MIIR) at Marshall University are seeking applicants for a tenure-track joint appointment at the Assistant or Associate Professor level. Applicants must have a PhD, MD, or equivalent degree and at least 2 years of postdoctoral experience. The successful candidate will establish a competitive, externally funded research program, participate in team teaching to medical and graduate students as assigned by the department chair, and provide service within the Department, Medical School, and University. Preference will be given to individuals with research interests and experience in the causes and treatment of chronic human diseases. A competitive, state-funded salary and start-up package commensurate with experience will be provided. Additional information about the Department and the MIIR can be found at their websites (*jcesom.marshall.edu/departments/biochemistrymicrobiology*; *www.marshall.edu/miir*). Interested candidates should send a curriculum vitae, representative reprints, summary of past experience, statement of teaching philosophy, statement of research interests and future plans, contact information, and a list of three references. All materials should be submitted online to the Marshall University Human Resources website *https://marshall.peopleadmin.com/postings/4913*. Applications will be considered on a rolling basis until the position is filled. Marshall University is an equal opportunity/affirmative action employer and strongly encourages applications from women and minority candidates.

Assistant/Associate/Full Professor: The Oklahoma Medical Research Foundation (OMRF, *www.omrf.org*) is seeking established investigators with expertise in aging skeletal muscle physiology or neurobiology of aging to join the Aging and Metabolism Research Program. Successful candidates will be expected to maintain a vigorous independent research program that addresses issues relevant to mechanisms of sarcopenia and frailty, neuromuscular junction maintenance, aspects of age-related changes in muscle physiology, or mechanisms of age-related neurodegeneration. Candidates with significant knowledge of peripheral motoneuron biology and diseases of neuromuscular degeneration, such Amyotrophic Lateral Sclerosis (ALS) or age-related neurodegenerative diseases (Alzheimer's or Parkinson's disease), are of special interest. Successful candidates will receive an appointment at the Associate or Full Member level (Associate and Full Professor equivalents) in the Program as well as a generous, multi-year start-up package with significant sustained salary and research support. The Aging and Metabolism Research program is comprised of seven faculty members studying a variety of disease-related areas of investigation associated with the aging process, including musculoskeletal, neurological, metabolic, and cardiovascular disorders. The program is supported by significant equipment resources including two LC-tandem mass spectrometry systems, equipment for metabolite analysis, an electron spin resonance instrument, and a small animal metabolic screening system. OMRF also supports a state-of-the-art AAALAC accredited animal facility, a small animal MRI and MRS core facility, an imaging core, and a DNA sequencing facility. Additional information on the Aging and Metabolism Research Program can be found at the Program's website (*http://omrf.org/programs/aging-and-metabolism-research-program/*). Other attractive aspects include a generous startup package, institutional support for competitive salaries, comprehensive benefits, and a collegial work environment. Overall, OMRF is committed to creating an attractive position suitable for applicants with a proven track record of high-quality funded research. OMRF is an independent, not-for-profit, biomedical research institute supporting four major interdisciplinary research programs. Our facilities are located adjacent to the campus of the University of Oklahoma Health Sciences Center (OUHSC) in Oklahoma City and the Oklahoma City VA Medical Center. OMRF investigators enjoy close scientific interactions with OUHSC faculty and participate in OUHSC graduate programs. To apply, please send a CV, a letter describing your interest in the position and concise research prospectus, and the names of three references via *AMfaculty@omrf.org*. Visit *jobs.omrf.org* for more information. [EOE/AA]

Assistant/Associate/Full Professor: Gonzaga University's Department of Human Physiology seeks to fill a 9-month tenure-track faculty position, full-time, beginning in fall 2016. We are particularly interested in individuals who are able to teach any lower division course in our undergraduate curriculum, including anatomy and physiology lecture and lab, experimental research design and data analysis, and scientific writing. The candidate must also have experience and a specialty in an area that compliments the expertise of the current faculty and must provide evidence of a willingness and ability to mentor undergraduate students in research.

Essential functions: Teach 9 credits per semester (Fall & Spring) in the program; advise students, guiding them in meeting degree requirements and career goals; participate in departmental councils/committees as well as School of Nursing and Human Physiology and university committees; participate in curriculum review/revision; pursue relevant professional development, presentations at professional meetings, research, and publications in peer-reviewed journals to meet requirements for tenure/promotion. *Required qualifications:* PhD in physiology or related degree; at least 1 year previous teaching experience with undergraduate students; evidence of scholarly productivity; demonstrated ability to articulate a specific agenda for scholarly work and/or research. *Desired qualifications:* An expertise in integrative physiology, including the molecular and genetic basis of exercise, nutrient metabolism, pathophysiology, and/or aging. *Starting date:* September 2016. *Application procedure:* The required application materials include: cover letter, curriculum vitae (CV), statement of teaching philosophy (1 page), list of courses the candidate is interested in teaching or would like to develop that are not currently in the curriculum and articulate how their research interests compliment the research agenda of the department's current faculty, graduate transcripts, three current letters of reference, and contact details for three professional references. To apply, please visit the Gonzaga University Human Resources website at *https://gonzaga.peopleadmin.com/*. Applications must be received by midnight PDT on Tuesday, January 12, 2016. Gonzaga University is a Jesuit, Catholic, humanistic institution and is therefore interested in candidates who will contribute to its distinctive mission. Gonzaga University is a committed EEO/AA employer, and diversity candidates are encouraged to apply. All qualified applicants will receive consideration.

Chair: The Oregon Health & Science University (OHSU) School of Medicine invites applications and nominations for the position of Chair of the Department of Physiology and Pharmacology (PHPH). The Department of Physiology and Pharmacology provides an extraordinary, multidisciplinary research and educational environment, with particular research strengths in sensory and autonomic neuroscience, neuroendocrinology, and chemical biology. The Department strategically merges systems physiology with cutting-edge chemical biology, spanning from the molecule to the organism. The next Chair of PHPH will take over a well respected department within a thriving university that is deeply invested in advancing neuroscience, cardiovascular science, cancer biology, infectious disease research, and chemical biology in conjunction with advanced 'omic, imaging, and computational technologies that support basic research. OHSU is seeking a creative leader and visionary for the Department. S/he will think innovatively about science and strategically build and expand faculty expertise in the Department's emergent programmatic emphasis of Chemical Biology, while sustaining excellence in systems physiology. Reporting to the Dean of the School of Medicine, the Chair will plan, lead, organize, and direct the academic, research, and business affairs of the Department and represent PHPH in interactions with the University, School of Medicine, other departments, affiliated hospitals, donors, and external constituents. The Chair will also be directly involved in teaching and research. With this in mind, OHSU will consider any outstanding PhD, MD, or MD/PhD scientist with a thriving research program in an area relevant to the broad field of physiology, pharmacology, or chemical biology. The successful candidate will share our vision that the Department of Physiology and Pharmacology can play a major role in assisting OHSU scientists in basic science research and in translating basic science discoveries into improved human disease management. Furthermore, the next Chair of the Physiology and Pharmacology Department will support university-wide efforts toward drug discovery, drug optimization, candidate selection, and basic and translational research. Additionally, the candidate must have a strong track record in the education and training of doctoral students. A cover letter and current CV should be sent via OHSU's website: *https://goo.gl/v9R6xC*. OHSU is an equal-opportunity, affirmative-action institution. Women, minorities, individuals with disabilities, and veterans are encouraged to apply. AA/EOE.

Chair: American University of Antigua, College of Medicine invites nominations and applications for the position of Chairperson of the Department of Physiology. The Department is mostly engaged in teaching activities, although scientific research is encouraged. The mission of American University of Antigua College of Medicine is to provide students with an excellent education that is compatible with the most innovative teaching methods

available at the leading U.S. and Canadian medical schools. At the same time, AUA is dedicated to breaking down the barriers that have prevented underrepresented minorities from obtaining a medical education in the U.S. and subsequent licensure. In alignment with this mission, AUA's students are highly diverse, representing many international backgrounds. The curriculum is designed to provide students with the knowledge and skills necessary to prepare them to successfully practice medicine in the U.S. The successful candidate will have the skills to develop a strategic vision for the Department, a strong record of achievement in scholarship and teaching, and demonstrated administrative/managerial skills. Qualifications include an MD and/or PhD degree, and academic credentials meriting appointment at the rank of professor. Candidates who have teaching experience and research interests in education and how effective teaching techniques are adapted to different learning styles are of particular interest. This position requires an understanding of U.S. MD education requirements, strategic planning, finances, including budgets and the basic principles of faculty practice plans, and operations improvement. Candidates must have demonstrated experience working in and fostering a diverse faculty, staff, and student environment or a commitment to do so as a faculty member at AUA. *Duties & responsibilities: 1) Administration:* leads the department and the physiology faculty in achieving AUA's mission. Be responsible for working within and managing the departmental budget. Manages and develops faculty, performs faculty evaluations, and assists with the selection of additional faculty. Participates on faculty committees as appropriate or as assigned by the Executive Dean. Represents the university at community, campus, and professional meetings and events as assigned by the Executive Dean. Confers with other academic staff to explain and formulate admission requirements and course credit policies. *2) Student education:* Teaches courses within the department. Interacts directly with students in classroom lecture and lab settings to ensure that all students possess the physiology knowledge necessary to be successful in AUA's program of medical education, including passing licensing exams. Provides interventions with students to support learning process to maximize their adjustment. Provide academic counseling to students on a one-to-one or small-group basis. Participates in student selection and admission, making admissions recommendations as assigned by the Executive Dean. *3) Program development & implementation:* leads the planning, development, implementation, and evaluation of the physiology program, including course material, lecture, and lab sessions based on AUA's desired outcomes. Develops curricula and recommends curricula revisions and additions. Determines course schedules and coordinates teaching assignments to ensure optimum use of resources. Prepares, administers, scores, and reports on examinations. Additional duties and responsibilities that may be assigned by the Executive Dean. *Minimum experience, skills, training/education: 1)* MD, DO, or MBBS and/or PhD in human physiology. *2)* Demonstrated ability to communicate effectively with students, faculty, and administration. *3)* 2+ years of experience in a similar position within the U.S., Canada, or UK institution of higher education or medical training. *4)* A minimum of 3 years teaching experience. *5)* Computer proficiency utilizing current and new computerized teaching technology and familiarity with communicating platform such as Black Board. Applications including a CV, cover letter, and compensation requirements should be submitted via e-mail to Dr. Reza Sanii (*RSanii@auamed.net*); subject line should read "Chair Physiology - Applicant Name." AUA is an equal-opportunity employer.

Lecturer: The Department of Kinesiology at the University of Toledo is one of three departments in the University's College of Health Sciences. Approximately 600 students are actively pursuing majors in the department at the BS, MS, and PhD levels. Undergraduate students may choose to major in Athletic Training, Exercise Science, or Respiratory Care. Specializations include, Human Performance and Fitness Promotion, and Pre-Health Care professions (Pre-PT/Pre-OT/Pre-PA, and Pre-Med). The department also provides instruction in microbiology, pathophysiology, and anatomy and physiology to many students in non-kinesiology majors. Areas of emphasis at the MS level include Athletic Training and Exercise Physiology. At the doctoral level, students develop an individualized program that reflects a combination of their professional goals and the research specialization of their advisor. Additional information on the department can be found on the college website: *http://www.utoledo.edu/healthsciences/depts/kinesiology/index.html*. *Required qualifications: 1)* Master's degree in Exercise Science or closely associated program; *2)* clinical certification/s, credentials from NSCA, and/or ACSM; *3)* previous instructional experience. *Preferred*

qualifications: 1) Terminal degree, earned doctorate in Exercise Physiology or closely associated program; *2)* emphasis in one or more of the following areas: Exercise Physiology, Clinical Exercise Physiology, Biomechanics, or Neuromuscular Control; *3)* 1-2 years of collegiate-level teaching experience, with innovative teaching technologies, including development of online or hybrid courses; *4)* knowledge of, or experience in, working with diverse populations; *5)* professional experience in clinical science. *Responsibilities: 1)* Teach undergraduate courses in one or more of the following related areas: Strength and Conditioning, Exercise Testing and Programming, Biomechanics, Neuromuscular Control; *2)* collaborate with students and faculty in the Department of Kinesiology, as well as other appropriate departments across the College and University; *3)* participate in department, college, and university service. *Applications:* All applications must be submitted through *https://jobs.utoledo.edu*. Click on "Faculty" and select posting no. 0600344. There will be an option to "Apply for this posting." Applicants must electronically submit their curriculum vitae, statement of research interest, letter of interest, and a list references through this system. If there are any questions, they can be directed to Sue Wambold, search committee chair, at *Suzanne.Wambold@UToledo.Edu*. Submissions will be accepted until the position is filled. A concerted effort is underway in the College of Health Sciences to ensure and enhance culturally diverse representation among our faculty, students, and staff. Interest from underrepresented groups is strongly encouraged. The University of Toledo is an Equal Opportunity Employer committed to excellence through diversity. An EEO/AA/Title IX employer.

Postdoctoral Fellowship: Sanford Burnham Prebys Medical Discovery Institute is dedicated to discovering the fundamental molecular causes of disease and devising the innovative therapies of tomorrow. SBP takes a unique, collaborative approach to medical research and has established major research programs in cancer, neurodegeneration, diabetes, and infectious, inflammatory, and childhood diseases. The Institute is especially known for its world-class capabilities in stem cell research and drug discovery technologies. The site at Lake Nona (Orlando) is dedicated to advancing the frontiers of scientific knowledge in life sciences and medicine, with an emphasis on diabetes and obesity research. SBP resources include 175,000 sq. ft. of state-of-the-art laboratory facilities for over 200 faculty and staff. The Lake Nona site has several outstanding technology cores for modern molecular and metabolic research, with a strong interdisciplinary collaborative approach to scientific study. *Job description:* We are seeking a talented, dynamic, and self-motivated Postdoctoral Research Associate with experience in muscle cell biology/biochemistry to train in Dr. Muthu Periasamy's lab. A major focus of our research is on SR Ca^{2+} cycling and how alterations in Ca^{2+} cycling impact mitochondrial metabolism. The candidate will investigate the role of Sarcolipin, a regulator of SERCA pump, and its impact on mitochondrial metabolism, especially under different pathophysiological states. He/she will be expected to work with genetically altered mouse models to analyze muscle energetics/function. The candidate will also be expected to become proficient in muscle biochemistry, physiology, and mitochondrial biology. The candidate will be encouraged to work independently and become proficient in manuscript/grant writing. There are opportunities to develop ideas, seek independent funding, and get promoted in rank. *Required skills/experience:* Requires a PhD degree in biological sciences. Candidates who have research experience in mitochondrial biology/energy metabolism are strongly preferred. Ideal candidate must be motivated, have excellent written/oral communication skills, have a strong desire and ability to work as a team in a collaborative environment. Interested candidates should apply with full CV, statement of research interests, and list of references to *itiu@SBPdiscovery.org*. *Employer name:* Sanford Burnham Prebys Medical Discovery Institute. *Position location:* Orlando, FL.

Postdoctoral Fellowship: A postdoctoral research fellowship position is available to study bladder/lower urinary tract physiology and pathophysiology at Beth Israel Deaconess Medical Center & Harvard Medical School, Boston, MA. The laboratory is studying signaling pathways involved in regulating bladder epithelial mechanosensory function and bladder smooth muscle motility. Candidates must have a doctoral degree with 0-2 years of postdoctoral experience. Preference will be given to candidates with previous experience with genetic modified mouse model, smooth muscle biology, epithelial biology, or imaging skills. Candidates are expected to be self-motivated and have excellent writing and communication skills. Please send a cover letter with a brief statement of professional goals, a CV with

a list of prior publications, and names of 3 references to: Weiqun Yu, Ph.D. Assistant Professor of Medicine Beth Israel Deaconess Medical Center & Harvard Medical School Boston, MA 02215. E-mail: *wyu2@bidmc.harvard.edu*. The Beth Israel Deaconess Medical Center/Harvard Medical School is an Affirmative Action/Equal Opportunity Employer.

Postdoctoral Fellowship: The University of Washington, Division of Nephrology, is seeking applicants for the UW Nephrology Training Program, under the direction of Drs. Stuart Shankland and Ying Zheng. This is a 12-month, full-time position with the title of Senior Fellow Trainee (job class code 442). The successful candidate must have a PhD in the biomedical fields or bioengineering, an MD, or equivalent degree. A strong record of productivity and interest in nephrology and/or bioengineering is desirable. A successful applicant must also be eligible for a NIH T32 Fellowship. In order to be eligible for the NIH T32 Fellowship, per NIH regulations, the individual to be trained must be a citizen or noncitizen national of the United States or have been lawfully admitted for permanent residence at the time of appointment. University of Washington is an affirmative-action and equal-opportunity employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, age, protected veteran or disabled status, or genetic information. Interested applicants should send the following application materials by e-mail to Drs. Stuart Shankland (*stuartjs@uw.edu*) and Ying Zheng (*yingzy@uw.edu*): current curriculum vitae, statement of research interest (1-2 pages maximum), copy of graduate school or medical school transcript, and three references with names and contact information.

Postdoctoral Fellowship: The National Space Biomedical Research Institute (NSBRI)-sponsored Mentored Research Program in Space Life Sciences at Texas A&M University (TAMU) is currently accepting applications for Fall 2016. Students participating in this program work toward a PhD in Biomedical Engineering, Genetics, Nutrition, Kinesiology, or Nuclear Engineering (Health Physics), or a MD/PhD or PhD in Medical Sciences from the Texas A&M University Health Sciences Center. Students will focus their research on space environment-induced bone loss, muscle wasting, cosmic radiation damage, and/or changes in metabolism. The Space Life Sciences graduate program at TAMU is designed with immersive components including fundamental courses in space life sciences, individual research, and an experiential component with work at Johnson Space Center, Brookhaven National Laboratory, and/or University of Texas Medical Branch. All trainees participate in outreach by teaching elements of space life sciences in a K-12 setting. To learn more about the program, please visit *http://SLSGraduateProgram.tamu.edu*. The deadline for submitting an application package is February 15, 2016. For more information, please contact Dr. Nancy Turner, Texas A&M University Director, Mentored Research Program in Space Life Sciences, 214 Cater-Mattil 2253 TAMU, College Station, TX 77843; phone: 979-845-4426; e-mail: *n-turner@tamu.edu*.

Postdoctoral Fellowship: Brown University is seeking a well published PhD or MD/PhD postdoctoral fellow, to be supported by a recently funded grant, to investigate the role of natriuretic peptide receptor-C (NPR-C) protecting against lung vascular injury using genetically altered mice models and in vitro approaches. Experience in cell biology, physiology, and biochemistry are desired. Send your resume and contact references as pdf to *Elizabeth_Harrington@brown.edu* and *James_Klinger@brown.edu*. Brown University is an equal-opportunity and affirmative-action employer.

Meetings & Congresses

2016

January 13-16

Genomics of Neurodegenerative Disorders, Cairo, Egypt. *Information:* internet: *http://www.goldenhelix.org/index.php/education/golden-helix-conferences/symposia/upcoming-symposium/222-2016-golden-helix-symposium-cairo-egypt#welcome*

January 16-18

International 3rd Caribbean Biomedical Research Days Conference (CBRD-2016), Rodney Bay, St. Lucia, West Indies. *Information:* e-mail: *info@stressandbehavior.com*; internet: *http://www.stressandbehavior.com/Years/2016/Carribean/Carribean2016.html*

March 6-8

Biomedical Basis of Elite Performance 2016, Nottingham, United Kingdom. *Information:* internet: *http://www.physoc.org/bbep2016/*

April 2-6

2016 Experimental Biology, San Diego, CA.

June 20-24

APS Institute on Teaching and Learning, Madison, Wisconsin. *#ITLPhysiology*

July 21-25

12th International Congress of Cell Biology, Prague, Czech Republic. *Information:* internet: *http://www.cscb.cz/*

July 29-31

APS/TPS Joint Meeting: Physiology 2016, Dublin, Ireland. #Physiology2016

August 24-27

APS Conference: Inflammation, Immunity and Cardiovascular Disease, Westminster, Colorado.

November 2-4

APS Intersociety Meeting: The Integrative Biology of Exercise VII, Phoenix, Arizonia.

2017

April 22-26

2017 Experimental Biology, San Francisco, CA.

May 27-June 1

International Neuromodulation Society 13th World Congress, Edinburgh, Scotland. *Information:* internet: *http://www.neuromodulation.com/ins-congress*.

August 1-5

IUPS 38th World Congress: Rhythms of Life, Rio de Janeiro, Brazil. *Information:* internet: *http://iups2017.com/*.

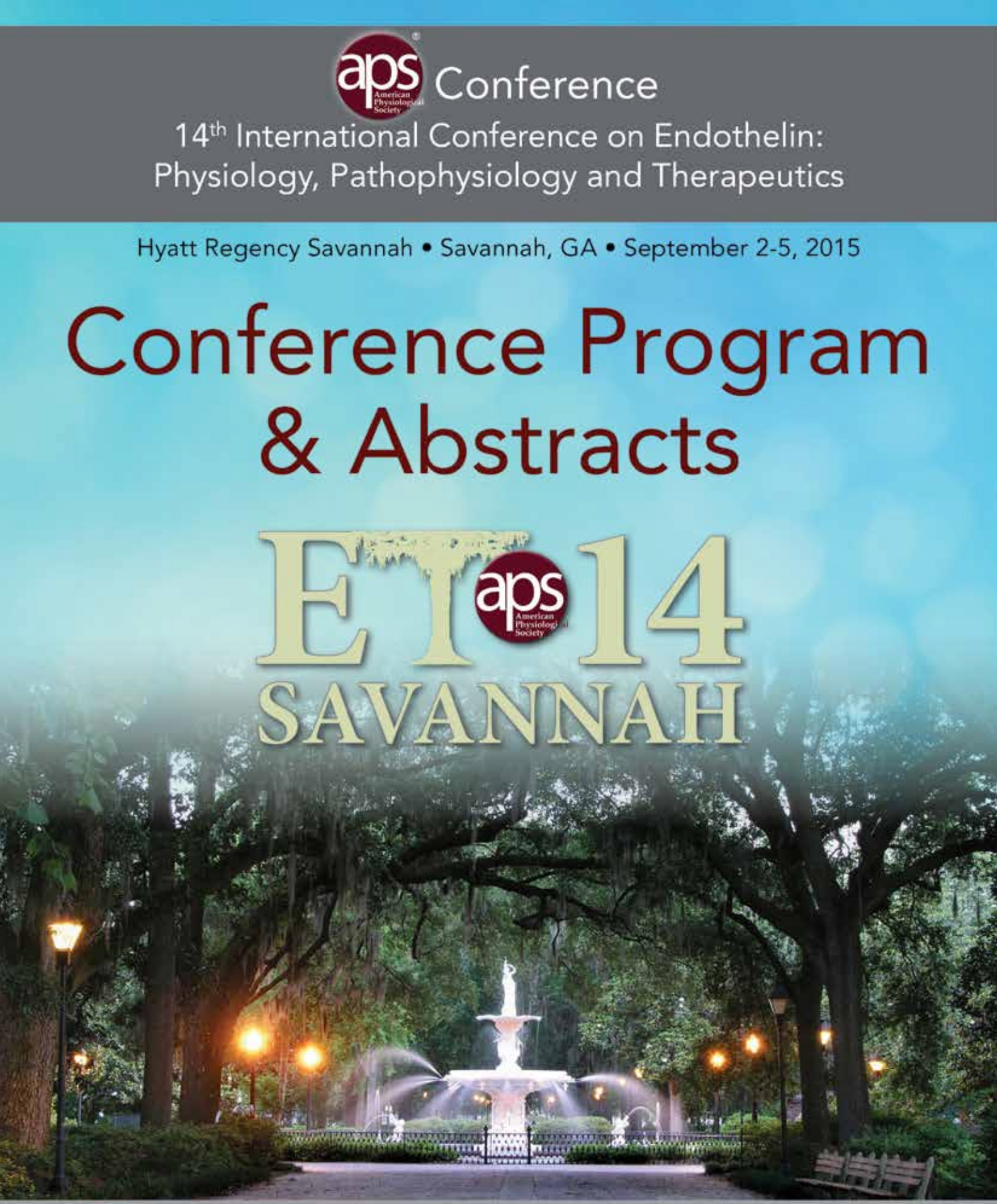
aps Conference

14th International Conference on Endothelin:
Physiology, Pathophysiology and Therapeutics

Hyatt Regency Savannah • Savannah, GA • September 2-5, 2015

Conference Program & Abstracts

ET aps 14 SAVANNAH



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www.the-aps.org/et-14

#Endothelin14

2015 APS/ET-14: International Conference on Endothelin: Physiology, Pathophysiology and Therapeutics

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Acknowledgements

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APS 14th International Conference on Endothelin: Physiology, Pathophysiology and Therapeutics
September 2—5, 2015, Savannah, Georgia, USA
Week-At-A-Glance

Wednesday, September 2	Thursday, September 3	Friday, September 4	Saturday, September 5
3:00 PM **Registration**	7:00 AM **Registration**	7:30 AM **Registration**	7:30 AM **Registration**
	8:00—10:00 AM **Symposia I** Novel Aspects of the Endothelin System **Ivana Vaneckova** **Anthony Davenport**	8:00—10:00 AM **Symposia V** Endothelin and End-Organ Injury **Noriaki Emoto** **Matthias Barton**	8:00—10:00 AM **Symposia IX** Central Nervous System **Constantino Iadecola** **Janet Maguire**
	10:30 AM—12:00 Noon **Symposia II** The Immune System and Endothelin **Ariela Benigni** **John Pernow**	10:30 AM—12:00 Noon **Symposia VI** Endothelin, Angiotensin and Vascular Function **Anna Bagnato** **Marilena Loizidou**	10:30 AM—12:00 Noon **Symposia X** Novel Integration **David Webb** **Adviye Ergul**
	12:00 Noon—1:00 PM **Lunch** 1:00—2:30 PM **Poster Session 1** **Odd Numbered Posters**	12:00 Noon—1:00 PM **Lunch** 1:00—2:30 PM **Poster Session 2** **Even Numbered Posters**	12:00 Noon—1:30 PM **Lunch**
	2:30—4:00 PM **Symposia III** ET, Sex, and Pregnancy **Rita Tostes** **Donald Kohan**	2:30—4:00 PM **Symposia VII** Endothelin and Fluid-Electrolyte Balance **Yasuo Matsumura** **David M. Pollock**	1:30—3:30 PM **Symposia XI** Endothelin Therapeutics—Where Are We? **Jennifer Pollock** **Jennifer Sullivan**
	4:30—5:30 PM **Symposia IV** Role of ET in the Vasculature **Joey Granger** **Anil Gulati**	4:30—5:30 PM **Symposia VIII** Pulmonary Function **Martine Clozel** **Pedro D'Orleans-Juste**	3:30—4:30 PM **Conference Summary and Highlights** Participant Discussion and Feedback Closing Remarks
6:00—8:00 PM **Welcome Address and Opening Reception**	5:30—7:00 PM **Trainee Mixer** Trainee Hot Topics Happy Hour **Kelly Hyndman** **Joshua Speed**	7:00—10:00 PM **Special Event Banquet** **Mansion on Forsythe Park** Ticket needed for entry (see registration desk for details)	

GENERAL INFORMATION

Location:

The 2015 APS Conference: 14th International Conference on Endothelin, Physiology, Pathophysiology and Therapeutics will be held September 2—5, 2015 at the Hyatt Regency Savannah Hotel, 2 West Bay Street, Savannah, GA 31401, USA, telephone (912) 238-1234, FAX: (912) 721-4671.

Onsite Registration Hours:

Wednesday, September 2……3:00—8:00 PM
Thursday, September 3……7:00 AM—6:00 PM
Friday, September 4……..7:30 AM—6:00 PM
Saturday, September 5……..7:30 AM—3:30 PM

On-Site Registration Fees:

APS Member	$600
APS Retired Member	$450
Nonmember	$750
Postdoctoral	$500
Student	$450

The registration fee includes entry into all scientific sessions, opening reception, and the special event at the Mansion on Forsyth Park.*

*Must have a ticket for entry.

Payment Information:

Registrants may pay by institutional or personal check, traveler's check, MasterCard, VISA or American Express or in United States Dollars. Checks must be payable to *"The American Physiological Society"* and drawn on a United States bank payable in US dollars.

Student Registration:

Any student member or regularly matriculated student working toward a degree in one of the biomedical sciences is eligible to register at the student fee. Nonmember postdoctoral fellows, hospital residents and interns, and laboratory technicians do not qualify as students. Nonmember students who register onsite must provide a valid university student ID card. APS student members should present their current APS membership card indicating their student category status.

Postdoctoral Registration:

Any person who has received a Ph.D. degree in physiology or related field, **within four years** of this meeting, as attested to by the department head is eligible to register at the postdoctoral fee. **A statement signed by the department head must accompany the registration form and remittance when registering.**

Press:

Press badges will be issued at the APS registration desk, only to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public affairs, etc.) must register as nonmembers.

Special Ticketed Event:

Join your colleagues for a special evening event and southern hospitality at the Mansion on Forsyth Park. Enjoy authentic southern cuisine while catching up with old and new acquaintances. The cost of the event is included in your registration fee. If you haven't already signed up for the event, please visit the registration desk on the second floor of the hotel. Tickets are limited and are on first come, first-served basis. Transportation is provided and will begin boarding at 6:45 PM in front of the hotel.

Program Objective:

Upon completing the program, participants should gain more knowledge in the physiology and pathophysiology of endothelin. The goal of the conference is to accumulate together a critical mass of scientists and those in industry who have interests in the important role of endothelin to promote the exchange of ideas and potential collaborations in the future.

Target Audience:

The intended audience for this conference includes all levels of researchers working in the field of endothelin.

Photography is *not* permitted during the scientific sessions or in the poster room

Don't forget to join us at the ET-14 Welcome Reception

Harborside Center East (street level of hotel)

6:00—8:00 PM

THURSDAY, SEPTEMBER 3, 2015

Symposia I

1.0 NOVEL ASPECTS OF THE ENDOTHELIN SYSTEM

Thurs., 8:00—10:00 AM, Ballroom A.

Chairs: **Ivana Vaneckova,** *Inst. of Physiology, Prague, Czech Rep.*
Anthony Davenport, *Univ. of Cambridge, UK.*

8:00 AM **1.1** New Insights in ET Receptor Pharmacology. **Janet Maguire.** *Univ. of Cambridge, UK.*

8:30 AM **1.2** Identification of EDN1-AS: A Novel Long, Non-coding RNA in the Regulation of Endothelin1. **Kristin Solocinski.** *Univ. of Florida, Gainesville.* **(3.20).**

8:45 AM **1.3** Autocrine Endothelin 1 Signaling Promotes Osteoblast Growth and Mineral Deposition Via Induction of miR 126-3p. **Michael G. Johnson.** *Univ. of Wisconsin, Madison.* **(3.59).**

9:00 AM **1.4** Novel UVR-induced Melanoma Mouse Model Based on Endothelin 3 Overexpression in Conjunction with Deficiency of the Nucleotide Excision Repair Pathway. **Diana Cardero.** *Florida Intnl. Univ., Miami.* **(3.67).**

9:15 AM **1.5** Endothelin A Receptor Drives Invadopodia Function and Cell Motility Through β-arrestin/PDZ-RhoGEF Pathway in Ovarian Carcinoma.**Laura Rosanò.** *Regina Elena Natl. Cancer Inst., Rome, Italy.* **(3.49).**

9:30 AM **1.6** Early-life Stress Induces Epigenetic Regulation of the ET System in Adult Male Mice. **Dao Ho.** *Univ. of Alabama at Birmingham.* **(3.40).**

Symposia II

2.0 THE IMMUNE SYSTEM AND ENDOTHELIN

Thurs., 10:30 AM—12:00 Noon, Ballroom A.

Chairs: **Ariela Benigni,** *Mario Negri Inst. for Pharmacological Res., Bergamo, Italy.*
John Pernow, *Karolinska Inst., Stockholm, Sweden.*

10:30 AM **2.1** Inflammation, Immunity and Hypertension. **David Harrison.** *Vanderbilt Univ.*

11:00 AM **2.2** Macrophage Endothelin-B Receptors Clear Endothelin-1 & Regulate Blood Pressure. **Neeraj Dhaun.** *Univ. of Edinburgh, UK.* **(3.61).**

11:15 AM **2.3** Long-term High Salt Diet Delays Development of Proteinuria in Murine Systemic Lupus Erythematosus (SLE). **Hanna Broome.** *Mississippi Coll., Clinton.* **(3.62).**

11:30 AM **2.4** Role of the Myeloid Endothelin-B Receptor in Angiotensin II Mediated End-organ Damage. **Lea Guyonnet,** *INSERM, PARCC, Paris, France.* **(3.34).**

Photography is *not* permitted during the scientific sessions or in the poster room

1:00 PM **POSTER SESSION I**
Ballroom BCDEF
Thursday: 1:00—2:30 PM, Odd numbered poster boards presenting. **Friday:** 1:00—2:30 PM, Even numbered poster boards presenting.

Symposia III

4.0 ET, SEX, AND PREGNANCY

Thurs., 2:30—4:00 PM, Ballroom A.

Chairs: **Rita Tostes,** *Univ. of Sao Paulo, Ribeirao Preto, Brazil.*
Donald Kohan, *Univ. of Utah Hlth. Sci. Ctr.*

2:30 PM **4.1** Sex and Hypertension. **Jennifer Sullivan,** *Georgia Regents Univ.*

3:00 PM **4.2** Endothelin-1 (ET-1) Regulates the Expression of Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of MMPs in Human First Trimester Trophoblasts via ETB Receptor: A Possible Role in Trophoblast Invasion. **Alejandro Majali-Martinez,** *Med. Univ. of Graz, Austria.* **(3.56).**

3:15 PM **4.3** Attenuation of Endothelin-1-induced Cardiomyocyte Hypertrophy Through Estrogen Pretreatment. **Nobutake Shimojo,** *Univ. of Tsukuba, Japan.* **(3.54).**

3:30 PM **4.4** Endotehlin-1: A Final Common Pathway Linking Placental Ischemia to Endothelial Dysfunction and Hypertension During Preeclampsia. **Joey Granger,** *Univ. of Mississippi Med. Ctr.* **(3.30).**

3:45 PM **4.5** Data-listed Endothelin Receptor Type B (ETB) Deficiency Results in Greater Blood Pressure Levels During Pregnancy and in Response to Placental Ischemia-induced Hypertension in Rats. **F. Spradley.** *Univ. of Mississippi Med. Ctr.* **(3.11).**

Symposia IV

5.0 ROLE OF ET IN THE VASCULATURE

Thurs., 4:30—5:30 PM, Ballroom A.

Chairs: **Joey Granger,** *Univ. of Mississippi Med. Ctr.*
Anil Gulati, *Midwestern Univ.*

4:30 PM **5.1** ET-1 in the Heart in Health and Disease. **Noriaki Emoto,** *Kobe Univ., Japan.*

5:00 PM **5.2** Endothelin-1 Overexpression Exaggerates Type 1 Diabetes-induced Endothelial Dysfunction by Altering Oxidative Stress Balance. **Pierre Paradis,** *McGill Univ., Montreal, Canada.* **(3.32).**

5:15 PM **5.3** Linagliptin Provides Cerebrovascular Protection via Upregulation of Endothelial ET-1 and ETB Receptors in Diabetes. **Mohammed Abdelsaid,** *Georgia Regents Univ.* **(3.29).**

Trainee Mixer

6.0 TRAINEE MIXER

Thurs., 5:30—7:00 PM, Ballroom A.

Chairs: **Kelly Hyndman,** *Univ. of Alabama at Birmingham.*
Joshua Speed, *Univ. of Alabama at Birmingham.*

5:40 PM **6.1** Endothelial-Derived Endothelin-1 Contributes to Renal Dysfunction and Mortality in Sickle Cell Mice. **Brandon Fox,** *Univ. of Alabama at Birmingham.* **(3.19).**

5:42 PM **6.2** Endothelin-1 Increases Glomerular Permeability in Sickle Cell Mice. **Malgorzata Kasztan,** *Univ. of Alabama at Birmingham.* **(3.14).**

5:44 PM **6.3** Gender Comparison of Recovery from Intravenous and Inhalational Anaesthetics Among Adult Patients in South-West Nigeria. **Yewande Okunoren-Oyekenu.** *Univ. of Leicester, UK.* **(3.71).**

5:46 PM **6.4** Evaluation of Endothelin A Receptor (ETA) Blockade on the Progression of Renal Injury in Various Models of Metabolic Disorders with Pre-existing Renal Disease. **Kasi McPherson,** *Univ. of Mississippi Med. Ctr.* **(3.22).**

5:48 PM **6.5** Renal Endothelin and Purinergic Systems Contribute to Sexual Dimorphism in Sodium Excretion. **Eman Y. Gohar,** *Univ. of Alabama at Birmingham.* **(3.7).**

5:50 PM **6.6** The Role of Endothelin System in Renal Structure and Function During the Postnatal Development of the Rat Kidney. **Maria Florencia Albertoni,** *Univ. of Buenos Aires, Argentina.* **(3.3).**

5:52 PM **6.7** TUDCA Attenuates High Salt-Induced Renal Cortical Injury in ETB Receptor Deficient Rats by Decreasing Apoptosis. **Randee Sedaka,** *Univ. of Alabama at Birmingham.* **(3.5).**

5:52 PM **6.8** ETA Receptor Blockade Improves the Differential Diurnal Natriuretic Response to an Acute Salt Load in Male and Female ETB Deficient Rats. **Jermaine Johnston,** *Univ. of Alabama at Birmingham.* **(3.13).**

5:54 PM **6.9** Hypoglycemic Effect of the Methyl Chloride-Methanolic Extract of the Fresh Fruits of the Gongronema Latifolia in Normoglycemic and Alloxan-induced Diabetic Rats. **Ifeoma Okoli,** *Imo State Univ., Owerri, Nigeria.* **(3.74).**

5:56 PM **6.10** Clinical use of Serum Big Endothelin-1 Levels as a Tumour Marker for Haemangiosarcoma. **Shinya Fukumoto,** *Rakuno Gakuen Univ., Ebetsu, Japan.* **(3.50).**

5:58 PM **6.11** Treatment with DPPIV Inhibitor Linagliptin Reduces Plasma ET-1 and ET-1-induced Cerebrovascular Hyper-reactivity in Diabetes. **Trevor Hardigan,** *Georgia Regents Univ.* **(3.41).**

6:00 PM **6.12** Endothelins as Markers of Cardiovascular Protection in Adults with Isolated Deficiency of Growth Hormone (IDGH). **Sydney Leao,** *Federal Univ. of São Paulo, Brazil.* **(3.52).**

6:02 PM **6.13** Endothelin 3 Regulates Pigment Production and Coat Color in Mice. **Javier Pino,** *Florida Intnl. Univ., Miami.* **(3.68).**

Trainee Mixer and Reception
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FRIDAY, SEPTEMBER 4, 2015

Symposia V

7.0 ENDOTHELIN AND END-ORGAN INJURY

Fri., 8:00—10:00 AM, Ballroom A.

Chairs: **Noriaki Emoto,** *Kobe Univ., Japan.*
Matthias Barton, *Univ. of Zurich, Switzerland.*

8:00 AM **7.1** Endothelin and Diabetic Complications. **John Pernow,** *Karolinska Inst., Stockholm, Sweden.*

8:30 AM **7.2** Knockout of Endothelin-1 in Vascular Endothelial Cells Ameliorates Cardiac Mitochondria Dysfunction After Myocardial Infarction in Diabetes Type 2 Mice. **Hary S. Muliawan,** *Kobe Univ., Japan.* **(3.53).**

8:45 AM **7.3** The Role of Endothelin in the Regulation of Blood Pressure in Early Diabetes Mellitus. **Geoff Culshaw,** *Univ. of Edinburgh, UK.* **(3.4).**

9:00 AM **7.4** The Endothelin System Mediates Renal Endoplasmic Reticulum Stress Development. **Carmen De Miguel.** *Univ. of Alabama at Birmingham.* **(3.10).**

9:15AM **7.5** p66 Shc Regulates ET-1-mediated Intracellular Calcium Handling in Renal Resistance Arteries and Contributes to Renal Glomerular Injury in Hypertension. **Oleg Palygin,** *Med. Coll. of Wisconsin, Milwaukee.* **(3.27).**

9:30 AM **7.6** Renal Vascular Regeneration by Angiotensin II Antagonism is Due to Abrogation of ET-1/ETAR Signaling. **Ariela Benigni,** *Inst. di Ric. Farmacol. Mario Negri, Bergamo, Italy.* **(3.1).**

Symposia VI

8.0 ENDOTHELIN, ANGIOTENSIN AND VASCULAR FUNCTION

Fri., 10:30 AM—12:00 Noon, Ballroom A.

Chairs: **Anna Bagnato,** *Inst. Natl. Tumori Regina Elena-IFO, Rome, Italy.*
Marilena Loizidou, *Univ. Coll., London, UK.*

10:30 AM **8.1** ET and Anti-angiogenic Therapy. **Anton H. van den Meiracker,** *Erasmus MC, Rotterdam, The Netherlands.*

11:00 AM **8.2** Endothelin-1 Stimulates Endothelial-derived Microparticle Release. **Philip J. Kavlich,** *Univ. of Colorado, Boulder.* **(3.39).**

11:15 AM **8.3** Endothelin Receptor Signaling and Age Related Deregulation of Cerebral Artery Myogenic Tone. **Adel Zrein,** *Dalhousie Univ., Halifax, Canada.* **(3.75).**

11:30 AM **8.4** High Dietary Fat Intake is Associated with Enhanced Endothelin-1 Vasoconstrictor Tone. **Caitlin Dow,** *Univ. of Colorado, Boulder.* **(3.35).**

1:00 PM **POSTER SESSION II**
Ballroom BCDEF
Friday: 1:00—2:30 PM
Even numbered poster boards presenting.

Symposia VII

9.0 ENDOTHELIN AND FLUID-ELECTROLYTE BALANCE

Fri., 2:30—4:00 PM, Ballroom A.

Chairs: **Yasuo Matsumura,** *Osaka Univ. Pharma. Sci., Japan.*
David M. Pollock, *Univ. of Alabama at Birmingham.*

2:30 PM **9.1** Endothelin Antagonism as a Therapeutic Strategy in Kidney Disease. **Neeraj Dhaun,** *Univ. of Edinburgh, UK.*

3:00 PM **9.2** Medullary Histone Deacetylase Enzymes are Critical for Water Balance During High Salt Feeding. **Kelly Hyndman,** *Univ. of Alabama at Birmingham.* **(3.6).**

3:15 PM **9.3** Regulation of Collecting Duct Endothelin-1 Production by Flow and Osmolality. **Yang Gao,** *Univ. of Utah.* **(3.2).**

3:30 PM **9.4** The Role of a Renal Aldosterone-Endothelin Feedback System in Total Na Balance and Mineralocorticoid Escape. **Charles Wingo,** *Univ. of Florida, Gainesville.* **(3.16).**

3:45 PM **9.5** Circadian Regulation of Renal Endothelin-1. **Joshua Speed,** *Univ. of Alabama at Birmingham.* **(3.12).**

Symposia VIII

10.0 PULMONARY FUNCTION

Fri., 4:30—6:00 PM, Ballroom A.

Chairs: **Martine Clozel,** *Actelion Pharma., Ltd., Allschwil, Switzerland.*
Pedro D'Orleans-Juste, *Univ. of Sherbrooke, Canada.*

4:30 PM **10.1** Chronic Hypoxia in Endothelin-1 Transgenic (ETTG) Mice Generates Moderate Pulmonary Hypertension, Not Severe Pulmonary Hypertension and Its Plexiform Lesions. **Muhammed Satwiko,** *Kobe Univ., Japan.* **(3.81).**

5:00 PM **10.2** Postnatal Ece1 Ablation Causes Severe, Progressive Pulmonary Disease. **Jasmin Kristianto,** *Univ. of Wisconsin, Madison.* **(3.84).**

5:15 PM **10.3** The Evaluation of Endothelin Receptor Antagonist for Pulmonary Hypertension with Lung Disease. **Kazuhiko Nakayama,** *Kobe Univ. Japan.* **(3.88).**

SATURDAY, SEPTEMBER 5, 2015

Symposia IX

11.0 CENTRAL NERVOUS SYSTEM

Sat., 8:00—10:00 AM, Ballroom A.

Chairs: **Constantino Iadecola,** *Weill Cornell Med. Coll.*
Janet Maguire, *Univ. of Cambridge, UK.*

8:00 AM **11.1** Mechanisms of ET in Pain. **Wolfgang Liedtke,** *Duke Univ. Med. Ctr.*

8:30 AM **11.2** Differential Role of ETA and ETB Receptors in CNS Parameters. **Yogendra Gupta,** *All India Inst. of Med. Sci., New Delhi, India.* **(3.78).**

8:45 AM **11.3** Significant Contribution of the Mast Cell-derived Chymase, mMCP-4, in Early Phases of Multiple Sclerosis in Mice. **Louisane Desbiens,** *Univ. of Sherbrooke, Canada.* **(3.66).**

9:00 AM **11.4** The Endothelin System in Amyotrophic Lateral Sclerosis (ALS). **Lyle Ostrow,** *Johns Hopkins Univ. Sch. of Med.* **(3.65).**

9:15 AM **11.5** Central Endogenous Endothelins (ETs) are Involved in the DOCA-Salt Hypertension. Interactions Between ETs Receptor A (ETA) Blockade and Tyrosine Hydroxylase (TH) in the Anterior (AH) and Posterior Hypothalamus (PH). **Maria Guil,** *Univ. of Buenos Aires, Argentina.* **(3.64).**

9:30 AM **11.6** Endothelin B Receptor Agonist, IRL-1620, Provides Neuroprotection and Enhances Angiogenesis in Diabetic Rats with Cerebral Ischemia. **Anil Gulati,** *Midwestern Univ.* **(3.23).**

Symposia X

12.0 NOVEL INTEGRATION

Sat., 10:30 AM—12:00 Noon, Ballroom A.

Chairs: **David Webb,** *Univ. of Edinburgh, UK.*
Adviye Ergul, *Georgia Regents Univ.*

10:30 AM **12.1** ET-1 and Neurovascular Coupling. **Constantino Iadecola,** *Weill Cornell Med. Coll.*

11:00 AM **12.2** Relationship of Endothelin-1 and NLRP3 Activation in HT22 Hippocampal Cells: Relevance to Cognitive Decline in Diabetes. **Rebecca Ward,** *Georgia Regents Univ.* **(3.63).**

11:15 AM **12.3** Endothelin 3 Regulates Pigment Production and Coat Color in Mice. **Javier Pino,** *Florida Intnl. Univ., Miami.* **(3.68).**

11:30 AM **12.4** Endothelin Receptor Antagonism in Sickle Cell Nephropathy. **O. Lenoir,** *INSERM, Paris, France.* **(3.18).**

11:45 AM **12.5** ETA Receptor Blockade Inhibits Leukocyte Activation and Adhesion in Sickle Cell Disease. **D. Gutsaeva,** *Georgia Regents Univ.* **(3.47).**

Symposia XI

13.0 ENDOTHELIN THERAPEUTICS-WHERE ARE WE?

Sat., 1:30—3:00 PM, Ballroom A.

Chairs: **Jennifer S. Pollock,** *Univ. of Alabama at Birmingham.*
Jennifer Sullivan, *Georgia Regents Univ.*

1:30 PM **13.1** Endothelin Therapeutics in Cancer-Where Are We? **Anna Bagnato,** *Inst. Natl. Tumori Regina Elena-IFO, Rome, Italy.*

1:55 PM **13.2** Endothelin Antagonists in Diabetic Nephropathy. **Donald Kohan,** *Univ. of Utah Hlth. Sci. Ctr.*

2:20 PM **13.3** Endothelin Antagonism, Where Next? **Pierre-Louis Tharaux,** *INSERM, Paris, France.*

2:45 PM **13.4** Review of Clinical Development of Sparsentan, a Dual-acting Angiotensin and Endothelin Receptor Antagonist. **Radko Komers,** *Retrophin, Inc., Cambridge, MA.*

3:10 PM **13.5** Endothelin Research and Drug Discovery. **Martine Clozel,** *Actelion Pharma., Ltd., Allschwil, Switzerland.*

Summary

14.0 CONFERENCE SUMMARY AND HIGHLIGHTS

Sat., 3:30—4:30 PM, Ballroom A.

Panelists: **Ariela Benigni,** *Mario Negri Inst. for Pharma. Res., Bergamo, Italy*
Pedro D'Orleans-Juste, *Univ. of Sherbrooke, Canada.*
Anthony Davenport, *Univ.of Cambridge, UK.*
David Webb, *Univ. of Edinburgh, UK.*
Masashi Yanagisawa, *Univ. of Tsukuba, Japan.*

POSTERS

POSTER SESSIONS

Ballroom BCDEF
Thursday: 1:00—2:30 PM, Odd numbered poster boards presenting. **Friday:** 1:00—2:30 PM, Even numbered poster boards presenting.

Poster Board

1 **3.1** Renal Vascular Regeneration by Angiotensin II Antagonism is Due to Abrogation of ET-1/ETAR Signaling. **A. Benigni, A. Remuzzi, F. Sangalli, D. Macconi, S. Tomasoni, I. Cattaneo, P. Rizzo, B. Bonandrini, E. Bresciani, L. Longaretti, E. Gagliardini, S. Conti, and G. Remuzzi.** *Mario Negri Inst. for Pharmacological Res., Bergamo Univ., and Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy.*

2 **3.2** Regulation of Collecting Duct Endothelin-1 Production by Flow and Osmolality. **Y. Gao, M. Pandit, and D. Kohan.** *Univ. of Utah.*

3 **3.3** The Role of Endothelin System in Renal Structure and Function During the Postnatal Development of the Rat Kidney. **M. F. Albertoni Borghese, M. C. Ortiz, S. Balonga, A. Lavagna, A. Filipuzzi, M. Barchuk, A. Schneider, R. M. Szokalo, and M. Majowicz.** *Univ. of Buenos Aires, Argentina.*

4 **3.4** The Role of Endothelin in the Regulation of Blood Pressure in Early Diabetes Mellitus. **G. Culshaw, M. Bailey, P. Hadoke, and D. Webb.** *Univ. of Edinburgh, UK.*

5 **3.5** TUDCA Attenuates High Salt-Induced Renal Cortical Injury in ETB Receptor Deficient Rats by Decreasing Apoptosis. **R. Sedaka, C. De Miguel, J. L. Hobbs, D. M. Pollock, and J. S. Pollock.** *Univ. of Alabama at Birmingham.*

6 **3.6** Medullary Histone Deacetylase Enzymes are Critical for Water Balance During High Salt Feeding. **K. Hyndman, J. Speed, C. Jin, D. M. Pollock, and J. S. Pollock.** *Univ. of Alabama at Birmingham.*

7 **3.7** Renal Endothelin and Purinergic Systems Contribute to Sexual Dimorphism in Sodium Excretion. **E. Y. Gohar, and D. M. Pollock.** *Univ. of Alabama at Birmingham.*

8 **3.8** Endothelin Receptor Antagonist Protects Against Ischemia/Reperfusion-induced Acute Kidney Injury in Male but not in Female Rats. **R. Tanaka, M. Ohkita, and Y. Matsumura.** *Osaka Univ. of Pharmaceutical Sci., Japan.*

Poster Board

9 **3.9** Effects of Combined Endothelin A Receptor and Renin-Angiotensin System Blockade on the Regression of Chronic Kidney Disease in 5/6 Nephrectomized Ren-2 Transgenic Rats. **V. C. Chabova, L. Sedlakova, Z. Huskova, L. Kopkan, P. Skaroupkova, S. Dolezelova, L. Cervenkova, Z. Vanourkova, L. Cervenka, and I. Vaneckova.** *Charles Univ., IKEM, and Academy of Sci., Praha, Czech Rep.*

10 **3.10** The Endothelin System Mediates Renal Endoplasmic Reticulum Stress Development. **C. De Miguel, W. C. Hamrick, J. L. Hobbs, M. Yanagisawa, D. M. Pollock, and J. S. Pollock.** *Univ. of Alabama at Birmingham, Univ. of Texas Southwestern Med. Ctr., and Univ. of Tsukuba, Japan.*

11 **3.11** Data-listid Endothelin Receptor Type B (ETB) Deficiency Results in Greater Blood Pressure Levels During Pregnancy and in Response to Placental Ischemia-induced Hypertension in Rats. **F. Spradley.** *Univ. of Mississippi Med. Ctr.*

12 **3.12** Circadian Regulation of Renal Endothelin-1. **J. Speed, and D. M. Pollock.** *Univ. of Alabama at Birmingham.*

13 **3.13** ETA Receptor Blockade Improves the Differential Diurnal Natriuretic Response to an Acute Salt Load in Male and Female ETB Deficient Rats. **J. Johnston, J. Speed, C. Jin, and D. M. Pollock.** *Univ. of Alabama at Birmingham.*

14 **3.14** Endothelin-1 Increases Glomerular Permeability in Sickle Cell Mice. **M. Kasztan, C-W. Sun, T. M. Townes, and D. M. Pollock.** *Univ. of Alabama at Birmingham.*

15 **3.15** Selective Endothelin-A Receptor Antagonism Prevents the Progression of Acute Kidney Injury to Chronic Kidney Disease. **R. Moorhouse, A. Czopek, L. Guyonnet, O. Lenoir, P. Tharaux, D. Webb, D. Kluth, and N. Dhaun.** *Univ. of Edinburgh, UK., and INSERM PARCC, Paris, France.*

16 **3.16** The Role of a Renal Aldosterone-Endothelin Feedback System in Total Na Balance and Mineralocorticoid Escape. **C. Wingo, I. J. Lynch, A. Welch, M. Gumz, B. Cain, and D. Kohan.** *Univ. of Florida, North Florida/South Georgia Hlth. Sys., Gainesville, FL, and Univ. of Utah Hlth. Sci. Ctr.*

17 **3.17** High Salt Intake Increases ET-1 Mediated Natriuresis and Diuresis via the ETB Receptor in Rats. **C. Jin, and D. M. Pollock.** *Univ. of Alabama at Birmingham.*

18 **3.18** Endothelin Receptor Antagonism in Sickle Cell Nephropathy. **O. Lenoir, N. Sabaa, C. Henique, L. Guyonnet, C. Fligny, V. Audard, and P. Tharaux.** *INSERM, PARCC, Paris, France, and Henri Mondor Hosp., Créteil, France.*

19 **3.19** Endothelial-Derived Endothelin-1 Contributes to Renal Dysfunction and Mortality in Sickle Cell Mice . **B. Fox, J. Heimlich, C. Sun, T. Townes, M. Yanagisawa, D. M. Pollock, and J.**

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S. Pollock. *Univ. of Alabama at Birmingham, Georgia Regents Univ., and the Univ. of Tsukuba, Japan.*

20 **3.20** Identification of EDN1-AS: A Novel Long, Non-coding RNA in the Regulation of Endothelin1. **K. Solocinski, S. Barilovits, A. Welch, C. Wingo, B. Cain, and M. Gumz.** *Univ. of Florida, and North Florida/South Georgia Vet. Hlth. Care Sys., Gainesville, FL.*

21 **3.21** ETA Receptor Activation Contributes to T Cell Infiltration Following Renal Ischemia-reperfusion Injury. **E. Boesen.** *Univ. of Nebraska Med. Ctr.*

22 **3.22** Evaluation of Endothelin A Receptor (ETA) Blockade on the Progression of Renal Injury in Various Models of Metabolic Disorders with Pre-existing Renal Disease. **K. McPherson, D. Spires, L. Taylor, A. Szabo-Johnson, J. and M. Williams.** *Univ. of Mississippi Med. Ctr.*

23 **3.23** Endothelin B Receptor Agonist, IRL-1620, Provides Neuroprotection and Enhances Angiogenesis in Diabetic Rats with Cerebral Ischemia. **A. Gulati, M. Husby, and M. Leonard.** *Midwestern Univ.*

24 **3.24** The Apoptotic Pathway Mediates the Neuroprotective Effect of IRL-1620 in a Rat Model of Focal Cerebral Ischemia. **A. Gulati, S. Briyal, A. Puppala, and L. Thanh.** *Midwestern Univ.*

25 **3.25** Neuroprotective Effect of Apilimod in Ischemia Reperfusion Injury in Rats. **S. Tiwari, D. Tripathi, and A. Verma.** *King George's Med. Univ., Lucknow, India.*

26 **3.26** Neuroprotective Potential of Endothelin ETA Receptor Antagonist in Cerebral Ischemia Models. **S. Sharma, T. Deshpande, and A. Gulati.** *Natl. Inst. of Pharma. Edu. and Res. (NIPER), Na-gar, India, and Midwestern Univ.*

27 **3.27** p66 Shc Regulates ET-1-mediated Intracellular Calcium Handling in Renal Resistance Arteries and Contributes to Renal Glomerular Injury in Hypertension. **O. Palygin, B. Miller, A. Chong, and A. Staruschenko, and A. Sorokin.** *Med. Coll. of Wisconsin.*

28 **3.28** The Dominance of Renin-angiotensin System Blockade Over Endothelin Receptor A Blockade in Lowering of Blood Pressure in Heterozygous Ren-2 Transgenic Rats. **I. Vaneckova, and J. Zicha.** *Inst. of Physiology, Prague, Czech Rep.*

29 **3.29** Linagliptin Provides Cerebrovascular Protection via Upregulation of Endothelial ET-1 and ETB Receptors in Diabetes. **M. Abdelsaid, T. Hardigan, and A. Ergul.** *Georgia Regents Univ., and VA Med. Ctr., Augusta, GA.*

30 **3.30** Endotehlin-1: A Final Common Pathway Linking Placental Ischemia to Endothelial Dysfunction and Hypertension During Preeclampsia. **J. Granger.** *Univ. of Mississippi Med. Ctr.*

31 **3.31** Induction of Long-term Endothelin-1 Overexpression Causes Blood Pressure Rise and Small

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Artery Stiffening. **P. Paradis, S. C. Coelho, S. Ouerd, J. C. Fraulob-Aquino, S. Offermanns, and E. L. Schiffrin.** *McGill Univ., Montreal, Canada, and Max-Planck-Inst. for Heart and Lung Res., Bad Nauheim, Germany.*

32 **3.32** Endothelin-1 Overexpression Exaggerates Type 1 Diabetes-induced Endothelial Dysfunction by Altering Oxidative Stress Balance. **P. Paradis, N. Idris-Khodja, S. Ouerd, M. O. Rehman Mian, J. Gornitsky, T. Barhoumi, and E. L. Schiffrin.** *McGill Univ., Montreal, Canada.*

33 **3.33** Endothelin-1 Overexpression Preserves Endothelial Function in Mice with Vascular Smooth Muscle Cell-restricted Pparγ Knockout. **P. Paradis, N. Idris-Khodja, S. Ouerd, M. Trindade, J. Gornitsky, A. Rehman, T. Barhoumi, S. Offermanns, F. J. Gonzalez, and E. L. Schiffrin.** *McGill Univ., Montreal, Canada, Max-Planck-Inst. for Heart and Lung Res., Bad Nauheim, Germany, and Natl. Cancer Inst.*

34 **3.34** Role of the Myeloid Endothelin-B Receptor in Angiotensin II Mediated End-organ Damage. **L. Guyonnet, N. Dhaun, P. Bonnin, V. Baudrie, R. Moorhouse, A. Czopek, O. Lenoir, D. Webb, D. Kluth, and P. Tharaux.** *INSERM PARCC, Paris, France, and Univ. of Edinburgh, UK.*

35 **3.35** High Dietary Fat Intake is Associated with Enhanced Endothelin-1 Vasoconstrictor Tone. **C. Dow, J. Greiner, N. Schuette, B. Stauffer, and C. DeSouza.** *Univ. of Colorado, Boulder, and Univ. of Colorado, Denver.*

36 **3.36** Vitamin C Supplementation Reduces ET-1 System Activity in Overweight and Obese Adults. **C. Dow, J. Greiner, D. Templeton, B. Stauffer, and C. A. DeSouza.** *Univ. of Colorado, Boulder, and Univ. of Colorado, Denver.*

37 **3.37** Borderline-high Triglycerides and Endothelin-1 Vasoconstrictor Tone. **C. Dow, J. J. Greiner, K. J. Diehl, B. Stauffer, and C. A. DeSouza.** *Univ.of Colorado, Boulder, and Univ. of Colorado, Denver.*

38 **3.38** C-reactive Protein Does Not Influence Endothelin-1 System Activity in Healthy Adults. **C. Dow, J. Greiner, G. Lincenberg, B. Stauffer, and C. A. DeSouza.** *Univ. of Colorado, Boulder, and Univ.of Colorado, Denver.*

39 **3.39** Endothelin-1 Stimulates Endothelial-derived Microparticle Release. **P. J. Kavlich, T. D. Bammert, J. G. Hijmans, K. J. Diehl, G. M. Lincenberg, R. T. Fay, W. N. Riaekvam, J. J. Greiner, and C. A. DeSouza.** *Univ. of Colorado, Boulder.*

40 **3.40** Early-life Stress Induces Epigenetic Regulation of the ET System in Adult Male Mice. **D. Ho, M. Burch, D. M. Pollock, and J. S. Pollock.** *Univ. of Alabama at Birmingham.*

41 **3.41** Treatment with DPPIV Inhibitor Linagliptin Reduces Plasma ET-1 and ET-1-inducedCerebrovascular Hyper-reactivity in

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Diabetes. **T. Hardigan, Y. Abdul, and A. Ergul.** *Georgia Regents University.*

42 **3.42** High Glucose-mediated Increase in Perinuclear ETA and ETB Expression in Human Brain Vascular Smooth Muscle Cells is not Ameliorated by Linagliptin. **Y. Abdul, T. Hardigan, and A. Ergul.** *Georgia Regents Univ.*

43 **3.43** Potential Association of Circulatory Level of Endothelin-1 and Diabetes in Rural Women in Bangladesh. **S. Jesmin, Y. Matsuishi, A. Rahman, M. Islam, N. Shimojo, S. N. Sultana, S. Zaedi, S. Akhtar, A. A. Habib, O. Okazaki, N. Yamaguchi, T. Miyauchi, S. Kawano, and T. Mizutani.** *Univ. of Tsukuba, Japan, Shaheed Ziaur Rahman Med. Coll., Bogra, Bangladesh, Natl. Ctr. for Global Hlth. and Med., Tokyo, Japan, and Ibaraki Prefectural Univ., Japan.*

44 **3.44** Amelioration of Acute Liver Injury with the Blockade of Protease Activated Receptor (PAR)-2 Through the Suppression of Upregulated Levels of Endothelin-1 and TNF-α in a Rat Model of Endotoxemia. **S. Jesmin, S. Zaedi, N. Shimojo, S. Akhtar, A. Rahman, Y. Matsuishi, N. Yamaguchi, S. Sultana, S. Gando, S. Sakai, S. Kawano, T. Mizutani, and T. Miyauchi.** *Univ. of Tsukuba, Japan.*

45 **3.45** Effects of Endothelin Antagonism on Microvascular Complications Such as Diabetic Erectile Dysfunction and Diabetic Retinopathy are Partly Mediated Through Restoration of Altered VEGF Signaling in Rats. **S. Jesmin, S. Sakai, S. Zaedi, M. Islam, S. Kawano, N. Shimojo, S. Homma, Y. Miyauchi, K. Aonuma, T. Mizutani, and T. Miyauchi.** *Univ. of Tsukuba, Japan.*

46 **3.46** High Fat and High Glucose Synergistically Impair Brain Microvascular Endothelial Cell Survival and Angiogenic Potential Independent of ET-1. **J. P. Valenzuela, T. Hardigan, M. Abdelsaid, Y. Abdul, and A. Ergul.** *Georgia Regents Univ., Univ. of Georgia Coll. of Pharmacy, Athens, GA, and VA Med. Ctr., Augusta, GA.*

47 **3.47** ETA Receptor Blockade Inhibits Leukocyte Activation and Adhesion in Sickle Cell Disease. **D. Gutsaeva, H. Xiao, J. Parkerson, C. Dickerson, S. Yerigenahally, J. S. Pollock, D. M. Pollock, and S. Meiler.** *Georgia Regents Univ., and Univ. of Alabama at Birmingham.*

48 **3.48** Stimulation of ETB Receptors by IRL-1620 Modulates the Progression of Alzheimers Disease. **S. Briyal, M. Leonard, A. Gulati, C. Nguyen, and C. Shepard.** *Midwestern Univ.*

49 **3.49** Endothelin A Receptor Drives Invadopodia Function and Cell Motility Through β-arrestin/PDZ-RhoGEFPathway in Ovarian Carcinoma. **L. Rosanò, E. Semprucci, P. Tocci, V. Caprara, R. Cianfrocca, R. Sestito, V. Di Castro, G. Ferrandina, and A. Bagnato.** *Regina Elena Natl. Cancer Inst., Rome, Italy, and Catholic Univ. of Rome, Italy.*

50 **3.50** Clinical use of Serum Big Endothelin-1 Levels as a Tumour Marker for Haemangiosarcoma. **S. Fukumoto, K. Saida, T. Miyasho, T. Kadosawa, H. Iwano, and T. Uchide.** *Rakuno Gakuen Univ., Ebetsu, Japan, and Natl. Inst. of Advanced Ind. Sci. and Tech., Tsukuba, Japan.*

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51 **3.51** Regulation of the Cardiac Endothelin System and Cardiomyocyte Hypertrophy by GPER. **M. Meyer, N. Fredette, C. Daniel, K. Amann, M. Barton, and E. Prossnitz.** *Univ. of New Mexico Hlth. Sci. Ctr., Univ. of Erlangen-Nurnberg, Germany, and Univ. of Zurich, Switzerland.*

52 **3.52** Endothelins as Markers of Cardiovascular Protection in Adults with Isolated Deficiency of Growth Hormone (IDGH). **S. Leao, C. A. Santos Aragão, M. S. de Freitas, J. V. Lima Dantas, W. B. Souza, S. L. Mattos, H. M. do Nascimento, M. H. Aguiar-Oliveira, M. R. Dashwood, and T. M. de Andrade Rodrigues.** *Fed. Univ. of São Paulo, Brazil, Fed. Univ. of Sergipe, São Cristóvão, and Univ. Coll. London, UK.*

53 **3.53** Knockout of Endothelin-1 in Vascular Endothelial Cells Ameliorates Cardiac Mitochondria Dysfunction After Myocardial Infarction in Diabetes Type 2 Mice. **H. S. Muliawan, K. I. Hirata, K. Nakayama, K. Ikeda, K. Yagi, and N. Emoto.** *Kobe Univ., Japan, and Kobe Pharma. Univ., Japan.*

54 **3.54** Attenuation of Endothelin-1-induced Cardiomyocyte Hypertrophy Through Estrogen Pretreatment Via Non-genomic Pathway: Potential Involvement with VEGF System. **N. Shimojo, S. Jesmin, Y. Matsuishi, S. Zaedi, S. Akhtar, A. Rahman, S. N. Sultana, K. Aonuma, T. Miyauchi, and S. Kawano.** *Univ. of Tsukuba, Japan, and Shaheed Ziaur Rahman Med. Coll., Bogra, Bangladesh.*

55 **3.55** Withdrawn.

56 **3.56** Endothelin-1 (ET-1) Regulates the Expression of Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of MMPs in Human First Trimester Trophoblasts via ETB Receptor: A Possible Role in Trophoblast Invasion. **A. Majali-Martinez, P. Velicky, J. Pollheimer, M. Knöfler, G. Desoye, and M. Dieber-Rotheneder.** *Med. Univ. of Graz, Austria, and Med. Univ. of Vienna, Austria.*

57 **3.57** Plasma and Urinary Endothelin-1 Levels in Neonates and Renal Function. **G. Pais, G. Stefanov, B. Puppala, L. Schweig, and A. Gulati.** *Midwestern Univ., and Advocate Children's Hosp. Park Ridge, IL.*

58 **3.58** Maternal Ethanol and Oxycodone Exposure Delay CNS Development as Determined by Endothelin Receptor Expression in Neonatal Rat Brains. **M. Leonard, S. Briyal, M. Ansari, M. Devarapalli, L. Schweig, B. Puppala, and A. Gulati.** *Midwestern Univ., and Advocate Children's Hosp, Park Ridge, IL.*

59 **3.59** Autocrine Endothelin 1 Signaling Promotes Osteoblast Growth and Mineral Deposition Via Induction of miR 126-3p. **M. G. Johnson, J. Kris-**

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tianto, X. Wang, K. Konicke, B. Yuan, and R. Blank. *Univ. of Wisconsin, Madison, William S. Middleton Vet. Hosp., Madison, WI, Med. Coll. of Wisconsin, Milwaukee, and Clement J. Zablocki VAMC, Milwaukee.*

60 **3.60** Functional Significance of Endothelin in Periodontitis. **I. Chang, G. Son, S. Y. Oh, D. and M. Shin.** *Yonsei Univ. Coll. of Dentistry, Seoul, Rep. of Korea.*

61 **3.61** Macrophage Endothelin-B Receptors Clear Endothelin-1 & Regulate Blood Pressure. **N. Dhaun, R. Moorhouse, L. Guyonnet, A. Czopek, O. Lenoir, V. Baudrie, D. Webb, M. Bailey, P. Tharaux, and D. Kluth.** *Univ. of Edinburgh, UK., and INSERM PARCC, Paris, France.*

62 **3.62** Long-term High Salt Diet Delays Development of Proteinuria in Murine Systemic Lupus Erythematosus (SLE). **H. Broome, J. Sasser, and M. Ryan.** *Mississippi Coll., Clinton, and Univ. of Mississippi Med. Ctr.*

63 **3.63** Relationship of Endothelin-1 and NLRP3 Activation in HT22 Hippocampal Cells: Relevance to Cognitive Decline in Diabetes. **R. Ward, and A. Ergul.** *Georgia Regents Univ.*

64 **3.64** Central Endogenous Endothelins (ETs) are Involved in the DOCA-Salt Hypertension. Interactions Between ETs Receptor A (ETA) Blockade and Tyrosine Hydroxylase (TH) in the Anterior (AH) and Posterior Hypothalamus (PH). **M. Guil, V. Morales, L. Cassinotti, C. Alvarez, L. Bianciotti, and M. Vatta.** *Univ. of Buenos Aires, Argentina.*

65 **3.65** The Endothelin System in Amyotrophic Lateral Sclerosis (ALS). **L. Ostrow, K. Russell, S. Vidensky, C. Donnelly, J. Johnson, B. Traynor, and J. Rothstein.** *Johns Hopkins Univ. Sch. of Med., and Natl. Inst. on Aging, NIH.*

66 **3.66** Significant Contribution of the Mast Cell-derived Chymase, mMCP-4, in Early Phases of Multiple Sclerosis in Mice. **L. Desbiens, C. Lapointe, D. Gris, and P. D'Orléans-Juste.** *Univ. de Sherbrooke, Canada.*

67 **3.67** Novel UVR-induced Melanoma Mouse Model Based on Endothelin 3 Overexpression in Conjunction with Deficiency of the Nucleotide Excision Repair Pathway. **D. Cardero, A. P. Benaduce, D. Batista, G. Grilo, K. Jorge, C. Milikowski, and L. Kos.** *Florida Intl. Univ., Miami, FL, and Univ. of Miami.*

68 **3.68** Endothelin 3 Regulates Pigment Production and Coat Color in Mice. **J. Pino, S. Ito, K. Wakamatsu, and L. Kos.** *Florida Intl.l Univ., Miami, FL, and Fujita Hlth. Univ. Sch. of Hlth. Sci., Aichi, Japan.*

69 **3.69** Effective Management of Sickle Cell Anaemia and Thalassaemia: Lessons from NHS England. **M. Dawodu, O. Okunoren, A. Olawuyi, A. Agboola, and Y. Okunoren-Oyekenu.** *Aston Univ., Birmingham, UK, Babcock Univ., Remo, Nigeria, Univ. of Ibadan, Nigeria, Ondo*

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State Trauma and Surgical Ctr. Nigeria, and Univ. of Leicester, UK.

70 **3.70** Differential Potentiation of Opioid Analgesia by Endothelin ETA Receptor Antagonist BMS182874. **S. Andurkar, A. Gulati, and S. Bhalla.** *Midwestern Univ.*

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83 **3.83** Three Hours Treatment of Landiolol Hydrocholoride, an Ultra-short-acting β-blocker, is not Effective to Reverse Altered Pulmonary Endothelin-1 System in Acute Lung Injury in a Rat Model of Early Hours of Endotoxemia. **Y. Matsuishi, S. Jesmin, S. Hideaki, N. Shimojo, S. Akhtar, S. Zaedi, T. Khatun, S. Kawano, and T. Mizutani.** *Univ. of Tsukuba, Japan.*

84 **3.84** Postnatal ECE1 Ablation Causes Severe, Progressive Pulmonary Disease. **J. Kristianto, M. G. Johnson, A. Radcliff, R. Zastrow, J. M. Koch, F. Patel, X. Wang, B. Yuan, and R. Blank.** *Univ. of Wisconsin, Madison, William S. Middleton Vet. Hosp., Madison, WI, Med. Coll. of Wisconsin, Milwaukee, and Clement J. Zablocki VAMC, Milwaukee, WI.*

85 **3.85** The Utility of the Pulse Oximeter for Pulmonary Hypertension During the Six Minute Walk Test. **N. Tamada, K. Nakayama, H. Kinutani, Y. Tsuboi, Y. Shinkura, Y. Suzuki, N. Emoto, and K. Hirata.** *Kobe Univ., Japan.*

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87 **3.87** Endothelin Receptor Blockade Attenuates Thrombin- and hypoxia-stimulated Intracapillary Neutrophil Retention in Lungs of Sickle Cell Disease Mice. **X. Hu, H. Chen, C. Dickerson, S. Meiler, and S. Wu.** *Georgia Regents Univ.*

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Abstracts of Invited and Contributed Presentations

1.0 NOVEL ASPECTS OF THE ENDOTHELIN SYSTEM

1.1
NEW INSIGHTS IN ET RECEPTOR PHARMACOLOGY

Janet Maguire[1], Rhoda Kuc[1], and Anthony Davenport[1]
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Understanding endothelin receptor pharmacology is essential to unravelling the role of these important peptides in health and disease. Recently, comprehension of how G-protein coupled receptors (GPCRs) interact with their ligands to transmit extracellular signals into cellular responses has expanded[1,2], with some of the basic tenets of pharmacology requiring re-evaluation. This should prompt researchers to look at published data with a new perspective and to rethink the design of future experiments. The two endothelin receptors, ET_A and ET_B, are defined by their rank order of potency for the three endogenous peptides, ET-1, ET-2 and ET-3. Selective agonists are available for the ET_B receptor but, interestingly, not the ET_A receptor. Receptor selective and non-selective antagonists have been developed as important research tools for defining receptor function and as clinically significant drugs for pulmonary arterial hypertension. However, some of the pharmacology of the two receptors has been difficult to reconcile, for example differences in ligand affinity for cloned and native receptors and ligand dependence of antagonist affinities. The aim of this talk is to revisit what we know about the pharmacology of endothelin receptors and to re-evaluate these data in the light of recent structural studies[1] and the discovery of GPCR biased signalling[2]. 1. Rosenbaum DM, Rasmussen SG, Kobilka BK. 2009. The structure and function of G-protein-coupled receptors. *Nature* **459**:356–363. 2. Wisler JW, Xiao K, Thomsen AR, Lefkowitz RJ. 2014. Recent developments in biased agonism. *Curr Opin Cell Biol* **27**:18–24.

2.0 THE IMMUNE SYSTEM AND ENDOTHELIN

2.1
INFLAMMATION, IMMUNITY AND HYPERTENSION

David Harrison[1]
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Hypertension remains an enormous health care burden that affects 30% of Western populations. Despite its prevalence the cause of most cases of hypertension remain unknown. Our laboratory has defined a novel mechanism for hypertension involving adaptive immunity. We found that mice lacking lymphocytes (RAG-1$^{-/-}$ mice) develop blunted hypertensive responses to a variety of stimuli including chronic angiotensin II infusion, DOCA-salt challenge and norepinephrine infusion. Adoptive transfer of T cells, but not B cells, restores the hypertensive responses to these stimuli. Hypertension is associated with the infiltration of T cells into the kidney and vasculature, where they release cytokines, including IFN-γ, IL-17A, and TNFα, which promote sodium retention, vasoconstriction and oxidative injury. Recently, we have found that angiotensin II has striking effects on dendritic cells (DCs), promoting their propensity to activate T cells. Our data indicate that angiotensin II infusion increases DC superoxide production by 5-fold and causes a striking accumulation isoketals, oxidized products of arachidonic acid in these cells. These form covalent bonds to lysines of proteins and these modified proteins become immunogenic. Several isoketal scavengers, including 2-hydroxybenzylamine (2-HOBA) prevent DC activation, the ability of DCs to stimulate T cell proliferation and prevent hypertension. A major impetus for immune cell activation seems to be increased sympathetic outflow, stimulated by the central actions of angiotensin II. By lesioning the AV3V region of the forebrain of mice or inactivating the NADPH oxidase in the subfornical organ using Cre Lox technology, we have prevented the central actions of angiotensin II and found that this inhibits both T cell activation and hypertension. Renal denervation likewise prevents activation of DCs in the kidney and the accumulation of activated DCs in the spleen. Thus, the kidney seems to be a major site of DC activation in hypertension. In summary, we have identified a new mechanism underlying hypertension and a potential new therapy for this common and yet difficult to manage disease.

3.0 POSTERS

3.1
RENAL VASCULAR REGENERATION BY ANGIOTEN-SIN II ANTAGONISM IS DUE TO ABROGATION OF ET-1/ET_AR SIGNALING

Ariela Benigni[1], Andrea Remuzzi[1,2], Fabio Sangalli[1], Daniela Mac-coni[1], Susanna Tomasoni[1], Irene Cattaneo[1], Paola Rizzo[1], Barbara Bonandrini[1], Elena Bresciani[1], Lorena Longaretti[1], Elena Gagliar-dini[1], Sara Conti[1], and Giuseppe Remuzzi[1,3]
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Endothelial dysfunction and vascular rarefaction play an important role in the progression of renal fibrosis. Treatment of Munich Wistar Fronter (MWF) rats with advanced nephropathy with an angiotensin converting enzyme inhibitor showed regression of established renal lesions and substantial glomerular tuft repair. Here we investigated whether this effect was associated with protection of the kidney vasculature. The whole kidney vasculature was analyzed by micro-computed tomography (microCT) in untreated and lisinopril- or losartan-treated MWF rats and in Wistar rats as controls. Drugs were given at 50 week-old animals with established renal damage for 10 weeks. The 3D reconstruction of the vascular network showed a progressive rarefaction affecting intermediate and small size vessels in kidneys from aged MWF rats as compared to controls. These changes were associated with endothelial mesenchymal transition (EndMT) and apoptosis concomitant with the overexpression of pro-fibrotic genes including endothelin-1 (ET-1). Within the glomerulus, ET-1 protein was highly expressed by both endothelial cells (EC) and podocytes as documented by co-staining of RECA-1 and a-actinin-4. Renal ET_AR expression in the vascular endothelium of MWF rats was also increased in a time-dependent manner. Renin angiotensin system (RAS) inhibition halted vascular rarefaction and even increased the volume density of kidney vessels as compared to pre-treatment suggesting a regenerative process. The treatment normalized ET-1/ ET_AR renal endothelial expression and significantly reduced EndMT and apoptosis while increased EC proliferation. Our data suggest that ET-1/ ET_AR deregulation contribute to renal EC damage and vascular rarefaction and that restoration of total kidney vasculature by RAS inhibition relates in part to abrogation of ET-1/ ET_AR signaling pathway.

3.2
REGULATION OF COLLECTING DUCT ENDOTHELIN-1 PRODUCTION BY FLOW AND OSMOLALITY

Yang Gao[1], Meghana Pandit[1], and Donald Kohan[1]
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Background: Endothelin-1 (ET-1) produced by the renal collecting duct (CD) is an important regulator of blood pressure and urinary sodium and water excretion. CD ET-1 production is increased by high salt intake; since ET-1 acts as an autocrine inhibitor of CD sodium and water reabsorption, this process facilitates normalization of body fluid volume. The mechanisms coupling salt intake to CD ET-1 synthesis are incompletely understood. Herein, we have investigated the role of tubule fluid flow and tubule fluid solute delivery in stimulating CD ET-1 production since both of these factors are augmented by a high salt diet. **Methods:** A mouse inner medullary collecting duct cell line (IMCD3) was exposed to stationary conditions or laminar flow (using Hanks Balanced Salt Solution) at a shear stress of 2 dyne/cm^2 for 2 hr at 37ºC (conditions determined to maximize the ET-1 flow response). The ratio of IMCD3 ET-1 to GAPDH mRNA levels was determined; since ET-1 protein is below detection levels due to the small number of cells and since ET-1 mRNA almost always parallels ET-1 protein levels, ET-1 mRNA content was taken as an index of ET-1 ET-1 protein levels. For all studies, N≥10 per data point. **Results:** ET-1 mRNA increased by 219 ± 21% in response to flow (compared to cells not exposed to flow). When perfusate osmolality was increased from 300 to 450 mOsm/L with NaCl, urea or mannitol, the ET-1 flow response increased to 450-500% over that seen in cells not exposed to flow (but containing 450 mOsm/L). This heightened flow response to osmolality was not altered by inhibition of the epithelial sodium channel (using 0.2 μM benzamil). While the ET-1 flow response under 300 mOsm/L conditions was blocked by chelation of intracellular calcium (50 μM BAPTA-AM), calcineurin inhibition (3 μg/ml cyclosporine A), purinergic receptor blockade (30 μM PPADS), or genetic deletion of polycystin-2, the augmented flow response in the presence of increasing solutes to 450 mOsm/L was not affected by these maneuvers. In contrast, inhibition of NFAT5 with 10 μM rottlerin abolished the ET-1 flow response under 300 or 450 mOsm/L conditions. Since rottlerin can have off-target effects, more specific evaluation of NFAT5 was performed. NFAT5 siRNA (68% knockdown of NFAT5 mRNA) completely blocked the heighted ET-1 flow response seen with 450 mOsm/L perfusate. **Conclusions:** Tubule fluid flow increases IMCD ET-1 production via a calcium, calcineurin, purinergic receptor and polycystin-dependent mechanism. Increased perfusate osmolality increases the IMCD ET-1 flow response via an NFAT5-dependent pathway. The flow and osmolality pathways work in concert to augment CD ET-1 production, providing evidence that both tubule fluid flow and solute delivery are involved in augmenting CD ET-1 production in response to salt loading. Funding source: NIH P01 HL095499.

3.3
THE ROLE OF ENDOTHELIN SYSTEM IN RENAL STRUCTURE AND FUNCTION DURING THE POST-NATAL DEVELOPMENT OF THE RAT KIDNEY

M. F. Albertoni Borghese[1], M. C. Ortiz[1], S. Balonga[1], A. Lavagna[1], A. Filipuzzi[1], M. Barchuk[1], A. Schneider[1], R. Moreira Szokalo[1], and M. Majowicz[1]

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Renal development in rodents, unlike in humans, continues during early postnatal period. We aimed to evaluate whether the pharmacological inhibition of ET system during this period affects renal development, both at structural and functional level in male and female rats. Newborn rats were treated orally from postnatal day 1 to 20 with vehicle or bosentan (Actelion, 20 mg/kg/day), a dual endothelin receptor antagonist (ERA). The animals were divided in 4 groups: control males (Cm), control females (Cf), ERA males (ERAm) and ERA females (ERAf). At day 21, one kidney was used to assess the glomerular number by a maceration method, and the other was used to perform morphometric analysis with Image Pro Plus software. Results are mean ± SEM ($n \geq 6$). Two-way ANOVA was used for the statistical analysis. The body weight of ERAm and ERAf decreased when compared with Cm and Cf respectively. However, neither femur length nor kidney weight/100g bw showed differences between groups. The number of total glomeruli (maceration method) decreased in ERAm vs Cm (Cm: 101499 ± 3526; ERAm: 84734 ±2709*; Cf: 89225±7032; ERAf: 88762 ±3359). The morphometric evaluation showed that the number of glomeruli/mm2 decreased in the juxtamedullary (JM) area in ERAm and ERAf vs Cm and Cf respectively (Cm: 12.9±0.8; ERAm: 10.2±0.8**; Cf: 13.4±0.9; ERAf: 11.2±0.9##). The JM renal filtration surface area (μm^2) decreased in ERA groups (Cm: 55406±3496; ERAm: 44297±3720*; Cf: 61697±5208*; ERAf: 52496±4108#&). There was a decrease in the ratio Capilar Glomerular Area/Total Glomerular Area (%) of the JM nephrons in ERAf, whereas in ERAm there was a tendency to decrease this parameter (Cm: 80.5±0.9; ERAm: 78.7±1.0; Cf: 83.5±1.4; ERAf: 75.7±0.8###). There were no changes in the same parameters in the cortical area; although there was a tendency to decrease those parameters in ERAm and ERAf. The creatinine clearance (ml/min/100g), decreased in ERAm and ERAf vs Cm and Cf respectively (Cm= 0,33±0,03; ERAm: 0,26±0,02*; Cf: 0,34±0,04; ERAf: 0,24±0,04#). There was an increase in proteinuria (mg/24h/100g) in ERAm and ERAf vs Cm and Cf respectively (Cm: 1.96 ± 0.30; ERAm: 3.16±0.29*; Cf: 1.92±0.32; ERAf: 2.40±0.39#) and a tendency to increase diuresis in ERA groups. *p<0.05, **p<0.01 vs Cm; #p<0.05, ## p<0.01, ### p<0.005 vs Cf; &p<0.05 vs ERAm. These results suggest that ET has an important role in rat renal postnatal development. The observed decrease in nephron number and the increase in proteinuria are factors associated with susceptibility to develop hypertension and renal diseases[1,2]. However, these results do not imply that the same could happen in humans, since human renal development is complete at birth. This work was supported by UBACyT grant 20020130200184BA and 20020090200685. 1. Murawski; 2010. 2. Loria; 2007.

3.4
THE ROLE OF ENDOTHELIN IN THE REGULATION OF BLOOD PRESSURE IN EARLY DIABETES MELLITUS

Geoff Culshaw[1], Matthew Bailey[1], Patrick Hadoke[1], and David Webb[1]

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Introduction: Diabetes mellitus (DM) is associated with sodium and water retention, loss of diurnal variation in arterial blood pressure (BP), and hypertension. Endothelin-1 (ET-1) regulates BP by vasoconstriction via ETA receptors and natriuresis via ETB receptors in the renal collecting duct. We hypothesised that in early DM, BP is increased and diurnal variation in BP is lost through enhanced ETA receptor signalling and reduced ETB receptor signalling. **Methods:** Heart rate (HR), systolic, diastolic and mean BPs (SBP, DBP, MBP) and diurnal variation in BP were recorded continuously by radiotelemetry in 8 adult male Sprague Dawley rats (322g SEM 4). Recording started 2 weeks after intraperitoneal injection of vehicle or streptozotocin (30-45mg/kg) to induce DM (n=4 per group). There were 4 recording periods of 7 days: baseline, salt supplementation, salt + ETA antagonist (oral atrasentan 5mg/kg/day), and salt + ETA (atrasentan) and ETB antagonist (oral A-192621 10mg/kg/day). **Results:** At baseline, diabetic rats had higher DBP (97±1mmHg) and MBP (110±1mmHg) than controls (93±1mmHg and 108±1mmHg respectively; P<0.001). Otherwise, BP did not differ between the 2 groups during the whole study despite a lower HR in diabetics (326±2 beats/min vs 354±2 beats/min control; P<0.001). BP in diabetics was unaffected by salt supplementation. In controls, salt increased SBP (126±1mmHg to 130±1mmHg; P<0.001) and MBP (107±1mmHg to 112±1mmHg; P<0.001). ETA antagonism reduced SBP, DBP and MBP in both groups (all P<0.001) with no difference in the effect size. Reductions in BP were countered by ETB antagonism (all P<0.001). Diurnal dipping in BP was less in diabetic rats than controls at baseline (DBP 2.3% vs 7.2%, P <0.001; MBP 2.7% vs 5.1%, P=0.011) and during ETA antagonism (DBP 1.4% vs 4.4%, P=0.006; MBP 1.5% vs 3.3%, P=0.06). 24 hour periodicity in DBP and MBP was lost in diabetics but not controls (DBP P<0.001; MBP P<0.044) during ETA antagonism, but was present in both groups during mixed ET receptor antagonism (all P<0.044). **Conclusion:** BP was increased and diurnal variation in BP was reduced in this model of early DM. Selective ETA antagonism, but not mixed ET receptor antagonism, reduced BP in controls and diabetic rats but did not recapitulate diurnal variation in BP in diabetics. ETA receptors but not ETB receptors may represent a therapeutic target for hypertension in early DM. Funded by Kidney Research UK, The British Heart Foundation and The Roslin Institute.

3.5
TUDCA ATTENUATES HIGH SALT-INDUCED RENAL CORTICAL INJURY IN ET_B RECEPTOR DEFICIENT RATS BY DECREASING APOPTOSIS

Randee Sedaka[1], Carmen De Miguel[1], Janet L. Hobbs[1], David M. Pollock[1], and Jennifer S. Pollock[1]

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ET_B receptor deficient (ET_B def) rats have a loss of functional ET_B receptors with higher circulating ET-1 displaying salt-sensitive hypertension and renal injury. It is unknown if apoptosis via cellular stressors are involved in high salt-induced renal injury. We hypothesized that high salt-induced renal injury in ET_B def rats occurs via apoptosis. The chemical chaperone, tauroursodeoxycholic acid (TUDCA), nullifies cellular stress pathways. To test our hypothesis, we utilized ET_B def and transgenic (TG) control rats on normal (1% NaCl, NSD) or high salt (8% NaCl, HSD) diet for three weeks (n=5-7/group) given daily TUDCA (400 mg/kg/day; i.p.) or vehicle. TUDCA did not influence blood pressure in either group. Urinary renal injury biomarkers (NGAL, KIM-1, albumin, nephrin) were assessed. HSD significantly increased renal injury in ET_B def rats (NSD vs. HSD; KIM-1: 14.2±3.4 vs. 104.1±20.6 pg/day; NGAL: 43.6±17.6 vs. 215.6±27.7 pg/day; albumin: 0.12±0.04 vs. 10.69±3.43 ng/day, p<0.001), while HSD did not elicit any change in TG rats. TUDCA significantly decreased injury markers (KIM-1: 55.7±13.8 pg/day; NGAL: 114.2±18.0 pg/day; albumin: 2.27±1.54 ng/day, p<0.05) in ET_B def rats. Renal cortical tissue KIM-1 levels mirrored the urinary KIM-1 excretion in ET_B def rats (KIM-1: NSD 10.2±3.0 pg/day vs. HSD 77.5±8.8 pg/day vs. TUDCA 25.4±7.6 pg/day, p<0.001). Renal glomerular injury assessed by nephrin excretion significantly increased in both ET_B def and TG rats on HSD, however TUDCA failed to attenuate this increase in either group. Apoptosis was increased in the renal cortex of ET_B def rats on HSD (NSD vs. HSD: 4.0±1.0 vs. 18.8±4.2 $TUNEL^+$ cells/field), while TUDCA decreased the high salt-induced apoptosis (2.1±0.1 $TUNEL^+$ cells/field). No significant apoptosis was detected in the renal cortex in TG rats. Neither diet nor TUDCA changed renal medullary apoptosis in ET_B def rats suggesting that medullary and cortical apoptosis may be mediated via distinct pathways. In conclusion, loss of functional ET_B receptors leads to exaggerated renal cortical tubular injury and apoptosis. These findings indicate a high salt-induced reno-protective role for ET_B receptor activation that may be blood pressure-independent. Supported by NIH T32 DK007545 to CDM and P01 HL95499 to DMP and JSP.

3.6
MEDULLARY HISTONE DEACETYLASE ENZYMES ARE CRITICAL FOR WATER BALANCE DURING HIGH SALT FEEDING

Kelly Hyndman[1], Joshua Speed[1], Chunhua Jin[1], David M. Pollock[1], and Jennifer S. Pollock[1]

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Histone deacetylases (HDAC) play a pivotal role in modifying lysines of histone and nonhistone proteins, thereby regulating transcription and protein function. We determined that a high salt diet increases expression of HDAC1 in the rat renal medulla. Moreover, overexpression of HDAC1 in collecting duct cells increases nitric oxide synthase-1, and decreases vasopressin V2 receptor expression. Thus, we hypothesized that HDAC1 functions as a pro-natriuretic/diuretic factor during high salt feeding. To test this hypothesis, male uninephrectomized Sprague Dawley rats were implanted with an iPrecio® perfusion pump to facilitate interstitial infusion into the medulla of the remaining kidney. Pumps infused vehicle (33% DMSO in saline, N = 5) or the HDAC inhibitor, MS275 (1 mg/kg/day, N =10). Rats were given 4.0% NaCl diet for 7 days. Food and water intake were similar between controls and MS275 infused rats. However, urinary osmolality was significantly increased in MS275 infused rats (1441.8± 86 vs 1001 ± 64 mOsm/kg H_2O). MS275 infusion led to a significant 19.2 ± 4.0 g increase in body mass compared to controls (3.4 ± 1.4 g). Sodium excretion was similar between the groups. Urinary nitrite/nitrate excretion was significantly reduced in MS275 rats compared to vehicle (4.3 ± 0.8 vs 8.6 ± 2.6 µmol/day, P < 0.05), while urinary ET-1 excretion was similar (3.4± 0.6 vs 5.0 ± 1.7 pg/day, P > 0.05). These data suggest that inhibition of Class 1 HDACs are critical for medullary nitric oxide production and regulation of water homeostasis.

3.7
RENAL ENDOTHELIN AND PURINERGIC SYSTEMS CONTRIBUTE TO SEXUAL DIMORPHISM IN SODIUM EXCRETION

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Renal endothelin-1 (ET-1) and purinergic systems are important regulators of Na homeostasis and may account for sex differences in cardiovascular and renal function. A link between these two systems has been recently demonstrated *in vitro*, however, the *in vivo* interaction is not clear. Therefore, we tested the hypotheses that (1) Na loading has sexually dimorphic effects on renal ET-1 production/release, and (2) purinergic signaling is involved in the renal ET-1 dependent response to a Na load. Our results showed that female Sprague Dawley rats on a normal Na diet had a 2.5-fold higher ET-1 excretion than males (14.2 ± 3.0 vs. 5.6 ± 1.0 pg/day/kg, $p<0.05$). Urinary ET-1 increased 2-fold in males with increasing dietary Na (11.2 ± 0.8 vs. 5.6 ± 1.0 pg/day/kg, $p<0.05$), but remained unchanged in females, although diuresis and natriuresis were more robust in females compared with males. Furthermore, in males only, renal intramedullary infusion of suramin (purinergic (P2) receptor blocker) significantly blunted the increase in Na excretion and inner medullary ET-1 gene expression induced by intramedullary Na loading. In contrast, ET-1 gene expression in females did not change with intramedullary Na in the presence or absence of suramin. These data indicate that an activation of inner medullary purinergic (P2) and ET-1 signaling systems could play a more important role in the natriuretic response to Na loading in male compared to female rats. These studies were funded by NIH grants P01 HL69999 and P01 HL95499.

3.8
ENDOTHELIN RECEPTOR ANTAGONIST PROTECTS AGAINST ISCHEMIA/REPERFUSION-INDUCED ACUTE KIDNEY INJURY IN MALE BUT NOT IN FEMALE RATS

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Endothelin (ET)-1/ETA receptor system has been shown to play an important role in the pathogenesis of ischemia/reperfusion-induced acute kidney injury (AKI) and we have reported that ABT-627, a selective ETA receptor antagonist, markedly attenuated AKI in male rats. On the other hand, sex differences in AKI have been established in humans and experimental animals, and there are consistent findings that females are more resistant to the renal injury than males. Müller et al. have shown that the expression of prepro-ET mRNA in kidneys subjected to ischemia was significantly higher in males and the administration of ETA receptor antagonist abolished the differences in survival between sexes (Kidney Int 2002; 62: 1364-1371). However, this area has not been extensively studied, and further studies are needed to confirm the role of ET-1/ETA receptor system in the sex differences of AKI. In the present study, we examined the protective effects of ABT-627 on AKI, using male and female Sprague-Dawley rats. AKI was achieved by clamping the left renal artery and vein for 45 minutes followed by reperfusion, 2 weeks after contralateral nephrectomy. Although renal function in both male and female vehicle-treated AKI rats significantly decreased 1 day after reperfusion, these renal dysfunction were more severe in male than in female rats. In comparison to female rats, males exhibited much more severe renal injury, characterized by proteinaceous casts in tubuli and tubular necrosis. Since female rats have very mild injury in the above experiment condition, it may not be enough to show the protective effect of ABT-627. Therefore, female rats were subjected to a longer ischemic period (60-minute ischemia) to make severe injury, which is comparable to 45-minute ischemia-induced kidney injury in males. Intravenous bolus injection of ABT-627 (1 mg/kg) 5 minutes before ischemia markedly attenuated AKI in males, but not in females. Furthermore, the sex difference in AKI was abolished by ovariectomy and ABT-627 administration attenuated AKI in ovariectomized female rats. These findings suggest that ET-1/ETA receptor system is contributive to the sex difference in the pathogenesis of AKI.

3.9
EFFECTS OF COMBINED ENDOTHELIN A RECEPTOR AND RENIN-ANGIOTENSIN SYSTEM BLOCKADE ON THE REGRESSION OF CHRONIC KIDNEY DISEASE IN 5/6 NEPHRECTOMIZED REN-2 TRANSGENIC RATS

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Study objective: We tested the hypothesis whether combined renin-angiotensin system (RAS) and endothelin 1 (ET_A) receptor blockade could be more effective in the slowing of progression of chronic kidney disease (CKD) with already established sings of renal injury than antihypertensive treatment based on isolated RAS inhibition in 5/6 nephrectomized (NX) Ren-2 transgenic hypertensive rats (TGR). **Methods:** In five groups of rats: sham-operated TGR; untreated 5/6 NX TGR; 5/6 NX TGR with dual RAS blockade (trandolapril and losartan); 5/6 NX TGR with combined treatment with RAS and ET_A receptor blockade (atrasentan), 5/6 NX was done at the age of 6 weeks and the treatments were initiated 6 weeks after 5/6 NX. Albuminuria was determined at weeks 6, 8, 12, 16, 20, 30. In other groups, concentrations of ANG II and ET1 were evaluated in the kidneys after 2 weeks of treatments. **Results:** Mortality of untreated 5/6 NX TGR was 100% at 17th week compared to sham-operated rats with mortality 0% at 30th week. Mortality of 5/6 NX TGR with RAS blockade was 32 % and in group with RAS and ET_A receptor blockade 37 % at the end of 30th week. In 5/6 NX TGR, both treatments reduced enhanced albuminuria, plasma and kidney ANG II and cortical ET-1 concentrations similarly. **Conclusion:** There is no additive effect of combined ET_A receptor and RAS blockade on the regression of CKD in 5/6 NX TGR most likely due to efficient reduction of ET-1 in the renal cortex by the dual RAS inhibition.

3.10
THE ENDOTHELIN SYSTEM MEDIATES RENAL ENDOPLASMIC RETICULUM STRESS DEVELOPMENT

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Endothelin-1 (ET-1) promotes renal damage during cardiovascular disease; yet, the exact molecular mechanisms involved remain unknown. Endoplasmic reticulum (ER) stress, triggered by unfolded protein accumulation in the ER, contributes to apoptosis and renal injury. These studies aimed to determine the role of ET-1 in renal ER stress development. Vascular endothelial cell ET-1 knockout (VEET KO) and flox control mice were used to study the role of ET-1 in renal vascular ER stress development in response to the ER stressor, tunicamycin (TM; i.p.). ER stress marker expression and renal apoptosis were determined by qRT-PCR and TUNEL assay, respectively. In flox mice, TM significantly increased relative ER stress marker expression in renal vessels (saline vs. TM, n=6-9/group, $p<0.05$; GRP94: 1.0±0.3 vs. 16.5±6.6, ATF-6: 1.0±0.5 vs. 6.8±3.5, and CHOP: 1.4±0.4 vs. 12.5±1.9) and led to increased outer medullary, non-vascular apoptosis (saline vs. TM, n=5-6/group, $p<0.05$: 0.4±0.1 vs. 5.9±1.8 $TUNEL^+$cells/field). Interestingly, TM failed to increase renal vascular ER stress or renal apoptosis in VEET KO mice. The role of ET-1 receptors in renal ER stress was assessed using ET_B deficient (sl/sl) or transgenic control (TG) rats. TM similarly increased cortical ER stress in both rat genotypes. However, in the outer medulla, TM led to a 13 to 22 fold increase from baseline only in sl/sl rats for sXBP-1, GRP78 and CHOP (n=7-8/group). Pre-treatment of TG control rats with ABT-627 (ET_A antagonist; 5mg/kg/day) for 1 week prior to TM injection significantly reduced the ER stress response to TM in cortex (7 to 50 fold decrease for GRP78, sXBP-1, CHOP and caspase-12; n=3-4/group) and medulla (7 to 25 fold decrease for GRP78, ATF-4, spliced XBP-1, CHOP and ATF-6; n=3-4/group), also inhibiting renal apoptosis. ABT-627 pre-treatment failed to reduce renal ER stress development and apoptosis in sl/sl rats, indicating that a functional ET_B receptor is key for the anti-ER stress and anti-apoptosis actions of ABT-627. In conclusion, endothelial-derived ET-1 is critical for the development of TM-induced renal ER stress and apoptosis. ET_A receptor activation induces renal ER stress genes and apoptosis, while ET_B receptor activation has reno-protective effects. These results highlight the possibility of targeting the ET-1 system as a therapeutic approach against ER stress-induced kidney damage. Funded by NIH T32 DK007545 to CDM and P01 HL95499 and P01 HL69999 to DMP and JSP.

3.11
DATA-LISTED ENDOTHELIN RECEPTOR TYPE B (ETB) DEFICIENCY RESULTS IN GREATER BLOOD PRESSURE LEVELS DURING PREGNANCY AND IN RESPONSE TO PLACENTAL ISCHEMIA-INDUCED HYPERTENSION IN RATS

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Preeclampsia (PE) is a pregnancy-specific disorder of new-onset hypertension during pregnancy that threatens the lives of both mother and fetus. The mechanisms mediating this hypertension are unclear, but studies have shown that it most likely originates from placental ischemia. Indeed, placental ischemia/hypoxia induced by reduced uterine perfusion pressure (RUPP) in experimental animals stimulates the release of soluble factors into the maternal circulation where they cause vascular dysfunction and hypertension. Importantly, blockade of the vasoconstrictive endothelin type A receptor (ETA) abolishes RUPP-induced hypertension implicating this receptor in PE. Although it is has been reported that RUPP reduces ETB-mediated blunting of ET-1-induced vasoconstriction and ETB expression in small mesenteric arteries, direct evidence implicating ETB in blood pressure regulation during pregnancy or in response to placental ischemia is scarce. Thusly, we tested the hypothesis that

ETB deficiency would result in increased blood pressure levels during pregnancy and even greater levels in response to placental ischemia. At eighteen weeks old, ETB deficient (def) and transgenic control (Tg) timed-pregnant rats were generated. Rats remained either in the normal pregnant (NP) group or RUPP surgeries performed at gestational day 14 with assessment of mean arterial blood pressure (MAP, carotid catheter) and pregnancy weights at day 19. This resulted in 4 groups: NP Tg (N=5); RUPP TG (N=4); NP ETB def (N=4); and RUPP ETB def (N=4). MAP was greater in NP ETB def than NP Tg (117±9 vs. 75±1, P<0.05). MAP levels were increased by RUPP in Tg (100±2, P<0.05) and to even greater levels in ETB def (146±6, P<0.05). Fetal weights were similar between NP ETB def and Tg (1.99±0.09 vs. 2.05±0.06) but were reduced significantly in RUPP ETB def (1.53±0.13, P<0.05) vs. Tg (1.68±0.15). Placental weights were similar in NP ETB def and Tg (0.53±0.02 vs. 0.54±0.02), and RUPP reduced these weights only in Tg (0.47<A name=OLE_LINK1 data-listid>±</A>0.03, P<0.05) not ETB def (0.58±0.01). Placental sufficiency (fetal weight divided by placental weight, a marker of placental function) was similar in both NP groups (3.79±0.26 vs. 3.86±0.19) whereas RUPP reduced this in ETB def (2.62±0.21, P<0.05) but not Tg (4.08±0.65). In conclusion, these data indicate that the ETB receptor is important for blood pressure regulation during pregnancy and blunts the hypertension and placental dysfunction found in PE. Funding: T32HL105324-01.

3.12
CIRCADIAN REGULATION OF RENAL ENDOTHELIN-1

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Rats lacking endothelin type B receptor function (ET_B def) have an exacerbated circadian blood pressure rhythm. Endothelin-1 (ET-1) promotes renal excretion of Na^+ and our lab has recently shown that endothelin-1 (ET-1) facilitates the storage and clearance of Na^+ by the skin interstitium, providing a buffer for Na^+ when salt intake is elevated. The goal of the current study is to determine if lack of ET_B impairs the ability of the skin to store Na^+, and if this impairment plays a role exacerbated circadian blood pressure rhythm in ET_B def rats. Transgenic control (Tg con) or ET_B def rats were maintained on normal (0.8% NaCl) or high salt diet (4% NaCl) and urine was collected in 12-hour intervals (active and inactive periods). In a separate group of animals, rats were euthanized at 4-hour intervals beginning at zeitgeber time 0 (lights on), and skin was taken for the measurement of Na^+ and water content. In Tg con rats, urinary excretion of ET-1, an indication of renal production, was significantly higher during the active period vs. inactive period in rats on NS (3.6±1.1 vs. 0.8±0.2 pg/12hr respectively), an effect that was more pronounced in HS fed rats (9.2±4.1 vs. 1.6±0.3 pg/12hr respectively). There was no difference in active vs. inactive period ET-1 excretion in ET_B def rats on NS (6.6±2.2 vs. 4.6±1.7 pg/12hr respectively) suggesting an altered circadian pattern of renal ET-1 production. Interestingly, the pattern was restored in ET_B def rats fed HS (2.2±1.0 vs. 9.2±2.5 pg/12hr inactive vs. active). In addition, rats fed HS had an increase in skin Na^+:H_2O compared to NS fed rats; however, there was no difference between genotypes. These data suggest that ET-1 control of blood pressure rhythms occurs mainly through activation of renal ET_B receptors to promote Na^+ excretion, and not through a reduction in the ability of skin to buffer Na^+. This research was supported by NIH grants P01 HL95499, P0169999, and T32DK007545.

3.13
ET_A RECEPTOR BLOCKADE IMPROVES THE DIFFERENTIAL DIURNAL NATRIURETIC RESPONSE TO AN ACUTE SALT LOAD IN MALE AND FEMALE ET_B DEFICIENT RATS

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We have previously shown that rats lacking ET_B receptors in non-neuronal tissues (ET_B def) have an impaired ability to handle an acute sodium load that is time-of-day dependent. Subsequent studies suggested that the attenuated natriuretic response was more evident in male compared to female ET_B def rats. The loss of the ET_B receptor not only causes salt-dependent hypertension, but also results in elevated plasma ET-1 and uncontested ET_A dependent vasoconstriction that can reduce sodium excretion. Therefore, we hypothesized that ET_A receptor blockade (ABT-627, 5mg/kg/day, po) would improve the attenuated natriuretic response to acute salt loading. Male and female ET_B def rats and littermate controls were implanted with telemetry transmitters to monitor mean arterial pressure (MAP). After a recovery period of at least a week, baseline urine samples were collected in 12 hr light/dark intervals. Rats were then given a single 900µEq Na salt load (NaCl) in 1 mL H_2O by oral gavage at the beginning of their active (7pm-7am, dark) or inactive (7am-7pm, light) period. Control rats of both sexes given ABT-627 did not show any change in the pattern of urinary sodium excretion and excreted the majority of the salt load during the first 12 hrs regardless of time of day similar to untreated rats. ET_B def males treated with ABT-627 showed a significantly improved natriuretic response to a salt load given during the inactive period after the first 12 hrs (456 ± 74 treated, n=4; 50 ± 91µEq Na/12hr untreated, n=5; P<0.05). ET_B def females treated with ABT-627 also showed an improved natriuretic response during the first 12 hrs of the inactive period as well, though this improvement was not statistically significant (474 ± 34 treated, n=4; 183 ± 44µEq Na/12hr untreated, n=6; NS). MAP was unchanged in response to salt loading although ABT-627 significantly reduced MAP throughout the entire treatment period in both male and female rats (P<0.05). These results show that ET_A receptor activation contributes to the impaired natriuretic response to salt loading in both male and female rats but does not diminish the time–related sex difference in sodium handling. This research was supported in part by NIH grants: P01 HL95499, P01 HL69999, and T32 DK007545.

3.14
ENDOTHELIN-1 INCREASES GLOMERULAR PERMEABILITY IN SICKLE CELL MICE

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Sickle cell disease (SCD) extensively alters renal structure and function, leading to nephropathy manifested by increased permeability of filtration barrier and albuminuria/proteinuria. The endothelium-derived peptide, endothelin-1 (ET-1), with its powerful vasoconstrictor and pro-inflammatory effects mediated primarily through ET_A receptors, is elevated in plasma and urine of SCD patients and may contribute to the development of sickle cell glomerulopathy. Therefore, the aim of the study was to determine whether ET-1 contributes to increased glomerular permeability to albumin in SCD and if ET_A receptors blockade ameliorates glomerular damage. Furthermore, because our preliminary studies showed sex differences in the vasoconstrictor response to ET-1 in sickle cell mice the study was designed to determine if sex differences exist in this response. Experiments utilized 12 week old humanized sickle cell mice (HbSS) and genetic controls (HbAA) recently developed by the Townes' lab. Ambrisentan (ET_A antagonist), A-182086 (ET_{AB} antagonist) or vehicle was administrated via drinking water and the concentration adjusted daily according to the intake (10mg/kg/day) for 2 weeks. Glomeruli were isolated for direct permeability measurements as a volume response of glomerular capillaries to an oncopressive medium generated by defined concentrations of albumin. Urinary protein excretion was determined using Bradford colorimetric method. Urinary albumin excretion was measured using enzyme immunoassay kit (GenWay). Glomerular permeability to albumin (P_{alb}) was significantly higher in glomeruli from sickle mice (both in males and females) than control mice (0.50±0.07 and 0.47±0.06 vs. 0.13±0.02 and 0.10±0.2, respectively). Ambrisentan treatment significantly reduced the elevated P_{alb} in glomeruli from male (0.24±0.05 vs. 0.50±0.07) and female (0.20±0.03 vs. 0.47±0.06) HbSS mice. ET_{AB} receptors antagonism with A-182086 also significantly decreased the P_{alb} in glomeruli from male (0.28±0.06 vs. 0.50±0.07) and female (0.24±0.03 vs. 0.47±0.06) HbSS mice. However, there was no effect on albumin or protein excretion after the treatment with both antagonists. Treatment with both antagonists did not alter P_{alb} in HbAA mice. These data support the hypothesis that ET-1 may play an important role in the development of sickle cell nephropathy and support the use of chronic ET_A antagonism as a prospective treatment for sickle cell nephropathy. This work was supported by the program project grant on The Role of Endothelin-1 in Sickle Cell Disease (U01 HL117684), and UAB- USCD O'Brien Center (grant DK079337).

3.15
SELECTIVE ENDOTHELIN-A RECEPTOR ANTAGONISM PREVENTS THE PROGRESSION OF ACUTE KIDNEY INJURY TO CHRONIC KIDNEY DISEASE

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Introduction: Acute kidney injury (AKI) is very common and associated with significant morbidity and mortality. AKI often progresses to chronic kidney disease (CKD) and endothelin-1 (ET-1) contributes to this. We hypothesized that therapeutic administration of selective ET_A receptor antagonism would protect from the transition of AKI to CKD. **Methods:** 28 FVB mice underwent prolonged (50min) unilateral ischemia-reperfusion injury (IRI) with 28 days recovery. 14 mice received daily selective ET_A antagonism (sitaxentan) starting 24h after IRI. We assessed blood pressure (BP) *via* telemetry, vascular function, renal injury and measures of the ET system. **Results:** Systolic BP increased by ~5mmHg after IRI and was associated with vascular dysfunction in both resistance and conduit vessels. Sitaxentan partly

prevented both of these. At 28d after IRI kidney weight was reduced (-55%) and associated with significant macrophage infiltration and fibrosis compared to the contralateral control kidney. Mice treated with sitaxentan had normal kidney weight, reduced macrophage infiltration and less fibrosis: IRI kidney *vs.* control kidney *vs.* IRI kidney with sitaxentan: F4/80 stain/high power field: 2.5 *vs.* 0.2 *vs.* 0.8%; picosirius red stain/high power field: 8.6 *vs.* 0.48 *vs.* 3.1%. For both macrophage infiltration and fibrosis, $p<0.05$ for IRI *vs.* control and for IRI *vs.* IRI with sitaxentan, p=ns for control *vs.* IRI with sitaxentan. Furthermore, an up-regulation of both the ET_A (28-fold) and ET_B (2-fold) receptors as well as pre-pro-ET-1 (10-fold) mRNA was seen in both the cortex and medulla of the IRI kidney relative to control. Angiotensinogen and renin mRNA was unchanged. With sitaxentan treatment ET_A/ET_B receptor and pre-pro-ET-1 mRNA remained similar to baseline levels. Finally, renal ET-1 production increased following IRI and this was prevented by ET_A receptor antagonism (fractional excretion of ET-1: IRI *vs.* IRI with sitaxentan: 47 *vs.* 16%, $p<0.05$). **Conclusions:** In an *in vivo* model of AKI progressing to CKD, ET_A receptor antagonism reduced BP and vascular dysfunction and prevented progression of renal injury and ET system activation after AKI. Therefore, selective ET_A receptor antagonism offers a potentially novel therapy for AKI. Translational studies are now warranted. *Funded by a British Heart Foundation PhD studentship (FS/11/7829328).*

3.16
THE ROLE OF A RENAL ALDOSTERONE-ENDOTHELIN FEEDBACK SYSTEM IN TOTAL NA BALANCE AND MINERALOCORTICOID ESCAPE

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Aldosterone increases blood pressure (BP) by stimulating sodium (Na) reabsorption in the collecting duct (CD) and, by negative feedback, stimulates CD endothelin-1 (ET-1) that acts to inhibit Na and water reabsorption. We have previously provided evidence for a renal aldosterone-endothelin feedback system. We tested the hypothesis that this system is necessary for proper Na balance and BP control by comparing the effect of a high NaCl diet (2% gelled diet with saline to drink) and mineralocorticoid stimulation on wild-type mice (WT) and CD-specific ET-1 knockout mice (CD ET-1 KO). Metabolic balance and radiotelemetric BP were measured before and after treatment with desoxycorticosterone pivalate (DOCP, 0.07mg/g, IM). CD ET-1 KO mice consumed more high NaCl diet and saline and had greater urine output than WT. WT mice did not increase fluid intake or retention, body weight (BW), or systolic BP until during DOCP treatment. In contrast, the CD ET-1 KO mice increased fluid retention, BW, and systolic BP on the high NaCl diet alone. DOCP further increased systolic BP in the CD ET-1 KO, which exhibited greater Na and water retention and BW gain than WT. Unlike WT, CD ET-1 KO mice failed to return to neutral Na balance during 19 days of DOCP administration (mineralocorticoid escape). Thus, the absence of CD ET-1 expression impairs renal response to Na loading, which results in abnormal fluid and electrolyte handling when challenged with a high NaCl diet and DOCP treatment. We conclude that CD expressed ET-1 functions as an essential element necessary for mineralocorticoid escape. This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-82680 to BDC & CSW, NHLBI grant P01 HL095499 to DEK, NIH postdoctoral fellowship 2T32HL083810 to AKW, and by funds from the Department of Veterans Affairs to CSW.

3.17
HIGH SALT INTAKE INCREASES ET-1 MEDIATED NATRIURESIS AND DIURESIS VIA THE ET_B RECEPTOR IN RATS

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Endothelin 1 (ET-1) causes vasoconstriction (anti-natriuresis) through ET_A receptors and promotes natriuresis via ET_B within the renal medulla, specifically in the collecting ducts. Direct intramedullary infusion of ET-1 has no effect on renal sodium and water excretion but is associated with ET_A-dependent reductions in medullary blood flow. We have data to suggest that chronic high salt (HS; 4%NaCl) intake reduces renal medullary expression of ET_A receptors, therefore leading us to hypothesize that HS intake would increase the natriuretic and diuretic response to renal medullary infusion of ET-1. Male Sprague Dawley (SD) rats were fed a normal salt (NS; 0.4% NaCl) or HS diet for 7 days. Rats were anesthetized and a catheter implanted in the renal medulla for interstitial infusion along with a ureteral catheter for urine collection. An adjustable occlude was placed around the aorta proximal to the left renal artery to maintain renal perfusion pressure throughout the protocol. After equilibration and a 40 min control period, the urine flow (UV) and sodium excretion (UNaV) responses to medullary infusion of a low dose of the ET_B receptor agonist, sarafotoxin S6c (0.15 μg/kg/h), or non-selective agonist ET-1 (0.45 μg/kg/h) were determined over two 40 min infusion periods. Intramedullary infusion of S6c markedly increased UV and UNaV in the high salt treated rats (UV: 5.0±0.5 to 28.3±3.4 μl/min; UNaV: 0.87±0.12 to 1.98±0.18 μmol/min), but not in the NS treated rats. In HS treated rats, intramedullary infusion of ET-1 (0.45 μg/kg/h) induced a significant natriuretic responses compared to NS treated rats. (HS: 0.90±0.21 to 1.50±0.23; NS: 0.40±0.08 to 0.69±0.17 μmol/min). We conclude that high salt intake enhances the diuretic and natriuretic effects of ET_B receptor activation in vivo consistent with a role for the ET_B receptor in maintaining fluid-electrolyte balance that is counterbalanced by ET_A activity. This work was supported by the program project grant on the Endothelin Control of Renal Hemodynamic and Excretory Function (PO1 HL-95499).

3.18
ENDOTHELIN RECEPTOR ANTAGONISM IN SICKLE CELL NEPHROPATHY

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Introduction: Sickle-cell disease (SCD) is characterized by chronic hemolysis and recurrent episodes of vaso-occlusive events that affect the microcirculation and lead to ischemic tissue injury with multi-organ dysfunction. Sickle cell nephropathy (SCN), a major mortality risk factor in SCD, is characterized by an early increase in glomerular filtration rate with subsequent progressive decline of renal function. Focal and segmental glomerulosclerosis (FSGS) and hypertrophied glomeruli with distended capillaries are the major hallmarks of glomerular lesions. We investigated the effects of chronic mixed ET receptor antagonism in a model of SCD-mediated FSGS. **Methods & Results:** We used SAD1 (SAD) Hbβsingle/single hemizygous mice on the C57BL/6J background. At 3 months of age SAD mice displayed little evidence of chronic renal damage but significant glomerulomegaly compared to controls (average glomerular section area: 1733±155 *vs.* 1540±70μm^2, $p<0.01$). Glomerulomegaly persisted, and was worse, at 6 months of age (average glomerular section area: 2372±207 *vs.* 1519±180μm^2, $p<0.001$). In addition, SAD mice had increased glomerulosclerosis than controls (SAD *vs.* WT: 21±9 *vs.* 10±8%, $p<0.05$). Masson trichrome and Marinot silver staining showed marked thickening of glomerular basement membranes, mesangiolysis and sclerosis with diminished podocyte density as observed with podoplanin and WT-1 immunostaining ($p<0.01$ *vs.* controls). Based on these data we treated SAD mice and controls aged 3 months with the mixed ET receptor antagonist bosentan for 9 months in a preventative study, and 6 months old SAD mice for 6 months in a therapeutic study. We assessed blood pressure, kidney structure and function after 6 and 9 months of continuous treatment. In the preventative study, 6 months of bosentan therapy was associated with ~4-fold less glomerulosclerosis compared to untreated SAD mice (22±8 *vs.* 86±4%, $p<0.001$). Additionally, there was an 80% reduction in mean glomerular surface area ($p<0.05$). In the therapeutic study, there was a significant reduction in glomerulosclerosis ($p<0.01$) and glomerulomegaly ($p<0.01$) compared to untreated mice but this was less effective than in the preventative study ($p<0.05$). **Conclusions:** ET receptor antagonism is a potentially useful preventative or therapeutic approach in SCN. Based on these data clinical trials are warranted.

3.19
ENDOTHELIAL-DERIVED ENDOTHELIN-1 CONTRIBUTES TO RENAL DYSFUNCTION AND MORTALITY IN SICKLE CELL MICE

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Sickle cell disease (SCD) is a common genetic hematologic disease that causes progressive multi-organ pathology including nephropathy beginning as early as childhood. Endothelin-1 (ET-1) is known to be elevated in the plasma and urine of SCD patients and mice. A growing body of evidence using ET receptor antagonists in SCD mice suggests that ET-1 plays a role in the pathophysiology of SCD. However, the cellular source of increased ET-1 production in SCD has yet to be determined. We hypothesized that endothelial-derived ET-1 contributes to renal dysfunction and mortality in SCD mice. Vascular endothelial-specific ET-1 knockout (VEETKO) mice and genotype controls (flox) underwent bone marrow transplantation (BMT) with marrow derived from humanized SCD mice (HbSS). This method resulted in the de-

velopment of SCD in mice lacking endothelial ET-1 production (HbSS-VEETKO^{BMT}) and mice with endothelial ET-1 production (HbSS-flox^{BMT}). Full induction of the sickle hemoglobin phenotype requires approximately 12 weeks. The HbSS-VEETKO^{BMT} group showed a significant survival advantage over the HbSS-flox^{BMT} group (p=0.026) with all HbSS-VEETKO^{BMT} mice surviving and only 58% of HbSS-flox^{BMT} mice surviving at 18 weeks post-BMT. Dysfunctional urine-concentrating ability exists in SCD patients and mice, most likely, secondary to progressive renal injury. To determine whether endothelial-derived ET-1 participates in the SCD mediated loss of urine-concentrating ability, osmolality was measured in both spot urines and 24- hour metabolic cage urine collections. At five weeks post-BMT, prior to the onset of the SCD phenotype, there was no difference in spot urine osmolality between HbSS-flox^{BMT} and HbSS-VEETKO^{BMT} mice (3244±268 vs. 3096±165 mOsm/kg, p>0.05). Following the onset of the SCD phenotype, mortality in the HbSS-flox^{BMT} group left only a single mouse for the assessment 24-hour urine osmolality, and this mouse showed a low urine osmolality (930 mOsm/kg). However, when compared to HbSS mice, HbSS-VEETKO^{BMT} mice demonstrated preserved urine-concentrating ability as indicated by significantly higher 24-hour urine osmolality (584±11 vs. 2512±361 mOsm/kg, p=0.006). These data indicate that endothelial-derived ET-1 is a major mediator of SCD pathophysiology and that lack of endothelial ET-1 production is sufficient to prevent renal dysfunction and mortality in SCD mice. This work was supported by U01 HL117684 to DMP and JSP and T32 HL007918 to BMF.

3.20
IDENTIFICATION OF EDN1-AS: A NOVEL LONG, NON-CODING RNA IN THE REGULATION OF ENDOTHELIN-1

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Long, non-coding (lnc)RNAs regulate gene expression via diverse mechanisms and can either activate or silence genes. Endothelin-1 (ET-1) is a peptide hormone that contributes to blood pressure regulation in a tissue-specific manner. We have identified a novel lncRNA transcribed from the human ET-1 gene (EDN1) locus. This antisense lncRNA, designated EDN1-AS, was first identified in human bronchial epithelial (S9) cells. We have also detected expression of EDN1-AS in human umbilical vein endothelial (HUVEC) cells, human mammary epithelial (HMEC) cells, and whole human kidney. These samples were derived from males and females, thus, EDN1-AS appears to be expressed in both sexes. The EDN1-AS lncRNA was also shown to oscillate over the course of 24 hours in synchronized human renal proximal tubule cells (HK2), suggesting that its expression may be regulated by the circadian clock mechanism. The clock is made of four core proteins: Bmal1, CLOCK, Cry (homologs 1 and 2), and Per (homologs 1-3). Interestingly, Per1 inhibits ET-1 expression in the kidney and Per1 was detected at the EDN1-AS promoter in HK2 cells. Furthermore, we have recently identified a murine homolog of EDN1-AS. Preliminary data indicate that expression of mEDN1-AS is higher in mouse kidney medulla compared to the cortex. Together these data demonstrate the identification of a novel ET-1-related lncRNA expressed in mice and humans, detectable in several cell types in both sexes, and may represent a previously unknown mechanism for regulation of ET-1 expression. Funding: This work was supported by NIH DK085193 and DK098460, and the ASN Foundation for Kidney Research to MLG, 2T32HL083810 to KS, 2T32HL083810 to AKW, and NIH DK082680 to BDC and CSW.

3.21
ET_A RECEPTOR ACTIVATION CONTRIBUTES TO T CELL INFILTRATION FOLLOWING RENAL ISCHEMIA-REPERFUSION INJURY

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Endothelin-1 (ET-1) and the ET_A receptor undergo rapid and sustained upregulation in the kidney following ischemia and reperfusion (IR) injury, but data on the long-term consequences of this on renal function are limited. Renal IR injury increases the risk of hypertension, and while both the endothelin and immune systems have been implicated in hypertension, the contribution of the endothelin system to the inflammatory response following renal IR injury is poorly understood. The current study tested whether ET_A receptor activation mediates adhesion molecule and chemotactic protein expression, and subsequent T cell infiltration following renal IR injury. Male C57BL/6 mice were treated with the selective ET_A receptor antagonist ABT-627 (10 mg/kg/d p.o.) or vehicle (drinking water), from 2 days prior to 45 min unilateral renal IR. Treatment continued during recovery and mice were sacrificed at 24 h or 10 days post-IR (n=5-8 per group). The concentration of monocyte chemoattractant protein (MCP)-1 was significantly increased in the renal cortex of the ischemic versus contralateral kidney at 24 h post-IR (10-12 fold; P_{kidney}<0.001), but there was no significant difference between vehicle and ABT-627-treated mice. A similar increase was seen in the ischemic outer medulla, with no difference between vehicle and ABT-627-treated mice (MCP-1: 47±3 and 45±5 pg/mg protein respectively P>0.05). The mRNA expression of the adhesion molecule E-Selectin was significantly increased in the cortex of the ischemic kidney in both groups at 24 h (P_{kidney}<0.01), but this was not significantly affected by ABT-627. At 10 days post-IR, $CD3^+$ T cell numbers were dramatically increased in the ischemic versus contralateral cortex and outer medulla of both groups (P_{kidney}<0.001). ET_A blockade significantly blunted the rise in T cell number in the outer medulla ($P_{kidney*treatment}$<0.05; 250±60 cells/mm^2 in ABT-627-treated mice, 422±37 cells/mm^2 in vehicle), but this effect did not reach statistical significance in the cortex ($P_{kidney*treatment}$=0.1; 333±64cells/mm^2 in ABT-627-treated mice, 490±71cells/mm^2 in vehicle). These data suggest that ET-1 acting via the ET_A receptor contributes to T cell infiltration of the outer medulla post-IR injury. This may have important implications for long-term blood pressure control following acute kidney injury, an area which awaits further investigation. Funding: American Heart Association Scientist Development Grant 12SDG8960028.

3.22
EVALUATION OF ENDOTHELIN A RECEPTOR (ETA) BLOCKADE ON THE PROGRESSION OF RENAL INJURY IN VARIOUS MODELS OF METABOLIC DISORDERS WITH PRE-EXISTING RENAL DISEASE

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The endothelin (ET) system has been shown to play an important role in the development of renal injury via the endothelin A (ET_A) receptor. Therefore, the current study examined whether chronic ET_A blockade with ABT-627 would prevent the progression of renal injury in various animals models of metabolic disorders with pre-existing renal disease. In our type-1 diabetic model of renal disease (the streptozotocin (STZ) treated Dahl salt-sensitive (SS) rat), proteinuria increased to 353±34 mg/day and the rats were then separated into two groups: (1) vehicle and (2) ET_A antagonist, ABT-627 (5mg/kg/day). After 6 weeks of treatment, MAP (via telemetry) decreased by 20% (139±10 vs. 108±6 mmHg, respectively, n=5) and proteinuria was markedly reduced in ABT-627 treated STZ-SS rats versus vehicle treated rats (310±32 vs. 517±68 mg/day, respectively, n=8). The degree of glomerular injury and renal interstitial fibrosis was significantly reduced in the kidneys of ABT-627 treated STZ-SS rats compared to vehicle STZ-SS rats. Next, we determined whether treatment with ABT-627 for 6 weeks would be beneficial in a type-2 diabetic model (T2DN rat) with pre-existing renal injury. While chronic ET_A blockade did not have an effect on arterial pressure or proteinuria in T2DN rats, ABT-627 prevented the rise in plasma creatinine (Pcr) levels (1.4±0.2 vs. 0.9±0.1 mg/dL, respectively, n=6). Since the contribution of ET during the progression of renal disease associated with non-diabetic obesity has not been thoroughly investigated, we examined whether ET_A blockade would be beneficial in preventing the progression of renal injury associated with obesity in the obese leptin receptor mutant Dahl salt-sensitive (SS^{LepR}mutant) strain derived from Zinc-finger nucleases. After 9 weeks of treatment, MAP was reduced by 20% (215±8 vs. 173±6 mmHg, respectively, n=3-5). However, we did not observe any differences in proteinuria between the groups. Similar to T2DN rats, chronic ET_A blockade attenuated the increase in Pcr (1.3±0.2 vs. 0.7±0.1 mg/dL). Interestingly, treatment with ABT-627 increased the survival rate of SS^{LepR}mutant rats (83%, 5 of 6 vs. 50%, 3 of 6). In conclusion, these data indicate that ET_A blockade prevents the decline in renal function in animal models suffering from hypertension, diabetes and/or obesity with pre-existing renal disease. This research supported by NIGMS NIH P20GM104357 and AHA 12SDG9440034.

3.23
ENDOTHELIN B RECEPTOR AGONIST, IRL-1620, PROVIDES NEUROPROTECTION AND ENHANCES ANGIOGENESIS IN DIABETIC RATS WITH CEREBRAL ISCHEMIA

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The incidence of Type 2 Diabetes Mellitus (T2DM) and its associated cardiovascular complications are on the rise. Endothelin B (ET_B) receptor agonist, IRL-1620, has been shown to provide neuroprotection and enhance neurovascular repair following cerebral ischemia, however its efficacy in comorbid models of ischemia and T2DM is unknown. T2DM was induced in rats using a HFD/STZ protocol, followed by permanent occlusion of the right middle cerebral artery (MCAO). IRL-1620 (5µg/kg,IV) was administered at 4, 6, and 8 hr on days 0, 3, and 6 post-MCAO. Survival and neurological function for the IRL-1620 group were significantly higher in

both non-diabetic (ND) and diabetic (D) rats compared to vehicle. A significant reduction in infarct volume was seen with IRL-1620 in both ND (80.8%) and D (69.6%) rats. There were significantly more PECAM-positive vessels/30μm brain slice in the ND and D IRL-1620 rats (9.1±0.5; 9.0±0.4) versus vehicle (5.9±0.4; 7.1±0.4). Immunostaining revealed a significant increase in ET_B expression for ND ($p<0.0001$) and D IRL-1620 rats ($p=0.04$), compared to vehicle. Co-localization of VEGF- and PECAM-positive endothelial cells was significant higher in D IRL-1620 rats (15.5±1.1%) compared to vehicle (10.2±0.8%). Results indicate that IRL-1620 is effective in cerebral ischemia in rats with T2DM by significantly reducing infarct volume, improving neurological/motor function, and enhancing angiogenesis. This study was supported by Midwestern University.

3.24
THE APOPTOTIC PATHWAY MEDIATES THE NEUROPROTECTIVE EFFECT OF IRL-1620 IN A RAT MODEL OF FOCAL CEREBRAL ISCHEMIA

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Previous studies have shown that IRL-1620 enhances neurovascular remodeling following cerebral ischemia. It is possible that IRL-1620 provides protection to neurons by inhibiting the apoptotic pathway. Following middle cerebral artery occlusion (MCAO), rats received 3 injections of vehicle or IRL-1620 (5μg/kg,IV) at 2, 4 and 6 hr. Behavioral evaluation confirmed the induction of stroke. Rats were sacrificed and brains processed to evaluate protein expression of apoptotic markers. All procedures were approved by Midwestern University IACUC. Rats treated with IRL-1620 showed significant improvement in neurological and motor function tests compared to vehicle. In addition, there was a significant decrease in infarct volume in IRL-1620 treated rats ($24.47\pm4.37mm^3$) versus vehicle group ($153.23\pm32.18mm^3$). Anti-apoptotic protein Bcl-2 expression was decreased and pro-apoptotic protein Bax expression was increased in vehicle-treated compared to sham ($p<0.0001$). IRL-1620 treatment showed significantly ($p<0.01$) increased expression of Bcl-2 and decreased expression of Bax. There were no changes in total Akt expression in sham, vehicle and IRL-1620 treated rats, however, there was an increase in pAkt in IRL-1620 treated rats compared to vehicle group ($P<0.05$) post-occlusion. The results demonstrate that IRL-1620 is a neuroprotective agent and attenuates the neuronal damage following cerebral ischemia in rats by preventing apoptosis. Study was supported by Midwestern University.

3.25
NEUROPROTECTIVE EFFECT OF APILIMOD IN ISCHEMIA REPERFUSION INJURY IN RATS

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Background: Cerebral stroke is a neurodegenerative disease and it is a major cause of death and disability throughout the world. Mitochondrial reactive oxygen species (ROS) generation and Lipid peroxidation level is increase during ischemia, which is responsible for neuronal death in cerebral stroke. Higher levels of ROS cause state of oxidative stress. Reactive oxygen species damage the DNA and play an important role in apoptosis. Lipid peroxidation is a well-established mechanism of cellular injury in animals, and refers to the oxidative degradation of lipids. So, Lipid peroxidation is an important factor in the pathophysiology of ischemia. **Purpose:** The aim of this study was to investigate the neuroprotective effects of apilimod (IL-12 inhibitor) on middle cerebral artery occlusion model of stroke in rats. **Methods:** Focal cerebral ischemia was induced in male S.D. rats (250 ± 20g) by occlusion of middle cerebral artery Apilimod was administered prior and post induction of ischemia to assess its therapeutic window. Neurological deficit were determined by Longa's score. Lipid peroxidation levels were evaluated by malondialdehyde assay and Glutathione (GSH) level was evaluated by 5,5-dithiobis-2-nitrobenzoic acid (DTNB) which is readily reduced by sulfhydryls forming a yellow substance which is measured spectrophotometrically at 412 nm. **Result:** Apilimod significantly ameliorated the neurological deficit. The biomarker of oxidative stress malondialdehyde (MDA) was also found to be significantly reduced following apilimod administration and increased the level of the antioxidant enzyme glutathione (GSH). **Conclusion**: These results show that apilimod has a preventive effect against ischemic stroke in animal model. **Acknowledgement:** We thanks the Defence Research & Development Organisation (DRDO), New Delhi, India for providing a grant (no. ERIP/ER/1103953M/01/1361).

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3.26
NEUROPROTECTIVE POTENTIAL OF ENDOTHELIN ET_A RECEPTOR ANTAGONIST IN CEREBRAL ISCHEMIA MODELS

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Stroke is the second most common cause of death and the leading cause of disability, worldwide. The pathophysiology of stroke involves a complex multifactorial cascades. There is increasing evidence of involvement of endothelin in the development and progression of ischemic stroke. Therefore, endothelin is considered as one of the target for the treatment of cerebral ischemia-reperfusion injury. In this study we have investigated the effect of ET_A receptor antagonist in two models viz. rat model of focal cerebral ischemia and gerbil model of global cerebral ischemia. Effect of endothelin receptor antagonist was assessed on cerebral blood flow, neurological score, neurological damage and biochemical changes after induction of cerebral ischemia. Middle cerebral artery occlusion (MCAO) resulted in drastic decrease (80%) in ipsilateral cerebral blood flow as measured by Laser Doppler flowmetry. Contralateral blood flow remained unaltered. Significant neurological deficits and damage was observed with MCAO. BMS 182874 showed neuroprotection in both animal models. BMS 182874 treatment significantly reduced ischaemic damage in focal cerebral ischemia model. This was associated with reduction in TNF α levels and MPO activity. BMS 182874 treatment (5 mg/kg) in gerbil model significantly decreased the locomotor activity as compared with control group. The response latency in passive avoidance test was also increased. This improvement in neurological parameters was well reflected in histopathology of brain. CA1 region of hippocampus was examined for number of survived neurons. BMS 182874 treatment increased the number of survived cells in CA1 region. Results of this study suggested the potential of pharmacological interventions targeted at endothelin receptors in cerebral ischemia models.

3.27
P66 SHC REGULATES ET-1-MEDIATED INTRACELLULAR CALCIUM HANDLING IN RENAL RESISTANCE ARTERIES AND CONTRIBUTES TO RENAL GLOMERULAR INJURY IN HYPERTENSION

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The pathogenesis of hypertension-induced nephropathy is associated with increased renal vascular resistance and loss of vascular responsiveness. Even though microvascular injuries are detected in the majority of patients with hypertension and are among causes of diseases leading to end stage renal disease, the pathophysiological mechanisms mediating renal microvascular dysfunction remain unknown. Excessive signaling via adaptor protein p66 Shc is likely to be one of the mechanisms of oxidative stress-related pathologies and the purpose of this study was to assess the contribution of p66 Shc to regulation of vascular responsiveness associated with hypertension-induced nephropathy. Dahl salt-sensitive (SS) rats exhibit many traits associated with salt-sensitive hypertension in man and became a well-established model for the study of salt-sensitive hypertension and accompanying cardiovascular disorders. By combining the precise modification of rat *Shc1* gene with *in vivo* knock-in strategy we have generated a panel of mutant rat strains on the genetic background of SS rats. Endothelin-1 (ET-1), an important player in hypertension-related kidney diseases, induces the phosphorylation of p66 Shc serine 36 residue which is essential for p66 Shc to promote oxidative stress-related pathologies. ET-1-mediated elevation of intracellular Ca^{2+} is strongly linked to renal microvascular contraction and is crucial for ET-1-induced contraction of smooth muscle cells. We used two-photon imaging of intracellular Ca^{2+} handling in renal resistance arteries isolated either from wild type or from p66 Shc rat knockouts to investigate the role of p66 Shc in ET-1-mediated calcium signaling. We here report that overexpression of p66 Shc, observed in SS rats maintained on high-salt diet, partially impairs ET-1-mediated intracellular calcium handling in renal resistance arteries. ET-1-treated renal microvessels isolated from p66 Shc knockout rats demonstrate an increased elevation of intracellular calcium concentration when compared to renal vessels isolated from wild type SS rats. Since glomerular damage is one of direct consequences of renal vascular dysfunction, we have carried out glomerular injury scoring on PAS-stained paraffin sections. The comparison of glomerular damage in p66 Shc knockout and wild type SS animals revealed the mitigation of glomerular damage in the absence of p66 Shc. Our data suggest that p66 Shc knockout restores the calcium handling by ET-1 in smooth muscle cells of

isolated rat renal microvessels and mitigates renal damage in rats with hypertension-induced nephropathy.

3.28
THE DOMINANCE OF RENIN-ANGIOTENSIN SYSTEM BLOCKADE OVER ENDOTHELIN RECEPTOR A BLOCKADE IN LOWERING OF BLOOD PRESSURE IN HETEROZYGOUS REN-2 TRANSGENIC RATS

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Objective: We tested the hypothesis whether the addition of ET_A blockade to renin-angiotensin system (RAS) blockade would have other effects on principal vasoactive systems contributing to blood pressure (BP) maintenance in Ren-2 transgenic rats (TGR). **Design and Methods**: 5-week-old TGR rats were given either atrasentan, or a combination of atrasentan with angiotensin receptor blocker losartan, angiotensin converting enzyme inhibitor captopril, or direct renin inhibitor aliskiren for 4 weeks. At the end of the study, basal BP and acute BP responses to consecutive blockade of renin-angiotensin (RAS), sympathetic nervous (SNS), and nitric oxide (NO) systems were determined in conscious rats. Moreover, BP responses to acute inhibition of nifedipine-sensitive calcium influx through voltage-dependent calcium channels (L-VDCC) were measured. **Results**: Atrasentan alone partially lowered BP, while in combination with all three RAS blockers BP was fully normalized. The BP lowering effects of all three RAS-blocking agents was dependent on the attenuation of both RAS- and SNS-dependent vasoconstriction. In all atrasentan-treated groups, NO-dependent vasodilation was substantially reduced and calcium influx through L-VDCC significantly decreased. **Conclusion:** Although the BP-lowering effects of combined ET_A blockade and RAS blockade is predominantly dependent on the effects mediated by RAS blockade, further effects are attributable to ET_A blockade. Grant 304/12/0259-Czech Science Foundation.

3.29
LINAGLIPTIN PROVIDES CEREBROVASCULAR PROTECTION VIA UPREGULATION OF ENDOTHELIAL ET-1 AND ETB RECEPTORS IN DIABETES.

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We have shown that glycemic control with metformin or endothelin-1 (ET-1) inhibition with bosentan prevents AND restores diabetes-mediated pathological remodeling and neovascularization of the cerebrovasculature. Our recent data suggest that linagliptin, a member of the dipeptidyl peptidase-4 (DPP-4) inhibitor class of oral hypoglycemic agents, prevents cerebrovascular remodeling and dysfunction independent of its blood glucose lowering effects. We hypothesized that linagliptin provides cerebrovascular protection via modulation of the ET-1 system. **Methods:** 24 week old diabetic (Hemoglobin A1C >6.5%) and nondiabetic Wistar rats were treated for 4 weeks with either vehicle chow or chow containing 166mg/kg linagliptin. Retinal acellular capillary formation was measures as a surrogate marker for pathological neovascularization in diabetes. Brain microvascular endothelial cells (BMVEC) isolated from control or diabetic rats were also treated with (100 nM) linagliptin or Bosentan (10 uM) and tested for angiogenic properties with cell migration and tube formation assays. Expression of ET-1 and ET-B receptor were assessed using ELISA, RT-PCR and immunoblotting after treatment of BMVEC with linagliptin. **Results:** Linagliptin treatment significantly decreased retinal acellular capillaries in diabetes. BMVEC from diabetic animals showed a significant reduction in ET-B receptor. Linagliptin significantly increased ET-1 mRNA expression in both control and diabetic BMVEC and increased ET-B receptor mRNA and protein levels only in BMVECs from diabetic animals. In addition, linagliptin normalized the augmented angiogenic properties of diabetic BMVEC, such effect was blocked with the dual ET-A/Et-B antagonist, bosentan. **Conclusions:** Our results suggest that linagliptin-mediated increases in ET-1 and ETB provide cerebrovascular protection in a feed-forward mechanism.

3.30
ENDOTEHLIN-1: A FINAL COMMON PATHWAY LINKING PLACENTAL ISCHEMIA TO ENDOTHELIAL DYSFUNCTION AND HYPERTENSION DURING PREECLAMPSIA

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Despite being one of the leading causes of maternal death and a major contributor of maternal and perinatal morbidity, the mechanisms responsible for the pathogenesis of preeclampsia (PE) has yet to be fully elucidated. Growing evidence supports the concept that the placenta plays a central role in the pathogenesis of PE and that reduced uteroplacental perfusion leads to release of soluble placental factors such as the antiangiogenuc factor (sFlt-1) and tumor necrosis factor (TNF-α) and increased formation of the agonistic autoantibody to the angiotensin II type 1 receptor (AT1-AA).There is growing evidence to suggest an important role for endothelin-1 (ET-1) in the pathophysiology of preeclampsia. Multiple studies have examined circulating levels of ET-1 and found elevated levels of plasma ET-1 in the preeclamptic group, with some studies indicating that the level of circulating ET-1 correlates with the severity of the disease symptoms. ET-1, however, is produced locally and plasma levels typically do not reflect tissue levels of the peptide. Animal studies from our lab has shown that a number of experimental models of preeclampsia (placental ischemia, sFlt-1 infusion, TNF-α infusion, and AT1-AA infusion) are associated with elevated tissue levels of ET-1. Moreover, we have reported that hypertension in pregnant rats, induced by placental ischemia or chronic infusion of sFlt-1, TNF-α, or AT1-AA can be completely attenuated by ET_A receptor antagonism, strongly suggests that ET-1 appears to be a final common pathway linking factors produced during placental ischemia to elevations in blood pressure. The findings that antagonism of the endothelin-A receptor is beneficial in numerous animal models of PE suggest that the ET system may be an intriguing target for pharmacological intervention in PE.

3.31
INDUCTION OF LONG-TERM ENDOTHELIN-1 OVEREXPRESSION CAUSES BLOOD PRESSURE RISE AND SMALL ARTERY STIFFENING

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Background: The mechanisms of blood pressure (BP) regulation by endothelin (ET)-1 produced by endothelial cells are complex and remain unclear. Recently, we developed a transgenic mouse with tamoxifen-inducible endothelium-restricted human ET-1 overexpression (ieET-1) using Cre/loxP technology. ieET-1 mice exhibited BP rise after three weeks of induction in an ET type A receptor-dependent manner, in absence of vascular and kidney injury. It is unknown whether long-term exposure to ET-1 overexpression results in elevated BP elevation and vascular injury. **Methods:** Nine to 12-week old male ieET-1 mice and control ieCre mice expressing a tamoxifen-inducible Cre recombinase ($CreER^{T2}$) under the control of EC-specific *Tie2* promoter, were treated with tamoxifen (1 mg/kg/day, s.c.) for 5 days and studied 3 months later. Metabolic cages were used to collect 24-hour urine for sodium, potassium and protein measurements. Renal artery flow (RAF) was assessed by ultrasonography. BP was determined by telemetry, plasma aldosterone by ELISA, and small mesenteric artery (MA) endothelial function and vascular remodeling by pressurized myography. **Results:** Systolic BP was increased in ieET-1 compared with ieCre mice (144±5 vs 117±3 mmHg, $P<0.001$). RAF was decreased in ieET-1 compared with control (1.9±0.2 vs 3.0±0.3 mL/min, $P<0.01$). The excretion of urinary sodium, potassium and protein was similar in both groups. Plasma aldosterone levels were increased in ieET-1 compared with ieCre mice (1.99±0.20 vs 1.29±0.12 ng/mL, $P<0.05$). MA endothelium-dependent relaxation responses to acetylcholine were impaired in ieET-1 compared to ieCre mice (36.3±4.7 vs 71.4±9.7%, $P<0.01$), whereas endothelium-independent relaxation responses to sodium nitroprusside were unchanged. MA media/lumen and media cross-sectional area were similar in both groups, but stiffness was increased in ieET-1 compared to ieCre mice, as indicated by leftward displacement of the stress-strain curves (strain at 140mmHg: 0.61±0.04 vs 0.71±0.02, $P<0.05$). **Conclusions:** The results demonstrate that long-term exposure to endothelial ET-1 overexpression caused sustained BP rise and small artery stiffening. This work was supported by Canadian Institutes of Health Research (CIHR) grants 37917 and 102606, a Canada Research Chair on Hypertension and Vascular Research and by the Canadian Fund for Innovation, all to ELS, and by a fellowship to SCC and to JCFA from the program of CsF/CNPq (Brazil).

3.32
ENDOTHELIN-1 OVEREXPRESSION EXAGGERATES TYPE 1 DIABETES-INDUCED ENDOTHELIAL DYSFUNCTION BY ALTERING OXIDATIVE STRESS BALANCE

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Objective: Increased endothelin (ET)-1 expression has been shown to cause endothelial dysfunction and oxidative stress. Plasma ET-1 is increased in patients with diabetes mellitus. Since endothelial dysfunction often precedes vascular complications in diabetes, we sought to determine whether ET-1 contributes to diabetes-induced endo-

thelial dysfunction. We hypothesized that overexpression of ET-1 in the endothelium will exaggerate diabetes-induced endothelial dysfunction. **Method:** Diabetes was induced by streptozotocin treatment (STZ, 55 mg/kg/day, ip) for 5 days in 6 week old male wild-type (WT) mice and in mice overexpressing human ET-1 restricted to the endothelium (eET-1). Mice were studied 14 weeks later. Blood glucose, plasma ET-1 levels, mesenteric artery (MA) reactivity, mitochondrial superoxide production in aorta and endothelial nitric oxide synthase (*Nos3*), superoxide dismutase 1 (*Sod1*) and 2 (*Sod2*) mRNA expression in MA were determined. **Results:** STZ-induced diabetes was confirmed by increased glycemia in WT (P<0.001). Plasma ET-1 was increased in vehicle- (15.9±4.6 vs 0.6±0.04 pg/mL, P<0.05) and STZ-treated eET-1 (4.9±0.6 vs 0.8±0.1 pg/mL, P<0.05) compared to respective WT controls. Diabetes caused a 27% reduction in vasodilatory responses to acetylcholine in WT (P<0.05), which was further decreased by 20% in eET-1 (P<0.05). Mitochondrial superoxide production was increased 1.8-fold by diabetes in WT (P<0.05) and further augmented by 31% in eET-1 (P<0.05). *Nos3* expression was increased by 43% in vehicle-treated eET-1 compared to WT (P<0.05). Diabetes reduced *Nos3* expression in eET-1 by 31% (P<0.05) but not in WT. Diabetes caused an increase in *Sod1* (52%, P<0.05) and *Sod2* (32%, P<0.05) expression in WT but not in eET-1. **Conclusions:** Increased levels of ET-1 exaggerate diabetes-induced endothelial dysfunction. This may be caused by a decrease in *Nos3* expression, an increase in mitochondrial oxidative stress and a decrease in antioxidant capacity. This work was supported by Canadian Institutes of Health Research (CIHR) grants 37917 and 102606, a Canada Research Chair on Hypertension and Vascular Research and by the Canadian Fund for Innovation, all to ELS, and by fellowships to NIK (Fonds de recherché en santé du Québec), SO (CIHR), MORM (Canadian Vascular Network and Lady Davis Institute/TD Bank studentship) and TL (Richard and Edith Strauss Postdoctoral Fellowship).

3.33
ENDOTHELIN-1 OVEREXPRESSION PRESERVES ENDOTHELIAL FUNCTION IN MICE WITH VASCULAR SMOOTH MUSCLE CELL-RESTRICTED *PPARΓ* KNOCKOUT

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Objective: Peroxisome proliferator-activated receptor γ (PPARγ) agonists reduce blood pressure (BP) and vascular injury in hypertensive rodents and humans. *Pparγ* inactivation in vascular smooth muscle cells (VSMC) using a tamoxifen inducible Cre-Lox system enhanced angiotensin II-induced vascular injury. Transgenic mice overexpressing endothelin (ET)-1 selectively in the endothelium (eET-1) exhibit endothelial dysfunction, increased oxidative stress and inflammation. We hypothesized that inactivation of *Pparγ* in VSMC (sm*Pparγ*$^{-/-}$) will exaggerate ET-1-induced vascular damage. **Methods and Results:** Eleven week-old male control, eET-1, sm*Pparγ*$^{-/-}$ and eET-1/sm*Pparγ*$^{-/-}$ mice were treated with tamoxifen (1 mg/kg/day, s.c.) for 5 days and sacrificed 4 weeks later. Systolic BP was 14 mmHg higher in eET-1 compared to control (P<0.05) and unaffected by *Pparγ* inactivation. Mesenteric artery (MA) vasodilatory responses to acetylcholine were reduced by 37% in sm*Pparγ*$^{-/-}$ (P<0.05) compared to control. Reactive oxygen species levels were increased in eET-1 (70%), sm*Pparγ*$^{-/-}$ (120%) and eET-1/sm*Pparγ*$^{-/-}$ (180%) compared to control (P<0.05). MA monocyte chemoattractant protein-1 expression was 70% higher in sm*Pparγ*$^{-/-}$ compared to control (P<0.05), and unaffected by ET-1 overexpression. Perivascular fat monocyte/macrophage infiltration was >2-fold higher in eET-1 and sm*Pparγ*$^{-/-}$ compared to control (P<0.05), and further increased by 68% in eET-1/sm*Pparγ*$^{-/-}$ (P<0.05). Nitric oxide synthase (*Nos*) 3 mRNA expression was increased by 21% in eET-1 compared to WT (P<0.05). *Nos2* expression was increased 3.7 and 2-fold in eET-1 and sm*Pparγ*$^{-/-}$ compared to WT, respectively (P<0.05). The *Ednra*/*Ednrb* mRNA ratio was decreased by 29% in eET-1/sm*Pparγ*$^{-/-}$ compared to sm*Pparγ*$^{-/-}$ (P<0.05).**Conclusion:** Increased ET-1 paradoxically preserves endothelial function in mice with sm*Pparγ* inactivation, despite enhanced oxidative stress and inflammation.This work was supported by Canadian Institutes of Health Research (CIHR) grants 37917 and 102606, a Canada Research Chair on Hypertension and Vascular Research and by the Canadian Fund for Innovation, all to ELS, and by fellowships to NIK (Fonds de recherche en santé du Québec), SO (CIHR), and TL (Richard and Edith Strauss Postdoctoral Fellowship).

3.34
ROLE OF THE MYELOID ENDOTHELIN-B RECEPTOR IN ANGIOTENSIN II MEDIATED END-ORGAN DAMAGE

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Introduction: Hypertension is common and in the majority of cases its cause remains unknown. Recent interest has focused on the role of macrophages (Mφ) in blood pressure (BP) regulation. Endothelin-1 (ET-1) is the most potent endogenous vasoconstrictor mediating its effects through two receptors – the endothelin-A receptor (ET_A) and endothelin-B (ET_B) receptor. The ET_B receptor has a specific role in ET-1 clearance. We investigated the role of the Mφ ET_B receptor in a model of angiotensin II (Ang II)-mediated end-organ damage. Methods: Mφ ET_B receptor deficient mice ($LysMET_B^{-/-}$) and controls were exposed to Ang II infusion for 6 weeks under a high salt diet. We assessed BP *via* telemetry, cardiac structure and function and endothelial function by Doppler ultrasound, end-organ injury and plasma and urine ET-1. Results: At baseline, components of BP did not differ between groups and increased similarly with Ang II. Whereas after 6 weeks of Ang II $LysMET_B^{-/-}$ and controls had similar left ventricular hypertrophy and cardiac insufficiency, endothelial function was better in $LysMET_B^{-/-}$ at both baseline and after Ang II (% dilation of basilar artery in response to CO_2, $LysMET_B^{-/-}$ *vs.* controls: baseline: 20 *vs.*11%, p<0.01; at 6 weeks: 11 *vs.*0%, p<0.01). Baseline renal function and proteinuria did not differ between groups. After Ang II, $LysMET_B^{-/-}$ showed similar renal function compared to controls but less proteinuria (urine albumin:creat, mg/mmol: 208 ± 10 *vs.* 530 ± 25, p<0.01), glomerulosclerosis (34 ± 2 *vs.* 61 ± 4%, p<0.001), and fewer renal Mφ compared to controls (F4/80 staining *per* high power field, $LysMET_B^{-/-}$ *vs.* controls: 1.1 ± 0.7 *vs.*3.2 ± 0.5%, p=0.02), although similar levels of $CD3^+$ T cells. Plasma ET-1 was no different at baseline but increased more in $LysMET_B^{-/-}$ with Ang II ($LysMET_B^{-/-}$ *vs.* controls after 6 weeks Ang II: 3.7 ± 0.7 *vs.*1.4 ± 0.2 pg/ml, p=0.03). Urine ET-1 was similar baseline and 6 weeks. Discussion: Deletion of the Mφ ET_BR is associated with a blunting of the effects of systemic Ang II infusion as reflected by less endothelial dysfunction, reduced inflammation and end-organ damage. The mechanisms for these effects are the focus of ongoing research. *Partly funded by a British Heart Foundation Intermediate Clinical Research Fellowship (FS/13/30/29994).*

3.35
HIGH DIETARY FAT INTAKE IS ASSOCIATED WITH ENHANCED ENDOTHELIN-1 VASOCONSTRICTOR TONE

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High dietary fat intake is associated with increased cardiovascular disease (CVD) risk. Endothelin (ET)-1 is a potent vasoconstrictor peptide synthesized and released by the endothelium that contributes to CVD risk. We hypothesized that high dietary fat intake is associated with enhanced ET-1 system activity. Fifty sedentary, non-obese adults (41-71 years) were studied: 30 (21M/9F) who habitually consumed a lower fat diet (LFD; 30±5% total calories from fat) and 20 (12M/8F) who habitually consumed a high fat diet (HFD; 40±5% total calories from fat). Dietary fat classifications were based on American Heart Association's recommendation to consume <35% of total calories from fat and were determined via 4-day diet records. Forearm blood flow (FBF; plethysmography) was determined in response to intra-arterial infusions of ET-1 (5 pmol/min for 20 min), a selective ET_A receptor blockade (BQ-123, 100 nmol/min for 60 min), and non-selective $ET_{A/B}$ receptor blockade (BQ-123+BQ-788 [50 nmol/min for 60 min]). The vasoconstrictor response to ET-1 was significantly blunted (~60%; P<0.05) in the HFD compared with the LFD group. ET_A receptor blockade resulted in greater vasodilation (~40%; P<0.05) in the HFD (19%) versus LFD (11%) group. There was no additional dilator response to non-selective $ET_{A/B}$ blockade in either group. These results indicate that habitual consumption of a diet high in fat is associated with enhanced ET_A-mediated ET-1 vasoconstrictor tone.

3.36
VITAMIN C SUPPLEMENTATION REDUCES ET-1 SYSTEM ACTIVITY IN OVERWEIGHT AND OBESE ADULTS

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Endothelin (ET)-1 system activity is elevated in overweight and obese (OW/OB) adults, contributing to vasomotor dysregulation and increased vascular risk. Regular aerobic exercise is a lifestyle strategy that reduces ET-1-mediated vasoconstrictor tone; however, >50% of OW/OB adults do not exercise. Vitamin C supplementation beneficially influences endothelial function. We determined whether daily vitamin C supplementation is as efficacious as regular aerobic exercise in lowering ET-1 system activity in OW/OB adults. 35 sedentary, OW/OB adults completed three months of

either vitamin C (500 mg/day, timed-release) supplementation (VC; n=20; 15M/5F; 58±2 yr; BMI: 31.4±0.6 kg/m^2) or aerobic (walking) exercise training (EX; n=15; 10/5 57±2 yr; 29.3±0.7 kg/m^2). Forearm blood flow (FBF; plethysmography) responses to intra-arterial infusion of ET-1 (5 pmol/min for 20 min) and selective ET_A receptor blockade (BQ-123, 100 nmol/min for 60 min) were determined before and after intervention. There were no anthropometric changes in response to either VC or EX. Vasoconstriction to ET-1 increased similarly (~2-fold; P<0.05) in response to both interventions. Prior to intervention, resting FBF to BQ-123 was significantly increased (~20%; P<0.05) in both groups. Similar to EX, after VC supplementation, BQ-123 did not elicit a significant change in resting FBF. Vitamin C supplementation represents an effective lifestyle strategy for reducing ET-1-mediated vasoconstrictor tone in OW/OB adults.

3.37
BORDERLINE-HIGH TRIGLYCERIDES AND ENDOTHELIN-1 VASOCONSTRICTOR TONE

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Borderline high fasting plasma triglyceride (TG) concentrations (150-199 mg/dL) are an independent risk factor for cardiovascular (CV) disease risk, though the mechanisms underlying this risk are unclear. Endothelin-1 (ET-1) is a potent endogenous vasoconstrictor synthesized and released by the endothelium. Enhanced ET-1 vasoconstrictor tone is linked to CV pathologies. We hypothesized that ET-1 system activity is elevated in adults with borderline-high plasma TG concentrations compared with those with normal plasma concentrations. Eighteen sedentary, overweight adults (43-70 years) were studied: 9 (7M/2F, BMI: 28.0±1.2 kg/m^2) with normal plasma TG concentrations (56-120 mg/dL); and 9 (7M/2F; BMI: 27.1±1.0 kg/m^2) with borderline-high plasma TG concentrations (150-192 mg/dL). Forearm blood flow (FBF; plethysmography) was determined in response to intra-arterial infusions of ET-1 (5 pmol/min for 20 min) and a selective ET_A receptor blocker (BQ-123, 100 nmol/min for 60 min). Vasoconstriction to ET-1 was ~10-fold lower (P<0.05) in the borderline-high TG compared with the normal TG group. In response to BQ-123, FBF increased ~25% in the borderline-high TG versus ~10% in the normal TG group (P<0.05). These results indicate that borderline-high TG concentrations are associated with increased ET-1 mediated vasoconstrictor tone. Enhanced ET-1 system activity may contribute to the increased cardiovascular burden associated with elevations in TG concentrations.

3.38
C-REACTIVE PROTEIN DOES NOT INFLUENCE ENDOTHELIN-1 SYSTEM ACTIVITY IN HEALTHY ADULTS

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C-reactive protein (CRP) is an inflammatory cytokine that has been shown to be an independent predictor of future atherothrombotic events. Endothelin-1 (ET-1) is a potent vasoconstrictor peptide synthesized and released from the endothelium. In addition to its role in vasoregulation, ET-1 hyper-bioactivity is atherogenic. ET-1 is sensitive to inflammatory stimuli; however, the influence of CRP on ET-1 system activity is unknown. We tested the hypothesis that ET-1-mediated vasoconstrictor tone is enhanced in adults with elevated plasma CRP concentrations. Sixty non-obese, sedentary adults (42-70 years) were studied: 20 (13M/7F; BMI: 26.5±0.5 kg/m^2) with CRP <1.0 mg/dL (low CRP; 0.5±0.1 mg/dL); 20 (13M/7F; BMI: 26.4±0.6 kg/m2) with CRP 1.0-3.0 mg/dL (moderate CRP; 2.0±0.1 mg/dL); and 20 (13M/7F; BMI: 27.9±0.8 kg/m2) with CRP >3.0 mg/dL (high CRP; 6.3±0.5 mg/dL). Forearm blood flow (FBF; plethysmography) was determined in response to intra-arterial infusions of ET-1 (5 pmol/min for 20 min) and selective ET_A receptor blockade (BQ-123, 100 nmol/min for 60 min). Vasoconstriction to exogenous ET-1 was not significantly different (P>0.05) between the low (10%), moderate (11%) and high (7%) CRP groups. FBF response to BQ-123 was almost identical between groups; all groups demonstrated a marginal (~10%) but significant vasodilator response. These results indicate that ET-1 system activity is not influenced by elevations in CRP.

3.39
ENDOTHELIN-1 STIMULATES ENDOTHELIAL-DERIVED MICROPARTICLE RELEASE

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Endothelial microparticles (EMPs) are vesicles shed from the endothelium and are a marker of endothelial injury. Elevated circulating EMPs are associated with several cardiovascular, inflammatory and metabolic diseases. Endothelin (ET)-1, a potent vasoconstrictor peptide, is associated with endothelial damage and atherogenesis. We tested the hypothesis that ET-1 increases the release of EMPs from arterial endothelial cells. In three separate experimental units, human aortic endothelial cells (HAECs) were grown to confluence; thereafter, cells were incubated for 24 hours in the absence and presence of ET-1 (100 and 200 pmol) and the selective ET_B receptor antagonist, BQ-788 (1μmol). Following incubation, media was collected, centrifuged (200 x g: 10 min). Prior to analysis, samples were centrifuged (13,000 x g: 2 min) and 100μL of the supernatant was collected and microparticles were labeled with anti-human CD31, a marker expressed on microparticles derived from endothelial cells, and CD42b, a non-specific surface marker. Samples were analyzed using flow cytometry and EMPs were defined as CD31+/CD42b- events less than 1.0 μm. EMP release into the media significantly increased from 8 MP/μL under basal conditions to 16 and 18 MP/μL with ET-1 stimulation at 100 and 200 pmol, respectively. The presence of BQ-788 blunted (~35%) ET-1 stimulated EMP release at both concentrations. Furthermore, boiled ET-1 had no effect on EMP release indicating that the response to ET-1 did not represent a general protein effect. These results indicate that: 1) ET-1 induces EMP release from endothelial cells; and 2) this effect is mediated, in large part, via an ET_B receptor process.

3.40
EARLY-LIFE STRESS INDUCES EPIGENETIC REGULATION OF THE ET SYSTEM IN ADULT MALE MICE

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Early-life stress (ELS) is a risk factor for cardiovascular diseases associated with a dysfunctional endothelin (ET) system; however, little is known of whether ELS epigenetically alters the ET system. We hypothesized that ELS induces vascular dysfunction and a dysregulated ET system in an epigenetic manner. Using adult male control mice (CON) and mice exposed to maternal separation with early weaning (MSEW), a model of ELS, we assessed the gene expression of 84 different chromatin remodeling enzymes and components of the endothelin system (ET-1, ETA and ETB receptor) in thoracic aorta, as well as *ex vivo* vascular function by wire myography. Mean arterial pressure, heart rate and body weight were not different between groups (n=6). However, plasma ET-1 level was elevated in MSEW compared to CON (1.5 vs 1.2 pg/ml, n=8, p=0.05). Aortic mRNA expression of histone deacetylase (HDAC) 1, 6 and 9 were significantly elevated in MSEW compared to CON (RT-qPCR; 1.17, 1.29 and 1.67 fold increase from CON, respectively, n=3, p<0.05). Western blot demonstrated that only HDAC9 protein levels were significantly elevated in aortas of MSEW (1.88 fold increase from CON, n=6, p=0.01). MSEW displayed aortic endothelial dysfunction when compared to CON (i.e., blunted acetylcholine relaxation; 67.6 vs 89.9% maximal relaxation, n=10, p=0.01) that was reversed by *ex vivo* treatment with pan-HDAC inhibitor, trichostatin A (TSA). MSEW had increased aortic ETB receptor mRNA expression (2.2 fold change from CON, n=6, p<0.05), which was reduced by *ex vivo* TSA treatment. Expression of ET-1 and ETA receptor were not different between groups. To assess the mechanism of MSEW-induced ETB receptor expression, we tested the hypothesis that HDAC9 overexpression in cultured mouse aortic endothelial cells (MAECs) increases ETB receptor expression. Interestingly, overexpression of GFP-tagged HDAC9 via plasmid transfection in MAECs did not increase ETB receptor mRNA expression when compared to vector-transfected control, suggesting that MSEW-induced ETB receptor expression may be localized in non-endothelial cells. We propose that MSEW may induce HDAC9-regulated ETB receptor expression in aorta as a compensatory response to pathological postnatal programming of vascular function. In conclusion, ELS induces HDAC-mediated endothelial dysfunction and regulation of the endothelin system, possibly contributing to the programming of increased cardiovascular disease risk. F32 HL116145; P01 HL69999.

3.41
TREATMENT WITH DPPIV INHIBITOR LINAGLIPTIN REDUCES PLASMA ET-1 AND ET-1-INDUCED CEREBROVASCULAR HYPER-REACTIVITY IN DIABETES

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Objective: Diabetes is associated with macrovascular and microvascular complications leading to cerebrovascular disease. We have previously shown that endothelin-1 (ET-1) contributes to cerebrovascular dysfunction and remodeling in the Goto-Kakizaki (GK) rat model of type 2 diabetes. We also reported that glycemic control with metformin prevented vascular changes and decreased plasma ET-1 levels in this model. Since metformin has direct antioxidant effects in addition to its insulin sensitizing actions, discerning whether the beneficial effects were due to glycemic control or due to direct effects of metformin remained unknown. Thus, we

used linagliptin (LINA), a member of the dipeptidyl peptidase-IV (DPP-IV) inhibitor class of glucose lowering agents, to further investigate the impact of glycemic control on ET-1 activation and vascular dysfunction in diabetes. We hypothesized that glycemic control with LINA treatment would decrease blood glucose levels, plasma levels of ET-1, and ameliorate the increase in ET-1-induced vascular hyperreactivity in the GK rat. **Methods and Results:** Male diabetic GK (HA1C%>6.5%) and Wistar rats (age 24 weeks) were fed either normal or LINA chow for 4 weeks at a concentration of 166 mg/kg of chow (n=5-6/group). Plasma was collected following treatment, and basilar arteries were mounted on a wire myograph where a dose response curve to ET-1 (10^{-10}-10^{-7} M) was performed. LINA treatment did not lower blood glucose in GK rats (HA1C%: GK: 8.02±0.27 vs. GK LINA: 8.20±0.33) but decreased plasma ET-1 levels (pg/ml) in diabetic GK rats (Wistar: 1.22±0.02, Wistar LINA: 1.21±0.02, GK: 1.85±0.02***, GK LINA: 1.75±0.01$^{\#\#}$, ***=p< 0.0001 vs. Wistar, $^{\#\#}$=p<0.01 vs. GK). LINA treatment decreased ET-1-induced contraction in basilar arteries from diabetic rats: (Area Under the Curve: Wistar: 562.2±111.1, Wistar LINA: 410.8±63.34, GK: 815.1± 62.65**, GK LINA: 548.7±34.86$^{\#}$, **=p<0.01 vs. Wistar, $^{\#}$=p< 0.05 vs. GK). **Conclusions**: Contrary to our hypothesis, linagliptin did not decrease blood glucose levels in the GK rats. This allowed us to examine the effects of linagliptin on the ET-1 system independent of glycemic control. We show here for the first time an effect of linagliptin on plasma ET-1 levels and ET-1 vascular hyper-reactivity in GK rats. DPP-IV inhibition with linagliptin holds potential as a possible therapy for diabetic vascular disease due to its reduction of ET-1 levels and consequent vasoprotection.

3.42

HIGH GLUCOSE-MEDIATED INCREASE IN PERINUCLEAR ETA AND ETB EXPRESSION IN HUMAN BRAIN VASCULAR SMOOTH MUSCLE CELLS IS NOT AMELIORATED BY LINAGLIPTIN

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Introduction: We have previously reported that endothelin-1 (ET-1) promotes cerebrovascular remodeling in diabetes via ETA and ETB receptor activation on vascular smooth muscle cells. Dipeptidyl peptidase-4 (DPP-4) inhibitors have emerged as a new class of anti-diabetic therapies. We recently observed that treatment with linagliptin (DPP-IV inhibitor) in type-2 diabetic Goto-Kakizaki (GK) rats prevents vascular remodeling and improve the contractile functions independent of its blood glucose lowering effect. Thus, the goal of the current study was to explore the effect of linagliptin on the brain vascular smooth muscle cell (BVSMC) ET system in an *in vitro* model. **Hypothesis:** Linagliptin treatment can prevent the high glucose-induced increase in secretion of ET-1 and expression of ET receptors in BVSMCs. **Methods:** Serum starved human BVSMCs were subjected to either normal glucose (5.5mM) or high glucose (25 mM) containing media and treated with the linagliptin (100nM) for 24 hours. Media was collected for measurement of ET-1 by ELISA and cell lysates were prepared for the measurement of ETA and ETB receptor expression. Cells were also cultured on glass slides and incubated with ETA and ETB receptor antibodies for the immunostaining. **Results:** Immunostaining showed a remarkable increase in expression of ETA receptor in peri-nuclear areas of high glucose treated cells, however there was no significant difference after linagliptin treatment. Similarly, ETB receptor expression was also increased in the high glucose treated cells but the linagliptin treatment did not change the expression. High glucose exposure increased (not significant) the expression of both ETA and ETB receptor in cell lysate of BVSMCs. The linagliptin treatment did not show any significant difference in the expression of both ETA and ETB receptors. The secretion of ET-1 measured in media was also not significantly different between the groups. **Conclusions:** The dose of linagliptin and the duration of exposure used in the present study did not show any significant effect of linagliptin on ET receptor system of BVSMCs. Thus, it is concluded that the attenuation of brain vascular remodeling by linagliptin could be attributed through the other cell types (like endothelial cells) of the vasculature and may be independent of the ET system. High glucose-induced perinuclear ET receptor localization needs to be further studied.

3.43

POTENTIAL ASSOCIATION OF CIRCULATORY LEVEL OF ENDOTHELIN-1 AND DIABETES IN RURAL WOMEN IN BANGLADESH

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Aims: Diabetes Mellitus (DM) is a global epidemic affecting approximately 285 million people and has a high (and rising) prevalence in both developed and, more recently, developing countries. DM has, in particular, become an important health concern in the South Asian region, with an estimated increase in the prevalence of diabetes of over 151% between the years 2000 and 2030. Endothelin-1 (ET-1), a potential marker of endothelial dysfunction has been shown to be elevated in diabetic subjects. However, to date, the circulatory profile of ET-1 and its association with diabetes mellitus (DM) have not been investigated in any South Asian country like in Bangladesh. The present study assessed circulating levels of ET-1 in subjects with or without DM and further examined the association of ET-1 with clinical and metabolic parameters in Bangladeshi rural women. **Main Methods:** A total of 2022 rural Bangladeshi women were studied using a cross-sectional survey. Then further analysis was done on a case control basis having DM (n=179) and non-DM (204). Multiple regressions were used to examine the association between circulatory ET-1 level and DM. We used the World Health Organization's (WHO) STEPS approach (modified), health-related behaviour (step 1), basic physical measures (step 2) and basic biochemical investigations, such as levels of blood glucose and cholesterol (step 3). **Key findings:** DM prevalence in the current study was 9.1%. ET-1 levels were significantly increased in diabetes subjects [DM vs. non-DM: 3.11 ± 0.16 vs. 1.97 ± 0.03, p<0.001]. In multivariable analyses, after adjusting for age, ET-1 had significant positive association with waist circumference (p=0.029), fasting plasma glucose levels (p=0.001), and HDL-C (p=0.016). In multiple regression analysis considering ET-1 level as dependent variable, we found plasma glucose level and HDL-C is the independent determinants for plasma ET-1 levels in Bangladeshi rural women. Through tertile analysis, we found mean ET-1 levels significantly increase as levels of blood glucose increases (p for trend<0.001). **Significance:** A higher concentration of ET-1 among DM subjects suggests the possible endothelial dysfunction in this apparently healthy population. The relation of ET-1 and DM needs further investigations to define the clinical predictive value of plasma ET-1 levels in DM for the South Asian population. This work has been supported by Ministry of Education and Science in Japan.

3.44

AMELIORATION OF ACUTE LIVER INJURY WITH THE BLOCKADE OF PROTEASE ACTIVATED RECEPTOR (PAR)-2 THROUGH THE SUPPRESSION OF UPREGULATED LEVELS OF ENDOTHELIN-1 AND TNF-A IN A RAT MODEL OF ENDOTOXEMIA

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Sepsis is associated with tissue hypoperfusion and metabolic impairment, which may contribute to the subsequent development of multiple organ failure. The liver is one of the organs that are normally damaged during the pathogenesis of sepsis and septic shock. Numerous studies on infection- or sepsis-induced liver injury have been reported, however, to date no effective treatment has been reported on this disorder. Some studies suggested that circulating endothelin (ET)-1 is elevated in sepsis. Here, we examined the time-dependent alterations of ET-1, NO and inflammatory cytokine, such as TNF-α in liver tissue in a septic rat model. Normal Wistar rats were administered with lipopolysaccharide (LPS: 15 mg/kg) and sacrificed at different time points (1h, 3h, 6h and 10h). The classical features of acute liver injury, such as infiltration of inflammatory cells, hepatocytic necrosis, were seen in LPS administered rats, and plasma bilirubin, GOT and GPT levels were also significantly changed. A 28-fold increase in ET-1 level was observed in liver tissue at 10 h after LPS administration, while a peak increase of 14-fold ET-1 mRNA level was seen 1 hour after LPS administration in liver tissue. Levels of hepatic TNF-α peaked (4.5-fold) at 1 hour of sepsis. Endotoxemia often triggers exuberant inflammatory responses and activation of the coagulation cascade, and interactions between inflammation and coagulation may be important in this setting. Protease-activated receptors (PARs) connect coagulation proteases to cellular responses and represent one mechanism by which coagulation might affect inflammation. Of the 4 mammalian PARs, PAR1, PAR3, and PAR4 are activated by thrombin, and PAR2 can be activated by coagulation proteases VIIa and Xa but not thrombin. Interestingly, PAR2 blocking peptide improved the status of liver injury, an effect that was associated with suppression of TNF-α elevation, and normalization of ET-1. **Conclusions:** The present study revealed a distinct chronological expression of ET-1 in LPS-mediated liver injury and suggested that blockade of PAR2 played a crucial role in treating liver injury in the septic rats, via a

mechanism of the normalization of inflammation, coagulation and vaso-active peptides including ET-1.

3.45

EFFECTS OF ENDOTHELIN ANTAGONISM ON MICRO-VASCULAR COMPLICATIONS SUCH AS DIABETIC ERECTILE DYSFUNCTION AND DIABETIC RETINOPATHY ARE PARTLY MEDIATED THROUGH RESTORATION OF ALTERED VEGF SIGNALING IN RATS

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(Purpose) Diabetes Mellitus (DM) causes both macrovascular and microvascular complications. The diabetic patients often cause erectile dysfunction (ED), which is considered as the microvascular and neuropathic complications, in which vascular endothelial growth factor (VEGF) has been documented for its pathogenic significance in diabetic complications. We studied the effects of endothelin (ET) antagonism on the diabetic microvascular complications such as diabetic ED and diabetic retinopathy, and also on expression of VEGF in these tissues in the DM rats. (Methods) We used 3 weeks duration of streptozotocin (STZ)-induced DM rats to assess the VEGF expression in penile tissue and retinal tissue, and the effects of ET antagonism has been studied on these changes. Male rats were administered saline vehicle or STZ (65 mg/kg IP). One week after the injection, the animals were divided into those receiving the ET-A/B dual receptor antagonist SB209670 (1 mg/kg/day), or saline for 2 weeks by osmotic mini-pump. The local ET-1 level in DM penis was higher by 20% than non-DM penis. A 30% decrease in VEGF expression in penile tissue was seen in DM rats, and SB209670 partly prevented erectile dysfunction through restoration of VEGF reduction. SB209670 also partly prevented the development of diabetic retinopathy through restoration of VEGF overexpression. (Conclusion) ET antagonism by SB209670 is effective in preventing diabetic microvascular complications such as diabetic erectile dysfunction and diabetic retinopathy by partly mediated through restoration of altered VEGF signaling in these DM rats.

3.46

HIGH FAT AND HIGH GLUCOSE SYNERGISTICALLY IMPAIR BRAIN MICROVASCULAR ENDOTHELIAL CELL SURVIVAL AND ANGIOGENIC POTENTIAL INDEPENDENT OF ET-1

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Introduction: We have shown that diabetes causes dysfunctional neovascularization in the brain and endothelin-1 (ET-1) receptor antagonism with bosentan prevents and restores this pathological angiogenesis in the Goto-Kakizaki (GK) model of diabetes. We now have evidence that diet induced metabolic disease also causes dysfunctional angiogenesis. Accordingly, in this study we tested the hypothesis that exposure of primary brain microvascular endothelial cells (BMVECs) to high glucose (HG) AND palmitate (Pal), an in vitro model for diet-induced metabolic disease, activates the ET system and resulting inflammation leads to poor cell survival and attenuates pro-angiogenic properties. Methods: BMVECs were cultured in normal glucose (Control), HG (12.5mM), Pal (200μM), or HG plus Pal for 14 hours before collecting the cell lysate and medium. Cell survival (MTT assay and cell counts), angiogenic properties (migration and tube formation), ET-1, ET-B receptors, and inflammatory mediators (TNFa and toll like receptor-TLR4) were measured. Results: HG plus Pal combination significantly reduced cell number, % viability, % migration and tube length and increased TNFa and TLR4 levels (Table). In contrast to our hypothesis, there was no difference in ET-1 secretion or ET-B receptor levels. Discussion: These results suggest that mild elevations in glucose and free fatty acids impair angiogenic properties of BMVECs. While these changes coincide with increased TLR-4 and TNFa levels, they are not associated with changes in ET system. Ongoing studies will determine whether ET receptor blockade will prevent downstream inflammation and impairment of angiogenic potential. Funding Support: VA Merit BX00347, NS070239 and NS083559.

	Control	HG	Pal	HG + Pal
Cell number (102/μl)	5.5± 0.1	4.0± 0.6*	4.5± 0.2	2.2± 0.1*#
% Viability	100± 4	78± 5*	82± 2*	51± 9
% Migration	24.5± 0.7	24.1± 2.2	22.2± 3.0	17.4± 1.0*#
Tube Length (μM)	36.9± 3.4	29.3± 3.7	21.7± 0.1*	17.4± 1.2*#
TLR4 (pixels)	129± 4	186± 8*	152± 5	201± 12
TNFα (pixels)	275± 16	343± 13	450± 38*	568± 62*
ET-1 (pg/ml)	1.4± 0.1	1.3± 0.1	1.4± 0.1	1.3± 0.1
ET-B (fold of control)	1	0.6± 0.2	0.7± 0.1	0.7± 0.1

*vs Control NG, # vs HG or Pal alone

3.47

ET_A RECEPTOR BLOCKADE INHIBITS LEUKOCYTE ACTIVATION AND ADHESION IN SICKLE CELL DISEASE

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Leukocytes (WBCs) in Sickle Cell Disease (SCD) are in an activated state characterized by increased ROS, cytokine production and adhesiveness. Endothelin-1 (ET-1) is elevated in SCD and is also known to mediate local inflammation and oxidative stress. Here we examined whether ET-1 contributes to WBC activation and endothelial cell adhesion in a transgenic mouse model of SCD. Cytokine and ROS production were measured at baseline and after ET-1 exposure in SCD and control WBCs. WBC-endothelium interactions were visualized by intravital microscopy in the vasculature of the calvarial bone marrow under normoxia or hypoxia/reoxygenation (H/R) conditions after 8 weeks of treatment with either Ambrisentan (AMB), the non-selective ET_{AB} antagonist Bosentan (BOS) or vehicle. Leukocytes from SCD mice produced higher ROS and cytokine levels at baseline compared to controls. At normoxia, blockade with BOS enhanced WBC rolling and adhesion, suggesting an important role for ET_B signaling at baseline. Following H/R, both antagonists were equally effective in decreasing WBC rolling/adhesion. Our results suggest that ET-1 promotes leukocyte adhesion to the vascular endothelium in SCD. Both selective (ET_A) and non-selective (ET_{AB}) receptor blockade abrogated this response, which may have important therapeutic implications for patients with SCD. Supported by HL-117684.

3.48

STIMULATION OF ET_B RECEPTORS BY IRL-1620 MODULATES THE PROGRESSION OF ALZHEIMER'S DISEASE

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Studies indicate that stimulation of ET_B receptors provide neuroprotection. The purpose of this study was to determine the effect of selectively activating the ET_B receptors following Aβ. Rats were treated with Aβ in the lateral cerebral ventricles. The rats were treated chronically with IRL-1620 for 14 days in one set of treatment and in other IRL-1620 was administered IV on day 8. Spatial memory, oxidative stress and brain ET_B, VEGF and NGF expression were assessed. All procedures were approved by Midwestern University IACUC. Aβ treatment produced a significant increase in MDA ($p<0.0001$) and a decrease in SOD ($p<0.0001$) and GSH ($p<0.001$) compared to sham. IRL-1620 treatment reversed these effects, indicating that ET_B receptor activation reduces oxidative stress injury following Aβ. Aβ treated rats showed a significant impairment in spatial memory, which was significantly reduced with IRL-1620 treatment. IRL-1620 treatment also increased the number of blood vessels labeled with VEGF compared to vehicle. Additionally, cells showed positive staining for NGF ($p<0.001$) in IRL-1620 treated rats. ET_B, VEGF and NGF protein expression significantly increased in IRL-1620 treated rats compared to vehicle ($p<0.001$). Results demonstrate that IRL-1620 improves memory, prevents oxidative stress and enhances neurovascular remodeling, it may be a novel therapeutic target for AD. Study was supported by Alzheimer's Drug Discovery Foundation.

3.49

ENDOTHELIN A RECEPTOR DRIVES INVADOPODIA FUNCTION AND CELL MOTILITY THROUGH B-ARRESTIN/PDZ-RHOGEF PATHWAY IN OVARIAN CARCINOMA

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The endothelin-1 (ET-1)/ET A receptor (ET_AR) signalling pathway is a well-established driver of epithelial ovarian cancer (EOC) progression. One key process promoted by ET-1 stimulation is tumor cell invasion, which requires the scaffolding functions of β-arrestin-1 (β-arr1); however, the potential role of ET-1 in inducing invadopodia formation/function, which are crucial for cellular invasion and tumor metastasis, is completely unknown. We set out to molecularly dissect the ET-1 signaling pathway that control EOC cell invasion through regulation of small Rho GTPase family members. Results of this work revealed that ET-1/ET_AR activates RhoA and RhoC GTPase, and downstrem ROCK1, in association with actin-based dynamic remodelling and enhanced cell invasion, that are inhibited by macitentan as well as by the ROCK inhibitor, Y-27632. Mechanically, we found that these effects are accomplished by the direct interaction of b-arrestin 1 (β-arr1) with PDZ-RhoGEF, a RhoA specific guanine nucleotide exchange factor, representing the predominant activator of RhoA and C downstream of ET_AR. Interestingly, ET_AR-mediated invasive properties are related to the regulation of invadopodia, as evaluated by colocalization of actin/cortactin with areas of matrix degradation, and activation of cofilin pathway, which is crucial for regulating invadopodia activity. Depletion of PDZ-RhoGEF, or β-arr1, or RhoC, as well as the treatment with macitentan, significantly impairs invadopodia function, matrix-metalloprotease activity, and invasion, demonstrating that β-arr1/PDZ-RhoGEF interaction mediates ET_AR-driven ROCK-LIMK-cofilin pathway through the control of RhoC GTPase activity. In vivo, macitentan is able to inhibit metastatic dissemination and Rho GTPase expression. Finally, analysis of EOC human tissues reveals that ET_AR overexpression, which is significantly associated with poor prognosis, positively correlates with RhoC expression. Overall, our results have uncovered a novel role for the complex β-arr1/PDZ-RhoGEF as regulator of ET-1/ ET_AR-induced motility and metastasis, unravelling ET-1 axis as regulator of invadopodia protrusions through RhoC/ROCK/LIMK/cofilin pathway during the initial steps of EOC invasion.

3.50

CLINICAL USE OF SERUM BIG ENDOTHELIN-1 LEVELS AS A TUMOUR MARKER FOR HAEMANGIOSARCOMA

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In veterinary, haemangiosarcoma (HSA) is an important malignant neoplasm of dogs that originates from vascular endothelial cells. HSA is the most common splenic malignant neoplasm in dogs, approximately 50% of splenic masses were HSA. This study explored the suitability of using serum big endothelin-1 (ET-1) as a tumour marker for canine spontaneous HSA. Serum big ET-1 was measured in dogs with splenic HSA ($n = 23$), splenic malignant tumours other than HSA ($n = 10$), benign splenic lesions ($n = 12$) and normal healthy dogs ($n = 17$) by ELISA. Serum big ET-1 levels in dogs with HSA were significantly ($P < 0.01$) higher than in other dogs. High sensitivity (100%, 95% confidence interval 92-100%) and specificity (95%, 95% confidence interval 87-95%) for HSA diagnosis were obtained using a cut-off of 17 pg/mL according to receiver operating characteristic (ROC) curves (area under ROC curve 0.95). *PPET1*, *ETA, VEGF* and *Hif1-α* mRNA expression, measured by real-time PCR, were elevated in HSA compared to normal tissues. These findings suggest that elevated serum big ET-1 could be used as a diagnostic marker for canine HSA.

3.51

REGULATION OF THE CARDIAC ENDOTHELIN SYSTEM AND CARDIOMYOCYTE HYPERTROPHY BY GPER

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Aging is a major risk factor for cardiac hypertrophy that is partly mediated by increased activity of the local endothelin system. Endothelin-1 potently induces cardiomyocyte hypertrophy through stimulus-dependent activation of ET_A or ET_B receptors, which are involved in a complex cardiac signaling network of G protein-coupled receptors that control cardiomyocyte adaptation. Because signaling pathways of the G protein-coupled estrogen receptor (GPER) and endothelin receptors functionally interact in vascular smooth muscle, we hypothesized that GPER may also play a role in the age-dependent activation of the cardiac endothelin system. Using hearts from senescent (24 month-old) GPER-deficient and wild-type mice, we analyzed myocardial mRNA expression of prepro-endothelin-1, endothelin converting enzymes (ECE), as well as ET_A and ET_B receptors by RT-PCR. In addition, cardiomyocyte size was determined. In hearts of GPER-deficient mice, we found reduced expression of ECE-2 (2.3-fold, n=6, $p<0.05$ vs. wild-type mice), a rate-limiting enzyme in endothelin-1 synthesis. Furthermore, ET_B receptor mRNA levels were decreased (1.7-fold, n=6, $p<0.05$ vs. wild-type mice), while ET_A receptor or prepro-endothelin-1 expression was unaffected by GPER deletion. Consistent with lower ECE-2 and ET_B receptor expression that may result in reduced myocardial endothelin-1 signaling, cardiomyocyte size was decreased 1.9-fold by GPER deletion (28±2 vs. 51±3 □m^2, n=6-8, $p<0.0001$ vs. wild-type mice). In conclusion, these findings indicate that endogenous GPER is partly required for activation of the endothelin system and cardiomyocyte hypertrophy during cardiac aging, suggesting a localized interaction between GPER, ECE-2, and endothelin receptor signaling that may be pharmacologically relevant to target age-dependent cardiac hypertrophy and resulting congestive heart failure. Supported by the National Institutes of Health (R01 CA127731 & CA 163890 to E.P.), the Swiss National Science Foundation (grants 135874 & 141501 to M.M. and grants 108258 & 122504 to M.B.), and the Interdisciplinary Center for Clinical Research (IZKF) Erlangen (project F1 to K.A.). N.F. was supported by NIH training grant HL07736.

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ENDOTHELINS AS MARKERS OF CARDIOVASCULAR PROTECTION IN ADULTS WITH ISOLATED DEFICIENCY OF GROWTH HORMONE (IDGH)

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Background: In the city of Itabaianinha, located on the southern state of Sergipe, Brazil, there is a group of 105 individuals affected by isolated GH deficiency (IDGH) due to a mutation of the GH receptor gene (GHRH). Previous studies in patients with GH deficiency acquired in adulthood demonstrated increased cardiovascular morbidity and mortality. However, the literature claims that patients with elevated serum Endothelins (ET) have Cardiovascular Risk (CVR) increased due to endothelial dysfunction caused by atherosclerosis. **Objective**: To evaluate the serum levels of ET in adults with IDGH and its correlation with CVR. **Methods:** It is a descriptive and prospective case-control study. A total of 34 patients, 20 patients with IDGH coming of Itabaianinha/SE and 14 healthy individuals residing in the city of Aracaju (state capital of Sergipe, Brazil.) comprised the control. They were submitted to dosage of serum ET through Elisa test. All experiments were conducted in accordance with the Declaration of Helsinki. Statistical analysis was performed by measures of central tendency and variance and comparisons between groups was performed by chi square test. The statistical significance level was less than 5% ($p < 0.05$). **Results:** The control group was composed of seven men (50%) and seven women (50%) with mean age of 42.2 ± 19.9 years; while the dwarves sample consisted of 11 men (55%) and nine women (45%) with mean age of 46 ± 15.5 years. The mean values of the serum ET from controls were ET 70.78 ± 39.02 pg / ml and for patients with IDGH was 25.15 ± 6.40 pg/ml ($p = 0.0003$ CI: 92%). **Conclusion:** It is concluded that the ET levels in patients with IDGH were inferior to controls, so it appears that people with IDGH have less cardiovascular events than healthy patients. But further studies are needed to corroborate this claim.

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KNOCKOUT OF ENDOTHELIN-1 IN VASCULAR ENDOTHELIAL CELLS AMELIORATES CARDIAC MITOCHONDRIA DYSFUNCTION AFTER MYOCARDIAL INFARCTION IN DIABETES TYPE 2 MICE

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Background: Persistent elevation of circulating endothelin-1 (ET-1) has been detected in diabetic patients and associates with low cardiac function post myocardial infarction. However, the role of ET-1 in linking diabetic heart and myocardial infarction is not well understood. Several studies suggest that ET-1 can induce mitochondria dysfunction which is an important feature of heart failure. Thus, we hypothesize that ET-1 might increase diabetic heart vulnerability to myocardial infarction through impairment of mitochondrial biogenesis and function. **Methods**: We induced diabetes mellitus type 2 in vascular endothelial cells specific-ET-1 knock out mice (VEETKO) and their wild type (WT) littermates using combination of streptozotocin injection and western diet. Six weeks after, we performed myocardial infarction using cryoinfarction procedure. Cardiac histology, function, gene expression, and mitochondrial biogenesis were evaluated one week after cryoinfarction. **Results**: Diabetic WT mice exhibited lower cardiac function, higher mitochondria structural abnormality, down-regulation of PGC-1α/NRF1/OXPHOS signaling, higher ADP/ATP ratio, and higher oxidative stress compared to diabetic VEETKO mice. In vitro study revealed lower mitochondrial area, size, PGC-1α, and OXPHOS gene down-regulation in H9C2 cell treated with combination of high glucose and ET-1. **Conclusion**: These results show that vascular ET-1 increase diabetic heart vulnerability post myocardial infarction by aggravating mitochondrial biogenesis impairment. Reducing circulating ET-1 level might be beneficial to protect diabetic heart from myocardial infarction by preserving mitochondrial biogenesis and function. Support: Grant-in-Aid for Scientific Research (C) 26460213 and 18590813 from the Japan Society for the Promotion of Science.

3.54
ATTENUATION OF ENDOTHELIN-1-INDUCED CARDIOMYOCYTE HYPERTROPHY THROUGH ESTROGEN PRE-TREATMENT VIA NON-GENOMIC PATHWAY: POTENTIAL INVOLVEMENT WITH VEGF SYSTEM

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Estrogen (β-estradiol), the female hormone, has been reported to inhibit cardiac hypertrophy and apoptosis by different research groups in recent days. Thus, although the anti-hypertrophic action of estrogen in heart has begun to be appreciated, the potential mechanism underlying the estrogen-mediated cardioprotection is unknown. Endothelin (ET)-1, a potent vasoconstrictor, induces hypertrophic changes in neonatal cardiomyocytes at both morphological and molecular levels. Targeting ET-1 in the prevention of heart failure is crucial to suppress the cardiovascular diseases. Indeed, pathological cardiac hypertrophy progressively leads to heart failure. In the first part of the current study, we investigated whether estrogen confers beneficial effect against ET-1-induced cardiomyocyte hypertrophy and if cardioprotective, then whether this estrogen mediated cardiac anti-hypertrophic action is genomic or non-genomic. The doses of estrogen and ET-1 were optimized based on preliminary dose- and time-dependent studies. At day 4 of culture, neonatal rat cardiomyocytes were divided into three groups: control, ET-1 (10nM) treated and estrogen-pre-treated (1μM) ET-1 groups. 2.0-fold increase in cardiomyocyte surface area, and 1.8-fold in protein synthesis rate in cardiomyocyte were observed after ET-1 administration and these changes were greatly prevented by estrogen pre-treatment. Estrogen could also normalize the upregulated ET-1 and ETA receptor mRNA expression in ET-1-induced hypertrophied cardiomyocyte. The pure estrogen receptor (ER) blocker, ICI-182,780, failed to reverse the estrogen-mediated anti-hypertrophic effect on ET-1-induced hypertrophied cardiomyocytes suggesting the non-genomic pathway of estrogen action. Moreover, we recently found that ET-1-mediated over-expression of VEGF contributes to the development of ET-1-induced cardiomyocyte hypertrophy. Thus, subsequently the present study investigated whether VEGF system would contribute to the anti-hypertrophic action of estrogen in ET-1-induced hypertrophied cardiomyocyte. Interestingly, we found that the upregulated VEGF system in ET-1-induced hypertrophied cardiomyocyte was greatly normalized by estrogen pre-treatment. The present results implied that estrogen (non-genomic action) may arrest the cardiomyocyte hypertrophy through the suppression of VEGF system and demonstrate for the first time the role of estrogen and VEGF system in the prevention of ET-1-induced cardiomyocyte hypertrophy.

3.55
WITHDRAWN

3.56
ENDOTHELIN-1 (ET-1) REGULATES THE EXPRESSION OF MATRIX METALLOPROTEINASES (MMPS) AND TISSUE INHIBITORS OF MMPS IN HUMAN FIRST TRIMESTER TROPHOBLASTS VIA ETB RECEPTOR: A POSSIBLE ROLE IN TROPHOBLAST INVASION

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Introduction: Pre-eclampsia (PE) and fetal growth restriction (FGR) are severe pregnancy complications associated with an impairment of trophoblast invasion during the first trimester of pregnancy. This process is mediated by members of the matrix metalloproteinase family (MMPs) such as MMP-2, MMP-14 and MMP-15. Since ET-1 can regulate cell proliferation, migration and invasion and is upregulated in PE and FGR, we hypothesize that increased levels of ET-1 modify the expression of MMPs and their natural inhibitors (TIMPs) during the first trimester of pregnancy. **Methods:** Primary human trophoblasts isolated from first trimester placentas (week 7 to 12 of gestation) were incubated in the absence or the presence of 10nM and 100nM ET-1. MMP-2, -14 and -15 and TIMP mRNA levels were determined by RT-qPCR. MMP-2, -14 and -15 protein levels were assessed by zymography and Western blotting. ETR involvement was determined using two selective antagonists for ETAR (BQ-123) and ETBR (BQ-788). ET-1 functional effects were tested in first trimester chorionic villous explants. **Results:** ET-1 had a dose-dependent effect on MMP expression after 24h, with 100nM ET-1 down-regulating MMP-2 (24% and 17%), MMP-14 (21% and 25%) and MMP-15 (27% and 26%) mRNA and protein levels, respectively ($p \leq 0.05$). ET-1 also up-regulated TIMP-3 (47%) and TIMP-4 (39%) mRNA levels in a dose-dependent manner ($p \leq 0.05$). Blocking of ETAR increased ET-1 effect on MMP-15 down-regulation by 17% ($p \leq 0.001$) whereas ETBR blockade partially abolished the ET-1 effect on MMP-15 expression. Preliminary data showed that ET-1 also inhibits trophoblast outgrowth in placental explants by 39%. **Conclusion:** ET-1 alters the balance between invasion promoting MMPs and invasion inhibiting TIMPs in human first trimester trophoblasts, leading to a decrease in trophoblast invasion. This effect is mediated via ETBR and might contribute to the impairment of trophoblast invasion observed in PE and FGR.

3.57
PLASMA AND URINARY ENDOTHELIN-1 LEVELS IN NEONATES AND RENAL FUNCTION

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Endothelin-1 (ET-1) is a vasoconstrictor implicated in hypoxia-induced lung and brain injury in neonates. Recent studies show increased urinary ET-1 (uET-1) excretion with renal dysfunction. Neonatal kidneys are vulnerable with 24% of newborns presenting with acute kidney injury (AKI). As uET-1 could be indicative of renal stress and a potential biomarker of AKI, we evaluated the correlation between plasma (pET-1) and uET-1 levels with parameters of renal function in neonates. 63 neonates were enrolled in 3 groups based upon gestational age (GA): preterm (PT) <31 wk gestation, 31-37 wk and full-term (FT) ≥37 wk. 1.5mL cord blood and 1mL blood, 3mL urine at 24h of life were obtained. ET-1 levels were estimated using a kit. Mean uET-1 levels (pg/mL) of PT groups <31 wk (2.4±0.3) and 31-37 wk (1.4±0.4) were higher ($p<0.05$) than those of FT (0.7±0.2). uET-1 negatively correlated with GA ($r=-0.4$, $p<0.01$). No correlation was found between uET-1 and pET-1 levels and uET-1 with creatinine, BUN or urinary output. However uET-1 negatively correlated with glomerular filtration rate (GFR) ($r=-0.3$, $p<0.05$). Plasma and uET-1 levels are independent of each other and uET-1 excretion is reflective of intrinsic renal ET-1 production. uET-1 levels negatively correlated with GA and GFR. In neonatal population, uET-1 may be indicative of renal injury. The study was funded by Advocate Children's Hospital and Midwestern University and approved by respective Institutional Review Boards.

3.58
MATERNAL ETHANOL AND OXYCODONE EXPOSURE DELAY CNS DEVELOPMENT AS DETERMINED BY ENDOTHELIN RECEPTOR EXPRESSION IN NEONATAL RAT BRAINS

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The incidence of combined opioid and alcohol abuse during pregnancy continues to rise, despite serious side effects to the fetus, such as reduced brain size and increased apoptosis. The ET system has been implicated in development of the CNS and may demonstrate changes in response to prenatal alcohol and/or oxycodone exposure.

Pregnant rats were administered an oral gavage of vehicle, ethanol (EtOH), oxycodone (OXY) or EtOH+OXY daily from gestational day 7 to 21. The brains of pups on post-natal day (PND) 1, 7, 14 and 28 were analyzed for expression of ET_A and ET_B receptors. Pups in the EtOH, OXY and combination groups presented with more congenital malformations (5.7, 5.9 & 27.8%, respectively) at birth than vehicle. ET_A expression was significantly higher in the OXY (23.3%) and EtOH+OXY (80.5%) groups at PND 1 as compared to vehicle and EtOH alone. ET_B receptor levels were significantly lower in all groups as compared to vehicle at PND 1 (EtOH:-59.8%; OXY:-65.0%; EtOH+OXY:-57.3%). ET_B expression in vehicle pups decreased with CNS development (-35.37%), whereas expression increased in all other groups between PND 1 and 28 (EtOH:+181.3%; OXY:+217.9%; EtOH+OXY:+177.1%). Maternal EtOH and OXY exposure during pregnancy result in decreased levels of brain ET_B receptors after birth, indicating a possible delay in CNS development. This project was supported by Advocate Children's Hospital and Midwestern University. All procedures were approved by Midwestern University IACUC.

3.59
AUTOCRINE ENDOTHELIN 1 SIGNALING PROMOTES OSTEOBLAST GROWTH AND MINERAL DEPOSITION VIA INDUCTION OF MIR 126-3P.

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Ece1, encoding endothelin (ET) converting enzyme 1 (ECE1), is a positional candidate for a pleiotropic quantitative trait locus affecting femoral size, shape, mineralization, and biomechanical performance and is responsible for 40% of the variation in bone biomechanical performance between recombinant congenic mouse strains HCB8 and HCB 23. To explore the mechanisms by which *Ece1* polymorphisms might affect bone phenotypes, we undertook *in vitro* studies of the ET axis in osteoblasts. When exposed to ascorbic acid and □ glycerol phosphate, cultured osteoblasts recapitulate maturation and mineralization over the course of 2 weeks. Treatment of TMOb osteoblasts with big ET1 increases mineralization and secretion of IGF1 while decreasing secretion of DKK1 and SOST, actions that can be blocked by pharmacologic inhibition of ECE1 or EDNRA. To confirm that ET1 signaling is vital for normal bone physiology in the absence of ET1 supplementation, we pharmacologically inhibited EDNRA and ECE1 in TMOb osteoblasts. Inhibition of either ENDRA (BQ-123) or ECE1 (phosphoramidon) reduced mineralization. Blockade of ENDRA showed the expected decrease in IGF1 secretion and increase in DKK1 and SOST secretion. We used *Ece1* siRNA to knock down *Ece1*. We confirmed knock down by western blot and saw similar results in mineralization, and decreased secretion of IGF1, and DKK1 and SOST. Big ET1 treatment increased expression of miR 126-3p approximately 120-fold relative to control. This miR is predicted to target murine SOST to decrease its expression. To test the hypothesis that ET1 signaling partially works through miR regulation, we transfected TMOb cells with a miR 126-3p mimic, a miR 126-3p inhibitor, and a negative control in the presence and absence of big ET1. We found that transfection of the mimic in the absence of big ET1 increased mineralization and decreased secretion of SOST. We found that transfection of the inhibitor decreased mineralization and increased secretion of SOST in the presence of ET1. Our data suggest that osteoblast maturation and bone mineralization is promoted by ET1 signaling and that this is mediated in part through control of miR126-3p expression. Our findings highlight the importance of ET1 signaling in extravascular tissues.

3.60
FUNCTIONAL SIGNIFICANCE OF ENDOTHELIN IN PERIODONTITIS

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Periodontitis is a very common oral inflammatory disease and results in the destruction of supporting connective and osseous tissues of tooth. Although the etiology is still unclear, Gram-negative bacteria, especially *Porphyromonas gingivalis* in subgingival pockets has been thought as one of the major etiologic agent. It has been known that endothelin is involved in the occurrence and progress of various inflammatory process and diseases. Expression of ET-1 and its receptors, ET_A and ET_B is detected in the periodontal tissues and the ET-1 levels in gingival crevicular fluid are increased in the periodontitis patients. However, functional roles of endothelin in periodontitis are still unclear. In this study, we explored cellular and molecular mechanisms of ET-1 actions in periodontitis using human gingival epithelial cells (hGECs) and human gingival fibroblasts (hGFs). ET-1 and ET_A, but not ET_B were abundantly expressed in both hGECs and hGFs. Stimulation of hGECs with *P. gingivalis* LPS increased the expression of ET-1 and ET_A suggesting the activation of endothelin signaling pathway. Production of pro-inflammatory cytokines, IL-1□, IL-6, and IL-8 was significantly enhanced by exogenous ET-1 treatment in both hGECs and hGFs. Moreover, ET-1 augmented the number of multinucleated osteoclasts implicating the acceleration of alveolar bone loss. Together, our study showed that activation of ET-1/ET_A signaling pathway by *P. gingivalis* may exacerbate periodontitis by stimulating production of pro-inflammatory cytokines in hGECs and hGFs and provoking the alveolar bone loss through the increment of multinucleated osteoclasts at the same time. To directly examine the endothelin antagonism as a potential therapeutic approach for periodontitis, bosentan treatment will be applied to the ligature-induced mouse periodontitis model. Infiltration of immune cells, production of pro-inflammatory cytokines, and alveolar bone loss will be evaluated.

3.61
MACROPHAGE ENDOTHELIN-B RECEPTORS CLEAR ENDOTHELIN-1 & REGULATE BLOOD PRESSURE

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Introduction: Hypertension is common. However, its cause remains unclear in the majority of those affected. Recent data suggest that macrophages (Mϕ)/monocytes contribute to, and protect from, hypertension. Endothelin-1 (ET-1) is the most potent endogenous vasoconstrictor with additional pro-inflammatory properties. However, the effects of ET-1 on Mϕ biology are not well studied. **Methods:** To examine the interactions between the ET and Mϕ systems we administered incremental doses of intravenous ET-1 to CD11b-diphtheria toxin receptor (DTR) mice given diphtheria toxin (DT; to deplete Mϕ/monocytes) and to mice lacking ET_B receptors solely on myeloid cells ($LysMET_B^{-/-}$). We also cultured bone marrow derived Mϕ (BMDM) from both these mice and human monocytes *in vitro*. Finally, we examined BP and the ET system in patients receiving Mϕ depleting and non-depleting immunotherapy. **Results:** Mϕ depletion or loss of function–Cd11b-DTR mice given DT *and* $LysMET_B^{-/-}$ mice – were not associated with a difference in baseline BP or endothelial dysfunction. In both groups of mice administration of ET-1 resulted in an exaggerated hypertensive response compared to controls. At a dose of ET-1 1nmol/kg the maximal change in BP was ~2-fold greater and the overall BP response ~3-fold greater in Mϕ deficient mice compared to control groups. *In vitro*, we show that mouse BMDM and human monocytes possess both ET_A and ET_B receptors (ET_B>>ET_A). Whereas stimulation of mouse and human Mϕ with exogenous ET-1 did not polarize Mϕ to a classical or alternative phenotype, both displayed chemokinesis to ET-1. This was reduced by selective ET_A (BQ123) and completely blocked by ET_B (BQ788) receptor antagonism. BMDM stimulation with LPS/INFγ (but not IL-4/IL-13) led to an increase in the concentration of ET-1 in their media at 24h, an effect that was blocked by phosphoramidon, an inhibitor of endothelin converting enzyme. Importantly, using pharmacological and gene targeting studies we show a novel clearance mechanism for ET-1 through ET_B receptor mediated dynamin-dependent endocytosis present in both murine and human Mϕ. Finally, in patients receiving Mϕ depleting immunotherapy we show that BP is higher and the ET system more activated than in those receiving non-depleting therapies. **Conclusions:** Overall, these data suggest that Mϕ and ET-1 may play an important role in BP control and potentially have a critical role as a therapeutic target in hypertension. *Funded by a British Heart Foundation Intermediate Clinical Res. Fellowship (FS/13/30/29994).*

3.62
LONG-TERM HIGH SALT DIET DELAYS DEVELOPMENT OF PROTEINURIA IN MURINE SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

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Recent evidence suggests that dietary salt may be an important environmental factor that promotes some autoimmune diseases. Therefore, we hypothesized that a long-term high salt diet would accelerate the progression of renal disease during SLE. In order to test this, an established experimental model of SLE (female NZBWF1 mice) was fed a standard (0.4% NaCl) or high salt (4% NaCl) diet starting at 10 weeks of age. Sodium intake was significantly greater in high salt fed animals (5.7±0.6 vs. 0.6±0.05, meq/day $p<0.01$). Urinary albumin was monitored by dipstick assay as a

marker of renal injury until 34 weeks of age at which time the mice were euthanized and the kidneys were harvested. Consistent with previous studies, 46% of mice (n=14) fed standard diet developed albuminuria over the course of the study. In contrast, only 6% of mice fed high salt developed albuminuria (n=17, p<0.02), suggesting a paradoxical renal protection. To provide mechanistic insight, inflammatory markers were assessed by qRT-PCR. Renal interleukin-2 (IL-2), a cytokine associated with T cell differentiation and with activation of the endothelin system, was significantly lower in high salt fed animals (0.26±0.14 relative to normal chow fed mice, p=0.03, n=4). Moreover, renal endothelin type A receptor protein expression was lower in high salt fed mice (0.61±0.14 vs. 1.0±0.21, p=0.05, n=6). These data suggest that, contrary to the original hypothesis, a long-term high salt diet may protect against the renal injury associated with SLE, possibly through down-regulation of renal inflammation and associated activation of the endothelin system.

3.63
RELATIONSHIP OF ENDOTHELIN-1 AND NLRP3 ACTIVATION IN HT22 HIPPOCAMPAL CELLS: RELEVANCE TO COGNITIVE DECLINE IN DIABETES

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Diabetes increases and worsens the progression of cognitive decline. Decreased cerebral blood flow and cerebrovascular remodeling is believed to precede the negative changes observed in diabetes-induced cognitive dysfunction. Diabetic rats treated with bosentan, an ET-A and ET-B antagonist, has been shown to improve hippocampal based cognitive deficits. We have shown that endothelin receptor antagonism restores vascular function and remodeling caused by diabetes. Inflammasome NLRP3 has been implicated in vascular complications of diabetes. The aim of the study was to elucidate the relationship between ET-1 and NLRP3-induced inflammation in hippocampal neurons in diabetes. An in vitro model was employed by exposing HT22 hippocampal neurons to regular control medium (C, 25 mM glucose) with and without palmitate (Pal, 200 μM) in the presence and absence of 10 μM bosentan for 24 hours. To mimic low nutrient state that occurs with vascular dysfunction, additional cells were grown in low glucose (LG, 5.5 mM). NLRP3 activity was measured by western blotting for ACS, cryopyrin and caspase-1. ET-1 and IL-1β in the media and cell lysate, respectively, was determined by ELISA. Palmitate decreased expression of ET-1 in neurons treated with and without bosentan (p<0.01). ET-1 was decreased in LG conditions after bosentan treatment (p<0.001). Bosentan and palmitate reduced expression of ASC (p<0.05), whereas expression was higher in LG treated cells with recovery bosentan treated neurons (p<0.05). Caspase-1 was decreased in neurons treated with both bosentan and palmitate (p<0.05). Cryopyrin and IL-1 β expression was increased in all but C+Pal condition when treated with bosentan (p<0.05). Low nutrient conditions stimulate inflammatory and ET-1 expression in neurons, which could account for neuronal dysfunction observed in diabetes. Understanding the relationship between endothelin and inflammation in the hippocampus could provide therapeutic targets in diabetes and cognitive decline.

3.64
CENTRAL ENDOGENOUS ENDOTHELINS (ETS) ARE INVOLVED IN THE DOCA-SALT HYPERTENSION. INTERACTIONS BETWEEN ETS RECEPTOR A (ETA) BLOCKADE AND TYROSINE HYDROXYLASE (TH) IN THE ANTERIOR (AH) AND POSTERIOR HYPOTHALAMUS (PH)

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In previous studies we have reported that the exogenous administration of ETs modified noradrenergic transmission in PH and AH of DOCA-salt hypertensive rats. Therefore in the present work we sought to establish the role of ETA receptor stimulation by endogenous ETs and its correlation with TH activity and expression in the AH and PH of DOCA-salt hypertensive rats. Normotensive and DOCA-salt hypertensive Sprague-Dawley male rats were prepared with a guide cannula placed in the brain lateral ventricle for the administration of artificial cerebrospinal fluid (CSFa) or BQ610 (ETA receptor antagonist). Following a recovery period of seven days, both groups were randomly sub-divided and icv administered with 1ul CSFa or 1ul BQ610 (20mM). BP was monitored for 60 min through a catheter placed in the femoral artery. Brain was then removed and the AH and PH dissected. The expression of TH and its phosphorylated forms were determined by immunoblotting and TH activity by a radioenzymatic assay. Results showed that BQ610 markedly reduced blood pressure in both normotensive and hypertensive animals, although a more prominent decrease was observed in systolic BP of DOCA-salt hypertensive rats (30 mmHg decrease following 30 min ETA exposure). No changes in TH expression or activity was observed in the PH and AH of normotensive rats either injected with vehicle or BQ610, or in the AH of DOCA-salt hypertensive rats. However in hypertensive rats PH, ETA blockade reduced TH phosphorylation at 40Ser and 19Ser sites (55.6% and 33.3%, respectively). Moreover, a Pearson correlation index showed that the amount of TH and TH-PSer40 expressed in this region correlated with SBP values (p<0.05). These results were in accordance with increased ETA expression (129%, p<0.05) found in the HP of DOCA-salt rats. Present findings shows that ETA receptor blockade reduces catecholamine activity in the PH of DOCA-salt hypertensive rats. Given that the PH is a well-characterized sympatho-excitatory area intimately involved in the regulation of cardiovascular activity, it allows us to conclude that brain ETs through ETA receptor activation strongly contribute to blood pressure elevation in DOCA-salt hypertension. These findings further support the relevance of the central endothelinergic system in a salt dependant hypertensive model such us DOCA-salt rats. Founding: ANPCyT, CONICET and UBA.

3.65
THE ENDOTHELIN SYSTEM IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Objective: ET-1 exerts negative effects on several pathways implicated in ALS pathogenesis, including glutamate transporter expression, heightened sensitivity to hypoxic stress, matrix metalloproteinase and growth factor expression, and oligodendrocyte development. These experiments examine whether ET-1 and ET-B receptor expression are altered in disease-relevant CNS regions and cell types in (1) ALS patient tissues, (2) patient-derived cell cultures, and (3) a well-characterized mouse model of ALS. **Methods**: We used Nanostring RNA counts, ELISA, Western blot, and immunohistochemistry (IHC) to examine ET-1 and ET-B receptor expression in postmortem CNS tissue samples from ALS patients and controls, ALS patient-derived fibroblast cultures, and SOD1-G93A ALS mice. We also surveyed an exome sequencing dataset comprised of 247 familial ALS (fALS) cases for variants in ET-system genes. **Results**: ET-1 (EDN1 gene) RNA and protein levels are increased in motor cortex from ALS patients compared to controls (P<0.05), and in ALS motor cortex compared to cerebellum from the same patients (P<0.01). IHC suggests ET-1 expression is increased in astrocytes in ALS motor cortex compared to both ALS occipital cortex and healthy control motor cortex. ET-B receptor staining is strikingly increased in motor neurons of ALS motor cortex compared to both other cortical regions and healthy control motor cortex. EDN1 RNA expression was significantly higher in motor cortex from ALS patients who possess the C9orf72 pathogenic ALS mutation compared to C9-negative cases. Fibroblasts cultured from C9+ vs. C9- ALS patients showed divergent ETB and ET-1 expression, and ET-1 ELISA of conditioned media samples readily distinguished the C9+ ALS cases. Endstage ALS mice had increased ET-1 protein in lumbar spinal cord compared to presymptomatic (p<0.01) and symptom onset (p<0.05) time points. In the exome analysis, 4% (10/247) of fALS patients had variants in ET-system genes that altered the coding sequence and were novel (ie not seen in ~7,000 controls). Six of these cases specifically had novel coding variants in ECE-2, ie 2.5% of total fALS cases. Interestingly, ECE-2 shares many functions with MMP-9, recently demonstrated to be characteristically upregulated in vulnerable motor neurons in ALS. **Conclusions:** These experiments demonstrate striking abnormalities in the CNS endothelin system in ALS patient tissue samples, patient-derived cell cultures, and ALS mice. There is upregulation of ET-1 expression by astrocytes and ET-B receptors on motor neurons in disease relevant CNS regions. The mounting data connecting the ET system to many pathways currently under investigation in ALS combined with the potent autocrine and paracrine effects of ET-1 in the CNS suggest that the endothelin system may represent a largely unexplored and potentially significant target for therapeutic intervention in ALS.

3.66
SIGNIFICANT CONTRIBUTION OF THE MAST CELL-DERIVED CHYMASE, MMCP-4, IN EARLY PHASES OF MULTIPLE SCLEROSIS IN MICE

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Multiple sclerosis (MS) is the most frequent neurodegenerative disease that affects humans between 20 and 45 years of age. To date, treatments only reduce symptoms related to spinal demyelination but do not reverse neurologic damages associated with MS. It has been suggested that MS has a vascular component associated with lym-

phocytes and mast cells infiltration (Wong *et al.*, Neurology, 2012). In addition, ET-1 plasma levels are elevated in MS patients (Hausfchild et al., *J. Neuroophtalmo*., 2001). Recently, our laboratory has reported that mast cell derived-chymase generates ET-1 in the mouse *in vitro* and *in vivo* (Houde *et al.*, *J Pharmacol Exp Ther*, 2013; Semaan *et al*, *Biochem Pharmacol*, 2015). Our principal aim was to assess the roles of a murine isoform of ET-1 producing chymase, namely mMCP-4, in the development of a murine model of experimental autoimmune encephalomyelitis (EAE). In the present study, we therefore evaluated, on a scale of 0 to 5, ascending paralysis in WT and mMCP-4 KO mice subjected to EAE. We also monitored ET-1 brain levels by ELISA, at various times following EAE induction or in healthy WT or mMCP-4 KO mice. When compared to WT littermates, mMCP-4 KO mice show a marked delay in onset and severity of EAE ($p < 0.05$). Furthermore, WT mice with induced EAE show a 3 fold increased level of ET-1 brain levels one week after inoculation of EAE when compared to healthy mice ($p < 0.01$). No such increases were observed in mMCP-4 KO mice induced by EAE. Basal brain levels of ET-1 were not different in naive WT or mMCP-4 KO mice. These results suggest that genetic repression of mMCP-4 improves neuromotor disabilities in a mouse model of MS. Also, ET-1 could be a marker of early phase of the disease due to the elevation of the level of this peptide in the CNS before the appearance of the first symptoms of EAE in mice. Whether mMCP-4-dependent production of ET-1 is involved in the neuromotor impairment prompted by EAE in the mouse model remains to be investigated. Funded by the Canadian Institutes of Health Research.

3.67

NOVEL UVR-INDUCED MELANOMA MOUSE MODEL BASED ON ENDOTHELIN 3 OVEREXPRESSION IN CONJUNCTION WITH DEFICIENCY OF THE NUCLEOTIDE EXCISION REPAIR PATHWAY

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Melanoma is the most aggressive type of skin cancer due to its high propensity to metastasize. Melanomagenesis is influenced by both genetic and environmental factors. Ultraviolet Radiation (UVR) exposure is widely accepted as the most important environmental factor leading to melanomagenesis. The development of melanocyte precursor cells is highly dependent on the Endothelin 3 (Edn3) pathway. In humans, this pathway has also been associated with melanoma progression and its high potential to metastasize. Congenital disorders that result in a disruption of the nucleotide excision repair (NER) pathway are some of many genetic factors that increase susceptibility to melanomas, as well as other skin cancers. The role of the NER pathway is to repair ultraviolet-induced DNA damage. Individuals lacking any of the Xeroderma Pigmentosum (XP) genes, which code for essential proteins of the NER pathway, are unable to successfully repair DNA damage caused by sunlight exposure. As a result, XP patients are extremely vulnerable to sunlight and, consequently, predisposed to skin cancer development. *Xpa*-deficient mice share many characteristics of XP patients, however do not develop melanomas upon UVR. The aim of this study was to develop a UV-induced melanoma mouse model with overexpression of the Edn3 pathway in conjunction with a targeted *Xpa* mutation. Three populations of transgenic mice with *Edn3* overexpression under the control of the keratin 5 promoter (K5-*Edn3*) and mutations in the *Xpa* gene ($Xpa^{-/-}$; K5-*Edn3, Xpa*$^{+/-}$; K5-*Edn3,* and $Xpa^{+/+}$; K5-*Edn3*) were exposed to a single suberythemal neonatal dose of UVR at 3.5 days of age, two doses of UVR (at 3.5 days and 6 weeks of age), or a single dose of UVR at 6 weeks of age. Histomorphology and immunostaining results confirmed the melanocytic origin of primary skin tumors and metastases. Melanomas were only found in transgenic K5-*Edn3* mice. A single suberythemal neonatal UVR dose at 3.5 days of age resulted in increased penetrance and decreased latency in $Xpa^{-/-}$; K5-*Edn3* mice (60%, n=10) when compared to $Xpa^{+/+}$; K5-*Edn3* (46%, n=13) and $Xpa^{+/+}$; K5-*Edn3* (19%, n=16). Animals exposed to two UVR doses did not reveal significant differences between *Xpa* null, heterozygous, or wild type; animals exposed to one dose of UVR at 6 weeks of age did not develop any melanomas. These results suggest that neonatal UVR exposure along with over-activation of the Edn3 pathway is sufficient for melanomagenesis in mice, and is enhanced by NER deficiency.

3.68

ENDOTHELIN 3 REGULATES PIGMENT PRODUCTION AND COAT COLOR IN MICE

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Skin and hair pigmentation plays an essential protective role against damages caused by UV irradiation. Humans with fair skin and light hair have a higher susceptibility to developing various skin cancers, including melanoma. The production of pigment involves several signaling molecules essential for the proper development and function of melanocytes, the pigment producing cells of the skin. α-Melanocyte Stimulating Hormone (α-MSH) regulates the production of both eumelanin (black/brown) and pheomelanin (yellow/red), while Endothelin 3 (Edn3) is required during melanocyte development. *Lethal yellow* mice (A^y) have a non-functional α-MSH pathway leading to the production of pheomelanin in the hair. Doxycycline (dox) inducible transgenic mice that express *Edn3* under the *Keratin 5* promoter (*K5-Edn3*)showed hyperpigmentation of the skin and coat. Transgenic *Edn3* darkened the coat color of A^y mice. The goal of this study is to understand the role of *Edn3* in pigment production. To test if continuous transgenic *Edn3* expression is required to maintain a dark pigmentation phenotype in A^y mice, dox was administered to newborn pups, deactivating the transgenic *Edn3* expression. After 6 weeks of dox treatment, the coat color of A^y; *K5-Edn3* mice became lighter and was similar to those of A^y littermates. This result indicates that Edn3 is required post-natally to maintain the increased levels of hair pigmentation. The comparative analysis of dorsal hairs from A^y and A^y; *K5*-Edn3 mice using high performance liquid chromatography showed that transgenic *Edn3* expression significantly increased both pigment types in A^y mice. The number of melanocytes in hair follicles of Edn3 transgenic mice as evidenced by immunofluorescence with an antibody against tyrosinase related protein 1 was similar to that of non-transgenic littermates. This indicates that the observed increase in melanin content in hairs of Edn3 transgenic mice was not due to the presence of more melanocytes. Our results indicate that the paracrine expression of *Edn3* from keratinocytes is capable of generating and maintaining a dark coat color in the absence of a functional α-MSH pathway. A better understanding of the pathways that regulate the process of pigment production can help in the development of more effective therapies for skin cancers and pigmentation disorders in humans. J. P. was supported by NIH/NIGMS R25 GM061347. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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EFFECTIVE MANAGEMENT OF SICKLE CELL ANAEMIA AND THALASSAEMIA; LESSONS FROM NHS ENGLAND

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Sickle Cell Anaemia and Thalassaemia are Haemoglobinopathies which are common among Asians and Blacks. They are genetic disorders and have been since the existence of man. However, the mortality rate is still high. According to NHS England there are 15,000 patients with Sickle Cell Disease (SCD) and 1,000 with Thalassaemia currently living in England. With therapeutic intervention, NHS England has been able to manage these patients with life expectancy of greater than 50years. However, as these patients are not able to engage in stressful work they are not employed by many organizations and they end up becoming depressed or suicidal in addition with the stigma associated with the disease. The aim of this study is to find a way of making Sickle Cell and Thalassaemia patients live longer in their home country where the disease originated from so as to enable more beds available for other critical cases in the A and E and on the wards in hospitals in England as many of them have become compulsory residents in developed countries due to access to healthcare. ***Grant Funding Source**: Self-Funded Research Students.* Full Article to be published in the British Journal of Clinical Pharmacology.

3.70

DIFFERENTIAL POTENTIATION OF OPIOID ANALGESIA BY ENDOTHELIN ET_A RECEPTOR ANTAGONIST BMS-182874

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Objective: To comparatively assess the potentiation of morphine, oxycodone and tramadol analgesia by ET_A antagonist BMS182874. **Methods:** Antinociceptive (tail-flick) latencies were measured in male Swiss Webster mice treated with vehicle or BMS182874 (50 µg, i.c.v.) plus morphine (6 mg/kg, s.c.), oxycodone (5 mg/kg, s.c.) or tramadol (50 mg/kg, s.c.). Tail flick latencies were measured before and 30, 60, 90, 120, 180, 240, 300 and 360 minutes after injection of the respective opioid. **Results:** Morphine produced a peak latency of 7.70±1.04 s 1 hour post injection while peak tail-flick latency in BMS182874 + morphine treated mice was 9.01±0.66 s. Oxycodone produced peak latency of 4.59 ± 0.17 s 30 min post injection and BMS182874 pretreatment increased oxycodone tail flick latency to 7.01±0.33 s. Tramadol produced peak latency of 8.2 ± 1.02 s 1 hour post injection while the BMS182874 + tramadol group yielded peak latency of 8.20 ± 1.02 s. Pretreatment with BMS182874

significantly increased latency to morphine ($P<0.05$) and oxycodone ($P<0.05$) but had no impact on latency to tramadol. **Conclusions:** BMS182874 differentially potentiated opioid analgesia, increasing morphine and oxycodone analgesia while having no impact on tramadol analgesia in mice. Morphine and oxycodone are considered low efficacy opioids with significantly different efficacy compared to tramadol, and potentiation may be dependent upon relative efficacy of the opioid. Opioids with a wide range of relative efficacies need to be tested in animals pretreated with BMS182874 to determine any correlation. Most clinically useful opioids produce their effects via μ-opioid receptor agonism. Different opioid agonists may act on the same receptor but yet produce distinctly unique downstream events. This functional selectivity of various opioids may determine the nature and extent of opioid-endothelin interaction and could be important in the differential potentiation of opioid analgesia by BMS182874. Funds for this work were provided by Midwestern University.

3.71
GENDER COMPARISON OF RECOVERY FROM INTRAVENOUS AND INHALATIONAL ANAESTHETICS AMONG ADULT PATIENTS IN SOUTH-WEST NIGERIA

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Gender is a known factor in recovery from general anaesthetics. This study reports such a difference in South-West Nigerian patients, and proposes gender-based differences in pharmacokinetic profiles as explanation. Two main strata (20 males and 20 females) were considered, and written informed consents were obtained for both strata. Intravenous general anaesthesia was induced with propofol or thiopental and maintained with inhalational agent halothane or isoflurane. Patient plasma samples were analyzed with high performance liquid chromatography (HPLC), for anaesthetic agents before induction; at 10, 30 60, 180 mins after induction of anaesthesia, and at recovery. The Elimination Half Life, Mean Resident Time, and Clearance measured in propofol patients maintained on halothane or isoflurane were 310.7 ± 138.4 min, 459.2 ± 199.4 min and 431.1 ± 154.1 ml/min, respectively for females versus 503.0 ± 312.2 min, 732.8 ± 448.3 min and 290.0 ± 157.8 ml/min for males. The corresponding values with thiopental induction were 148.0 ± 112.7 min, 220.9 ± 180.3 min and 544.6 ± 500.1 ml/min for females versus 76.8 ± 28.7 min, 115.4 ± 35.4 min and 533.7 ± 502.5 ml/min for males. Data was significantly different ($p<0.05$) across gender. Gender differences in recovery from anaesthetic agents in Southwest Nigerian patients is due to differences in pharmacokinetic profiles. *Anaesthesia, Recovery time, HPLC, Pharmacokinetics.* ***Grant Funding Source****: Self-Funded Research Student supported by Supervisors.* Full Article to be published in the British Journal of Clinical Pharmacology.

3.72
REVERSAL OF NALOXONE-PRECIPITATED OPIOID WITHDRAWAL IN MICE BY ENDOTHELIN ET_A RECEPTOR ANTAGONISTS

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Statement of Purpose: A major limitation of chronic use of opioid analgesics is their potential to cause adverse effects such as dependence, tolerance, and withdrawal. Our previous studies demonstrate that ET_A receptor antagonists significantly enhance opioid analgesia and reverse antinociceptive tolerance in mice and rats. It is also known that β-arrestin 2 regulates opioid receptors and is involved in opioid tolerance. The present study was designed to investigate the involvement of central endothelin (ET) and β-arrestin 2 mechanisms in opioid withdrawal. The effect of intracerebroventricular (i.c.v.) administration of ET_A receptor antagonist, BQ123, on morphine and oxycodone withdrawal was determined in male Swiss Webster mice. Methods and Materials: Opioid tolerance was induced by twice-daily injections of morphine for three days, and once-daily injections of oxycodone for five days. Withdrawal was precipitated by opioid receptor antagonist, naloxone, on day 4 and day 6 in morphine and oxycodone studies, respectively. Behavioral signs and symptoms of withdrawal were observed for 15-min post naloxone injection. Expression of ET_A receptors, ET_B receptors, vascular endothelial growth factor (VEGF), nerve growth factor (NGF), and β-arrestin 2 was determined in the brain using western blot analysis. Data and Results: BQ123 reversed hypothermia in mice following morphine and oxycodone withdrawal. Loss of body weight induced during withdrawal was also blocked by BQ123. Further, BQ123 attenuated number of wet shakes, rearing behavior and jumping behavior following opioid withdrawal. Western blot analysis indicated no change in expression of VEGF, ET_A, and ET_B receptors following administration of vehicle or BQ123 in mice undergoing morphine and oxycodone withdrawal. Brain NGF expression was not affected in morphine withdrawal but was significantly decreased in oxycodone withdrawal. This change in NGF expression was not altered by BQ123. No change in expression of β-arrestin 2 was observed following opioid withdrawal in the presence or absence of BQ123. Conclusion and Significance: These studies demonstrate that ET_A receptor antagonists mitigate withdrawal symptoms of morphine and oxycodone. The mechanism involved in attenuating opioid withdrawal appears to be different for morphine and oxycodone, because brain NGF expression decreased only with oxycodone and not with morphine. It appears that β-arrestin 2 is not involved in attenuation of morphine and oxycodone withdrawal by BQ123. *This study was funded by Midwestern University Chicago College of Pharmacy and College of Health Sciences.*

3.73
DEVELOPMENT AND VALIDATION OF A REVERSED-PHASE HPLC METHOD FOR THE ANALYSIS OF ENDOTHELIN-B RECEPTOR AGONIST, IRL-1620

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Endothelin-B (ET_B) receptors in the brain are being identified as vital in the development of CNS and have demonstrated potential application in the management of cerebral ischemia, Alzheimer's disease and other CNS disorders. Attempts are being made for the clinical development of ET_B receptor agonist, IRL-1620, in the management of cerebral ischemia. We have therefore embarked on validation of analytical method for the analysis of IRL-1620. A reverse phase HPLC method was developed and validated in terms of specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, robustness and ruggedness. Chromatographic separation of IRL-1620 was achieved on C_{18} column by isocratic elution. The mobile phase consisted of trifluoroacetic acid and acetonitrile in water and the quantitative assessment were performed at 215 nm. The method was specific, accurate and illustrated linearity from 50-400 ng/mL with the regression coefficient (r^2) of 0.998. IRL-1620 shows LOD and LOQ of 2.03 and 6.14 ng/mL, respectively. The intra- and inter-day precision (RSD) ranged from 0.48 to 1.95%. The recovery of IRL-1620 ranged between 97.0 to 100.0% with a relative standard deviation (RSD) of <2%. The method was robust at different variable conditions (RSD<2%). The developed method is simple, rapid, sensitive, precise, and accurate and was successfully applied for quantitative analysis of IRL-1620 in human plasma and finished products of IRL-1620.

3.74
HYPOGLYCEMIC EFFECT OF THE METHYL CHLORIDE-METHANOLIC EXTRACT OF THE FRESH FRUITS OF THE GONGRONEMA LATIFOLIA IN NORMOGLYCEMIC AND ALLOXAN-INDUCED DIABETIC RATS

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This study investigated the hypoglycemic effect of the fresh fruits extracts of *Gongronema latifolia* in both normoglycemic- and alloxan-induced glycemic animals. All were broken into small pieces. The extracts from the comminuted fruits were gotten by soxhlet extraction with a methylene chloride/methanol (1:1) system. Acute toxicity testing was done according to the method of Lorke (1983) using albino mice. Phytochemical screening was done according to standard methods. Diabetes was induced with alloxan monohydrate in groups of the rats. The rats were divided into groups of five animals each and fasted for 15 hours with free access to drinking water. For the normoglycemic animals, group 1 received 100 ml/kg of distilled water; group 2- 50mg/kg of extract; group 3- 100mg/kg of extract; group 4- 5mg/kg of glibenclamide; and group 5- 50mg/kg of extract plus 5mg/kg of glibenclamide. Blood samples were drawn by tail milking at 0 hr, 30 minus, 1hr, 2hrs, and 4 hrs, respectively and blood glucose concentration was determined using the glucometer. The acute toxicity results indicate that the extracts of the fresh fruits are safe for human consumption. The phytochemical results show, for the fresh fruits extract- flavonoids, starch and carbohydrates were absent; glycosides, resins and tannins were present in trace quantities; fats and oils were present in moderate quantities; alkaloids and saponins were present in large amounts; terpenoids, proteins, and steroids were present in very large quantities. Biochemical tests showed for the fresh fruits extracts to have significant ($p < 0.05$) dose-dependent hypoglycemic activity though in each case less than that of 5 mg/ kg glibenclamide, in both normoglycemic and alloxan-induced hyperglycemic rats. However, in each case, the co-administration of extract at 50 mg/kg and glibenclamide 5 mg/kg gave significantly ($p < 0.05$) increased hypoglycemic activity. In conclusion, the shade-dried fruits extract have more effect in reducing blood glucose.

3.75
ENDOTHELIN RECEPTOR SIGNALING AND AGE RELATED DEREGULATION OF CEREBRAL ARTERY MYOGENIC TONE

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Cerebral arterioles intrinsically maintain constant tissue perfusion despite variations in systemic blood pressures (myogenic tone). This process is deregulated with age and may have implications in various cerebral ischemic pathologies. The endothelin system (ES) contributes to regulation of myogenic tone and is comprised of two G-protein coupled receptor subtypes, ETA and ETB, and peptide ligands. In relation to vascular myogenic response, the net effect of ETA activation is vasoconstriction, whereas ETB activation is overall vasodilating. In vitro experiments have found that ETA and ETB physically interact and this dimerization may potentially affect the pharmacology and signaling of the ES. Our objectives were to examine: 1) The physical interactions between ETRA and ETRB and how these interactions affect pharmacology in terms of signaling and receptor trafficking, and 2) the contributions of ETA and ETB signaling to age-related dysfunction of cerebral arteriole myogenic tone. Receptor interactions were examined in a cell expression system using bioluminescent resonance energy transfer ($BRET^2$). ES regulation of myogenic responsiveness was examined using perfused cerebral arteries isolated from young and aged Fischer 344 rats, in compliance with local animal ethics committee approval. Vessel diameter in response to pressure +/- ETA and ETB antagonists was quantified using a video edge detector. Using the $BRET^2$ technology, we found that ETRA and ETRB form high affinity physical interactions. Our ex-vivo vascular experiments showed that, compared to young rats, arterioles from aged rats have significantly increased contractility through a range of pressures, and while ETRA responsiveness significantly increased with age, ETRB responsiveness declined. Ongoing experiments will determine how ETRA and ETRB dimerization affects the pharmacology of the ES and whether the changes we observed in aged cerebral vessels are the result of altered gene expression, deterioration of endothelial cell function, or changes in receptor coupling. Our results suggest that interactions between endothelin receptors may have consequences during aging and disease states, in which alterations in the expression and/or signaling of ETA and ETB receptors may occur. This research was supported by the Natural Sciences and Engineering Research Council of Canada grant NSERC-43526 and by the Nova Scotia Graduate Scholarship.

3.76
ATTENUATION OF ENDOTHELIN-1-INDUCED CARDIOMYOCYTE HYPERTROPHY THROUGH ESTROGEN PRE-TREATMENT

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Estrogen (β-estradiol), the female hormone, has been reported to inhibit cardiac hypertrophy and apoptosis by different research groups in recent days. Thus, although the anti-hypertrophic action of estrogen in heart has begun to be appreciated, the potential mechanism underlying the estrogen-mediated cardioprotection is unknown. Endothelin (ET)-1, a potent vasoconstrictor, induces hypertrophic changes in neonatal cardiomyocytes at both morphological and molecular levels. Targeting ET-1 in the prevention of heart failure is crucial to suppress the cardiovascular diseases. Indeed, pathological cardiac hypertrophy progressively leads to heart failure. In the first part of the current study, we investigated whether estrogen confers beneficial effect against ET-1-induced cardiomyocyte hypertrophy and if cardioprotective, then whether this estrogen mediated cardiac anti-hypertrophic action is genomic or non-genomic. The doses of estrogen and ET-1 were optimized based on preliminary dose- and time-dependent studies. At day 4 of culture, neonatal rat cardiomyocytes were divided into three groups: control, ET-1 (10nM) treated and estrogen-pre-treated (1μM) ET-1 groups. 2.0-fold increase in cardiomyocyte surface area, and 1.8-fold in protein synthesis rate in cardiomyocyte were observed after ET-1 administration and these changes were greatly prevented by estrogen pre-treatment. Estrogen could also normalize the upregulated ET-1 and ETA receptor mRNA expression in ET-1-induced hypertrophied cardiomyocyte. The pure estrogen receptor (ER) blocker, ICI-182,780, failed to reverse the estrogen-mediated anti-hypertrophic effect on ET-1-induced hypertrophied cardiomyocytes suggesting the non-genomic pathway of estrogen action. Moreover, we recently found that ET-1-mediated over-expression of VEGF contributes to the development of ET-1-induced cardiomyocyte hypertrophy. Thus, subsequently the present study investigated whether VEGF system would contribute to the anti-hypertrophic action of estrogen in ET-1-induced hypertrophied cardiomyocyte. Interestingly, we found that the upregulated VEGF system in ET-1-induced hypertrophied cardiomyocyte was greatly normalized by estrogen pre-treatment. The present results implied that estrogen (non-genomic action) may arrest the cardiomyocyte hypertrophy through the suppression of VEGF system and demonstrate for the first time the role of estrogen and VEGF system in the prevention of ET-1-induced cardiomyocyte hypertrophy.

3.77
ENDOTHELIN AND APELIN: THE YANG AND YIN PEPTIDES IN PULMONARY ARTERIAL HYPERTENSION

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Introduction: Increases in endothelin-1 (ET-1) contribute to elevated pulmonary vascular resistance in pulmonary arterial hypertension (PAH), which ultimately causes death by right ventricular (RV) heart failure. ET_A receptors are upregulated in the RV in PAH[1] but the beneficial effects of ET-1 as a positive inotropic agent are blocked by current therapies using ET_A receptor antagonists. The apelin peptides acting via a single G-protein coupled receptor have emerged as key physiological regulators in the cardiovascular system. In human hearts, [Pyr^1]-apelin-13 (apelin) is the most potent inotropic agent discovered to date[2] and the peptide also causes vasodilatation in humans and animals in vivo and opposes the vasoconstrictor actions of ET-1 in human vessels in vitro. The peptide is downregulated in PAH and we hypothesise that apelin would attenuate the development of PAH in the monocrotaline (MCT) rat model. We have recently developed a synthetic apelin agonist MM07, [3] biased towards the beneficial G-protein actions but with reduced recruitment of beta-arrestin and internalization, avoiding detrimental effects of GPCR agonists in desensitising and silencing the receptor. **Methods:** Sprague Dawley rats were randomly assigned to one of the following groups (n=8, mean weight 186 2 ±2). 1. A single subcutaneous dose of MCT (60 mg/kg) was followed with vehicle injections for 21 days. 2. MCT (60 mg/kg) followed by daily ip injections of MM07 (1mg/kg for 21 days) 3. MM07 ip 1mg/kg for 21 days. 4. Control vehicle injected ip for 21 days. Animals were imaged using in vivo magnetic resonance (MR) imaging before and at the end of the experiment; right ventricular systolic pressure was measure using a catheter under anaesthesia. Animal experiments were performed according to local ethics committee and Home Office (UK) guidelines under the 1986 Scientific Procedures Act. **Results:** MR imaging in vivo in transverse and longitudinal sections of rat heart showed that MCT resulted in the enlargement of the right ventricle compared with the control and this was attenuated by MM07. There was no effect of MM07. As expected the Fulton index (wet wt of right ventricle (RV) divided left ventricle & septum) was significantly elevated in MCT rats compared with control, but this increase was significantly attenuated by MM07 (One way ANOVA, Tukey's multiple comparisons $p<0.001$). Similar results were obtained for right ventricular systolic pressure as a surrogate of pulmonary arterial pressure. **Conclusions**. The results show a biased apelin agonist attenuates the increase in right ventricular hypertrophy and systolic pressure. These results suggest apelin agonists could be used in combination with ET antagonists to reduce remodeling and improve cardiac output. 1. Kuc RE et al(2014). Modulation of endothelin receptors in the failing right ventricle of the heart and vasculature of the lung in human pulmonary arterial hypertension. Life Sci. 118, 391-396. 2. Maguire JJ et al (2009) [Pyr^1]apelin-13 identified as the predominant apelin isoform in human heart: vasoactive mechanisms and inotropic action in disease. Hypertension, 54:598-604. 3. Brame et al. (2015). Design, characterization and first-in-human study of the vascular actions of a novel 'biased' apelin receptor agonist. Hypertension, 65, 834-840.

3.78
DIFFERENTIAL ROLE OF ET_A AND ET_B RECEPTORS IN CNS PARAMETERS

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Endothelin-1 peptide predominantly acts through two main receptors ET_A and ET_B. On vascular smooth muscles, ET_A mediates vasoconstriction whereas ET_B mediates vasodilatation. In addition, Endothelin-1 *via* ET_A and ET_B receptors also plays a vital role in the modulation of central pain. In brain, the differential role of ET_A and ET_B is not clear. We have reported earlier that nonspecific ET_A/ET_B receptor antagonist TAK-004 has neuroprotective effect in MCAo model of cerebral infarction. Further it also demonstrated neuroprotective effect against neuronal damage caused by hypoxia induced in neuronal culture. Recently, it has been reported that specific ET_B receptor agonist IRL-1620, prevented beta amyloid induced oxidative stress and cognitive im-

pairment in rats. In the present study, we studied the activity of ET_B agonist IRL-1620, at a dose of 20μg/kg, i.p. in seizure, amnesia and nociceptive animal models. Pretreatment with the ET_B agonist IRL-1620 did not protect against seizures induced by PTZ and MES in rats. There was no potentiation in anticonvulsant effect when combined with sub-therapeutic dose of sodium valproate and phenytoin in PTZ and MES induced seizures respectively. On the other hand, it showed dose dependent (5, 10 and 20 μg/kg) effect in scopolamine-induced (3 mg/kg, i.p.) amnesia with complete reversal at 20 μg/kg dose, as assessed by elevated plus maze (EPM) and passive avoidance (PA) test. The retention transfer latency in EPM were found to be increased to 24.0 ± 3.1s in scopolamine treated rats as compared to control 12.3 ± 0.9s which was reversed after drug treatment (20 μg/kg) to 17.7 ± 0.9s. Similarly, in passive avoidance test the retention latency decreased to 111.0 ± 13.4s in scopolamine treated rats as compared to control 300 ± 0.0s which was reversed after drug treatment to 300 ± 0.0s. Further, antinociceptive activity was quantified by hot plate latency test which is reported to mediate effect primarily *via* supraspinal mechanism. Anti-nociceptive activity of ET_B agonist (IRL-1620) (expressed as percentage maximum possible effect, % MPE) was assessed at 15, 30, 45, 60 and 90 min after administration of drug and in combination with sub-analgesic dose of morphine (3 mg/kg, i.p.) in rats. Antinociceptive activity exceeded to 20% MPE in drug treated group sustaining till 30 min and potentiated upon combination with morphine to 41% MPE at 15 min. The anti-nociceptive activity was further blocked by naloxone (1 mg/kg, s.c.) in combination group of morphine and IRL-1620. In conclusion, IRL-1620 showed antiamnesic and antinociceptive activity, but no anticonvulsant activity was observed in PTZ and MES induced seizures.

3.79

EFFECTS OF ENDOTHELIN RECEPTOR ANTAGONISTS ON DOXORUBICIN-INDUCED APOPTOSIS IN VASCULAR SMOOTH MUSCLE CELLS DERIVED FROM HUMAN PULMONARY ARTERY

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Purpose: Vascular remodeling is an aberration of the normal balance between cell proliferation and death. Proliferation of pulmonary arterial smooth muscle cells (PASMCs) is the major phenotype of pulmonary arterial hypertension, in which the endothelin (ET) receptor signaling is augmented and therefore ET-1 is thought to be involved in the development of PASMC proliferation. However, it remains unclear how the ET receptor system contributes to the apoptosis of PASMCs, another aspect for affecting vascular remodeling. The aim of this study was to investigate the effects of ET receptor blockade on apoptosis in human PASMCs. **Methods & Results:** Doxorubicin (Dox) induced apoptosis in human PASMCs in a dose (0.1-1 μM) and time (0-36 hrs.) dependent manner. Apoptosis was evaluated by Western blot (an increase of cleaved Caspase-3) and by FACS (annexin V (+)). ET-1 (10 nM), BQ-123 (ET_A antagonist, 100 nM), A-192621 (ET_B antagonist, 100 nM) was treated prior to Dox administration. Dox markedly induced apoptosis and ET-1 significantly attenuated Dox-induced apoptosis in PASMCs, suggesting that ET-1 has an anti-apoptotic effect. Pretreatment with BQ-123 did not change the extent of Dox-induced apoptosis, whereas A-192621 significantly augmented the Dox-induced apoptosis. **Conclusion and Discussion:** It was revealed that the ET_B receptor blockade by A-192621 enhances apoptosis of PASMCs, suggesting that ET_B receptor blockade might contribute to ameliorating vascular thickening through enhancing apoptosis of PASMCs in the diseased pulmonary arteries. Because we and others have reported that blockade of ET_A receptor is effective in ameliorating vascular thickening by inhibiting the proliferation of PASMCs, the present findings would imply us that $ET_{A/B}$ dual receptor blockade might be preferable for PAH treatment rather than only ET_A blockade from the viewpoint of inhibition of vascular remodeling through the two mechanisms, proliferation and apoptosis of PASMCs.

3.80

THE IMPORTANCE OF CELL CYCLE STRETCH IN COUNTER REGULATING CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION BY SUPPRESSING ERK1/2 SIGNALING

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Introduction: Chronic thromboembolic pulmonary hypertension (CTEPH) is one type of the pulmonary hypertension. It has been hypothesized that chronic obstruction of pulmonary artery by organic thromboembolic materials would induce pulmonary vessel remodeling and lead to increase of pulmonary pressure. However, the mechanism of this remodeling affecting vascular signaling, tone, and cycle stretch is still elusive. **Objective:** Since CTEPH patient displays an increase of circulatory endothelin-1 (ET-1) level, we aimed to investigate the effect of ET-1 and cell cycle stretch (CCS) in CTEPH pulmonary artery. **Methods:** We isolated pulmonary arterial smooth muscle cells from the samples of pulmonary endarterectomy obtained from the patients with CTEPH (CTEPH-SMC). We introduced stretch culture system (10% or 20% CCS), ET-1 (100nM), and combination of CCS and ET-1 into CTEPH-SMC and human pulmonary artery smooth muscle cells (HPASMC), then measured ERK1/2 protein expression. **Results:** Western blot analyses revealed that ERK1/2 protein expression was reduced after CCS treatment in HPASMC but was increased in CTEPH group. On the contrary, ERK1/2 expression was increased in both groups after ET-1 stimulation. Interestingly, combination of CCS and ET-1 significantly decreased ERK1/2 protein expression in both group. **Conclusion:** Our preliminary study showed that CCS negatively regulates ERK1/2 expression induced by ET-1. This result suggests the importance of CCS in counter regulating deleterious effects induced by ET-1 on pulmonary artery smooth muscle. This study is supported by Grant-in-Aid for Scientific Research (C) 26460213 and 18590813 from the Japan Society for the Promotion of Science.

3.81

CHRONIC HYPOXIA IN ENDOTHELIN-1 TRANSGENIC (ETTG) MICE GENERATES MODERATE PULMONARY HYPERTENSION, NOT SEVERE PULMONARY HYPERTENSION AND ITS PLEXIFORM LESIONS.

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Background: Pulmonary hypertension (PH) is a disease which affects the vascularizetion of the lungs. PH is a rare disease, however the mortality rate is still high, with the most cause of deaths dominated by heart failure. During the progression of PH, endothelial damage may participate in creating more severe vasoconstriction. Endothelin-1 (ET-1) which is mostly produced by endothelial cells is already known to be involved in the progression of PH, especially through its strong vasoconstriction effects. Furthermore ET-1 is also known to be involved in worse degree of PH, inducing plexiform lesions, a hallmark of severe PH. **Objective:** We aimed to create a model of severe PH in mice using ETTG mice combined with chronic hypoxia condition. **Methods:** We used ET-1 transgenic mice (ETTG) which have higher level of ET-1. We induced PH in ETTG mice and wild type littermates by putting them on hypoxic chamber containing 10% of O2. Ten-week old mice were put in hypoxic chamber and normoxic condition mice were used as controls. Mice were examined and sacrificed after 6 weeks in hypoxic chamber, a chronic condition of hypoxic, in order to get irreversible and more severe condition of PH. Right heart hemodynamic was assessed using Right Ventricular Systolic Pressure (RVSP) by transducer. Level of expression of ET-1, its receptor ETA (ETAR) and ETB (ETBR), Interleukin-1 Beta (IL-1 Beta), eNOS, iNOS, were evaluated by real time PCR or western blot. Vessel mean wall thickness was analyzed using Hematoxylin Eosin (HE) staining. **Results:** Abundance of ET-1 combined with chronic hypoxia increased ET-1, ETAR and ETBR expression. IL-1 Beta, eNOS and iNOS increased expression were also found. This was accompanied by the increase of wall thickening in small pulmonary vessels and inflammation. However, plexiform lesions as the hallmark of severe PH could not be found. **Conclusion:** Our results suggest that the abundance of ET-1 combined with chronic hypoxia condition could increase severity of PH moderately. Furthermore, plexiform lesions as the hallmark of severe PH could not be found, suggesting there might be a counter regulatory mechanism toward increasing ET-1 level through Nitric Oxide Synthases (NOS) induced by the increase of eNOS and iNOS. This study is funded by Grant-in-Aid for Scientific Research (C) 26460213 and 18590813 from the Japan Society for the Promotion of Science.

3.82

BOSENTAN REVERSES THE HYPOXIA-INDUCED DOWN-REGULATION OF THE BONE MORPHOGENETIC PROTEIN SIGNALING IN PULMONARY ARTERY SMOOTH MUSCLE CELLS

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Aims: Pulmonary hypertension (PH) is a common complication of chronic hypoxic lung diseases. Bone morphogenetic protein (BMP) and endothelin-1 signalling path-

ways have been shown to be altered in hypoxic PH and to play crucial roles in the associated pulmonary artery remodeling. We, therefore, aim to study the potential link between hypoxia and the alteration of BMP and endothelin-1 signaling in pulmonary artery smooth muscle cells (PA-SMCs). **Methods:** Primary cultured human PA-SMCs were treated with hypoxia-mimetic agent cobalt chloride (CoCl2; 100 μM), with or without pre-treatment with a dual endothelin receptor antagonist bosentan (1 μM). Expressions of preproendothelin-1, endothelin converting enzyme-1 (ECE1), BMP type 2 receptor (BMPR-2), BMP agonist BMP4 and antagonist noggin and BMP signalling target gene, the inhibitor of DNA binding 1 (ID1) were, then, evaluated by real time quantitative polymerase chain reaction. **Results:** In PA-SMCs, preproendothelin-1 expression increased after CoCl2 treatment, while ECE1 expression did not change. Hypoxia-mimetic agent CoCl2 decreased the expressions of BMPR-2 and ID1 maximally after 3- and 6-hour treatment respectively, while CoCl2 treatment progressively increased the expression of noggin. Pretreatment with bosentan restored the decrease in ID1 expression induced by CoCl2 treatment to basal level, while bosentan had no effects on noggin, BMPR-2 and BMP4 expressions. **Conclusions:** Hypoxia seems to induce the downregulation of the BMP signalling, partly through the endothelin system in PA-SMCs.

3.83

THREE HOURES TREATMENT OF LANDIOLOL HYDROCHLORIDE, AN ULTRA-SHORT-ACTING B-BLOCKER, IS NOT EFFECTIVE TO REVERSE ALTERED PULMONARY ENDOTHELIN-1 SYSTEM IN ACUTE LUNG INJURY IN A RAT MODEL OF EARLY HOURS OF ENDOTOXEMIA

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Among multiple organs failure and dysfunction associated with sepsis, molecular mechanisms of sepsis-associated acute lung injury (ALI) are poorly defined. Endothelin (ET)-1, a potent vasoconstrictor has been found to be involved in the pathogenesis of ALI in a rat model of sepsis. Here we studied whether landiolol hydrochloride, an ultra-short-acting β-blocker, can play an important role in ameliorating LPS-induced ALI through the normalization of ET-1 system. Male Wistar rats at 8 weeks of age were administered with either saline or lipopolysaccharide (LPS) for three hours and some LPS-administered rats were continuously treated with landiolol for three hours. The features of acute lung injury were observed at sepsis model. At 3h after LPS administration, both circulatory and pulmonary TNF-alpha and iNOS levels (inflammatory cytokines) increased and PaO_2 was significantly decreased. LPS induced a time-dependent expression of ET-1 in the lungs compared to control, peaking and increasing by 3 fold at 6 h after induction of endotoxemia with the upregulation of ET (A) receptor, whereas levels of ET (B) receptor, which has vasodilating effects, were remarkably down regulated time-dependently with the progression of sepsis. We conclude that time-dependent increase of ET-1 and ET (A) receptor with the down regulation of ET (B) receptor may play a role in the pathogenesis of acute lung injury in endotoxemia. Finally, treatment of LPS-administered rats with landiolol for three hours failed to normalize the upregulated pulmonary ET-1 and ET (A) receptors (although there was a normalizing trend) while caused further significant downregulation of ET (B) receptor in lung tissues at the early hours of endotoxemia. Of note, parallel to ET system, landiolol also failed to reverse the upregulated inflammatory mediators (TNF, iNOS) in lung tissues in endotoxemia. Landiolol hydrochloride, an ultra-short-acting and highly cardio-selective beta-1 blocker, has already been approved as an emergency treatment of supraventricular tachyarrhythmia in patients in Japan. In a recent study, landiolol exerted lung protective effect in sepsis. These data taken together, led us to conclude that landiolol mediated ALI improvement in sepsis may not involve pulmonary ET system and potential inflammatory cytokines, although a dose and time dependent study is needed in future to have more specific conclusion. This study has been supported by Ministry of Education and Science in Japan.

3.84

POSTNATAL *ECE1* ABLATION CAUSES SEVERE, PROGRESSIVE PULMONARY DISEASE

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Endothelin converting enzyme-1 (ECE1) catalyzes the conversion of inactive big endothelin 1 (ET1) to active ET1. Homozygous *Ece1* knock out (KO) mice die *in utero* or at birth, displaying multiple abnormalities recapitulating the phenotypes of *Edn1*, *Edn3*, *Ednra* and *Ednrb* KOs. These include malformations of the cardiac outflow tract, mandibular hypoplasia, and intestinal aganglionosis, and these abnormalities occur in spite of the presence of ample tissue ET. However, increased ECE1 activity and circulating and/or tissue ET1 are associated with many adult cardiovascular diseases, including idiopathic pulmonary fibrosis (IPF), a chronic and fatal lung disease. There is an apparent paradox between the need for ET1 in development and its harmful effects in adult disease. To study the role of ECE1 in postnatal life, our lab developed a conditional *Ece1* KO mouse, in which *Ece1* is ablated following tamoxifen (tam) administration. We hypothesized that ECE1 serves to localize ET1 signals to specific cell populations and is essential in normal adult physiology. We studied the following groups: mice given vehicle rather than tam, mice lacking tam-inducible Cre recombinase, mice harboring a normal *Ece1* allele (*Ece1*$^{+/flox}$), and the experimental animals (Cre *Ece1*$^{-/flox}$). Mice were treated with vehicle or tam at 8-9 weeks of age. Cre *Ece1*$^{-/flox}$ mice showed 85-100% mRNA and protein knock-down efficiency 8 weeks after tam treatment. Beginning several weeks following tam treatment, Cre *Ece1*$^{-/flox}$ mice develop a progressive respiratory illness manifested by tachypnea, decreased activity, and weight loss, requiring euthanasia for humane considerations within 3 months of tam treatment. Associated features include decreased adipose tissue mass, lower blood pressure, pectus excavatum, and right heart failure of variable severity. The right sided CHF is manifested by right heart enlargement, reduced stroke volume, and reduced cardiac output as measured by echocardiography. Histological examination revealed eosinophilic crystalline pneumonia and increased collagen deposition in the lung and heart. These findings are consistent with development of IPF in the experimental mice. Our findings show that *Ece1* ablation in postnatal animal results in a severe cardiorespiratory disease, suggesting that ectopic activation of ET1 by tissue proteases rather than by ECE1 is the primary mechanism underlying the observed association of increased ET1 signaling in disease states.

3.85

THE UTILITY OF THE PULSE OXIMETER FOR PULMONARY HYPERTENSION DURING THE SIX MINUTE WALK TEST

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Objective: The six minute walk test (6MWT) has widely been performed to evaluate exercise tolerance on the patients of pulmonary hypertension (PH). But some studies suggested that six minute walk distance (6MWD) are not well correlated with the prognosis and hemodynamics. We evaluated the dynamics of vital sign during 6MWT to figure out more sensitive parameters for evaluating exercise tolerance in PH. **Method:** We utilized the pulse oximeter (Konica Minorta PULSOX-300i) on 10 PH patients and 13 healthy volunteers during 6MWT, and compared the data of blood oxygen saturation (SpO2) and pulse rate between these groups. Furthermore, we evaluated these parameters by examined their correlation with hemodynamics obtained right heart catheterization and indices from cardiopulmonary exercise test (CPX). **Results:** 10 PH patients (9 females) consisted of 7 chronic thromboembolic pulmonary hypertension, 2 lung diseases, and 1 limited systemic sclerosis. 5 of them were treated by endothelin receptor antagonists (ERA), 4 by soluble guanylate cyclases (sGC), 1 by phosphodiesterase type5 inhibitor (PDE5-I), and 1 by oral epoprostenol analogue. Compared with 13 healthy volunteers (4 females), the patients walked shorter (368±34 vs 489±10m; $p<0.01$), have more severe dyspnea (New Borg Scale: 3.1±0.9 vs 0.2±0.2; $p=0.01$), showed lower SpO2 (95.3±0.4% vs 98.0±0.2% at baseline; $p<0.01$, 91.3±0.6% vs 96.3±0.3% on average, $p<0.01$, 88.3±0.9% vs 94.1±0.6% at a minimum point; $p<0.01$), and higher pulse rate at baseline (87.8±4.4 bpm vs 71.1±3.1; $p<0.01$). We also examined the correlation between mean pulmonary arterial pressure (mPAP) and these vital signs with other clinical paramenters. Pulse rate (PR) didn't show any significant correlation with mPAP (baseline PR: $r=0.09$, average PR : $r=0.17$, maximum PR: $r=0.12$). 6MWD, maximal VO_2 , SpO2 at baseline had also no correlation with mPAP (6MWD: $r=0.24$, maximal VO_2: $r=0.35$, SpO2 at baseline: $r=0.29$). New Borg scales and VE/VCO_2 slope show significant correlation with mPAP (New Borg scale: $r=0.65$, VE/VCO_2: $r=0.50$). SpO2 on average and at a minimum point show strong correlation with mPAP (on average: $r=0.88$, at a minimum point 0.95). **Conclusion:** We documented SpO2 during 6MWT has much stronger correlation with hemodynamics than 6MWD and CPX parameters for the first time in PH patients. Monitoring SpO2 in exercise provide us much information. Clinical utility of this parameter should be further elucidated.

3.86

ET_A RECEPTOR BLOCKADE PROTECTS AGAINST PNEUMOLYSIN-INDUCED BARRIER DYSFUNCTION IN SICKLE CELL DISEASE

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Acute lung injury is a common and life-threatening complication in sickle cell disease (SCD). Endothelin-1 (ET-1) is elevated in SCD and is known to mediate local inflammation and oxidative stress. We established a novel, clinically relevant lung injury model in SCD mice based on pneumolysin (PLY), a pore-forming toxin released from *S. Pneumoniae*, a common pathogen in SCD patients. The goal of this study was to investigate the role of ET-1 in the PLY injury pathway. Transgenic SCD mice were treated with an intra-tracheal administration of PLY (1.5μg/kg). PLY-induced barrier dysfunction was assessed by Evans Blue Dye (EBD) incorporation with or without pretreatment with the ET_A blocker Ambrisentan (AMB). Additionally, NADPH oxidase isoforms, ET-1 and its cognate receptors were measured in lungs from SCD and control mice. PLY exposure induced a two-fold greater EBD leak in SCD mouse lungs compared to controls. Pretreatment with AMB blocked this SCD-related step of the injury response. Further, the SCD lung expressed higher mRNA levels of ET-1 and ET_A and NOX2 protein in pulmonary vascular endothelium. PLY-induced barrier dysfunction, a clinically relevant form of lung injury, is enhanced in SCD. ET-1 signaling through the ET_A receptor significantly augmented the injury response, which is likely mediated by increased oxidative stress. Clinically available ET_A receptor blockers, like Ambrisentan, may provide a novel therapeutic option in patients with SCD. Supported by HL-117684.

3.87
ENDOTHELIN RECEPTOR BLOCKADE ATTENUATES THROMBIN- AND HYPOXIA-STIMULATED INTRACAPILLARY NEUTROPHIL RETENTION IN LUNGS OF SICKLE CELL DISEASE MICE

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Endothelin-1 (ET-1) has been implicated in a number of critical pathogennic events underlying a variety of vasculopathies including sickle cell disease (SCD) however, the physiologic significance of ET-1 elevations in SCD remains poorly understood. Here we sought to determine whether ET-1 mediates an augmented agonist-stimulated intracapillary neutrophil retention (INR) in SCD lungs. We employed an *ex vivo* polymorphonuclear neutrophil (PMN)-perfused lung model. Lungs isolated from 12 week transgenic humanized SCD homo-(SCD-/-) and hetero-(SCD+/-) zygous mice, were assessed for intravascular PMN sequestration using IHC for leukocyte myeloperoxidase following either thrombin perfusion (3unit/mL/15min) or hypoxic ventilation (5%O_2/30min) followed by perfusion of non-stimulated rat PMNs (1.0´10^5/mL/20min). Thrombin- and hypoxia-stimulated INR were observed to be more pronounced in both groups of mice. To examine if the ET-1 signaling pathway is involved in this process, we treated both groups with antagonists to either ET_A, ambrisentan (20 mg/kg/ip/3days), or ET_{AB}, bosentan (20 or 50 mg/kg/ip/3days), or vehicle prior to lung isolation. ET_A or ET_{AB} blocking comparably reduced thrombin- and hypoxia-stimulated INR in SCD-/- lungs, but had a minimal effect on SCD+/- lungs. These results suggest a chronic alveolar capillary endothelial pro-inflammatory phenotype that involves a potentiated ET-1 signaling pathway. *Supported by HL-117684 (to S. Meiler) and HL-066299 (to S. Wu).*

3.88
THE EVALUATION OF ENDOTHELIN RECEPTOR ANTAGONIST FOR PULMONARY HYPERTENSION WITH LUNG DISEASE

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Objective: Although clinical efficacy of endothelin receptor antagonist (ERA) is established in the patients with pulmonary arterial hypertension, an application of ERA to pulmonary hypertension (PH) with lung disease is still under debate. This study is designed to analyze retrospectively the effect of ERA on hemodynamics, oxygenation, and symptom in PH patients with mild and severe lung diseases, separately. **Method:** We analyzed 46 PH patients with lung diseases separately depending on the severity of PH and lung disease. Severe PH is defined as more than 35mmHg of mean pulmonary arterial pressure (mPAP) which is measured by right heart catheterization (RHC), and mild PH is as 25 to 35 mmHg of mPAP. Besides, we defined the patients with less than 60% of percent vital capacity (%VC) and/or less than 50% of forced expiratory volume% in 1 second (FEV1%) as severe lung disease, and 60 to 80% of %VC and/or 50 to 70% of FEV1% as mild lung disease. We performed RHC before/after ERA and analyzed the clinical efficacy. **Results:** In 22 patients with mild lung disease, 11 are mild PH (MLD-MPH) and 11 are severe PH (MLD-SPH). In 23 patients with severe lung disease, 12 are mild PH (SLD-MPH) and 11 are severe PH (SLD-SPH). In mild lung disease groups, both MLD-MPH and MLD-SPH groups have shown the improvement of mPAP (MLD-MPH: 28.5±0.3 to 26.6±0.7mmHg, MLD-SPH: 44.8±2.4 to 38.3±5.0 mmHg), blood oxygenation saturation (SpO2) (MLD-MPH:93.3±0.4 to 94.7±0.3%, MLD-SPH: 92.0±1.6 to 92.9±1.5%), and WHO functional class (MLD-MPH:2.7±0.1 to 2.6±0.1, MLD-SPH:3.0±0.2 to 2.6±0.2). On the other hands, in the severe lung disease groups, SpO2 in SLD-MPH become worse in contrast of the improvement in SLD-SPH (SLD-MPH: 91.2±3.0 to 89.8±3.1%, SLD-SPH: 86.0±2.3 to 89.6±1.9%). WHO functional class has also shown same tendency (SLD-MPH:2.8±0.2 to 3.0±0.1, SLD-SPH 3.3±0.1 to 3.3±0.2) although both SLD-MPH and SLD-SPH have shown the improvement of mPAP (SLD-MPH:30.1±0.9 to 26.0±2.1mmHg, SLD-SPH: 42.0±1.8 to 39.3±3.6mmHg). **Conclusion:** We reported the beneficial effect of ERA on the patients of mild lung disease with all PH and of severe lung disease with severe PH. But, it should be noted that ERA on the patients of severe lung disease with mild PH has shown worsening symptom and oxygenation in spite of hemodynamic improvement.

3.89
IMPACT OF URINARY ENDOTHELIN-1 ON DERANGEMENTS IN STRESS-INDUCED PRESSURE NATRIURESIS

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Sodium retention during stress (retainer) is known to increase the risk of hypertension and other diseases. Our group has previously shown that angiotensin II type I receptor blockade improves natriuresis in retainers. Since our pre-clinical studies demonstrate that angiotensin II inhibits endothelin-1 (ET-1) dependent natriuresis, we predict that ET-1 may be linked to the response. We hypothesize that reduction in urinary (renal) ET-1 accounts for derangements in sodium handling under stress, a link never before explored in a large human cohort. We evaluated urinary ET-1 and albumin excretion in 4 studies of stress-induced pressure natriuresis, of which 3 were observational studies with 776 healthy youth (15-19 years) enrolled in a 5 hr protocol (1 hr of mental stress before and after 2 hrs of rest). The 4th study involved 213 African American adults (18-54 years) in a double blind crossover trial comparing irbesartan (angiotensin II type I receptor antagonist) to placebo. The protocol entailed 7 days of vehicle (placebo or irbesartan 150 mg P.O.) followed by a 3 hr protocol (1 hr of rest before and after 1 hr of mental stress). In all studies, 60 min urine samples were obtained. Subjects were grouped as retainers or excreters if they retained or excreted sodium under stress. In the observational studies, the mean change in ET-1 between stress and baseline was significant ($p<0.001$), being negative (mean= -0.0154 pmol/min) in retainers but positive (mean= 0.0194 pmol/min) in excreters. ET-1 excretion was significantly higher ($p<0.028$) in retainers than excreters at baseline but significantly lower in retainers under stress ($p<0.0001$). ET-1 excretion continued to decline in retainers during recovery but returned to pre-stress levels in excreters. Albumin excretion and albumin to creatinine ratio were significantly higher in retainers ($p<0.046$, $p<0.008$, respectively). During stress, the irbesartan group had significantly higher ET-1 excretion than placebo ($p<0.001$). Loss of ET-1-dependent natriuresis may account for sodium retention during stress and correction of sodium handling re-establishes ET-1-mediated natriuresis. Retainers have both lower ET-1 excretion and increased albuminuria, suggesting renal impairment and risk for future diseases.

4.0 ET, SEX, AND PREGNANCY

4.1
SEX AND HYPERTENSION

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Hypertension is well-recognized as having distinct sex differences in the prevalence, absolute blood pressure values, and molecular mechanisms contributing to the pathophysiology of the disease. Hypertension is a complex and multifaceted disease, and sex differences in the molecular mechanisms regulating blood pressure likely underlie the above observations. Numerous vasoactive pathways have been implicated in blood pressure control in hypertension, as well as in contributing to sex differences in blood pressure, including the renin angiotensin system, the nitric oxide pathway, oxidative stress, inflammation and the endothelin (ET) system. Endothelin (ET)-1 has been described as the most potent vasoconstrictor substance identified to date, and over-activation or dysfunction of the ET system contributes to the development and progression of hypertension. There are numerous sex differences in the ET system and these differences have been linked to sex differences in blood pressure control, including sex differences in ET-1 levels, sensitivity to ET-1 induced-vasoconstriction, and ET receptor expression. Due to the prominent role played by the ET system in maintaining cardiovascular homeostasis, coupled with the ability of the ET system to interact with numerous other pathways involved in blood pressure control more studies are needed to better define how the ET system regulates blood pressure in both males and females. References: Zimmerman and Sullivan. 2013. Hypertension:

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5.0 ROLE OF ET IN THE VASCULATURE

5.1

ET-1 IN THE HEART IN HEALTH AND DISEASE

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Endothelin-1 (ET-1) is a multifunctional peptide with complex effects on the cardiac function. Pharmacological blocking of the endothelin system was effective in the treatment of heart failure in rodent models. However, despite convincing experimental evidence of a pathogenetic role for endothelin in heart failure, many initial clinical studies failed to show beneficial effect of endothelin receptor antagonists in patients. Our group and others have generated and analyzed a series of the endothelin-related genes-deficient mice to investigate the possible cause of these contradictive results. Some animal models have demonstrated that ET-1 has hypertrophic and profibrotic effects in the heart as expected. On the other hand, ET-1 appears to have cardio-protective properties through its anti-apoptotic effects. Thus, ET-1 induces numerous cellular responses in the heart that may be contradictory depending on the situation. Based on the knowledge obtained from the studies with the genetically modified animals, the question whether chronic blockade of the endothelin system, by endothelin receptor antagonists or endothelin-converting enzyme inhibitors, might be beneficial in particular cardiac conditions will be discussed. Support: Grant-in-Aid for Scientific Research (C) 26460213 and 18590813 from the Japan Society for the Promotion of Science. REFERENCES: Vignon-Zellweger N, Heiden S, Miyauchi T, Emoto N. Molecular Biology of Endothelin-1 in the Renal and Cardiovascular Systems. Life Sci. (2012) 91, 490; Heiden S, Vignon-Zellweger N, Masuda S, Yagi K, Nakayama K, Yanagisawa M, Emoto N. Vascular endothelium derived endothelin-1 is required for normal heart function after chronic pressure overload in mice. PLoS One. (2014) 9, e88730.

7.0 ENDOTHELIN AND END-ORGAN INJURY

7.1

ENDOTHELIN AND DIABETIC COMPLICATIONS

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Endothelin-1 (ET-1) is a vasoconstrictor, proinflammatory and proliferative endothelial cell-derived peptide that is of significant importance in the regulation of vascular function. The production and function of ET-1 acting on its receptors ETA and ETB are altered during development of cardiovascular and metabolic diseases including diabetes mellitus. These alterations include increased production of ET-1 as well as changes in the expression of ETA and ETB receptors. The vascular homeostatsis in diabetes is altered to a pro-inflammatory, pro-oxidative and pro-thrombotic state favouring atherothrombosis with microvascular and macrovascular complications. These changes are both driven by and further promoting endothelial cell dysfunction. A central mechanism is the negative effect of glucose that results in accumulation of reactive oxygen species that stimulates ET-1 production and reduces nitric oxide production and increases its inactivation. The increased production of ET-1 and its receptors further stimulates oxidative stress creating a vicious cycle in diabetes. The increased vasoconstrictor response to ET-1 in diabetes is the result of increased expression of both ETA and ETB receptors on vascular smooth muscle cells and due to reduced endothelium-dependent and nitric oxide-mediated dilatation. Administration of both selective ETA and dual ETA/ETB receptor antagonists improve endothelium-dependent vasodilatation in experimental models of diabetes as well as in clinical studies of patients with type 2 diabetes mellitus. Interestingly, ET receptor antagonists not only improves vascular function but also increases insulin sensitivity and insulin resistant states via an effect that seems to be related to both insulin delivery and facilitation of glucose uptake. Collectively, available data suggest that ET-1 plays an important pathophysiological role in complications associated with diabetes and that ET receptor antagonists may provide beneficial effects as a therapeutic target for treatment of these complications.

8.0 ENDOTHELIN, ANGIOTENSIN AND VASCULAR FUNCTION

8.1

ET AND ANTI-ANGIOGENIC THERAPY

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Vascular endothelial growth factor (VEGF) secreted by tumor cells targets endothelial cells (ECs) to promote angiogenesis. The recognition that angiogenesis is critical to tumor growth has led to the development of treatments to inhibit VEGF-signaling, like anti-VEGF antibodies, small molecule VEGFR inhibitors (RTKIs) and soluble VEGFR to trap VEGF. The endogenous VEGF inhibitor soluble Fms-like tyrosine kinase (sFlt-1) is markedly increased in preeclampsia. Soon after their introduction inhibitors of the VEGF-signaling pathway appeared to be associated with hypertension and renal injury. In patients with renal cancer exposed to the RTKI sunitinib we observed that the rise in blood pressure (BP) was associated with increased circulating ET-1 levels and renin suppression. In subsequent rodent studies sunitinib administration was associated with a dose-dependent increase in BP, proteinuria and circulating ET-1 levels. In preeclamptic patients we found that plasma levels of ET-1 and sFlt-1 strongly correlated, indicating ET-axis activation, by both exogenous and endogenous VEGF inhibition. Proof that ET activation is involved in the rise in BP and proteinuria during anti-angiogenic treatment was obtained with the ET-receptor antagonist macitentan. Administration of macitentan with sunitinib could largely prevent the rise in BP and proteinuria in rats and normalize the rise in BP to pretreatment values in swine. The mechanism by which VEGF-inhibition results in activation of the ET-axis requires clarification. Studies in cultured ECs have provided contrasting findings, i.e. both increased and decreased ET-1 production in response to VEGF. VEGF-inhibition causes a decrease in NO production, which potentially contributes to the rise in ET-1 during anti-angiogenic treatment. Furthermore, since VEGF is essential for the maintenance of a healthy endothelium, EC activation, contributing to increased ET production, may occur during antiangiogenic treatment.

9.0 ENDOTHELIN AND FLUID ELECTROLYTE BALANCE

9.1

ENDOTHELIN ANTAGONISM AS A THERAPEUTIC STRATEGY IN KIDNEY DISEASE

Neeraj Dhaun[1]
[1]Univ. of Edinburgh.

The role of endothelin-1 (ET-1) in the physiology and pathophysiology of the kidney continues to be defined. It is well-established that ET-1 contributes to the development and progression diabetic nephropathy and phase III trials assessing the role of ET receptor antagonists in both reducing proteinuria and extending time to end-stage renal failure are on-going. Encouragingly, industry appears to have learnt its lessons. They now appear to accept that fluid retention is a feature of treatment with both selective and mixed ET receptor antagonists and formulated protocols to manage this. It will be particularly interesting to see if these trials show any evidence of regression of kidney disease and by extension potential regression of fibrosis, the Holy Grail for many chronic inflammatory conditions. Diabetic nephropathy has also been the focus for the use of combined endothelin converting enzyme and neutral endopeptidase inhibitors. This single trial showed that despite a fall in blood pressure remarkably no antiproteinuric effect was seen. Future trials of ET blocking strategies may well combine these agents with those blocking the renin-angiotensin system, vasopeptidase or, as seen here, neutral endopeptidase. The role of ET-1 in relation to inflammation and the kidney remains relatively unexplored. We have recently shown that macrophages (Mϕ) express *both* ET_A and ET_B receptors and display chemokinesis to ET-1. However, stimulation of Mϕ with exogenous ET-1 does not polarize Mϕ phenotype. Importantly, we show a novel clearance mechanism for ET-1 through ET_B receptor mediated dynamin-dependent endocytosis present in both murine and human Mϕ. Interstingly, systemic depletion of Mϕ results in an augmented pressor response to ET-1. In patients with kidney disease receiving Mϕ depleting immunotherapy blood pressure is higher and the ET system more activated than in those receiving non-depleting therapies. These data suggest that Mϕ and ET-1 may play an important role in BP control and potentially have a critical role as a therapeutic target in the hypertension associated with kidney disease. There remain a number of areas in kidney disease where ET-1 may play an important role and where current therapies are either limited or non-existent. These included ischemia-reperfusion injury – commonly seen and an important cause of acute kidney injury, sickle cell nephropathy, post-transplant glomerulopathy and scleroderma renal crisis.

12.0 NOVEL INTEGRATION

12.1

ET-1 AND NEUROVASCULAR COUPLING

Costantino Iadecola[1]
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The brain has no fuel reserves and requires a continuous and well-regulated delivery of oxygen and glucose through the blood supply to maintain its functional and structural integrity. Critical to the delivery of blood flow to the brain are key regulatory mechanisms controlling the cerebral circulation. Thus, brain activation leads to increases in cerebral blood flow (CBF) to match the increased energy demands

(functional hyperemia), whereas endothelial cells regulate the distribution of microvascular flow by releasing vasoactive agents (endothelium-dependent vasodilation). These control mechanisms are compromised by stroke risk factors, such as hypertension or obstructive sleep apnea, resulting in an increased susceptibility to brain dysfunction and ischemic brain injury. Endothelin-1 (ET-1) plays a key role in the cerebrovascular dysfunction associated with these conditions. In a mouse model of hypertension produced by chronic administration of low doses of angiotensin-2 (slow pressor hypertension), there is impairment of functional hyperemia and endothelium-dependent responses caused by NOX2-dependent vascular oxidative stress. The dysfunction is associated with increased ET1 expression in pial arterioles. Both the cerebrovascular dysfunction and the increase in radicals are counteracted by topical superfusion of ET type A receptor inhibitors on the cerebral cortex, implicating ET1 in its mechanisms. The cerebrovascular ET1 upregulation is mediated by vasopressin acting on V1a receptors. Similarly, in a mouse model of sleep apnea produced by chronic intermittent hypoxia (CIH), there is massive upregulation of ET1 in cerebral resistance vessels, which is associated with cerebrovascular dysfunction and is counteracted by ETA inhibitors. The mechanisms of ET1 upregulation are likely to involve activation of the hypoxia sensitive transcription factor HIF1alpha. The cerebrovascular dysfunction observed in these conditions can be reproduced by systemic infusions of ET1 in normal mice. In vitro studies indicate that ET1 suppresses endothelial production of nitric oxide by promoting the inhibitory phosphorylation of endothelial nitric oxide synthase. Thus, ET1 has emerged as an important mediator in the pathophysiology of the cerebral circulation. ETA receptors may represent a therapeutic target to counter the deleterious cerebrovascular effects of major stroke risk factors, such as hypertension and sleep apnea.

13.0 ENDOTHELIN THERAPEUTICS-WHERE ARE WE?

13.1
ENDOTHELIN THERAPEUTICS IN CANCER-WHERE ARE WE?

Anna Bagnato[1]
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Activation of autocrine and paracrine signalling by endothelin-1 (ET-1) binding to its receptors elicits pleiotropic effects on tumour cells and on the host microenvironment, providing a strong rationale for targeting ET1 receptors in cancer. This article describe the latest preclinical and clinical progress that has been made using antagonists of ET1 receptors, as well as personal perspectives on how to proceed with further clinical trial. The previous disappointments in clinical development of ET-1 therapeutics should not prevent us from exploring the potential of this class of drugs using carefully designed clinical trials. The preclinical data obtained with the dual ETAR and ETBR antagonist macitentan indicate that this class of drugs, that target not only cancer cells (which typically express ETAR) but also tumour-associated stromal elements, such as vascular, lymphatic and inflammatory cells and fibroblasts, which all express ETBR, could be a cancer therapeutic option. The field of ET-1 cancer therapeutics is poised for transformation in the next decade, facilitated by the new knowledge on the genomic landscape of the human microenvironment and tumor, by next-generation sequencing. The knowledge of pathobiology will also bring with it the noninvasively detection of biomarkers able to predict risk and diagnose diseases early and also to predict response to therapy in individual patients. The genomic information obtained with efforts of The Cancer Genome Atlas will fuel much of future cancer research. With these advances, there is the possibility that ET-1 therapeutics might be effective as therapeutics in a variety of tumors, either alone or coupled with new targeted and/or immunotherapy approaches.

13.2
ENDOTHELIN ANTAGONISTS IN DIABETIC NEPHROPATHY

Donald Kohan[1]
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Numerous pre-clinical studies have implicated endothelin-1 (ET-1) in the pathogenesis of diabetic chronic kidney disease (CKD). Renal ET-1 production is increased in diabetic kidneys. ET-1, likely via the endothelin A receptor (ETA), causes vasoconstriction, proteinuria, inflammation, cellular injury and fibrosis. ETA antagonism alone, and/or combined ETA/B blockade, reduces renal disease progression in experimental models of diabetic kidney disease. Based on this strong pre-clinical data, several clinical trials using ETA antagonists in diabetic CKD have been conducted. A large phase 3 trial (ASCEND) examined the effects of high doses of avosentan, a relatively ETA selective antagonist, on renal disease progression in diabetic nephropathy. Proteinuria was reduced after 3-6 months of treatment, however the study was prematurely halted due to increased cardiovascular morbidity and mortality associated with avosentan-induced fluid retention. Subsequent phase 2 trials found that ETA blockade, at concentrations that caused minimal fluid retention, reduced proteinuria even on top of maximally tolerated labeled doses of renin-angiotensin system inhibitors. Based on these promising results, the largest phase 3 trial (SONAR) ever conducted in diabetic kidney disease is now underway that examines the effect of atrasentan, an ETA antagonist, on renal disease progression. Reference: Kohan DE, Barton M. Endothelin and endothelin antagonists in chronic kidney disease. Kidney Int 86:896-904, 2014.

13.3
ENDOTHELIN ANTAGONISM, WHERE NEXT?

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Endothelin (ET) is not merely a vasoconstrictor, but a multifunctional peptide. Despite initial disappointment in several trials such as those in the field of congestive great failure, PAH became the first licensed indication for ET receptor antagonists in 2000. Some of the early studies, although adequately executed, demonstrated lack of efficacy for these agents. Others, through lack of rigorous trial design, showed an unacceptably high incidence of side effects. Unfortunately, there is a global trend to encourage too rapid a progression of basic scientific discoveries into clinically relevant strategies, a concept referred to as 'translational research'. We must question whether we simply move too fast from the bench to the bedside without the requisite knowledge or do we simply ignore some important available pre-clinical data? Preclinical studies suggest that chronic conditions such as proteinuric glomerular diseases, solid tumors, connective tissue diseases and vasculitides and the chronic microvascular damage associated with sickle cell anemia all represent attractive clinical targets for ET receptor antagonists (ETRA). Studies that have moved fastest into the clinical arena are those in diabetic nephropathy and focal and segmental glomerulosclerosis. However, treatment with ETRAs also effectively interferes with development of graft arteriosclerosis, fibrosis and glomerulosclerosis and, to date, there remain no clinical studies investigating the therapeutic potential of ETRAs in transplantation medicine. Appropriate selection of patients and diseases should ensure safety with an acceptable side effect profile. In addition to transplantation we will discuss other potential indications for ETRAs in this lecture. In addition to its vasoconstrictor and tissue-remodeling actions, the endothelin peptides should also be considered as cytokine-like. The pro-inflammatory role of the ET system is clear, although not well deciphered. Its involvement in the development and progression of autoimmune diseases deserves further attention. A strong body of evidence suggests that acute and severe conditions such as ischemia/reperfusion injury should be treated with anti-endothelin strategies, which might prevent their progression to chronic damage. This includes a variety of conditions such as acute kidney injury, post-transplant ischemia/reperfusion injury, sickle cell disease vaso-occlusive crises. For ET blockers to realize their potential further information needs to be obtained regarding the basic mechanisms of how they work which will inform the optimal time window for their dosing. Despite the vast volume of basic research on the ET system, the clinical trials have gone on to emphasise the need to return to the bench to further our understanding of the fundamental mechanisms. This is important for the development of maximally effective therapeutic strategies with minimal adverse effects.

13.4
REVIEW OF CLINICAL DEVELOPMENT OF SPARSENTAN, A DUAL-ACTING ANGIOTENSIN AND ENDOTHELIN RECEPTOR ANTAGONIST

Radko Komers, Meghan Kelly, Jennifer Hunt and Horacio Plotkin
Retrophin, Inc., Cambridge, MA, USA.

Endothelin 1 (ET) has been well established as an important player in renal physiology and pathophysiology. Similar to inhibitors of renin-angiotensin system (RASi), ET type A (ET_A) receptor blockers (ERA) have demonstrated a spectrum of beneficial effects in models of kidney diseases. Moreover, these agents could provide additive protective effects to RASi in proteinuric diseases, a hypothesis being currently tested in clinical trials. This presentation will provide an overview of Sparsentan, a first-in-class, orally-active, dual-acting angiotensin receptor Type 1 receptor blocker (ARB) and highly selective ERA, and the rationale for its clinical development in primary focal segmental glomerulosclerosis (FSGS). FSGS is a disorder of the podocyte and a common cause of non-diabetic nephrotic syndrome leading to end-stage renal disease in a large proportion of patients with this disorder. There are no approved therapies for FSGS in the US, and standard treatments often fail to reduce proteinuria. RASi are used in most patients for their non-immune antiproteinuric actions. In patients with FSGS at high risk of progression the treatment relies on immunosuppressive agents such as steroids and calcineurin inhibitors (CNI). Other agents that have been tried with marginal success include mycophenolate mofetil and rituximab. ET has been recently identified as an important player in the pathophysiology of podocyte disorders, including FSGS. Therefore, dual RASi and ET inhibition may combine non-specific protective actions in the kidney with targeting specific molecular pathogenic steps in the pathophysiology of FSGS. DUET trial has been designed to test this hypothesis. It is a randomized, active-control (Irbesartan), dose-escalation study with an initial 8-week fixed dose double-blind period followed by 144 weeks of open-label Sparsentan treatment. The primary efficacy analysis will

test whether at least one Sparsentan dose (200, 400, or 800 mg) is superior to Irbesartan (300 mg/day) in decreasing proteinuria, as measured by the change from baseline. As secondary objectives, the trial will evaluate a spectrum of additional parameters including the proportion of patients in each dose group that achieve pre-specified targets of Up/c reduction after 8 weeks; time to and durability of these effects; quality of life parameters; and detailed safety analysis including adverse actions of ERAs.

13.5

ENDOTHELIN RESEARCH AND DRUG DISCOVERY

Martine Clozel[1], Marc Iglarz[1], Martin Bolli[1], John Gatfield[1], and Patrick Hess[1]
[1]Actelion Pharmaceuticals, Ltd., Gewerbestrasse 16, Allschwil, CH-4123, Switzerland.

Dual endothelin receptor antagonists (ERA) have been registered for the treatment of pulmonary arterial hypertension. The last one to be approved, macitentan, was discovered by a tailored research aimed at improving efficacy and safety. The goal of increased efficacy was based on the study of the different roles between physiology and pathology of endothelial and smooth muscle ETB receptors, and on understanding of receptor binding kinetics of macitentan. The goal of increased safety was based on the discovery of the role of ETB receptors in the regulation of vascular permeability and vasopressin release, and on elucidation of the mechanism of aminotransferase increases due to bosentan. Macitentan binds very deeply into a subpocket of the ETA receptor and is an insurmountable antagonist. It also antagonizes the ETB receptors. In hypertensive rats macitentan had an additional effect on top of maximal effect of bosentan or ambrisentan. Macitentan had major effects on cardiac protection and improved endothelial function. Macitentan did not cause vascular permeability increase in rat vessels and did not increase bile salts. In patients with pulmonary arterial hypertension, macitentan reduced morbidity-mortality events by 45 % and showed a favorable safety and tolerability profile.

NOTES

*Indicates Invited Speaker

*Indicates Invited Speaker

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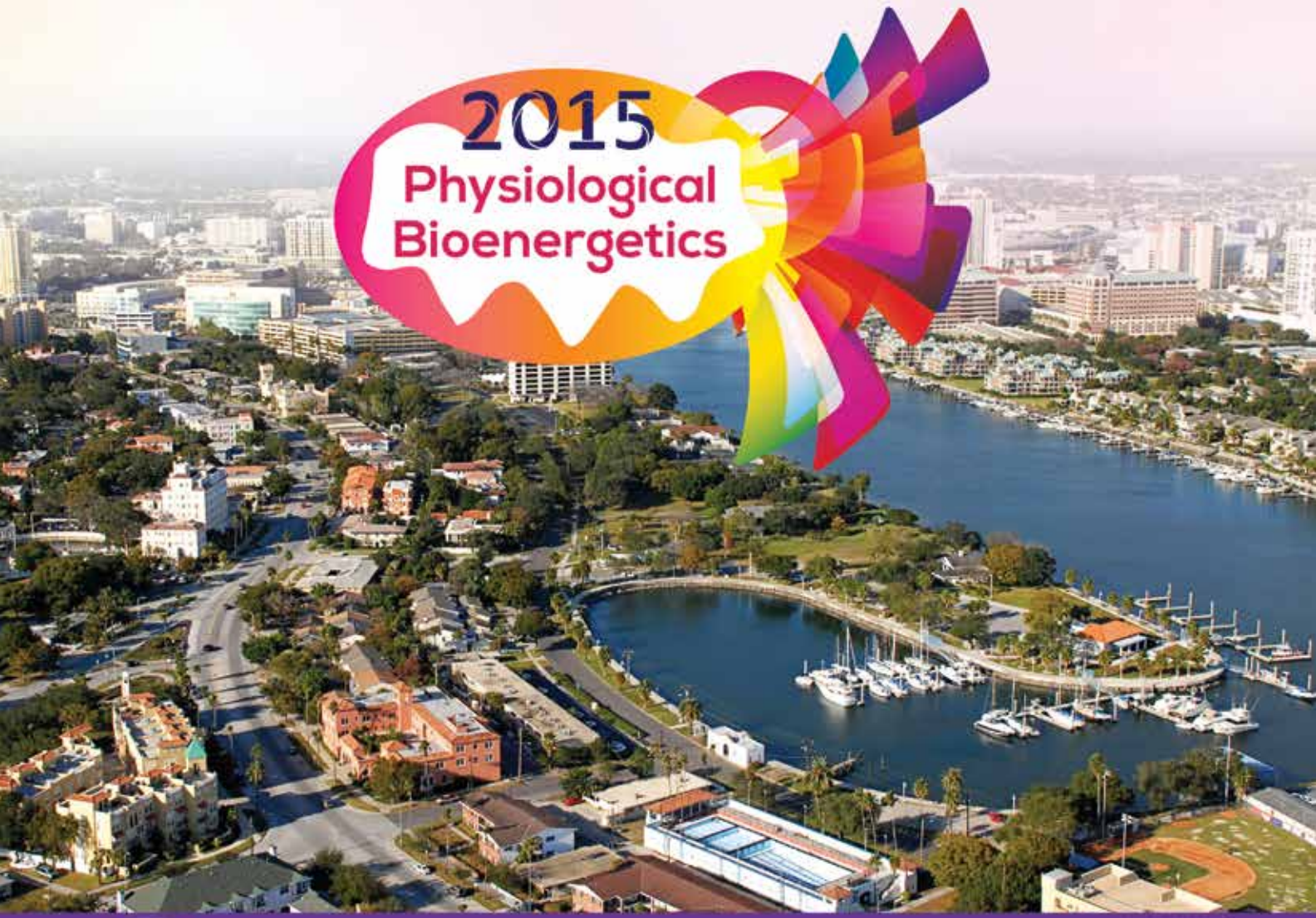
Physiological Bioenergetics: From Bench to Bedside

Westin Tampa Harbour Island • Tampa, FL • September 9-12, 2015

Conference Program & Abstracts



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2015 APS Conference:
Physiological Bioenergetics: From Bench to Bedside
September 9—12, 2015, Tampa, Florida, USA
Week-At-A-Glance

Wednesday, September 9	Thursday, September 10	Friday, September 11	Saturday, September 12
3:00 PM **Registration**	7:00 AM **Registration**	7:30 AM **Registration**	8:00 AM **Registration**
	8:00—9:00 AM **Symposia I** Energy School I **Brad Hill** **Jianhua Zhang**	8:00—9:00 AM **Symposia IV** Energy School II **Yan Burelle** **Afshan Malik**	9:00 AM—10:50 AM **Symposia VII** Mitochondrial Genetic and Metabolic Programs **David Lee** **Scott Ballinger** **Janine Santos** **Hannele Ruohola-Baker**
	9:00—11:30 AM **Symposia II** Mitochondria on the Move: Networking in Health and Disease **Yisang Yoon,** **Roberta Gottlieb,** **Gyorgy Hajnoczky**	9:00—11:30 AM **Symposia V** Mitochondrial Adaptation and Susceptibility to Stress **Paul Brookes** **Nika Danial**	10:50—11:00 AM **Closing Remarks**
	11:30 AM—12:30 PM **Lunch** 12:30—1:30 PM Career Symposia: How to Succeed: A Research Scientist and Entrepreneur in Bioenergetics **Brian Dranka**	12:00 Noon—1:00 PM **Lunch**	
	1:30—2:00 PM Plenary Lecture II **Martin Brand**	1:15—2:00 PM Plenary Lecture III **Orian Shirihai**	
5:00—5:10 PM **Welcome and Opening Remarks** 5:10—6:30 PM Plenary Lecture I **Doug Wallace** **John Lemasters**	2:30—5:30 PM **Symposia III** Translational Bioenergetics **Victor Darley-Usmar** **Sruti Shiva** **Russ Swerdlow** **Brian Dranka** **Anthony Molina**	2:20—4:30 PM **Symposia VI** It's Not Just the ATP! Signaling and Mitochondrial Function **Ben van Houten** **Shannon Bailey** **Andreas Beyer**	
6:30—8:30 PM **Welcome and Opening Reception**	5:30—7:30 PM **Poster Session Social**	5:00—7:00 PM **Poster Session Social** 7:00—9:30 PM **Banquet and Awards Ceremony**	

GENERAL INFORMATION

Location:

The 2015 APS Conference: Physiological Bioenergetics: From Bench to Bedside will be held September 9—12, 2015 at the Westin Tampa Harbour Island Hotel, 725 South Harbour Island Blvd., Tampa, FL 33602, USA, telephone (813) 229-5000, FAX: (813) 229-5022.

Onsite Registration Hours:

Wednesday, September 9……...3:00—8:00 PM
Thursday, September 10…….7:00 AM—6:00 PM
Friday, September 11……..7:30 AM—6:00 PM
Saturday, September 12……..8:00—10:30 AM

On-Site Registration Fees:

APS Member	$650
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Program Objective:

This meeting will serve as a cross disciplinary bridge, allowing the sharing of knowledge and the establishment of collaborations among investigators who may otherwise be confined within the discipline/pathology they study. Ultimately, the goals of this meeting are to advance the study of mitochondria, particularly in the realm of clinical studies and to catalyze collaboration/conversation across disciplines to understand the role of the mitochondrion in human health and disease.

Target Audience:

The goal of the "Physiological Bioenergetics-from Bench to Bedside" conference is to bring together experts studying varied facets of bioenergetics across disciplines and in the context of different pathologies to share their most recent findings and to discuss strategies to advance the field of "mitochondriology" into translational and clinical studies.

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Ballroom Foyer

6:30—8:30 PM

WEDNESDAY, SEPTEMBER 9, 2015

Plenary I

1.0 PLENARY I

Wednes., 5:00—6:30 PM, Harbour Island Ballroom.

Chair: **Scott Ballinger,** *Univ. of Alabama at Birmingham.*

5:10 PM **1.1** The Ongoing Evolution of the Mitochondrial Paradigm of Disease. **Douglas Wallace.** *Children's Hosp. of Philadelphia.*

5:50 PM **1.2** Variants of Mitophagy: Type 1, Type 2 and Micromitophagy (Type 3). **John Lemasters.** *Med. Univ. of South Carolina, Charleston.*

THURSDAY, SEPTEMBER 10, 2015

Symposia I

2.0 ENERGY SCHOOL I

Thurs., 8:00—9:00 AM, Harbour Island Ballroom.
Session partly sponsored by Seahorse Bioscience.

Chair: **Hannele Ruohola-Baker,** *Univ. of Washington.*

8:00 AM **2.1** Integrating Mitochondrial Activity Measurements with High Resolution Central Carbon Metabolomics Data. **Brad Hill.** *Univ. of Louisville.*

8:30 AM **2.2** How to Measure Autophagy and Mitophagy. **Jianhua Zhang.** *Univ. of Alabama at Birmingham.*

Symposia II

3.0 MITOCHONDRIA ON THE MOVE: NETWORKING IN HEALTH AND DISEASE

Thurs., 9:10—11:30 AM, Harbour Island Ballroom.

Chairs: **Brian Dranka,** *Seahorse Bioscience.*
Russell Swerdlow, *Univ. of Kansas Med. Ctr.*

9:10 AM **3.1** Targeting Mitochondrial Fission for Oxidative Pathology. **Yisang Yoon.** *Georgia Regents Univ.*

9:35 AM **3.2** Mitochondrial Autophagy and Biogenesis: The Cycle of Life. **Roberta Gottlieb.** *Cedars-Sinai Med. Ctr., Los Angeles.*

10:00 AM Break

10:30 AM **3.3** Mitochondrial Motility and Fusion Dynamics and Calcium. **Gyorgy Hajnoczky.** *Thomas Jefferson Univ., Philadelphia.*

10:55 AM **3.4** Mitochondrial Motility Response to Nutrient Environment in the Pancreatic Beta-Cell: Role of Milton 1 Nutrient-sensing Through OGlcNAc Modification. **Kyle Trudeau.** *Boston Univ. Sch. of Med.* **(12.15).**

11:10 AM **3.5** Knockdown of Voltage-dependant Anion Channels 1 and 2 Inhibits Mitochondrial Fission by Decreasing Binding of Dynamin-related Protein 1 to Mitochondria. **Eduardo Maldonado.** *Med. Univ. of South Carolina, Charleston.* **(12.18).**

11:25 AM **3.6** The Liver Molecular Circadian Clock in Chronic Alcohol-induced Mitochondrial Dysfunction. **Jennifer Valcin.** *Univ. of Alabama at Birmingham.* **(7.3).**

Career Session

4.0 CAREER SESSION

Thurs., 12:30—1:30 PM, Harbour Island Ballroom.

Chairs: **Brian Dranka,** *Seahorse Bioscience.*

12:30 PM **4.1** How to Suceed: A Research Scientist and Entrepreneur in Bioenergetics. **Brian Dranka,** *Seahorse Bioenergetics.*

Plenary II

5.0 PLENARY II

Thurs., 1:30—2:00 PM, Harbour Island Ballroom.

Chair: **John Lemasters,** *Med. Univ. of South Carolina, Charleston.*

1:30 PM **5.1** Sites of Production of Mitochondrial ROS: Mechanism and Physiological Function. **Martin Brand.** *Buck Inst. on Aging.*

2:00 PM Break

Symposia III

6.0 TRANSLATIONAL BIOENERGETICS

Thurs., 2:30—5:30 PM, Harbour Island Ballroom.

Chairs: **Gyorgy Hajnoczky,** *Thomas Jefferson Univ., Philadelphia.*
Martin Brand, *Buck Inst. on Aging.*

2:30 PM **6.1** Measuring Bioenergetic Health in Human Populations. **Victor Darley-Usmar.** *Univ. of Alabama at Birmingham.*

2:55 PM **6.2** Platelet Mitochondria: From Biomarker to Biological Mechanism in Sickle Cell Patients. **Sruti Shiva.** *Univ. of Pittsburgh.*

3:20 PM **6.3** Mitochondrial Biomarkers for Neurodegenerative Diseases. **Russell Swerdlow.** *Univ. of Kanas Med. Ctr.*

3:45 PM **6.4** Translational Bioenergetics in Cancer. **Brian Dranka.** *Seahorse Bioscience.*

4:10 PM **6.5** Using Machine Learning to Advance Blood Based Bioenergetic Profiling: A Focus on Geriatric Health. **Anthony Molina.** *Wake Forest Baptist Med. Ctr.*

4:35 PM **6.6** Mitochondrial Respiratory Capacity and Coupling Control Decline with Age in Human Skeletal Muscle. **Craig Porter.** *Univ. of Texas Med. Branch, Galveston.* **(12.22).**

4:50 PM **6.7** High Intensity Training Increases Mitochondrial Respiratory Capacity in Old Males But Not Females. **Steen Larsen.** *Univ. of Copenhagen, Denmark.* **(7.20).**

5:05 PM **6.8** Mitochondria DNA is Damaged in Military Veterans with Fatiguing Conditions. **Yang Chen.** *New Jersey Med. Sch., Rutgers Univ.* **(7.25).**

Photography is *not* permitted during the scientific sessions or in the poster session room

Poster Session

7.0 POSTER SESSION I

Thurs., 5:30—7:30 PM, Terrace.

Poster Board

1 **7.1** Transgenic Redox-indicator Mice Expressing Cytosolic and Mitochondrial roGFP1. **K. W. Wagener, B. K. Kolbrink, K. C. Can, B. K. Kempkes, and M. M. Müller.** *Univ. Göttingen, Germany.*

2 **7.2** Effects of Skeletal Muscle Aging on Mitochondrial Morphology and Dynamics. **J. L. Leduc-Gaudet, M. P. Picard, F. St-Jean Pelletier, N. S. Sgarioto, M. A. Auger, J. V. Vallée, R. R. Robitaille, D. H. St-Pierre, and G. G. Gouspillou.** *Univ. de Québec à Montréal, Univ. de Montréal, Canada, Children's Hosp. of Philadelphia, Univ. of Pittsburgh, Philadelphia, and Univ. of Gériatrie à Montréal, Canada.*

3 **7.3** The Liver Molecular Circadian Clock in Chronic Alcohol-induced Mitochondrial Dysfunction. **J. V. Valcin, U. U. Udoh, T. S. Swain, C. O. Oliva, and S. B. Bailey.** *Univ. of Alabama at Birmingham.*

4 **7.4** Rett Syndrome Provokes a Cytosolic and Mitochondrial Redox Imbalance in Neonatal Neurons. **K. C. Can, J. T. Tolö, C. M. Menzfeld, S. K. Kügler, and M. M. Müller.** *Univ. of Göttingen, Germany.*

5 **7.5** Bioenergetic Influence on APP Production and Processing. **H. W. Wilkins, S. C. Carl, I. W. Weidling, S. R. Ramanujan, S. W. Weber, and R. S. Swerdlow.** *Univ. of Kansas Med. Ctr.*

6 **7.6** Modulation of Mitochondrial Adenine Nucleotide Translocase (ANT) Regulation with Aging. **P. D. Diolez, I. B. Bourdel-Marchasson, P. P. Pasdois, D. D. Detaille, R. R. Rouland, G. C. Calmettes, and G. G. Gouspillou.** *Univ. de Bordeaux, Pessac, France, Univ. de Bordeaux, France, Univ. of California, Los Angeles, and Univ. du Québec à Montréal, Canada.*

7 **7.7** Mitochondrial Reserve Capacity is Driven by Glutamine in Lung Cancer Cells with Mesenchymal Phenotype. **Y. S. Si, D. B. Ulanet, J. B. Hurov, M. D. Dorsch, and K. M. Marks.** *Agios Pharma., Cambridge, MA.*

8 **7.8** L-OPA1 Functions Independently of S-OPA1 by Forming Separate Structural Entities. **H. L. Lee, and Y. Y. Yoon.** *Georgia Regents Univ.*

9 **7.9** Withdrawn.

10 **7.10** Bioenergetic Properties of Human Renal Tubular and Mesangial Cells in Normal and Diabetic Conditions. **A. C. Czajka, and A. M. Malik.** *King's Coll. London, UK.*

11 **7.11** Regulation of Bioenergetics and Angiogenic Response in Vasa Vasorum Endothelial Cells by Extracellular Purines and Hypoxia. **M. L. Lapel, P. P. Paucek, T. L. Lyubchenko, P. W. Weston, K. S. Stenmark, and E. G. Gerasimovskaya.** *Univ. of Colorado, Denver, and Univ. of Colorado, Boulder.*

Poster Board

12 **7.12** Increased Autophagy is Required for Mechanical Ventilation-induced Diaphragm Mitochondrial Dysfunction. **A. J. Smuder, K. J. Sollanek, W. B. Nelson, K. M. Min, E. E. Talbert, and S. K. Powers.** *Univ. of Florida, Gainesville.*

13 **7.13** Mitochondrial Respiratory Capacity is Decreased in Rat Cardiomyocytes Following Exposure to Maternal Diabetes and High Fat Diet. **K. S. Mdaki, T. D. Larsen, and M. L. Baack.** *Sanford Res., Sioux Falls, SD, and Univ. of South Dakota.*

14 **7.14** ATP Production and Oxygen Consumption in Isolated Mitochondria from H9c2 Cells. **P. A. Albrycht.** *Warsaw Univ. of Life Sci., Poland.*

15 **7.15** Withdrawn.

16 **7.16** Mitochondrial Dysfunction in Heart of Coronary Artery Disease: Correlation with Telomerase Activity. **K. A. Ait-Aissa, J. K. Kim, G. M. Morgan, J. H. Santos, A. K. Camara, D. D. Gutterman, D. H. Betts, T. D. Donato, and A. M. Beyer.** *Med. Coll. of Wisconsin, Milwaukee, Western Univ., London, ON, Canada, Univ.of Utah., and NIHES.*

17 **7.17** High-thoughput Screening Reveals the Mitochondrial Complex I Inhibitor Nornicotine is Cardioprotective in Ischemia-reperfusion Injury when Delivered at Reperfusion. **J. Z. Zhang, M. K. Karcz, S. N. Nadtochiy, and P. B. Brookes.** *Univ. of Rochester Med. Ctr.*

18 **7.18** Mitochondrial Chaperone GRP75 Haploinsufficiency Promotes Liver Tumorigenesis by Adapted Metabolism. **Y. W. Wang, X. J. Jin, N. M. Mivechi, and D. M. Moskophidis.** *Georgia Regents Univ.*

19 **7.19** Mitochondrial Energy Deficiency Leads to Hyperproliferation of Skeletal Muscle Mitochondria and Enhanced Insulin Sensitivity. **R. M. Morrow, M. P. Picard, O. D. Derbeneva, J. L. Leipzig, G. G. Gouspillou, R. H. Hepple, and D. W. Wallace.** *Children's Hosp. of Philadelphia, Univ. du Quebec a Montreal, Canada, and McGill Univ., Montreal, Canada.*

20 **7.20** High Intensity Training Increases Mitochondrial Respiratory Capacity in Old Males but not Females. **S. L. Larsen, T. D. Dohlmann, D. S. Søgaaard, F. D. Dela, and J. W. Helge.** *Univ. of Copenhagen, Denmark.*

21 **7.21** Aged Muscle Exhibits Blunted Cardiolipin and Ceramide Remodeling During Hindlimb Unloading Induced Atrophy and a Lack of Muscle Hypertrophy Following Reloading. **X. Z. Zhang, T. L. Leone, R. V. Vega, B. G. Goodpaster, D. K. Kelly, X. H. Han, and P. C. Coen.** *Florida Hosp., Orlando, Sanford-Burnham Med. Res. Inst., Orlando, FL.*

22 **7.22** Mitochondrial DNA Changes and Dysfunction in Diabetic Nephropathy. **S. A. Ajaz, A. C. Czajka, L. G. Gnudi, and A. M. Malik.** *Kings Coll. London, UK.*

Poster Board
23 **7.23** Combined AMPK and PPARδ Agonism Improves Exercise Performance in Trained Mice. **M. C. Manio, K. I. Inoue, M. F. Fujitani, S. M. Matsumura, and T. F. Fushiki.** *Kyoto Univ., Japan.*

24 **7.24** Lipid Droplets Interact with an Exclusive Sub-population of Mitochondria in Brown Adipocyte. **K. M. Mahdaviani, I. B. Benadore, G. T. Twig, J. W. Wikstrom, M. L. Liesa, D. C. Chess, K. T. Trudeau, N. M. Miller, M. F. de Oliveira, and O. S. Shirihai.** *Boston Univ., Chaim Sheba Med. Ctr., Tel-Hashomer, Israel, Stockhom Univ., Sweden, and Univ. Federal do Rio de Janei-ro, Brazil.*

25 **7.25** Mitochondria DNA is Damaged in Military Veterans with Fatiguing Conditions. **Y. C. Chen, X. J. Jiao, H. H. Hill, J. K. Klein, D. N. Ndirangu, and M. F. Falvo.** *New Jersey Med. Sch. Rutgers Univ., and VA New Jersey Hlth. Care System.*

26 **7.26** Statin Myalgic Patients have Impaired Mitochondrial Respiratory Function in Skeletal Muscle. **T. D. Dohlmann, J. W. Helge, F. D. Dela, and S. L. Larsen.** *Inst. of Bio-medical Sci, København, Denmark.*

FRIDAY, SEPTEMBER 11, 2015

Symposia IV
8.0 ENERGY SCHOOL II
Fri., 8:00—9:00 AM, Harbour Island Ballroom.

Chair: **Brian Dranka,** *Seahorse Bioscience.*

8:00 AM **8.1** The Lactic Acidosis Consortium: A Multidisciplinary Research Effort to Translate Gene Discovery into Better Management and Treatment for Patients with Mitochondrial Disorders. **Yan Burelle.** *Univ. of Montreal, Canada.*

8:30 AM **8.2** Mitochondrial DNA Content: Accurate Measurement and Evaluation as an Early Biomarker of Mitochondrial Dysfunction. **Afshan Malik.** *King's Coll., London, UK.*

Symposia V
9.0 MITOCHONDRIAL ADAPTATION AND SUSCEPTIBILITY TO STRESS
Fri., 9:10—11:30 AM, Harbour Island Ballroom.

Chairs: **Sruti Shiva,** *Univ. of Pittsburgh.*
Andreas Beyer, *Med. Coll. of Wisconsin.*

9:10 AM **9.1** Withdrawn.

9:35 AM **9.2** A Unifying Hypothesis for the Mitochondrial Contribution to Ischemia-reperfusion. **Paul Brookes.** *Univ. of Rochester.*

10:00 AM Break

10:30 AM **9.3** Mitochondrial Fuel Substrate Switching and the Excitable Brain. **Nika Danial.** *Dana Farber Cancer Inst., Boston, MA.*

10:55 AM **9.4** Regulation of Bioenergetics and Angiogenic Response in Vasa Vasorum Endothelial Cells by Extracellular Purines and Hypoxia. **Martin Lapel.** *Univ. of Colorado, Denver.* **(7.11).**

11:10 AM **9.5** Increased Autophagy is Required for Mechanical Ventilation-induced Diaphragm Mitochondrial Dysfunction. **Ashley Smuder.** *Univ. of Florida, Gainesville.* **(7.12).**

11:25 AM **9.6** Screening Ascites-derived Ovarian Cancer Cells for Histological Subtype-specific Bioenergetic Signatures and Mitochondrial Dysfunction. **Nadine Hempel,** *Penn State Coll. of Med., Hershey.* **(12.20)**.

Don't forget to visit the exhibitors during the conference

Plenary III
10.0 PLENARY III
Fri., 1:15—2:00 PM, Harbour Island Ballroom.

Chair: **Victor Darley-Usmar,** *Univ. of Alabama at Birmingham.*

1:15 PM **10.1** The Sugar Disconnection in Diabetic Mitochondrial Networks. **Orian Shirihai.** *Boston Univ.*

1:50 PM Break

Symposia VI
11.0 IT'S NOT JUST THE ATP! SIGNALING AND MITOCHONDRIAL FUNCTION
Fri., 2:20—4:30 PM, Harbour Island Ballroom.

Chairs: **Janine Santos,** *Natl. Inst. of Environmental Hlth. Sci., Res. Triangle Park, NC.*
Brad Hill, *Univ. of Louisville.*

2:20 PM **11.1** Mitochondria Matter: Targeting Mitochondrial Function in Tumor Cells. **Ben van Houten.** *Univ. of Pittsburgh.*

2:45 PM **11.2** Tick, Tock: The Biological Clock Controls the Powerhouse. **Shannon Bailey.** *Univ. of Alabama at Birmingham.*

3:10 PM **11.3** Mitochondrial Telomerase and Vasodilation. **Andreas Beyer.** *Med. Coll. of Wisconsin.*

3:35 PM **11.4** Evidence for Involvement of Mitochondrial Matrix ROS and Hypoxia-inducible Factor-1 in the Growth Inhibitory Effect of Resveratrol. **Joao Fonseca.** *Brock Univ., St. Catherines, ON, Canada.* **(12.14).**

3:50 PM **11.5** Mitochondrial Energy Deficiency Leads to Hyperproliferation of Skeletal Muscle Mitochondria and Enhanced Insulin Sensitivity. **Ryan Morrow.** *Children's Hosp. of Philadelphia.***(7.19).**

4:05 PM **11.6** Withdrawn.

Poster Session
12.0 POSTER SESSION II
Fri., 5:00—7:00 PM, Terrace.

Poster Board
27 **12.1** Assessment of Peripheral Mitochondrial DNA Damage and Dysfunction as a Biomarker of Parkinson's Disease. **C. C. Corey, N. J. Jensen, E. H. Howlett, A. W. Weinstein, K. E. Erickson, J.**

Poster Board

G. Greenamyre, S. J. Jain, S. S. Shiva, and L. S. Sanders. *Univ. of Pittsburgh.*

28 **12.2** The DRP-1 Inhibitor Mdivi-1 Prevents Compensatory Mitochondrial H202-mediated Vasodilation Induced by Ceramide Treatment in Human Adipose Arterioles. **M. D. Durand, J. F. Freed, J. H. Hockenberry, and D. G. Gutterman.** *Med. Coll. of Wisconsin, Milwaukee.*

29 **12.3** Mitochondrial Oxygen Consumption is Reduced in Cerebral Arteries by Distant Ischemia. **I. R. Rutkai, S. D. Dutta, K. W. Walter, P. K. Katakam, and D. B. Busija.** *Tulane Univ.*

30 **12.4** Role of O-GlcNAcylation in Regulating Mitophagy and Mitochondrial Function in Cardiomyocytes. **J. W. Wright, P. K. Kramer, V. D. Darley-Usmar, and J. C. Chatham.** *Univ. of Alabama at Birmingham.*

31 **12.5** Impaired Cardio-skeletal Muscle Energetics in Children with Barth Syndrome: A 31P MRS Study. **W. C. Cade, K. B. Bohnert, D. R. Reeds, L. P. Peterson, R. T. Tinius, A. B. Bittel, D. B. Bittel, L. de las Fuentes, B. B. Byrne, and A. B. Bashir.** *Washington Univ. Sch. of Med.,St. Louis, MO, and Univ. of Florida, Gainesville.*

32 **12.6** Metabolic and Bioenergetic Characterization of a Non-ischemic Mouse Model of Heart Failure. **A. G. Gupte, A. Z. Zhang, S. L. Li, A. C. Cordero-Reyes, K. Y. Youker, G. T. Torre-Amione, and D. H. Hamilton.** *Houston Methodist Res. Inst., and Tecnologico de Monterrey, Mexico.*

33 **12.7** Mitochondrial Functions in the Regulation of Effector Macrophage in Coronary Artery Disease. **R. N. Nazarewicz, T. S. Shirai, D. H. Harrison, and C. W. Weyand.** *Vanderbilt Univ., and Stanford Univ.*

34 **12.8** Mitochondrial Permeability Transition Drives ROS Generation Associated with Degradation of Electron Transfer Chain Supercomplexes in Heart Ischemia-reperfusion. **S. J. Jang, and S. J. Javadov.** *Univ. of Puerto Rico Sch. of Med.*

35 **12.9** Mitochondrial Respiration and Calcium Activation are Maintained in the Presence of Heart Failure Levels of Extramitochondrial Sodium. **S. K. Kuzmiak-Glancy, B. G. Glancy, and M. K. Kay.** *George Washington Univ., and NHLBI, NIH.*

36 **12.10** An Electrically Conductive Mitochondrial Reticulum in Skeletal Muscle. **B. G. Glancy, L. M. Hartnell, D. M. Malide, Z. Y. Yu, C. A. Combs, P. S. Connelly, S. S. Subramaniam, and R. S. Balaban.** *NHLBI, NIH.*

37 **12.11** Diet-induced Ketosis Protects Against Focal Cerebral Ischemia in Mouse. **M. P. Pucho-wicz, Y. J. Jin, T. C. Caldwell, Y. L. Luo, K. X. Xu, and J. L. LaManna.** *Case Western Res. Univ.*

38 **12.12** Role of Mitochondrial Structure, Function and Redox Signaling in Megakaryopoiesis. **T. C. Cole, G. B. Bullock, and S. S. Shiva.** *Univ. of Pittsburgh.*

Poster Board

39 **12.13** Effector T Cells Upregulate Mitochondrial Metabolism During Graft-Versus-Host Disease. **P. C. Chiaranunt, J. G. Grekin, V. T. Tkachev, and C. B. Byersdorfer.** *Univ. of Pittsburgh, Univ. of Michigan, and Seattle Children's Hosp.*

40 **12.14** Evidence for Involvement of Mitochondrial Matrix ROS and Hypoxia-inducible Factor-1 in the Growth Inhibitory Effect of Resveratrol. **J. F. Fonseca, and J. S. Stuart.** *Brock Univ., St. Catherines, ON, Canada.*

41 **12.15** Mitochondrial Motility Response to Nutrient Environment in the Pancreatic Beta-Cell: Role of Milton 1 Nutrient-sensing Through OGlcNAc Modification. **K. T. Trudeau, G. P. Pekkurnaz, S. S. Sereda, T. S. Schwarz, and O. S. Shirihai.** *Boston Univ. Sch. of Med., and Harvard Med. Sch.*

42 **12.16** Mitochondrial Fragmentation in Response to Glucolipotoxicity Represents a Compensatory Adaptation to Maintain Beta-cell Function. **K. T. Trudeau, S. S. Sereda, N. M. Miller, P. M. MacDonald, and O. S. Shirihai.** *Boston Univ. Sch. of Med., and Univ. of Alberta, AB, Canada.*

43 **12.17** Molecular Mechanisms Behind the Accumulation of Lipids that Occur After Skeletal Muscle Injury. **J. G. Gumucio, and C. M. Mendias.** *Univ. of Michigan.*

44 **12.18** Knockdown of Voltage-dependant Anion Channels 1 and 2 Inhibits Mitochondrial Fission by Decreasing Binding of Dynamin-related Protein 1 to Mitochondria. **E. M. Maldonado, D. D. DeHart, M. Beck Gooz, H. R. Rodebaugh, and J. L. Lemasters.** *Med. Univ. of South Carolina, and Inst. of Theoretical & Experimental Biophysics, Puschino, Russia.*

45 **12.19** Effects of Low Level Laser Therapy on Tenocytes in High Glucose Environment. **Y. C. Chen, C. C. Chen, Y. W. Wu, C. L. Lee, and M. H. Huang.** *Kaohsiung Municipal Ta-Tung Hosp., Taiwan, Kaohsiung Med. Univ. Hosp., Taiwan, Meiho Univ., Pingtung, Taiwan, Kaohsiung Municipal Hsiao-Kang, Taiwan,*

46 **12.20** Screening Ascites-derived Ovarian Cancer Cells for Histological Subtype-specific Bioenergetic Signatures and Mitochondrial Dysfunction. **U. D. Dier, P. T. Timmins, and N. H. Hempel.** *SUNY Poly. Inst., Albany, NY, Albany Med. Coll., and Penn State Hershey Coll. of Med.*

47 **12.21** Bioenergetic Reprogramming in Monocytes in Chronic Kidney Disease. **B. C. Chacko, G. A. Benavides, T. M. Mitchell, D. V. Rizk, and V. D. Darley-Usmar.** *Univ. of Alabama at Birmingham.*

48 **12.22** Mitochondrial Respiratory Capacity and Coupling Control Decline with Age in Human Skeletal Muscle. **C. P. Porter, N. H. Hurren, M. C. Cotter, N. B. Bhattarai, P. R. Reidy, E. D. Dillon, W. D. Durham, D. T. Tuvdendorj, M. S. Sheffield-Moore, E. V. Volpi, L. S. Sidossis, B. R. Rasmussen, and E. B. Børsheim.** *Univ. of Texas Med. Branch, Galveston, Univ. of Arkansas*

Poster Board

for Med. Sci., Little Rock, and Arkansas Children's Hosp. Res. Inst., Little Rock.

49 **12.23** Interference with Microchondrial Bioenergetics by TPP-IOA, A Mitochondria-targeted Anti-apoptotic Inhibitor of Cytochrome c Peroxidase Activity. **L. M. Maddalena, J. A. Atkinson, and J. S. Stuart.** *Brock Univ., St. Catherines, ON, Canada.*

50 **12.24** Chronic Alcohol Exposure Increases Susceptibility to Oxidate Stress in Hepatocytes. **G. A. Benavides, B. K. Chacko, S. M. Bailey, and V. Darley-Usmar.** *Univ. of Alabama at Birmingham.*

51 **12.25** Increase Mitochondrial Uncoupling in Stored Platelets. **H. S. Sawada, S. R. Ravi, M. J. Johnson, B. C. Chacko, P. K. Kramer, V. Darley-Usmar.** *Univ. of Alabama Birmingham.*

52 **12.26** Crosstalk Between Mitochondrial Acetyl-COA Metabolism, Cytoskeleton Modifcations and Autophagy. **M. S. Stoner, and I. S. Scott.** *Univ. of Pittsburgh.*

53 **12.27** Study on the Effects of Alcohol and Cannabinol Treatment on Hypothalamic Pituitary Gonadal Axis in Male Wistar Rats. **C. A. Akintayo, S. K. Karga, and M. A. Ayodele.** *Afe Babalola Univ., Ekiti State, Nigeria, and Bingham Univ., Jos Plateau State. Nigeria.*

54 **12.28** Regulation of Cardiac Autophagy by Adiponectin Under Hypoxic/Ischemic Stress. **J. W. S. Jahng, Y. K. Chan, H. K. Sung, H. H. Cho, and G. Sweeney.** *York Univ., Toronto, Canada.*

55 **12.29** Lipocalin-2 Regulates Cardiomyocyte Autophagy to Control Apoptosis and Insulin Sensitivity. **H. K. Sung, Y. K. Chan, M. Han, J. W. S. Jahng, and G. Sweeney.** *York Univ., Toronto, Canada.*

Thank you! Thank you! Thank you! for the generous support from

National Institute of General Medical Sciences, NIH

Seahorse Bioscience

University of Pittsburgh, Ctr. for Metabolism and Mitochondrial Medicine

SATURDAY, SEPTEMBER 12, 2015

Symposia VII

13.0 MITOCHONDRIAL GENETIC AND METABOLIC PROGRAMS

Sat., 9:00—10:50 AM Harbour Island Ballroom.

Chairs: **Martin Brand,** *Buck Inst. for Res. on Aging.* **Shannon Bailey,** *Univ. of Alabama at Birmingham.*

9:05 AM **13.1** Novel Signaling Peptides from the Mitochondrial Genome. **Changhan David Lee.** *Univ. of Southern California, Los Angeles.*

9:30 AM **13.2** Mitochondrial Nuclear Genetic Cross Talk and Disease: "Mito-Mendelian" Genetics. **Scott Ballinger.** *Univ. of Alabama at Birmingham.*

9:55 AM **13.3** The Crosstalk Between Mitochondrial Function, the Epigenome and Gene Expression. **Janine Santos.** *Natl. Inst. of Environmental Hlth. Sci., NIH.*

10:20 AM **13.4** Bioenergetics, Stem Cells and Hypoxia. **Hannele Ruohola-Baker.** *Univ. of Washington.*

Closing Remarks

14.0 CLOSING REMARKS

Sat., 10:50—11:00 AM Harbour Island Ballroom.

Chairs: **Victor Darley-Usmar,** *Univ. of Alabama at Birmingham.* **Sruti Shiva,** *Univ. of Pittsburgh.*

10:50 AM **14.1** Closing Remarks. **Victor Darley-Usmar.** *Univ. of Alabama at Birmingham* and **Sruti Shiva.** *Univ. of Pittsburgh.*

NOTES

2015 APS Conference
Physiological Bioenergetics: From Bench to Bedside

Abstracts of Invited and Contributed Presentations

1.0 PLENARY I

1.2
VARIANTS OF MITOPHAGY: TYPE 1, TYPE 2 AND MICROMITOPHAGY (TYPE 3)

John Lemasters[1,2,3]
[1]Drug Discovery & Biomedical Sci., Med. Univ. of South Carolina, DD504 Drug Discovery Bldg., 70 President St., MSC 140, Charleston, SC, 29425, [2]Biochemistry & Molecular Biology, Med. Univ. of South Carolina, DD504 Drug Discovery Bldg., 70 President St., MSC 140, Charleston, SC, 29425, [3]Inst. of Theoretical & Experimental Biophysics, Russian Academy of Sci., Pushchino, Russian Fed.

Mitochondrial autophagy, or mitophagy, removes damaged, effete and superfluous mitochondria and appears to have several distinct variants. During nutrient deprivation, preautophagic structures (PAS) grow into cup-shaped phagophores, or isolation membranes, that surround and sequester individual mitochondria into mitophagosomes, a process requiring phosphatidylinositol-3-kinase (PI3K) and frequently occurring in coordination with mitochondrial fission. After sequestration in such Type 1 mitophagy, the outer compartment of mitophagosomes acidifies, followed only then by mitochondrial depolarization and ultimately hydrolytic digestion after fusion with lysosomes. Another variant of mitophagy occurs after photodamage to single mitochondria. Here, mitochondrial depolarization initiates mitophagy. Mitophagophores, however, seem to form by a different mechanism, namely by decoration of mitochondrial surfaces with LC3-containing structures. After coalescence of these presumably membranous structures, vesicular acidification and fusion with lysosomes occurs. By contrast to Type 1 mitophagy, this Type 2 mitophagy is not blocked by PI3K inhibition and is not associated with phagophore formation or mitochondrial fission. Formation of mitochondria-derived vesicles (MDV) enriched in oxidized mitochondrial proteins that bud off and transit into multivesicular bodies represents a third form of mitophagy. Internalization of MDV by invagination of the surfaces of multivesicular bodies followed by vesicle scission into the lumen is microautophagy, or more specifically micromitophagy (Type 3 mitophagy). Future studies are needed to characterize the molecular and biochemical similarities and differences between Types 1, 2 and 3 mitophagy.

2.0 ENERGY SCHOOL I

2.1
INTEGRATING MITOCHONDRIAL ACTIVITY MEASUREMENTS WITH HIGH RESOLUTION CENTRAL CARBON METABOLOMICS DATA

Bradford Hill[1]
[1]Medicine/Cardiology, Univ. of Louisville, 580 S. Preston St., Rm. 404A, Louisville, KY, 40202.

Respirometry has been a cornerstone for understanding mitochondrial (dys)function in health and disease. In recent years, the use of high-throughput respirometry has increased collective knowledge of the role of intermediary metabolism in numerous biological processes. In addition, measurements of glycolytic activity provide essential information on glucose utilization in cells. Such information, while useful, is not sufficient to understand how other metabolic pathways—such as ancillary glucose or anaplerotic/cataplerotic metabolic pathways—are affected by pathologies or interventions. Integrating respirometry measurements with stable isotope-resolved metabolomics (SIRM) confers considerably more information regarding metabolism in general and allows for analysis of intracellular carbon flux in metabolic networks. The purpose of this talk is to present and discuss work flow plans and key data sets that integrate respirometry measurements with SIRM. Integration of these two techniques can divulge novel understanding of the metabolic underpinnings of cell growth, proliferation, adaptations to stress, and pathology.

2.2
HOW TO MEASURE AUTOPHAGY AND MITOPHAGY

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Autophagy and mitophagy are important cellular processes that are responsible for clearance of damaged biomolecules and organelles. These pathways are important for preserving organelle function and maintaining redox signaling. More than 30 proteins are involved in a highly regulated and multi-step mechanism. Perturbation of autophagy and mitophagy has been shown to contribute to many disease pathogenic mechanisms and therefore measurement of autophagy and mitophagy in different cell and tissue contexts and in response to physiological and pathological signals is essential to determine the roles of autophagy and mitophagy play in health and diseases. Biochemical, cell biological, histological and molecular methods are used for these measurements. These include using biochemical and microscopic methods to measure a lipid modified cytosolic protein LC3II to assess the amount of autophagosomes in a given cell, association of LC3II with lysosomes and its entrance into the lysosomal compartment, and degradation of long lived proteins. This workshop will provide an overview of these methods and discuss their usage and interpretations. (NIHR01-NS064090) Zhang J (2013) Autophagy and mitophagy in cellular damage control. Redox Biology 1:19-23; Zhang J (2015) Teaching the basics of autophagy and mitophagy to redox biologists-mechanisms and experimental approaches. Redox Biology 4:242-259.

3.0 MITOCHONDRIA ON THE MOVE: NETWORKING IN HEALTH AND DISEASE

3.1
TARGETING MITOCHONDRIAL FISSION FOR OXIDATIVE PATHOLOGY

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Mitochondrial morphology changes dynamically mainly through fission and fusion. Dynamin-like/related protein 1 (DLP1/Drp1) mediates mitochondrial fission. Mitofusin isoforms (Mfn1 & Mfn2) and optic atrophy 1 (OPA1) are associated with the outer and inner membranes, respectively, and mediate fusion of the respective membranes. Currently, the mechanisms linking mitochondrial morphology and energetic activity are ill defined. Mitochondrial electron transport chain is a major source of reactive oxygen species (ROS), contributing to oxidative stress development in metabolic excess conditions. Our in vitro and in vivo studies have demonstrated that decreasing mitochondrial fission normalizes ROS levels and alleviates oxidative stress in high glucose and high fat conditions. Mechanistically, we found that mitochondrial interconnection caused by DLP1 inhibition increases the inner membrane proton leak through the induction of the large-scale transient depolarization. We further identified a novel cellular process of transient contraction of the mitochondrial matrix coinciding with a reversible loss or decrease of the inner membrane potential. Our studies indicate that the inner membrane fusion dynamin OPA1 mediates depolarization through inner membrane leak during matrix contraction. Support: NIH Grant DK061991. Reference: Galloway, C.A., Lee, H., Nejjar, S. Jhun, B.S., Yu, T., Hsu, W., and Yoon, Y. 2012. Transgenic control of mitochondrial fission induces mitochondrial uncoupling and relieves diabetic oxidative stress. *Diabetes* 61: 2093-2104.

3.2
MITOCHONDRIAL AUTOPHAGY AND BIOGENESIS: THE CYCLE OF LIFE

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Specialized cells require mitochondria optimized to meet the metabolic requirements of the cell. We used the C2C12 cell line as a model to explore the process of metabolic remodeling during the differentiation of primitive myoblasts to mature myotubes. Myoblasts rely primarily on glycolysis whereas myotubes predominantly utilize fatty acid oxidation for ATP production. This metabolic remodeling requires mitophagy and biogenesis as well as dynamic regulation of fusion and fission. Early myogenic differentiation is characterized by mitochondrial fission, autophagy, and p62-dependent mitophagy. This is followed by PGC-1α-mediated mitochondrial biogenesis and fusion to form a highly connected mitochondrial network. Mitochondrial content is substantially increased, and the mitochondria themselves are more tightly coupled, have a higher maximal respiratory capacity than myoblast mitochondria, and are better-equipped to use fatty acid substrates. This program of metabolic remodeling precedes expression of contractile proteins characteristic of differentiated myotubes, and blocking autophagy interrupts all subsequent differentiation events. These findings reveal an essential role for mitochondrial metabolic reprogramming in myogenic differentiation.

3.3
MITOCHONDRIAL MOTILITY AND FUSION DYNAMICS AND CALCIUM

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Mitochondrial positioning and fusion-state are recognized as critical factors for many aspects of mitochondrial function such as ATP synthesis, calcium signaling, ROS production, and apoptosis. Furthermore, ATP production, [Ca2+] and ROS change either mitochondrial motility or fusion or both, creating positive and negative feed-

back loops, and homeostatic mechanisms in mitochondrial dynamics. In addition, the motility and fusion/fission components of mitochondrial dynamics are mutually coupled with each other in many paradigms. In this presentation, I will focus on the relevance and the mechanisms of the interactions of Ca2+ and ROS with mitochondrial motility and fusion dynamics.

5.0 PLENARY II

5.1
SITES OF PRODUCTION OF MITOCHONDRIAL ROS: MECHANISMS AND PHYSIOLOGICAL FUNCTION

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Superoxide and H_2O_2 are generated at ten or more mitochondrial sites. Sites III_{Qo} in complex III, I_Q in complex I, and II_F in complex II have the greatest capacities in skeletal muscle mitochondria; site I_F in complex I has low capacity. The rate of superoxide/H_2O_2 production at any site depends on its redox state, so we can assess rates at different sites from measured redox states. Surprisingly, in a substrate mix mimicking resting muscle cytosol, the major contributors were I_Q and II_F, with contributions from I_F and III_{Qo}. In medium mimicking contracting muscle, the total rate was fivefold less and site I_F was dominant, with contributions from I_Q, II_F, and III_{Qo}. These *ex vivo* results may mimic ROS production in muscle *in vivo*. By screening small molecule libraries against different sites, we identified novel suppressors of superoxide/H_2O_2 production at sites I_Q and III_{Qo} that do not affect oxidative phosphorylation. They suppress several physiological and pathological phenotypes, and provide new tools to identify the roles of mitochondrial ROS production in cells, and potential leads for pharmacological modifiers of ROS signaling and oxidative damage. **References:** Goncalves et al. (2015) Sites of superoxide and hydrogen peroxide production by muscle mitochondria assessed *ex vivo* under conditions mimicking rest and exercise. *J Biol Chem* 290, 209-227. Orr et al. (2013) Inhibitors of ROS production by the ubiquinone-binding site of mitochondrial complex I identified by chemical screening. *Free Radic Biol Med* 65, 1047-1059. Orr et al. (2015) Suppressors of superoxide production from mitochondrial complex III. In revision.

6.0 TRANSLATIONAL BIOENERGETICS

6.1
MEASURING BIOENERGETIC HEALTH IN HUMAN POPULATIONS

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Bioenergetics is now at the forefront of our understanding of pathological mechanisms, new therapies and as a biomarker for the susceptibility of disease progression in metabolic diseases, neurodegeneration, cancer and cardiovascular disease. A key concept is that the mitochondrion can act as the "canary in the coal mine" by serving as an early warning of bioenergetic crisis in patient populations. Furthermore, cellular mitochondrial function is known to vary between populations due to differences in genetic background and in response to lifestyle changes including diet and exercise. It is clear that we urgently need new clinical tests to monitor changes in bioenergetics in patient populations. This is now possible due to the development of high-throughput assays to measure cellular energetic function in the small numbers of cells that can be isolated from human blood or from tissue biopsy samples. The sequential addition of well characterized inhibitors of oxidative phosphorylation allows a bioenergetic profile to be measured in cells isolated from normal or pathological samples. This profile can define the extent to which these cells utilize mitochondrial oxygen consumption to produce ATP, are using protons for other processes or leak and the maximal respiration. Non-mitochondrial oxygen consuming pathways are also measured and are likely indicative of a pro-inflammatory state. Taken together we propose these parameters are a measure of bioenergetic health of a cell population. We therefore propose the development of the Bioenergetic Health Index (BHI), which is a single value that defines bioenergetic health based upon the analysis of cellular mitochondrial profiles in cells isolated from human subjects. Ultimately, BHI has the potential to be a new biomarker for assessing patient health of (or for) both prognostic and diagnostic value.

6.2
PLATELET MITOCHONDRIA: FROM BIOMARKER TO BIOLOGICAL MECHANISM

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While it is well established that bioenergetic dysfunction plays a role in the pathogenesis of numerous diseases, mitochondrial dysfunction remains uncharacterized in many patient populations because of the invasiveness of obtaining tissue for mitochondrial studies. Platelets are easily accessible and have long been recognized to contain fully functional mitochondria. However, it remains unclear whether platelets harbor the bioenergetic dysfunction observed in other organ systems during pathology or whether mitochondrial dysfunction contributes to platelet pathology. We hypothesize that platelet bioenergetics can serve as a biomarker of specific diseases and that mitochondrial function regulates platelet thrombotic and inflammatory function. We have recently shown that patients with Sickle Cell Disease have altered platelet bioenergetics due to an inhibition of mitochondrial complex V, leading to increased membrane potential and augmented reactive oxygen species (ROS) production. We have shown that this augmented ROS directly leads to platelet activation. We now extend this study to determine whether platelet mitochondrial function is differentially altered in other disease cohorts including asthma, pulmonary hypertension, Parkinson's Disease and cardiac arrhythmias. We show data demonstrating differential bioenergetic profile in patients with each of these pathologies and discuss the role of this altered mitochondrial function in disease progression.

6.3
MITOCHONDRIAL BIOMARKERS FOR NEURODEGENERATIVE DISEASES

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Mitochondrial dysfunction is observed across a spectrum of neurodegenerative diseases. This raises the question of whether mitochondrial-based biomarkers could be used to reveal the presence of disease or pre-disease, endophenotype states, and whether mitochondrial biomarkers could be used to guide the development of new therapies. Approaches with the ability to interrogate brain bioenergetics currently exist, although these approaches have limitations and more comprehensive and practical ways to assess brain mitochondrial function are needed. Interestingly, mitochondrial changes similar to those observed in the brains of patients with some neurodegenerative diseases are also detected in peripheral tissues, which suggests the possibility that mitochondrial function in peripheral tissues may be able to function as a surrogate for brain mitochondrial function. We have previously considered different options for the assessment of brain mitochondrial function and brain bioenergetics, as they pertain to studies of diagnosis, pathophysiology, and drug target engagement. When it comes to assessing these parameters, we are further considering the opportunities and limitations of adapting measures of peripheral tissue mitochondrial function and bioenergetics. (NIH P30AG035982; R03NS077852; R01FD003739; PCTR-15-330495). **Reference:** Swerdlow RH. Bioenergetic Medicine. BJP 2014;171:1854-1869.

6.5
USING MACHINE LEARNING TO ADVANCE BLOOD BASED BIOENERGETIC PROFILING: A FOCUS ON GERIATRIC HEALTH

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Blood based bioenergetic profiling is recognized to have potential diagnostic and prognostic applications. In primates, we have observed that the respirometric profile of blood cells can recapitulate the bioenergetic capacity of other tissues such as skeletal muscle. Our studies in older adults indicate that that the respiratory capacity of PBMCs is associated with multiple measures of physical function, including; gait speed, Short Physical Performance Battery score, upper and lower body strength, and muscle quality. These physical function measures are recognized to be excellent predictors of morbidity and mortality in this age group. PBMCs are comprised of multiple cell types and do not encompass all cells accessible for blood based profiling. It is likely that different cell types and respirometric parameters will have variable utility with regard to prognostic and diagnostic applications. To address this, we are utilizing Machine Learning methods designed for high dimensional data analysis to identify respirometric signatures and patterns across multiple cell types that are most closely associated with clinical outcomes. This branch of artificial intelligence utilizes algorithms that can be trained by example to distinguish between groups or predict outcomes. Random Forests is an ensemble learning approach that can build powerful predictive models and detect subtle multivariate gait patterns. The strengths of this approach are: it does not over fit; it is robust to noise; it estimates error rates; it provides indices of variable importance; it works with mixes of continuous and categorical variables; it can be used for data imputation and cluster analysis; and it can deal with issues stemming from a large number of variables and a small sample size.

7.0 POSTER SESSION I

7.1
TRANSGENIC REDOX-INDICATOR MICE EXPRESSING CYTOSOLIC AND MITOCHONDRIAL ROGFP1

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Reactive oxygen species (ROS) and related redox changes contribute to cellular signaling and are linked to neuropathology and mitochondrial dysfunction. For long, redox imaging was limited by a lack of reliable optical probes. Genetically-encoded, fluorescent protein derived optical redox sensors bridge this gap. Demanding is, however, the delivery of coding DNA to the tissue of interest. This requires transfection/transduction of cultured preparations or viral injections into each individual animal. To extend reliable redox imaging to adult and complex preparations while circumventing surgical procedures, we generated transgenic redox indicator mice. They express roGFP1 under the Thy1 promoter in the cytosol or the mitochondrial matrix almost throughout the brain. NeuN labeling confirmed neuronal expression of cytosolic and mitochondrial roGFP1, and Mitotracker staining verified its proper targeting to mitochondria. RoGFP1 is functional at all postnatal stages; any negative effects of the transgene can be ruled out. Detailed response calibrations of roGFP1 already detected regional differences in redox conditions among the hippocampal subfields. In conclusion, roGFP1 mice are valuable to analyze ROS/redox signaling in various preparations during maturation and aging. Their crossbreeding with disease mouse models will unveil details on ROS formation and redox imbalance in the onset and progression of various neuronal disorders, degenerative conditions, and mitochondriopathies. *Supported by the Cluster of Excellence and the DFG Research Center Nanomicroscopy and Molecular Physiology of the Brain (CNMPB).*

7.2
EFFECTS OF SKELETAL MUSCLE AGING ON MITOCHONDRIAL MORPHOLOGY AND DYNAMICS

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Background: Skeletal muscle aging is associated with a progressive decline in muscle mass and strength, a process named sarcopenia. Strong evidence points towards a causal role played by accumulation of mitochondrial dysfunctions in the development of sarcopenia, a process that could be triggered by impaired mitophagy. It is now recognized that mitochondrial function, mitophagy and mitochondrial morphology are interconnected. However, the impact of muscle aging on mitochondrial morphology remains unknown. **Methods:** To address this issue, we assessed the morphology of SubSarcolemmal (SSm) and InterMyoFibrillar (IMFm) mitochondria in skeletal muscle of young (8-12wk-old) and old mice (88-96wk-old) using a quantitative transmission electron microscopy approach. Protein contents of OPA1, Mfn1, Mfn2, Drp1 and key protein of the oxidative phosphorylation system were quantified in muscle homogenates using western blots. **Results and Conclusions:** We show that aging-related muscle atrophy is associated with larger and less circular SSm, and more complex (increased length and branching) IMFm. In line with these morphological changes, and although no difference in the content of proteins regulating mitochondrial dynamics (Mfn1, Mfn2, Opa1 and Drp1) was observed, a mitochondrial fusion index (Mfn2-to-Drp1 ratio) was significantly increased in aged muscles. Our results reveal that muscle aging is associated with complex changes in mitochondrial morphology that could interfere with mitochondrial function and mitophagy, and thus contribute to aging-related accumulation of mitochondrial dysfunction and sarcopenia.

7.3
THE LIVER MOLECULAR CIRCADIAN CLOCK IN CHRONIC ALCOHOL-INDUCED MITOCHONDRIAL DYSFUNCTION

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Mitochondrial bioenergetics is compromised by alcohol consumption. Studies suggest that hepatic beta oxidation is regulated by clock-controlled rhythms in protein acetylation. The extent to which these or other mitochondrial processes are clock regulated is unknown. To determine the interaction of the clock and alcohol on mitochondrial function we used a model of hepatocyte clock dysfunction; hepatocyte-specific BMAL1 knockout (HBK) mice. HBK and wild type (WT) mice were kept under a 12:12 h L-D cycle and fed control and alcohol-containing diets. Livers were collected every 4h for 24h. Data showed that mtDNA content was rhythmic in liver of control WT mice, and *Pgc1a* and *Nrf1* were rhythmic in WT, but not HBK liver. These results suggest that mitochondrial content and bioenergetics are regulated by the clock. Diurnal rhythms in *Pgc1a*, *Pgc1b*, *Pdk4*, and *Sirt3* were decreased in livers of alcohol-fed mice. Activity of cytochrome c oxidase (CcO) was rhythmic in livers of control mice with peak activity in the dark/active phase. Notably, the CcO rhythm was lost in livers of alcohol-fed mice. In summary, these results support the idea that mitochondria adapt to changing metabolic demands of the cell during the day by clock-regulated mechanisms. Conversely, the lack of flexibility in mitochondrial metabolism in alcohol-exposed liver may lead to bioenergetic stress. Thus, a failure in clock-driven adaptive processes in mitochondrial function contributes to alcoholic liver disease.

7.4
RETT SYNDROME PROVOKES A CYTOSOLIC AND MITOCHONDRIAL REDOX IMBALANCE IN NEONATAL NEURONS

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Rett syndrome is a neurodevelopmental disorder associated with mitochondrial impairment and redox imbalance. Mitochondria of MeCP2-deficient (*Mecp2*$^{-/y}$) mouse brain are partly uncoupled and show increased respiratory rates. Previously, we confirmed more oxidized baseline conditions and exaggerated responses of *Mecp2*$^{-/y}$ hippocampus to redox challenge. To unveil the molecular causes of this imbalance, we generated viral vectors expressing the redox sensor roGFP1 in cytosol or mitochondrial matrix of neurons. This probe responds to oxidation/reduction and enables quantitative live-cell imaging of subcellular redox dynamics. Genotypic differences were evident in organotypic slices; both mitochondria and cytosol showed more oxidized redox baselines in *Mecp2*$^{-/y}$ neurons. Blocking superoxide dismutase caused a less intense oxidation in *Mecp2*$^{-/y}$ cytosol and mitochondria, suggesting a decreased efficiency of this scavenging enzyme. Challenge by H_2O_2 and severe hypoxia elicited intensified oxidizing and reducing transients in *Mecp2*$^{-/y}$ neurons, respectively. Cuvette tests on isolated mitochondria showed increased ROS formation also in adult *Mecp2*$^{-/y}$ hippocampus. Interestingly, the differences among WT and *Mecp2*$^{-/y}$ mice already manifest at neonatal stages and involve mitochondria and cytosol. Since mitochondria are a primary source of ROS, this supports our hypothesis that the mitochondrial dysfunction underlies the oxidative burden in Rett syndrome and drives disease progression. *Supported by the DFG Research Center Molecular Physiology of the Brain (CMPB) and the International Rett Syndrome Foundation (IRSF).*

7.5
BIOENERGETIC INFLUENCE ON APP PRODUCTION AND PROCESSING

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Existing data suggest relationships exist between mitochondrial function, APP processing, and beta amyloid (Aβ) deposition. If correct, a better understanding of the relationship between mitochondrial function, cell bioenergetics, and Aβ could enhance our understanding of AD. To test the impact of bioenergetics on APP processing we measured APP mRNA, APP protein, and APP derivatives (soluble APPα, sAPPα; Aβ) in human neuronal SH-SY5Y cells with different bioenergetic manipulations. These manipulations include depletion of mitochondrial DNA (ρ0), glycolysis inhibition (2-deoxyglucose; 2DG), and varying medium glucose concentrations (0, 2.5, 25 mM). Endpoints were measured at 24 and 72 hours for the 2DG and variable glucose experiments. The effects of these manipulations on respiration and glycolysis were determined using a Seahorse XF24 analyzer. Relative to SH-SY5Y cells, SH-SY5Y ρ0 cells (which have a high glycolysis flux and negligible respiratory chain flux) had comparable full-length APP protein and mRNA levels, but lower medium sAPPα and Aβ levels. At both the 24 and 72 hour time points, 2DG treatment reduced glycolysis with no change in respiration. At 24 hours no changes were observed with any APP processing endpoints following 2DG treatment. At 72 hours, the 2DG treatment showed unchanged APP mRNA levels, reduced full length APP protein, medium sAPPα and Aβ levels. Relative to cells maintained at a high glucose level (25 mM), 0 mM glucose showed reduced glycolysis and increased respiration, while cells in 2.5 mM glucose showed increased respiration and comparable glycolysis. At 24 hours, cells maintained in 0 and 2.5 mM glucose had reduced medium sAPPα, but all other endpoints were unchanged. With 0 mM glucose, APP mRNA was unchanged, full length APP protein and medium sAPPα were reduced, while medium Aβ levels were increased at 72 hours. Cells maintained in 2.5 mM glucose appeared to show intermediate changes to APP endpoints. Results suggest bioenergetically-stressed cells reduce APP translation, or alter processing, compartmentalization, or solubility of APP and its derivatives. Results from ρ0 cells are perhaps more consistent with this latter view. Experiments to resolve these questions are underway.

7.6
MODULATION OF MITOCHONDRIAL ADENINE NUCLEOTIDE TRANSLOCASE (ANT) REGULATION WITH AGEING

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By studying bioenergetic parameters (oxidation and phosphorylation rates, membrane potential) in isolated mitochondria from aged rat muscle (gastrocnemius) we observed a decrease in mitochondrial affinity for ADP, and a change in ANT response to atractyloside[1]. These age-induced modifications of ANT result in an increase in the ADP concentration required to sustain the same ATP turnover as compared to young muscle, and thus lower membrane potential and higher coupling efficiency under conditions of low ATP turnover[1,2], due to the down-regulation of basal proton leak caused by membrane potential decrease[3]. The decrease in membrane potential caused by ANT alteration during ageing may also decrease reactive oxygen species (ROS) production as compared to young muscles for equivalent ATP turnover. ANT alteration with ageing may be the result of oxidative damage caused by ROS and may appear like a virtuous circle where ROS induce a mechanism that reduces their production. Because of the importance of mitochondrial ROS as therapeutic targets, we believe that this new mechanism deserves further studies. All experiments are in agreement with the European Guide for animal use. PD has a permanent license to conduct experiments on animals (03/17/1999, license 3308010). [1] G. Gouspillou et al., Biochim Biophys Acta, 1797 (2010) 143-15. [2] 1. G. Gouspillou et al., Aging cell, 13 (2014) 39-48. [3] M.D. Brand, L.F. Chien, P. Diolez, Biochem J, 297 (Pt 1) (1994) 27-29.

7.7
MITOCHONDRIAL RESERVE CAPACITY IS DRIVEN BY GLUTAMINE IN LUNG CANCER CELLS WITH MESENCHYMAL PHENOTYPE

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Metastasis is the main cause of cancer mortality, and its initiation is enabled by a process known as the epithelial-to-mesenchymal transition (EMT). It is thus desirable to identify specific drug targets for cancer cells with mesenchymal phenotype. Previously we have shown that lung cancers with mesenchymal phenotype are more sensitive to inhibition of glutaminase (GLS). As EMT can lead to changes in both the glycolytic and glutaminolytic pathways, we sought to investigate the importance of these fuels for mitochondrial respiration, and to understand the impact of GLS inhibition on mitochondrial function. We developed a cell-based assay to profile substrate preference under basal and FCCP-stimulated conditions. We first showed that transforming growth factor beta 3 (TGFβ3)-induced EMT was accompanied by the loss of glucose-driven reserve capacity. As a result, small molecule inhibition of GLS abolished reserve capacity and blocked proliferation in a TGFβ3-induced mesenchymal line without affecting the epithelial parental line. We further applied this assay to a lung cancer cell line panel, and demonstrated that cell lines with high sensitivity to GLS inhibitor were solely dependent on glutamine-driven reserve capacity. Taken together, our data demonstrate EMT is associated with a change in substrate utilization for mitochondrial reserve capacity in lung cancer cells, and reserve capacity may play a key mechanistic link between GLS inhibition and impaired cell proliferation.

7.8
L-OPA1 FUNCTIONS INDEPENDENTLY OF S-OPA1 BY FORMING SEPARATE STRUCTURAL ENTITIES

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Optic atrophy 1 (OPA1) is a dynamin-related membrane-remodeling protein that functions in mitochondrial fusion and cristae remodeling. Loss of OPA1 has been shown to cause defects in inner membrane fusion and oxidative phosphorylation (OXPHOS). OPA1 is expressed in multiple splice variants produced by alternative splicing at the N-terminal exons downstream of a transmembrane (TM) domain. These splice variants undergo partial or full proteolytic cleavage depending on exon composition after alternative splicing, resulting in membrane-anchored long forms (L-OPA1) and TM-free short forms (S-OPA1). In this study, we expressed S-OPA1, membrane-anchored non-cleavable L-OPA1, and cleavable L-OPA1 in OPA1-KO mouse embryonic fibroblasts (MEFs) and examined their capacities to restore OXPHOS function and mitochondrial fusion. We found that, while OPA1-KO cells failed to grow in galactose medium which forces cells to use OXPHOS to generate ATP, expression of L-OPA1 or S-OPA1 alone was sufficient to support cell growth in galactose medium. Similarly, L-OPA1 or S-OPA1 alone restored respiration in OPA1-KO MEFs. Analyses of respiration complexes using blue-native gel electrophoresis (BNGE) indicated that OPA1-KO cells showed greatly diminished levels of complexes III, IV, and V, which was restored by L- or S-OPA1 alone indistinguishably. However, we observed that L-OPA1 was more effective than S-OPA1 in inducing mitochondrial elongation when fission was inhibited, similar to previous observations in the conditions of nutrient starvation or cycloheximide treatment. Interestingly, analyses of oligomeric state of L- and S-OPA1 showed that, while non-cleavable L-OPA formed mostly hexamers, the majority of S-OPA1 was in dimers. In wild-type cells and cells expressing a cleavable L-OPA1 in OPA1 KO cells, L- and S-OPA1 also exhibited similar hexameric and dimeric patterns, respectively, as examined by 2-dimensional BNGE (BNGE followed by SDS-PAGE). These results suggest that although L-OPA1 is required for mitochondria fusion, cristae maintenance for proper OXPHOS function can be supported by S-OPA1 or L-OPA1 alone.

7.9
WITHDRAWN

7.10
BIOENERGETIC PROPERTIES OF HUMAN RENAL TUBULAR AND MESANGIAL CELLS IN NORMAL AND DIABETIC CONDITIONS

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Background: We previously reported altered circulating mitochondrial DNA and mitochondrial respiration in human and *in-vitro* studies and proposed that hyperglycemia can affect mitochondrial biogenesis and function in diabetic kidney disease. In the current study we examined the effect of hyperglycemia on cellular bioenergetics in cultured renal mesangial (HMCs) and tubular (HK-2) cells. **Methods:** HMCs and HK-2 cells were cultured in normal (5mM, NG), high (25mM, HG) glucose and osmolarity control (5mM glucose + 20mM mannitol, NGM) for 4-12 days. Cellular bioenergetics was measured using XF^{e}96 Seahorse analyzer. **Results:** Comparison of cellular bioenergetics of HMCs and HK-2 cells cultured in NG showed that HK-2 cells have significantly lower basal, ATP-linked and maximal respiration rates ($P<0.001$) and reserve capacity ($P<0.001$). 4-day culture in HG caused a significant down-regulation of all respiratory parameters in the HK-2 cells ($P<0.01$) while

HMCs started to show reduced maximal respiration after 8 day exposure to HG ($P<0.05$) and reduced basal, maximal and ATP-linked respiration after 12 days. There was a compensatory up-regulation of glycolysis in HK-2 cells cultured in HG for 8 days ($P<0.05$). **Conclusion:** These results show that hyperglycemia leads to the decrease in energy metabolism in renal cells by down-regulation of their respiratory function and that distinct cell within one organ have different bioenergetic profiles and bioenergetic reserve. AC is funded by KCL.

7.11
REGULATION OF BIOENERGETICS AND ANGIOGENIC RESPONSE IN VASA VASORUM ENDOTHELIAL CELLS BY EXTRACELLULAR PURINES AND HYPOXIA

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Cell proliferation is an energy taxing process, however, both the role of cellular metabolism in angiogenic endothelial cells and the regulation of cellular energy pathways by extracellular stimuli remain unexplored. Extracellular purines are widely accepted as important regulators of endothelial cell function. Our group has previously shown their autocrine/paracrine role in pulmonary artery vasa vasorum angiogenesis in a neonatal model of hypoxic pulmonary hypertension. By using glycolytic and mitochondrial respiration inhibitors, in this study we demonstrated that glycolysis and oxidative phosphorylation (OXPHOS) are both vital for ATP-stimulated vasa vasorum endothelial cell (VVEC) mitogenesis. We also showed that VVEC isolated from control animals exhibited higher rates of OXPHOS compared to those isolated from chronically hypoxic complements. Measurement of OXPHOS in digitonin-permeabilized VVEC showed that chronic hypoxia both in vivo and in vitro significantly decreased basal, Complex I and Complex II mitochondrial respiration. Additionally, F_1F_0 ATP-synthase β-subunit and Cytochrome C oxidase subunit IV expression levels were decreased, suggesting persistent hypoxia-induced phenotypical changes in VVEC bioenergetics. Cells cultured 7 days in Galactose [20mM] and Glucose [5mM] displayed augmented intracellular ATP production along with a significant increase in basal and maximal respiration rates. Furthermore, a one-hour nucleotide treatment [100uM] increased maximal respiration rates under said conditions. Interestingly, glycolysis experiments displayed a unique response to oligomycin [0.4,0.8,1&2uM] wherein a decrease in extracellular acidification rate (ECAR) was observed despite exposure time [1,4,8 & 24h]. ATP stimulation increased ECAR while 2-deoxyglucose (2DG) also yielded an unorthodox response resulting in a marked decrease in ECAR followed by immediate recovery to pre-injection levels within 10 minutes. In parallel, lactate measurements showed an insignificant increase in response to oligomycin, while ATP concentrations spiked at the 4 hour mark in control and hypoxic VVEC not coinciding with OXPHOS changes. Finally, consistent with observed increases in OXPHOS rates, ATP was shown to induce a transient increase in $[Ca^{2+}]$ in VVEC mitochondria. Therefore, purinergic and metabolic regulation of VVEC energy pathways may present a novel strategy for the treatment of vasa vasorum pathologic angiogenesis in hypoxic pulmonary hypertension. **Funding:** R01 HL086783 (E.V. Gerasimovskaya).

7.12
INCREASED AUTOPHAGY IS REQUIRED FOR MECHANICAL VENTILATION-INDUCED DIAPHRAGM MITOCHONDRIAL DYSFUNCTION

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Mechanical ventilation (MV) is a life-saving intervention for patients in respiratory failure. However, prolonged MV results in diaphragm weakness. While the mechanisms controlling MV-induced diaphragm atrophy are not fully elucidated, it has been demonstrated that mitochondrial function plays an important role in regulating skeletal muscle mass. Evidence in mechanically ventilated patients indicates that the autophagy/lysosomal proteolytic pathway is upregulated in the diaphragm. However, it is unknown if MV-induced increased autophagy occurs as a protective mechanism to degrade dysfunctional mitochondria or if increased autophagy exacerbates mitochondrial dysfunction. Therefore, these experiments were designed to determine the effects of accelerated autophagy on diaphragm mitochondrial function during MV. Cause and effect was determined by inhibiting MV-induced autophagy via adeno-associated virus overexpression of mutated autophagy-related protein 5 (ATG5) in the diaphragm of rats. Our results reveal that inhibiting autophagy prevented the MV-induced reduction in mitochondrial oxygen consumption. Further, transduction of mutated ATG5 prevented MV-induced increase in both mitochondrial ROS emission and caspase-3 activation. Finally, inhibiting autophagy prevented MV-induced increased expression of PINK1 and the fission/fusion proteins OPA1 and DLP1. Therefore, our data indicate inhibition of MV-induced autophagy is sufficient to protect against diaphragm mitochondrial dysfunction. Supported by NIH R21 AR064956 awarded to SKP.

7.13
MITOCHONDRIAL RESPIRATORY CAPACITY IS DECREASED IN RAT CARDIOMYOCYTES FOLLOWING EXPOSURE TO MATERNAL DIABETES AND HIGH FAT DIET

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Background: Offspring of diabetic mothers (ODM) are at risk of cardiovascular disease at birth and throughout life. Diabetic pregnancy is associated with increased circulating fuels that may cross the placenta to incite influences on the developing fetal heart. Emerging evidence suggests that bioenergetic reprogramming at the mitochondrial level plays a pivotal role in the pathophysiology of adult cardiac disease. However, the short and long-term consequences of exaggerated fuel exposure on mitochondrial function in the developing fetal heart are unknown. **Objective:** To determine the effects of maternal diabetes and high-fat (HF) diet on developmental programming of cardiac metabolism. **Methods**: Sprague Dawley rats received controlled (CD) or HF diet throughout the study. At gestational day 14 dams were injected with either citrate buffer (CB) placebo or streptozotocin (STZ) to induce diabetes producing offspring from the following groups: controls (CD-CB), diabetic exposed (CD-STZ), HF exposed (HF-CB), and double exposed (HF-STZ). Cellular bioenergetics of primarily isolated cardiomyocytes (CM) from each litter were investigated using a Seahorse XF24 analyzer which measures oxygen consumption rate and extracellular acidification rate as markers of mitochondrial respiration and anaerobic glycolysis respectively. Pairwise analyses were performed and significance was set at $p<0.05$. **Results**: Basal respiration was significantly decreased in the HF-STZ suggesting changes in either ATP turnover, substrate oxidation or proton leak. Maximal respiratory capacity was significantly decreased in HF-STZ exposed CMs suggestive of mitochondrial substrate uptake and processing dysfunction. Moreover, a decrease in reserve capacity was also detected in the HF-STZ exposed CMs suggestive of inability to respond to an increase in energy demand. The diabetic and double exposed CMs also had limited glycolytic capacity. **Conclusion**: Late gestation diabetes reprograms fuel metabolism in the developing heart of offspring. These effects are exacerbated with a maternal HF diet. Additionally a reduced reserve capacity, especially with inability of fuel switch in diabetic exposed CMs suggests inability to produce sufficient ATP during elevated demands. Together, our findings have important implications in preventing developmentally programmed cardiac disease in ODMs. **Funding**: NIH K08HD078504, NICHD, SSOM-USD Faculty Grant, Sanford Health–SDSU Collaborative Research Seed Grant.

7.14
ATP PRODUCTION AND OXYGEN CONSUMPTION IN ISOLATED MITOCHONDRIA FROM H9C2 CELLS

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ATP in animal cells is first produced in cytoplasm in the process of glycolysis which is based on breaking glucose into two pyruvate molecules. Than pyruvate can be converted into lactic acid in the process of fermentation or can be involved in the respiration which takes place in the mitochondria. This study addresses the question whether the ATP generation is strictly correlated to oxygen consumption. For the purpose mitochondria were isolated from myocardial cells H9c2. Utilization of pure (isolated) mitochondria helps to avoid aberration of ATP measurements associated with the production of ATP in the glycolysis process, which impairs performance. ATP production was measured using the Luciferase-Luciferin method, which is based on the luminescence reaction between luciferin and ATP – catalyzed by luciferase. Oxygen consumption was measured with Oxygraph-2 OROBOROS. Glutamate, malate and succinate were used as respiratory chain substrates. To check the background we used blockers such as rotenone and oligomycin. Two major findings were noted: After substrate are added either both ATP production and oxygen consumption increases significantly or only ATP production rises rapidly while oxygen consumption is not so much noticeable. This may be due to the process of iso-

lation, where the outer mitochondrial membrane may have been damaged losing its cytochrome c from the surface. Cytochrome c is responsible for the transfer of electrons from the III to the IV complex. This way oxygen consumption is not observed or the results are understated, since only electrons coming out of the complex IV reduce oxygen. Therefore, one of the key steps in this research is the isolation of mitochondria from cardiomyocytes. This work was supported by a grant from UDA-POKL-04.03.00-00-042/12-00 and partially by financial resources of WULS-SGGW.

7.15
WITHDRAWN

7.16
MITOCHONDRIAL DYSFUNCTION IN HEART OF CORONARY ARTERY DISEASE: CORRELATION WITH TELOMERASE ACTIVITY

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Rational: Heart disease is the primer cause of death worldwide. Mitochondrial defects have been related to cardiomyopathy, heart failure, endothelial dysfunction and coronary artery disease (CAD). However the direct role and mechanism of the mitochondrial dysfunction in the development of CAD is not fully understood. Recently, we have shown that the catalytic subunit of telomerase (TERT) plays an important role as a regulator of mitochondrial integrity in the human microcirculation. An increased level of TERT has been shown to be cardioportective in rodent models of myocardial infarct, yet the direct contributions of TERT are ill defined. The dominant negative splice variant of TERT (β-del) is increased under pathological conditions and mainly localized to the mitochondria. Therefore, we hypothesize that TERT (β-del) decreases mitochondrial telomerase activity in the human heart resulting in mitochondrial damage that contributes to an environment that promotes the development of CAD. **Methods:** Fresh and frozen tissue samples of discarded heart tissue from subjects with and without CAD were used. Protein, cell lysate or mitochondria were isolated using standard techniques. Mitochondrial DNA (mtDNA) integrity, levels of NAD+ and ATP as well as mitochondrial oxidative phosphorylation were evaluated. **Results:** Western blot analysis revealed increased expression of β-del TERT in the Left Ventricle (LV) from subjects with CAD. Using PCR analysis, we found an increased frequency of mitochondrial common deletion, an established marker for mitochondrial abnormalities (0.9±0.2 in CAD; vs 1.5±0.2 in non-CAD; N=8; P<0.05). A 5 fold elevation of mtDNA lesions was also observed in the LV from subjects with CAD compared to non-CAD. NAD+ and ATP levels were significantly decreased in CAD subjects compared to non-CAD (291±62 and 0.5±0.1 RLU/mg protein in CAD vs. 4203±336 and 84.1±24.8 pmol/mg protein in non-CAD respectively; N=15; P<0.005). Decrease respiration control index (RCI) in the presence of either complex I substrate K (+)-pyruvate/malate (PM) or complex II substrate K (+)-succinate (SUC) was observed in freshly isolated mitochondria from subject hearts with CAD (KPM-RCI: 2.9±1.3; SUC-RCI: 7.6± 1.9 in CAD vs KPM-RCI: 8.5±1.9; SUC-RCI: 19.1± 8.3 in non-CAD; N=3; P<0.05). **Conclusions:** In summary these results indicate an impaired mitochondrial function in subjects with CAD correlated with a reduction in mitochondrial telomerase activity. Grants: R01-HL-113612, R21-OD-018306.

7.17
HIGH-THROUGHPUT SCREENING REVEALS THE MITOCHONDRIAL COMPLEX I INHIBITOR NORNICOTINE IS CARDIOPROTECTIVE IN ISCHEMIA-REPERFUSION INJURY WHEN DELIVERED AT REPERFUSION

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Background: To date, there are no FDA-approved therapies for the reduction of infarct size in acute myocardial infarction. Previously, we developed a cell-based phenotypic assay of ischemia-reperfusion (IR) injury, which was used to identify novel cytoprotective agents delivered prior to ischemia. Herein, we sought to identify cytoprotective agents in a more clinically relevant model: drug delivery at reperfusion, and to investigate possible underlying mechanisms of protection. **Methods:** Primary adult mouse cardiomyocytes were subjected to simulated IR injury using a modified Seahorse XF24 apparatus with drug addition at the onset of reperfusion. Cell death was estimated using LDH release. Drugs which protected cardiomyocytes *in vitro* were tested in a Langendorff model of IR injury, measuring functional recovery and infarct size. In separate experiments, metabolites extracted from perfused hearts were resolved by HPLC. **Results:** Nornicotine was identified as a cardioprotective agent in the screen. In perfused hearts, 10 nM nornicotine injected at the onset of reperfusion improved functional recovery and decreased in infarct size (13.1% ± 2.4 vs 49.2% ± 2.5 in non-treated hearts, p<0.05, n=16-20). Nornicotine also exhibited profound inhibitory effects on mitochondrial complex I activity. Succinate is known to accumulate in ischemia, and its rapid consumption during early reperfusion exacerbates reperfusion injury via ROS generation from electron backflow through complex I [PMID: 25383517]. In non-treated hearts, we confirmed that high post ischemic levels of succinate rapidly declined during the first 2 min of reperfusion. In contrast, nornicotine slowed post-ischemic succinate consumption, suggesting that electron backflow through complex I is the major pathway driving succinate consumption. **Conclusions:** Herein, we demonstrated that nornicotine was cardioprotective when delivered at early reperfusion *in vitro* and *ex vivo*. The mechanism of cardioprotection may be due to inhibition of rapid succinate consumption during early reperfusion via reverse electron flow back through complex I. **Acknowledgements:** Work in the lab' of PSB is funded by a grant from the US National Institutes of Health (R01 HL-071158). JZ is supported by the University of Rochester Medical Scientist Training Program (NIH T32GM007356).

7.18
MITOCHONDRIAL CHAPERONE GRP75 HAPLOINSUFFICIENCY PROMOTES LIVER TUMORIGENESIS BY ADAPTED METABOLISM

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The reprogramming of energy metabolism is one of the hallmarks of cancer; however, underlying mechanism of mitochondria's role in tumorigenesis remains unclarified. To determine whether and how mitochondria attributes to tumorigenesis, we investigated the effects of genetic inactivation of GRP75, a mitochondrial HSP70 chaperone, using a well-established cancer mouse model in which injection of precarcinogen diethylnitrosamine (DEN) at 15 days of age induces liver tumor within 7 months with a 100% prevalence. Our study revealed that hepatocytes of *grp75*+/- mice, compared to *grp75*+/+ (WT) controls, exhibited distinct metabolic alterations associated with a lower respiratory capacity (oxygen consumption rate), higher glycolysis, decreased mitochondria membrane potential (MMP), and higher ROS production. Although both strains were sensitive to DEN-induced liver tumorigenesis, the *grp75*+/- mice exhibited higher tumor burdens and accelerated tumor growth. Thus, partial inactivation of GRP75 promotes liver tumorigenesis by causing mitochondrial dysfunction, increasing ROS production, and engaging metabolic adaptive pathways promoting malignant transformation. Further detailed studies on the metabolic and molecular signaling pathways driving tumor progression in *grp75*+/- mice may provide potential targets for liver cancer treatment. Funding resources: The research was supported from the National Institutes of Health CA121951 (D.M.) and CA062130 (N.M.).

7.19
MITOCHONDRIAL ENERGY DEFICIENCY LEADS TO HYPERPROLIFERATION OF SKELETAL MUSCLE MITOCHONDRIA AND ENHANCED INSULIN SENSITIVITY

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Mitochondrial dysfunction is known to be associated with type II diabetes, but whether it is a cause or consequence is still unknown. The adenine nucleotide translocase (ANT1) is a mitochondrial protein that exchanges cytosolic ADP for ATP produced by oxidative phosphorylation. Previous studies have shown that mice deficient in ANT1 have reduced muscle mitochondrial function and develop cardiomyopathy. Our goal was to study the relationship of mitochondrial energy production to insulin sensitivity by using *Ant1* -/- and *Ant1* +/+ mice fed a high fat diet. The deletion of *Ant1* resulted in a hyperproliferation of dysfunctional mitochondria in the gastroc-

nemius muscle as determined by electron microscopy, mitochondrial DNA copy number, and enzyme activity of citrate synthase (CS), succinate dehydrogenase and cytochrome c oxidase. This was accompanied by a greater proportion of oxidative-like myofibers showing increased staining for myosin heavy chain isoforms IIa and IIx in the *Ant1* -/- mouse. Next we measured oxygen consumption and reactive oxygen species (ROS) production in permeabilized myofibers from gastrocnemius muscle. The *Ant1* -/- mouse had a 60% and 80% decrease in state II and III respiration, respectively, when normalized to CS activity. However, because of mitochondrial hyperproliferation there is a 260% and 140% increase in state II and III respiration, respectively, when normalized to tissue mass. ROS production follows a similar trend where the amount of H_2O_2 produced is decreased in *Ant1* -/- myofibers when normalized to CS activity, but increased when normalized to tissue mass. Finally, *Ant1* -/- mice showed signs of improved insulin sensitivity. *Ant1* -/- mice were found to be significantly more glucose tolerant and have an increased glucose infusion rate during a hyperinsulinemic-euglycemic clamp. This enhanced insulin sensitivity seems to happening independent of the PI3K-Akt signaling pathway as shown by downregulated gene expression in a RNA-seq profile of *Ant1* -/- muscle. Overall, ANT1 deficiency leads to a compensatory increase in mitochondrial number and oxidative metabolism that result in enhanced insulin sensitivity. Funding supported by NIH grants.

7.20
HIGH INTENSITY TRAINING INCREASES MITOCHONDRIAL RESPIRATORY CAPACITY IN OLD MALES BUT NOT FEMALES

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High intensity training (HIT) has been shown to increase maximal oxygen uptake (VO2max) and mitochondrial oxidative phosphorylation (OXPHOS) capacity in young subjects. The objective of the study was to investigate the effect of six weeks of HIT in older male and females, to see if the response is similar to what is seen in young subjects and if the improvement is similar between genders. This training intervention has never been investigated in old subjects before. Old obese males (n=10; age: 64±2 years; BMI: 31±1 kg/m2) and females (n=9; age: 64±1 years; BMI: 31±1 kg/m2) were recruited for the study. OXPHOS capacity was measured in permeabilized skeletal muscle fibers and subcutaneous abdominal adipose tissue before and after six weeks of supervised HIT training (3 times per week; 5 times 1 minute at approximately 125% of VO2max) using high-resolution respirometry (Oxygraph-2k, Austria). The following protocol was used: Malate, glutamate, ADP (CI*P*), succinate (CI+II*P*) and FCCP (ETS). VO2max, glycosylated hemoglobin (HbA1c) and body composition was measured as well. Males increased VO2max after HIT (P=0.09), with no difference seen in females. Body composition was similar in females after HIT, whereas males reduced their body fat percentage. HbA1c increased in females and decreased in males after HIT. No difference was seen at baseline between males and females in skeletal muscle OXPHOS capacity with the three different substrate / uncoupler combinations (CIP; CI+IIP; ETS). Six weeks of HIT did not change OXPHOS capacity in females (CI*P*: 24±1 vs. 23±1; CI+II*P*: 60±3 vs. 66±5; ETS: 71±2 vs. 76±5 pmol/mg/sec), whereas males increased OXPHOS capacity (CI*P*: 22±2 vs. 28±3; CI+II*P*: 62±4 vs. 86±8; ETS: 70±4 vs. 94±8 pmol/mg/sec). OXPHOS capacity in adipose tissue was higher at baseline in females compared with males (CI*P*: 0.50±0.05 vs. 0.36±0.03; CI+II*P*: 1.71±0.11 vs. 1.30±0.08; ETS: 1.91±0.11 vs. 1.41±0.12 pmol/mg/sec, respectively), the training intervention did not change OXPHOS capacity in either of the genders. In conclusion old males increase VO2max and OXPHOS capacity in skeletal muscle after six weeks of HIT, whereas no improvements are seen in females. No difference was seen after training in adipose tissue, but females have a higher OXPHOS capacity at baseline compared with males. Further analysis is needed to explain these gender differences.The study was supported by Oda and Hans Svenningsens Foundation.

7.21
AGED MUSCLE EXHIBITS BLUNTED CARDIOLIPIN AND CERAMIDE REMODELING DURING HINDLIMB UNLOADING INDUCED ATROPHY AND A LACK OF MUSCLE HYPERTROPHY FOLLOWING RELOADING

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Background: Aging is associated with a progressive loss of muscle mass (sarcopenia) and a loss of adaptive response to mechanical loading and unloading. The molecular mechanisms that underlie the loss of adaptive response are unknown. Recent evidence suggests that mitochondrial function and mitophagy may be important factors. Moreover, cardiolipin (CL) and ceramide (CER) remodeling are emerging as important players in mitophagy, but have not been examined in the context of mechanical unloading and recovery in aging. **Purpose:** To evaluate the response of aged sarcopenic muscle to hindlimb unloading and recovery with respect to mitochondrial function (respiration; H_2O_2 emission; calcium retention capacity, CRC) and changes in content of individual molecular species of CL and CER. We hypothesized that the loss of adaptive response in sarcopenic muscle, specifically the soleus, will be evident at the level of mitochondria function, and remodeling of CL and CER species. **Methods:** Young (Y, 6-mo old) and aged (O, 26-mo old) C57BL/6J mice were divided into 3 groups: control (C), 10-day hind limb unloading (H) and H with 3-days of recovery/reloading (R). Following treatments, mice were sacrificed by CO_2 asphyxiation. Hind limb muscle groups were harvested, weighed and snap frozen. Quantification of CL and CER species in the left soleus was performed by mass spectrometry-based shotgun lipidomics. Permeabilized fiber bundles were prepared from the right soleus to measure mitochondrial respiration, H_2O_2 emission and CRC. **Results:** OC mice were sarcopenic, as evidenced by lower soleus, gastrocnemius and quadriceps mass, compared to YC. A similar degree of atrophy and mitochondrial dysfunction was evident in the soleus of both OH and YH groups following 10-days of unloading. However, a decrease in total cardiolipin content and distinct remodeling of individual molecular species was only evident in the YH group. Moreover, C18:0 CER content increased in the YH group, and C18:0, C23:0, and C24:1 CER content was greater in YH, compared to the OH group. Following 3 days of reloading, the YR group recovered soleus mass. There was no recovery of soleus mass for OR. Mitochondrial respiration, H_2O_2 emission, CRC, and total cardiolipin content also recovered in the soleus of YR, but not OR. **Conclusion:** Alterations in profile of CL and CER species, recently identified as important mediators of mitophagy, occur in soleus from young mice during unloading. This in turn accompanies and perhaps facilitates effective recovery of muscle mass and mitochondrial function with reloading. This adaptive response was not evident in sarcopenic muscle. **Grant Support:** This research was supported by grants from National Institute of Aging (AG044437-PMC) and Diversified Translational Laboratory funding from Sanford-Burnham.

7.22
MITOCHONDRIAL DNA CHANGES AND DYSFUNCTION IN DIABETIC NEPHROPATHY

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The hypothesis underlying this work is that changes in circulating mitochondrial DNA (MtDNA) and subsequent mitochondrial dysfunction are key players in the pathophysiology of diabetic nephropathy (DN). To investigate this we examined the quantity and function of circulating MtDNA in patients with DN. In a cross-sectional study (N=168), the samples were studied as 3 groups: Healthy controls (HC, N=40), Diabetics without DN with more than 20 years duration of diabetes, no history of albuminuria or signs of diabetic retinopathy (DC, N=45) and diabetic nephropathy patients with a history of or current albuminuria (DN, N=83). Whole blood DNA was isolated, MtDNA content was determined as the mitochondrial to nuclear genome ratio (Mt/N) using real time qPCR. PBMCs were used for bioenergetic assessment profile using Seahorse XF^{e}96 analyser. Circulating MtDNA was increased in DC (64 ±75) compared to HC (34 ± 9, P<0.05) whereas the DN patients had reduced MtDNA (43 ± 52) compared to DC (P<0.001). PBMCs from DN patients had reduced reserve capacity and maximal respiration, loss of metabolic flexibility and reduced Bioenergetic Health Index (BHI). Our data shows that patients with DN have impaired mitochondrial metabolism and support the hypothesis that alterations in MtDNA content and mitochondrial dysfunction are involved in the development of DN.

7.23
COMBINED AMPK AND PPARA AGONISM IMPROVES EXERCISE PERFORMANCE IN TRAINED MICE

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Exercise training improves muscle function and fatigue resistance during endurance exercise. AMPK and PPARδ agonists have been shown to mimic these effects in

sedentary and mdx model mouse however, their effects together with endurance exercise training in healthy animals have not been reported. We determined the effects of the said agonists in conjunction with exercise training on endurance exercise performance, energy substrates, gene transcription, and mitochondrial density in healthy mice. **General methods:** Male 7 week-old Balb/c mice underwent treadmill training and were administered the following for 1mo: Vehicle (V), PPARδ agonist GW0742 (G), AMPK agonist AICAR (A) or both (A+G). A non-exercised control (SED) received the vehicle. Groups were further divided into before-exercise and exercise-to-exhaustion groups. Indirect calorimetry in sedentary state (24h) and during exercise was conducted 2 and 3days, respectively after the final treatment. Mice were sacrificed after indirect calorimetry and biochemical assays conducted. **Results:** Exercise groups had improved running performance (work output). Both A and A+G had better performance with the latter significantly exceeding all groups. Treatments influenced neither the sedentary nor early-phase running indirect calorimetry parameters. Analysis of indirect calorimetry data 30min before the point of exhaustion onward showed significant changes in RQ, an increase in fatty acid oxidation with a concomitant decrease in carbohydrate oxidation in A+G but without affecting total energy expenditure. Serum glucose was elevated in A+G before exercise and depletion was observed in exercise groups at exhaustion. Serum triglycerides and non-esterified fatty acids were relatively similar among groups before and after exhaustion. Muscle glycogen was elevated in A+G before exercise and a general decrease was observed in all groups at exhaustion. Interestingly, muscle glycogen in A and A+G did not decrease significantly. Muscle FFA was similar before exercise but an increase especially in A+G was observed at exhaustion. Absolute liver glycogen before exercise tended to decrease in exercise groups except for A+G which was similar to SED. This was depleted in exercise groups but not in SED at exhaustion. Muscle PGC1α, LPL, and PDK4 mRNA were elevated while increased PGC1α and decreased CHREBP mRNA in the liver was observed in A+G. PGC1α protein in the muscle was increased in all exercise groups. Finally, mitochondrial DNA copy number was similar among groups but mitochondrial density as measured by citrate synthase activity was elevated in both A and A+G. **Conclusion:** Combined AMPK and PPARδ agonism improves exercise performance in trained mice by increasing mitochondrial density, increasing available energy substrates with improved fuel switching to fat thereby delaying the onset of hypoglycemia known to cause exercise cessation.

7.24
LIPID DROPLETS INTERACT WITH AN EXCLUSIVE SUB-POPULATION OF MITOCHONDRIA IN BROWN ADIPOCYTE

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We have recently shown that mitochondrial dynamics is a physiological regulator of adrenergically-induced changes in energy expenditure and lipid metabolism in brown adipocyte (BA). In this study we addressed the hypothesis that local changes to mitochondrial dynamics at subcellular levels play a role in generating sub-populations of mitochondria with specialized functions. Confocal microscopy of brown adipocytes harvested from wild-type C57BL6/J mice identifies the mitochondria surrounding the lipid droplets as an exclusive sub-population of mitochondria. Peri-droplret mitochondria (PDM) in BA are more elongated as compared to cytosolic-mitochondria (CM) (average length of PDM and CM are 4.9 and 3.2μm respectively). This form of mitochondria is more commonly associated with coupled respiration. Indeed, measurements of membrane potential and NADH show that PDM have higher mitochondrial membrane potential and higher NADH content. Interestingly the heterogeneity in membrane potential can be manipulated by mitochondrial dynamic protein expression and by hormonal stimulation. As the level of coupling and ATP synthesis in BA may be influenced by the levels of ATP synthase, which is expressed at very low levels in this tissue, we tested the ATP synthase expression in this specific set of mitochondria. Immune fluorescence and super-resolution microscopy indicated that ATP synthase in the brown adipocyte is concentrated in PDM. Since mitochondrial composition has been shown in other cells to be equilibrated and homogenized by continuous mitochondrial fusion and fission, we next measured the levels of fusion and equilibration across the mitochondrial population in the BA. Using fusion assay and matrix-targeted PAGFP we found that following photo-conversion the fluorescent intensity of PAGFP in the matrix of PDM stays ~10% higher, 65 min after photo activation in PDM, indicating lower fusion activity. Ratiometric analysis of MitoTimer probe showed that PDM have higher protein imports and protein content, but similar rate of protein turnover, indicating that PDM do not equilibrate their content with the rest of the mitochondrial population. This finding is also supported by their higher Tomm20 content. In conclusion, PDM represent a sub-population of mitochondria with unique protein composition, architecture and activity that is likely maintained due to their reduced level of interaction with the rest of the mitochondrial population.

7.25
MITOCHONDRIA DNA IS DAMAGED IN MILITARY VETERANS WITH FATIGUING CONDITIONS

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Background: Gulf War illness (GWI) is the most prominent health issue affecting veterans of the 1990-1991 Gulf War, with 1 in 4 Gulf War Veterans suffering from GWI. GWI is characterized by multiple diverse symptoms of fatigue, muscle pain and cognitive dysfunction that are suggestive of mitochondrial impairment. Military exposures during deployment have been put forward in the etiology of GWI, many of which have been identified as genotoxins. To date, mitochondrial dysfunction in veterans with GWI has only been described indirectly. **Objective:** The goal of this study was to access more direct evidence of mitochondrial dysfunction in GWI, by measuring mitochondrial DNA (mtDNA) content and mtDNA damage in peripheral blood mononuclear cells (PBMCs) in veterans with GWI. **Methods:** Twenty-five veterans with GWI and six non-deployed healthy controls were recruited and provided PMBCs for total DNA extraction. Relative mtDNA copy number (i.e. mtDNA content) and mtDNA damage were determined by quantitative polymerase chain reaction. **Results:** Veterans with GWI had significantly higher mtDNAcn ($t=2.820$, $p=0.008$) and mtDNA damage ($t=2.037$, $p=0.051$) than non-deployed controls. **Conclusions:** This is the first study to report direct evidence of higher mtDNAcn and mtDNA damage in veterans with GWI, supporting prior indirect evidence of mitochondrial dysfunction in this group. Future studies are necessary to confirm these findings and determine their association with mitochondrial function. In addition, work in this area may guide new diagnostic testing and treatments for veterans suffering from GWI.

7.26
STATIN MYALGIC PATIENTS HAVE IMPAIRED MITOCHONDRIAL RESPIRATORY FUNCTION IN SKELETAL MUSCLE

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Introduction: Statins reduce endogenous cholesterol synthesis, and is widely consumed to decrease the risk of cardiovascular events. However, statin therapy is associated with development of side effects, such as muscle ache and pain (myalgia), but the mechanism is unknown. It has been shown that statins decrease the mitochondrial function in skeletal muscle, but the aim of this study was to investigate if statin induced myalgia is coupled with impaired mitochondrial respiratory function in human skeletal muscle and adipose tissue. **Methods:** Two groups of healthy adults in continuous simvastatin treatment (40 mg/day) were recruited for this study. One group (n=11, 5m/6f) experienced myalgia (SM) with a VAS score of 4.7±0.7, and the other group (n=13, 6m/ 7f) without side effects (VAS 0.3±0.2) served as controls (SC). The groups were matched for age, BMI, and VO2max (59±2 vs 61±2 yrs, 29±2 vs 29±1 kg/m2 and 26±5 vs 28±6 ml O2/min/kg, respectively). Mitochondrial respiration was measured in permeabilized muscle fibers and subcutaneous adipose tissue, using high-resolution respirometry. The protocols investigated maximal mitochondrial respiration with electron flow through complex (C) I, CII, CI+II (OXPHOS capacity), and electron transport system (ETS) capacity. **Results:** In skeletal muscle, maximal mitochondrial CII respiration and ETS capacity were lower in SM compared to SC (49±2 vs 55±1 (P=0.004) and 72±3 vs 84±3 (P=0.004) pmol O_2/mg/s, respectively), and OXPHOS capacity tended to be lower in SM (63±3 vs 70±3 (P=0.076) pmol O_2/mg/s, respectively). In adipose tissue, ETS capacity was lower in SM compared to SC (2.1±0.1 vs 2.4±0.1 (P=0.036) pmol O_2/mg/s, respectively), and females (n=13) had higher mitochondrial respiration through CI, and CII, OXPHOS capacity and ETS capacity, compared to men (n=11). The myalgic females (n=6) had a lower CII respiration, OXPHOS capacity and ETS capacity, compared to female controls (n=7). **Discussion:** We demonstrate, that statin induced myalgia is coupled to impaired mitochondrial function in skeletal muscle. Interestingly, mitochondrial respiration in adipose tissue was impaired in statin myalgic females, but not males. Since statins reduce synthesis of Ubiquinone (Q10) as well as cholesterol, reduced mito-

chondrial Q10 may the responsible for statin induced impaired mitochondrial function. However, more research is needed to establish the exact mechanism behind statin induced myalgia. **Funding:** This study is funded by the UCPH 2016 Funds.

8.0 ENERGY SCHOOL II

8.1
THE LACTIC ACIDOSIS CONSORTIUM: A MULTI-DISCIPLINARY RESEARCH EFFORT TO TRANSLATE GENE DISCOVERY INTO BETTER MANAGEMENT AND TREATMENT FOR PATIENTS WITH MITO-CHONDRIAL DISORDERS

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Mitochondrial dysfunction is involved in a large number of human pathologies, including a broad spectrum of rare but usually severe genetic mitochondrial diseases. While recent advances in next generation sequencing have led to considerable progresses in the identification of the molecular and biochemical defects underlying a number of these genetic diseases, they have not translated into major improvements of patient management. This can be explained by the existence of numerous barriers including: *i)* limited information on the impact of the various mutations affecting the oxidative phosphorylation system (OXPHOS) on the mitochondrial, metabolic, and signaling phenotype, and hence, on the underlying pathogenic mechanisms, *ii)* difficulty in choosing therapeutic strategies in absence of a detailed phenotypic signature, and *iii)* lack of clinical tools or biomarkers, which are indicative of disease progression, clinically relevant outcomes, and impact of treatments. This presentation will provide an overview of the research effort made by our multidisciplinary research consortium to meet these challenges in patients with Leigh Syndrome French Canadian (LSFC), a severe disease prevalent in the Saguenay-Lac-St-Jean region in Quebec, which is caused by mutation of LRPPRC, a protein involved in the translation of mitochondria-encoded polypeptides of the OXPHOS system. (Funded by CIHR. References: Burelle et al. & LSFC consortium (2015). PLoS ONE, 10(3), e0120767; Sasarman, et al. & LSFC consortium (2015). Human Molecular Genetics, 24(2)).

8.2
MITOCHONDRIAL DNA CONTENT: ACCURATE MEASUREMENT AND EVALUATION AS AN EARLY BIOMARKER OF MITOCHONDRIAL DYSFUNCTION

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The amount of mitochondrial DNA per cell is dependent on the cell's bioenergetic requirements, under normal conditions there is a correlation between MtDNA content, MtmRNA and mitochondrial function. However we found that in conditions of disease, for example in diabetic cells, there is a disconnect between the amount of MtDNA and MtmRNA and cellular bioenergetics (1). Increasingly greater numbers of studies are reporting alterations in MtDNA in disease conditions (2), however the methodology being employed is leading to confusion in the field because of two major issues, the presence of nuclear pseudogenes with high homology to the mitochondrial genome and dilution bias caused by the differing sizes of the nuclear and mitochondrial genomes. In this talk I will explain how to accurately measure MtDNA content and describe data showing that adaptive changes MtDNA are an early event in disease suggesting that MtDNA content could be an early biomarker of mitochondrial dysfunction. References: 1. Czajka, Ajaz, Gnudi , and Malik (2015) Altered mitochondrial function, mitochondrial DNA and reduced metabolic flexibility in patients with diabetic nephropathy. EBiomedicine, in Press. 2. Malik and Czajka (2012) Is mitochondrial DNA content a biomarker of mitochondrial dysfunction. Mitochondrion. 3. Malik et al., (2011) Mitochondrial DNA as a non-invasive biomarker: Accurate quantification using real time quantitative PCR without co-amplification of pseudogenes and dilution bias Biochemical and Biophysical Research Communications 412 (2011) 1–7.

9.0 MITOCHONDRIAL ADAPTATION AND SUSCEPTIBILITY TO STRESS

9.3
MITOCHONDRIAL FUEL SUBSTRATE SWITCHING AND THE EXCITABLE BRAIN

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Altered fuel utilization by the brain has profound effects on neuronal activity as evident from the seizure protective effect of diets that reduce glucose utilization and promote ketone body metabolism[1]. However, the molecular underpinnings of this glucose-to-ketone body fuel switch have remained elusive due to the complex effects of dietary manipulations. Understanding the molecular details underlying metabolic control of neuronal activity will be greatly aided by identification of cell-intrinsic regulators that can trigger a glucose-to-ketone body fuel switch in the brain without any dietary manipulation. We have found that BAD, a protein with dual roles in metabolism and apoptosis, imparts cell-autonomous reciprocal effects on glucose and ketone body consumption in neurons and astrocytes independent of its apoptotic function[2]. BAD modifications that trigger a glucose-to-ketone body fuel switch produce a marked increase in the activity of metabolically sensitive K_{ATP} channels and resistance to behavioral and electrographic seizures *in vivo*[2]. Seizure resistance is reversed by ablation of the K_{ATP} channel, implicating the BAD-K_{ATP} axis in metabolic control of neuronal excitation. Studies are underway to define the BAD-dependent metabolic alterations that mediate changes in neural fuel preference. This will provide a molecular handle on regulation of fuel choice in the brain and potentially reveal therapeutic strategies for the control of neuronal excitation in seizure disorders. This work is funded by NIH grant R01 NS083844. 1. Lutas A, and Yellen G, Trends Neurosci, 2013. 36:32-40. 2. Gimenez-Cassina, A., et al., Neuron, 2012, 74:719-30.

11.0 IT'S NOT JUST THE ATP! SIGNALING AND MITOCHONDRIAL FUNCTION

11.1
MITOCHONDRIA MATTER: TARGETING MITOCHONDRIAL FUNCTION IN TUMOR CELLS

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The tumor cell Warburg effect is associated with increased glycolysis even in the presence of ample oxygen, and has dominated the cancer metabolism field for the last two decades. More recently it has been found that tumor cells our metabolically heterogeneous and can switch from glycolysis to oxidative phosphorylation depending on environmental conditions surrounding the tumor. Some forms of cancers, such as ovarian tumor cells, utilize fatty acids from adipocytes to fuel beta-oxidation in the mitochondria. We therefore have developed several approaches to attack mitochondrial function and inhibit tumor cell growth. We have found that the CPT1 inhibitor, etomoxir, greatly decreases oxygen consumption in melanoma, breast and ovarian cancer cells, and given after cisplatin treatment acts synergistically to inhibit ovarian cancer cell growth. We have also found that mitochondrial division inhibitor-1 (midivi-1), which has been suggested to inhibit Drp1 and therefore block mitochondrial fission, causes G2/M arrest in tumor cells, but not normal cells. Furthermore we have found that mdivi-1 synergistically increases cisplatin potency by causing both a Drp1- and Bax/Bak- independent release of cytochrome C and subsequent cell death. Tumor cells also display increased reactive oxygen species (ROS) and are in a pro-oxidant state and we have developed a proof of principle approach based on specific targeting of ROS to the mitochondria by fusing reactive oxygen generating fluorescent protein, KillerRed to TFAM. While this approach is effective, creating stable cell lines has been difficult. To overcome this problem we have developed a novel protein-dye system, that when activated by light can directly deliver singlet oxygen into the mitochondrial matrix. This approach greatly diminishes mitochondrial function within hours of damage. Finally we have developed a novel mitochondria-targeted lapachone using the alkene peptide isostere segment of the antibiotic gramicidin S (XJB-peptide). We have found that XJB-lapachone causes rapid loss of mitochondrial function and catastrophic vascularization of the cellular contents leading to rapid cell death in tumor cells. Together these results suggest that mitochondria are critical targets in cancer therapy. Work supported by GM102989 and GM067082 (PW) and funding from the Univ. of Pittsburgh Cancer Institute (BVH) and the PA CURE (BVH).

11.2
TICK, TOCK – THE BIOLOGICAL CLOCK CONTROLS THE POWERHOUSE

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Many metabolic processes oscillate during the day, enabling organisms/tissues/cells to remain in synchrony with their environment. Studies report that 5-20% of the liver transcriptome oscillates during the day, affecting many metabolic networks. Daily rhythms in metabolism tend to align with, but are not dependent on, sleep/wake and feeding/fasting cycles. In fact, many metabolic rhythms are partially controlled by a cell autonomous intrinsic circadian oscillator or 'clock'. The molecular clock allows cells to rapidly respond to environmental stimuli or stresses by adapting metabolism in a temporally appropriate manner. Because of the tight connection between circadian rhythms and metabolism, it has been proposed that mitochondrial functions may also be regulated by the clock. While some mitochondrial functions (e.g., carbohydrate and fatty acid oxidation) may be circadian regulated, very little is known regarding time-of-day dependent changes in bioenergetics, and the importance of the molecular clock in this vital process. New data will be presented showing that integral bioenergetic components (i.e., respiratory complex activities) exhibit diurnal rhythms in the liver; thus, supporting the concept that the bioenergetic machinery of the mitochondrion is regulated by the molecular clock. To underscore the importance of the molecular clock in regulating these processes, results will be presented from studies using a hepatocyte-specific BMAL1 knockout mouse model, in which the clock is nonfunctional in hepatocytes. Finally, Dr. Bailey will provide data supporting the concept that a critical mechanism underpinning alcohol-induced mitochondrial dysfunction and tissue injury is a failure in fundamental clock-driven adaptive processes in the alcohol-exposed liver.

11.3
MITOCHONDRIAL TELOMERASE AND VASODILATION

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TERT, the catalytic subunit of telomerase elongates telomeres to prevent cellular aging. A potential role in the development of cardiovascular disease (CVD), especially the vascular endothelium has not been described. A mitochondrial role for TERT in regulating reactive oxygen species ($_{mt}$ROS) has been shown positioning TERT as a key regulator of oxidative stress. Under physiological conditions blood flow stimulates endothelial release of nitric oxide (NO), mediating flow-mediated dilation (FMD) suppressing vascular smooth muscle proliferation and inflammation. In subjects with coronary artery disease (CAD) arterioles no longer produce NO during shear but $_{mt}$ROS (H_2O_2). Interestingly acute exposure to increased intraluminal pressure (IILP) also triggers this compensatory switch whereas exposure to the physiological stressor ANG II only evokes reduced NO mediated dilation without a compensatory rise in $_{mt}H_2O_2$. We tested whether TERT plays a critical role in maintaining physiological NO levels and in term preventing the transition from NO to $_{mt}H_2O_2$ as the mediator of FMD during vascular stress. a protective effect of telomerase is underlined by recant work showing that acute up-regulation of TERT is sufficient to reduce damage caused by myocardial infarction[1], while depletion of telomerase leads to systolic hypertension concomitant with telomere shortening[2]. The natural occurring dominant-negative splice variant of TERT (b-del) is increased in most human cancers[3], which predominantly produce energy by glycolysis rather than mitochondrial oxidative phosphorylation. We have found and increase in b-del TERT in heart tissue from subjects with CAD generating a direct link to mitochondrial dysfunction and $_{mt}$ROS production. We propose an inverse mechanistic relationship between $_{mt}$ROS production and telomerase activity. Grants: R01-HL-113612, R21-OD-018306. Reference: Bär C et al. Nature Communications. 2014;5; Perez-Rivero G., et al. Circulation. 2006;114:309; Listerman I, et al. Cancer Res. 2013;73:2817-2828.

12.0 POSTER SESSION II

12.1
ASSESSMENT OF PERIPHERAL MITOCHONDRIAL DNA DAMAGE AND DYSFUNCTION AS A BIOMARKER OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder. Even with expert treatment, PD patients typically deteriorate over time and endure considerable motor and non-motor disability in the years after diagnosis. Currently, no cures or disease modifying therapies exist for PD. This is partially due to the inability to detect the disease before it has progressed to a stage of significant dopaminergic neuronal loss resulting in movement symptoms; this highlights the critical unmet need to identify and validate biomarkers to measure disease status and progression. PD is increasingly being viewed as a systemic illness that affects tissues outside the brain and nervous system. While it is generally accepted that mitochondrial alterations in the brain play a role in the pathogenesis of PD, systemic mitochondrial defects have also been strongly implicated. The over-arching goal of this work is to explore and validate blood mitochondrial DNA (mtDNA) damage and mitochondrial bioenergetics as potential biomarkers of PD – which has not been previously been assessed or considered. In blood buffy-coat derived samples from a pilot study, using a quantitative polymerase chain reaction (QPCR)-based assay that measures multiple forms of mtDNA damage, we found significantly increased mtDNA damage in PD subjects compared to age-matched healthy controls. Strikingly, levels of mtDNA damage in PD subjects correlated with parameters of clinical motor signs. In a separate study evaluating platelets, a significant decrease in mitochondrial reserve capacity was observed in PD subjects, and this correlated with clinical non-motor symptoms. Currently in an expanded study, mtDNA damage and bioenergetics from enriched platelets and leukocytes samples in control and PD subjects will be assessed along with clinical features longitudinally. This will allow us to simultaneously evaluate multiple mitochondrial markers and clinical symptoms within a single individual, compare between control and PD groups and evaluate the utility of each cell-type as a biomarker of PD. Funding sources include: William N. and Bernice E. Bumpus Foundation Innovation Award, Mitochondria, Aging and Metabolism/Basic Biology Aging Pilot Project Program, and Pittsburgh Claude D. Pepper OAIC.

12.2
THE DRP-1 INHIBITOR MDIVI-1 PREVENTS COMPENSATORY MITOCHONDRIAL H_2O_2-MEDIATED VASODILATION INDUCED BY CERAMIDE TREATMENT IN HUMAN ADIPOSE ARTERIOLES

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Background: The sphingolipid ceramide has been shown to increase mitochondrial reactive oxygen species and is elevated in the plasma of patients at risk for heart disease. Previously we have shown that incubating human adipose arterioles with C2-ceramide inhibits nitric oxide (NO) as the mediator of flow induced dilation (FID), allowing mitochondrial-derived hydrogen peroxide (H_2O_2) to compensate. Ceramide also induces mitochondrial fission in cell culture by upregulating dynamin related protein 1 (DRP-1), however it is not known if ceramide-induced fission is critical for the switch in mediator of FID to occur. **Hypothesis:** Inhibiting DRP-1 in human adipose microvessels prevents the compensatory H_2O_2-mediated dilation that occurs when NO is suppressed following ceramide treatment alone. **Methods:** Discarded human adipose tissue was obtained at the time of surgery, microvessels (100-200 μm in diameter) were cannulated in an organ chamber, and the internal diameter was measured via video microscopy. FID was assessed during graded increases in intraluminal flow ± the NO synthase inhibitor L-NAME (100 μM) or the H_2O_2 scavenger polyethylene (PEG) catalase (500 U/mL). Vessels were incubated for 16-20 hours at 37°C with C-2 ceramide (10 μM) ± Mdivi-1 (50 μM). MitoPY1, a fluorescent probe specific for mitochondrial H_2O_2, was used to quantify changes in mitochondrial H_2O_2 during flow. **Results:** Immunohistochemistry showed increased DRP-1 expression in microvessels treated with ceramide vs. vehicle. Mdivi-1 treatment alone had no effect on FID (max dilation 75 ± 5.6% vs. vehicle 76 ± 5.1%) which was mediated by NO since L-NAME abolished dilation (max dilation 4 ± 2.6%) and PEG-catalase had no effect (max dilation 91 ± 2.2%). Vessels treated with Mdivi-1 + ceramide did not dilate to flow (max dilation 6 ± 5.6%) and reduced generation of mitochondrial H_2O_2 was observed (the increase in Mito-PY1 fluorescence with ceramide alone was 86 ± 31% compared to baseline, but with Mdivi1 + ceramide only an 11 ± 14% increase was seen). **Conclusion:** Treating human adipose vessels with the DRP-1 inhibitor Mdivi-1 prevented compensatory, mitochondrial H_2O_2 – mediated dilation from occurring in response to ceramide treatment, suggesting that mitochondrial fission may be necessary for this process to occur. This work was supported by National Institutes of Health HL113612-02 (DDG) and American Heart Association 14POST18780022 (MJD).

12.3
MITOCHONDRIAL OXYGEN CONSUMPTION IS REDUCED IN CEREBRAL ARTERIES BY DISTANT ISCHEMIA.

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Ischemic stroke is a leading cause of morbidity and mortality. Several studies have indicated that mitochondrial dysfunction plays an important role in the pathophysiology of stroke but the exact mechanisms are not clear. Our laboratory has made the novel and surprising finding that both mitochondrial protein mass and mitochondrial-derived vasodilation of the middle cerebral artery are intact on the side ipsilateral (Ipsi) to transient middle cerebral artery occlusion (MCAO) while severely reduced on the side contralateral (Contra) to MCAO. However, mitochondrial respiration following MCAO is unknown. We examined mitochondrial oxygen consumption rate (OCR) in MCAs following MCAO or sham operation and correlated it with mitochondrial DNA encoded proteins and MnSOD expression. Eight to ten week old, male, Sprague-Dawley rats were exposed to 90 min of ischemia and 48 h of reperfusion using the filament method, while the sham animals received anesthesia without filament insertion. The Seahorse Bioscience XFe24 analyzer was used to measure OCR in isolated MCAs following experimental stroke or sham operations using oligomycin, FCCP, antimycin, and rotenone. Western blotting was used to determine protein expression in the arteries. The protein normalized OCR (pmol/min/μg protein) was significantly ($p < 0.05$) decreased in Contra MCAs compared with Ipsi and sham, with no statistical differences between Ipsi and sham. The basal respiration (128 ± 15), ATP production (49 ± 6), proton leak (68 ±10), maximal respiration (238 ± 23), and the non-mitochondrial respiration (31 ± 3) were decreased in Contra compared with Ipsi and sham MCAs (196 ± 13; 83 ± 6; 114 ± 10; 333 ± 14; and 56 ± 3, respectively). All of the beta-actin normalized protein levels of Complex-II, III, IV, and MnSOD but not Complex-I, were higher in Ipsi MCAs (136 ± 3; 104 ± 10; 35 ± 4; and 149 ± 15, respectively) compared with Contra (121 ± 6; 90 ± 3; 13 ± 2; and 82 ± 16, respectively). These results extend our previous findings that mitochondrial function in Ipsi MCAs is preserved while it is severely impaired in Contra MCAs. Furthermore, our results indicate side specific therapies may be appropriate. This work was supported by NIH grants HL-077731 and HL093554, AHA grants 14SDG20490359 and 15POST23040005, and the Louisiana Board of Regents Support Fund-Research Competitiveness Subprogram LEQSF(2014-17)-RD-A-11.

12.4

ROLE OF O-GLCNACYLATION IN REGULATING MITOPHAGY AND MITOCHONDRIAL FUNCTION IN CARDIOMYOCYTES

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The post-translational modification of proteins by O-linked-N-acetylglucosamine (O-GlcNAc) has been implicated to play a role in cardiovascular disease and mitochondrial function. Acute increases in O-GlcNAcylation have been shown to protect cardiomyocytes against oxidative stress which has been associated with O-GlcNAcylation of mitochondrial proteins. We have previously shown that acute increases in O-GlcNAc attenuate loss in mitochondrial membrane potential. Autophagy, a highly conserved cell survival mechanism, is activated in the heart in response to stress. O-GlcNAc has also been reported to increase in response to cellular stress. Mitophagy, a specific type of cargo-mediated autophagy, is critical in removing damaged mitochondria; therefore the goal of this study was to determine whether O-GlcNAcylation was involved in regulating mitophagy. AC16 cells, an immortalized human cardiac cell line, were treated with 2,3-dimethoxy-1,4-napthoquinone (DMNQ, 20 μM), a redox cycling agent which increases mitochondrial reactive oxygen species (ROS) to induce mitophagy. Protein levels of PINK1, a mitochondrial kinase which signals Parkin recruitment to damaged mitochondria for mitophagy initiation, were measured following 3 hours of DMNQ treatment. Initial studies revealed that DMNQ treatment decreased O-GlcNAc levels and increased levels of total PINK1. Increasing basal O-GlcNAc levels for 2 hours with Thiamet-G (3 μM), a highly selective inhibitor for O-GlcNAcase, the enzyme that catalyzes the removal of O-GlcNAc moieties from proteins, attenuated the loss of O-GlcNAc induced by DMNQ treatment and increased basal PINK1 levels. Preliminary studies have shown PINK1 is O-GlcNAc modified and Thiamet-G treatment increases this O-GlcNAc modification. Using the Seahorse Extracellular Flux Analyzer, to measure mitochondrial function, we demonstrated that DMNQ treatment increases basal and maximal oxygen consumption but decreases the reserve capacity of the mitochondria. Treatment with Thiamet-G following DMNQ and 4-Hydroxnonenal (HNE, 30μM), lipid peroxidation product, yielded an increase in reserve capacity of the mitochondrial oxygen consumption. These results support the concept that protein O-GlcNAcylation may play a role in regulating mitophagy and protection against oxidative stress. I would like to thank the members of the Chatham and Darley-Usmar labs for their assistance and support with this project. This work is supported by NIH grant (HL101192).

12.5

IMPAIRED CARDIO-SKELETAL MUSCLE ENERGETICS IN CHILDREN WITH BARTH SYNDROME: A 31P MRS STUDY

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Background: Barth syndrome (BTHS) is a rare inherited disease caused by mutations in the tafazzin gene resulting in cardiolipin remodeling, mitochondrial abnormalities, cardio-skeletal myopathy and early mortality. Whole-body oxidative metabolism and cardiac function have been previously reported in BTHS; however, in vivo cardio-skeletal energetics and the relationship to heart and skeletal muscle function in children with BTHS are not known. Objective: To quantify cardio-skeletal energetics and examine the relationship with cardio-skeletal muscle function in children with BTHS. Methods: Eleven participants with BTHS and 12 healthy, age-matched controls underwent 31P magnetic resonance spectroscopy (MRS). Myocardial energetic reserve was determined by phosphocreatine to adenosine triphosphate ratio (PCr/ATP). For skeletal muscle energetics, participants performed plantar flexion exercise at a frequency of 0.5Hz at 30% maximal voluntary contraction for 60 sec. Skeletal muscle (gastroc-soleus) phosphocreatine (PCr) recovery kinetics were used to determine maximal oxidative capacity. Student's t-tests were used to compare the two groups and Pearson's product correlations in all participants were used to examine the relationships between cardio-skeletal energetics and function. Results: Myocardial and skeletal muscle energetics were lower in children with BTHS compared to age-matched controls. Lower myocardial PCr/ATP ratio tended to correlate with lower cardiac fractional shortening ($r=0.40$, $p=0.065$). Lower skeletal muscle oxidative ATP production was significantly associated with lower whole-body peak oxygen consumption during graded exercise testing ($r=0.73$, $p<0.001$).

	Control (n=12)	BTHS (n=11)	p value
Age (yrs)	12.8 ± 2.7	12.2 ± 2.4	0.60
Weight (kg)	52.1 ± 19.0	32.3 ± 9.1	0.01
Fat-free mass (kg)	43.0 ± 15.7	28.8 ± 13.7	0.04
PCr/ATP	2.1 ± 0.3	1.5 ± 0.2	< 0.001
Fractional Shortening (%)	40.3 ± 5.9	30.8 ± 10.2	0.01
Qmax-linear (mM ATP/s)	1.0 ± 0.2	0.6 ± 0.1	< 0.001
Peak VO_2 (ml/kg/min)	35.8 ± 8.8	14.2 ± 3.2	< 0.001

Conclusions: Cardio-skeletal oxidative function in children with BTHS is significantly impaired. This likely contributes to decreased cardiac function, exercise intolerance and fatigue in these individuals. Interventions to augment oxidative function may improve heart function, exercise tolerance and reduce fatigue in BTHS.

12.6

METABOLIC AND BIOENERGETIC CHARACTERIZATION OF A NON-ISCHEMIC MOUSE MODEL OF HEART FAILURE

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Mitochondrial dysfunction has been implicated as a cause for energy deprivation in heart failure (HF). Much of our understanding of the metabolism and bioenergetics in HF derives from animal models of myocardial ischemia. In this study, we characterized the metabolic and cardiac mitochondrial function in a mouse model of non-ischemic HF. Male C57BL6/J mice (12 weeks old) were administered L-N^G-Nitroarginine methyl ester (L-NAME, 0.3 mg/ml with 1% NaCl) in the drinking water, or Angiotensin II (AngII, 0.7 mg/kg/day) via subcutaneous osmotic pumps or a combination of both (L-NAME+AngII) for 5 weeks. Cardiac function, protein expression, mitochondrial reactive oxygen species production and mitochondria function in isolated mitochondria and permeabilized fibers in response to pyruvate-malate (PM), palmitoylcarnitine-malate (PC), succinate and glutamate-malate (GM) substrates were measured. ADP-independent, substrate-dependent respiration rates (state 2),

ADP-supported respiration rates (state 3) and respiratory control ratio (RCR=state 3/state2) were calculated. Compared with L-NAME or AngII treatment alone, L-NAME+AngII induced the most severe phenotype of HF characterized by edema, hypertrophy (increased heart weight:tibia length ratio), fibrosis, increased blood pressure and reduced ejection fractions to <40%. L-NAME+AngII treated mice had robust deterioration of cardiac mitochondrial function, as observed by reduced RCR for PM, PC and GM but not for succinate in subsarcolemmal mitochondria. In interfibrillar mitochondria, only state 3 rates were significantly reduced in the L-NAME+AngII group versus controls for PM, PC and GM but not for succinate. However, mitochondrial membrane potential was not significantly different among the 4 groups. Cardiac myofibrils from L-NAME+AngII mice had reduced ADP-supported oxygen consumption, uncoupled respiration and oligomycin rates for PM+succinate. Further, mitochondrial DNA content was reduced in AngII and L-NAME+AngII hearts. Production of reactive oxygen species (H2O2) was the highest in AngII and L-NAME+AngII groups. Phospho-AMPK/AMPK was reduced in hearts of L-NAME and L-NAME+AngII groups. We conclude that combination of L-NAME+AngII exacerbates cardiac contractile and mitochondrial functional deregulation compared with L-NAME and AngII alone, resulting in non-ischemic HF.

12.7

MITOCHONDRIAL FUNCTIONS IN THE REGULATION OF EFFECTOR MACROPHAGE IN CORONARY ARTERY DISEASE

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Macrophages play a pivotal role in coronary artery disease (CAD). Cytokine production is one of the main effector functions of the macrophages. Cytokines trigger local inflammation in arteries and thus contribute to the progression of CAD. There is evidence that mitochondrial functions play a regulatory role in effector macrophages. We hypothesized that mitochondrial reactive oxygen species (ROS)-dependent signaling regulates cytokine production in hyperinflammatory CAD macrophages. We used monocyte-derived macrophages from CAD patients and healthy subjects to characterize basic mitochondrial functions, including cellular respiration, mitochondrial membrane potential, ROS production and mitochondrial morphology. We found that CAD macrophages have distinctive fragmented mitochondria when compared to controls indicating functional alterations. In fact, we found that resting (M0) and activated (M1) macrophages show significant differences in metabolic and mitochondrial functions. CAD macrophages had a higher metabolic rate associated with a higher uptake of glucose. This resulted in higher basal and maximal mitochondrial respiration. In line with this observation, we found over 30% higher membrane potential and mitochondrial ROS production in CAD. Increased mitochondrial ROS production in CAD macrophages depleted reduced glutathione by over 50%. Next, we analyzed detailed molecular mechanisms behind observed dysfunctions and identified a main regulatory mechanism in the mitochondria responsible for the ROS overproduction. We also investigated what are molecular targets for ROS released from the mitochondria and its functional consequences. Increased ROS production and mitochondrial metabolism induced a proinflammatory phenotype in CAD macrophages, including intensified Il-6 and Il-1b production. Manipulations of mitochondrial ROS by targeting hydrogen peroxide with mitoEbelen reversed changes in effector functions of macrophages. Correcting mitochondrial dysfunctions by pharmacological intervention also reversed the proinflammatory phenotype in CAD macrophages. Taken together our study indicates a critical role for mitochondria in the regulation of effector functions and a proinflammatory phenotype in CAD macrophages.

12.8

MITOCHONDRIAL PERMEABILITY TRANSITION DRIVES ROS GENERATION ASSOCIATED WITH DEGRADATION OF ELECTRON TRANSFER CHAIN SUPERCOMPLEXES IN HEART ISCHEMIA-REPERFUSION

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Background: Sustained ischemia-reperfusion (IR) induces Ca^{2+} overload and enhances generation of reactive oxygen species (ROS) predominantly by damaged mitochondrial electron transfer chain (ETC) complexes. These alterations and high [Pi] due to increased ATP depletion induce mitochondrial permeability transition (MPT) accompanied by the opening of non-specific MPT pores (MPTP) in the inner mitochondrial membrane. In addition, oxidative stress caused by IR increases oxidation of cardiolipin, a unique mitochondrial phospholipid, and leads to destabilization of ETC supercomplexes (SCs). SCs are the large supramolecular assembly of ETC complexes that provide highly efficient flux of electrons through the ETC. Consequently, SCs increase ATP synthesis and significantly reduces electron leakage and ROS production due to short diffusion distances between ETC complexes. Both opening of MPTP and degradation of SCs are central players to initiate mitochondria-mediated cell death, however the contribution of MPTP to SCs disassembly remains unclear. **Hypothesis:** MPT-induced ROS is associated with SCs dissociation in cardiac IR. **Methods:** The relationship between MPT, ROS, and SCs were investigated using Langendorff rat hearts with or without 25 min of warm ischemia followed by 5 min or 60 min reperfusion in the presence or absence of the MPT inhibitor, sanglifehrin A (SfA). Calcium-induced swelling of mitochondria was monitored to assess MPTP opening. SCs were analyzed by Blue native electrophoresis followed by 2D SDS-PAGE was used to analyze individual ETC complexes and SCs. ROS levels were measured with Amplex Red. **Results:** Cardiac ischemia followed by both 5 and 60 min reperfusion induced MPTP opening and ROS generation. The production of ROS was inhibited by SfA indicating that it is MPT-dependent. 2D SDS PAGE revealed that over 50% of complex I was involved in the SC I+III+IV while only ~10% of the complex remained unbound. Percent distributions of SCs were significantly affected by IR and the effects were depend on the reperfusion time. We found a high SC I+IV and low SC I+III associated with increased complex I proportion at early (5-min) reperfusion. The changes remained after 60 min of reperfusion. **Conclusion:** Cardiac IR exerts various effects on mitochondrial SCs depending on reperfusion time. MPT-induced ROS presumably plays a key role in SCs disassembly. **Funding sources:** NHLBI NIH Grant SC1HL118669 (to SJ).

12.9

MITOCHONDRIAL RESPIRATION AND CALCIUM ACTIVATION ARE MAINTAINED IN THE PRESENCE OF HEART FAILURE LEVELS OF EXTRAMITOCHONDRIAL SODIUM

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An increase in cytosolic Na^+ is a hallmark of heart failure. Elevated Na^+ is thought to decrease mitochondrial matrix Ca^{2+} via the Na^+-Ca^{2+} exchanger, inhibiting the Ca^{2+} activation of mitochondrial ATP production. However, the impact of elevated Na^+ on mitochondrial respiration, matrix redox potential, and membrane potential ($\Delta\Psi$) during an acute increase in Ca^{2+} remains unknown. Isolated adult male rat ventricular mitochondria were respired at maximal (State 3) and intermediate oxygen consumption rates (J_O), achieved by modifying the Gibbs free energy of ATP (ΔG_{ATP}). $\Delta\Psi$ was measured with a TPP^+ electrode and matrix redox potential (NADH) was monitored using UV excitation and measuring emission with a spectrometer. Mitochondria were incubated with normal (5 mM) or heart failure (15 mM) levels of NaCl, with and without added Ca^{2+} (840 nM free Ca^{2+}). Maximal respiration rate was the same whether mitochondria were incubated with 5 or 15 mM Na^+ (216±28 vs 244±66 nmol O_2/mg/min), with the addition of Ca^{2+} increasing respiration to the same level with either Na^+ concentration (360±40 vs 342±10 nmol O_2/mg/min). Additionally, the slope of ΔG_{ATP}:J_O relationship, a measure of whole mitochondrial conductance when fuel is saturating, did not differ between 5 and 15 mM Na^+ (53±14 and 53±17). Ca^{2+} increased the slope of ΔG_{ATP}:J_O with either 5 or 15 mM Na^+ (95±7 and 100±22). At either Na^+ concentration, the matrix redox potential was constant across all values of ΔG_{ATP}, with the NAD^+/NADH pool 20±4% reduced at a ΔG_{ATP} of -13.1 kcal/mol and 21±5% reduced at a ΔG_{ATP} of -14.4 kcal/mol. With Ca^{2+}, the NAD^+/NADH pool was 24±3% reduced at a ΔG_{ATP} of -13.1 kcal/mol, but reduction increased to 41±1% at a ΔG_{ATP} of -14.4 kcal/mol at either Na^+ level. The addition of Ca^{2+} to either Na^+ concentration increased conductance (the effective activity) of the electron transport chain, shown by a 2.5-fold increase in the slope of the relationship between J_O and the free energy difference between NADH and $\Delta\Psi$, as well as conductance of mitochondrial ATP production and transport ($\Delta\Psi$ to ΔG_{ATP}). Healthy or failing [Na^+] decreases respiration, and acute increases in Ca^{2+} activate respiration and increase the conductance of the oxidative phosphorylation pathway to the same level regardless of Na^+ concentration. This implicates the importance of Ca^{2+} import via the mitochondrial Ca^{2+} uniporter, potentially compensating for Na^+-impairments. NIH (R01-HL095828A) to MWK; AHA (14POST20490181) to SKG.

12.10

AN ELECTRICALLY CONDUCTIVE MITOCHONDRIAL RETICULUM IN SKELETAL MUSCLE

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The mechanism of intracellular energy distribution has been proposed to occur in skeletal muscle via metabolite-facilitated diffusion. However, both myoglobin and creatine kinase knockout mice survive with near normal skeletal muscle performance and only modest adaptations. Thus, our goal was to re-examine the energy distribution mechanisms in skeletal muscle. We hypothesized that muscle mitochondrial structure minimizes diffusion distances. Focused ion beam scanning electron microscopy (FIB/SEM) was used to visualize fixed mouse skeletal muscle volumes with 15 nm 3D resolution and was validated by super-resolution microscopy of live, single muscle fibers. Mitochondria formed a highly connected reticulum as nearly all mitochondria were coupled either directly through a continuous outer mitochondrial membrane or through electron dense contact sites (EDCS) between adjacent mitochondria. 99.9±0.1% of the largely ellipsoidal shaped paravascular mitochondria (PVM) were connected to adjacent PVM by EDCS and 21.2±3.2% of PVM were directly coupled to thin mitochondrial tubules projecting into the intrafibrillar space along the I-bands (IBM). 99.7±0.3% of the thick mitochondrial tubules running parallel to fibers (FPM) were coupled to adjacent FPM through EDCS while 81.7±3.6% of FPM branched directly into IBM. To test the electrical connectivity of this reticulum, we used a photoactivatable mitochondrial uncoupler to depolarize the membrane potential ($\Delta\Psi$) of a small interior region of an isolated muscle fiber and observed whether the fall in $\Delta\Psi$ propagated to mitochondria in neighboring regions. Upon a mild depolarization, a rapid (<200 msec) and near uniform drop in $\Delta\Psi$ occurred in mitochondria across the cell including a homogenous decrease in the PVM. Further, using dual labeling immunofluorescence, we found that mitochondrial proteins associated with $\Delta\Psi$ production (cytochrome oxidase) are relatively higher in abundance near the cell boundary and proteins that utilize $\Delta\Psi$ for ATP production (ATP synthase) are preferentially located in the cell interior near contractile and transport ATPases. Together, these data demonstrate a mitochondrial reticulum that allows for a rapid, coordinated distribution of energy throughout the cell without the requirement of extensive metabolite diffusion. We propose that membrane potential conduction via the mitochondrial reticulum is the dominate pathway for energy distribution in this cell. Funding: NIH Intramural Research Program.

12.11
DIET-INDUCED KETOSIS PROTECTS AGAINST FOCAL CEREBRAL ISCHEMIA IN MOUSE

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Introduction: Over the past decade our research has consistently shown that ketosis is neuroprotective against ischemic insults in rats. Diet-induced ketotic rats had a significant reduction in infarct volume when subjected to middle cerebral artery occlusion (MCAO). Ketotic rats showed improved survival and recovery after cardiac arrest and resuscitation. Ketones can act as alternate energy substrates to glucose, especially during metabolic derangements of glucose metabolism. Supply of substrate to the mitochondria is critical, especially during conditions of high energy demand where glucose metabolism may be deficient, such as with oxidative injury. Under these circumstances, ketone bodies can restore energy balance via stabilization of glucose metabolism and reduced oxidative damage though upregulation of salvation pathways. One of the mechanisms involves succinate-induced stabilization of hypoxic inducible factor-1alpha (HIF-1α) and its downstream effects on intermediary metabolism. HIF1, acting as a metabolic sensor, is important for cell survival, especially under acute "metabolic stress conditions", such as with ischemia reperfusion injury. In this study we investigated the effect of diet-inducted ketosis on HIF1α accumulation and infarct volume following transient focal cerebral ischemia in mice. **Methods:** Mice (11 weeks old) were randomly assigned to two groups, ketogenic (high fat, carbohydrate restricted; KG) or standard lab-chow (STD) diet for 3 weeks before MCAO. Mice underwent 60 minutes of MCAO and reperfusion. The total brain infarct volume was evaluated by Giemsa staining 48 hours after reperfusion. In a separate group of mice, HIF-1α levels were measured in brains by Western Blot analysis. **Results:** After 3 weeks of ketogenic diet, plasma ketone bodies (beta-hydroxyburyrate, BHB; mM) were increased (range: 1.1 -2.6). The infarct volume was decreased in the KG group compared to the STD group (4.2 ± 0.6 vs 7.8 ± 2.2 mm^3, mean ± SEM, n = 8 and 4 for KG and STD groups, respectively); there was a proportional decrease in infarct volume with increased plasma BHB levels. The HIF-1α accumulation was increased significantly in the KG-diet group as previously reported in rats. **Conclusion:** Our results showed that ketosis can be induced in mice by ketogenic diet and that diet-induced ketosis was neuroprotective against focal cerebral ischemia. One potential mechanism may be related to the upregulation of HIF-1α through redox modulation by ketosis.

12.12
ROLE OF MITOCHONDRIAL STRUCTURE, FUNCTION AND REDOX SIGNALING IN MEGAKARYOPOIESIS

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Platelets are circulating cellular fragments that play an important role in hemostasis, thrombosis and inflammation. Platelets contain low numbers of fully functional mitochondria and we have recently shown that these mitochondria play an important role in platelet thrombotic function. Specifically, we have shown that platelets from patients with Sickle Cell Disease (SCD) show inhibited mitochondrial complex V activity, which leads to increased mitochondrial reactive oxygen species production and subsequent thrombotic activation. Notably, while mitochondria contribute to mature platelet thrombotic function, it is unknown whether mitochondrial function determines platelet maturation and differentiation. Megakaryopoiesis is the complex process of producing mature megakaryocytes from hematopoietic stem cells. Platelets are shed from the tips of specialized projections of megakaryocyte cytoplasm called proplatelets. Importantly, the role of mitochondrial function in megakaryopoiesis is unknown. We hypothesize that early bioenergetic function is required for megakaryocyte differentiation into platelets and that mitochondrial function changes as megakaryocytes differentiate into platelets. Here, we measure mitochondrial function in human megakaryocytes as they differentiate into a platelet phenotype and find that there are distinct differences in oxidative phosphorylation over the differentiation process. We present preliminary results showing the bioenergetics profile of primary human megakaryocytes undergoing megakaryopoiesis. This work is significant since understanding the role of mitochondria in megakaryopoiesis will provide potential new targets and therapeutic approaches to modulate thrombocytopenia and thrombocytosis. In addition, these studies will help us further define the general applicability of platelet mitochondrial functional assays in SCD and other diseases that affect systemic or circulatory oxidant stress. Funding Source: Center for Metabolism and Mitochondrial Medicine.

12.13
EFFECTOR T CELLS UPREGULATE MITOCHONDRIAL METABOLISM DURING GRAFT-VERSUS-HOST DISEASE

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Hypothesis: The current paradigm of T cell metabolism posits that T cells up-regulate glycolysis to meet the increased demands of activation. However, we have shown that effector T cells mediating graft-versus-host disease (GVHD) increase oxidative metabolism, and in particular fatty acid oxidation (FAO). We therefore hypothesized that effector T cells during GVHD would require both increased mitochondrial mass and mitochondrial metabolism. **Methods:** On day 7 following a major histocompatibility (MHC)-mismatched murine bone marrow transplant, donor T cells were analyzed by flow cytometry for mitochondrial mass, reactive oxygen species (ROS), and mitochondrial H_2O_2 levels. In addition, mitochondrial protein levels were quantitated in purified donor cells by immunoblot and mitochondrial to nuclear (mt/nuc) DNA ratios were measured by quantitative PCR. Finally, PGC-1$\alpha^{-/-}$ T cells were assayed for proliferation and survival in a mixed leukocyte reaction against MHC-mismatched splenocytes. **Results**: During GVHD, effector T cells increased their mitochondrial mass as early as day 3 post-transplant compared to naïve cells (MFI 3.0 vs. 0.9, $p < 0.0001$). This increase was substantiated by a concomitant elevation in mt/nuc DNA ratios (2.7 ± 0.52 vs. 1.0 ± 0.07, $p < 0.0001$). Alloreactive cells also increased protein levels of the mitochondrial protein VDAC and the co-activator PGC-1α (1.78 vs. 0.36, relative density vs. naïve cells, $p < 0.001$). Notably, PGC-1α levels were minimally increased in T cells undergoing homeostatic proliferation. GVHD T cells also enhanced their mitochondrial metabolism, with increased cellular and mitochondrial ROS levels versus naïve/homeostatic cells (relative MFI 3.1 vs. 1.0, $p < 0.0001$). Increased ROS levels were directly tied to FAO, as ROS levels fell with etomoxir treatment. Finally, when PGC-1$\alpha^{-/-}$ T cells were stimulated *in vitro* in a mixed leukocyte reaction, there was a marked decrease in well-divided PGC-1$\alpha^{-/-}$ T cells, while undivided T cell numbers remained similar between wildtype and PGC-1$\alpha^{-/-}$ groups. **Conclusions:** During GVHD, effector T cells up-regulate both mitochondrial mass

and mitochondrial metabolism. In addition, PGC-1α, a driver of mitochondrial biogenesis, is required for survival of robustly proliferating alloreactive T cells *in vitro*. Future studies will determine if modulation of PGC-1α, or its downstream targets, can mitigate GVHD and make blood and marrow transplantation a safer and more effective therapy.

12.14
EVIDENCE FOR INVOLVEMENT OF MITOCHONDRIAL MATRIX ROS AND HYPOXIA-INDUCIBLE FACTOR-1 IN THE GROWTH INHIBITORY EFFECT OF RESVERATROL

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Despite the well-known inhibitory effect of RES on cell growth, the molecular mechanism(s) behind it is not fully understood. We have shown that RES's inhibition of cell growth is dependent upon Mn- superoxide dismutase (MnSOD) induction and an active mitochondrial respiratory chain (Robb, E.L. and Stuart, J.A. 2011; Robb E.L. and Stuart, J.A. 2014). These results suggest that mitochondrial matrix ROS is involved in the inhibition of cell growth caused by RES and similar molecules. A possible downstream target of mitochondrial ROS that could link the modulation of mitochondrial matrix ROS to growth inhibition is the hypoxia inducible factor (HIF1). HIF1 is a heterodimeric transcription factor, which is redox-regulated via its HIF-1α subunit. Mitochondrial ROS, including MnSOD levels specifically, have been implicated in HIF-1α stabilization (Kaewpila, S. et al., 2008), and HIF-1 stabilization has in turn been implicated in the growth of various cancer cells. We found that RES's inhibition of PC3 (pancreatic cancer) cell growth was abolished when HIF-1 is stabilized by $CoCl_2$ (a hypoxia mimetic) or IOX2 (a prolyl hydroxylase inhibitor). This may be linked to HIF-1's induction of glycolytic machinery, as the expression of some HIF-1 gene targets was reduced in cells treated with RES. Interestingly, growth of PC3 cells in galactose media, which forces greater reliance on oxidative phosphorylation and prevents reliance on the glucose fermentation promoted by HIF-1, was not inhibited by RES. Also, the effects of RES on PC3 cell growth were substantially greater in cells grown under hypoxic conditions (as low as 0.4% O_2). Together, these results are consistent with a role for mitochondrial matrix ROS, MnSOD, and HIF-1 in the cell growth inhibitory effects of RES. Research funding was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant.

12.15
MITOCHONDRIAL MOTILITY RESPONSE TO NUTRIENT ENVIRONMENT IN THE PANCREATIC BETA-CELL: ROLE OF MILTON1 NUTRIENT-SENSING THROUGH O-GLCNAC MODIFICATION

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Our previous work has shown that chronic exposure to high glucose and fatty acids, termed glucolipotoxicity (GLT), inhibits mitochondrial fusion and networking in the pancreatic beta-cell. However it remains unclear how GLT affects mitochondrial motility in the beta-cell. Importantly, it is not fully understood what is the mechanism connecting nutrient availability to changes in mitochondrial motility and dynamics. Therefore we hypothesized that availability of glucose and fatty acids would modulate mitochondrial motility in the pancreatic beta-cell. We further hypothesize that changes to activity of Milton1, a key mitochondrial adaptor protein essential for motility, may be an essential mediator connecting nutrient availability to mitochondrial motility and dynamics in the pancreatic beta-cell. To assess changes to mitochondrial motility in INS1 cells (beta-cell line), we performed time-lapse imaging and analyzed mitochondrial displacement. Exposure to GLT decreased mitochondrial motility, as assessed by reduction in average mitochondrial displacement. We found that O-GlcNAcylation, a regulatory mechanism which links protein activity to nutrient status, of Milton1 was increased by high glucose, fatty acids, or treatment with O-GlcNAcase inhibitor, PUGNAc. When wild-type Milton1 (Mil-WT) was over-expressed in INS1 or mouse islets, mitochondrial morphology was altered towards more elongated and aggregated architecture. Increasing cellular O-GlcNAc levels by high glucose or PUGNAc reversed this Mil-WT-induced phenotype, suggesting decreased Milton1 activity upon O-GlcNAcylation. Over-expressing a mutated form of Milton1 that is resistant to O-GlcNAcylation (Mil-Qmut) showed similar effect on mitochondrial elongation and aggregation, but was insensitive to high nutrient exposure. Accordingly Mil-Qmut preserved mitochondrial connectivity and protected from GLT-induced cell death. However, Mil-Qmut expression in INS1 cells or mouse islets inhibited acute (<1 hour) glucose-induced stimulation of mitochondrial oxygen consumption and insulin secretion, suggesting O-GlcNAcylation of Milton1 may also play a regulatory role during glucose-stimulated insulin secretion in the beta-cell. Collectively, our findings suggest that changes to mitochondrial motility under a chronic high nutrient environment may contribute to mitochondrial and beta-cell dysfunction. Importantly, nutrient-sensing of Milton1 activity via O-GlcNAc modification is a key player connecting nutrient status to mitochondrial motility and dynamics in the pancreatic beta-cell. K.T. was supported by a National Science Foundation Graduate Research Fellowship under Grant No. DGE-0741448, and Levinsky Fellowship from Boston University School of Medicine. O.S. is funded by NIH grants RO1 DK35914, R01 DK56690, and R01 DK074778.

12.16
MITOCHONDRIAL FRAGMENTATION IN RESPONSE TO GLUCOLIPOTOXICITY REPRESENTS A COMPENSATORY ADAPTATION TO MAINTAIN BETA-CELL FUNCTION

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We have previously reported that high glucose and fatty acids, termed glucolipotoxicity (GLT), causes mitochondrial fragmentation and is concurrent with beta-cell dysfunction in cultured insulinoma (INS1) cells and mouse islets. However, it remains unclear if GLT causes mitochondrial fragmentation in human islets and whether this fragmentation plays a compensatory or pathological role during GLT-induced beta-cell dysfunction. To address if GLT alters mitochondrial shape in human beta-cells, islets were cultured in media with normal nutrients (5mM glucose, 10% BSA) or media with high glucose (11.2 mM) and fatty acids (1mM oleate and palmitate) for 4 days. Assessing mitochondrial morphology and membrane potential (MMP) by confocal imaging revealed that GLT significantly fragments mitochondria and increases MMP heterogeneity in human beta-cells. Concomitantly, glucose-stimulated insulin secretion (GSIS) was inhibited. Genetically inducing mitochondrial fragmentation by decreasing levels of mitochondrial fusion protein, Mfn2, in INS1 cells protected from GLT-induced cell death. Conversely, preventing GLT-induced fragmentation by expressing dominant-negative construct of mitochondrial fission protein, DRP1 (DRP1-DN), further disrupts MMP and causes accumulation of depolarized mitochondria in human beta-cells. Moreover, GLT-induced inhibition of GSIS is further decreased in human islets expressing DRP1-DN. In conclusion, mitochondrial fragmentation in response to GLT represents a compensatory adaptation to GLT insult, which works to preserve mitochondrial and beta-cell function. K.T. was supported by a National Science Foundation Graduate Research Fellowship under Grant No. DGE-0741448, and Levinsky Fellowship from the department of Medicine at Boston University School of Medicine. O.S. is funded by NIH grants RO1 DK35914, R01 DK56690, and R01 DK074778.

12.17
MOLECULAR MECHANISMS BEHIND THE ACCUMULATION OF LIPIDS THAT OCCUR AFTER SKELETAL MUSCLE INJURY

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Myosteatosis is the accumulation of lipid that occurs after skeletal muscle injury or in certain chronic neuromuscular or metabolic diseases. Muscle fiber atrophy and fibrosis often accompany myosteatosis. The amount of lipid accumulation is negatively correlated with skeletal muscle functional capacity, but the causes of myosteatosis are unknown. To gain a greater understanding of the ontogeny of myosteatosis, we induced a severe injury to the rotator cuff musculature in rats, isolated tissues 10, 30 or 60 days after tear, and used a combination of RNA sequencing and shotgun lipidomics to identify global changes in gene expression and lipid content in injured muscles. The RNA sequencing data and shotgun lipidomics results were then analyzed with MetScape and IPA software to determine the biochemical pathways involved in myosteatosis. After injury, there was a time dependent increase in total lipid, which was primarily due to a dramatic rise in triglyceride (TAG) content over each time point. While the expression of the rate-limiting TAG synthesis enzymes, DGAT1 and DGAT2, were not increased, major lipases responsible for the breakdown of TAG, including ATGL, LPL, and HSL, were downregulated following injury. There was also a decrease in cardiolipin content and a reduction in the expression

of components of Complex II and IV after injury, indicating reduced mitochondrial content. Pathways that were predicted by IPA software to be involved in the development of myosteatosis include mitochondrial dysfunction, oxidative stress response, and production of NO and ROS. Interestingly, the biochemical pathways that were most highly downregulated included oxidation of lipid and beta-oxidation of fatty acid. Combined, these data suggest that the accumulation of lipid that occurs after muscle injury is due to a decrease in lipid utilization and breakdown rather than an increase in lipid synthesis. Sustained mitochondrial dysfunction and oxidative stress may result in the increased lipid infiltration and atrophy of muscle fibers over time. While further investigation is necessary, this study provided important insight into the development of myosteatosis and associated chronic muscle dysfunction.

12.18
KNOCKDOWN OF VOLTAGE-DEPENDENT ANION CHANNELS 1 AND 2 INHIBITS MITOCHONDRIAL FISSION BY DECREASING BINDING OF DYNAMIN-RELATED PROTEIN 1 TO MITOCHONDRIA

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Background: In cancer cells, mitochondria continuously undergo fusion and fission. Mitochondrial fission involves the binding of dynamin-related protein-1 (Drp1) with mitochondrial fission factor (Mff) and fission 1 protein (Fis1) located in the mitochondrial outer membrane (MOM). Voltage dependent anion channels (VDAC), the most abundant proteins in MOM, comprises 3 isoforms - VDAC1, VDAC2 and VDAC3. Previously in HepG2 cells, VDAC1/2 double knockdown promoted mitochondrial fusion. Here, we hypothesize that VDAC1 and VDAC2 are anchors for Drp1 and that VDAC1/2 double knockdown inhibits mitochondrial fission by preventing Drp1 binding to mitochondria. Our aim was to evaluate the effects of single and double VDAC knockdowns on mitochondrial Drp1 binding, membrane potential ($\Delta\Psi$) and morphology. **Methods:** HepG2 hepatoma cells were treated with siRNA to generate single and double knockdowns of VDAC1/2/3 in all possible combinations. As positive control, mitochondrial fission was induced by the uncoupler CCCP (5 μM). Mitochondrial $\Delta\Psi$ was assessed by confocal mi-croscopy of tetramethylrhodamine methylester (TMRM). Immunofluorescence using primary antibodies against Fis1, Drp1 and Tom20 assessed subcellular localization of Fis1 and Drp1.The Duolink proximity ligation assay was used to determine interactions of Drp1 with VDAC1 or VDAC2. **Results**: In wild type cells, mitochondria were short, branched and filamentous and indistinguishable from non-target siRNA and single knockdown of each VDAC isoform. After double VDAC1/2 knockdown but not VDAC2/3 or VDAC1/3 knockdown, mitochondrial filaments became longer and larger in diameter. Additionally, $\Delta\Psi$ increased after VDAC1/2 double knockdown. Localization of Drp1 by inmunocytochemistry was mainly cytosolic and was not different between non-target and double VDAC1/2 knockdown cells. Fis1 distribution was mitochondrial and also not affected by VDAC knockdown. In cells transfected with non-target siRNA but not in VDAC1/2 double knockdown cells, CCCP increased Drp1 binding to mitochondria. Knockdown of VDAC1/2 also decreased Duolink interaction of Drp1 with VDAC1 and VDAC2 both before and after treatment with CCCP. **Conclusion**: VDAC1/2 double knockdown promotes mitochondrial fusion by inhibiting Drp1-driven mitochondrial fission, and VDAC1 and VDAC2 appear to serve as anchors for Drp1 translocation to mitochondria. ACS 13-043-01 and COBRE Pilot GM103542 (ENM), T32DK083262 (DND); AA022815, AA021191 and 14.Z50.31.0028 (JJL).

12.19
EFFECTS OF LOW LEVEL LASER THERAPY ON TENOCYTES IN HIGH GLUCOSE ENVIRONMENT

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Tendons consist of parallel collagen fibers embedded within an extracellular matrix (ECM). Diabetes mellitus (DM) is one of the risk factors of musculoskeletal damage and may interfere with tendon healing process. Low-level laser therapy (LLLT) has been extensively applied in the treatment of tissue injury. The beneficial effects of LLLT on tendinopathy have been reported, however the effect of LLLT on injured tendons in DM population is less discussed, and the effect of LLLT and their molecular mechanisms on the tenocytes in high glucose environment remain undetermined. We hypothesized that LLLT alter the expression of transforming growth factor beta 1 (TGF-β1) and ECM proteins in high glucose-treated tenocytes, which have the potential to improve tendon properties for accelerating tendon healing. Tenocytes were isolated from porcine Achilles tendon with type I collagenase digestion. Tenocytes were cultured in DMEM-F12 medium with 0 and 30mM glucose concentrations, and subjected to 808nm laser with total fluence of 0 J/cm^2, 1 J/cm^2, 2 J/cm^2 and 3 J/cm^2. The MTT assay was used to evaluate the viability of laser-treated cells. The mRNA and protein expressions of TGF-β1 and ECM proteins, type I and III collagen, were assessed by real-time PCR and Western blot analysis, respectively. The comparative proteomic approach was used to determine alternation in protein expressions of high glucose-treated tenocytes with or without laser irradiation, and differentially expressed proteins were identified through mass spectrometry. The most effective dosage of laser irradiation in facilitating tenocyte proliferation was 1 J/cm^2 energy density. In high glucose environment, the mRNA and protein expression levels of major ECM proteins, type I and type III collagen, were significantly increased after laser irradiation, compared to that without laser irradiation. In addition, the increased TNF-α and TGF-β1 under high glucose environment were significantly reduced after laser irradiation. Compared to tenocytes in high glucose environment without laser irradiation, several proteins with underexpression and overexpression were identified in cells treated with laser irradiation. In conclusion, low level laser irradiation acts as an anabolic stimulus of tenocytes in alteration of TGF-β1 and ECM protein expressions, which is potential in improving tendon properties under high glucose-induced tendon injury. Source of Funding: kmtth-102-009.

12.20
SCREENING ASCITES-DERIVED OVARIAN CANCER CELLS FOR HISTOLOGICAL SUBTYPE-SPECIFIC BIOENERGETIC SIGNATURES AND MITOCHONDRIAL DYSFUNCTION

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We have previous established that ovarian cancer cell lines of different histological origins are characterized by distinct bioenergetics signatures (Dier *et al.* 2014 PLoS ONE). In particular, Ovarian Clear Cell Carcinomas (OCCC) display high oxygen consumption rate (OCR) and glycolytic flux compared to the more common high grade serous adenocarcinoma (HGSA) subtype. To assess if these findings are clinically relevant we used extracellular flux analysis to screen the bioenergetics profile of ovarian cancer patient ascites derived tumor cells. Similar to OCCC, high metabolic activity was associated with tumor cells isolated from a patient diagnosed with endometrioid ovarian cancer, a histological subtype that shares similarities in tissues of origin with OCCC. These data add to the notion that the term ovarian cancer comprises distinct diseases, with different gene expression, as well as metabolic signature. Further, using extracellular flux analysis a HGSA cell line (OVCA420) was identified with compromised oxygen consumption. One of six ascites specimen similarly shared a lack of mitochondrial OCR and inability to respond to OCR stimulation by the mitochondrial uncoupler FCCP, suggesting that mitochondrial dysfunction may be associated with a sub-population of ovarian cancer adenocarcinomas. OVCA420 cells and the HGSA specimen with compromised mitochondrial function displayed mitochondrial morphology changes, indicative of dysfunction in mitochondrial fusion/fission. Assessing the expression of the mitochondrial fission protein Drp1 revealed that these cells display high levels of a lower molecular weight splice variant of Drp1 (~70kDa). Current studies are underway to identify the molecular identity of the shorter Drp1 variant and its potential effects on compromised mitochondrial slicing observed in these cells. Further, larger scale population studies will reveal if mitochondrial dysfunction plays a significant role in HGSA. This may aid in the development of targeted glycolysis-based therapeutics for a sub-population of ovarian cancer cases.

12.21
BIOENERGETIC REPROGRAMING IN MONOCYTES IN CHRONIC KIDNEY DISEASE

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Mitochondria control cellular homeostasis through maintaining proper bioenergetic programs and signaling mechanisms. Mitochondrial dysfunction in chronic metabolic and inflammatory diseases such as obesity, diabetes, chronic kidney disease etc. underscore the significance of this organelle in maintaining normal health. Mitochondria integrate the cellular bioenergetic program, a unique set of bioenergetic relationships between the individual parameters of the oxidative phosphorylation (basal, ATP-linked, proton-leak, maximal, reserve capacity and non-mitochondrial respiration). These parameters demonstrate distinct aspects of mitochondrial function and their interdependence. Alteration in this program causes cellular stress and diseases, which suggests that identifying the defects associated with the bioenergetic program can be used to explain mechanisms of mitochondrial dysfunction in chronic inflammatory diseases. The objective of the study is to determine the bioenergetic health index (BHI, a single number that integrates the bioenergetic parameters) and relationships between the parameters that form the bioenergetic program in healthy subjects and chronic kidney disease patients. **Methods**: Using the mitochondrial stress test, bioenergetic parameters were determined in peripheral blood monocytes that are freshly isolated from healthy subjects (n=50) and chronic kidney disease patients (n=40) using the extracellular flux analyzer. The BHI was determined using the formula BHI = (Reserve Capacity x ATP-Linked Respiration)/(Proton Leak x Non-Mitochondrial Respiration). The bioenergetic parameters were compared using multivariate analysis and linear regression methods. **Results:** Compared to the healthy subjects, chronic kidney disease patients demonstrate a significantly lower BHI. Multivariate analysis of the bioenergetic parameters in healthy subjects show a high correlation between basal mitochondrial respiration and ATP-linked respiration (R^2=0.92, p=<0.0001). Basal respiration is also correlated well with maximal (R^2=0.428, p<0.0001) and with non-mitochondrial respiration (R^2=0.437, p<0.0002). In chronic kidney disease, the correlation between basal respiration and ATP-linked respiration remains strong, but the correlation that existed between other bioenergetic parameters weakened considerably. **Conclusion:** These novel findings suggest that BHI can be used to determine the bioenergetic health of individual subjects. The distinct relationships between mitochondrial bioenergetic parameters suggest their potential utility in gaining insights into the mechanism of diseases with bioenergetic dysfunction.

12.22
MITOCHONDRIAL RESPIRATORY CAPACITY AND COUPLING CONTROL DECLINE WITH AGE IN HUMAN SKELETAL MUSCLE

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Mitochondrial health is critical to physiological function, particularly in tissues with high ATP turnover, such as striated muscle. It has been postulated that derangements in skeletal muscle mitochondrial function contribute to impaired physical function in older adults. Here, we determined mitochondrial respiratory capacity and coupling control in skeletal muscle biopsies obtained from young and older adults. Twenty four young (28±7 yrs) and thirty one older (62±8 yrs) adults were studied. Mitochondrial respiration was determined in permeabilized myofibers from the *m. vastus lateralis* after the addition of substrates followed by either oligomycin or cyanide *m*-chlorophenyl hydrazine (CCCP). Thereafter, mitochondrial coupling control was calculated from the flux control ratios for CCCP and the coupling control ratio and factor for oligomycin. Maximal coupled respiration (respiration linked to ATP production) was lower in muscle from older vs. young subjects (55.3±4.2 vs. 40.9±3.1 pmol/sec/mg; P<0.01), as was maximal uncoupled respiration (61.5±4.6 vs. 50.7±3.4 pmol/sec/mg; P<0.06). Coupling control in response to the ATP synthase inhibitor oligomycin was lower in older adults (P<0.05), as was the mitochondria flux control ratio, coupled respiration normalized to maximal uncoupled respiration (P<0.05). Calculation of respiratory function revealed lower respiration linked to ATP production (34.0±.36 vs. 17.7±2.0 pmol/sec/mg; P<0.001) and greater reserve respiration (6.2±1.5 vs. 10.1±1.1 pmol/sec/mg; P<0.01), i.e., respiratory capacity not used for phosphorylation in muscle from older adults. We conclude that skeletal muscle mitochondrial respiratory capacity and coupling control decline with age. Lower respiratory capacity and coupling efficiency result in a reduced capacity for ATP production in skeletal muscle of older adults. This work was supported by the National Institutes of Health and Shriners of America.

12.23
INTERFERENCE WITH MITOCHONDRIAL BIOENERGETICS BY TPP-IOA, A MITOCHONDRIA-TARGETED ANTI-APOPTOTIC INHIBITOR OF CYTOCHROME *C* PEROXIDASE ACTIVITY

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Recently, 3-hydroxypropyl-triphenylphosphonium-conjugated imidazole-substituted oleic acid (TPP-IOA) was designed as an anti-apoptotic molecule targeting cytochrome *c*'s redox-active catalytic site and thereby inhibiting a peroxidase activity that has been linked to apoptosis (Atkinson et al., Nature Comm. 2011; 2:497). TPP-IOA effectively mitigates radiation-induced death in both cell culture and animal models and thus may have therapeutic potential in pathological scenarios involving apoptotic cell death. However, many such possible scenarios require that TPP-IOA not be toxic to mitochondrial ATP production, and therefore it is essential to understand TPP-IOA's potential effects on oxidative phosphorylation. Using purified cytochrome *c*, isolated mitochondria, and cultured cells, we determined whether TPP-IOA can inhibit pro-apoptotic events and cell death without impairing mitochondrial bioenergetics. Assessments with pure cytochrome *c* revealed that TPP-IOA inhibits cytochrome *c* peroxidase activity at doses marginally lower than those that interfered with cytochrome *c* electron transfer rates. However, in isolated rat liver mitochondria TPP-IOA inhibited peroxidase activity at doses similar to those that perturbed electron transport and apparent proton leak. Since TPP-IOA affected oxidative phosphorylation at concentrations similar to those inhibiting cytochrome *c* peroxidase activity, we compared the protection against cell death conferred to cells grown in galactose/glutamine media (promoting oxidative phosphorylation) versus those grown in glucose media (allowing glucose fermentation). Initial findings suggest that TPP-IOA is effective at inhibiting cell death in cells that are less reliant on oxidative phosphorylation. These findings suggest that therapeutic applications of TPP-IOA may be limited to pathologies involving more glycolytic cell types (e.g. stem cells affected by radiation). Additionally, this work highlights the general importance of evaluating the efficacy of mitochondria-targeted small molecules using cell culture models that are more reliant on mitochondrial respiration.

12.24
CHRONIC ALCOHOL EXPOSURE INCREASES SUSCEPTIBILITY TO OXIDATIVE STRESS IN HEPATOCYTES

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Chronic alcohol consumption decreases the expression and activity of the proteins in oxidative phosphorylation system resulting in differential sensitivity to mitochondrial specific modulators including nitric oxide. It is known that the lipid peroxidation product 4-hydroxynonenal (4-HNE) is elevated in hepatocytes in response to chronic alcohol consumption and in other cell types and that it can cause mitochondrial dysfunction. We hypothesized that the decreased bioenergetic capacity in hepatocytes exposed to chronic alcohol will be more susceptible to 4-HNE and other hepatotoxicants such as acetaminophen (APAP), an important over-the-counter pain reliever with high hepatotoxic potential. This was tested in a rat model of chronic alcohol consumption using the Lieber-DeCarli pair fed control and ethanol feeding regimen. Hepatocytes were isolated from the control and alcohol-fed rats and the mitochondrial stress test was performed to establish the basal bioenergetic profile. Under control conditions we found that maximal respiration and reserve capacity was decreased in the hepatocytes from the chronic alcohol consuming animals. Next, we assessed the susceptibility to increasing concentrations of 4-HNE and APAP. In control heaptocytes there was a concentration dependent decrease initially in reserve capacity but only at a 100μM concentration was a profound change in bioenergetics observed. At this concentration, basal respiration was substantially stimulated but this effect was not persistent and at 2 hr bioenergetic parameters were completely lost. APAP at 10 mM dose caused an immediate decrease in basal respiration and suppression of maximal respiration. When hepatocytes from the alcohol treated animals were exposed to 4-HNE and APAP they were more susceptible to both 4-HNE and APAP. Taken together, these data indicate that the impact of chronic alcohol consumption on bioenergetics increases the susceptibility to the pathologically relevant toxicants, 4-HNE

and APAP. *This work was supported by National Institute of Health grant R01 AA018841 (to SM Bailey) and AA013395 (to VM Darley-Usmar).*

12.25
INCREASE MITOCHONDRIAL UNCOUPLING IN STORED PLATELETS

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Stored platelet concentrates have been shown to have decreased quality which could ultimately lead to worsening patient outcomes. This phenomenon termed the platelet storage lesion is characterized by the change in cell morphology, decreased aggregation, an increased glycolytic rate, and decreased mitochondrial function, the mechanisms of which are not clearly understood. In the present study, we measured the functional changes in mitochondrial and glycolytic function between freshly isolated and stored platelet concentrates. We used platelets between storage days 6–9 and measured mitochondrial and glycolytic bioenergetics using the Seahorse XF technology. Stored platelets showed decreased recovery after hypotonic stress compared to freshly isolated platelets although stored platelets did not show any differences in thrombin-mediated aggregation. The bioenergetic health index (BHI), an index of overall health of the platelets was decreased in stored platelets which were ascribed to a 10% decrease in basal oxygen rate, a 190% increase in proton leak and no change in maximal oxygen consumption compared to the freshly isolated cells. When mitochondrial ATP production was inhibited, the glycolytic rate was increased in stored platelets. In summary, stored platelet concentrates showed a decrease in oxidative phosphorylation that was predominately driven by an increase in mitochondrial proton leak.

12.26
CROSSTALK BETWEEN MITOCHONDRIAL ACETYL-COA METABOLISM, CYTOSKELETON MODIFICATIONS AND AUTOPHAGY

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Lysine acetylation, a well characterized post-translational modification, is tightly coupled to the nutritional status of the cell. This connection occurs as the availability of the main substrate for acetylation, acetyl-CoA, fluctuates greatly with changing metabolic conditions. Recent studies have demonstrated that acetyl-CoA levels act as an indicator of cellular nutrient status, and increased abundance of this metabolite can block the induction of cellular recycling programs. Here we investigated the crosstalk between mitochondrial metabolic pathways and autophagy, using biochemical inducers of mitochondrial acetyl-CoA production. Treatment of cells with one compound, a co-factor of several mitochondrial metabolic protein complexes, led to the unexpected hyperacetylation of α-Tubulin in the cytosol. This acetylation was catalyzed by the α-Tubulin acetyltransferase, αTAT; and was dependent on a loss in function of the cytosolic histone deacetylase, HDAC6. Finally, we show that α-Tubulin hyperacetylation alters the flux of substrates through autophagy-related pathways, which may limit the ability of cells to remove dysfunctional mitochondria through autophagic mechanisms. Based on these results, we hypothesize that acetyl-CoA derived from mitochondrial sources may act as a modulator of cellular recycling pathways, by regulating the cytoskeletal transport of substrates to the autophagy machinery. This work is funded in part by National Institutes of Health Award HL116728.

12.27
STUDY ON THE EFFECTS OF ALCOHOL AND CANNABINOL TREATMENT ON HYPOTHALAMIC PITUITARY GONADAL AXIS IN MALE WISTAR RATS

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This study investigated the effects of oral administration of alcohol and cannabinol on the hypothalamic pituitary gonadal system in male adult rats. Twenty five male rats were purchased from NIPRD and were divided into five groups containing five rats each and were treated for a period of 48 days. Ethical approval was obtained from National Institute for Pharmaceutical Research and Development (NIPRD) and the experiment conducted was in conformance with guidelines for experimental procedures as set forth in the Declaration of Helsinki and the APS Guiding Principals in the care and Use of Animals. Group one serves as the control, group two was administered 5mg/kg body weight methanol, group three was given 3g/kg body weight as 25%v/v alcohol, group four was given 10mg/kg body weight cannabinol and group five was treated with alcohol (3g/kg body weight as 20%v/v) and cannabinol (10mg/kg body weight). Drug administration was via oral route throughout the experimental period. At the end of the experimental period, blood was collected via the retro-orbital sinus under ether anaesthesia and was allowed to clot for hormonal assay and the brain was dissected and immediately fixed. Semen analysis was carried out by exposing the testis together with the epididymis and the epididymis was carefully separated and caput was removed. The caput was then transferred unto a pre-warmed slide and lacerated to release some semen unto the slide surface. The animals were anesthetized and sacrificed cervical dislocation and their reproductive organs were removed and weighed immediately. There was no significant change in the body weight, however, there was a significant change in the percentage weight difference in the experimental groups when compared with the control group. Serum level of testosterone of the groups treated with alcohol, cannabinol, alcohol plus cannabinol were significantly decreased ($p<0.05$) when compared with the control rats. However, there were reduction in sperm motility and sperm count of rats exposed to alcohol, cannabinol, alcohol plus cannabinol treated rats in comparison to the control rats. The histological section showed alteration in the hypothalamic and testicular cyto-architecture in groups treated with alcohol or/and cannabinol treated rats, while there was reversal of this in the groups co-treated with quercetin. The results suggest that alcohol and cannabinol administration have deleterious effect on male reproductive activities (system) in rats. Keywords: alcohol, cannabinol, HPG-axis, histomorphology, sperm content, hormone profile.

12.28
REGULATION OF CARDIAC AUTOPHAGY BY ADIPONECTIN UNDER HYPOXIC/ISCHEMIC STRESS

James Won Suk Jahng, Yee Kwan Chan, Hye Kyoung Sung, Hee Ho Cho & Gary Sweeney.
Dept. of Biology, York Univ., Toronto, Canada.

Adiponectin is a hormone secreted from adipose tissue which confers anti-inflammatory, anti-diabetic and cardioprotective effects. The role of autophagy in metabolic dysfunction and heart failure is now apparent, and recent work indicates regulation of autophagy by adiponectin is of functional significance. We used Adiponectin knockout (Ad-KO) mice ± ischemia induced by coronary artery ligation (CAL) and H9c2 cardiomyoblasts ± hypoxia to investigate the significance of adiponectin in regulating autophagy. We used Western blotting for LC3-II and H9c2 cells stably expressing tandem RFP/GFP-LC3 to show increased autophagic flux in response to adiponectin. This was confirmed by analysis of DQ-BSA degradation and transmission electron microscopy. Using the mouse model of cardiac ischemia, Western blotting analysis of LC3 and p62 indicated less autophagic clearance after CAL in Ad-KO versus wild type mice. Importantly, these changes in autophagy corresponded with enhanced CAL-induced necrosis and apoptosis in Ad-KO mice, as shown by HMGB-1 and cleaved caspase-3 levels, respectively. We also found higher beclin1:Bcl-2 ratio in ischemic Ad-KO hearts and levels of the pro-apoptotic protein Bax were induced to a greater extent by ischemia in Ad-KO versus wild type mice. Echocardiography analysis showed that CAL-induced cardiac dysfunction was exaggerated in Ad-KO mice. In conclusion, our data suggests that adiponectin is an important mediator of autophagic flux in cardiomyocytes and that lack of autophagic activity in hearts of Ad-KO mice after ischemia contributes to enhanced cell death and cardiac dysfunction.

12.29
LIPOCALIN-2 REGULATES CARDIOMYOCYTE AUTOPHAGY TO CONTROL APOPTOSIS AND INSULIN SENSITIVY

Hye Kyoung Sung, Yee Kwan Chan, Meng Han, James Won Suk Jahng & Gary Sweeney.
Dept. of Biology, York Univ., Toronto, Canada.

Lipocalin-2 (Lcn2; also termed neutrophil gelatinase-associated lipocalin (NGAL)) is a proinflammatory adipokine which has become established as an important biomarker for kidney disease. It has also been implicated in the pathogenesis of heart failure and as a potential biomarker. Here we investigated it's direct effects on autophagy in H9c2 cardiomyocytes and the functional consequences. After treating H9c2 cells with recombinant Lcn2 (1 μg/ml, 1 hour) we used transmission electron microscopy, Western blotting and immunofluorescence for LC3-II, stable overexpresion of tandem fluorescent RFP/GFP-LC3, DQ-BSA degradation and MagicRed assay for lysosomal cathepsin activity to show that Lcn2 reduced autophagic flux. Lcn2 also reduced phosphoULK1 S555, increased phosphoULK1 S757. Importantly, this correlated with reduced insulin sensitivity. We then created an autophagy-deficient H9c2 cell model by overexpressing a dominant-negative Atg5 mutant and found that reduced autophagy levels also induced insulin resistance, and that adding rapamycin after Lcn2 could stimulate autophagy and recover insulin sensitivity. We also observed that long-term Lcn2 treatment contributed to hypoxia/reoxygenation-induced apoptosis in H9c2 cells via reducing autophagy. We have also shown that Lcn2 in-

creased intracellular iron levels, and reactive oxygen species production, to mediate pro-apoptotic effects. In conclusion, our study indicated that Lcn2 treatment caused insulin resistance and apoptosis and the use of gain and loss of function approaches elucidated a causative link between autophagy and these effects of Lcn2.

13.0 MITOCHONDRIAL GENETIC AND METABOLIC PROGRAMS

13.1
NOVEL SIGNALING PEPTIDES FROM THE MITOCHONDRIAL GENOME

Changhan David Lee[1]
[1]Gerontology, Univ. of Southern California, 3715 McClintock Ave., Rm. B12, Los Angeles, CA, 90089.

Mitochondria are known to be functional organelles, but their role as a signaling unit is increasingly being appreciated. We have recently identified a short open reading frame (sORF) within the mitochondrial 12S rRNA encoding a 16 amino acid peptide named MOTS-c (mitochondrial open-reading-frame of the twelve S rRNA -c) that regulates insulin sensitivity and metabolic homeostasis [1-3]. Its primary target organ appears to be the skeletal muscle and its cellular actions inhibit the folate cycle and its tethered *de novo* purine biosynthesis, causing a significant accumulation of AICAR levels concomitantly with AMPK activation. MOTS-c treatment in mice prevented age-dependent and high-fat diet-induced insulin resistance, as well as diet-induced obesity. These results suggest that mitochondria may be more actively engaged in regulating metabolic homeostasis than previously recognized, through the production of peptides encoded within its genome that act at the cellular and organismal level.

References: 1. Lee, C., et al., *The Mitochondrial-Derived Peptide MOTS-c Promotes Metabolic Homeostasis and Reduces Obesity and Insulin Resistance.* Cell Metab, 2015. **21**(3): p. 443-54. 2. Zarse, K. and M. Ristow, *A mitochondrially encoded hormone ameliorates obesity and insulin resistance.* Cell Metab, 2015. **21**(3): p. 355-6. 3. Alis, R., et al., *The Role of Mitochondrial Derived Peptides (MDPs) in Metabolism.* J Cell Physiol, 2015.

13.2
MITOCHONDRIAL NUCLEAR GENETIC CROSS TALK AND DISEASE: "MITO-MENDELIAN" GENETICS

Scott Ballinger[1]
[1]Pathology, Univ. of Alabama at Birmingham, BMR2 530, 1720 2nd Ave. S., Birmingham, AL, 35294.

The genetic basis for common disease is generally thought to be "complex" involving both environmental – genetic interactions. Interestingly, the evolution and origins of the eukaryotic cell are thought to be the consequence of an estimated 1.5 billion years of endosymbiosis and adaptation to the environment involving mitochondrial-nuclear, or "Mito-Mendelian" genetic interactions. Herein, we suggest that Mito – Mendelian genetics, plays a major role in influencing cellular metabolism and response to disease risk factors and thus, susceptibility to disease development. These concepts will be discussed in the context of cardiovascular and metabolic disease.

Funding was provided by the U.S. Army Medical Research & Material Command (W81XWH-07-1-0540d); National Institutes of Health (HL94518 and HL103859); and the Diabetes Research Center Bioanalytical Redox Biology Core (P30 DK079626).

13.3
THE CROSSTALK BETWEEN MITOCHONDRIAL FUNCTION, THE EPIGENOME AND GENE EXPRESSION

Janine Santos[1]
[1]Epigenetics & Stem Cell Biology, NIEHS, 111 TW Alexander Dr., Durham, NC, 27709.

Mitochondria are organelles known for their role in energy production through the process of oxidative phosphorylation (OXPHOS), a byproduct of which is reactive oxygen species (ROS). Mitochondrial function also gives rise to a diverse range of metabolic products that are known to function as co-factors of enzymes that epigenetically regulate the nuclear genome. We hypothesize that some of these metabolites may be rate-limiting for epigenetic reactions that regulate gene expression in the nucleus. The data obtained so far indicate that loss of OXPHOS function is accompanied by changes in some mitochondrial metabolites, decreases in histone acetylation, modulation of DNA methylation and changes in gene expression. Further experiments are ongoing to further tease out this crosstalk.

This research was supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

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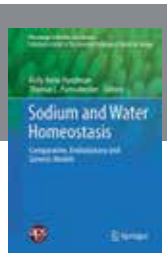

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2015 APS Conference:
Cardiovascular, Renal and Metabolic Diseases: Physiology and Gender
November 17—20, 2015
Annapolis, Maryland
Week-At-A-Glance

Tuesday, November 17	Wednesday, November 18	Thursday, November 19	Friday, November 20
3:00 PM **Registration**	7:00 AM **Registration**	7:30 AM **Registration**	7:30 AM **Registration**
	7:50—8:00 AM **Welcome** **S. Ananth Karumanchi** 8:00—10:00 AM **Symposia I** Immune System and Regenerative Medicine—Impact of Gender/Sex **Heddwen Brooks**	8:00—10:00 AM **Symposia V** Developmental Programming of Cardiovascular, Renal and Metabolic Diseases: Roles of Gender/Sex **Javier Salazar**	8:00—10:00 AM **Symposia VIII** Pregnancy and Pre-eclampsia **Christine Maric-Bilkan**
	10:00—10:30 AM **Break** 10:30 AM—12:30 PM **Symposia II** Non-reproductive Actions of Sex Hormones/Receptors—A **Rolando J. Ramirez**	10:00—10:30 AM **Break** 10:30 AM—12:30 PM **Symposia VI** Non-reproductive Effects of Sex Hormones/Receptors—B **Kate M. Denton**	10:00—10:30 AM **Break** 10:30 AM—11:30 AM **Symposia IX** Population Studies—Gender/Sex in CVD, Renal Disease, and Metabolic Syndrome **Rita Tostes**
	12:30—1:30 PM **Lunch** 1:30—2:30 PM **Poster Session I**	12:30—1:30 PM **Lunch** 1:30—2:30 PM **Poster Session II**	11:35—11:45 AM **Closing Remarks**
	2:30—3:50 PM **Symposia III** Neuro Control of Cardiovascular, Renal and Metabolic Diseases: Impact of Gender/Sex **Willis K. Samson** 3:55-4:30 Distinguished Investigator Award **Jennifer Sullivan, Chair** **Chris Baylis, Speaker**	2:30—3:00 PM **Plenary Lecture** **Kathryn Sandberg (Chair)** Janine Clayton (Speaker) 3:00—5:00 PM **Symposia VII** Obesity, Metabolic Syndrome, Gender/Sex **James R. Sowers**	
6:30—8:30 PM **Welcome and Opening Reception**	5:00—6:00 PM **Career Development Session** **Jennifer Sasser** **Erica Wehrwein**	7:00—9:30 PM **Banquet and Awards Ceremony**	

GENERAL INFORMATION

Location:

The 2015 APS Conference: Cardiovascular, Renal and Metabolic Diseases will be held November 17—20, 2015 at the Crowne Plaza Annapolis Hotel, 173 Jennifer Rd., Annapolis, MD 21401, USA, telephone (410) 266-3131, FAX: (410) 266-6247.

Onsite Registration Hours:

Tuesday, November 17…….....3:00—8:00 PM
Wednesday, November 18……..7:00 AM—5:30 PM
Thursday, November 19…….7:30 AM—5:30 PM
Friday, November 20……..7:30 —11:00 AM

On-Site Registration Fees:

APS Member.....$650
APS Retired Member.....$450
Nonmember $800
Postdoctoral.....$500
Student.....$450

*The registration fee includes entry into all scientific sessions, poster socials, opening reception, and the closing conference banquet**.

*Must have a ticket for entry.

Payment Information:

Registrants may pay by institutional or personal check, traveler's check, MasterCard, VISA or American Express or in United States Dollars. Checks must be payable to *"The American Physiological Society"* and drawn on a United States bank payable in US dollars.

Student Registration:

Any student member or regularly matriculated student working toward a degree in one of the biomedical sciences is eligible to register at the student fee. Nonmember postdoctoral fellows, hospital residents and interns, and laboratory technicians do not qualify as students. Nonmember students who register onsite must provide a valid university student ID card. APS student members should present their current APS membership card indicating their student category status.

Postdoctoral Registration:

Any person who has received a Ph.D. degree in physiology or related field, **within four years** of this meeting, as attested to by the department head is eligible to register at the postdoctoral fee. **A statement signed by the department head must accompany the registration form and remittance when registering.**

Press:

Press badges will be issued at the APS registration desk, only to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public affairs, etc.) must register as nonmembers.

Program Objective:

The role that sex steroids and gender play in the physiology and pathophysiology of cardiovascular and renal disease (CVRD) is becoming an increasingly more important area of research. The program will be balanced to include both basic science and clinical studies, ranging from the gene to the whole animal or human. The global aspect of the conference is to gather a critical mass of scientists with interests and expertise in the role of sex steroids and/or the gender differences in the physiology of CVRD, and to promote an exchange of ideas to foster collaboration that will further advance this important line of scientific investigation. In addition, this conference will be to increase the awareness of sex disparities in CVRD that need to be understood in order to ultimately improve clinical outcomes for men and women and promote individualized health care.

Target Audience:

The intended audience for this conference includes all levels of researchers working in the field of gender disparities in cardiovascular, renal and metabolic diseases. This conference will provide a diverse program that covers many of the organ systems in which sex steroids and gender have been shown to be important in cardiovascular diseases.

Photography is *not* permitted during the scientific sessions or in the poster room

Don't forget to join us at the Welcome and Opening Reception

Admiral's Ballroom

Tuesday, November 17
6:30—8:30 PM

WEDNESDAY, NOVEMBER 18, 2015

Welcome

1.0 WELCOME ANNOUCEMENT

Wednes., 7:50—8:00 AM, Wye Room.

Chairs: **S. Ananth Karumanchi,** *Harvard Med. Sch.*
Jane F. Reckelhoff, *Univ. of MississippiMed. Ctr.*

Symposia I

2.0 IMMUNE SYSTEM AND REGENERATIVE MEDICINE-IMPACT OF GENDER AND SEX

Wednes., 8:00—10:00 AM, Wye Room.

Chair: **Heddwen Brooks,** *Univ. of Arizona, Tucson.*

8:00 AM **2.1** Estrogen Receptor Alpha Enhances Loss of Tolerance to Nuclear Antigens and Immune Cell Activation Induced by the *Sle*1 Lupus Susceptibility Allele and is Responsible for the Sex Bias Associated with *Sle*1. **Karen Gould.** *Univ. of Nebraska, Omaha.*

8:20 AM **2.2** Role of T Cells In Development of Cardiovascular Disease and Hypertension. **Jennifer Sullivan.** *Georgia Regents Univ.*

8:40 AM **2.3** Estrogen and its Effects on Women with Lupus Erythematosus. **Michael Ryan.** *Univ. of Mississippi Med. Ctr., Jackson.*

9:00 AM **2.4** Lower Levels of Interleukin-6 in Female Mice at Days 1 and 3 Post-myocardial Infarction Attenuate Neutrophil Infiltration, Rupture, and Left Ventricular Dilation. **Kristine DeLeon-Pennell.** *Univ. of Mississippi Med. Ctr., Jackson.* **(5.11).**

9:15 AM **2.5** The Effects of Testosterone and Oxidative Stress on Neuroinflammatory Signaling in Dopamine Neurons. **Shaletha Holmes.** *Univ. of North Texas Hlth. Sci. Ctr., Forth Worth.* **(14.6).**

9:30 AM **2.6** Sexually Dimorphic Myeloid Inflammatory and Metabolic Responses to Diet-induced Obesity. **Kanakadurga Singer.** *Univ. of Michigan.* **(6.17).**

9:45 AM **2.7** Doxorubicin Reduces Proinflammatory Mediator Expression in Brain and Pial Arteries from Ovariectomized Female Rats. **Rayna Gonzales.** *Univ. of Arizona, Phoenix.* **(14.7).**

Symposia II

3.0 NON-REPRODUCTIVE ACTIONS OF SEX HORMONES AND RECEPTORS-A

Wednes., 10:30 AM—12:30 PM, Wye Room.

Chair: **Rolando J. Ramirez,** *Univ. of Akron.*

10:30 AM **3.1** Testosterone Therapy in Men with Testosterone Deficiency (TD): Advances and Controversies. **Abdulmaged M. Traish.** *Boston Univ. Sch. of Med.*

10:50 AM **3.2** Differential Body Weight and Blood Pressure Responses to Normal Versus High-fat Diet in Melanocortin-4 Receptor-deficient Preganant Rats. **Frank Spradley.** *Univ. of Mississippi Med. Ctr.*

11:10 AM **3.3** GPER and Vascular Function. **Sarah Lindsey.** *Tulane Univ.*

11:30 AM **3.4** Contribution of the Nuclear Progesterone Receptor (nPR) to Breathing Stability and Hypercapnic Ventilatory Response in Adult Male Mice. **Sofien Laouafa.** *Univ. of Laval, Quebec, Canada.* **(13.7).**

11:45 AM **3.5** Functional and Structural Changes in Internal Pudendal Arteries Underlie Erectile Dysfunction Induced by Androgen Deprivation. **Rheure Lopes.** *Univ. of São Paulo, Brazil.* **(4.8).**

12:00 Noon **3.6** 6β-Hydroxytestosterone, A Cytochrome P450 1B1-Derived Metabolite of Testosterone Plays an Important Role in Renal Dysfunction Associated with Angiotensin II-Induced Hypertension in Male Mice. **Ajeeth Pingili.** *Univ. of Tennessee Hlth. Sci. Ctr., Memphis.***(7.13).**

12:15 PM **3.7** Attenuation of Cardiac Aging and Leptin-dependant Cardioprotection in Long-lived αMUPA Mice. **Edith Hochhauser.** *Rabin Med. Ctr., Israel.* **(5.9).**

Poster Session I

4.0 CARDIOVASCULAR DISEASE

Wednes., 1:30—2:30 PM, Rhode/Severn Room.

Poster Board

1 **4.1** Matrix Metalloproteinase-9 is Critical for 2-Methoxyestradiol Mediated Angiotensin Type 1 Receptor Down-Regulation. **B. Ogola, Y. Zang, and T. Thekkumkara** *Texas Tech Univ. Hlth. Sci. Ctr. Sch. of Pharmacy, Amarillo, TX.*

2 **4.2** Underrepresentation of Sex in Reporting Traditional and Emerging Biomarkers for Primary Prevention of Cardiovascular Disease: A Systematic Review. **A. Gohar, R. Schnabel, M. Hughes, T. Zeller, S. Blankenberg, G. Pasterkamp, and Hester den Ruijter.** *Univ. Med. Ctr., Utrecht, The Netherlands, Univ. Heart Ctr., Hamburg, Germany, German Ctr. for Cardiovascular Res., Hamburg, Germany, and UKCRC Ctr. of Excellence for Public Hlth. Res., Belfast, UK.*

3 **4.3** Loss of the Y Chromosome in Men Undergoing Carotid Endarterectomy. **S. Haitjema, D. Kofink, Jessica. van Setten, S. W. van der Laan, S. C. A. de Jager, P. I. W. de Bakker, G. Pasterkamp, F. W. Asselbergs, and H. M. den Ruijter.** *Univ. Med. Ctr., Utrecht, The Netherlands, ICN-Netherlands Heart Inst., Amsterdam, The Netherlands, and Univ. Coll. London, UK.*

4 **4.4** Circulating GDF-15 Levels are Explicitly Valuable for the Prediction for Future Cardiovascular Complications in Women. **A. Gohar, J. Vrijenhoek, G. Pasterkamp, H. M. den Ruijter, and S. C. A. de Jager** *Univ. Med. Ctr., Utrecht, The Netherlands.*

5 **4.5** A Study of the Potential Risk Factors of Cardiovascular Diseases in Young Saudi females. **L. Al-Asoom** *Univ. of Dammam, Saudi Arabia.*

6 **4.6** Assessment of Gender and Age-dependent Patterns of Cardiovascular Remodeling in Spontaneously Hypertensive Rats (SHR). **S. Al-Gburi, I. Kopaliani, B. Zatschler, R. Galli, M. Kasper, and A. Deussen.** *Tech. Univ. of Dresden, Germany.*

7 **4.7** Indices of Cardiovascular Function Derived from Peripheral Pulse Wave Analysis Using Radial Applanation Tonometry in HIV Positive Patients from Mthatha District of South Africa. **K. Awotedu, R. Erasmus, A. Awotedu, and A. Namugowa .** *Walter Sisulu Univ., Mthatha, South Africa, and Univ. of Stellenbosch, Cape Town, South Africa.*

8 **4.8** Functional and Structural Changes in Internal Pudendal Arteries Underlie Erectile Dysfunction Induced by Androgen Deprivation. **R. Lopes, K. Neves, M. Barbosa, V. Olivon, S. Ruginsk, J. Antunes, L. Ramalho, F. Carneiro, and R. Tostes** *Univ. of São Paulo, Ribeirao Preto, Brazil.*

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Poster Board

9 **4.9** Effects ofAerobic Exercise Training on Renin-angiotensin System Components in Hypertensive Women. **A. Jarrete, R. Esposti, M. Ferreira, C. Sponton, C. Anaruma, T. Fernandes, F. Fernandes, E. Oliveira, D. Casarini, and A. Zanesco.** *Campinas State Univ., São Paulo, Brazil, São Paulo State Univ., Brazil, Univ. of São Paulo, Brazil, and Fed. Univ. of Sao Paulo, Brazil.*

10 **4.10** Gender and Circulating Vascular MicroRNAs in Middle-Aged Adults. **J. Hijmans, T. Bammert, G. Lincenberg, K. Diehl, W. Reiakvam, C. Dow, J. Greiner, B. Stauffer, and C. DeSouza.** *Univ. of Colorado, Boulder.*

Poster Session II

5.0 CARDIAC

Wednes., 1:30—2:30 PM, Rhode/Severn Room.

Poster Board

11 **5.1** Soluble Guanylyl Cyclase Exerts Opposite Effects in the Myocardium of Male and Female Endotoxemic Mice. **I. Hobai, K. Aziz, D. Siwik, and W. Colucci.** *Boston Univ. Med. Ctr., and Massachusetts Genl. Hosp.*

12 **5.2** Haemostatic and Rheologic Factorials as Determinants of Acute Myocardial Infarctions in Nigerians. **E. Nwali, and O. Ajayi.** *Univ. of Benin, Benin City, Nigeria.*

13 **5.3** Increased Prevalence of Atrial Fibrillation in Male Mice is Associated with Lower Expression of Connexin 40 and 43. **A-H. Ton, A. Ducharme, and C. Fiset.** *Montreal Heart Inst., Canada.*

14 **5.4** Indices of Cardiac Sympathetic Activity During Lower Body Negative Pressure in Men and Women Throughout the Menstrual Cycle. **H. Edgell, and R. Hughson.** *York Univ., Toronto, Canada, and Univ. of Waterloo, Canada.*

15 **5.5** In Vivo Electrocardiograms in a Murine Model of Chagasic Cardiomyopathy Specify First Degree Atrioventricular Block as a Predictor for Severe Disease. **J. Respress, M. Barry, K. Jones, M. E. Bottazzi, and P. Hotez.** *Southwest Electronic Energy Med. Res. Inst., Missouri City, TX, and Baylor Coll. of Med.*

16 **5.6** Angiotensin II Modulates Sex Steroid Metabolizing Enzyme and Receptor Expression in Cardiac Fibroblasts From Male and Female Rats. **L. Madhavpeddi, R. Gonzales, and T. Hale.** *Univ. of Arizona, Phoenix.*

17 **5.7** Cardiac Remodeling in Female Hearts by Kvβ1 Subunit. **J. Tur, K. Chapalamadugu, T. Padawer, and S. Tipparaju.** *Univ. of South Florida.*

18 **5.8** Impaired Diastolic Function Following Acute Starvation in Men but not Premenopausal Women. **M. Nelson, L. Szczepaniak, D. Clegg, D. Li, and C. N. Bairey Merz.** *Cedars-Sinai Med. Ctr., Los Angeles.*

19 **5.9** Attenuation of Cardiac Aging and Leptin-dependent Cardioprotection in Long-lived αMUPA Mice. **E. Hochhauser, E. Levy, R. Gavrieli, I. Fratty, G. Greenberg, M. Waldman, E. Birk, A. Shainberg, R. Miskin, and R. Kornowski.** *Rabin Med. Ctr. & Tel-Aviv Univ., Israel, Bar Ilan Univ., Ramat Gan, Israel, Schneider Med. Ctr. & Tel-Aviv Univ., Israel, and Weizmann Inst., Rehovot, Israel.*

20 **5.10** The Characterization of Auxotonic Twitch of Right Ventricular Cardiomyocytes from Non-failing and Failing Hearts of Impuberal Male and Female Rats. **O. Lookin, and Y. Protsenko.** *Ural Branch of Russian Academy of Sci., Yekaterinburg, Russian Fed.*

21 **5.11** Lower Levels of Interleukin-6 in Female Mice at Days 1 and 3 Post-myocardial Infarction Attenuate Neutrophil Infiltration, Rupture, and Left Ventricular Dilation. **K. DeLeon-Pennell, R. P. Iyer, Y. Ma, A. Yabluchanskiy, G. V. Halade, and M. L. Lindsey.** *Univ. of Mississippi Med. Ctr., Jackson, and San Antonio Cardiovascular Proteomics Ctr., Univ. of Alabama at Birmingham, and G. V. Montgomery Vet. Affairs Med. Ctr., Jackson, MS.*

Poster Session III

6.0 METABOLISM AND DIABETES

Wednes., 1:30—2:30 PM, Rhode/Severn Room.

Poster Board

22 **6.1** Augmentation of Urinary Angiotensinogen Levels in Young Men and Women with Type-1 Diabetes Mellitus. **L. G. Navar, A. Katsurada, V. Fonseca, M. C. Prieto, S. Chalew, and H. Kobori.** *Tulane Univ., Louisiana State Univ., and Intl. Univ. of Hlth. & Welfare, Tokyo, Japan.*

23 **6.2** Estrogen Treatment Restores Muscle Mitochondrial Capacity and Reverses Pro-Diabetogenic State induced by Ovariectomy. **M. Torres, L. Reese, L. Gilliam, K. Buddo, C. Smith, and P. D. Neufer.** *East Carolina Univ.*

24 **6.3** Withdrawn.

25 **6.4** Increased Orexigenic Innervation of Dopamine Neurons Reduces Prolactin Secretion in Obese Female Rats. **N. Toporikova, M. Knabe, V. Pogrebna, T. Barrett, P. Ozark, and S. Blythe.** *Washington & Lee Univ., Lexington, VA.*

26 **6.5** Diet-Induced Obesity Impairs Estrous Cycle Regularity in Female Rats. **S. Blythe, J. Roberts, K. Sarfert, J. Wu, and N. Toporikova.** *Washington & Lee Univ., Lexington, VA.*

27 **6.6** Influences of Diet on Serum C-Reactive Protein in Unobstructured and Obstructed Bladders of Male Wistar Rats. **T. Adedeji, A. Fasanmade, and E. Olapade-Olaopa.** *Univ.of Ibadan, Nigeria, and Univ. Coll. Hosp., Ibadan, Nigeria.*

28 **6.7** A High-Fat Diet Impacts Glucose and Blood Pressure in Female and Male Dahl Salt-Sensitive Rats. **J. Sullivan, E. Ralph, P. T. Menk, and E. J. Belin de Chantemèle.** *Georgia Regents Univ.*

29 **6.8** High Fructose Intake Exacerbates the Impairment of Mesenteric Arterial Function Compared to Glucose in Female Rats: Possible Involvement of EDHF Contribution in Modulating Vascular Reactivity. **S. Shaligram, G. Sangüesa, F. Akther, M. Alegret, J. C. Laguna, and R. Rahimian.** *Univ. of the Pacific, and Univ. of Barcelona, Spain.*

30 **6.9** The Impact of HIV Infection on Body Composition, Lipid Profile, Adiponectin Level and Resting Energy Expenditure in Mthatha District, A Semi Urban South African Community. **S. Zono, K. Awotedu, and B. Longo Mbeza.** *Walter Sisulu Univ.Mthatha, South Africa.*

31 **6.10** Alterations in Fatty Acid Signaling Pathways Differentially Affect Fat Intake in Male and Female Mice. **T. Gilbertson, M. Fillmore, N. Dahir, and D. Minaya.** *Utah State Univ., and Univ. of Florida, Gainesville.*

Poster presenters…don't forget that your poster is displayed *only* on the day you present.

Please remove your poster at the end of your presentation day. Unclaimed posters will be removed and stored by APS until the conclusion of the conference. Any unclaimed posters will be recycled.

Poster Board

32 **6.11** Withdrawn.

33 **6.12** A Pilot Study Exploring Metabolic Dysfunction in Trans-sexual Women: Novel Insight From Magnetic Resonance Spectroscopy. **M. Nelson, D. Clegg, L. Szczepaniak, J. Broussard, R. Bergman, and C. N. Bairey Merz** *Cedars-Sinai Med. Ctr., Los Angeles.*

34 **6.13** Do Women Need to Lose More Weight than Men to Increase Circulating Adiponectin? **X. Wang.** *Univ. of South Carolina, Columbia.*

35 **6.14** Sex, Leptin Status, and Obesity Modulate Buprenorphine-induced Respiratory Depression in Mice. **C. Angel, W. Alami, S. Mihalko, H. A. Baghdoyan, and R. Lydic.** *Univ. of Tennessee, Knoxville.*

36 **6.15** Increasing Leptin Sensitivity with Protein Tyrosine Phosphatase 1B Deletion Leads to More Severe Cardiac Alterations in Female than Male Mice. **A-C. Huby, and E. J. Belin de Chantemèle.** *Georgia Regents Univ.*

37 **6.16** Sex Differences in Renal Sodium Handling in Mice on High-fructose and High-salt Diet. **A. Rouch, L. Fan, B. Swar, and C. Waturuocha.** *Oklahoma State Univ., Tulsa.*

38 **6.17** Sexually Dimorphic Myeloid Inflammatory and Metabolic Responses to Diet-induced Obesity. **K. Singer, N. Maley, J. DelProposto, B. Zamarron, and C. Lumeng.** *Univ. of Michigan.*

39 **6.18** Sex Dimorphism In Plasma Soluble Prorenin Receptor (sPRR) Levels in Obese Patients Is Associated With Type 2 Diabetes Mellitus in Women But Not in Men. **C. B. Rosales, D. Y. Arita, T. Thethi, V. Fonseca, L. G. Navar, and M. C. Prieto.** *Tulane Univ. Sch. of Med.*

40 **6.19** Sex Differences in Renal Gene Expression in a Diet Induced Obesity Model of Diabetic Nephropathy (DN). **V. Halperin Kuhns, and J. Pluznick.** *Johns Hopkins Univ. Sch.of Med.*

41 **6.20** Leptin-Mediated Aldosterone Secretion Causes Hypertension in Obese Females. **A-C. Huby, M. Cortez-Cooper, J. Cannon, and E. J. Belin de Chantemèle.** *Georgia Regents Univ.*

42 **6.21** Effects of Diet-induced Obesity on Reproductive Hormone Signaling and Gene Expression. **J. Bowman, T. Caldwell, J. Guider, J. Lee, P. Ozark, S. Blythe, N. Toporikova, and G. Whitworth.** *Washington & Lee Univ., Lexington, VA.*

Poster Session IV

7.0 RENAL

Wednes., 1:30—2:30 PM, Rhode/Severn Room.

Poster Board

43 **7.1** Molecular Mechanisms for Lower Plasma Potassium Set Point in Females. **L. Veiras, A. Tran, D. Ralph, and A. McDonough.** *Univ. of Southern California, Los Angeles.*

44 **7.2** Long-term Estrogen Treatment Increases Renal Tubular Casts and TGFβ in Aged Ovariectomized Long Evans Rats. **M. Zimmerman, D. Hutson, B. Murphy, S. Kashyap, E. Trimmer, J. Daniel, and S. Lindsey.** *Tulane Univ.*

45 **7.3** Alterations in 20-HETE Production Contribute to End Organ Damage in Dahl S rats. **F. Fan, W. Wu, and S. Murphy.** *Univ. of Mississippi Med. Ctr., Jackson.*

46 **7.4** Apoptotic Cell Death In Renal Ischemia-Reperfusion Injury in Male and Female Spontaneously Hypertensive Rats (SHR). **R. Crislip, and J. Sullivan.** *Georgia Regents Univ.*

Poster Board

47 **7.5** Withdrawn.

48 **7.6** Kidney Epithelium-specific Knockout of SHP-1 enhances Urinary Concentration in Female Mice. **H. Wang, L. A. MacMillan-Crow, and X. Zhou.** *Uniformed Services Univ., Bethesda, MD, and Univ. of Arkansas for Med. Sci., Little Rock.*

49 **7.7** Progesterone Synergizes Estradiol-Induced Natriuresis in Response to Increased Dietary Sodium Intake. **E. Gohar and D. M. Pollock.** *Univ. of Alabama at Birmingham.*

50 **7.8** Withdrawn.

51 **7.9** High Salt Alters Cellular Transcriptional Milieu And Human Angiotensinogen Expressionin a Gender-Dependent Manner: An Effect Exacerbated by a Risk Haplotype. **M. Kaw, N. Puri, and A. Kumar.** *Univ. of Toledo Hlth. Sci.*

52 **7.10** Different Response to Dopamine or to Bradykinin Inhibition in Ovariectomized Adult Wistar Rats Under High Sodium Intake. **F. R. Ibarra, S. G. Vlachovsky, G. Moiron, P. J. Azurmendi, E. M. Oddo, E. E. Arrizurieta, S. Nowicki, and L. A. Di Ciano.** *Buenos Aires Univ., Argentina, Inst. de Investigaciones Medicas A Lanari, Buenos Aires, Argentina, and CEDIE-CONICET, Buenos Aires, Argentina.*

53 **7.11** Participation of CYP4AΩ-hydroxylase/20-HETE in Blood Pressure Regulation of Hyperandrogenemic Female Rats. **C. Dalmasso, R. O. Maranon, C. N. Patil, A. Harris, H. Zhang, and J. F. Reckelhoff.** *Univ. of Mississippi Med. Ctr., Jackson.*

54 **7.12** Multiple Estrogen Receptor Subtypes Selectively Influence Fluid Intake in Female Rats. **J. Santollo, and D. Daniels.** *Univ. at Buffalo.*

55 **7.13** 6β-Hydroxytestosterone, A Cytochrome P450 1B1-Derived Metabolite of Testosterone Plays an Important Role in Renal Dysfunction Associated with Angiotensin II-Induced Hypertension in Male Mice. **A. Pingili, S. Thirunavukkarasu, M. Kara, and K. Malik.** *Univ. of Tennessee Hlth. Sci. Ctr.*

Symposia III

8.0 NEURO CONTROL OF CARDIOVASCULAR, RENAL AND METABOLIC DISEASES: IMPACT OF GENDER AND SEX

Wednes., 2:30—3:50 PM, Wye Room.

Chair: **Willis K. Samson,** *St. Louis Univ.*

2:30 PM **8.1** Autonomic Regulation of Blood Pressure in Adult Humans: Effects of Sex and Age. **Michael Joyner.** *Mayo Clinic, Rochester, MN.*

2:50 PM **8.2** Sex Differences in Desensitization of the Dipsogenic Effect of Angiotensin II. **Derek Daniels.** *Univ. of Buffalo, SUNY.*

3:10 PM **8.3** Adipokines, Obesity, and Sex: Implications for Cardiovascular Function. **Gina Yosten.** *St. Louis Univ.*

3:30 PM **8.4** Characterizing the Gender Differences of Multidrug-resistance Peptide (MRP) Transporter Expression in Mouse Blood-brain Interfaces. **Katira Flores.** *Univ. of Connecticut.* **(14.3).**

Join us for the Opening Reception on Tuesday, November 17, 2015 from 6:30—8:30 PM

DAILY SCHEDULE

Plenary Lecture

9.0 DISTINGUISHED INVESTIGATOR AWARD

Wednes., 3:55—4:30 PM, Wye Room.

Chair: **Jennifer Sullivan,** *Georgia Regents Univ.*

3:55 PM **9.1** The Enigma of the Maternal Plasma Volume Expansion During Nnormal Pregnancy. **Chris Baylis.** *Univ. of Florida, Gainesville.*

Career Session

10.0 CAREER DEVELOPMENT SESSION

Wednes., 5:00—6:00 PM, Wye Room.

Chairs: **Jennifer Sasser,** *Univ. of Mississippi Med. Ctr., Jackson.* **Erica Wehrwein,** *Michigan State Univ.*

THURSDAY, NOVEMBER 19, 2015

Symposia V

11.0 DEVELOPMENTAL PROGRAMMING OF CARDIOVASCULAR, RENAL AND METABOLIC DISEASES: ROLES OF GENDER AND SEX

Thurs., 8:00—10:00 AM, Wye Room.

Chair: **Javier Salazar,** *Univ. of Murcia, Spain.*

8:00 AM **11.1** Effect of Estrogen in Gender-dependent Fetal Programming of Adult Cardiovascular Dysfunction. **Daliao Xiao.** *Loma Linda Univ. Sch. of Med.*

8:20 AM **11.2** Sex Differences in Cardiovascular and Metabolic Risks Due to Early Life Stress. **Analia Loria.** *Univ. of Kentucky, Lexington.*

8:40 AM **11.3** Maternal Undernutrition Significantly Impacts Ovarian Follicle Number and Increases Ovarian Oxidative Stress in Adult Rat Offspring. **Deborah Sloboda.** *McMaster Univ., Hamilton, Canada.*

9:00 AM **11.4** Reduced Sleep Time During Pregnancy-Effects on Renal Morphology and Function of Female Offspring. **Guiomar N. Gomes.** *Univ. of São Paulo, Brazil.* **(16.5).**

9:15 AM **11.5** Delayed Effects of Perinatal Hypoxia on Adult Rats Pulmonary Vessels Structure and Reactivity. **Martin Vizek.** *Charles Univ., Prague, Czech. Rep.* **(16.6).**

9:30 AM **11.6** Sex Difference in Sensitization of Angiotensin (ANG) II-elicited Hypertension in Offspring of Hypertensive Pregnant Rats. **Baojian Xue.** *Univ. of Iowa.* **(16.7).**

9:45 AM **11.7** Sex Differences in Cardiovascular Responses to Stress in Adult Rats Prenatally Exposed to Dexamethasone. **Taben Hale.** *Univ. of Arizona, Phoenix.* **(16.8).**

Symposia VI

12.0 NON-REPRODUCTIVE EFFECTS OF SEX HORMONES AND RECEPTORS-B

Thurs., 10:30 AM—12:30 PM, Wye Room.

Chair: **Kate M. Denton,** *Monash Univ., Melbourne, Australia.*

10:30 AM **12.1** Androgen Effects on Endothelial Function in Women in Polycystic Ovary Syndrome. **Nina Stachenfeld.** *Yale Univ.*

10:50 AM **12.2** Mechanisms Involved in Cardioprotection in Females: Role of Estrogen and Estrogen Receptors (ERs). **Elizabeth Murphy.** *NIH, NHLBI.*

11:10 AM **12.3** Sex and Sex Hormone Effects in Cardiovascular Pathophysiology. **Vera Regitz-Zagrosek.** *Charite Univ., Berlin, Germany.*

11:30 AM **12.4** Effects ofAerobic Exercise Training on Renin-angiotensin System Components in Hypertensive Women. **Aline Jarrete.** *Campinas State Univ., Brazil.* **(4.9).**

11:45 AM **12.5** Gender and Circulating Vascular MicroRNAs in Middle-Aged Adults. **Jamie Hijmans.** *Univ. of Colorado, Boulder.* **(4.10).**

12:00 Noon **12.6** Cerebral Blood Flow Regulation is Affected Throughout the Menstrual Cycle in Young Women. **Michelle Favre.** *Rutgers Univ. Biomed. Hlth. Sci., Newark.* **(14.8).**

12:15 PM **12.7** Effects of Menopause and Acute Exercise on Brachial Artery Flow Mediated Dilation and Plasma Endothelial Microparticles. **Corinna Serviente.** *Univ. of Massachusetts, Amhearst.* **(12.7).**

Poster Session II

13.0 RESPIRATORY

Thurs., 1:30—2:30 PM, Rhode/Severn Room.

Poster Board

1 **13.1** Sex Differences in Diet and Inhaled Ozone (O_3) Induced Metabolic Impairment. **U. Kodavanti, V. Bass, M. Schldaweiler, C. Gordon, K. Jarema, P. Phillips, A. Ledbetter, D. Miller, S. Snow, and J. Richards.** *U.S. Environ. Protection Agency, Res. Triangle Pk., NC, and Univ. of North Carolina, Chapel Hill.*

2 **13.2** Lung Antioxidant Levels in Neonatal Rats and Response to Air Pollution: Influence of Sex and Strain. **E. Gibbs-Flournoy, J. Richards, E. Hines, K. Kraft, J. Norwood, G. Hatch, M. Madden, and J. Dye.** *U.S. Environ. Protection Agency, Res. Triangle Pk., NC, and Chevron Energy Tech., Co., Houston, TX.*

3 **13.3** Estradiol Prevents Cardio-respiratory Dysfunctions Induced by Chronic IntermittentH hypoxia in Female Rats. **S. Laouafa, F. Marcouiller, D. Roussel, A. Bairam, and V. Joseph.** *Univ. of Laval, Quebec, Canada, and Univ. of Claude Bernard Lyon, Villeurbanne, France.*

4 **13.4** Potential Role of Estrogen in 15-hydroxyeicosatetranoic Acid Production and Activity in Human Pulmonary Artery Endothelial Cells. **S. Pfister.** *Med. Coll. of Wisconsin, Milwaukee.*

5 **13.5** Muscular and Cardiorespiratory Adaptations to Treadmill Training with Aging are Blunted in Female Compared to Male Mice. **K. Huey, T. Drake, G. Dillon, and C. Lee.** *Drake Univ., Des Moines, IA.*

6 **13.6** Effect of Exercise on Red Blood Cells Variables in Highly Trained Female Athletes. **D. Akther.** *Holy Family Red Cres. Med. Coll., Dhaka, Bangladesh.*

7 **13.7** Contribution of the Nuclear Progesterone Receptor (nPR) to Breathing Stability and Hypercapnic Ventilatory Response in Adult Male Mice. **S. Laouafa, F. Marcouiller, and V. Joseph.** *Univ. of Laval, Quebec, Canada.*

Poster Session II

14.0 NEUROCONTROL

Thurs., 1:30—2:30 PM, Rhode/Severn Room.

Poster Board

8 **14.1** The Important Role of Nitric Oxide Synthase in Controlling Mitochondrial Respiration of Large Cerebral Arteries in Female and Male Rats. **I. Rutkai, S. Dutta, P. Katakam, and D. Busija.** *Tulane Univ.*

Poster Board

9 **14.2** Sex Differences in the Cerebral Vascular Function and K Channel Role. **M. Pabbidi.** *Univ. of Mississippi Med. Ctr., Jackson.*

10 **14.3** Characterizing the Gender Differences of Multidrug-resistance Peptide (MRP) Transporter Expression in Mouse Blood-brain Interfaces. **K. Flores, J. L. Renfro, and J. Manautou.** *Univ. of Connecticut.*

11 **14.4** Sex and Genotype Differences to Epinephrine Infusions in Humans. **A. Eugene, and M. Joyner.** *Mayo Clinic, Rochester, MN.*

12 **14.5** Sex Differences in the Effect of Hypoglycemia on Baroreflex Sensitivity in Patients with Type 1 Diabetes Mellitus. **J. Limberg, S. Dube, M. Mozer, A. Basu, R. Basu, and M. Joyner.** *Mayo Clinic, Rochester, MN.*

13 **14.6** The Effects of Testosterone and Oxidative Stress on Neuroinflammatory Signaling in Dopamine Neurons. **S. Holmes, and R. Cunningham.** *Univ. of North Texas Hlth. Sci. Ctr., Forth Worth.*

14 **14.7** Doxorubicin Reduces Proinflammatory Mediator Expression in Brain and Pial Arteries from Ovariectomized Female Rats. **R. Gonzales, P. Raman, N. Vijayavel, C. Kerrigan, J. Echeverria, J. Dickinson, C. Carroll, T. Hale, and S. Angati.** *Univ. of Arizona, Phoenix, Arizona State Univ., Phoenix, Midwestern Univ., and Mayo Clinic, Phoenix, AZ.*

15 **14.8** Cerebral Blood Flow Regulation is Affected Throughout the Menstrual Cycle in Young Women. **M. Favre, L. A. Reyes, A. Fox, and J. M. Serrador.** *Rutgers Univ. Biomed. Hlth. Sci., and Vet. Affairs Hlth. Care Sys., East Orange, NJ.*

Poster Session II

15.0 PREGNANCY

Thurs., 1:30—2:30 PM, Rhode/Severn Room.

Poster Board

16 **15.1** Placental Ischemia Increases Sensitivity to Pentylenetetrazol-induced Seizures and Cerebrospinal Fluid Inflammation. **J. Warrington.** *Univ. of Mississippi Med. Ctr., Jackson.*

17 **15.2** Vitamin D Supplementation Inhibits Blood Pressure and Uterine Artery Resistance Induced by Autoantibodies to the AT1 Receptor. **J. Faulkner, L. Amaral, D. Cornelius, T. Ibrahim, M. Cunningham, Jr., D. Thomas, G. Wallukat, R. Dechend, and B. LaMarca.** *Univ. of Mississippi Med. Ctr., Jackson, and HELIOS Clinic, Berlin, Germany.*

18 **15.3** Role of Obese-Related Metabolic Factors on Blood Pressure Regulation in Pregnant Rats. **A. Palei, F. Spradley, and J. Granger.** *Univ. of Mississippi Med. Ctr., Jackson.*

19 **15.4** The Increased Endothelium-dependent Vasodilatory Response of Healthy Pregnancy is Absent in the Preeclamptic Dahl Salt-sensitive Rat. **E. Gillis, T. Coleman, F. Spradley, J. Granger, M. Garrett, M. Ryan, and J. Sasser.** *Univ. of Mississippi Med. Ctr., Jackson.*

20 **15.5** Decreased Uterine Artery Blood Flow and Enhanced Myogenic Tone in RGS2-deficient Mice. **L. Jie, E. Owens, and P. Osei-Owusu.** *Drexel Univ., Philadelphia, PA.*

21 **15.6** Impact of Obesity on Nitric Oxide Synthase (NOS)-mediated Regulation of Blood Pressure During Pregnancy in Rats. **F. Spradley, A. Palei, and J. Granger.** *Univ. of Mississippi Med. Ctr., Jackson.*

Poster Board

22 **15.7** Agonistic Autoantibodies to the Angiotensin II Type 1 Receptor Enhances ANG II Induced Renal Vascular Sensitivity and Reduces Renal Function During Pregnancy. **M. Cunningham, Jr., J. Williams, G. Wallukat, R. Dechend, and B. LaMarca.** *Univ. of Mississippi Med. Ctr., Jackson, and HELIOS Clinic, Berlin, Germany.*

23 **15.8** A Novel, Master Switch for Ovarian Cyclicity: The Impact on Cardiometabolic Health. **L. Stein, S. Mathews, W. K. Samson, and G. Yosten.** *St. Louis Univ.*

24 **15.9** Blood Pressure Responses to Isometric Handgrip Exercise and Post-exercise Ischemia in Women with a History of Hypertensive Pregnancy. **S. Ranadive, R. Harvey, M. Joyner, V. Miller, and J. Barnes.** *Mayo Clinic, Rochester, MN, and Univ. of Wisconsin at Madison.*

25 **15.10** Up-regulation of VEGFR2 Improves Uterine Artery Myogenic Response and Maternal Hypertension Altered by Uterine Perfusion Rressure Reductions. **B. Balser, R. Ramirez, D. Crowder, J. Reho, Y. Yun, and J. Novak.** *Univ. of Akron, Univ. of Iowa, Iowa City, IA, and Walsh Univ., North Canton, OH.*

26 **15.11** Effects of High-sucrose Diet on Blood Pressure Regulation During Pregnancy in Rats. **F. Spradley, A. Palei, and J. Granger.** *Univ. of Mississippi Med. Ctr., Jackson.*

27 **15.12** Mechanisms of Renal and Colonic Potassium Retention during Late Pregnancy. **C. West, P. Welling, T. DuBose, C. Baylis, and M. Gumz.** *Georgetown Univ., Univ. of Maryland Sch. of Med., Baltimore, Wake Forest Sch. of Med., and Univ. of Florida, Gainesville.*

28 **15.13** Impaired Flow-Mediated Dilation Before, During and After Preeclampsia: A Systematic Review and Meta-analysis. **T. Weissgerber, N. Milic, J. Milin-Lazovic, and G. Vesna.** *Mayo Clinic, Rochester, MN, and Univ. of Belgrade, Yugoslavia.*

Poster Session II

16.0 DEVELOPMENTAL PROGRAMMING

Thurs., 1:30—2:30 PM Rhode/Severn Room.

Poster Board

29 **16.1** Vendor-specific Effect on Sex Differences in the Developmental Programming of Blood Pressure in the Sprague Dawley Rat. **J. H. Dasinger, S. Intapad, M. Backstrom, and B. Alexander.** *Univ. of Mississippi Med. Ctr., Jackson.*

30 **16.2** Is There a Sex Difference Between Hypertension Risk and Low Birth Weight in Healthy Young Japanese Adults? **S. Bao, E. Kanno, H. Tanno, and R. Maruyama.** *Tohoku Univ. Grad. Sch. of Med., Sendai, Japan.*

31 **16.3** Sex Differences in High Fat Diet-induced Adipocyte Morphology and Fat Distribution Due to Early Life Stress. **M. Murphy, L. Schmuckie, D. Powell, and A. Loria.** *Univ. of Kentucky, Lexington.*

32 **16.4** Sphingosine-1-phosphate Receptor Type 3 Plays a Role in the Etiology of High Blood Pressure Programmed by Intrauterine Growth Restriction in the Male but not the Female Mouse. **S. Intapad.** *Univ. of Mississippi Med. Ctr., Jackson.*

33 **16.5** Reduced Sleep Time During Pregnancy-Effects on Renal Morphology and Function of Female Offspring. **G. N. Gomes, R. Argeri, and S. Tufik.** *Univ. of São Paulo, Brazil.*

Poster Board

34 **16.6** Delayed Effects of Perinatal Hypoxia on Adult Rats Pulmonary Vessels Structure and Reactivity. **M. Vizek, V. Hampl, J. Novotna, J. Herget, and V. Sedivy.** *Charles Univ., Prague, Czech. Rep.*

35 **16.7** Sex Difference in Sensitization of Angiotensin (ANG) II-elicited Hypertension in Offspring of Hypertensive Pregnant Rats. **B. Xue, F. Gao, T. Beltz, R. Thunhorst, and A. Johnson.** *Univ. of Iowa, Iowa City, IA.*

36 **16.8** Sex Differences in Cardiovascular Responses to Stress in Adult Rats Prenatally Exposed to Dexamethasone. **T. Hale, D. Carbone, L. Madhavpeddi, M. Thompson, and R. Handa.** *Univ. of Arizona, Phoenix.*

Poster Session II

17.0 AGING AND MENOPAUSE

Thurs., 1:30—2:30 PM Rhode/Severn Room.

Poster Board

37 **17.1** Prehypertension and Endothelial Fibrinolytic Function in Middle-Aged Women. **K. Diehl, T. Bammert, B. Weil, J. Greiner, B. Stauffer, and C. DeSouza.** *Univ. of Colorado, Boulder.*

38 **17.2** Gender Differences in Circulating Microparticles in Middle-Aged Adults. **T. Bammert, J. Hijmans, C. Dow, W. Reiakvam, G. Lincenberg, J. Greiner, B. Stauffer, C. DeSouza.** *Univ. of Colorado, Boulder.*

39 **17.3** Forearm Vascular Conductance Responses to Terbutaline, a β_2-adrenergic Receptor Agonist, Differ in Premenopausal Versus Postmenopausal Women. **R. E. Harvey, J. K. Limberg, W. T. Nicholson, T. B. Curry, J. N. Barnes, and M. J. Joyner.** *Mayo Clinic, Rochester, MN, and Univ. of Wisconsin at Madison.*

40 **17.4** ET_A Receptor Antagonism Prevents Ang II-Induced Hypertension in VCD-Treated Postmenopausal Female Mice. **D. Pollow, Jr., M. J. Romero-Aleshire, and H. L. Brooks.** *Univ. of Arizona, Tucson.*

41 **17.5** Myogenic Tone is Increased in Resistance-sized Arteries Isolated From Rat Models of Post-menopausal Physiology. **J. Novak, S. Dennis, L. Woodward, Z. Thomas, M. Thomas, J. McCarthy, A. Underwood, and R. Ramirez.** *Walsh Univ., North Canton, OH, Univ. of Akron, and Tufts Med. Ctr.*

42 **17.6** Circulating Steroid Hormones have no Influence on the Cardiovascular Beneficial Effect in Trained Hypertensive Postmenopausal Women. **I. Novais, A. Jarrete, G. Puga, H. Agaujo, M. Delbin, and A. Zanesco.** *São Paulo State Univ., Brazil, Campinas Univ., São Paulo, Brazil, and Fed. Univ. of Uberlândia, Brazil.*

43 **17.7** Renal Function in Aging Hyperandrogenemic Female Rats. **C. N. Patil, C. Dalmasso, R. O. Maranon, A. Harris, H. Zhang, and Jane. F. Reckelhoff.** *Univ. of Mississippi Med. Ctr., Jackson.*

44 **17.8** Effect of Estradiol Replacement in Hypertension in the Aging Female Dahl Salt Sensitive Rat. **L. L. Yanes Cardozo, D. G. Romero, and J. F. Reckelhoff.** *Univ. of Mississippi Med. Ctr., Jackson.*

45 **17.9** Role of the Renal Nerves and Angiotensin II in a Model of Postmenopausal Hypertension. **R. E. Maranon, C. Dalmasso, C. N. Patil, L. L. Yanes Cardozo, and J. F. Reckelhoff.** *Univ. of Mississippi Med. Ctr., Jackson.*

46 **17.10** Elderly Women Maintain Better Cerebral Blood Flow Regulation to Both Pressure and Carbon Dioxide than Elderly Men. **J. Serrador, L. Reyes, F. Sorond, and L. Lipsitz.** *Rutgers Univ., Vet. Affairs NJ Hlth. Care. Sys., East Orange, NJ, and Harvard Med. Sch.*

Poster Board

47 **17.11** Estrogenic Phytochemicals Reduce Bone Adiposity and Improves Bone Quality Following Ovariectomy. **C. Miller, S. Ambati, N. Hohos, D. Hartzell, E. Bass, E. England, T. Avra, M. A. Della-Fera, C. Baile, and S. Rayalam.** *U.S., Environ. Protection Agency., Res. Triangle Pk, NC, Univ. of Georgia, Athens, and Philadelphia Coll. of Osteopathic Med., Suwanee, GA.*

48 **17.12** Effects of Menopause and Acute Exercise on Brachial Artery Flow Mediated Dilation and Plasma Endothelial Microparticles. **C. Serviente, D. Shill, K. Lansford, N. Jenkins, and S. Witkowski.** *Univ. of Massachusetts, Amhearst, and Univ. of Georgia, Athens.*

Plenary Lecture

18.0 PLENARY LECTURE

Thursday, 2:30—3:00 PM, Wye Room.

Chair: **Kathryn Sandberg,** *Georgetown Univ.*

2:30 PM **18.1** Studying Both Sexes: A New Frontier for Discovery. **Janine Clayton.** *NIH, Office of Res. in Women's Hlth., Bethesda, MD.*

Symposia VII

19.0 OBESITY, METABOLIC SYNDROME, GENDER AND SEX

Thurs., 3:00—5:00 PM, Wye Room.

Chair: **James R. Sowers,** *Univ. of Missouri.*

3:00 PM **19.1** In Utero Consequences of Rodent Vertical Sleeve Gastrectomy on Maternal Health and Feto-placental Development. **Bernadette Grayson.** *Univ. of Mississippi Med. Ctr.*

3:20 PM **19.2** Nutrient Sensing Mechanisms in Hypothalamic Cell Models: Neuropeptide Regulation and Neuroinflammation. **Denise Belsham.** *Univ. of Toronto, Canada.*

3:40 PM **19.3** The Role of Estrogens and Androgen in Control of Glucose Homeostasis. **Franck Mauvais-Jarvis.** *Tulane Univ.*

4:00 PM **19.4** Sex Dimorphism In Plasma Soluble Prorenin Receptor (sPRR) Levels In Obese Patients is Associated With Type 2 Diabetes Mellitus in Women But Not in Men. **Carla B. Rosales.** *Tulane Univ.* **(6.18).**

4:15 PM **19.5** Loss of the Y Chromosome in Men Undergoing Carotid Endarterectomy. **Hester Den Ruijter.** *Univ. Med. Ctr., Utrecht, The Netherlands.* **(4.3).**

4:30 PM **19.6** Leptin-Mediated Aldosterone Secretion Causes Hypertension in Obese Females. **Eric J. Belin De Chantemele.** *Georgia Regents Univ.* **(6.20).**

4:45 PM **19.7** Effects of Diet-induced Obesity on Reproductive Hormone Signaling and Gene Expression. **John Bowman.** *Washington & Lee Univ., Lexington, VA.* **(6.21).**

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FRIDAY, NOVEMBER 20, 2015

Symposia VIII

20.0 PREGNANCY AND PRE-ECLAMPSIA

Fri., 8:00—10:00 AM, Wye Room.

Chair: **Christine Maric,** *NIH, NHLBI.*

8:00 AM **20.1** Mechanisms of Maternal Uterine Vascular remodeling During Gestation. **George Osol.** *Univ. of Vermont Coll. Med., Burlington.*

8:20 AM **20.2** Spontaneous Superimposed Preeclampsia in Dahl Salt Sensitive Rats. **Jennifer Sasser.** *Univ. of Mississippi Med. Ctr, Jackson.*

8:40 AM **20.3** Vasopressin: A New Beginning for the End of Preeclampsia? **Mark Santillan.** *Univ. of Iowa.*

9:00 AM **20.4** Up-regulation of VEGFR2 Improves Uterine Artery Myogenic Response and Maternal Hypertension Altered by Uterine Perfusion Rressure Reductions. **Brittany Balser.** *Univ. of Akron.* **(15.10).**

9:15 AM **20.5** Effects of High-sucrose Diet on Blood Pressure Regulation During Pregnancy in Rats. **Frank Spradley.** *Univ. of Mississippi Med. Ctr, Jackson.* **(15.11).**

9:30 AM **20.6** Mechanisms of Renal and Colonic Potassium Retention during Late Pregnancy. **Crystal West.** *Georgetown Univ.* **(15.12).**

9:45 AM **20.7** Impaired Flow-Mediated Dilation Before, During and After Preeclampsia: A Systematic Review and Meta-analysis. **Tracey Weissgerber.** *Mayo Clinic, Rochester, MN.* **(15.13).**

Symposia IX

21.0 POPULATION STUDIES-GENDER AND SEX IN CVD, RENAL DISEASE, AND METABOLIC SYNDROME

Fri., 10:30—11:30 AM, Wye Room.

Chair: **Rita Tostes.** *Univ. of São Paulo, Brazil.*

10:30 AM **21.1** Sex Differences in Risk Factors for Stroke in Women. **Kathryn Rexrode.** *Harvard Med. Sch.*

10:50 AM **21.2** Gender Differences in Hypertension and Health Behaviors. **Marie Krousel-Wood.** *Tulane Univ.*

11:10 AM **21.3** Tobacco Smoking Exposure from Childhood to Adulthood and Adult Subclinical Vascular Disease. **Shengxu Li.** *Tulane Univ.*

Closing Remarks

22.0 CLOSING REMARKS

Fri., 11:35—11:45 AM, Wye Room.

Chairs: **Jane F. Reckelhoff.** *Univ. of Mississippi Med. Ctr.*
S. Ananth Karumanchi. *Harvard Med. Sch.*

Join us at the Closing Banquet and Award Presentation

Thursday, November 19, 2015
7:00—9:30 PM

Get your complimentary ticket at the registration desk

NOTES

Abstracts of Invited and Contributed Presentations

2.0 IMMUNE SYSTEM AND REGENERATIVE MEDICINE-IMPACT OF GENDER AND SEX

2.1
ESTROGEN RECEPTOR ALPHA ENHANCES LOSS OF TOLERANCE TO NUCLEAR ANTIGENS AND IMMUNE CELL ACTIVATION INDUCED BY THE *SLE*1 LUPUS SUSCEPTIBILITY ALLELE AND IS RESPONSIBLE FOR THE SEX BIAS ASSOCIATED WITH *SLE*1

Karen Gould[1], and Shayla Yoachim[1]
[1]Genetics, Cell Biology & Anatomy, Univ. of Nebraska Med. Ctr., 985805 Nebraska Med. Ctr., Omaha, NE, 68198-5805.

Lupus is an autoimmune disease characterized by the development of anti-nuclear autoantibodies and immune complex mediated nephritis. ~90% of lupus patients are women, and this sex bias is thought to be driven largely by estrogens. Previously, we showed that estrogens promote lupus via estrogen receptor α (ERα). The Sle1 lupus susceptibility allele promotes the development of anti-nuclear autoantibodies and immune cell activation. The phenotype associated with Sle1 is more robust in females than males, suggesting that estrogens, acting via ERα, may enhance the effect of Sle1. To test this hypothesis, we examined the impact of a targeted disruption of ERα on the development of anti-nuclear autoantibodies and immune cell activation B6.Sle1 congenic mice. ERα deficiency attenuated the development of autoantibodies in B6.Sle1 congenic females but not males. ERα deficiency decreased Sle1-induced immune cell activation in females, and to a lesser extent, in males. Altogether, these data demonstrate that the sex bias in Sle1-induced loss of tolerance to nuclear antigens and immune cell activation is ERα-dependent. Support: NIH R01 AI075167 References: Yoachim S.D.,Nuxoll J,S.,Bynoté K.K.,Gould K.A., Estrogen receptor alpha signaling promotes Sle1-induced loss of tolerance and immune cell activation and is responsible for sex bias in B6.Sle1 congenic mice. Clin Immunol. 2015, 158(2):153-66.

2.2
ROLE OF T CELLS IN THE DEVELOPMENT OF CARDIOVASCULAR DISEASE AND HYPERTENSION

Jennifer Sullivan[1]
[1]Physiology, Georgia Regents Univ., 1459 Laney Walker Blvd., Augusta, GA, 30912.

Hypertension is now considered a state of low-grade inflammation. While T cells have broadly been implicated in blood pressure control, the most is known regarding the role of Th17 cells and T regulatory cells (Tregs). Th17 cells mediate pro-inflammatory responses through the secretion of the pro-inflammatory cytokine, IL-17 and a role for Th17 cells in hypertension has been indirectly surmised based on studies manipulating IL-17 levels. In contrast, Tregs are crucial in maintaining immunologic self-tolerance and protection from auto-immune disease as well as regulating immune responses to pathogens by impacting effector T cell function. Adoptive transfer studies have conclusively linked Tregs with decreases in blood pressure and improved cardiovascular outcomes. Despite an ever expanding literature base supporting a causal role of T cells to hypertension and related end-organ damage in both the basic sciences and clinically, the majority of this literature has been performed exclusively in males despite the fact that both men and women develop hypertension. Recent studies by our group and others, have highlighted important sex differences in the immune profile and blood pressure responses to T cells. These results highlight the need to better understand the influence of sex on the immune system and underline the potential complexity of immune system regulation of blood pressure and cardiovascular function. More work is needed to define the physiological impact of sex differences in immune system components, but also how each of these components may impact overall cardiovascular health. References: McMaster, W.G., Kirabo A., Madhur M. S., Harrison, D. G., Inflammation, immunity, and hypertensive end-organ damage. Circ Res. 2015 Mar 13;116(6):1022-33; Tipton AJ and Sullivan JC. Sex and gender differences in T cells in hypertension. Clinical Therapeutics, 36(12):1882-1900; 2014.

2.3
ESTROGEN AND ITS EFFECTS ON WOMEN WITH LUPUS ERYTHEMATOSUS

Michael Ryan[1]
[1]Physiology & Biophysics, Univ. of Mississippi Med. Ctr., 2500 N. State St., Jackson, MS, 39216-4505.

Systemic lupus erythematosus (SLE) is an autoimmune disorder that predominantly affects women of childbearing age. Women with SLE have a marked increase in the risk for developing hypertension, cardiovascular, and renal disease. Because of the strong bias towards women, estrogens are commonly implicated in the pathogenesis of SLE. Indeed, numerous studies in experimental models of SLE show that removing estrogens, or their receptors, early in life has a profound effect to delay the production of autoantibodies, renal pathology, and mortality. However, the role of estrogens and how they impact SLE disease progression and the associated cardiovascular risk factors like hypertension remain surprisingly unclear in adult women. Recent data from our laboratory suggest that there may be distinct temporal effects of estrogen in an established experimental mouse model of SLE (female NZBWF1 mice). Whereas early life (8 weeks of age) removal of estrogens by ovariectomy causes the expected delay in the onset of autoantibody production and renal injury, removal of estrogens in adulthood (30 weeks of age) exacerbates the hypertension and renal injury associated with SLE without impacting autoantibody production. Further studies are needed in women to better understand the role of estrogens in their disease progression, and experimental animal models may be useful to understand the complex role that estrogens have in this disease.

3.0 NON-REPRODUCTIVE ACTIONS OF SEX HORMONES AND RECEPTORS-A

3.1
TESTOSTERONE THERAPY IN MEN WITH TESTOSTERONE DEFICIENCY (TD): ADVANCES AND CONTROVERSIES

Abdulmaged Traish [1]
[1]Urology, Boston Univ. Sch. of Med., 715 Albany St., Boston, MA, 02118.

Testosterone (T) deficiency is a medical condition which has been recognized for more than a century. Since 1940s T therapy was reported to improve overall health in hypogonadal men with no serious adverse effects. Data from a number of recent studies demonstrate that T therapy is associated with: **a**) reduced body weight and waist circumference in overweight and obese men, **b**) increased lean body mass, **c**) reduced fat mass, and **d**) improved in glycemic control, **e**) improvements in lipid profiles, **f**) improvement in metabolic syndrome components, including blood pressure, reduced inflammation, and **g**) improved sexual function. T therapy is also shown in several studies to be associated with amelioration of diabetes and reduced mortality. A recent meta-analysis revealed no increase in cardiovascular (CV) risks in men receiving T therapy. However, few studies, with serious methodological and analytical flaws, suggested that T therapy is associated with increased CV risk. A thorough and critical analyses of these studies showed that the risks purported are unsubstantiated[1]. Also recent studies showed that no evidence exists that T therapy increases the risk of prostate cancer. In summary, there is no convincing evidence-based data to suggest increased CV risks with T therapy. In fact, the literature is replete with studies demonstrating beneficial effects of T therapy on CV and overall health. [1]Reference: Morgentaler A, Miner MM, Caliber M, Guay AT, Khera M, Traish AM. Testosterone therapy and cardiovascular risk: advances and controversies. Mayo Clin. Proc. 2015 Feb; 90 (2):224-51.

3.2
ESTROGEN REGULATES ADIPOGENESIS AND LIPID SYNTHESIS THROUGH MEMBRANE AND NUCLEAR ERALPHA

Ellis Levin[1]
[1]Med., Univ. of California, Irvine & Long Beach VA, 5901 E. 7th St., Long Beach, CA, 90822.

Estrogen has multiple, usually favorable metabolic effects, including suppression of appetite and adipogenesis, lipid synthesis, and stimulation of fatty acid oxidation and glucose homeostasis. The mechanisms are often incompletely understood and the collaboration or participation of various estrogen receptor cellular pools and isoforms is poorly understood. We have recently focused on the ability of estrogen to act through both nuclear and membrane ERalpha, using transgenic NOER and MOER mice that lack either membrane or nuclear ERalpha, to investigate adipogenesis. Both receptors are required for suppression of the genes that are important for the bone-marrow derived pleuri-potent stem cell to differentiate into the adipocyte lineage. This is also true for preventing adipocyte hypertrophy and proliferation. In contrast, lipid synthesis by the mature adipocyte is inhibited by estrogen only through engaging the membrane ERalpha through mechanisms to be discussed at the conference. The latter findings are consistent with our describing the sufficiency of membrane ERalpha to inhibit all forms of lipid synthesis in the liver (in-vivo) and isolated hepatocytes (Science Signaling, 2013). These and other studies to be mentioned defines a new concept in ER action to suppress pathology, resulting from signaling exclusively from membrane ER to post-transcriptional modification of multiple transcription factors.

3.3
GPER AND VASCULAR FUNCTION

Sarah Lindsey[1]
[1]Pharmacology, Tulane Univ., 1430 Tulane Ave., Mailbox 8683, New Orleans, LA, 70112.

While estrogen receptors ERα and ERβ are known to induce transcriptional effects, a new membrane-bound, G protein-coupled estrogen receptor (GPER) was recently identified as a possible mediator of estrogen's nongenomic effects. A growing body of evidence from our lab and others shows that GPER elicits many of the beneficial actions of estrogen in the cardiovascular system. Using the mRen2 congenic model of angiotensin II-induced hypertension, we showed that activation of GPER alone emulates the protective effects of estrogen on blood pressure via regulation of the vascular renin-angiotensin system and vascular tone. Further studies to determine the signaling mechanisms for GPER in the vasculature found that this receptor induces nitric oxide release from endothelial cells and activates the cyclic AMP pathway in smooth muscle cells. In salt-sensitive hypertension, GPER counteracts proteinuria and oxidative stress in the kidney and opposes vascular remodeling in large conduit vessels. GPER expression and function is reduced in males and aging females, which has important clinical implications. We hope that research on the cardiovascular effects of estrogen and the receptors and mechanisms involved will lead to improvements in hormone therapy for postmenopausal women. (NIH 103471). Lindsey et al 2009. Endocrinology. 150:3753-58. Lindsey et al 2011. Hypertension. 58:665-671. Lindsey et al 2011. J Cardiovasc Pharmacol. 57:598-603. Lindsey et al 2013. Am J Physiol Endocrinol Metab. 305(1):E113-8. Lindsey et al 2014. Steroids. 81:99-102.

4.0 CARDIOVASCULAR DISEASE

4.1
MATRIX METALLOPROTEINASE-9 IS CRITICAL FOR 2-METHOXYESTRADIOL MEDIATED ANGIOTENSIN TYPE 1 RECEPTOR DOWN-REGULATION

Benard Ogola[1], Yong Zang[1], and Thomas Thekkumkara[1]
[1]Dept. of Biomedical Sci., Texas Tech. Univ. Hlth. Sci. Ctr. Sch. of Pharmacy, 1300 S. Coulter, Amarillo, TX, 79106.

Recently, studies have demonstrated that one of the final end products of estrogen metabolism 2-methoxyestradiol (2ME2) has the therapeutic potential in a number of cardiovascular disorders, including hypertension. However, the exact mechanism(s) remains unknown. Inhibiting angiotensin type 1 receptor (AT1R) has been shown to be critical for controlling hypertension and associated disorders. Ongoing studies in our laboratory show that epithelial and smooth muscle cells exposed to 2ME2 down-regulates AT1R protein and mRNA in a concentration and time dependent manner. In this study, continuously passaged epithelial cells expressing native AT1R were exposed to 2ME2, and angiotensin II radio ligand binding and signaling pathways were assessed. In the presence of 2ME2, cells exhibited significant phosphorylation and nuclear translocation of ERK1/2 and down-regulation of AT1R. Using GM6001, a broad-based matrix metalloproteinases (MMPs) inhibitor, and AG1478, an epidermal growth factor receptor (EGFR) selective inhibitor, we demonstrated that 2ME2 mediated phosphorylation of ERK1/2 is depended on the activation of MMPs and transactivation of EGF receptor. Furthermore, marimastat, a matrix metalloproteinase-9 (MMP9) specific inhibitor attenuated 2ME2 induced phosphorylation of EGFR and ERK1/2 and reversed AT1R down-regulation. Under similar conditions stimulation of G-protein coupled estrogen receptor -1 (GPER-1) with the selective agonist G1 elicited similar signaling pathway and down-regulated the AT1R expression. Moreover, immunoprecipitation studies show that 2ME2 and G1 phosphorylate EGFR at tyrosine 1173, which is a critical residue on EGFR to interact with Src homology 2 domain-containing tyrosine phosphatase 1 (SHP1), which controls the level of EGFR phosphorylation. Collectively, our study demonstrates for the first time that 2ME2 mediated activation of MMP9 results in EGFR phosphorylation at tyrosine 1173 leading to the ERK1/2 activation; a signaling pathway essential for AT1R down-regulation. Furthermore, our study results suggest a potential mechanism for the observed effects of estrogen against cardiovascular disorders in premenopausal women.

4.2
UNDERREPRESENTATION OF SEX IN REPORTING TRADITIONAL AND EMERGING BIOMARKERS FOR PRIMARY PREVENTION OF CARDIOVASCULAR DISEASE: A SYSTEMATIC REVIEW

Aisha Gohar[1], Renate Schnabel[2,3], Maria Hughes[3,4], Tanja Zeller[2,3], Stefan Blankenberg[2,3], Gerard Pasterkamp[1], and Hester den Ruijter[1]
[1]Lab. for Experimental Cardiology, Univ. Med. Ctr., Utrecht, Heidelberglaan 100, Utrecht, 3508 GA, The Netherlands, [2]Dept. of General & Interventional Cardiology, Univ. Heart Ctr., Hamburg, Martinistr. 52, Hamburg, 20246, Germany, [3]Dept. of General & Interventional Cardiology, German Ctr. for Cardiovascular Res., Oudenarder Straße 16, Hamburg, Germany, [4]UKCRC Ctr. of Excellence for Public Hlth. Res., Inst. of Clinical Sci., Grosvenor Rd., Belfast, United Kingdom.

Objectives: Primary prevention of cardiovascular disease (CVD) relies on the identification of individuals at increased risk of developing cardiovascular events. Circulating biomarkers mirroring the (subclinical) disease process are valuable tools for CVD risk prediction. Evidence is accumulating that the clinical presentation and the mechanisms for CVD development and progression differ between men and women. To what extent this is reflected in biomarker profiles is unknown. We performed a systematic review of sex-specific data on established and emerging biomarker levels and their association with CVD in the setting of primary prevention. **Methods:** Pubmed MEDLINE and Embase were searched on 2nd February 2014 and updated on 15th January 2015. Biomarkers included represented pathophysiological pathways of lipids, inflammation, kidney function and of the heart. Data on patient characteristics, sex-specific biomarker levels, biomarker association with future CVD events and clinical value were extracted. **Results:** Only 55 studies out of 5,374 publications provided sex-specific information. The majority of these 55 studies only corrected for sex in multivariable models without presenting sex-specific results. All the biomarkers under study show a similar direction of the association with CVD between men and women. The magnitude of the association between the biomarker and outcome varied by study and sex. The cardiac specific biomarkers troponin and B type natriuretic peptide (BNP) show the most prominent differences in baseline levels between men and women. Troponin was more likely to be detected in men and women have higher levels of BNP. The predictive values of troponin levels are similar between men and women however for BNP the data is inconsistent with one study reporting a stronger association for men. The additional clinical utility of novel biomarkers was reported in seven publications, only one of which was stratified by sex. **Conclusions:** Sex-specific data on biomarkers for CVD in the general population exists but is under-reported. There is inconsistency in sex-specific differences in levels of traditional biomarkers and their relation to CVD. To improve personalized cardiovascular diagnoses and care for men and women, reporting sex-specific data on clinical utility of biomarkers is crucial and should be encouraged in publications of sufficiently powered studies. This project has received funding under the Marie Curie grant agreement No 289903 via EUTRAIN.

4.3
LOSS OF THE Y CHROMOSOME IN MEN UNDERGOING CAROTID ENDARTERECTOMY

Saskia Haitjema[1], Daniel Kofink[2], Jessica van Setten[1], Sander W. van der Laan[1], Saskia C.A. de Jager[1], Paul I.W. de Bakker[2,3], Gerard Pasterkamp[1,4], Folkert W. Asselbergs[5,6,7], and Hester M. den Ruijter[1]
[1]Lab. of Experimental Cardiology, Univ. Med. Ctr. Utrecht, Heidelberglaan 100, Utrecht, 3584 CX, The Netherlands, [2]Dept. of Med. Genetics, Univ. Med. Ctr. Utrecht, Heidelberglaan 100, Utrecht, 3584 CX, The Netherlands, [3]Julius Ctr. for Hlth. Sci. & Primary Care, Univ. Med. Ctr. Utrecht, Heidelberglaan 100, Utrecht, 3584 CX, The Netherlands, [4]Lab. of Clinical Chemistry & Haematology, Univ. Med. Ctr. Utrecht, Heidelberglaan 100, Utrecht, 3584 CX, The Netherlands, [5]Dept. of Cardiology, Univ. Med. Ctr. Utrecht, Heidelberglaan 100, Utrecht, 3584 CX, The Netherlands, [6]Durrer Ctr. for Cardiogenetic Res., ICIN-Netherlands Heart Inst., Meibergdreef 9, Amsterdam, 1105 AZ, The Netherlands, [7]Inst. of Cardiovascular Sci., Univ. Coll. London, Gower St., London, WC1E 6BT, United Kingdom.

Introduction: The Y chromosome has long been considered genomic wasteland with few genes only implicated in sex determination. However, recent studies found Y-chromosomal dosage-sensitive whole-genome regulators, an immunoregulatory role for Y, and a relation between loss of Y (LOY) and a higher risk of cancer and mortality. Given the involvement of immune cells in atherosclerosis, we hypothesized that LOY is associated with specific cardiovascular disease (CVD) phenotypes in men undergoing carotid endarterectomy (CEA). **Materials and Methods:** LOY was quantified in blood from raw intensity genotyping data in a cohort of 368 men within the Athero-Express biobank study. Atherosclerotic plaques were dissected, and the culprit lesions were used for histological characterisation and the measurement of various inflammatory proteins. We tested LOY for association with measures of cardiovascular disease severity and inflammatory atherosclerotic plaque phenotypes (macrophage content, IL6, IL10, IL12, TNFα, IFNγ and TGFβ levels). In addition we assessed the association of LOY with secondary major cardiovascular events during 3-year follow-up. The study was conducted in accordance with the declaration of Helsinki. **Results:** Out of 368 CEA patients, 61 exhibited LOY. Loss of Y in blood was negatively associated with age (β=-0.03/10yr, r2=0.08, p=2.2*10-8). Loss of Y was not associated with history of coronary artery disease, stroke, contralateral carotid stenosis or peripheral arterial disease of the lower limbs. Likewise we found no association of LOY with macrophage content or inflammatory cytokines in the plaque. Interestingly LOY was independently associated with secondary major cardiovascular events during three-year follow-up (p=0.032) in a Cox regression model corrected for confounders. **Conclusion:** LOY in circulating blood cells is independently associated with secondary major cardiovascular events in a severely atherosclerotic population.

Our data support that LOY is associated with an increased risk for the occurrence of secondary cardiovascular events. However, we did not observe an association with inflammatory plaque characteristics that could explain this result, suggesting that LOY affects secondary outcome by alternate mechanisms. **Funding:** Saskia Haitjema is supported by the FP7 EU project CVgenes@target (HEALTH-F2-2013-601456).

4.4

CIRCULATING GDF-15 LEVELS ARE EXPLICITLY VALUABLE FOR THE PREDICTION FOR FUTURE CARDIOVASCULAR COMPLICATIONS IN WOMEN

Aisha Gohar[1], Joyce Vrijenhoek[1], Gerard Pasterkamp[1], Hester den Ruijter[1], and Saskia de Jager[1]
[1]Lab. for Experimental Cardiology, Univ. Med. Ctr., Utrecht, Heidelberglaan 100, Utrecht, 3508 GA, The Netherlands.

Background: Cardiovascular disease (CVD) remains a major contributor to global morbidity and mortality. The underlying mechanisms for CVD and clinical presentations of diseases have been found to differ between men and women. Growth differentiation factor (GDF) 15 is a member of the transforming growth factor (TGF-β), which operates in acute phase responses. Elevated GDF-15 serum levels are an established risk factor for several cardiovascular diseases ranging from early chest pain to acute coronary syndromes and heart failure. In this study we aimed to evaluate the predictive value of GDF-15 as a biomarker for secondary cardiovascular events in men and women undergoing carotid endarterectomy. **Methods:** Circulating GDF-15 levels were determined by ELISA in a subcohort of 1064 patients from the Athero Express Biobank. Multiple linear regression models were used to investigate the associations between GDF-15 and clinical risk factors. Multivariable cox regression models were performed to analyze secondary events. **Results:** The Median GDF-15 level was 104206 ng/L (51803, 182296) for the entire cohort, which is higher than previously observed levels in CVD. We did not discern a difference in baseline GDF-15 levels between men and women (Men: 106375 [51182, 182596 vs. Women: 99042 [52094, 173273], p value for difference 0.241). High levels of GDF-15 were associated with increasing age, reducing renal function, and a history of diabetes. However in women, only increasing age was found to be associated with GDF-15 levels. Interestingly, we show that a high level of circulating GDF-15 is a strong predictor for secondary cardiovascular events specifically in women (composite events: Quantile 4: HR 2.69 95% CI 1.25-5.81 p=0.01 in women vs. HR 0.96 95% CI 0.66-1.40 p=0.82 in men) and more precisely for peripheral events (Quantile 4: HR 3.41 95% CI 1.11-10.47, p=0.03 in women vs. HR 0.68 95% CI 0.40-1.17 p=0.16 in men). **Conclusions:** High circulating GDF-15 is predictive of secondary outcome in women but not men, suggesting a potential role for GDF-15 as a biomarker for secondary prevention in women. In addition, this again illustrates the differences in atherosclerotic disease mechanisms in women, where the role of GDF-15 clearly deserves further interest. **Funding:** AG is supported by EUTRAIN. This project has received funding under the Marie Curie grant agreement No 289903.

4.5

A STUDY OF THE POTENTIAL RISK FACTORS OF CARDIOVASCULAR DISEASES IN YOUNG SAUDI FEMALES

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[1]Physiology, Univ. of Dammam, King Saud, Dammam, Saudi Arabia.

Background: Multiple risk factors have been blamed to precipitate wide range of cardiovascular diseases such as hypertension, ischemic heart diseases, stroke, and heart failure. These risk factors might differ between different selected age, sex and ethnic groups. **Aim and objectives:** In the current study, we aim to find out indicators of cardiovascular risk in young Saudi females by studying the correlation of three main risk factors i.e. body adiposity, physical fitness, and plasma level of 25-OH-vitamin D and haemodynamic parameters. **Subject and methods:** Convenient sample of 88 young Saudi females was recruited from University of Dammam, Dammam, Saudi Arabia in the period from November 2014-April 2015. All participants were healthy with no history of pregnancy or lactation in the last two years, no contraindication for exercise stress test, no endocrine diseases and no vitamin D supplement in the last six weeks. Weight, height, BMI, waist and hip circumference were determined. Bruce treadmill exercise stress test was performed. ECG, systolic, diastolic blood pressure, pulse rate were determined at rest, during and at maximum exercise. Plasma 25-OH-vitamin D was determined using HPLC method. **Statistical analysis:** Data were analyzed using SPSS 20. Data were normally distributed. Multivariate linear regression model was used to predict the relationship between multiple risk factors and haemodynamic parameters. The following haemodynamic parameters were used separately as a dependent variable: resting pulse rate, maximum pulse rate, resting diastolic blood pressure, maximum diastolic blood pressure, resting systolic blood pressure, maximum systolic blood pressure. The independent variables were: body weight, waist circumference, VO_{2max}, plasma 25-OH-vitamin D. **Results:** Mean age was 20.8±2.4 years, mean weight= 58.1±14.8 Kg, mean BMI= 23.0±4.8 Kg/m^2, mean VO_{2max}= 33.7±11.0 ml/kg.min, mean plasma 25-OH-vitamin D= 15.10±0.73 ng/ml**.** Multivariate linear regression model revealed significant positive linear relationship between body weight and resting diastolic (y_1), and resting systolic blood pressure(y_2) with p and R^2 values (0.041, 0.006) (0.121, 0.107) respectively, and linear equation y_1=0.244x+85.3, y_2=0.706x+127.1 respectively. Negative linear regression was demonstrated between VO_{2max} and maximum diastolic blood pressure (y_3), resting pulse(y_4) and maximum pulse rate(y_5) with p and R^2 values (0.017, 0.018, 0.001)(0.153, 0.113,0.185) and linear equations y_3=–0.237x+79.3, y_4=–0.398x+130.128, y_5=–0.805x+214.94. Vitamin D level showed no significant correlation with any of the haemodynamic parameters. **Conclusion:** The present study demonstrated that body adiposity and reduced physical fitness appeared to be the most important risk factors toward developing future cardiovascular abnormalities in young Saudi females. Body weight and reduced physical fitness can directly and independently predict the increment in arterial blood pressure and pulse rate in this studied group.

4.6

ASSESSMENT OF GENDER- AND AGE-DEPENDENT PATTERNS OF CARDIOVASCULAR REMODELING IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

S. Al-Gburi[1], I. Kopaliani[1], B. Zatschler[1], R. Galli[2], M. Kasper[3], and A. Deussen[1]
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Cardiovascular diseases are the leading cause of death worldwide. Whereas men are more prone to develop hypertensive disorders, the death rate due to cardiovascular events is much higher among women. Despite this disparity, experimental and clinical long-term studies are still lacking to better understand the contribution of gender to age-dependent progression of hypertensive cardiovascular diseases. Here, we investigated the impact of gender in progression of cardiovascular remodeling in female and male SHR. 5-, 14-, 29- and 36-week-old female and male SHR, age and gender matched with Wistar Kyoto rats (WKY) were studied. Animals were handled with permission (No.: 24-9168.24-1/2012-16) of institutional committee and the local authorities**.** Systolic blood pressure (SBP) was measured weekly with the tail-cuff method. Vessel function of aortic rings was quantified using Mulvany Myograph. Structural changes of aorta and heart were assessed by histological staining and CARS microscopy. Compared to WKY, all SHR showed significantly (P<0.01) higher SBP, except age of 5 weeks. Interestingly, at 14 weeks, SBP of female SHR was ~40 mmHg lower than that of male SHR. At this age, isolated aorta of female SHR showed significantly (P<0.01) lower vasoconstrictive response to norepinephrine stimulation compared to male SHR. While 5- and 14-week-old SHR showed normal endothelial function, this was deteriorated in male SHR at 29 weeks. In female SHR endothelial function was still preserved until 36 weeks. At 36 weeks SMC relaxation was strongly impaired. This was associated with distinct alterations in vessel structure. A massive degradation of elastin and increased degree of fibrosis was objectified particularly in male and to a lesser degree in female SHR. Adverse functional and structural changes in aorta were accompanied by concentric hypertrophy of the heart, starting at 29 weeks in male and at 36 weeks in female SHR. Cardiac fibrosis was much stronger in male than in female SHR at the age of 36 weeks. An age-dependent upregulation of ACE2 and AT_2 receptor expression was found in female as compared to male SHR. This study shows that the SHR model is a valuable tool to address gender-specific age-dependent changes of the cardiovascular system. As gender related differences are overt, the model may be well suited to improve our understanding of causal mechanisms. This project was financed by the Else Kröner-Fresenius Foundation.

4.7

INDICES OF CARDIOVASCULAR FUNCTION DERIVED FROM PERIPHERAL PULSE WAVE ANALYSIS USING RADIAL APPLANATION TONOMETRY IN HIV POSITIVE PATIENTS FROM MTHATHA DISTRICT OF SOUTH AFRICA

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Background: The objective of the study was to see if there is increased arterial stiffness or cardiac dysfunction in HIV patients by using applanation tonometry. **Methods:** A cross sectional study. 169 participants took part in the study between December 2012 and June 2013. There were 63 HIV positive participants, 52 HIV negative participants, and 54 HIV treatment naive participants. Augmentation index (AIx (75),

Ejection duration index (ED %) and subendocardial variability ratio (SEVR) and other parameters of interest were measured using arterial wave reflection in these participants. **Results:** SEVR was highest in the HIV negative participants and lowest in HAART naïve HIV participants ($p<0.001$). In both groups, the HIV positive participants had significant arterial stiffness compared to HIV negative participants ($p=0.024$). The HIV positive participants also had higher ejection duration index (ED %) with the highest values being observed in those that were not on treatment ($p<0.001$). SEVR had negative correlation with HR using Pearson's correlation and Stepwise Linear regression $p<0.001$. **Conclusion:** HIV patients are prone to having systolic dysfunction which may lead to myocardial ischemia. **Keywords:** HIV, subendocardial variability ratio, ejection duration index, arterial stiffness, cardiac function.

4.8
FUNCTIONAL AND STRUCTURAL CHANGES IN INTERNAL PUDENDAL ARTERIES UNDERLIE ERECTILE DYSFUNCTION INDUCED BY ANDROGEN DEPRIVATION

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Androgen deficiency is strongly associated with erectile dysfunction (ED). Inadequate penile arterial blood flow is one of the major causes of ED. The blood flow to the corpus cavernosum is mainly derived from the internal pudendal arteries (IPAs); however, no study has evaluated the effects of androgen deprivation on IPAs function. We hypothesized that castration impairs IPAs reactivity and structure, contributing to ED. Wistar male rats, 8 weeks-old, were castrated and studied 30 days after orchiectomy. Functional and structural properties of rat IPAs were determined using wire and pressure myograph systems, respectively. Protein expression was determined by western blot and immunohistochemistry. Plasma testosterone levels were determined using the IMMULITE 1000 Immunoassay System. Castrated rats exhibited impaired erectile function, represented by decreased intracavernosal pressure/mean arterial pressure ratio. IPAs from castrated rats exhibited decreased phenylephrine- and electrical field stimulation (EFS)-induced contraction and decreased acetylcholine- and EFS-induced vasodilatation. IPAs from castrated rats exhibited decreased internal diameter, external diameter, thickness of the arterial wall and cross-sectional area. Castration decreased nNOS and alpha actin expression and increased collagen expression, p38 (Thr180/Tyr182) phosphorylation, as well as caspase 3 cleavage. In conclusion, androgen deficiency is associated with impairment of IPA reactivity and structure and increased apoptosis signaling markers. Our findings suggest that androgen deficiency-induced vascular dysfunction is an event involving hypotrophic vascular remodeling of IPAs. Financial support: CRID, FAPESP, Brazil. Key words: Androgen, castration and internal pudendal artery.

4.9
EFFECTS OF AEROBIC EXERCISE TRAINING ON RENIN-ANGIOTENSIN SYSTEM COMPONENTS IN HYPERTENSIVE WOMEN

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The renin-angiotensin system (RAS) plays a major role in the pathogenesis of hypertension mainly through the classic axis, composed by angiotensin-enzyme converting (ACE), angiotensin II (Ang II) and AT1 receptor. Evidence has shown that RAS is influenced by age and sex hormones. Experimental studies have shown an inhibitory role of estrogen on ACE/Ang II-AT1-receptor axis with lower concentration of Ang II in female rats compared with male. However, the protective effect of estrogen in women, especially in climacteric phase, it is not fully understood. Indeed, it has demonstrated that menopause hormone therapy increases cardiovascular risk. Thus alternative strategies, like physical exercise, with well-controlled studies should be performed in an attempt to improve the health of this population. Therefore, the objectives of this study were a) to examine angiotensin peptides Ang I, Ang II and Ang-(1-7) and ACE activity in hypertensive (HT) women comparing with normotensive (NT) at baseline; b) whether aerobic exercise training (AET) exerts beneficial effects on RAS components as well as on blood pressure (BP) in both groups. This study was approved by Ethics Committee. Twenty-eight HT (55±1yrs) and sixty-six NT (52±1yrs) women were evaluated at baseline. Blood samples were collected after 12 hours of overnight fast. The components of RAS were quantified by High Performance Liquid Chromatography (HPLC). ACE activity was determined using fluorescent substrates. BP was measured by auscultation with aneroid sphygmomanometer after 15 minutes of rest. A subgroup of women, participated in AET, at moderate intensity, for 30-40 min, 3 times/week, for 24 sessions. At baseline, HT women showed higher concentrations of Ang I (240%), Ang II (90%) and Ang-(1-7), (140%) compared with NT. No differences were found in ACE activity. In HT (n=16) women, AET was effective in lowering BP (about 5%) that was accompanied by decrease in Ang I and Ang II levels, without changes in ACE activity. In NT (n=34) women, we found a reduction in systolic BP accompanied by a decrease in Ang II and ACE activity (approximately 2%) after AET. Our findings show that AET promoted a reduction in BP that was associated with decrease in RAS components in HT women, without affecting ACE activity. The striking of our study is that the beneficial effects of AET on RAS components might be the link between physical exercise and sympathetic activity in human population. Financial support Fapesp, Capes.

4.10
GENDER AND CIRCULATING VASCULAR MICRORNAS IN MIDDLE-AGED ADULTS

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MicroRNAs (miRNAs) are single stranded noncoding RNAs that are involved in the regulation of a number of physiological and pathological processes. miRNAs down regulate their target genes post-transcriptionally by degrading messenger RNA and/or by blocking their translation. It is now recognized that miRNAs play a key role in cardiovascular disease and its clinical consequences. Moreover, miRNAs are actively secreted into the circulation and specific circulating plasma miRNA signatures have been shown to be indicative of tissue expression and associated with vascular dysfunction and disease. Between the ages of 45 and 65 years, men have a significantly higher risk and prevalence of cardiovascular and cerebrovascular events compared with women, in spite of the absence of gender-related differences in the prevalence of traditional CVD risk factors. Endothelial dysfunction is considered to be a contributing factor in the gender-related disparity in CVD in middle-aged adults. Several circulating miRNAs have been linked to endovascular health including: miRNA-17, miRNA-92a, miRNA-126, miRNA-145, and miRNA-150. Reduced expression of these miRNAs in circulation is associated with endothelial dysfunction and development of vascular disease. The aim of this study was to determine whether the expression of circulating vascular miRNAs differ between middle-aged men and women. To address this aim 30 healthy, sedentary, non-obese, middle-aged adults were studied: 15 men (age: 54±2 yr; BMI 24.7±0.7 kg/m^2) and 15 women (56±1 yr; 25.6±0.7 kg/m^2). All women were at least 1 year post-menopausal and not taking hormone replacement therapy. Circulating miRNA was isolated from plasma and expression was assayed using real time reverse transcription polymerase chain reaction (RT-PCR). All values were normalized to exogenous *C. elegans* miRNA-39 and reported as relative expression (arbitrary units). There was no gender-related difference in the circulating vascular miRNA profile. Circulating levels of miRNA-17 (0.059±0.013 vs 0.061±0.01), miRNA-92a (0.558±0.121 vs 0.606±0.173), miRNA-126 (0.193±0.04 vs 0.183±0.031), miRNA-145 (0.008±0.002 vs 0.009±0.001) and miRNA-150 (0.059±0.014 vs 0.071±0.021) were not significantly different between the men and women. In summary, circulating expression of specific vascular-related miRNAs is not influenced by gender in healthy, middle-aged adults.

5.0 CARDIAC

5.1
SOLUBLE GUANYLYL CYCLASE EXERTS OPPOSITE EFFECTS IN THE MYOCARDIUM OF MALE AND FEMALE ENDOTOXEMIC MICE

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Sepsis induced cardiomyopathy (SIC) develops as the result of a decrease in cardiomyocyte contractile function. A central role is thought to be played by nitric oxide (NO) synthesized by inducible NO synthase (NOS2). NO acts by activating cGMP production by the enzyme soluble guanylyl cyclase (sGC), and also by causing oxidative modifications of calcium (Ca^{2+}) transporters. Despite a wealth of *in vitro* data that indicated a pathological role for cGMP, we found that in male (M) mice, cGMP plays a protective role by antagonizing the redox-mediated decrease in cellular

Ca^{2+}transients (ΔCa_i). No data is available about the effect of cGMP in septic female (F) hearts.We studied M and F C57Bl/6 mice (WT), as well as mice deficient in sGC activity ($sGC\alpha_1^{-/-}$). Lipopolysaccharide (LPS) administration (ip) induced an inflammatory shock syndrome and cardiomyopathy. Consistent with previous data, LPS -induced mortality was higher in male $sGC\alpha_1^{-/-}$ mice (60% for a dose of 4 µg/g, n = 5 mice) vs. male WT (0%, n=4 mice). In contrast, female $sGC\alpha_1^{-/-}$ had lower mortality (25% after a dose of 20 µg/g LPS, n = 8 mice) than female WT mice (100%, n = 4 mice). We measured sarcomere shortening (SS) and ΔCa_i in isolated, externally paced cardiomyocytes (5 Hz), at 37°C, 14h after challenge with 25 µg/g LPS: WT M mice had decreased SS and ΔCa_i, to 60 ± 7 and 78 ± 4% of baseline (bl), respecttively (n > 60 cells from 8 mice for all groups, 60/8). In $sGC\alpha_1^{-/-}$ M, the decrease in SS and ΔCa_i was more pronounced than in WT (to 26 ± 3 and 53 ± 3% of bl, respectively, n>60/8). In WT F, LPS induced a decrease in SS (to 41 ± 6% of bl, n>20/4), but not in ΔCa_i, suggesting a myofilament dysfunction. SS decrease was less in $sGC\alpha_1^{-/-}$ F mice (61 ± 10%, n>20/4) than in WT F. In conclusion, sGC-released cGMP plays opposite roles in the pathophysiology of LPS-induced cardimyopathy in M and F mice. In M, cGMP is protective, and mitigates ΔCa_i decrease. In contrast, in F, cGMP contributes to the development of myofilament dysfunction. Different therapeutic approaches may thus be required in septic M and F patients. Funded by K08GM096082 (NIH, to IAH).

5.2
HAEMOSTATIC AND RHEOLOGIC FACTORIALS AS DETERMINANTS OF ACUTE MYOCARDIAL INFARCTIONS IN NIGERIANS

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Background: Myocardial infarction (MI) is defined as necrosis of a portion of cardiac muscle caused by obstruction in coronary artery through either artheriosclerosis, a thrombus or spasm. The causative factors have been well documented and its risk has been reduced to the barest minimum in advanced countries of the world while in developing countries such as Nigeria, the advent of MI as a major cardiovascular problem is moderately recent. Therefore, researches into the responses of of rheological and fibrinolytic parameters are modestly new and ongoing. **Objectives:** We aimed to highlight basic information on pattern of presentation of haemorheologic and fibrinolytic parameters with a view to indicate their possible use as management indices in MI. **Methods:** We investigated longitudinally, 10 acute myocardial infarction (AMI) patients (5males and 5 females) together with 20 age and sex – matched apparently healthy subjects as controls. Blood samples were taken at the point of admission (Day 0), on the 4th and 7th day respectively after treatment has commenced. Rheologic and fibrinolytic indices such as hematocrit (HCT), Erythrocyte sedimentation rate (ESR), Plasma Fibrinogen concentration (PFC), D-dimer concentration (DDC), Euglobulin lysis time test (ELT) and Plasma viscosity (PV) were measured using standard laboratory methods. **Results:** We recorded a significantly reduced values of Haematocrit and fibrinolytic activity coupled with significantly increased D-dimer levels, PFC, ESR and PV in AMI patients on admission compared with controls (P<0.05, respectively). However, PFC, DDC and PV became significantly lowered from the 4th day of admission while all the parameters became significantly reduced from the 7th day of admission and treatment (P<0.05, respectively). There were no significant sex variations in all the parameters except haematocrit and whole blood viscosity which were lower in females than in males (P<0.05, respecttively. Platelet counts remained significantly high throughout the study period. **Conclusion:** We conclude therefore that hyperfibrinogenaemia coupled with hypofibrinolytic activity and high plasma viscosities could be likely associated risk factors of thrombosis in Nigerians with AMI and their reduction during treatment are positive indicators and as possible factorials in its pathogenesis.

5.3
INCREASED PREVALENCE OF ATRIAL FIBRILLATION IN MALE MICE IS ASSOCIATED WITH LOWER EXPRESSION OF CONNEXIN 40 AND 43

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The risk of developing *atrial fibrillation (AF)* is more prevalent in men than women, *with a 2:1 male preponderance.* The electrical remodelling of the atria is one of the critical processes involved in the development of AF and is characterized by a decrease in conduction velocity and shortening of the atrial action potential duration. Even though AF is the most common sustained cardiac arrhythmia, the basis of the sex-related difference has not been explored. Therefore, the objective of this study is to identify the sex differences in electrophysiological AF substrates responsible for the increased male susceptibility to AF. Accordingly, we used electrical programmed stimulations (EPS) to compare the inducibility of AF in adult male and female CD1 mice. Results obtained reveal that, similarly to humans, the probability of inducing AF in males was significantly higher compared to females (Males: 11 mice out of 21 (52%); Females: 6 mice out of 24 (25%)). Since the left atrium is particularly vulnerable to the development of AF we then used voltage-clamp technique to compare the ionic currents in left atrial myocytes isolated from mice of both sexes. The density of Na^+ current (I_{Na}) (at -35 mV; males: -20.3 ± 2.1 pA/pF, n=11; females: -19.1 ± 2.2 pA/pF, n=13; p=NS) and total K^+ current (I_{peak}) (at +30 mV; males: 20.5 ± 2.8 pA/pF, n=13; females: 21.4 ± 1.0 pA/pF, n=26; p=NS) is similar between both sexes. Also, qPCR data revealed that the mRNA expression level of the underlying Na^+ and K^+ ion channels in left atria of male and female mice was comparable. However, relative mRNA levels of connexin 40 and 43 (Cx40 and Cx43) were more than 3 times lower in the left atrial tissues obtained from male mice. These changes in Cx40 and Cx43 are consistent with a slower atrial conduction in males that could promote AF. In conclusion, our study suggests that atrial ionic currents are comparable between males and females however; our results suggest that there might be an important role for lower connexin expression in male mice. These findings contribute to explain the cellular mechanisms responsible for the higher incidence of AF reported in men. This research was funded by the Canadian Institutes of Health Research.

5.4
INDICES OF CARDIAC SYMPATHETIC ACTIVITY DURING LOWER BODY NEGATIVE PRESSURE IN MEN AND WOMEN THROUGHOUT THE MENSTRUAL CYCLE

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Women experience orthostatic intolerance to a greater degree than men and recent studies have begun to investigate the role of sex and the menstrual cycle on muscle sympathetic nerve activity (MSNA) during orthostatic stress (Stickford et al. (2015); Yang et al. (2012); Fu et al. (2009)). However, MSNA does not necessarily equate to cardiac sympathetic activity. Eleven women (5 not taking oral contraceptives (OC), 2 taking cyclic OC, and 4 taking non-cyclic OC) and eleven men were recruited and a standard electrocardiogram was recorded throughout a lower body negative pressure (LBNP) protocol. This protocol consisted of 5 minutes at -10, -20, -30 and -40mmHg. Women were investigated during the low-hormone phase (LH phase; day 8-11) and high-hormone phase (HH phase; day 18-24) of the menstrual cycle. At baseline and -40mmHg, heart-rate variability (HRV; time and frequency domains) and T-wave amplitude were determined. T-wave amplitude was investigated as Baumert et al. (2011) suggested that it may provide a tool to assess sympathetic outflow to the heart during orthostatic stress. Indeed, the percent change in T-wave amplitude due to LBNP was found to be significantly correlated with the percent change in low-frequency power (LF) (p=0.0003) and the percent change in the ratio of low frequency to high frequency power (LF/HF; p=0.0005) in these participants. LBNP resulted in: 1) decreased T-wave amplitude in all groups (Men: -18.9±3.3%, HH phase: -17.3±5.6%, LH phase: -15.6±4.1%), 2) increased LF in all groups (Men: +74.7±17.7%, HH phase: +87.1±33.2%, LH phase: +43.3±13.9%), 3) increased LF/HF in all groups (Men: +375±132%, HH phase: +617±376%, LH phase: +198±77%), and 4) decreased SDNN in men and women in the LH phase (Men: -17.7±6.7%, HH phase: -1.1±7.9%, LH phase: -19.6±5.2%). When comparing men to the HH phase, there were no significant differences in the responses of T-wave amplitude (p=0.815), LF (p=0.743), LF/HF (p=0.793) or SDNN (p=0.124) to LBNP. When comparing men to the LH phase, there were no significant differences in the responses of T-wave amplitude (p=0.554), LF (p=0.179), LF/HF (p=0.293), or SDNN (p=0.824) to LBNP. When comparing the two phases of the menstrual cycle, there were no significant differences in the responses of T-wave amplitude (p=0.807), LF (p=0.278) or LF/HF to LBNP (p=0.365); however, women in the LH phase had a greater decrease in SDNN (p=0.018). These results indicate that men and women have similar cardiac sympathetic responses to LBNP.

5.5
IN VIVO ELECTROCARDIOGRAMS IN A MURINE MODEL OF CHAGASIC CARDIOMYOPATHY SPECIFY FIRST DEGREE ATRIOVENTRICULAR BLOCK AS A PREDICTOR FOR SEVERE DISEASE

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Rationale: Chagas disease (CD) is a neglected tropical disease caused by infection with the protozoan parasite *Trypanosoma cruzi (T. cruzi)*. The disease afflicts millions

of people in Latin America, with over 40,000 new cases per year and approximately 14,000 cases of congenital transmission. In the USA and Europe alone, it is estimated that 1 million immigrants suffer from CD. Though many infected individuals will remain asymptomatic indefinitely, 20-30% of CD patients will progress to the symptomatic, chronic phase of disease within a period of 10-30 years after infection. This symptomatic phase, known as Chronic Chagasic Cardiomyopathy (CCC), is the most frequent and severe manifestation of CD. Several risk factors for CCC include myocardial inflammation, myocytolysis, fibrosis, and cardiomyopathy. Studies have shown that these negative effects may also manifest into cardiac rhythm disturbances, as identified in humans and various animal models of *T. cruzi* infection. However, it remains unknown whether chronic *T. cruzi* H1 infection: 1) impairs cardiac performance and reproduces human CCC or 2) has greater susceptibility based on gender. **Objective**: Determine the effects of *T. cruzi* H1 strain on cardiac function in mice. **Methods and Results**: To evaluate CCC, male and female ICR mice (Taconic Biosciences, Inc.) were infected intraperitoneally with a low dose of 500 *T. cruzi* H1 (Yucatán, Mexico) blood trypomastigotes (bt) for a period of 180 days post infection (DPI) and monitored by electrocardiography (ECG) and echocardiography. By 50 DPI, infected male mice showed high mortality rates (84%) and low survival curves, whereas, 70% of female mice survived beyond the acute phase (past 50 DPI) and entered into the chronic phase of disease. By 70 DPI, ECG analysis revealed a significant delay in the conduction of electrical impulses from the sinoatrial (SA) node to the atrioventricular (AV) node, indicated by prolonged P-R intervals (1st-degree AV block) in 20% of mice. In addition, 2nd-degree AV block was evident in 20% of mice. As surviving mice progressed to chronic infection (180 DPI), ~30% of mice displayed severe 2nd and 3rd-degree AV blocks, while another 30% began to display 1st-degree AV block or AV dissociation, indicating that prolonged PR intervals precede severe AV block and heart failure in murine CCC. **Conclusion:** Our results suggest that *T. cruzi* H1 infection in ICR mice serves as a model to study the pathology and mechanisms of human CCC.

5.6
ANGIOTENSIN II MODULATES SEX STEROID METABOLIZING ENZYME AND RECEPTOR EXPRESSION IN CARDIAC FIBROBLASTS FROM MALE AND FEMALE RATS

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Pathological cardiac remodeling involving fibrosis is a major underlying feature of progressive heart disease leading to heart failure. Gonadal sex steroids have been shown to attenuate angiotensin II (AngII)-induced cardiac fibrosis and fibroblast activation. Given that AngII has been shown to influence androgen and estrogen receptor expression in non-cardiac tissues, in the present study we investigated the impact of AngII on sex steroid receptor and enzyme expression in primary rat cardiac fibroblasts. Cardiac fibroblasts were isolated from adult male and female rats and treated at passage 1 in 2% charcoal-stripped FBS for 24 hours with AngII or vehicle (Veh). Gene expression of aromatase, 5α-reductase, androgen receptor (AR), and estrogen receptors (ERα, ERβ) were determined by qRT-PCR. Cardiac fibroblasts express ERα, ERβ, and AR, as well as the metabolizing enzymes 5α-reductase and aromatase; however, levels of expression were not influenced by sex. AngII significantly and equivalently reduced mRNA expression levels of ERβ, ERα, AR, and 5α-reductase in both male and female cardiac fibroblasts. Aromatase was expressed at low levels in male and female fibroblasts and was not altered by AngII. In separate studies the impact of testosterone, a potential substrate for local 17β-estradiol production via aromatase, was assessed to indirectly determine if AngII alters local aromatase activity. Fibroblasts isolated from male rats were treated with testosterone for the final 6 hours of AngII incubation. However, the addition of testosterone did not alter AngII effects on sex steroid receptor or enzyme gene expression, nor levels of 17β-estradiol in the culture media. These findings demonstrate that AngII downregulates the local sex steroid receptor expression and at least one enzyme involved in gonadal sex steroid metabolism. Given the previously-described protective effects of testosterone and 17β-estradiol, the downregulation of androgen and estrogen receptors in cardiac fibroblasts may contribute to the cardiac fibrosis induced by AngII in both males and females. Funding: AHA 13BGIA14720053.

5.7
CARDIAC REMODELING IN FEMALE HEARTS BY KVB1 SUBUNIT

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Cardiovascular disease remains the leading cause of death for women in the US. The etiology of the disease largely remains unknown in addition symptoms can remain silent for many years. The hallmarks for the disease demonstrate cardiac functional alterations including higher heart rates, longer QTc duration and a greater propensity for arrhythmias. These symptoms can be caused by cardiac remodeling leading to repolarization defects. Potassium channels play a major role in maintaining the repolarization reserve and the Kvβ1 subunits are uniquely positioned to modulate Kv (Kv4 and Kv1) channels. The present study investigates the physiological function and roles of Kvβ1.We utilized a Kvβ1.1-deficient female mouse line (Kvβ1.1 KO) and noted enlarged hearts compared with WT female controls (C57BL6/NJ). The physical and morphometric data showed increased heart weight, surface area, and left ventricular internal dimensions (LVID; S/D) (by echocardiography). KO females further demonstrated greater mean pressure and velocity in the ascending aorta (1.5±0.16mmHg vs. 0.88±0.17mmHg and 617±32mm/s vs. 456±42mm/s). KO females demonstrated significant prolongation in both monophasic action potentials (APD90: 57±1.7ms vs. 49±2.5ms) and QTc duration (51±2ms vs. 45±2ms).Taken together Kvβ1.1 KO female mice demonstrate enlarged hearts, systolic dysfunction, and electrical defects. This report clearly demonstrates that the Kvβ1 subunit is involved in cardiac growth and electrical remodeling. Funding source: NIH 1R01HL102171-01A1.

5.8
IMPAIRED DIASTOLIC FUNCTION FOLLOWING ACUTE STARVATION IN MEN BUT NOT PREMENOPAUSAL WOMEN

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Alterations in cardiac fatty acid uptake and metabolism have been implicated in the development of myocardial diastolic dysfunction. The majority of this work however remains limited to transgenic mice or rodent models of dietary obesity. We therefore sought to translate these preclinical findings into human subjects. To augment fatty acid uptake and metabolism, we performed an acute (48 hours) starvation intervention in ten healthy volunteers (6 men/4 women, age: 29±4 yrs). Myocardial triglyceride content and left ventricular diastolic function were measured by magnetic resonance spectroscopy and imaging, respectively; at baseline (BL), immediately after the 48 hour fast, and 48-72 hours following re-feeding with the subjects normal diet. As expected, acute starvation caused a significant, but transient, mean elevation in circulating free fatty acids (ΔBL: 162±11%, P=0.02), ketone bodies (ΔBL: 2387±168%, P<0.001), and myocardial triglyceride content (ΔBL: 396±139%, P<0.001), returning to baseline upon follow-up. Remarkably, left ventricular relaxation rate was reduced in each of the men following the 48 hour fast (ΔBL: -19±3%, $P < 0.05$), but remained unchanged in the female subjects (ΔBL: 4±2%, P=0.1916). Sex specific analysis also revealed significantly greater elevations in ketone bodies in females than males (ΔBL: 4235±651% vs. 1877±399%, respectively), despite a similar increase in circulating free fatty acids (ΔBL: 147±14% vs. 213±29%, female vs. male, respectively). Because ketone bodies are known to be anti-inflammatory, we speculate that premenopausal women may be protected against metabolic fatty acid-induced inflammation through this specific pathway. Further work in a larger sample size including post-menopausal women is warranted to further understand the role of estrogen on sex differences in metabolism and cardiac health and disease.

5.9
ATTENUATION OF CARDIAC AGING AND LEPTIN-DEPENDENT CARDIOPROTECTION IN LONG-LIVED AMUPA MICE

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Susceptibility of the heart to ischemia increases with age in man and rodents.αMUPA transgenic mice and mice treated for caloric restriction (CR) are two longevity models. αMUPA spontaneously consume less food compared with their wild type (WT) ancestors, and show endogenously increased levels of leptin, a satiety hormone known to decline under CR. To understand mechanisms linked to cardiac aging and protection, female αMUPA and WT mice were subjected to myocardial infarction (MI) in vivo at several ages and to ischemia/reperfusion ex vivo. Compared to WT mice, αMUPA showed functional and histological advantages under all experimental conditions. By 24 months, none of the WT mice survived the first ischemic day while αMUPA mice demonstrated 50% survival after 7 ischemic days. At baseline and post MI, leptin levels almost doubled in the αMUPA sera. Pretreatment with leptin neutralizing antibodies, or with inhibitors of leptin signaling (AG-490 and Wortmannin),

abrogated the αMUPA benefits. The antibodies also reduced phosphorylation of the leptin signaling components STAT3 and AKT specifically in the αMUPA myocardium. αMUPA mice did not show elevation in adiponectin previously implicated in CR-induced cardioprotection. These results demonstrate a life-long increased ischemic tolerance in αMUPA mice, indicating the attenuation of cardiac aging. αMUPA cardioprotection is mediated through endogenous leptin, suggesting a protective pathway distinct from that elicited under CR. This study was funded by the Israel Science Foundation.

5.10
THE CHARACTERIZATION OF AUXOTONIC TWITCH OF RIGHT VENTRICULAR CARDIOMYOCYTES FROM NON-FAILING AND FAILING HEARTS OF IMPUBERAL MALE AND FEMALE RATS

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The myocardial contractility in heart failure is impaired in adult males but preserved in adult females. This is associated with the protective effect of sex hormones. In impuberty, the protection is limited. We characterized auxotonic twitch of isolated right ventricular (RV) cardiomyocytes from young male/female healthy and RV failing rats. The experiments have been conducted on 2-month Wistar rats in conformance with the Declaration of Helsinki and the APS "Guiding Principals in the care and Use of Animals". RV cardiomyocytes were obtained from non-failing males/females (NF-m, NF-f) and monocrotaline-treated males/females (MCT-m, MCT-f); n=4 in each group. Auxotonic twitches (>20 cells/group) were measured at 25^{0}C and 1 Hz pacing rate under different preloads using carbon fiber technique. Data presented as mean±SE, difference is significant at P<0.05. At low preloads (<110% of slack length, L_0), end-systolic tension was two-fold larger in males vs. females in NF or MCT and was significantly larger in MCT-m/MCT-f vs. NF-m/NF-f (by 42.6±1.4/70.0±1.7%, respectively). The normalized rate of tension development did not differ in NF-m vs. NF-f (12.2±0.3 vs. 12.3±0.2 1/s) but was significantly lower in MCT-m vs. MCT-f (12.8±0.1 vs. 13.7±0.2 1/s). The time-to-peak tension and twitch duration were sex-independent in NF or MCT but both parameters were significantly lower in MCT-m vs. NF-m (by 7.7±0.7% and 7.5±0.3%, respectively) and in MCT-f vs. NF-f (by 17.4±0.1% and 16.5±0.7%). These proportions in general remain under increased preloads (115-130% L_0). End-systolic tension was higher in MCT-f vs. NF-f (by 75.1±2.8%, significant) and in MCT-m vs. NF-m (by 17.6±6.3%). The normalized rate of tension development was significantly lower in NF-m vs. NF-f (10.6±0.3 vs. 11.2±0.2 1/s) and in MCT-m vs. MCT-f (11.8±0.2 vs. 12.8±0.1 1/s); this parameter was substantially higher in MCT vs. same-sex NF. Time-to-peak tension and twitch duration were significantly shortened in MCT vs. same-sex NF. In conclusion, the characteristics of auxotonic twitch of RV cardiomyocytes of impuberal male and female rats with monocrotaline-induced RV heart failure display similar changes from those observed in the same-sex healthy animals. The minor gender-specific differences were found both at low and physiological preloads. In contrast to adult animals, the protective effect of sex hormones in female myocardium is not in action yet in young rats. The study is supported by RFBR #13-04-00367.

5.11
LOWER LEVELS OF INTERLEUKIN-6 IN FEMALE MICE AT DAYS 1 AND 3 POST-MYOCARDIAL INFARCTION ATTENUATE NEUTROPHIL INFILTRATION, RUPTURE, AND LEFT VENTRICULAR DILATION

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Survival after myocardial infarction (MI) is improved in female compared to male mice of the same age, yet the mechanisms to explain this phenotype remain undefined. We hypothesized that female mice have lower acute systemic pro-inflammatory cytokine production leading to improved survival and cardiac function post-MI. We used C57BL/6 male and female mice (3-7 months old; n=93) for this study. Females had better day (D) 7 survival (73%; 26 out of 34) compared to males (40%; 29 out of 59; p<0.05). In addition, rupture rate (rupture/total deaths) was reduced in females (1/9; 11%) compared to males (16/35; 46%; p<0.05). Out of 52 analytes measured at D1, D3, D5 or D7 days post-MI (n=10-12/sex/day), 5 were identified as possible regulators of sex related differences post-MI: immunoglobin A (IgA), interleukin-6 (IL-6), macrophage inflammatory protein (MIP) 1-g, plasminogen activator inhibitor-1 (PAI-1), and tissue inhibitor of metalloproteinase (TIMP)-1). Regression analysis showed only IL-6 levels positively correlated with end diastolic dimension (EDD) in both males (R^2=0.47; p<0.05) and females (R^2= 0.32 p<0.05). By time course analysis, IL-6 increased early post-MI in males, peaking at D3, and quickly decreased to baseline levels by D7. Females, on the other hand, had a subtle yet significant increase early post-MI that remained steady until D7 such that IL-6 was elevated in females at D7 compared to males (p<0.05). Interestingly, despite higher IL-6 at D7 post-MI in female mice, EDD was decreased in females (5.41 ± 0.10 mm) compared to males (5.94 ± 0.24 mm; p<0.05; n=10-12/sex/day) highlighting the importance of early post-MI events. Since IL-6 is known to regulate neutrophil infiltration early post-MI, we evaluated neutrophil numbers and found females had a 2-fold reduction compared to males at D1 and 3 post-MI (p<0.05 for both days; n=6/sex/day). In conclusion, females had reduced circulating IL-6 at D1 and D3 post-MI, which led to decreased neutrophil infiltration and attenuated cardiac rupture and LV dilation.

6.0 METABOLISM AND DIABETES

6.1
AUGMENTATION OF URINARY ANGIOTENSINOGEN LEVELS IN YOUNG MEN AND WOMEN WITH TYPE-1 DIABETES MELLITUS

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We previously reported that augmented urinary angiotensinogen (uAGT) reflects early activation of intrarenal renin-angiotensin system (RAS) in subjects with type-1 diabetes mellitus (T1DM). The uAGT levels increase prior to the development of albuminuria and are correlated with serum hemoglobin A1c (HbA1C) levels and urinary 8-isoprostane excretion rates. The present study explored the separate uAGT responses in young diabetic men and women and their relationships with HbA1C and urinary 8-isoprostane excretion rates in blood and urine samples from control subjects (21 men, 19 women) and short duration (6.1±.7 yr for men and 6.4±.4 yr for women) T1DM subjects (49 men, 37 women) with similar body weights, BMI and age. The T1DM subjects were normotensive (124±2/69±1 mmHg in men and 119±2/69±1 mmHg in women) and were only on insulin treatment. Serum glucose levels and HbA1c in T1DM remained significantly elevated above control but were not significantly different between men (178±15 mg/dl and 9.1±.2%) and women (203±14 mg/dl and 9.2±.3%) subjects. Urinary albuminuria/creatinine excretion rates were not significantly higher in T1DM subjects from their respective controls in either sex. There was no evidence of hyperfiltration as estimated GFR values did not differ between the T1DM men and women (82±3 ml/min/1.73m^2 in men vs. 88±5 ml/min/1.73m^2 in women) or from their respective control values (87.8 ±6.2 ml/min/1.73m^2 in men and 89±5 ml/min/1.73m^2 in women). uAGT/creatinine values were significantly greater in the diabetic subjects (15.3±1.7 μg/g in men vs. 15.2±2.5 μg/g in women) compared to controls (6.0±0.9 μg/g in men vs. 7.9±1.4 μg/g in women), but not different between men and women. Correlation analysis revealed significant relationships of uAGT with both urinary 8-isoprostane excretion (R=.45, P<.001 for men and R=.60, P<.001 for women) and HbA1c (R=.37, P<.01 for men and R=.53, P<.001 for women) in both men and women with T1DM. The results indicate that uAGT excretion rates are increased in some diabetic men and women with T1DM on insulin even before the development of proteinuria or hypertension suggesting that activation of the intrarenal RAS is an early event. The monitoring of uAGT levels in diabetic men and women may be potentially useful in determining therapeutic measures to block augmentation of the intrarenal RAS and prevent the development of proteinuria and diabetic nephropathy. This work was supported by NIDDK (1R21DK094006).

6.2
ESTROGEN TREATMENT RESTORES MUSCLE MITOCHONDRIAL CAPACITY AND REVERSES PRO-DIABETOGENIC STATE INDUCED BY OVARIECTOMY

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17β-estradiol (E2) is a key regulator of energy and glucose homeostasis. Menopause comes with a significant decline in E2 production and increases a woman's risk for developing cardiovascular disease and type-2 diabetes, while hormone replacement therapy decreases the incidence of type-2 diabetes. Mitochondrial function in skeletal muscle (SM) has been linked to the control of insulin sensitivity. To examine the potential underlying mechanism(s) by which E2 regulates insulin sensitivity in SM, young sexually mature (12 week-old) C57BL-female mice were studied 2 weeks after ovariectomy (OVX-2w) followed by 2 weeks of E2 treatment (OVX+E2, 1μg/day) administered via a subcutaneous miniosmotic pump. Control groups included normally cycling females (NC) in the Proestrus stage (high physiological E2 levels) and an OVX group implanted with a saline control pump (OVX-ctl). E2 treatment effectively reversed fasting hyperglycemia developed in the OVX-2w and OVX-ctl groups (+25%, $p<0.0001$ and +12%, $p<0.05$ vs NC), decreased fat mass by 48% ($p<0.01$ vs OVX-ctl) and restored ex-vivo insulin-stimulated muscle glucose uptake back to NC values ($p<0.05$ vs. OVX-2w and OVX-ctl). In permeabilized fibers from red gastrocnemius, maximal state-3 respiration with carbohydrate-derived substrates (pyruvate/malate) was reduced in OVX-ctl (-26%, $p<0.05$ vs NC) but fully restored in the OVX+E2. Surprisingly, maximal state-3 fatty acid-supported respiration was reduced in OVX+/- E2 treatment ($p<0.05$). Spectrophotometric assessment of the relative specific activities of OXPHOS complexes in isolated mitochondria revealed a significantly impaired Complex I activity in the OVX -2w and -ctl groups ($p<0.05$, $p<0.005$ vs NC) that was fully restored in the OVX+E2. While Complex II and III activities were not altered, transfer of electrons between Complex I and III, as well as between II and III, was also decreased in OVX-ctl ($p<0.005$ and $p<0.05$ vs NC, respectively), but completely restored in OVX+E2. Mitochondrial membrane fluidity was decreased in OVX -2w and –ctl ($p<0.05$ vs NC), but restored in OVX+E2. Expression of OXPHOS complexes and citrate synthase activity were not affected across all groups. These findings provide evidence that E2 restores mitochondrial function in skeletal muscle via non-genomic pathways, by promoting electron transfer efficiency between complexes I/II and III, thus offering new insights into the mechanism(s) by which menopause sets, and E2 therapy reverses a pro-diabetogenic state. NIH DK096907.

6.3
WITHDRAWN

6.4
INCREASED OREXIGENIC INNERVATION OF DOPAMINE NEURONS REDUCES PROLACTIN SECRETION IN OBESE FEMALE RATS

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Obesity adversely affects reproductive health in women causing menstrual irregularity, anovulation, miscarriages, and decreased conception. This suggests that an individual's metabolic state is pivotal for reproductive success. Neural circuits underlying feeding behavior and reproductive cycling are both found in the hypothalamus, specifically within the arcuate nucleus (ARC). Dopaminergic (DA) neurons within the ARC send inhibitory projections to lactotrophs in the pituitary which release prolactin (PRL), a hormone critical for lactation and maintenance of pregnancy. Dopamine release is inhibited by kisspeptin (KISS), a neuropeptide of the HPG axis, and stimulated by Orexin-A (ORX), an appetite stimulant. Thus, the objective of the current study was to determine the effects of diet-induced obesity on prolactin release and identify putative changes is the underlying neuroanatomy. Our study utilized female Sprague-Dawley rats fed either a high-fat, high-sugar (HFHS) diet or control chow from weaning. The HFHS animals received food containing 60% fat and a 32% sucrose solution. To assess the central effect of diet on reproductive hormone signaling, animals were ovariectomized (OVX) and half received a slow-release estrogen pellet (OVXE) to induce circadian prolactin and luteinizing hormone (LH) surges. Two weeks following OVX surgeries, animals were sacrificed. Blood samples were collected at specific time points, and PRL/LH levels were assessed using radioimmunoassay. Upon termination, HFHS animals weighed significantly more than their control counterparts and exhibited greater fat pad mass. Similarly, OVXE animals weighed significantly more than OVX animals. OVX females lacked LH/PRL surges, while OVXE females displayed daily surges. HFHS OVXE animals, on the other hand, exhibited a blunted PRL surge. Finally, we used immunofluorescence to assess the spatial relationship between dopaminergic neurons and KISS- or ORX-positive terminals. Analysis revealed a higher degree of co-localization between dopamine and ORX in HFHS animals, suggesting augmented levels of ORX may account for blunted prolactin secretion as a result of greater dopamine stimulation. Additionally, *in silico* models confirmed this relationship. These observations further elucidate the mechanism between diet-induced obesity and aberrant reproduction, targeting both ORX and KISS as central actors in successful PRL secretion and pregnancy.

6.5
DIET-INDUCED OBESITY IMPAIRS ESTROUS CYCLE REGULARITY IN FEMALE RATS

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Currently, 60% of women in the United States are overweight or obese. This condition can lead to high rates of menstrual irregularity and infertility. Therefore, the objective of this study is to determine the relationship between obesity and reproduction using female rats as a model. At 23 days old, Sprague-Dawley rats were split into two groups: control chow and high fat, high sugar (HFHS) diet. The HFHS diet consisted of a 32% sucrose solution and food containing 60% calories from fat. After three weeks of diet consumption, HFHS animals weighed significantly more than control-fed rats and continued to weigh more for the remainder of the experiment. Additionally, insulin sensitivity was assessed with fasting blood samples and the HOMA-IR calculation. When the rats reached sexual maturity at ten weeks of age, daily vaginal smears were taken over the course of five weeks in order to assess the effect of diet and weight gain on estrous cycling. While over 50% of the cycles occurring in control rats lasted for the normal four-day duration, only about 40% of HFHS rats exhibited the normal four-day pattern. Furthermore, HFHS rats experienced an increased number of days spent in consecutive estrus compared to their control counterparts. It was noted that these days spent in consecutive estrus occurred in the obese subjects after weight gain had occurred, therefore suggesting that obesity induces estrous cycle irregularity in previously normally cycling animals. Rats were ovariectomized, and ovaries were assessed for follicle development. In conclusion, our findings suggest that diet-induced obesity leads to a disruption in the regularity of estrous cycling, which may result in reduced fertility.

6.6
INFLUENCES OF DIET ON SERUM C-REACTIVE PROTEIN IN UNOBSTRUCTED AND OBSTRUCTED BLADDERS OF MALE WISTAR RATS

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Introduction: Serum C-reactive protein (CRP) is a marker for inflammation produced by the liver in response to factors released by adipocytes and macrophages. Its level in circulation is linked with benign prostatic hyperplasia (BPH), the primary cause of bladder outlet obstruction (BOO) in adult males. It is also directly related to the severity of lower urinary tract symptoms (LUTS). Diet has been strongly associated with inflammation and some diets have been related to chronic inflamemation. We evaluated the effects of diets of varying macronutrient composition on inflammation in the unobstructed bladder and BOO, by assessing its influences on Serum CRP levels. **Materials and Methods:** Appropriate institutional ethical approval for use of animals in laboratory research was obtained from the Ethical committee of the College of Medicine, University of Ibadan and all protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals. Partial BOO was surgically induced in male wistar rats. Animals were prefed on various diets which were continued for 4 weeks after surgery. Rats were divided into sham-operated and BOO groups each with the following: control (normal rats' feeds), high-carbohydrate (HCD), high-fat (HFD) and high-protein (HPD) dietary groups. After the experimental feeding period, blood was collected and Serum CRP level was assessed using Enzyme-linked immunosorbent assay (ELISA). **Results:** In the unobstructed bladder, serum CRP was elevated only in animals fed on the HFD ($P<0.05$). In the obstructed groups also, only the animals fed on the HFD showed an increase in CRP, an increase that was higher ($P<0.05$) than that in the HFD without obstruction. **Conclusion:** A high fat diet results in an increase in serum-CRP in both the unobstructed and obstructed rat bladder. As obesity and BOO are independently associated with the severity of LUTS in both sexes, these findings indicate that the worsening of LUTS seen with BOO and in obese patients may be due in part to increased inflammation.

6.7
A HIGH-FAT DIET IMPACTS GLUCOSE AND BLOOD PRESSURE IN FEMALE AND MALE DAHL SALT-SENSITIVE RATS

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There are numerous reported sex differences in metabolic parameters and blood pressure (BP), although many fewer studies have examined the molecular mechanisms driving high-fat (HF)-induced increases in BP and metabolic disorders in males vs. females. Obesity and a HF diet are risk factors for hypertension, and male Dahl salt-sensitive rats (DSS) exhibit an increase in free fatty acids and BP in response to a HF diet; nothing is known in females. The current study was designed to determine the impact of a HF diet on blood glucose levels, metabolic parameters, and BP in male and female DSS (n=4-6). At baseline, females had a lower BP (125±1 vs. 132±2 mmHg; p=0.008) and were smaller than males (220±4 vs. 324±9 g; p<0.01), although females have greater percent body fat (10±0.7 vs. 7±0.4%; p=0.005). DSS were implanted with a PhysioTel HD-XG glucose telemeter for the continuous measurement of blood glucose. A glucose tolerance test was performed and revealed that females have a better glucose tolerance at baseline than males (AUC: 2034±206 vs. 2381±216; p<0.05). Rats were then placed on a HF diet (36% fat; Bio-Serv). After 1 week on the HF diet, both female and male rats gained weight (247±3 vs. 385±12 g, respectively), although blood glucose levels were comparable between female and male rats (105±0.2 vs. 103±3 mg/dL; p=0.45). The HF diet also significantly increased BP in both female (145±4) and male rats (154±3; p<0.001 for both sexes vs. baseline BP), however, the increase in BP was comparable between the sexes (14% increase for both). To date, our studies indicate that female and male DSS rats exhibit fat-induced alterations in metabolic and cardiovascular parameters. Rats will continue to be followed for an additional 3 weeks on the HF-diet to determine if sex impacts the trajectory of fat-induced increases in glucose handling or BP. We would like to offer a special thanks to DSI for the glucose implants and technical support.

6.8
HIGH FRUCTOSE INTAKE EXACERBATES THE IMPAIRMENT OF MESENTERIC ARTERIAL FUNCTION COMPARED TO GLUCOSE IN FEMALE RATS: POSSIBLE INVOLVEMENT OF EDHF CONTRIBUTION IN MODULATING VASCULAR REACTIVITY

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Intake of high fructose in diet has shown to contribute to variety of metabolic disorders such as obesity and diabetes. Limited data is available on the relative effects of different dietary sugar intake on vascular reactivity. The aim of current study was to investigate and compare the effects of high glucose (HG) and high fructose (HF) consumption on mesenteric arterial (MA) functions in female rats. Sprague-Dawley female rats were supplemented with 20% w/v glucose or fructose in drinking water for 8 weeks. Control rats received no sugar supplement. Blood pressure was measured every 2 weeks by tail-cuff method (LE-5001, Panlab, Harvard). Endothelium-dependent vasodilation (EDV) to acetylcholine (ACh) and bradykinin (BK) and endothelium-independent vasodilation to sodium nitroprusside (SNP) were measured in MA pre-contracted with phenylephrine (PE) or U446619. In addition, EDV to ACh were measured before and after pretreatment with indomethacin (cyclooxygenase inhibitor), L-NAME (nitric oxide synthase (NOS) inhibitor), or barium chloride (KIR blocker) plus ouabain (Na+-K+-ATPase inhibitor). Contractile responses to endothelin-1 (ET-1) were also measured. Systolic BP was significantly elevated in both HG and HF groups. HF ingestion, but not HG, decreased relaxation responses to ACh and BK. Accordingly, the relative importance of endothelium derived hyperpolarizing factor (EDHF) to vascular regulation was reduced in MA of HF-fed rats only. The relaxation to SNP was impaired in MA from both HF- and HG groups. However, the extent of impairment in SNP-induced relaxation was significantly greater in HF compared to HG groups. Finally, we demonstrated that the ET-1-induced contraction was augmented in MA of both HG and HF groups. Our data suggest that a decrease in the sensitivity of smooth muscle to NO may in part contribute to the increased ET-1 contractile responsiveness and high blood pressure in HG and HF groups. Furthermore, a reduction in the relative contribution of EDHF to vascular reactivity may partially contribute to the impairment of ACh response in MA of HF rats. The impaired EDV along with a greater decrease in the sensitivity of smooth muscle to NO in HF group suggest that the fructose ingestion may have a higher impact in inducing vascular dysfunction compared to glucose ingestion (supported by NIDCR).

6.9
THE IMPACT OF HIV INFECTION ON BODY COMPOSITION, LIPID PROFILE, ADIPONECTIN LEVEL AND RESTING ENERGY EXPENDITURE IN MTHATHA DISTRICT, A SEMI URBAN SOUTH AFRICAN COMMUNITY

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Aim and objective: The aim of this study was to determine the impact the highly active antiretroviral therapy (HAART) has on; lipid profile, body composition indices, adiponectin levels and resting energy expenditure. **Methods:** This was a descriptive and comparative study made up of 81 participants recruited from the public clinics in Mthatha, South Africa. They were categorized into the following three equal groups: 27 HAART treated HIV participants (group A), 27 HAART naïve HIV participants (group B) and 27 healthy non HIV patients (group C). Anthropometric measurements were used to determine basal metabolic index (BMI) and body composition indices. Biochemical tests such as analysis of serum lipids and adiponectin were performed. **Results:** The participants with normal nutritional status (BMI of 18.5-24.9 kg/m^2) in the three groups had significant variation in the following parameters: resting energy expenditure, (REE) adiponectin level, lipid profile and ideal weight. (P<0.05 **Conclusion:** The treatment of HIV infection with first line antiretrovirals reduces the level of adiponectin, increased the lipid profiles with the exception of HDL making them more susceptible to atherosclerosis. **Key words:** HIV-infection, Highly Active Ante Retroviral Therapy (HAART), Adiponectin, Lipid profile, Resting Energy Expenditure (REE).

6.10
ALTERATIONS IN FATTY ACID SIGNALING PATHWAYS DIFFERENTIALLY AFFECT FAT INTAKE IN MALE AND FEMALE MICE

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Excessive dietary fat intake has been linked with the current epidemic of obesity. The pathway underlying the recognition of fatty acids, the prototypical fat stimulus, by chemosensory cells in the oral cavity and the digestive system has been at least partially elucidated. This transduction pathway for polyunsaturated fatty acids (PUFAs) involves PUFA binding to CD36 and/or fatty acid activated GPCRs, and downstream activation of PLCβ, IP_3 and TrpM5. To determine the contribution of this pathway to dietary fat intake, we have utilized several model organisms that lack individual elements in this pathway and monitored food intake and body composition on high fat, high carbohydrate and control diets. Our initial study looked at the effects of TrpM5 deletion ($TrpM5^{-/-}$) in mice compared to wild-type mice ($TrpM5^{+/+}$) while on a high-fat diet (60% fat diet for 46 days). KO male mice took in significantly less calories than their WT counterparts and subsequently gained significantly less body weight while on the 60% high fat diet. Similar, though less dramatic, effects were seen in mice lacking the IP_3 receptor (IP_3R3) or the fatty acid transport protein, CD36, which are implicated in the fatty acid pathway. Since both pre- and post-ingestively, the sweet (carbohydrate-sensing) pathway involves the same transduction elements, we compared the same metrics on a high sucrose diet. Despite the importance of TrpM5 in this pathway, no differences were seen, tying this phenomenon to high fat diets. The effects of TrpM5 deletion were gender specific – female mice lacking TrpM5 show similar levels of fat intake, body fat and body weights on a high fat diet as wild type females. As with males, no differences in food intake or body composition between $TrpM5^{-/-}$ and $TrpM5^{+/+}$ female mice were seen on high sucrose diets. Our data are consistent with the interpretation that alterations in fatty acid signaling, pre- and/or post-ingestively, lead to specific changes in the intake of dietary fat in male mice and that this effect is gender specific. We are currently exploring the mechanistic nature of these gender differences in fatty acid signaling pathways and their regulation. Supported by NIH DC013194 and NIH DC013318 (TAG).

6.11
WITHDRAWN

6.12
A PILOT STUDY EXPLORING METABOLIC DYSFUNCTION IN TRANS-SEXUAL WOMEN: NOVEL INSIGHT FROM MAGNETIC RESONANCE SPECTROSCOPY

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Trans-sexual women (male to female - MTF) experience significant changes in adipose fat distribution after sex re-assignment surgery, suffer from increased metabolic risk and premature mortality. The exact mechanism by which sex re-assignment surgery and/or female hormone treatment leads to metabolic impairment remains incompletely understood. To begin to address this question, we recruited 12 trans-sexual women, who had undergone bi-lateral orchiectomy (n = 4) or had not (n = 8). Both groups were using female hormones. Glucose tolerance was assessed using a standard 75g oral glucose tolerance test (OGTT). Hepatic steatosis was assessed by ^{1}H magnetic resonance spectroscopy. The major novel findings were three-fold: First, the hormone only group were insulin resistant, as evidenced by a marked increase in plasma insulin during the OGTT compared to the orchiectomy group (AUC glucose: 20,380 $\pm$ 1263 vs. 17,823 $\pm$ 859; AUC insulin: 14,235 $\pm$ 4694 vs. 5,491 $\pm$ 1538; respectively). Second, hepatic steatosis was markedly elevated in the hormone only group compared to the orchiectomy group (8.9$\pm$2.4 vs. 1.5$\pm$0.3 %fat/water, respectively). Third, hepatic steatosis was associated with insulin resistance (n=12, R^2: 0.4482). These pilot data provide novel mechanistic insight, and suggest hepatic steatosis and insulin resistance are prevalent in trans-sexual women treated with cross-sex hormones, and that orchiectomy may be "protective".Future studies will focus on increasing sample size and investigating the hormonal milieu and impact on metabolic dysfunction.

6.13
DO WOMEN NEED TO LOSE MORE WEIGHT THAN MEN TO INCREASE CIRCULATING ADIPONECTIN?

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Adiponectin is an anti-inflammatory protein and plays a protective role in the development of atherosclerosis. Obese individuals have lower circulating concentrations than their lean counterparts. However, previous studies do not consistently show increased adiponectin concentrations with weight loss induced by caloric restriction and/or exercise. The purpose of this review is to determine whether sex is a factor in explaining the different study results. **Methods**: Previous studies that involve caloric restriction and/or exercise-induced weight loss, and have reported adiponectin concentrations before and after weight loss are examined. Percentage of weight lost, method of weight loss (caloric restriction only, exercise only, or combined), number and proportion of participants of each sex, and circulating adiponectin concentration changes are summarized. **Results**: In studies involving mostly men, approximately 10% of weight loss is associated with an increase in adiponectin concentration. In studies involving only women or women as the majority of the study participants, adiponectin does not significantly increase with up to 11% of weight loss; adiponectin increases with greater than 15% of weight loss. With the addition of exercise, less than 15% of weight loss significantly reduces adiponectin concentration. **Conclusion**: It appears greater weight loss is needed for women than men to show an increase in adiponectin concentration. This may be related to the greater body fat percentage in women than men. Exercise may help reduce the amount of weight loss needed to induce an increase in adiponectin. This work is partially supported by NIH AG031297.

6.14
SEX, LEPTIN STATUS, AND OBESITY MODULATE BUPRENORPHINE-INDUCED RESPIRATORY DEPRESSION IN MICE

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Opiates cause sex-specific differences in modulation of pain (*Pain* 155:388, 2014; *Biol Psych* 76:213, 2014) and respiratory depression (*Br J Anaesthesia* 100:747, 2008). The mechanisms contributing to the foregoing differences are not understood, yet are clinically relevant for efforts to elucidate sex-specific differences in response to opiate therapy (*Pain Res Manag* 20:23, 2015). Previous studies using mice showed that leptin levels are sexually dimorphic (*Obesity Res* 12:1481, 2004) and contribute to the regulation of both breathing (*Resp Physiol* 119:173, 2000) and nociception (*Neuroscience* 275:531, 2014). This ongoing study is testing the hypothesis that buprenorphine (bupe) causes dose-dependent alterations in breathing as a function of sex, leptin status, and obesity. Subjects include three groups of male and female mice from the Jackson Laboratory that 1) lack leptin and are obese (Lep^{ob}); 2) lack leptin receptors, are obese, and are diabetic ($Lepr^{db}$); and 3) have normal leptin levels and normal body weight (B6). Bupe (0.1, 0.3, 0.5, 1, 3, and 10 mg/kg) or saline were administered intraperitoneally and breathing was measured for 1 h using whole body plethysmography. All data are reported as percent change. Bupe caused dose-dependent changes in breathing for all three genotypes. An antinociceptive dose (0.3 mg/kg) decreased rate of breathing in the Lep^{ob} (-10.7%) and the $Lepr^{db}$ (-11%) mice relative to the B6 mice. The largest dose of bupe (10 mg/kg) decreased rate of breathing relative to rates after saline injection (B6 =-0.8%, Lep^{ob}=-22.3%, $Lepr^{db}$=-9.5%). Tidal volume (V_T, ml/g body weight) was increased by the 0.3 mg/kg dose (B6=31.5%, Lep^{ob}=48.5%, $Lepr^{db}$=14.8%). Minute ventilation (V_E, ml/min/g body weight) also varied by genotype after 0.3 mg/kg bupe (B6=24.7%, Lep^{ob}=37.3%, $Lepr^{db}$=11%). Male (M) versus Female (F) differences in V_E (saline vs 0.3 mg/kg bupe) were ($B6_M$=48%, $B6_F$=1.4%); ($Lep^{ob}{}_M$=69.5%, $Lep^{ob}{}_F$=5.2%); ($Lepr^{db}{}_M$=11%, $Lepr^{db}{}_F$=34.2%). These results encourage efforts to determine the extent to which leptin modulates increases in opiate-induced adverse events associated with female sex and obesity (*Anesthesiology* 122: 659, 2015). Support: 5-R01-HL065272-12 and University of Tennessee.

6.15
INCREASING LEPTIN SENSITIVITY WITH PROTEIN TYROSINE PHOSPHATASE 1B DELETION LEADS TO MORE SEVERE CARDIAC ALTERATIONS IN FEMALE THAN MALE MICE

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High circulating levels of the adipocyte-derived hormone leptin, contributes to the development of cardiac dysfunction in males. Despite the fact that females secrete 3-4 times more leptin than males and that left ventricular dysfunction correlates to fat content and BMI in women only, the interaction between leptin and cardiac dysfunction has not been studied in women. Here we hypothesized that female mice are more prone to leptin-mediated cardiac alterations than males. To test this hypothesis, we characterized the cardiac phenotype of lean male and female mice rendered hypersensitive to leptin via the deletion of the molecular brake on the leptin signaling, the protein tyrosine phosphatase 1B. Leptin sensitization in lean animals similarly increased blood pressure in male and female KO (124±4 vs 126±4 respectively; WT 102±5 mmHg) but induced a higher increase in fibrosis (Masson's trichrome) in the ventricle of female (WT: 2.7%, KO: 7.3%) compared to male mice (WT: 3.8%, KO: 4.2%). Red oil staining revealed deposition of fat droplets in the myocardium, which was significantly higher in KO females (14-fold) compared to males (3-fold,), noatbly in the intra-ventricular septum. Quantification of gene expression via quantitative real-time RT-PCR, showed an increase in hypertrophic and cardiac stress markers: β-myosin and ANP in the ventricle of KO female only. Furthermore, fibrotic (CTGF) and inflammatory markers (COX2) were highly up-regulated in KO female only. Metabolic factors involved in cardiac energy metabolism were differentially regulated in leptin-sensitized mice. In particular, VLCAD was decreased, when GLUT4 was increased in KO females only, revealing a shift in energy metabolism.Together these data showed that leptin sensitization induced a more severe increase in cardiac fibrosis, fat deposition and metabolic changes, in females than males, for the same level of hypertension. These data could explain the rise of cardiovascular diseases in young obese women. This work was supported by a Scientist Development Grant from the American Heart Association (11SDG5060006 to EJ.BdC) and Start-up funds from Georgia Regents University.

6.16
SEX DIFFERENCES IN RENAL SODIUM HANDLING IN MICE ON HIGH-FRUCTOSE AND HIGH-SALT DIET

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Many studies suggest a protective element associated with female sex under various conditions that increase blood pressure. Metabolic syndrome and hypertension are linked to high fructose and high salt consumption, and studies indicate sex differences in the physiological effects of these diets. Maintaining sodium balance is of major concern. The goal of this study was to investigate sex differences in mice consuming high levels of both fructose and salt (F+S). Female and male 5-week-old CD-1 intact mice (n=6/group) were placed in metabolic cages and consumed a normal (0.4% salt) diet and water for 4 days followed by 30 days on the F+S diet consisting of a 20% fructose and 1% salt solution and a powdered 4% salt chow. Measurements included blood pressure via the tail-cuff method and urinary sodium excretion. Separate mice kept in plastic bins and maintained on the same dietary protocol were used for molecular analysis of the renal sodium transporters via real-time PCR using custom-made PCR arrays (QIAGEN). Results demonstrated that mean blood pressure (MBP,

mmHg) was not different between females and males in the control period (72.3 ± 2.6 and 73.4 ± 1.3, respectively). No change in MBP occurred after the first week of F+S diet; however, at the end of the second week, MBP increased in both females and males (86.4 ± 2.2 vs 77.2 ± 1.0, respectively, p<0.01 from control week) with the female MBP > male MBP (p<0.01). At the end of the 4th week of F+S consumption, female MBP and male MBP were not different (90.1 ± 3.2 vs 89.4 ± 2.6, respectively). Sex differences in mRNA expression of renal sodium transporters were observed with female kidneys showing significantly higher expression of NCC, NKCC2, NHE3, and each of the three ENaC subunits whereas higher expression of Na-phosphate transporter was found in male kidneys. Moreover, females consistently demonstrated lower sodium excretion-to-sodium intake ratio (%) compared to males during the F+S period (62.0 ± 4.4 vs 74.8 ± 3.7, respectively, p<0.03). We conclude that consuming the F+S diet for two weeks increased blood pressure in both female and male mice with higher increase occurring in females. We propose the estrogen-induced stimulation on the renal handling of sodium plays a key role in the increased blood pressure in female mice under the F+S diet and studies are underway to test this proposal. This study was funded by NIH-sponsored Oklahoma INBRE summer research program (PA-12-313).

6.17
SEXUALLY DIMORPHIC MYELOID INFLAMMATORY AND METABOLIC RESPONSES TO DIET-INDUCED OBESITY

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Background: It is well known in clinical and animal studies that women and men have different disease risk as well as different disease physiology. Women of reproductive age are protected from metabolic and cardiovascular disease compared to post-menopausal women and men. Most murine studies are skewed towards the use of male mice to study obesity-induced metabolic dysfunction because of similar protection in female mice. We have investigated dietary obesity in a mouse model and have directly compared inflammatory responses in males and females. **Objective:** To understand if sex differences in obesity-induced inflammation contribute to differences in metabolic disease risk. **Design/Methods**: Male and female C57Bl/6J mice were fed a 60% high fat diet (HFD). Assessments for glucose metabolism were performed as well as evaluations of inflammatory responses in leukocyte activation in bone marrow, blood, and adipose tissue as well as pre-adipocyte popoulations. BM was cultured from male and female animals and stimulated with LPS to investigate sex differences in inflammatory responses. TLR4$^{-/-}$ animals were also challenged to understand the dependence of the inflammatory changes to the presence of TLR4. Monocyte transfer and reciprocal bone marrow transplant experiments were performed to further assess sex differences in bone marrow myeloid responses to obesity independent of host-sex. **Results**: Males and females both gained adiposity after high fat diet but females had higher energy expenditure rates and dampened inflammatory responses with reduced CD11c^{+} adipose tissue macrophage populations and inflamematory cytokines. *Ex vivo* female marrow produced reduced cytokines after LPS stimulation. TLR4 $^{-/-}$ males had attenuated but persistent macrophage accumulation while females remained protected. Male BM cells continued to remain primed for a pro-inflammatory responses after monocyte transfer experiments into female host and bone marrow transplantation. **Conclusion**: Sex differences in high fat diet induced inflammatory activation are due to cell intrinsic differences in hematopoietic responses to obesogenic cues. This work was supported, in whole or in part, by American Heart Association Scientist Development Grant 14SDG17890004 and Department of Pediatrics Janette Ferrantino Investigator Award.

6.18
SEX DIMORPHISM IN PLASMA SOLUBLE PRORENIN RECEPTOR (SPRR) LEVELS IN OBESE PATIENTS IS ASSOCIATED WITH TYPE 2 DIABETES MELLITUS IN WOMEN BUT NOT IN MEN

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Obesity markedly increases the occurrence of Type 2 diabetes mellitus (T2D). Adipose tissue expresses all components of the renin-angiotensin system (RAS), which may contribute to inappropriate RAS activation and increased risk of end-stage organ damage (ESOD) in T2D. Increased circulating levels of soluble prorenin receptor (sPRR) in cardiovascular patients suggest that plasma sPRR might be a potential biomarker of RAS activation. While women with T2D exhibit disproportionately greater burdens of ESOD than men; sex differences in the RAS during T2D are poorly understood. To test the hypothesis that plasma sPRR levels are associated with T2D in obese patients and differ between men and women, we examined plasma samples from 201 patients (mean age, 41 ± 13 years; 39% men), including 107 controls (Ct; BMI<30), 66 obese (Ob; BMI≥30) and 28 obese with T2D (Ob+T2D) patients. The waist to hip ratio (WHR) was used as a measurement of abdominal adiposity. Plasma sPRR levels, measured by ELISA, were significantly higher in Ob+T2D patients (21.5 ± 1.6ng/mL) compared to Ct (16.5 ± 0.4ng/mL) and Ob (16.6 ± 0.5 ng/mL; P <.0001). Urine Albumin/Cre ratio showed a similar trend (Ob: 31.0 ±4, Ob+T2D: 53.1±8 vs. Ct: 24.9±2 mg/g uCr; P <0.0001). Plasma sPRR levels negatively correlated with WHR in the Ob+T2D (r=-40.62, P =0.0395) but not with Ct or Ob patients. Control lean men patients exhibited significantly higher plasma sPRR levels compared to women (18.1 ± 0.8 vs. 15.4 ± 0.4 ng/mL; P<0.01). Interestingly, the plasma sPRR differences among groups of same sex were greater in Ob+T2D women compared to Ct (20.9 ± 1.7 ng/mL vs. 15.4 ± 0.4 ng/mL; P<0.0001) and Ob (15.8 ± 0.6 ng/mL; P<0.0001) patients, but did not differ among men groups. The interaction between sex and group was significant (p=0.036) suggesting that the increase of plasma sPRR levels in T2D patients is greater in women than men. Multiple regression analysis, adjusted by age, WHR, and groups indicated a significant association between plasma sPRR levels and T2D status in women (P<.0001) but not men. Our data indicate that plasma sPRR levels are associated with T2D in women but not in men, and that this effect is independent of obesity. The results indicate that plasma sPRR may serve as a biomarker of RAS activation allowing for a better understanding of the association between obesity, T2D, and its complications. Supported in part by 1 U54 GM104940 from the General Medical Sciences of the National Institutes of Health, which funds the Louisiana Clinical and Translational Science Center (LA CaTS).

6.19
SEX DIFFERENCES IN RENAL GENE EXPRESSION IN A DIET INDUCED OBESITY MODEL OF DIABETIC NEPHROPATHY (DN)

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DN is a serious and common complication of diabetes mellitus. Our objective was to identify novel genes differentially regulated in the early stages of DN using an unbiased approach. To this end, male (M) (n=10) and female (F) (n=6) C57bl/6 mice were fed high fat diet (HFD, 60% kcal fat) or matched control diet (CD, 10% kcal fat) for 12 weeks starting from weaning; this induces metabolic syndrome and DN in M but not F. Renal cortex RNA was isolated from all samples, and RNA Seq was performed on 4 M samples from each diet. We identified 1134 differentially expressed genes; of the 22 genes with the greatest fold increase or decrease, only 2 (Lipg, Slc7a12) have been previously been studied in a diabetic context. We then utilized a Taqman real-time (RT) PCR array to confirm our initial findings and examine potential sex differences. The array included the 9 most upregulated and 11 most downregulated genes from the RNA Seq data, with an additional 11 genes altered by RNA Seq and of interest to our group (primarily sensory receptors and G proteins). These arrays (31 genes + 18S control) were used to screen F samples (n=3 CD, n=3 HFD), and a second cohort of M samples (n=3 CD, n=3 HFD). All 9 of the genes significantly upregulated with HFD by RNA Seq were also upregulated in the M samples by RT-PCR (Atp12a, Ccl28, Ctxn3, Cyp2b10, Lhx2, Popdc3, Ptpn5, Sorcs1, Synpr; p<0.05). However, of the remaining 22 genes (downregulated + other genes of interest) only 3 were confirmed in M by RT-PCR (Gpr12, Gpr146, Tpra1; p<0.05). Furthermore, none of the genes were altered in F by HFD diet (vs. CD). When comparing M vs. F, we found that 10 of the 31 genes were differentially expressed between the sexes both on CD and on HFD (Bhmt, Ccl28, Ces1g, Ctxn3, Cyp2b10, Lhx2, Popdc3, Slc22a29, Sorcs1, Synpr; p<0.05); 6 additional genes (Atp12a, Cyp2a5, Gpr12, Gpr146, Ptpn5, Tpra1; p<0.05) were altered between M and F on HFD but not CD. These data demonstrate that our RNA Seq data regarding upregulation were more reproducible than those regarding downregulation, and that changes are sex-specific. The fact that the 9 upregulated genes in M do not change in F (which do not develop metabolic syndrome) implies that renal changes are downstream of metabolic changes, and not non-specific alternations due to an alteration in diet. Thus, we have identified novel renal genes associated with DN, and have demonstrated sex differences in renal gene expression both in control conditions and in DN.

6.20
LEPTIN-MEDIATED ALDOSTERONE SECRETION CAUSES HYPERTENSION IN OBESE FEMALES

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Obesity causes hypertension in males and females. While leptin contributes to obesity-induced hypertension by increasing sympathetic activity, in males, it is unknown whether similar mechanisms trigger hypertension in obese females. Females secrete 3 to 4 times more leptin than males, but do not exhibit high sympathetic tone with obesity. They however show inappropriately high aldosterone levels that positively correlate with adiposity and blood pressure (BP). Here we hypothesized that leptin induces hypertension by increasing aldosterone production in obese females. Hypersensitivity to leptin, in lean mice deficient in protein tyrosine phosphatase 1B (PTP1B) or high leptin levels, in obese Agouti (Ay/a) mice induced hypertension (WT: 115±2; KO: 124±2; a/a: 113±1; Ay/a: 128±7mmHg, $p<0.05$) but did not increase sympathetic control of BP (response to ganglionic blockade). Leptin sensitization and obesity however elevated plasma aldosterone levels and adrenal aldosterone synthase (CYP11B2) expression, in females. Chronic leptin (KO+AA: 115±5; Ay/a+AA: 114±5mmHg) or mineralocorticoid (KO+spiro:111±5; Ay/a+spiro: 121±6mmHg) receptors inhibition restored BP to baseline levels in females PTP1B KO and obese agouti mice. Leptin or leptin receptor deficiency in female ob/ob and db/db mice, abolished obesity-induced increases in adrenal CYP11B2 and plasma aldosterone while chronic leptin infusion in female mice triggered a dose-dependent increase in adrenal CYP11B2 and plasma aldosterone levels. Leptin-mediated aldosterone secretion was independent of changes in plasma angiotensin II, potassium and corticosterone (index of ACTH levels) and preserved in the presence of losartan or alpha and beta adrenergic receptors antagonists. Stimulation of human adrenocortical cells with leptin dose-dependently increased CYP11B2 expression and aldosterone production. While investigating the interaction between percentage of body fat, leptin and aldosterone levels in young healthy adult Caucasians we reported a positive correlation between adiposity and aldosterone, and between leptin and aldosterone in adult women only. Together these data suggest that leptin directly regulates aldosterone secretion and that leptin induces hypertension via aldosterone dependent mechanisms in obese females. This work was supported by a Scientist Development Grant from the American Heart Association (11SDG5060006 to EJ.BdC) and Start-up funds from Georgia Regents University.

6.21
EFFECTS OF DIET-INDUCED OBESITY ON REPRODUCTIVE HORMONE SIGNALING AND GENE EXPRESSION

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Although diet-induced obesity has been shown to disrupt female reproductive hormone signaling and reduce fertility, the mechanics underlying the effects of obesity on central nervous system and peripheral reproductive signaling remain unclear. To investigate this, we have established a female rat model in which half one group of animals is fed a either a high-fat, high-sugar diet or a control chow diet starting at weening for a 12 week period. Moreover, to allow us to control estrogen levels, all rats are ovariectomized with basal levels restored in half the animals by implantation of a slow release estradiol pellet. We observed a significant effect of both diet and estrogen on animal weight gain, with animals fed a high fat diet without supplemental estrogen demonstrating a >2-fold increase in weight gain compared to control animals that received estradiol pellets. Using RNA purified from hypothalamus, pituitary, abdominal fat, and liver tissues, we are quantifying global changes in gene expression between our experimental groups with the goal of identifying signaling systems and cellular pathways that are adversely affected by diet induced obesity. In addition to addressing the specific aims of our study, this large gene expression dataset generated by RNA-Seq (2.25 billion 125bp paired-end reads) represents a useful resource for the community because it substantially increases the resolution of sequenced transcripts from female rats. Moreover, this dataset is of sufficient depth to enable us to quantify alternative splicing isoforms between experimental groups. In addition, the allelic diversity of our Sprague-Dawley line allows us to identify allele-specific effects on expression levels. The localization of candidate signaling systems is also being investigated using immunofluorescence and FISH staining. We will discuss implications the for candidate factors showing significant changes gene expression with diet and estrogen replacement in our initial analysis.

7.0 RENAL

7.1
MOLECULAR MECHANISMS FOR LOWER PLASMA POTASSIUM SET POINT IN FEMALES

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We recently discovered that female rats display lower proximal tubule (PT) Na reabsorption compared to males, i.e. Na/H exchanger isoform 3 retracted to the base of the microvilli, less Na-Pi cotransporter isoform 2, increased renal clearance of lithium and more rapid excretion of a saline challenge. In the distal tubule we detected higher levels of Na-Cl-cotransporter (NCC), its activation by phosphorylation (NCC-P) and evidence for epithelial Na channel (ENaC) activation in females vs. males.These findings suggest that lower PT Na reabsorption drives a volume load dependent activation of NCC and ENaC. ENaC activation drives potassium (K) secretion in principal cells. Dietary K rapidly reduces NCC-P, shifting Na downstream for reabsorption by ENaC which drives K secretion. Based on these findings, this study aimed to test the hypothesis that females have a lower plasma K set point than males. Female and male Sprague Dawley rats (n=6) were fasted overnight (16 hr) with free access to water, and then fed a 3 hr meal containing either 0%K or 2%K. Overnight urine volume (metabolic cages), Na and K (flame photometry) were similar between sexes. Food consumed during the 3 hr meal was similar in all four groups. After the 0%K meal, supporting our hypothesis, plasma K, Na and osmolality were all significantly lower in females vs. males: [K]: 3.9±0.2 vs. 4.5±0.1 mM, [Na]: 133±1 vs. 135±1 mM, Osm: 296±3 vs. 306±2 mOsm. Differences appeared independent of the estrous cycle (vaginal smear). After the 2%K meal, plasma K increased in both sexes: to 4.6±0.1 mM in females and to 5.8±0.4 mM in males, associated with 7 fold increases in urinary K (mmol/3 hr): from 0.12±0.03 to 0.9±0.1 in females, and from 0.10±0.03 to 0.7±0.2 in males. Plasma Na (mM) was unchanged in both sexes after meals, but urinary Na (mmol/3hr) increased in females from 0.3±0.1 to 0.5±0.1, evidence for lower NCC activation. In response to the K rich meal, NCC total protein decreased 20% in females, not males, and NCC-P decreased 50% in both females and males (all $p<0.05$). In summary, lower baseline plasma K set point is unmasked in females after an overnight fast. Despite lower plasma K, the kaliuretic response to a K rich meal are indistinguishable between sexes. Females actively adapt to maintain their plasma K set point at a lower level than males, suggesting that they could be protected from hyperkalemia. NIH DK 083785.

7.2
LONG-TERM ESTROGEN TREATMENT INCREASES RENAL TUBULAR CASTS AND TGFB IN AGED OVARIECTOMIZED LONG EVANS RATS

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Our lab previously reported that long-term (80 days) estradiol (E2) treatment initiated immediately after midlife ovariectomy (OVX) in Long Evans rats increases proteinuria and renal hypertrophy compared to short-term (40 days) E2 treatment. Therefore, the beneficial effects of E2 on renal health may be dependent on treatment duration. The goal of the current study was to determine why long-term but not short-term E2 was detrimental to the kidney. We hypothesized that long-term E2 had a negative impact on glomerular filtration, glomerulosclerosis, renal fibrosis, and TGFß expression. Urine, serum, and formalin-fixed renal sections were obtained from ovariectomized Long Evans retired breeders that received an implant of E2 or vehicle (veh) for 40 days followed by a new treatment for an additional 40 days (groups: veh>veh, E2>E2, E2>veh). Estimated glomerular filtration rate (eGFR) was measured by creatinine clearance and renal pathology was assessed through histological staining. Neither short-term nor long-term E2 impacted eGFR as compared to vehicle controls (veh>veh: 0.36 ± 0.04 ml/min/g kidney weight; E2>veh: 0.39 ± 0.05; E2>E2: 0.36 ± 0.04; $p=0.87$). There was no difference in the glomerulosclerosis index (GSI) as assessed by Periodic acid–Schiff staining (veh>veh: 1.76 ± 0.12; E2>veh: 1.79 ± 0.13; E2>E2: 1.83 ± 0.15; $p=0.94$). Renal interstitial collagen formation assessed by Gomori's trichrome staining also revealed no changes (veh>veh: 9.20 ± 0.34%; E2>veh: 8.60 ± 0.47%; E2>E2: 9.50 ± 0.85%; $p=0.50$). Interestingly, the percentage of tubular casts was significantly decreased by short-term E2 and increased in the long-term E2 group (veh>veh: 3.73 ± 0.93%; E2>veh: 1.29 ± 0.23%; E2>E2: 3.76 ± 0.60%; $p=0.02$). Additional immunofluorescence studies revealed upregulation of renal cortical transforming growth factor ß (TGFβ) by long-term E2 treatment (veh>veh 10.20 ± 0.58%, E2>veh 10.49 ± 1.09%, E2>E2 14.26 ± 0.32%). These results indicate that long-term E2 treatment may promote an increase in TGFß and associated renal injury in the aging ovariectomized Long Evans rat. Our findings highlight the importance of understanding how E2 treatment duration influences postmenopausal renal health. Research supported by NIH grant 4R00HL103974 awarded to S.H.L.

7.3
ALTERATIONS IN 20-HETE PRODUCTION CONTRIBUTE TO END ORGAN DAMAGE IN DAHL S RATS

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It is well documented that a sexual dimorphism exists in the regulation of blood pressure in both the human population as well as experimental animal models, such that males have higher blood pressures than females of the same age. While there is a clear disparity in the development of hypertension and progression of renal injury in many rodent models, evidence of a sex difference is lacking in the Dahl S rat. While the current reports present conflicting data, we hypothesize that alterations in CYP450 expression and 20-HETE production contributes to the relative resistance of female Dahl S rats to target organ damage compared to males. Consistent to what we have previously reported, the time course for the development of proteinuria and renal injury were significantly reduced in female Dahl SSJr rats challenged with a high salt diet relative to male rats. In addition, renal cortical 20-HETE production was elevated in female (120.8±4.2 pmol/min/mg) versus male rats (45.1±11.78 pmol/min/mg), while no difference was noted in the outer medulla (19.6±1.8 vs 17.15± 3.9 pmol/min/mg). Introgression of the CYP4A1 gene into the Dahl S genetic background, resulted in significant elevations in 20-HETE production both on low salt and high salt diets. Furthermore the rise in mean arterial pressure was attenuated in CYP4A1 overexpressing rats in both sexes (Δ19mmHg in CYP females vs 61mmg in Dahl S females; 20mmHg in CYP males vs 50mmHg in Dahl S males). Moreover, the degree of glomerular injury was reduced in CYP rats, both male and female, compared to Dahl S rats in response to a high salt diet. Therefore, increases in the CYP gene expression and 20-HETE production prevent the rise in mean arterial pressure and kidney injury in male Dahl S rats. AHA 14SDG20160020.

7.4
APOPTOTIC CELL DEATH IN RENAL ISCHEMIA-REPERFUSION INJURY IN MALE AND FEMALE SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

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Males develop a greater extent of ischemia-reperfusion (IR) induced injury than females. Recent studies have shown that renal IR injury is primarily mediated by necrosis in male mice, and pilot studies in our lab indicate a sex difference in renal cell death in SHR with females having more apoptotic cell death than males under control conditions. Based on the potential protective role of apoptosis vs. necrosis, the goal of this study was to test the hypothesis that female SHR exhibit greater apoptotic cell death following renal IR compared to male. 13 week old male and female SHR were studied: control and 45 minute warm bilateral renal ischemia followed by reperfusion (N=5-6). 24 hours later, kidneys and plasma were collected to quantify apoptotic cell death via TUNEL assay and assess renal injury by measuring plasma creatinine (Cr). Control female SHR have more apoptotic cells compared to male (M: 1.6±0.6; F: 5.0±1.0 cells per area; p=0.04). Following IR, apoptotic cells significantly increased in each sex, however the sex difference was abolished (M: 12.0±3.9; F: 18.3±3.9 cells per area; effect of treatment: p=0.01; effect of sex: p=0.1). IR induced injury was confirmed with an increase of Cr in both sexes (M: 0.22±0.06 vs. 4.1±0.6; p=0.01; F: 0.25±0.06 vs. 2.3±0.6; p=0.04). These data do not support the hypothesis that more apoptotic cell death that occurs in females following IR contributes to less IR injury compared to males. More studies will need to be performed to measure what the ratio of apoptotic cell death to total cell death is in each sex. Better understanding of the type of cell death in IR may offer novel insight into treatments for acute kidney injury in both sexes.

7.5
WITHDRAWN

7.6
KIDNEY EPITHELIUM-SPECIFIC KNOCKOUT OF SHP-1 ENHANCES URINARY CONCENTRATION IN FEMALE MICE

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Urinary concentration by the kidney medulla is a primary mechanism to maintain body fluid balance. The transcription factor NFAT5 is essential for urinary concentration, because it activates expression of osmoprotective genes like betaine/glycine transporter 1 and aldose reductase, which are necessary for the kidney medulla to survive and function under hypertonicity, and because it contributes to expression of aquaporin-2, possible aquaporin-1, and urea transporter. Despite the importance of NFAT5 in urinary concentration, how NFAT5 is regulated in the kidney medulla remains largely unknown. Through screening a genome-wide siRNA library against phosphatases in HEK293 cells, we previously identified the protein tyrosine phosphatase SHP-1 as a negative regulator of NFAT5. The viable mice with spontaneous mutation of SHP-1 (mev/mev) express ~30% of SHP-1 protein in the kidney inner medulla as compared with wild type. As the first step to test whether SHP-1 also regulates NFAT5 activity in the kidney inner medulla, we measured urinary osmolarity of the mutant and wild type mice under ad lib water and food intake and found that urinary osmolarity of mutants is 29% higher than that of wild type ($p<0.05$, n=4, males and females are roughly equal). We then generated the mice with the kidney epithelium-specific deletion of SHP-1. Under ad lib water and food intake, the urinary osmolarity of the knockouts is 69% higher than that of the control mice, which express Cre recombinase alone ($p<0.05$, 3 males and 3 females in each group). To determine whether the knockouts have increased urinary concentration and whether gender influences the concentration ability, we controlled water and food intake with gel diets under both water replete and water-restricted (reduced by 80%) conditions. There is no significant difference in urinary osmolarity under the water replete condition among each group. However, water restriction elevates urinary osmolarity more in the female knockouts than in the female control mice (147% vs 74%, $p<0.05$, n=6), whereas it has no significant difference in the male counterparts. We conclude that the kidney epithelium-specific knockout of SHP-1 increases urinary concentration only in female mice.

7.7
PROGESTERONE SYNERGIZES ESTRADIOL-INDUCED NATRIURESIS IN RESPONSE TO INCREASED DIETARY SODIUM INTAKE.

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Hypertension and renal diseases are more prevalent in postmenopausal women compared to premenopause, suggesting a central role for ovarian hormones in cardiovascular and renal protection. The renal endothelin (ET) system, which plays a critical role in Na regulation and blood pressure control, appears to function differently between the sexes. Recent preliminary data from our laboratory showed that 17β-estradiol (E_2) increases ET-1 gene expression in inner-medullary collecting duct cells. However, the role of ovarian hormones in regulating renal ET control of Na balance is not known. Therefore, we determined the effects of supplementation with E_2 and/or progesterone (P) on renal Na handling and urinary ET levels in OVX Sprague-Dawley rats on normal NaCl (NS) and high NaCl (HS) diets. Female rats were ovariectomized (OVX) and implanted with 21-day controlled release pellets containing 0.35 mg E_2 (OVX+E_2), 25 mg P (OVX+P), both (OVX+E_2+P) or placebo (OVX). On NS (0.4% NaCl) diet, OVX+E_2 and OVX+E_2+P showed significant increases in urinary Na excretion ($U_{Na}V$) compared to OVX rats (3.5±0.3 and 4.0±0.2 vs 2.1+0.2 µmol/min/kg, respectively, $p<0.05$), whereas P supplementation of OVX rats did not affect $U_{Na}V$. Interestingly, urinary ET-1 excretion was significantly enhanced by 3 fold in OVX+E_2+P rats compared to OVX rats, but was not affected by E_2 or P alone in OVX rats fed NS. After 24-hrs of HS diet, $U_{Na}V$ was enhanced by 3.5 fold in OVX and OVX+P, 6 fold in OVX+E_2 and 9 fold in OVX+E_2+P rats. Urine flow followed the same pattern as $U_{Na}V$. No significant changes were detected in urinary ET-1 with HS except in OVX+E_2 rats were it was doubled. These data indicate that P synergizes the effect of E_2 to facilitate the natriuretic and diuretic response to HS. The renal ET-1 system and possibly other natriuretic pathways appear to be playing a role in the synergistic effects of P on renal Na handling.These studies were funded by NIH grants P01 HL69999 and P01 HL95499.

7.8
WITHDRAWN

7.9
HIGH SALT ALTERS CELLULAR TRANSCRIPTIONAL MILIEU AND HUMAN ANGIOTENSINOGEN EXPRESSION IN A GENDER-DEPENDENT MANNER: AN EFFECT EXACERBATED BY A RISK HAPLOTYPE

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Angiotensinogen is the substrate for the entire RAS cascade and polymorphisms leading to its overexpression are linked to hypertension. Studies have shown that SNPs in the promoter of the hAGT gene are associated with hypertension. Importantly, these SNPs can further modulate the gene of interest in various physiological/environmental settings like gender or high-sodium diet. In this regard, the human angiotensinogen gene (hAGT) gene has polymorphisms in its 2.5Kb promoter that form two haplotype (Hap) blocks: -6A/G (-1670A/G, -1562C/T, -1561T/C) and -217A/G (-532T/C, -793A/G, -1074T/C, and -1178G/A). Hap -6A/-217A is associated with human hypertension whereas Hap -6G/-217G reduces cardiovascular risk. We have engineered transgenic (TG) mice with these haplotypes (Hap -6A: -6A/-

217A and Hap -6G: -6G/-217G) so as to examine the transcriptional regulation of the hAGT in an in vivo setting. This study is designed to study the effects of a high-sodium diet on the transcriptional milieu of hepatic and renal tissues with consequential effects on the hAGT expression in our two haplotypes. Male and female TG mice were placed on 4% Na^+ for a period of 8 weeks. High-salt diet upregulates the hAGT expression in both liver and kidney tissues ($p<0.05$); however, this effect is observed only in male mice with no effect in adult females. Interestingly, the hAGT activation observed was significantly ($p<0.05$) greater in the -6A haplotype males as compared to -6G males. High-salt increased the expression of transcriptional regulators including, CEBPβ, HNF4 and GR. This effect was also limited to the males of our two TG lines suggestive of a gender-dependent effect of Na^+ on the cellular transcriptional apparatus. Complementary ChIP assay confirmed enhanced transcription factor (TF) binding to the chromatin of male -6A TG mice as compared to their -6G counterparts after high-salt diet treatment. Thus, for the first time we show an effect of high-salt on cellular transcriptional apparatus that is gender-dependent, with consequential activation of the hAGT in male TG mice only. Crucially, increased TF affinity of the chromatin in -6A TG mice leads to higher salt-induced AGT levels in this haplotype. These observations could partly account for increased salt-sensitivity of adult males that, in turn, is governed by the 'risk" haplotype. Identifying these -6A haplotype individuals will help guide therapeutic lifestyle changes in patients with essential hypertension.

7.10
DIFFERENT RESPONSE TO DOPAMINE OR TO BRADYKININ INHIBITION IN OVARIECTOMIZED ADULT WISTAR RATS UNDER HIGH SODIUM INTAKE

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In previous work, we have shown that ovariectomized (oVx) adult Wistar rats develop high blood pressure upon high sodium intake (HS). Among other facts, oVx rats have a lower sodium excretion and renal overexpression of total and dephosphorylated Na+, K+-ATPase (NKA) as compared with intact female (IF) (1, 2). oVx rats also have a decreased expression of dopamine D1 receptor (D1R) (2) and a higher urinary kallikrein excretion than IF rats (3). With the aim to compare the relative contribution of dopamine and bradykinin-kinin systems to the deranged regulation of sodium balance and blood pressure control in ovariectomy, we studied IF and oVx rats on HS after both dopamine or bradykinin blockade. Ovariectomy was performed in Wistar rats at 60 days of life and rats were studied 90 days post oVx. The rats received 1% NaCl in drinking water on the final 5 days. D1R (SCH 23390, 1mg/kg bwt/day, sc) or bradykinin B2 receptor (HOE 140, 7μg/100 g bwt/day, sc) were blocked the last two days. In IF rats, D1R blockade caused a decrease in urinary sodium (UNa^+V = 3.14±0.03 vs 1.65±0.09 mmol/100g bwt /day; $p<0.01$), in volume excretion (V = 11.52±0.6 vs 6.13±0.19 ml/100g bwt /day; $p<0.01$), higher mean blood pressure (112±2 vs 140±2 mmHg; $p<0.05$) and more dephosphorylated NKA ($p<0.05$) compared to untreated rats. In opposition, D1R blockade did not cause any change in oVx rats. In IF rats, the bradykinin receptor (B2R) blockade had no effect on hydroelectrolyte excretion or NKA phosphorylation. In oVx rats, B2R blockade decreased urinary sodium (UNa^+V = 2.13±0.24 vs 1.48±0.33 mmol/100g bwt /day; $p<0.05$) and volume excretion (V = 8.50±1.66 vs 6.22±1.67 ml/100g bwt /day; $p<0.05$), while NKA phosphorylation state remained unaltered. No changes in glomerular filtration rate following D1R or B2R blockade were observed. Present results show that, as already described (2), dopamine system is unresponsive in oVx HS rats, whereas bradykinin through B2R, contributes to maintain sodium excretion in rats with absence of ovary hormones and HS intake. Funding source: UBACYT 20020120100379BA, Buenos Aires University, Argentina to FRI. References: 1) Clin. Exp. Hypertens. 2013;35(7):475-83; 2) Am J Physiol Renal Physiol. 2015 15;308(12):F1358-68; 3). Kidney Blood Press Res. 2009;32(5):342-8.

7.11
PARTICIPATION OF CYP4A Ω-HYDROXYLASE/ 20-HETE IN BLOOD PRESSURE REGULATION OF HYPERANDROGENEMIC FEMALE RATS

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Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects women during their reproductive age, and is associated with hyperandrogenemia, increased blood pressure (BP) and increased cardiovascular risk. Several studies have shown that elevated androgens increase cytochrome P450 (CYP) 4A expression and 20-hydroxyeicosatetraenoic acid (20-HETE) synthesis in rats. In particular, evidence from our laboratory, indicates that CYP4A2 expression is elevated in the renal vasculature of hyperandrogenemic female Sprague Dawley rats. Dahl Salt Sensitive (DS) rats have a deficiency in CYP4A ω-hydroxylase/20-HETE system in the kidneys compared with either Dahl Salt Resistant (DR) or $SS.5^{BN}$ consomic strain rats. Thus if an increase in 20-HETE, mediated via CYP4A, is necessary for the increase in BP in HAF rats, then DS rats that lack CYP4A may be resistant to hyperandrogenemic increases in BP. In the present study we tested the hypothesis that BP in DS rats maintained on low salt diet would be unresponsive to hyperandrogenemia. Four weeks old female DR, DS (from Harlan SD) and $SS.5^{BN}$ rats (from MCW colony) were implanted with dihydrotestosterone (DHT; 7.5mg/90d) or placebo pellets (n=6-8/grp). At 14 weeks of age, radiotelemetry transmitters were implanted, and after two weeks recovery, mean arterial pressure (MAP) was measured for 5 days. DHT significantly increased MAP in female SR rats (placebo: 84±4 vs. DHT: 95±1 mmHg, $p<0.05$) and female $SS.5^{BN}$ rats (placebo: 104±1 vs. DHT: 130±6 mmHg, $p<0.0005$). In contrast, DHT did not change MAP in female DS rats (placebo: 160±4 vs. DHT: 153±4 mmHg, p=NS). Interestingly, MAP in female SR was lower than in $SS.5^{BN}$ females, and with DHT there was a more robust increase in MAP in female $SS.5^{BN}$ than in female SR rats. In addition, placebo female DS rats, despite the low salt diet, had significantly higher MAP than the other groups ($p<0.001$). These data suggest that an active CYP4A ω-hydroxylase/20-HETE system is necessary for hyperandrogenemia to increase BP in our HAF model. The data also suggest alternative treatments, namely 20-HETE synthesis inhibition, to attenuate elevated BP in women with PCOS. Supported by NIH-R01HL66072, PO1HL51971 (JFR), 14POST18640015 (ROM).

7.12
MULTIPLE ESTROGEN RECEPTOR SUBTYPES SELECTIVELY INFLUENCE FLUID INTAKE IN FEMALE RATS

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Estradiol (E2) decreases fluid intake in female rats. Although this has been known for decades, the underlying mechanisms are still unknown. Our understanding of these mechanisms is complicated by the existence of five identified estrogen receptor (ER) subtypes including the classically recognized ERα and ERβ proteins and more recently discovered membrane-associated receptors: GPER-1, ERX and Gq-mER. In addition to the complexity offered by the existence of multiple subtypes, these receptors can act through multiple mechanisms (surface receptors or transcription factors) and can engage a variety of intracellular signaling pathways. In this series of experiments, we first tested the hypothesis that activation of membrane-associated ERs decreases fluid intake in ovariectomized rats. In support of this hypothesis, we found that angiotensin II (AngII)-stimulated fluid intake was decreased ($p<0.05$) after treatment with an estradiol-BSA conjugate that can only activate receptors on the cell surface. Follow up studies tested for a role of ERα, ERβ, and GPER-1 in mediating the antidipsogenic and anti-natriorexigenic effects of E2. Again, using AngII as a stimulus to consume fluid, we found unexpected receptor-selective effects on AngII-stimulated water and saline intake. Specifically, we found that AngII-stimulated water intake was decreased after selective activation of ERα and that AngII-stimulated saline intake was decreased after selective activation of ERβ or GPER-1 ($p<0.05$). Furthermore, analysis of drinking microstructure revealed differences in the underlying behavioral difference in the respective effects of ERα and ERβ on water and saline intakes. This analysis found that the ERα-mediated decrease in water intake was a function of a selective decrease in burst number ($p < 0.05$), suggesting a change in post-ingestive feedback. In contrast, the ERβ-mediated decrease in saline was a function of a change in burst size ($p < 0.05$), suggesting a change in the orosensory value of the fluid. Although activation of ERβ and GPER-1 similarly affected saline intake, without a concomitant effect on water intake, the decrease in saline intake after GPER-1 treatment was mediated by a change in burst number ($p < 0.05$), unlike the change in burst size that was underlying the ERβ-mediated change in intake. Together these findings demonstrate that specific ERs selectively influence water and saline intake through specific mechanisms in the female rat.

7.13
6B-HYDROXYTESTOSTERONE, A CYTOCHROME P450 1B1-DERIVED METABOLITE OF TESTOSTERONE PLAYS AN IMPORTANT ROLE IN RENAL DYSFUNCTION ASSOCIATED WITH ANGIOTENSIN II-INDUCED HYPERTENSION IN MALE MICE

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Recently, we showed that 6β-hydroxytestosterone (6β-OHT), a cytochrome P450 1B1 (CYP1B1)-derived metabolite of testosterone, contributes to the development of angiotensin II (Ang II)-induced hypertension and associated cardiovascular pathophysiology. In view of the critical role of Ang II in renal homeostasis and end organ damage, we determined the contribution of 6β-OHT to Ang II actions on water consumption and renal function in male *Cyp1b1*$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice. Eight weeks old male *Cyp1b1*$^{+/+}$ and *Cyp1b1*$^{-/-}$ intact or castrated mice were injected with 6β-OHT (15 μg/g, i.p. every 3rd day) or vehicle (DMSO, 50 μl), and infused with Ang II (700 ng/kg/min) or vehicle for 2 weeks. Urine was collected for 24 hours on the final day of experiment. Castration or *Cyp1b1*$^{-/-}$ gene disruption attenuated Ang II-induced increase in water consumption and urine output, proteinuria and decrease in osmolality in *Cyp1b1*$^{+/+}$ mice (Table 1). 6β-OHT did not alter Ang II-induced increase in water intake, urine output, proteinuria and decrease in osmolality in *Cyp1b1*$^{+/+}$ mice, but restored these effects of Ang II in *Cyp1b1*$^{-/-}$ or castrated mice (Table 1). *Cyp1b1* gene disruption or castration prevented Ang II-induced renal fibrosis, inflammation, oxidative stress and AT1 receptor mRNA expression. 6β-OHT-did not alter Ang II-induced renal fibrosis, inflammation or oxidative stress in *Cyp1b1*$^{+/+}$ mice, however in *Cyp1b1*$^{-/-}$ or castrated mice it restored these effects of Ang II. These data suggest that 6β-OHT, contributes to increased thirst, impairment of renal function and end organ damage associated with Ang II-induced hypertension in male mice, and that CYP1B1 could serve as a novel target for the treatment of renal disease and hypertension.

Table 1. AngII-induced increase in water intake and renal dysfunction is minimized by *Cyp1b1* gene disruption or castration and is restored by 6β-OHT

	Water Intake (ml/24 hours)		Urine Output (ml/24 hours)		Proteinuria (mg/24 hours)		Osmolality (mOsml/kg)	
	Veh	Ang II	Veh	Ang II	Veh	Ang II	Veh	Ang II
***Cyp1b1*$^{+/+}$**	4.0±0.2	8.6±0.7*	1.8±0.2	5.1±0.6*	4.5±0.3	13.2±2.2*	2645±175	1254±78*
***Cyp1b1*$^{-/-}$**	4.6±0.3	5.4±0.3*†	1.4±0.2	2.0±0.3†	3.6+0.6	4.5±0.7†	2409±59	2010±151*†
***Cyp1b1*$^{+/+}$ Cas**	4.4±0.8	5.1±1.2	0.9±0.3	3.5±0.7*	1.9±0.6	4.1±0.9*	2942±68	2292±219*
***Cyp1b1*$^{+/+}$ Cas+6β-OHT**	4.0±0.5	7.8±0.5*	1.3±0.2	4.4±0.3*	1.7±0.05	7.0±0.4*†	3122±149	1803±123*
***Cyp1b1*$^{+/+}$ +6β-OHT**	4.2±0.2	9.0±0.3*	1.4±0.3	4.5±0.3*	4.9±0.86	10.6±2.0*	2913±129	1161±81*
***Cyp1b1*$^{-/-}$ +6β-OHT**	4.6±0.3	10.0±0.1*	1.4±0.2	5.1±0.2*	5.2±0.62	10.1±0.6*	2613±79	1127±112*

Veh = Vehicle; Cas = Castration,
*$P < 0.05$, Vehicle vs. corresponding Ang II group
†$P < 0.05$, *Cyp1b1*$^{+/+}$ Ang II,vs. *Cyp1b1*$^{-/-}$ Ang II, and *Cyp1b1*$^{+/+}$ Cas+6β-OHT+ Ang II vs. *Cyp1b1*$^{+/+}$ Cas+Ang II

8.0 NEURO CONTROL OF CARDIOVASCULAR, RENAL AND METABOLIC DISEASES: IMPACT OF GENDER AND SEX

8.1
AUTONOMIC REGULATION OF BLOOD PRESSURE IN ADULT HUMANS: EFFECTS OF SEX & AGE

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Over the past 10 plus years my colleagues and I have made physiological measurements of the determinants of mean arterial blood pressure (MAP) in normotensive younger and older men and women. These measurements include muscle sympathetic nerve activity (MSNA), cardiac output (CO), and total peripheral resistance (TPR). In younger subjects of both sexes there is no relationship between MSNA and blood pressure. However, the relationships between MSNA, CO and TPR show divergent patterns. In young women there is no relationship between MSNA and TPR (or CO) largely because β-adrenergic vasodilator mechanisms offset α-adrenergic vasoconstriction. In young men there is a direct relationship between MSNA and TPR and no relationship with blood pressure because CO is lower in those with higher MSNA. In older men these relationships are less clear cut due to age related alterations in peripheral vasodilator function. In older women there is a loss of tonic β-adrenergic vasodilation and the relationship between MSNA and TPR seen in men emerges. These observations raise questions about sex specific causes and mechanisms of hypertension in human aging. Supported by HL83947.

8.2
SEX DIFFERENCES IN DESENSITIZATION OF THE DIPSOGENIC EFFECT OF ANGIOTENSIN II

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Angiotensin II (ANGII) is a critical regulator of body fluid homeostasis. Drinking after injection of ANGII has been an important model of the behavioral regulation of fluid homeostasis, and studying ANGII-induced drinking has led to findings that extend to the regulation of blood pressure. Although acute ANGII potently stimulates drinking, repeated injections of ANGII have bivalent effects; daily injections of ANGII sensitize responses, but more acute repeated injections cause a transient desensitization. This desensitization reduces water intake stimulated by ANGII, without reducing the natriorexigenic effect of the peptide. Moreover, we found sex differences in the desensitizing potency of AngII; females did not show the desensitization that is reliably observable in male rats. Preliminary studies suggest that this resistance to desensitization is not affected by ovarian hormones, and ongoing studies are testing the importance of testicular hormones. Additional studies found that the bivalent effects of ANGII can counter each other. Specifically, we found that the sensitization of intake normally occurring after daily ANGII administration is not induced if the daily injections are given with the timing of a desensitizing treatment, suggesting that desensitization can ameliorate sensitization. Given the highly conserved sex differences in blood pressure, and the role that ANGII-sensitization may play in the development of hypertension, it is tempting to speculate that properly timed increases in ANGII may help thwart sensitization and, therefore, could be used to prevent or treat hypertension. This would be a radical departure from current anti-hypertensive drugs that act by reducing angiotensinergic tone. Funding provided by NIH HL091911.

8.3
ADIPOKINES, OBESITY, AND SEX: IMPLICATIONS FOR CARDIOVASCULAR FUNCTION

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In addition to the storage of lipids, adipose tissue contributes to energy homeostasis by producing multiple adipokines, such as leptin and nesfatin-1, which regulate food intake and energy expenditure. Plasma levels of these adipokines, which inhibit appetite, increase as a function of adipocyte mass, thus decreasing food intake during times of energy excess. In addition to modulating energy intake, these adipokines also impact cardiovascular function, particularly through activation of the sympathetic nervous system. Like leptin, nesfatin-1 interacts with the central melanocortin system to exert its hypertensive effects. Interestingly, melanocortin neurons are heavily influenced by sex hormones, particularly estrogen, which regulates the responsiveness and activity of these neurons. The functional implication of this observation is that females may respond to the hypertensive effects of adipokines, like nesfatin-1, differently than males. We previously reported that male rats exhibit significant, dose-related increases in blood pressure following injection of nesfatin-1 into the lateral cerebroventricle, and that this effect could be blocked by pretreatment with a melanocortin receptor antagonist. In contrast, the hypertensive effect of nesfatin-1 in females appears to be dependent upon sex hormone levels, as the response to nesfatin-1 varied according to stage of the estrous cycle. We propose that this sex-related difference in the hypertensive effect of nesfatin-1 is due to the modulatory activity of estrogen on nesfatin-1-responsive melanocortin neurons, and that loss of estrogen, as observed in menopause, will lead to enhanced nesfatin-1 signaling and hypertension.

11.0 DEVELOPMENTAL PROGRAMMING OF CARDIOVASCULAR, RENAL AND METABOLIC DISEASES: ROLES OF GENDER AND SEX

11.1
EFFECT OF ESTROGEN IN GENDER-DEPENDENT FETAL PROGRAMMING OF ADULT CARDIOVASCULAR DYSFUNCTION

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Epidemiologic studies have demonstrated that intrauterine adverse environment increase the risk of cardiovascular disease in adulthood. However, previous studies in humans and different animal model have shown that whether individual develops a cardiovascular dysfunctional phenotype may dependent on its genetic background, postnatal life style, age, and gender. Recently, in maternal hypoxia and nicotine exposed pregnant rat model we have demonstrated a sex difference in fetal programming of adult hypertensive and heart ischemia-sensitive phenotype. We have further examined the mechanisms linking the fetal stress and increased risk for cardiovascular dysfunction in adulthood with an emphasis on gender differences and the potential role of estrogen in mediating sexual dimorphism. In perinatal nicotine exposed rat

model, our data support an important role of estrogen in the sex difference of perinatal nicotine-induced programming of vascular dysfunction, and suggest that estrogen may counteract heightened reactive oxygen species production, leading to protection of females from development programming of hypertensive phenotype in adulthood. Contrast to nicotine exposed animal model, the data in maternal hypoxia rat model indicate that estrogen is not directly responsible for the sex dimorphism in fetal programming of heart ischemic vulnerability but suggest a novel mechanism of estrogen in protecting female hearts against ischemia and reperfusion injury. (Supported by NIH/HL11861, NIH/DA032510, and by the regents of the University of California Tobacco Related Disease Research Program grant #22XT-0022).

11.2
SEX DIFFERENCES IN CARDIOVASCULAR AND METABOLIC RISKS DUE TO EARLY LIFE STRESS

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Clinical studies indicate that adults exposed to adverse childhood experiences or early life stress (ELS) develop several risk factors for cardiovascular and metabolic disease including higher systolic blood pressure, increased BMI, and clustering of metabolic risk biomarkers. Maternal separation is an established model of ELS during the early postnatal life in rodents ("first hit"). This procedure induces heightened reactivity to stresssors later in life ("second hit"), altering the normal physiological responses. Despite similar blood pressure and heart rate under baseline conditions, ELS enhances the angiotensin II (AngII)-induced hypertension in male and female rats. However, we found that impaired renal function and imbalanced plasma sex hormones were present in male but not female rats exposed to ELS. Additionally, *ex-vivo* studies revealed that AngII-mediated responsiveness in vasculature is exaggerated in male rats only. Both male and female rats demonstrate reduced baroreflex sensitivity; however, only male rats display signs of increased sympathetic outflow to the kidney including lower glomerular filtration rate which is normalized following bilateral renal denervation. In order to investigate the ELS-induced metabolic disease risk, we challenged maternally separated rodents with a chronic high fat diet (HFD, 60% kcal from fat). We found that females display a much more exaggerated rise in plasma insulin and leptin levels, impaired glucose tolerance and increased visceral fat mass compared to males. Taken together, these data indicate that ELS induces a sex-specific risk to develop chronic diseases that is dependent on the type of stressor. References: Loria AS, Yamamoto T, Pollock DM, Pollock JS. Early Life Stress induces renal dysfunction in adult male but not female rats. Am J. Physiol Regul Integr Comp Physiol, 15;304(2):R121. Murphy MO, Evans L, Mahanes T, Loria AS. Impaired baroreflex response correlates with reduced conduit vessel contractility in female maternally separated rats and reveals α-adrenergic receptor dysfunction. FASEB J, 29, 968.11.

11.3
MATERNAL UNDERNUTRITION SIGNIFICANTLY IMPACTS OVARIAN FOLLICLE NUMBER AND INCREASES OVARIAN OXIDATIVE STRESS IN ADULT RAT OFFSPRING

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There is now considerable epidemiological and experimental evidence indicating that early life environmental signals, including nutrition, affect development. A relationship exists between the periconceptional, fetal and early infant phases of life and the subsequent development of chronic diseases including obesity and Type 2 diabetes. This relationship, the "developmental origins of health and disease" (DOHaD), suggests that the embryo/fetus/neonate makes adaptations in response to early life cues, resulting in adjustments in homeostatic systems that are maladaptive in postnatal life, leading to an increased risk of chronic disease and/or the inheritance of risk factors across generations. Reproductive maturation and function is similarly influenced by early life events. This should not be surprising, since the primordial germ cell pool is established during embryonic life and is thus vulnerable to early life events. In both males and females, early life nutritional adversity accelerates pubertal onset. In males, prenatal events have been shown to modify sperm counts and fertility, and in females modify ovarian function. In females, a multitude of "modifying" cues inducing developmental adaptations have been identified that result in a decline in ovarian follicular reserve, changes in ovulation rates and altered age at onset of puberty. We have shown that fetal growth restriction induced by maternal caloric restriction, results in a premature loss of adult ovarian follicles, underpinned by an increase in apoptosis and increased ovarian oxidative stress levels. Critically, low birth weight offspring show impaired ovarian follicle function already as neonates, and demonstrate a loss of follicles and reproductive cycle impairment early in young adulthood, well before full adult reproductive maturity. Many pathways have been suggested to underpin these associations, where studies have investigated the maternal-fetal-placental relationship as well as events occurring in the early postnatal environment in modulating pubertal onset and ovarian function. But the underlying ovarian mechanisms regulating the relationship between the early life developmental environment and postnatal reproductive dysfunction remain unclear.

12.0 NON-REPRODUCTIVE EFFECTS OF SEX HORMONES AND RECEPTORS-B

12.1
ANDROGEN EFFECTS ON ENDOTHELIAL FUNCTION IN WOMEN IN POLYCYSTIC OVARY SYNDROME

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Polycystic ovary syndrome (PCOS) is the most common reproductive endocrinopathy in young women, affecting 6-10 % of women of reproductive age. Our studies focus on humans, and address the most common PCOS phenotype, androgen excess (AE)-PCOS. AE-PCOS is associated with insulin resistance and elevated endothelin-1 (ET-1) levels, indicating poor endothelial function. Endothelin-1 binds two receptor subtypes, endothelin A (ET_AR) and endothelin B (ET_BR). To control and isolate androgen effects on microvascular circulation in humans, we administer a gonadotropin-releasing hormone antagonist for 7-11 days in obese, otherwise healthy young women and obese, young women with AE-PCOS, adding methyl testosterone on days 8–11. We use cutaneous microdialysis to perfuse ET_AR and ET_BR blocking agents and use laser Doppler flowmetry to measure cutaneous microcirculatory responsiveness. These combined techniques enable us to examine the interaction of these subtype receptors with androgens on the microcirculation in women with AE-PCOS using mildly invasive methods, that are well tolerated by humans. With this model of microcirculation, we have demonstrated that ET_AR mediates vasoconstriction and ET_BR mediates vasodilation in women with and without AE-PCOS, but vasodilation is blunted in women. Only ET_BR are expressed in the endothelium, so our data suggest peripheral microvascular endothelial dysfunction in AE-PCOS. We have also demonstrated that the androgenic milieu is a key element to this endothelial dysfunction, and that the androgen effects on the endothelium are mediated by the ET_BR in AE-PCOS. These findings illustrate an interaction between androgens and the endothelin system on cardiovascular function and identify a potential new target for treatment in women with AE-PCOS.

12.2
MECHANISMS INVOLVED IN CARDIOPROTECTION IN FEMALES MECHANISMS INVOLVED IN CARDIOPROTECTION IN FEMALES: ROLE OF ESTROGEN AND ESTROGEN RECEPTORS (ERS)

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Our goal was to gain insight into the role of estrogen and ERs in reducing ischemia reperfusion (I/R) injury and hypertrophy in females. To examine the role of plasma membrane bound ERs, we used a non-nuclear selective ER modulator (estrogen-dendrimer conjugate, EDC). We treated ovariectomized WT mice with EDC, estradiol or dendrimer control using osmotic minipumps. Using a Langendorff perfused heart model of I/R we found that EDC reduced I/R injury. We studied cardiac-specific ERα–knockout (csERα-KO) mice, and found that EDC treatment significantly decreased infarct size and improved functional recovery compared to the vehicle-treated csERα-KO mice, suggesting that the protection is not mediated by plasma membrane ERα. To induce hypertrophy, male and female mice were treated with angiotensin II or saline via osmotic minipumps. At 3 weeks, females showed significantly less cardiac hypertrophy and better cardiac function than males. We also studied female and male mice with csER-□-KO and their WT littermates. The reduction in hypertrophy observed in the WT females was not altered by ablation of ERα. We also evaluated differences in long non coding RNA and miRNA between males and females that might contribute to these sex differences. Our findings show that females exhibit significantly less angiotensin II-induced hypertrophy than males at 3 weeks of treatment and the reduction in hypertrophy in females is retained in hearts lacking ERα, suggesting that ERα is not required for either the reduction in hypertrophy or cardioprotection. Funded by NIH intramural program.

12.3
SEX AND SEX HORMONE EFFECTS IN CARDIOVASCULAR PATHOPHYSIOLOGY

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Increased circulating volume, pressure overload and mineralocorticoid excess contribute differently to cardiovascular pathophysiology in women and men, in male and female rodents. In order to understand sex specific mechanisms, underlying protection or maladaptation in females and males, we analyse different stressors like exercise, pressure overload and myocardial ischemia and their sex specific effects on the heart. We are using animal models and cell culture models of hemodynamic and neurohormonal stress as well as animal models with modified sex hormone receptor expression- ER alpha and ER beta cell specific knock-outs and overexpression. We studied the interaction of the stressors with sex and sex hormone effects. Exercise leads to physiological myocardial hypertrophy. Females develop more physiological myocardial hypertrophy than males with better metabolic adaptation. Pressure overload and/or mineralocorticoid excess lead to pathological myocardial hypertrophy. Fibrosis, a hallmark of pathological myocardial hypertrophy, is more prominent in males than in females. Estrogen is protective in females but may be harmful in males in some conditions. Estrogen receptor alpha and beta activation have different effects on fibrosis and metabolism in females and males. Female animals under stress maintain energy metabolism better than males and have more favourable Calcium signalling. Women with aortic stenosis develop less excentric myocardial hypertrophy with less fibrosis than men and this is associated with better myocardial survival. Adaptation to cardiovascular stress and end organ damage are sex specific and sex specific approaches to treatment may lead to further benefit.

13.0 RESPIRATORY

13.1
SEX DIFFERENCES IN DIET AND INHALED OZONE (O_3) INDUCED METABOLIC IMPAIRMENT

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Diet and environmental stressors, including inhaled pollutants, have been implicated in the development and progression of metabolic diseases. Since metabolic processes of males and females are likely influenced by sex hormones, we hypothesized that high fat versus high fructose diet will produce differential metabolic impact in each sex, and that the injury induced by inhaled O_3 as an environmental stressor, will be influenced by sex and dietary interventions. Male and female Brown Norway rats were fed either normal, high fructose or high fat diet beginning 1 month of age for 3 months. At 4th months they were exposed to air or O_3 acutely (0.8 ppm) for 5 hours. The body fat composition and glucose tolerance (GT) were measured prior to O_3 exposure. GT was also examined immediately after air or O_3 exposure (n=10). Pulmonary toxicity and systemic metabolic changes were examined immediately after O_3 exposure in a separate group of rats (n=10). Compared to males, female BN rats fed a normal diet had relatively greater body fat %, higher levels of serum triglycerides, cholesterol and glucose, and lower leptin and insulin. At baseline, male rats fed high fat diet had increased body fat but not females. GT did not differ between males and females but high fat diet induced a small degree of glucose intolerance in both males and females. High fructose but not high fat diet induced marked increases in circulating triglycerides in both males and females. High fat but not high fructose diet increased circulating leptin in both males and females. O_3 exposure increased lung injury as determined by lavage fluid protein and albumin analysis in females fed all diets but only in high fat diet males. Both males and females had >10% of cells as eosinophils in the lung lavage fluid. No specific differences in BALF inflammatory cells were noted between air and O_3 exposed rats of either sex on any diet, however, both diets decreased baseline levels of neutrophils in each sex. O_3 induced glucose intolerance in each sex regardless of diet. O_3 also increased circulating leptin (females>males) regardless of diet. No O_3 effects occurred in circulating cholesterol or triglycerides in either sex. These data provide the evidence that although dietary interventions did not have major sex specific effects, female BN rats are more susceptible to O_3-induced pulmonary and metabolic effects. (Does not reflect US EPA policy).

13.2
LUNG ANTIOXIDANT LEVELS IN NEONATAL RATS AND RESPONSE TO AIR POLLUTION: INFLUENCE OF SEX AND STRAIN

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Emissions from biomass combustion in rudimentary cookstoves (CS) are causally linked with higher incidence of respiratory infections, especially in women and children. As with other air pollutants, oxidative insults are believed to play a major role in the etiology of CS-related lung pathologies. We are seeking to develop rodent models of respiratory infection for assessment of health benefits derived from use of more efficient CS. Previously, we infected neonatal Fischer (FIS) rats with a rat-adapted influenza virus, and showed that acutely, FIS pups developed minimal lung changes. We have also assessed ozone (O_3)-induced effects in adult FIS rats and found that they exhibited the least change compared to Sprague-Dawley (SD) or Wistar (W) rats. This pilot study evaluates lung antioxidant levels in air- and O_3-exposed neonatal FIS, SD, and W pups to determine which strain/sex was most susceptible to early life oxidative insult. Smaller litters in time-pregnant FIS rats dictated uneven group sizes. FIS were 30-40% smaller than SD or W pups. Subsets of female (F) and male (M) 14- and 21-d-old (pre- and weanling) pups were exposed to air, 0.5, or 1.0 ppm O_3 x2h. In controls, body wt increased ≈60% between 14-21 d. At weaning, no sex differences in body wt or lung antioxidants were apparent within strains. Except for increased uric acid (UA) in 14-d F W rats, no age/strain differences were apparent for lung UA, total protein, or glutathione (GSH) peroxidase/reductase (per gm of wet lung wt). At 14-d, FIS rats had 6-22% more GSH than SD or W rats, respectively. GSH decreased in all strains from 14-21 d. Lung SOD also decreased in all strains from 14-21 d, with FIS rats having 25-35% more than SD or W rats. Post-O_3, F 14-d rats of all strains had minor GSH decrements (≤20%); while M pups were unchanged. Relatedly, F SD and W rats had decreased GSH peroxidase (30-36%), GSH reductase (15-26%), SOD (13-30%), and UA (22-42%); while levels in F FIS were unchanged or increased. M 14-d rats had minimal changes. Conversely, F 21-d rats post-O_3 showed minimal change while M pups had increased SOD (25-31%); and M SD pups had increased GSH peroxidase/reductase (18%). In summary, FIS rats appear relatively resistant to lung insult, while neonatal F SD and W rats appear more prone to oxidative effects than M of the same age. We will pursue using non-FIS F pups to evaluate CS emission effects on susceptibility to early life infection. (Abstract does not reflect USEPA policy.).

13.3
ESTRADIOL PREVENTS CARDIO-RESPIRATORY DYSFUNCTIONS INDUCED BY CHRONIC INTERMITTENT HYPOXIA IN FEMALE RATS

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The prevalence of sleep-disordered breathing (SDB) and associated chronic intermittent hypoxia (CIH) increase after menopause in women. Despite evidences showing that hormonal replacement therapy can reduce apnea frequency, the potential protective roles of sex steroid hormones against the cardio-respiratory dysfunctions induced by SDB and CIH are unknown. We tested the hypothesis that estradiol protects female rats against the cardio-respiratory dysfunctions induced by CIH. Sprague-Dawley rats (230-250g) were ovariectomized (OVX) and implanted with osmotic pumps delivering vehicle or estradiol (E_2 - 0.5 mg/kg/day) for 21 days. After 14 days of recovery, the rats were exposed to CIH (21%-10% O_2; 8 hours/days; 6 cycles/hour) or room air (RA) for 7 consecutive days. At the end of CIH exposure, mean arterial pressure (MAP - tail-cuff) was measured, and the rats were placed in a whole body plethysmograph to record ventilation, breathing stability (Poincaré-plots), apnea frequency, and metabolic rate (O_2 consumption and CO_2 production rate) for 4 consecutive hours. All parameters were analyzed during sleep (determined by visual examination of the recordings). Then, the rats were exposed to hypercapnia (5% CO_2) and hypoxia (12% O_2 – 5 min each) to assess chemoreflex function. Sham-operated rats treated with vehicle and exposed to RA were used as a control group for the effects of endogenous estradiol. Compared to OVX-RA rats, OVX-CIH rats had higher MAP (93.4 +/- 0.8 vs 105.2 +/- 2.0 mmHg, $p<0.0001$), high instability of respiratory frequency, high frequency of apneas during sleep (9.5 +/- 1.5. vs 16.2 +/- 0.9 apneas/hour, $p=0.0001$), and a lower metabolic rate. The responses of respiratory frequency to hypoxia and hypercapnia were respectively 70% ($p=0.005$) and 50% ($p=0.03$) higher in OVX-CIH rats compared to OVX-RA. In OVX-E_2-CIH rats, MAP was lower (90.3 +/- 2.5 mmHg), apnea frequency was reduced (5.5 +/- 0.6 apnea/hour), metabolic rate higher, and respiratory responses to hypoxia and hyper-

capnia were lower. Furthermore, these values were similar when comparing OVX-E_2-CIH to sham-RA rats, likely indicating that the estradiol treatment remained in a physiological range. We conclude that estradiol efficiently prevents the cardio-respiratory dysfunctions induced by CIH in female rats. Funded by CIHR (MOP-102715).

13.4
POTENTIAL ROLE OF ESTROGEN IN 15-HYDROXYEICOSATETRANOIC ACID PRODUCTION AND ACTIVITY IN HUMAN PULMONARY ARTERY ENDOTHELIAL CELLS

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Pulmonary arterial hypertension (PAH) has a consistently higher risk occurrence in women compared to men. Mechanisms to explain the female predominance are scarce but likely relate to hormonal changes that contribute to the pathogenesis of the disease. The bioactive lipid arachidonic acid is metabolized to a variety of compounds that effect pulmonary vascular function. Key enzymes in the biosynthetic pathway of arachidonic acid are altered by estrogen. Our central hypothesis is that estrogen has a dual effect to increase 15-lipoxygenase (LO) gene transcription, and phosphorylation of 5-LO, which together result in increased production of the proliferative compound, 15-hydroxyeicosatetranoic acid (HETE). Human pulmonary artery endothelial cells (HPAEC) from a male donor were incubated with estrogen (17β-estradiol, 1 μM; 18 hrs). 15-LO and 5-LO protein expression increased compared to untreated cells. However, there was no evidence of 5-HETE production when cells were incubated with ^{14}C-arachidonic acid and analyzed by HPLC. 15-HETE was detected in the HPAECs, based on co-migration and similar retention time of authentic 15-HETE standard (17.8 min). Others showed that phosphorylation of Ser663 in 5-LO by ERK1/2 converts the enzyme to an active 15-LO (1). Cell lysates from estrogen-treated HPAECs were analyzed using the phosphorylated (p)-5-LO (Ser663) antibody. Expression of p-5-LO increased compared to control cells which supports a role for this enzyme in 15-HETE production. Functionally, the interaction of estrogen and the arachidonic acid/LO pathway in endothelial cell proliferation has important implications in understanding overall mechanisms for the vascular remodeling changes that occur in PAH. In the ^{3}H-thymidine proliferation assay, both 15-HETE (1 μM) and estrogen (1 μM) independently increased HPAEC proliferation. More importantly, when estrogen-treated cells were preincubated with the 15-LO Inhibitor 1 (10 μM), ERK1/2 inhibitor, FR180204 (1 μM) or the estrogen receptor antagonist, ICI182780 (1 μM) proliferation was attenuated. In summary, these studies suggest a novel mechanism whereby estrogen regulates the arachidonic acid pathway which may potentially contribute to alterations in vascular function in sex-based diseases like PAH. Supported by HL093181 and AHA-0151421Z. Reference: Gilbert NC, Rui Z, Neau DB, Waight MT, Bartlett SG, Boeglin WE, Brash AR, Newcomer ME. FASEB J. 8:3222-9, 2012.

13.5
MUSCULAR AND CARDIORESPIRATORY ADAPTATIONS TO TREADMILL TRAINING WITH AGING ARE BLUNTED IN FEMALE COMPARED TO MALE MICE

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Aging is associated with reductions in muscle strength and cardiovascular fitness that may be offset with regular exercise training. However, it is unclear if these exercise adaptations are affected by gender due to factors such as different hormonal and/or anatomical changes with aging. We tested the hypothesis that aging reduces muscle function and cardiorespiratory endurance; however, treadmill (TM) training exercise will attenuate these aging-associated decreases in strength and fitness in both male and female mice. In vivo plantarflexor maximal force and fatigue (% of max force after 10 contractions) were measured in young (4 mo. old) and aged (24 mo. old) sedentary (SED) male and female mice and following 2 wks of TM training (45 min/day, 5 day/wk). Cardiorespiratory adaptations were assessed with pre- and post-maximal treadmill tests. Maximal muscle force was lower in aged than young SED mice in both genders (1.1±0.04 vs. 1.6±0.07 g/g body mass for males and 1.0±0.03 vs. 1.5±0.01 g/g body mass for females, respectively, $p<0.05$). In young groups, TM did not increase force over SED in either gender. In aged male groups, TM was associated with 26% higher maximal force than SED mice ($p<0.05$), but was still lower than young groups. In contrast in female aged groups, TM was not associated with significant increases in maximal muscle force over SED (15% increase). Plantarflexor fatigue resistance was higher in aged than young SED male mice (50±3 vs. 35±3% of max, respectively, $p<0.05$) with no age differences in TM groups. In female mice there were no differences in fatigue resistance among all groups. In treadmill pre-tests, young groups ran longer than aged groups ($p<0.05$). Treadmill test time was not increased in any SED group. Similar increases in treadmill test time after TM training occurred in young and aged male mice with respective 1,059±130 and 1,133±138 second increases from pre to post test ($p<0.05$). In contrast in female mice, increases in treadmill test time with TM training from pre to post were greater in young than aged groups, 1,044±141 and 505±173 seconds, respectively ($p<0.05$). These findings suggest treadmill exercise training is more effective in attenuating age-associated reductions in muscle force and cardiorespiratory fitness in male than female mice. Funded by NIH R15AR060469.

13.6
EFFECT OF EXERCISE ON RED BLOOD CELLS VARIABLES IN HIGHLY TRAINED FEMALE ATHLETES

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Background: A suboptimal hematological status has often been recorded in athletes involved in intensive physical activity. A single bout of physical effort and even more repeated exercise may changes the morphological indices of blood and influence the erythropoietic process in the bone marrow. **Objectives:** To assess the basic red blood cell variables in highly trained female athletes and to compare the results with those for a control untrained groups. **Methods:** This was a cross sectional study was conducted in the Department of Physiology, Dhaka Medical College, Dhaka during the period of July 2005 to June 2006, on sixty apparently healthy female subjects aged 16 to 20 years. Thirty highly trained athletes as experimental group were recruited from Sultana Kamal Women Complex, whereas thirty non-athletes as control group were collected from different halls of Dhaka University. Venous blood samples were drawn from the cubital vein, and the red blood cell count, packed cell volume, hemoglobin concentration, were measured. The mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, were determined by equations. Statistical indices were computed for each group and for each variables. Statistical analysis was done by unpaired Student's 't' test. **Results:** The experimental group was found to have lower red blood cell count, packed cell volume, and hemoglobin ($p<0.05$) than that the control group. No significant differences were found in the mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration. **Conclusions:** Continuous high intensity sports training over more than one year decreases basic red blood cell (RBC) variables in female athletes, this being more pronounced for submaximal sports. Key words: Female athletes, exercise, RBC, PCV, MCV, MCH, MCHC.

13.7
CONTRIBUTION OF THE NUCLEAR PROGESTERONE RECEPTOR (NPR) TO BREATHING STABILITY AND HYPERCAPNIC VENTILATORY RESPONSE IN ADULT MALE MICE

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Progesterone is a potent respiratory stimulant that reduces the frequency of sleep-disordered breathing and apneas in women. Adult female mice carrying a mutation in the nPR gene (PRKO mice) have elevated apnea frequency during sleep, showing a role for nPR in respiratory regulation. So far it remains unknown if nPR has similar roles in males. Therefore, we tested the hypothesis that nPR contributes to respiratory regulation and mediates the respiratory effects of progesterone in adult male mice. Adult PRKO male mice and wild-type controls (WT) were implanted with an osmotic pump delivering vehicle or progesterone (4 mg/kg/day). 7 days after the surgery, the animals were placed in a whole body plethysmograph to record ventilation, the frequency of sighs (associated with micro awake) and post-sigh apneas for 4 consecutive hours. All parameters were analyzed during sleep (determined by visual examination of the recordings). Then the animals were exposed to hypercapnia (5% CO_2), hypoxia (12% O_2) and hypoxic-hypercapnia (5% CO_2 + 12% O_2 – 5 min each) to assess chemoreflex function. PRKO and WT mice treated with vehicle had the same level of minute ventilation during sleep, but PRKO mice had a slightly higher frequency of sighs than WT mice (29.1 +/- 1.0 vs. 23.9 +/- 0.9 sighs/hour, $p<0.0001$). The ventilatory response to hypoxic-hypercapnia was 34% lower in PRKO mice compared to WT ($p=0.016$). Progesterone treatment did not change ventilation recorded during sleep in WT or in PRKO mice. Progesterone treatment decreased the frequency of sighs (from 29.1 +/- 1.0 to 24.6 +/- 0.7 sighs/hour, $p=0.0006$) and increased the frequency of post-sigh apneas (from 8.3 +/- 1.4 to 14.8 +/- 2.3 apneas/hour, $p=0.009$) in PRKO, but not in WT mice. Contrastingly, progesterone treatment increased the tidal volume response to hypercapnia (+40% - $p=0.02$) in WT, but not in PRKO mice. In progesterone treated mice, the ventilatory responses to hyper-

capnia and hypoxic-hypercapnia were respectively 44% and 50% higher in WT than in PRKO mice. We conclude that, as previously observed in female mice, nPR contributes to the regulation of breathing in males. The effects of progesterone on apnea in PRKO males are probably related to other types of progesterone receptors, or to allopregnanolone, the neuroactive metabolite of progesterone. These results highlight the role of nPR and endogenous progesterone production on respiratory regulation in males. Funded by CIHR (MOP-102715).

14.0 NEUROCONTROL

14.1
THE IMPORTANT ROLE OF NITRIC OXIDE SYNTHASE IN CONTROLLING MITOCHONDRIAL RESPIRATION OF LARGE CEREBRAL ARTERIES IN FEMALE AND MALE RATS

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We have found that mitochondrial oxygen consumption (OCR) is substantially greater in large cerebral arteries in female compared to male rats. However, the underlying mechanisms underlying this sex-based difference have never been fully determined in intact cerebral arteries. Due to higher nitric oxide synthase (NOS) levels in female compared to male cerebral arteries, we tested the hypothesis that differences in NOS signaling mechanisms contribute to sex-based differences in mitochondrial respiration. The Seahorse XFe24 analyzer was used to examine the mitochondrial OCR (pM/min/μg protein) in isolated, large cerebral arteries (middle cerebral artery, circle of Willis, and basilar artery) from male and female Sprague Dawley rats in the absence and presence of the NOS inhibitor L-NAME. Western blots were used to determine both phosphorylated and total endothelial (eNOS) and neuronal NOS (nNOS). The components of mitochondrial respiration in arteries in the absence of L-NAME (vehicle) normalized to protein levels (pM/min/μg protein) including basal respiration (96.9 ± 15.2), ATP production (33 ± 5.3), proton leak (63.6 ± 10.5), maximal respiration (147.2 ± 21.6), and spare respiratory capacity (50.4 ± 8.4) were significantly ($p<0.05$) elevated in females compared with males (36.3 ± 8.5, 15.1 ± 4, 21.2 ± 4.6, 62.8 ± 16.4, 26 ± 7.3, respectively). Treatment with 100 μM L-NAME resulted in an increase over vehicle values in the OCR of both groups which was significant for all components of mitochondrial respiration in the male group: basal respiration (98.7 ± 8.8), ATP production (48.6 ± 8.6), proton leak (43.2 ± 11.7), maximal respiration (117.7 ± 16.7), and spare capacity (85.9 ± 9.7). However, L-NAME treatment in the female group caused a significant increase only in maximal respiretion and spare capacity (224.3 ± 25.8 and 125.6 ± 20.2, respectively) compared with vehicle. The ratios of phosphorylated eNOS and total eNOS and phosphorylated nNOS and total nNOS were significantly higher in the female (2.2 ± 0.6%, 1.2 ± 0.2%, respectively) compared with the male arteries (0.88 ± 0.2%, 0.5 ± 0.2%, respectively). Thus, NOS inhibition enhanced mitochondrial respiration in cerebral arteries from male and female rats but the relative effects of NOS inhibition were much greater in male than female arteries. Our findings support the concept that sex differences in mitochondrial respiration in cerebral arteries are in part due to involvement of NOS signaling pathways.

14.2
SEX DIFFERENCES IN THE CEREBRAL VASCULAR FUNCTION AND K CHANNEL ROLE

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Cerebrovascular incidence rate is lower in adult females compared to adult males but the role of vascular function and K channel is not clear. Using a combination of vascular and electrophysiological approaches we explored the hypothesis that "sex differences in the cerebral vascular function in adult Sprague Dawley (SD) rats is associated with differential K channel function in the vascular smooth muscle cells (VSMCs)". The diameter of female middle cerebral arteries (MCAs) increased with increase in the lumen pressure from 40 to 140mmHg in 20mmHg steps, whereas the diameter decreased in male MCAs (% change in diameter from 40 to 140mmHg: Females 16±8, Males -25±4, $p<0.05$, n=5-8). Female MCAs have ~1.76 fold lower diameter at 40mmHg compared to age matched males in the presence of calcium (Females 81±5, Males 143±13 μm, n=5-8). In contrast, passive dilation was similar (-Calcium, 2mM EGTA) (Females: 168±12, Males 167±10 μm, n=6). 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, $p<0.05$). 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, $p<0.05$). Spontaneous transient outward currents (STOC) that represent BK channel function are ~1.73 fold higher in VSMCs isolated from female SD rats compared to males (pA: Females 90±6, Males 53±5, n=5, $p<0.05$). In contrast, the mean amplitude of transient spontaneous hyperpolarization's (TSHs) that also represent BK channel role in membrane potential were ~0.8 fold lower in the female SD rats compared to males (mV: Females -22±3, Males -27±3, n=4). Together these results suggest that female MCAs may have higher myogenic tone but exhibit an attenuated pressure-mediated myogenic response compared to males. Higher BK channel function in VSMCs of adult female rats may contribute to the attenuated myogenic response and participate in the endothelium-independent exaggerated vasorelaxation. 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.

14.3
CHARACTERIZING THE GENDER DIFFERENCES OF MULTIDRUG-RESISTANCE PEPTIDE (MRP) TRANSPORTER EXPRESSION IN MOUSE BLOOD-BRAIN INTERFACES

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The choroid plexus (CP) epithelium and the capillary endothelium (blood brain barrier, BBB) are blood-brain interfaces with transporters that play important roles in clearing the brain of unwanted substances and preventing the entrance of potentially harmful material into the brain. Previous research has shown gender-specific patterns of the multidrug resistance peptide (MRP) efflux transporters, part of the ATP-Binding cassette (ABC) gene family. This is best documented in liver and kidney, where Mrp1, Mrp3 and Mrp4 have higher expression in females and Mrp5 and breast cancer resistance protein (Bcrp/ ABCG2), have higher expression in males. However, little is known about the Mrp gender differences and their function in brain. The aim of this study was to examine differences in mRNA and protein expression for ABC transporters in the brain and CP of naive wild type (WT) male and female C57 mice. We hypothesized that renal and hepatic gender-specific patterns of these transporters would also be present in the blood brain interfaces. mRNA and protein levels were measured by quantitative polymerase chain reaction (qPCR) and western blotting, respectively. Immunoblot analysis on CP and brain showed that Mrp4, Mrp6 and Bcrp are expressed in a gender specific pattern, and their expression correlates with the expression in the kidneys, supporting our hypothesis. In contrast, Mrp1 and Mrp2 expression had no gender pattern. Our findings indicate for the first time that significant differences in expression of these transporters at the blood-brain interfaces exist between male and female. These results will be helpful for understanding the physiological roles of individual transporters at both the blood-CSF barrier (CP) and BBB. Physiological barriers are known to influence many pharmacokinetic processes. Therefore, it is important to determine how gender can affect transport, metabolism and drug distribution. (Supported by NSF and NIH).

14.4
SEX AND GENOTYPE DIFFERENCES TO EPINEPHRINE INFUSIONS IN HUMANS

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Objective: We sought to identify any differences relative to gender and/or genotype on the cardiovascular responses to exogenous infusions of low dose epinephrine (5ng/kg/min). **Methods:** *Subjects: Ten males* (mean ± SD: age=27.8±6.6, height=178.5±8.0cm, weight=83.9 ±12.4kg, BMI=26.3±2.9kg/m^2, BSA=2.0±0.2) and *fourteen females* (mean ± SD: age=26.3±5.9, height=164.3±7.0cm, weight=60.8±6.2kg, BMI=22.5±2.1kg/m^2, BSA=1.7±0.1) were studied. Three males were homozygous for Arginine (Arg/Arg) and 7 males were homozygous for Glycine (Gly/Gly) at position 16. In the female cohort, eight females were Arg/Arg and six females were Gly/Gly, at position 16. *Procedure:* A 1-hour epinephrine infusion dosed at 5ng/kg/min was administered. BP was measured via an arterial catheter and HR via EKG. Stroke volume and Cardiac output were estimated via Modelflow and TPR was calculated. Plasma samples were collected at time: -10, 0, 30, 45, 60, 70, and 80 minutes and catecholamines measured using HPLC. *Statistics:* ANOVA with repeated measures was performed controlling for both gender and genotype during the epinephrine infusion at *time(s): 0, 30, 45, and 60 minutes*. All data were analyzed using the R software package with significance set if P was <0.05. **Results:** At baseline, mean values for MAP, HR, CO, SV, and TPR differed between sexes and genotype. At 30-minutes, mean values for MAP, HR, CO, and SV differed by sex as well as genotype. Significant, sex by genotype interactions were noted for MAP, SV, and TPR, while significant sex differences were identified for MAP, CO, and SV throughout the infusion. **Conclusion:** Our results indicate that the cardio-

vascular responses to epinephrine infusions are influenced by both sex and β2-adrenergic receptor genotype. These responses may explain why some of the responses to physiological stressors differ by sex and genotype.

Effect of Gender and Genotype on Human Cardiovascular Response

	Baseline (t=0)	30-min	Δ Delta
MAP (mmHg)			
Males	82±7	82±8	0
Females	81±9	77±10	4
Arg/Arg	79±9	77±12	2
Gly/Gly	82±8	81±7	1
HR (beats/min)			
Males	60±12	62±11	2
Females	70±14	74±11	4
Arg/Arg	67±12	70±12	3
Gly/Gly	65±15	68±13	3
CO (L/min)			
Males	6.4±0.7	7.7±1.1	1.3
Females	4.9±1.1	5.3±0.9	0.4
Arg/Arg	5.3±1.0	5.8±1.1	0.5
Gly/Gly	5.8±1.7	6.6±1.8	0.8
SV (mL/min)			
Males	110.0±25.7	126.7±29.5	16.7
Females	72.3±24.9	73.2±17.4	0.9
Arg/Arg	81.7±27.3	87.4±28.6	5.7
Gly/Gly	96.7±42.8	100.4±39.8	3.7
TPR (dyn*sec*cm^5)			
Males	12.9±1.5	10.9±2.0	2
Females	17.4±3.8	14.9±3.0	2.5
Arg/Arg	15.3±2.6	13.5±2.7	1.8
Gly/Gly	15.7±5.2	13.2±3.9	2.5

14.5
SEX DIFFERENCES IN THE EFFECT OF HYPOGLYCEMIA ON BAROREFLEX SENSITIVITY IN PATIENTS WITH TYPE 1 DIABETES MELLITUS

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Objective: Patients with type 1 diabetes mellitus (T1DM) exhibit impairments in autonomic function which are worsened with acute hypoglycemia and have been linked to an increased risk of cardiovascular events. Given women with T1DM have increased risk of cardiovascular morbidity and mortality when compared with men, we sought to examine whether sex differences exist in autonomic function during hypoglycemia in patients with T1DM. **Methods**: Thirteen adults with T1DM (6F/7M) completed a single 180-min hyperinsulinemic (2 mU/kg TBW/min), hypoglycemic (~3.3 μmol/mL) clamp. Measures of heart rate (electrocardiogram) and blood pressure (finger photoplethysmography) were analyzed at baseline and during the hypoglycemic clamp. The sequence method was used to derive measures of spontaneous cardiac baroreflex sensitivity (sCBRS). **Results:** Men and women were not different in regard to age, body mass index, HbA1c, duration of diabetes, nor baseline sCBRS ($p>0.05$). Heart rate increased during hypoglycemia and the rise was not different between the sexes ($p=0.82$). The effect of hypoglycemia on systolic blood pressure was sex-specific (Men: +13±6 mmHg, Women: -6±6 mmHg, $p=0.05$). When compared to euglycemia, male subjects exhibited significant reductions in sCBRS to rising (up-up) blood pressure ($p=0.02$) during hypoglycemia, whereas no change was observed in female subjects ($p=0.23$). A change in sCBRS to falling (down-down) blood pressure was not observed in either group during hypoglycemia ($p>0.05$). **Conclusions**: Changes in autonomic function during hypoglycemia differ between men and women with T1DM. sCBRS to rising blood pressure, a measure of parasympathetic control, is reduced during hypoglycemia in male patients with T1DM and these changes are not observed in women. In contrast, the rise in heart rate during hypoglycemia in women – while similar to that observed in men – appears insufficient to maintain blood pressure, suggesting impaired sympathetic control in women with T1DM. **Funding:** NIH DK090541, NIH HL120570.

14.6
THE EFFECTS OF TESTOSTERONE AND OXIDATIVE STRESS ON NEUROINFLAMMATORY SIGNALING IN DOPAMINE NEURONS

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Parkinson's disease, a progressive neurodegenerative disorder characterized by oxidative stress and neuroinflammation, is distinguished by the loss of dopamine neurons in the nigrostriatal pathway. Interestingly, men have a two-fold prevalence for Parkinson's disease than women. While the mechanisms underlying this sex difference remain elusive, we propose that the primary male sex hormone, testosterone, is involved. Our previous studies show that under oxidative stress conditions, testosterone increased oxidative stress generation and cell death in dopamine neurons. Oxidative stress can induce neuroinflammation, a prominent mechanism involved in the neurodegeneration of dopamine neurons. Pro-inflammatory mediators, NFkB and COX2, can increase oxidative stress in dopamine neurons and lead to apoptotic cell death. Thus, we hypothesize that under oxidative stress conditions, testosterone will increase COX2 mediated oxidative stress to induce alpha synuclein Lewy bodies and apoptosis in dopamine neurons. To test our hypothesis, we exposed a dopaminergic cell line (N27 cells) to a sublethal concentration of the pro-oxidant, tert-butyl hydrogen peroxide (H2O2) and assessed the role of testosterone on oxidative stress, cell viability, pro-inflammatory markers and apoptosis. Our results showed that under oxidative stress conditions, testosterone increased COX2 protein expression, alpha synuclein Lewy bodies, and apoptosis in dopamine neurons. Inhibiting COX2 blocked testosterone's negative effects on oxidative stress generation and apoptosis. Therefore, our results indicate that testosterone may mediate the sex differences observed in Parkinson's disease by increasing oxidative stress induced neuroinflammation and apoptosis in dopamine neurons.

14.7
DOXORUBICIN REDUCES PROINFLAMMATORY MEDIATOR EXPRESSION IN BRAIN AND PIAL ARTERIES FROM OVARIECTOMIZED FEMALE RATS

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Doxorubicin (DOX) is a highly effective chemotherapy agent. Its use is hampered however owing to severe dose-dependent cardiovascular toxicity in cancer survivors. Multiple mechanisms have been implicated in the pathogenesis of DOX cardiotoxicity, one of which involves inflammation mediated by activation of the NF□B/TLR4/COX-2 pathway in the heart. Knowledge regarding the toxic effects of DOX-induced inflammation in other organ systems such as the brain is sparse. Therefore, we explored the inflammatory potential of DOX by assessing TLR4 and COX-2 levels in cortex and pial arteries isolated from ovariectomized (OVX) female Sprague-Dawley rats. We hypothesized that DOX would promote inflammation by increasing COX-2 expression along with expression of its upstream innate immune receptor, TLR4, both of which are under the transcriptional regulation of NF□B. OVX rats were treated with three, bi-weekly, i.p. injections of DOX (4 mg/kg; cumulative dose 12mg/kg) or vehicle (saline) and euthanized 5 days after the last dose. Tissues were isolated, snap frozen, homogenized, and analyzed for COX-2, TLR4 and NF□Bp65 levels using standard western blotting. Although COX-2 is typically considered an inducible enzyme, it has been shown to be expressed under basal conditions. In cortex and pial arteries from vehicle-treated rats, measurable levels of COX-2 and TLR4 protein were detected. However, contrary to our hypothesis, levels of COX-2 and TLR4 were decreased following DOX. In pial arteries, DOX elicited a reduction in both the COX-2 72 kDa band 74 kDa band with the greatest reduction observed in the 72 kDa. The 72 kDa band corresponds to the partially glycosylated inactive form of enzyme, while the 74 kDa band has been shown to represent the fully glycosylated active form. Cytosolic levels of NF□Bp65 levels were detected in brain and pial vessel lysate, however levels were not altered by DOX. Similar to brain and pial arteries, basal expression of COX-2 and TLR4 were detected in left ventricle and DOX treatment attenuated expression. In conclusion, although others have suggested the involvement of the NF□B pathway during the development and progression of DOX-induced cardiomyopathy, our studies demonstrate a possible novel action for the anticancer agent implicating anti-inflammatory mechanisms, particularly in female cohorts with low circulating levels of gonadal hormones.

14.8
CEREBRAL BLOOD FLOW REGULATION IS AFFECTED THROUGHOUT THE MENSTRUAL CYCLE IN YOUNG WOMEN

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The objective was to determine if cerebral blood flow regulation is affected throughout the menstrual cycle in young, healthy women with naturally cycling hormones (NOC) compared to women on combined oral contraceptives (OC). Nine (4 NOC and 5 OC) healthy, young women (mean age 20.3 years) were tested during menstruation, the late follicular phase, and the mid-luteal phase. Each visit consisted of a cerebrovascular reactivity test, sit-to-stand tests, and squat-to-stand tests. Beat-by-beat blood pressure, heart rate, end-tidal CO_2, and transcranial Doppler ultrasonography of the anterior and middle cerebral arteries were measured for each subject. Results from the sit-to-stand maneuver indicate a significant reduction ($p= 0.05$) in the cerebral autoregulatory index of the middle cerebral artery during the late follicular phase (NOC: 3.7 ± 0.44; OC: 3.1 ± 0.39) compared to the menstrual phase (NOC: 4.2 ± 0.67; OC: 4.2 ± 0.60), but not a significant effect of oral contraceptives. There was a significant effect of oral contraceptives on both the resting mean arterial pressure ($p= 0.029$; menstruation NOC: 79.7 ± 4.9 mmHg; OC: 92.7 ± 4.4 mmHg; late follicular: NOC: 70.4 ± 6.2 mmHg; OC: 87.1 ± 5.6 mmHg; luteal: NOC: 66.1 ± 8.1 mmHg; OC: 93.0 ± 7.2 mmHg) and heart rate ($p= 0.027$; menstruation NOC: 60.7 ± 4.3 bpm; OC: 74.3 ± 4.3 bpm; late follicular: NOC: 66.4 ± 1.5 bpm; OC: 70.1 ±1.5 bpm; luteal: NOC: 65.8 ±3.5 bpm; OC: 72.8 ± 3.5 bpm), but no effect of menstrual cycle phase. There was not a significant effect of menstrual cycle phase or oral contraceptives on the decrease in mean flow velocity of the middle cerebral artery when going from a sitting to standing position, but there was a trend for a greater drop in steady-state flow velocity when standing in women on oral contraceptives (menstruation: NOC: 0.90 ± 4.3%; OC: -6.7 ± 3.8%; late follicular: NOC: -3.5 ± 3.4%; OC: -5.4 ± 3.0%; luteal: NOC: -0.35 ± 3.6%; OC: -5.8 ± 3.2%). While more data is necessary to interpret the findings, the preliminary results may indicate reduced cerebral vasodilation in women on oral contraceptives. However, this data is from a small number of young women. Additional participants are needed to support if menstrual cycle phase or oral contraceptive use effects cerebral blood flow regulation in young women. This work was supported by the War Related Illness and Injury Study Center and Dept of Veteran Affairs. This study was conducted in compliance with the Declaration of Helsinki.

15.0 PREGNANCY

15.1
PLACENTAL ISCHEMIA INCREASES SENSITIVITY TO PENTYLENETETRAZOL-INDUCED SEIZURES AND CEREBROSPINAL FLUID INFLAMMATION

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Eclampsia is diagnosed in preeclamptic patients who develop convulsions and/or unexplained coma during pregnancy or postpartum and accounts for ~13% of maternal deaths worldwide. The mechanisms contributing to the pathophysiology of eclampsia are not known, partly due to the lack of suitable animal models. The aim of this study was to test the hypothesis that placental ischemia, induced by reducing utero-placental perfusion pressure, increases susceptibility to seizures, cerebral edema, cerebrospinal fluid (CSF) cytokines/chemokines, and plasma neurokinin B (NKB). Pentylenetetrazol (PTZ), a pro-convulsive drug, was injected into pregnant and placental ischemic rats at the sub-convulsive dose of 40 mg/kg, i.p. on gestational day 19 followed by video monitoring for seizure activity for 30 minutes. Seizure scoring was blindly conducted. Placental ischemic rats (n=8) had reduced latency to the first seizure (264.5±78.7s compared to 835.7±246.6s in normal pregnant rats (n=7); $p<0.05$) and increased brain water content (78.8±0.1% vs. 78.4±0.1% in normal pregnant rats; $p<0.05$). PTZ treatment increased brain water content in both pregnant (78.8±0.1%; $p<0.05$) and placental ischemic rats (78.9±0.1%; $p<0.01$). Associated with reduced seizure latency, placental ischemia led to a significant increase in 4 out of 27 CSF cytokines/chemokines tested: IL-2, IL-17, IL-18, and eotaxin (CCL11). Placental ischemia had no effect on plasma NKB concentrations ($p>0.05$); however, PTZ increased plasma NKB in both pregnant and placental ischemic rats ($p<0.05$). NKB was strongly correlated with seizure susceptibility only in normal pregnant rats (R^2=0.88 vs. 0.02 in placental ischemic rats). These data demonstrate that placental ischemia increases seizure susceptibility potentially through increases in CSF cytokine levels and edema formation; thus, the rat model of placental ischemia is an excellent model for studying mechanisms contributing to eclampsia-like symptoms. Further studies are required to elucidate the role of CSF cytokines/chemokines in contributing to increased seizure susceptibility following placental ischemia. Funding: NIH: P20GM104357, P01HL051971, and AHA 13POST16240000.

15.2
VITAMIN D SUPPLEMENTATION INHIBITS BLOOD PRESSURE AND UTERINE ARTERY RESISTANCE INDUCED BY AUTOANTIBODIES TO THE AT1 RECEPTOR

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Studies in our lab have previously shown that Vitamin D supplementation in the RUPP rat model of preeclampsia lowers blood pressure and reduces autoantibodies to the AT1 receptor (AT1-AA). Therefore, we sought to determine the efficacy of Vitamin D supplementation to inhibit AT1-AA-induced endothelial dysfunction and hypertension during pregnancy. We hypothesized that Vitamin D supplementation to AT1-AA-induced hypertensive pregnant rats would reduce anti-angiogenic factor soluble FMS-like tyrosine kinase-1 (sFlt-1) and uterine artery resistance index (UARI) while improving blood pressure (MAP). Purified rat AT1-AA was infused (1:40) into Sprague-Dawley rats via miniosmotic pump from gestational day (GD) 12 to GD19. On GD14-18 we administered Vitamin D2 or D3 (VD2 or VD3) to AT1-AA rats (50ul/ml) by oral gavage. On GD18 indwelling carotid catheters were inserted and UARI assessed by Doppler sonography and MAP was measured on GD19. Consistent with previous studies, MAP was increased in AT1-AA infused pregnant rats (123.3±7.4 mmHg, n=3) compared to normal pregnant (NP) rats (101.2±1.2 mmHg, n=9, $p=0.0005$). MAP was reduced with VD2 treatment in AT1-AA-infused rats (105.0±2.3 mmHg, n=4, $p=0.04$) and AT1-AA+VD3 rats (110.4±1.5 mmHg, $p=0.06$). Our data indicated that UARI was increased in AT1-AA rats (0.55±0.02, n=4) compared to NP (0.44, n=1) and was unchanged with VD2 treatment (0.57, n=2) but reduced with VD3 (0.46±0.02, n=3, $p=0.03$). Plasma sFlt-1 levels were measured with ELISA and were greatly increased with AT1-AA infusion (>1050 pg/ml, n=3) compared to NP rats (74.91±10.71 pg/ml, n=4). sFlt-1 levels were reduced in AT1-AA+VD2 (42.3 pg/ml, n=2) and AT1-AA+VD3 rats (241.0±187.7 pg/ml, n=3). Our preliminary data demonstrate that Vitamin D supplementation improves uterine artery vascular resistance and sFlt-1 which are possible mechanisms for improved hypertension induced by AT1-AA during pregnancy. *This study was funded by NIH grants RO1HD067541 and T32HL105324.*

15.3
ROLE OF OBESE-RELATED METABOLIC FACTORS ON BLOOD PRESSURE REGULATION IN PREGNANT RATS

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Although the etiology of preeclampsia (PE) remains unclear, evidence indicates that impaired trophoblast invasion followed by placental ischemia/hypoxia promotes the release of placental anti-angiogenic, such as soluble fms-like tyrosine kinase (sFlt)-1, and pro-inflammatory, such as tumor necrosis factor (TNF)-α into the maternal circulation. These vasoactive factors elicit maternal endothelial dysfunction and ultimately hypertension. Obesity is a major risk factor for PE. In addition, increased circulating metabolic factors, including leptin and insulin have been associated with PE. However, the mechanisms whereby obesity and its related metabolic factors increase the risk for developing PE are unknown. Our aims were to determine the effects of chronic hyperleptinemia and euglycemic hyperinsulinemia on blood pressure regulation and placental sFlt-1 and TNF-α levels in pregnant rats. First, to examine the role of hyperleptinemia in PE, 12 weeks old Sprague-Dawley (SD) rats were randomly assigned to one of the following groups on gestational day (GD)14: normal pregnant with food intake ad libitum (NP, n=8); leptin (0.5 μg/Kg/min i.p. for 5 days)-treated pregnant with food intake ad libitum (P+Lep, n=8); and normal pregnant with food restriction adjusted to the P+Lep group's food intake (NP-FR, n=10). On GD19, mean arterial pressure (MAP) was assessed via carotid catheter and placentas were harvested. While P+Lep rats developed hypertension (121.3±8.1 mmHg, $P<0.05$), MAP was not statistically different between NP and NP-FR groups (102.4±2.4 vs 101.3±1.8 mmHg). Second, to examine the role of hyperinsulinemia in PE, SD rats were randomly assigned to either the NP group (n=8) or the insulin (1.5 mU/Kg/min s.c. for 5 days)-treated pregnant group supplemented with 20% glucose in drinking water (P+Ins, n=10) on GD14. On GD19, we observed that MAP was significantly increased in P+Ins rats compared to NP rats (114.0±2.3 vs 104.4±3.9 mmHg,

P<0.05). Furthermore, while placental TNF-α expression, but not sFlt-1, were increased in the P+Lep group compared with NP and NP-FR groups (1.6±0.1 vs 1.1±0.1 vs 1.2±0.1pg/mg, P<0.05), placental sFlt-1 and TNF-α levels were similar in P+Ins and NP rats. In summary, we showed that chronic hyperleptinemia and euglycemic hyperinsulinemia have significant effects to increase blood pressure during pregnancy through different pathways. Thus, elevated circulating metabolic factors such as leptin and insulin may contribute to the development of hypertension in PE women. Funding: 14POST18970005, HL051971, and 1T32HL105324.

15.4
THE INCREASED ENDOTHELIUM-DEPENDENT VASODILATORY RESPONSE OF HEALTHY PREGNANCY IS ABSENT IN THE PREECLAMPTIC DAHL SALT-SENSITIVE RAT

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Preeclampsia is a hypertensive disorder of pregnancy associated with renal injury and endothelial dysfunction. More specifically, in preeclampsia there is an absence of the well-characterized increase in endothelium-dependent vasorelaxation that occurs during normal pregnancy. Previously, our laboratory identified the Dahl salt-sensitive (Dahl S) rat strain as a spontaneous model of preeclampsia exhibiting hypertension and renal injury during late pregnancy; however, it is unknown whether this model also presents with endothelial dysfunction.Thus, in the present study, we hypothesized that the Dahl S rat would also exhibit impaired endothelium-dependent vasodilation during late pregnancy. Vascular rings were isolated from carotid arteries and third-order mesenteric arteries from pregnant Dahl S rats on gestational days 17-18 and age-matched virgin female rats (n=4/group). Endothelium-dependent vasorelaxation to acetylcholine and endothelium-independent vasorelaxation to the nitric oxide donor sodium nitroprusside were assessed. There was no significant difference in acetylcholine sensitivity (logEC50) in the pregnant rats compared to their virgin controls in carotid arteries (-6.57±0.51 M vs -6.30±0.13 M, respectively) or mesenteric arteries (-7.32±0.07 M vs -7.58±0.06 M, respectively). However, the maximum response to acetylcholine (at -log[4.5 M]) was significantly impaired in carotid arteries from pregnant Dahl S rats compared to virgins (85±1% vs 91±1%, p<0.05), with a similar trend observed in the mesenteric arteries (85±10% vs 91±4%). There were no differences in sensitivity or maximum vasorelaxation to sodium nitroprusside in pregnant or control rats in either vascular bed, indicating no changes in the vascular smooth muscle response to exogenous nitric oxide. These data support our hypothesis that the increased endothelium-dependent vasodilatory response that is characteristic of healthy pregnancy is absent in the Dahl S rat and that this failure of the normal cardiovascular adaptation to pregnancy contributes to the increased blood pressure and preeclamptic phenotype in the Dahl S rat.

15.5
DECREASED UTERINE ARTERY BLOOD FLOW AND ENHANCED MYOGENIC TONE IN RGS2-DEFICIENT MICE

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Uterine artery blood flow is critical to maintaining uteroplacental perfusion for delivery of nutrients and oxygen to the fetus during pregnancy. Impaired uterine artery blood flow is implicated in several pregnancy complications including fetal growth restriction, small for gestational age, and preeclampsia. The etiology of abnormal uterine artery blood flow is not known. Here we determined whether the loss of RGS2, a GTPase activating protein for Gq/11 and Gi/o class G proteins that regulates vascular smooth contraction, affects uterine artery blood flow during pregnancy. We used Doppler ultrasonography to assess uterine artery blood flow prior to and at three stages of gestation in wild type (WT) and Rgs2 null (Rgs2-/-) mice. Ex vivo video microscopy was used to examine myogenic tone in pressurized uterine artery segments. We found that baseline uterine artery blood flow velocity was markedly decreased while peak systolic velocity-to-least diastolic velocity ratio (PS/LD; WT: 2.45 ± 0.18 vs. Rgs2-/-: 3.85 ± 0.64, p<0.05), resistive index (RI; WT: 0.58 ± 0.04 vs. Rgs2-/-: 0.71 ± 0.03, p<0.01) and pulsatile index (PI; WT: 0.90 ± 0.06 vs. Rgs2-/-: 1.25 ± 0.11, p<0.01) were all increased in non-pregnant Rgs2-/- mice relative to WT controls. During pregnancy, PS/LD, RI and PI remained elevated and worsened by gestational day 15 in Rgs2-/- mice. Examination of uterine artery tone showed augmented myogenic response in both Rgs2+/- and Rgs2-/- mice, which was reduced to WT level following Gi/o inactivation with pertussis toxin (PTX). In contrast, PTX had no effect on myogenic response in WT uterine arteries. The data together indicate that RGS2 deficiency decreases uterine artery blood flow by increasing myogenic tone at least partly through prolonged Gi/o activation. Thus, mutations that decrease vascular RGS2 expression may be a predisposition to decreased uterine blood flow. Targeting Gi/o signaling therefore might improve uteroplacental underperfusion during pregnancy.

15.6
IMPACT OF OBESITY ON NITRIC OXIDE SYNTHASE (NOS)-MEDIATED REGULATION OF BLOOD PRESSURE DURING PREGNANCY IN RATS

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Although obesity is a major risk factor for preeclampsia, defined as new-onset hypertension during pregnancy, the mechanisms have yet to be elucidated. It is known that the dependency of blood pressure regulation on NOS is increases during normal pregnancy in lean rats. Whereas the role of NOS to control of blood pressure during obese pregnancies is less clear as human studies have shown both reductions and increases in NO bioavailability. Therefore, we examined the impact of obesity on NOS-mediated regulation of blood pressure during pregnancy. MC4R-deficient obese rats (MC4R+/-) and wild-type Wistar Hannover controls (MC4R+/+) were maintained on NIH31 standard chow; mated at 17 weeks old; and supplemented ad libitum in drinking water with the non-selective NOS inhibitor L-N^G-Nitroarginine methyl ester (L-NAME, 100 μg/L) starting at gestational day (GD) 14 until assessment of mean arterial blood pressure (MAP) and pregnancy weights at GD 19. Maternal body weight was greater in MC4R+/- (untreated: 366±10, N=12 vs. L-NAME: 359±9 g, N=10) than MC4R+/+ (untreated: 337<A name=OLE_LINK2 data-listid>±</A>8, N=16 vs. L-NAME: 332±8g; N=12) regardless of treatment (P<0.05). The same was true for visceral adipose tissue mass with MC4R+/- (untreated: 7.0±1.1 vs. L-NAME: 5.2±0.5g) being greater than MC4R+/+ (untreated: 3.4±0.3 vs. L-NAME: 3.5±0.4g) (P<0.05). Fetal weight was reduced by L-NAME only in MC4R+/- (1.98±0.03 vs. 1.82±0.06g, P<0.05) not MC4R+/+ (1.92±0.03 vs. 1.95±0.06g) while placental weights were similar among untreated and L-NAME-treated groups alike, respectively, (MC4R+/+: 0.50±0.01 vs. 0.50±0.03g) and (MC4R+/-: 0.56±0.02 vs. 0.55±0.02g). MAP was greater in untreated MC4R+/- vs. MC4R+/+ rats (P=0.005). The effect of NOS inhibition to raise MAP was statistically higher in <A name=OLE_LINK3 data-listid>MC4R+/+ (103</A>±2 vs. 130±3mmHg, P<0.0001) compared to MC4R+/- (112<A name=OLE_LINK4 data-listid>±</A>3 vs. 134±6mmHg, P<0.001). GFR was reduced (P<0.05) by L-NAME similarly in MC4R+/+ (1.3±0.1 vs. 1.1±0.1mL/min/100g) and MC4R+/- (1.5±0.2 vs. 1.3±0.1mL/min/100g). Circulating leptin (MC4R+/- untreated: 5.9±0.6 vs. L-NAME: 5.8±0.9ng/mL; MC4R+/+: untreated: 3.6±0.3 vs. L-NAME: 3.4±0.8ng/mL) and total cholesterol levels (MC4R+/- untreated: 123±5 vs. L-NAME: 137±23mg/dL; MC4R+/+ untreated: 93±5 vs. L-NAME: 113±11mg/dL) were greater in obese pregnant rats but unaltered by L-NAME. In conclusion, these data indicate that NOS-dependent regulation of MAP is reduced in obese pregnancies and may contribute to higher preeclampsia rates found in obese pregnant women.

15.7
AGONISTIC AUTOANTIBODIES TO THE ANGIOTENSIN II TYPE 1 RECEPTOR ENHANCES ANG II INDUCED RENAL VASCULAR SENSITIVITY AND REDUCES RENAL FUNCTION DURING PREGNANCY

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Preeclamptic women produce agonistic autoantibodies to the Angiotensin II type 1 receptor (AT1-AA) and exhibit increased blood pressure (BP) and vascular sensitivity to angiotensin II (ANG II). Although, together AT1-AAs and ANGII increase the BP, renal artery resistant index, and vasoconstriction of renal afferent arterioles in pregnant rats; the renal hemodynamics in the presence of the AT1-AAs during pregnancy has not been examined. Thus the objective of this study was to examine the changes in the glomerular filtration rate (GFR) and renal blood flow (RBF) during pregnancy in the presence of AT1-AAs and/or ANGII. **Methods:** Pregnant Sprague Dawley rats were divided into 4 groups: Normal Pregnant (NP, n=6), Pregnant + ANG II (Preg + ANG II, n=6), Pregnant + AT1-AA (Preg + AT1-AA, n=8), and Pregnant + ANG II + AT1-AA (Preg + ANGII + AT1-AA, n= 6). On day 13 of pregnancy, rats were implanted with mini-pumps infusing ANG II (50 ng/kg/min) and/or AT1-AA (1:40 dilution). On day 19 of pregnancy, rats were subjected to terminal renal function surgeries using FITC labeled Inulin. During the surgery, the

BP was recorded and a transonic flowmeter probe was placed on the left renal artery to measure RBF. **Results:** BP was elevated in all pregnant rats administered ANG II and/or the AT1-AA. Although GFR was reduced, it was not significant between Preg + ANG II and Preg + AT1-AA vs. NP rats (1.5 ±0.24, 1.60 ±0.17 vs. 1.90 ±0.16 ml/min). However, the GFR was further decreased in Preg + ANGII + AT1-AA rats (1.20 ±0.08). No difference was observed with the RBF between Preg + ANG II and Preg + AT1-AA vs NP rats (14.4 ±2.96, 14.4 ±1.48 vs. 15.4 ±1.75 ml/min). RBF was decreased in Preg + ANGII + AT1-AA vs NP rats (7.4 ±1.09 vs. 15.4 ±1.75 ml/min). No change in RVR between Preg + ANG II and Preg + AT1-AA vs. NP rats (9.7 ±2.69, 8.3 ±0.58 vs. 6.4 ±0.77). However, the RVR was drastically increased between Preg + ANGII + AT1-AA vs NP rats (18.4 ±2.91 vs. 6.4 ±0.77). **Conclusion:** Together ANG II and AT1-AA drastically decreases renal function by 37%, RBF by 50%, and caused a 3 fold increase in RVR vs NP rats. These data indicate the importance of AT1-AAs to drastically enhance ANG II induced renal vascular sensitivity and reduce renal function during preeclampsia. Research Supported by T32HL105324 and RO1HD067541.

15.8
A NOVEL, MASTER SWITCH FOR OVARIAN CYCLICITY: THE IMPACT ON CARDIOMETABOLIC HEALTH

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The increased risk for cardiovascular disease that follows menopause may reflect not only the cessation of ovarian hormone production but also previously unidentified, sex-specific factors whose presence or absence precipitates CVD. We identified a novel endogenous peptide called Phoenixin (PNX), which is robustly expressed in the hypothalamus where mRNA levels fluctuate during the estrous cycle. This plus our previous observation that knockdown of endogenous PNX levels using siRNA delayed the appearance of the next ovulatory event suggests a CNS site of action of PNX to control LH secretion. Here we describe our attempts to demonstrate the peptide's action to control GnRH release and identify its cognate receptor. Diestrous, female rats were administered saline, 1nmol, or 3nmol PNX i.c.v. for determination of plasma LH levels. We observed a significant dose-related increase in plasma LH levels suggesting that even under low estrogen conditions, PNX acts in CNS to activate the hypothalamo-pituitary-gonadal axis. We identified the orphan G-protein coupled receptor GPR173 as our top PNX receptor candidate. In a mouse pituitary cell line pretreated with a control siRNA, cFos mRNA expression increased upon PNX exposure. However, cells pretreated with siRNA against GPR173 exhibited an abrogated PNX response. This provided evidence of a potential interaction of PNX with GPR173 in vitro. We then tested whether siRNA mediated compromise of central GPR173 expression would also result in impaired estrous cyclicity. Female rats were treated i.c.v. with either siRNA against GPR173 or GFP as a control followed by daily monitoring of vaginal cytology. Rats administered GFP siRNA displayed the typical 4-day cycle, but animals given GPR173 siRNA exhibited a significant delay in the appearance of estrous with an average cycle length of 9 days. Taken together, these findings are the first to identify an interaction between GPR173 and PNX to regulate estrous cyclicity and therefore a potentially new target for treating ovulatory dysfunction. Age of onset and age of cessation of menstrual cycles are both correlated with increased risk of cardiovascular and metabolic disease. We hypothesize that PNX, by determining the onset and perhaps even cessation of ovulatory cycles may be an important target for the treatment of sex-specific disease risk, particularly under conditions such as primary amenorrhea, constitutional delay or premature onset of puberty, and menopause itself.

15.9
BLOOD PRESSURE RESPONSES TO ISOMETRIC HANDGRIP EXERCISE AND POST-EXERCISE ISCHEMIA IN WOMEN WITH A HISTORY OF HYPERTENSIVE PREGNANCY

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Objective: History of hypertensive pregnancy (HTNP) is considered a risk factor for cardiovascular disease. However, only some women with a history of HTNP become hypertensive later in life. An exaggerated blood pressure (BP) response to physical stressors (e.g. isometric handgrip and post exercise ischemia) is an independent marker of cardiovascular risk. Hence, the aim of the study was to compare BP responses to isometric handgrip exercise in normotensive women with a history of HTNP and women with a history of HTNP who are currently being treated for hypertension. **Methods:** Beat-to-beat BP (finger plethysmography) was recorded at rest and during first third phase, second third phase and final phase of isometric handgrip (HG) exercise (30% of maximal voluntary contraction) to fatigue in postmenopausal women (58 ± 1 years) with a history of HTNP. Isometric handgrip exercise was followed by 90 seconds of post-exercise ischemia on the exercising arm. BP was analyzed in three phases of 30 seconds each during cuff occlusion. **Results:** Women with a history HTNP women currently using anti-hypertensive medications (n=14) had a significantly higher rise in diastolic blood pressure (DBP) during the 1st and 2nd third of isometric HG (8 ± 1 and 12 ± 2 %) as compared to non-medicated (n=15) HTNP women (4 ± 1 and 8 ± 2%, p= 0.015 and p=0.036). Additionally, medicated women had a significantly higher rise in DBP during 1st cycle of cuff occlusion (12 ± 2%) as compared to non-medicated HTNP women (6 ± 2 %, p=0.028). Changes in systolic or mean arterial BP were not different between groups (p>0.05). **Conclusions:** These results identify differences in BP responses to physical stressors in women with a history of HTNP that are currently hypertensive versus normotensive. These data suggest the presence of two distinct phenotypes in women with a history of HTNP, which may be identified by the presence or absence of an altered muscle chemoreflex response along with an increased peripheral vascular resistance. Further investigation is needed to evaluate if these changes can be primarily attributed to a history of HTN pregnancy and how this affects overall cardiovascular risk. Funding: NIA 1P50AG044170-01, CTSA UL1 TR000135, HL 118154, HL83947.

15.10
UP-REGULATION OF VEGFR2 IMPROVES UTERINE ARTERY MYOGENIC RESPONSE AND MATERNAL HYPERTENSION ALTERED BY UTERINE PERFUSION RRESSURE REDUCTIONS

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Treatment options for the hypertensive disorder of pregnancy, preeclampsia (PE), remain limited. An imbalance in angiogenic factors (via the VEGF pathway) favoring a vasoconstrictory phenotype and inadequate vascular remodeling contribute to the maternal hypertension. Previous studies of VEGF signaling pathway have focused on either the soluble form of the VEGF receptor, or modifying VEGF release and/or production. The current study is unique in that it focuses on the VEGFR 2 receptor in the uterine vasculature. We hypothesize that increased uterine vasculature VEGFR2 receptor will improve uterine vascular behavior, maternal hypertension, and growth restriction in pregnant rats with PE pathology induced by surgical reductions in uteroplacental perfusion (RUPP). VEGFR 2 receptors are upregulated by a novel non-viral gene delivery system using L-tyrosine polyphosphate (LTP) nanoparticles (NP) seeded with DNA plasmid for VEGFR2. For the RUPP model, on day 14 of gestation, silver clips are placed on the abdominal aorta (0.2mm i.d.) and the utero-ovarian arteries (0.1mm i.d.) in pregnant Sprague-Dawley rats. SHAM rats undergo surgery without clip placement. On the same day as RUPP surgery, LTP nanoparticles (0.032673ug pDNA/ 2.5mg NP) are injected into the uterine wall. On day 21 of gestation an anesthetized blood pressure is measured via carotid catheter then resistance-sized uterine arteries (~ < 300μm) are harvested for study in an isobaric arteriograph. Uterine arteries from RUPP dams (n=8) display increased constriction to intraluminal pressure increases compared to SHAM pregnant rats (n=7; p<0.05). VEGFR2 LTP nanoparticle injection normalized the myogenic response in RUPP uterine arteries (p<0.05) so that the responses are similar to responses in arteries from SHAM rats. Maternal mean arterial pressure (MAP) is also normalized by VEGFR2 LTP injection. MAP is reduced from 99.0 mm Hg ±4.6 (RUPP) compared to 71.8±4.5 mmHg in injected RUPP dams (p<0.05). Finally, injection of VEGR2 LTP nanoparticles significantly increased fetal weights in RUPP to 4.8 ±0.72g vs. 2.85 ± 0.5g; p<0.05). In conclusion, uterine injection of LTP nanoparticles with DNA plasmid encoding for VEGFR2 improved the uterine arterial myogenic responsiveness, maternal blood pressure and fetal weights in RUPP animals. These data suggest a novel gene therapy to treat preeclamptic mothers and emphasize the importance of VEGFR2 receptor.

15.11
EFFECTS OF HIGH-SUCROSE DIET ON BLOOD PRESSURE REGULATION DURING PREGNANCY IN RATS

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While obesity increases the risk for developing preeclampsia, which is new-onset hypertension during pregnancy, the mechanisms are unclear. Although adverse diets such as high sucrose are thought to contribute to hypertension, human and animal studies have failed to demonstrate that high sucrose affects blood pressure during pregnancy. This could be due to the lack of high sucrose to produce frank obesity. However, it is unknown whether body weight, for example segregation of lower vs. higher body weights even within the normal weight range, is important <A name=_GoBack data-listid></A>to consider when examining blood pressure during high sucrose feeding in pregnancy. Therefore, we tested the hypothesis that higher vs. lower body weight status during a high-sucrose diet is accompanied by high blood pressure in pregnancy. Female Wistar hannover rats were started on a high-sucrose diet (HSD, 65% sucrose) or a controlled normal-sucrose diet (NSD, 5% sucrose) at 6 weeks old; time timed-pregnant rats generated at 17 weeks old; followed by assessment of mean arterial blood pressure (MAP) and pregnancy weights at gestational day (GD)19 while being maintained on respective diets. Maternal body weights at GD19 were segregated as lower (L) or higher (H) than the median for respective NSD (370g) and HSD (348g) groups. This resulted in 4 experimental groups: NSD-L (N=5), NSD-H (N=3), HSD-L (N=4) and HSD-H (N=5). Maternal body weights were greater (P=0.0002) in NSD-H and HSD-H (378±3 vs. 355±4g, respectively) over the NSD-L and HSD-L groups (345±7 vs. 325±12g, respectively). Body weight was greater in NSD-H than HSD-H (P<0.05). Visceral adipose tissue mass was greater (P=0.002) in the NSD-H and HSD-H groups (8.2±0.4 vs. 6.6±0.7g, respectively) than NSD-L and HSD-L groups (5.9±0.2 vs. 4.7±0.8g, respectively). Most interestingly, MAP was greatest (P<0.05) in HSD-H (120±2mmHg) over HSD-L (108±1mmHg) and NSD-H (113±1 mmHg, which was similar to NSD-L at 111±2mmHg). Fetal weights (g: NSD-L: 1.90±0.05, NSD-H: 1.94±0.04, HSD-L: 2.04±0.07, HSD-H: 1.92±0.1) and placental weights (g: NSD-L: 0.46±0.04, NSD-H: 0.54±0.02, HSD-L: 0.53±0.05, HSD-H: 0.57±0.0.3) were similar between all groups. These data suggest that the hypertensive response to HSD during pregnancy maybe dependent on the presence of increased body weight and visceral adiposity. In conclusion, pregnant women with higher body weight and visceral adiposity combined with an adverse diet may predict those most likely to develop hypertension during pregnancy.

15.12
MECHANISMS OF RENAL AND COLONIC POTASSIUM RETENTION DURING LATE PREGNANCY

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The fetus requires a large amount of potassium (K+) for normal development. To accommodate this need the normal pregnant rat accumulates considerable K+ over the course of gestation, most of which is retained during late pregnancy. This gestational K+ retention is essential for fetal development but the mechanism is unknown. The purpose of this study was to examine how renal and colonic K+ handling change in pregnancy in the setting of high circulating aldosterone and enhanced sodium reabsorption. We measured dietary K+ intake and urinary K+ excretion. K+ intake increased in MP and LP vs V (4.6±0.1, 5.2±0.1 vs 3.3±0.1 meq/24h, p<0.05) while renal K+ excretion also rose (4.3±0.1, 4.6±0.1 vs. 3.0±0.2 meq/24h, p<0.05). We also measured the mRNA expression of BK, ROMK, H+/K+ - ATPase type 1 (HKA1), H+/K+ - ATPase type 2 (HKA2), and H+-ATPase in the renal cortex, outer medulla, and inner medulla of virgin (V, n=6), mid pregnant (MP, n=6), and late pregnant (LP, n=6) rats using quantitative real-time PCR. We found an increase in HKA1 in the outer medulla in MP rats vs V and increased HKA2 expression in both cortex and outer medulla of LP rats vs. V. Furthermore, ROMK expression decreased in the inner medulla of MP and LP rats compared to V. BK mRNA increased in outer medulla and decreased in inner medulla at MP, and increased in cortex at LP. The expression level of the other genes tested did not differ with pregnancy stage. Although ROMK mRNA was unchanged in the CTX and decreased in the IM, the abundance detected by immunofluorescence was increased in both MP and LP vs V in the cortex and not different in the medulla. In the distal colon we found a fall in BK mRNA at MP, an increase in the HKA2 mRNA at LP and an increase in distal colon HKA2 protein abundance by western blot; HKA2 protein abundance was too low to be detected in kidney, even in LP rats. During pregnancy the kidney is receiving mixed signals with respect to K+ handling, with the changes in apical sodium channels/transporters and ROMK promoting K+ secretion, and the changes in HKA1 and HKA2 promoting K+ retention. Therefore the K+ retention of pregnancy is likely due both to increased collecting duct K+ reabsorption (via increased HKA1/HKA2) offsetting the increased K+ secretion, as well as increased colonic reabsorption via HKA2. Future studies will determine the signaling pathways involved in these mechanisms.

15.13
IMPAIRED FLOW-MEDIATED DILATION BEFORE, DURING AND AFTER PREECLAMPSIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Endothelial dysfunction is believed to play a critical role in preeclampsia, however it is unclear whether this dysfunction precedes the pregnancy or is caused by early pathophysiological events. It is also unclear whether vascular dysfunction resolves post-partum, or may be one mechanism linking preeclampsia with future cardiovascular disease. **Objective:** to determine whether women with preeclampsia, examined before, during and after a preeclamptic pregnancy, have worse vascular function compared to women who did not have preeclampsia. Vascular dysfunction was assessed by flow-mediated dilation (FMD). **Methods and Results:** We performed a systematic review and meta-analysis of studies examining FMD before, during and after preeclampsia published before January 27, 2015. Differences in FMD were evaluated by standardized mean differences. We searched 595 abstracts identified through PubMED, EMBASE and Web of Science. 32 studies were eligible for the meta-analysis. When compared to women who did not have preeclampsia, women who had preeclampsia had lower FMD prior to the development of preeclampsia (~20-29 weeks gestation), at the time of preeclampsia, and for three years post-partum. The estimated magnitude of the effect ranged between 0.5 and 3 standard deviations. Although statistically significant, the estimated effects had wide confidence intervals due to high heterogeneity. These differences were no longer evident by 10 years post-partum. **Conclusions:** Compared to women who do not develop preeclampsia, women who develop preeclampsia have worse vascular function from 20 weeks gestation until 3 years post-partum. This meta-analysis may over-estimate the effects of preeclampsia, as the small, observational studies included have a high risk of bias.

16.0 DEVELOPMENTAL PROGRAMMING

16.1
VENDOR-SPECIFIC EFFECT ON SEX DIFFERENCES IN THE DEVELOPMENTAL PROGRAMMING OF BLOOD PRESSURE IN THE SPRAGUE DAWLEY RAT

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Our laboratory uses a well-established model of intrauterine growth restriction (IUGR) induced by placental insufficiency that programs a sex difference in blood pressure (BP) in the Sprague Dawley (SD) rat. IUGR is induced by reduced uterine perfusion (RUP) initiated at day 14 of gestation in timed pregnant rats purchased from Harlan. Previously we reported that male IUGR rats exhibit hypertension at 16 weeks of age associated with a two-fold increase in testosterone relative to male control from sham operated dams whereas female IUGR rats remain normotensive. Hypertension is abolished by castration suggesting that IUGR programs a testosterone-dependent increase in BP in male IUGR. However, BP is significantly increased following ovariectomy (OVX) in female IUGR implicating estrogen is protective. Thus, these studies indicate that sex hormones play a vital role in BP control in Harlan SD IUGR rats. The aim of this study was to determine if the commercial vendor impacts the developmental programming of BP. Timed pregnant SD rats from Charles River underwent either RUP or sham surgery at day 14 of gestation. Birth weight was significantly reduced in male and female IUGR relative to same-sex controls (P<0.05). At 10 weeks of age animals underwent measure of body composition before and 6 weeks after gonadectomy or sham surgery. Prior to gonadectomy total fat mass did not differ between IUGR and control (Males: 26±3 vs.36 ±7g and Females: 19±3 vs. 17±3g; IUGR vs. control, respectively). However, OVX resulted in a significant increase in total fat mass in IUGR and control relative to intact (OVX: 45±6 vs. 37±5g and Intact: 20±3 vs. 22±2g; P<0.05, IUGR vs. control, respectively) while CTX had no effect on fat mass in male (data not shown). Baseline BP measured in conscious, chronically instrumented rats at 16 weeks of age did not differ in intact male control relative to intact male IUGR (137±3 vs. 137±3mmHg) or intact female control relative to intact female IUGR (119±5 vs. 123±4mmHg). Testosterone levels were not elevated in male IUGR versus male control; gonadectomy did not alter BP in IUGR rats relative to same-sex intact control (data not shown). Thus, these results suggest

that vendor-specific differences in the SD rat abolish the developmental programming of sex differences in BP and eliminate the effect of testosterone and estrogen on BP control in the IUGR rat. Dasinger: AHA 15PRE24700010; Alexander: HL074927, AHA GRNT19900004, P01-HL51971, GM104357.

16.2
IS THERE A SEX DIFFERENCE BETWEEN HYPERTENSION RISK AND LOW BIRTH WEIGHT IN HEALTHY YOUNG JAPANESE ADULTS?

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Low birth weight (LBW) was confirmed as a risk of high blood pressure (BP) in later stages of life. Low-grade inflammation and deterioration of autonomic regulation play an important role in hypertension. However, the association between birth weight and hypertension are poorly understood. We examined this association in healthy young Japanese adults, and investigated whether the relationship between LBW and hypertension risk factors differs between men and women. We measured the BP and heart rate variability at rest and during postural change from a supine to a sitting position in 26 healthy Japanese volunteers aged 18–23 years. Blood cell counts and levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, triglyceride (TG), and high sensitivity C-reactive protein were measured. Men were taller ($p < 0.01$), weighed more ($p < 0.01$), had a higher resting BP ($p < 0.01$), and had higher TG levels ($p < 0.05$) and lower HDL-C levels ($p < 0.05$) compared to women. In men, the HDL-C levels were lower in the LBW group compared to the normal birth weight (NBW) group ($p < 0.05$). In contrast, there were no significant differences in women considering any of the hypertension risk factors between the LBW and NBW groups. After the postural change, systolic blood pressure (SBP), diastolic blood pressure, and heart rate showed no significant increases in the LBW, whereas the NBW group had normal responses ($p < 0.01$). Women displayed an increase in SBP immediately after sitting ($p < 0.05$) and a decrease in SBP in the sitting position ($p < 0.01$), although no significant responses were observed in men. Similar to the results of earlier studies, our results showed that healthy young men have lower HDL-C and higher TG levels compared to healthy young women. Our results also show that healthy young men with a LBW have lower HDL-C levels compared to their counterparts with a normal birth weight. In addition, among healthy young Japanese adults, men may be less sensitive to postural changes in BP compared to women. In conclusion, we found that sex differences exist between LBW and hypertension risks in healthy young Japanese adults.This work was supported, in part, by a Grant-in-aid for Scientific Research (B) (25305018) from the Japan Society for the Promotion of Science.

16.3
SEX DIFFERENCES IN HIGH FAT DIET-INDUCED ADIPOCYTE MORPHOLOGY AND FAT DISTRIBUTION DUE TO EARLY LIFE STRESS

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Epidemiological studies indicate that adults exposed to early life stress (ELS) are at an increased risk of developing cardiometabolic disease. Previously, we have reported that females exposed to maternal separation (MSep), an established behavioral stress model, are glucose intolerant with no differences found in males. The aim of this study was to investigate the effect of ELS on adipocyte morphology and fat distribution. C57BL/6 mice were separated for 4 hours/day from postnatal day 2-5 and 8 hours/day from PND6 to 1 6 with early weaning at day 17. Normally reared litters serve as control (C). Upon weaning, mice were placed on a low-fat diet (LFD, 10% kcal from fat n=6-10) or high-fat diet (HFD, 60% kcal from fat, n=10) for 16 weeks. Although MSep did not lead to changes in body weight on mice fed a LFD, both male and female mice exposed to MSep gained a significant amount of weight on a HFD; however, this weight gain was greater in females. EchoMRI revealed that this increase in body weight was due to increased adiposity in both genders. Although male MSep mice showed significantly elevated fat mass through week 12, no differences were observed at week 16 compared to C (22.4± 0.8 vs.20.1 ± 0.8 g, respectively). Female MSep mice showed exaggerated adiposity beginning at week 4 that persists through the end of the study (18.2 ± 1.4 vs 7.6 ± 0.1 g, $p<0.01$). Because of these changes in adiposity, we examined morphological parameters from gonadal white adipose tissue in both sexes. Male MSep mice fed a LFD have elevated cell area (3268 ± 208vs. 2643± 141 μM^2, $p<0.05$) whereas no differences were present in females fed a LFD. Interestingly, when females were fed a HFD, MSep led to hypertrophy of adipocytes (6011± 503 vs. 4255± 282 μM^2, $p<0.05$) with increases in cell diameter (77.2 ± 4 vs. 64.8 ± 2.8 μM, $p<0.05$) compared to C; however, no differences were observed in male MSep cell area (2758.8 ± 190.1 vs 2863.9 ± 170 μM^2) or cell diameter (51.7 ± 2 vs 53.3 ± 1.9 μM) compared to C fed a HFD. Magnetic resonance spectroscopy revealed that female MSep mice fed a HFD have elevated levels of visceral fat compared to C (1550.7 ± 233.5 vs. 904.1 ± 104.5.6 mm^3, $p=0.08$) with no differences in subcutaneous levels. In addition, MSep increases serum cholesterol levels in both genders fed a HFD; however, MSep male mice display a greater response compared to MSep female mice ($p<0.05$). ELS worsens the fat morphology in female mice whereas it disturbs the lipid metabolism in male mice. These data suggest that the mechanisms by which ELS affects fat partitioning and adipocyte biology as well as cholesterol levels are sex-specific.

16.4
SPHINGOSINE-1-PHOSPHATE RECEPTOR TYPE 3 PLAYS A ROLE IN THE ETIOLOGY OF HIGH BLOOD PRESSURE PROGRAMMED BY INTRAUTERINE GROWTH RESTRICTION IN THE MALE BUT NOT THE FEMALE MOUSE

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Intrauterine growth restriction (IUGR) is a risk factor for hypertension and cardiovascular (CV) disease in later life, but the underlying mechanisms remain unclear. The bioactive sphingolipid metabolite sphingosine-1-phosphate (S1P) is critically involved in CV development in the fetus, and plays a significant role in the regulation of CV health in adulthood. S1P receptor (S1PR) type 1, 2 and 3 are widely expressed in CV system and S1PR3 is involved in the control of blood pressure (BP). We previously reported in IUGR induced by reduced uterine perfusion (RUP) in the mouse programs a significant increase in BP in male IUGR mice but not in female IUGR mice as compared to same-sex control counterparts. Hypertension in male IUGR is attenuated by the S1P receptors agonist. Yet, whether regulation of S1PR3 expression is sex-specific following IUGR is unknown. In the present study we tested the hypothesis that IUGR programs sex-specific renal expression of S1PR3 in IUGR mice. C57bl/6J mice underwent sham or RUP at day 13 of gestation with delivery at full term. IUGR offspring (from RUP dams) had a lower birth weight than control ($P<0.05$). Kidneys were isolated from 24 week old control and IUGR offspring after measure of BP. Male IUGR offspring had a significantly higher BP compared to male control via carotid catheter in the conscious state (control: 112.1±2.1, IUGR: 125.0±3.7 mmHg; N=7, $P<0.05$). MAP did not differ between female control and female IUGR (control: 113.8±3.8, IUGR: 117.8±2.8 mmHg; N=5). Kidney weight per body weight was not different between control versus IUGR same-sex counterparts. Renal S1PR3 gene expression levels were increased (2.5 fold vs. control, N=4, $P < 0.05$) whereas S1PR3 protein levels were decreased (0.75 fold vs. control, N=4, $P < 0.05$) in male IUGR. Renal gene and protein S1PR3 expression levels were not different between female control and female IUGR. Together our data suggest that IUGR programs a sex-specific alteration in renal S1PR3 expression which may contribute to an increase in BP programmed only in male IUGR but not female IUGR mice Thus, S1PR3 signaling is a potential putative mechanism underlying the sex-specific hypertension of IUGR mouse offspring. Dr. Intapad is supported by funding from NIH P20GM104357.

16.5
REDUCED SLEEP TIME DURING PREGNANCY EFFECTS ON RENAL MORPHOLOGY AND FUNCTION OF FEMALE OFFSPRING

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The shortening of sleeping time has become common in modern society. This alteration has been associated to several changes such as reduced glucose tolerance, increased blood pressure, and changes in hormonal pathways. Considering that changes in maternal environment may result in changes in the offspring, as shown in male offspring from different models of fetal programming, the aim of this study was to evaluate renal morphology and function of female offspring from rats sleep restricted during the last week of pregnancy. Methods: After confirmation of pregnancy, Wistar rats were divided into two groups: control and sleep restricted. Sleep restriction was performed between 14th and 20th day of pregnancy (20 hours/day). After birth, offspring was designated as C (control) and SR (sleep restricted). At two months, half of these were subjected to ovariectomy and the others to sham surgery. The groups were then designated Sham (CS and SRS) or ovariectomized (CO and SRO) and studied at 8 months of age. The parameters analyzed were: systolic blood pressure (BP), createnine clearance (CrCl), sodium excretion (ENa+), glomerular area (GA), number of glomeruli per field (NG), kidney cross-section area (KA) and kidney mass (KM). The results are shown as mean± SEM and number of measurements between parenthesis; Anova, $p \leq 0.05$. The SR groups presented increased BP [CS: 125±0.7(17); CO:

131±0.8 (17); SRS: 131±0.5(26); SRO: 138±0.55(26) mmHg] and decreased CrCl [CS: 4.6±0.3(17); CO: 4.1±0.3(17); SRS: 3.4±0.2(26); SRO: 2.5±0.2(26) ml/min/kg] compared to CS. The SRO group presented reduced ENa+ [CS: 1.1 ±0.05(17); CO: 1.1±0.06(17); SRS: 1.0±0.05(26); SRO: 0.9±0.07(26) mM/24h] compared to CS. Regarding the morphological parameters there was in RSO significant reduction in: GA [CS: 7936±106; CO: 7654±104; SRS: 7831±108; SRO: 7365±492 μm^2]; KA [CS: 104.6±2(6); CO: 97±2(6); SRS: 100.4±1(6); SRO: 90.3±2 (6) mm^2] and in KM [CS: 0.7±0.02(17); CO: 0.6±0.01(17); SRS: 0.7±0.01(26); SRO: 0.5±0.08(26) mg/100g bw]. Conclusion: Our data suggest that sleep restriction during pregnancy causes increased BP and changes in renal morphology and function of female offspring at adulthood. Moreover, ovariectomy aggravates the alterations in blood pressure and renal function, confirming the role of female sex hormones in regulation of arterial pressure and renal function during adult life. Financial support: FAPESP.

16.6
DELAYED EFFECTS OF PERINATAL HYPOXIA ON ADULT RATS PULMONARY VESSELS STRUCTURE AND REACTIVITY

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Perinatal hypoxia (PH) induces irreversible changes of lung circulation (2). Pulmonary vessels of adult rats that had been exposed to PH (1 wk before and 1 wk after birth, 12% O_2) and then lived in normoxia are more compliant and their vasoconstrictor response to acute hypoxia is increased. Adult females, but not males, with the perinatal experience of hypoxia have right ventricle hypertrophy. However, pulmonary arterial pressure of either male or female rats did not differ from that of controls. We also did not detect low molecular weight cleavages in the extracts of collagenous proteins from prealveolar pulmonary vessels typical for hypoxic pulmonary hypertension (3). The presence of right ventricle hypertrophy only in females led us to question the role of sexual hormones. Rats exposed to PH were therefore gonadectomized as newborns (1). Pulmonary arterial pressure was elevated in adult perinatally hypoxic, neonatally gonadectomized females (24.4 ±1.7 torr) but not males (17.2 ± 0.6 torr). In perinatally hypoxic, neonatally gonadectomized males the muscularization of peripheral pulmonary blood vessels (a reliable structural marker of pulmonary hypertension) in adulthood was greater than in intact, perinatally normoxic male controls. In gonadectomized females born in hypoxia the muscularization of prealveolar arteries was increased even more (5 times). Gonadectomy performed in adulthood did affect neither pulmonary vascular structure nor lung hemodynamics. Female pulmonary circulation is therefore more sensitive to the late effects of perinatal hypoxia, and these effects are blunted by the presence of ovaries during maturation. Because pulmonary vascular reactivity depends on transmembraneous K^+ current, we tested the response of pulmonary vasculature of male and female rats exposed to PH to K^+. In contrast to males, PH females have higher basal perfusion pressure and reactivity to K^+ than control females. The different effects of PH in male and female rats may result from different expression and/or activity of K+ channels. Supported by GACR 13-01710S and IGA NT/13358. References: 1. **Hampl V et al.** *Am J Physiol Lung Cell Mol Physiol* 285: L386-392, 2003. 2. **Hampl V, and Herget J**. *Am Rev Respir Dis* 142: 619-624, 1990. 3. **Novotná J, and Herget J**. *Life Sci* 62: 1 - 12, 1998.

16.7
SEX DIFFERENCE IN SENSITIZATION OF ANGIOTENSIN (ANG) II-ELICITED HYPERTENSION IN OFFSPRING OF HYPERTENSIVE PREGNANT RATS

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Recent studies demonstrate that there is association between maternal health status during pregnancy and cardiovascular disease of adult offspring. The present study test whether maternal hypertension produced by angiotensin (ANG II) infusion (250 ng/kg/min, sc) during pregnancy sensitizes ANG II-induced increase in blood pressure (BP) in adult offspring, and whether there are sex differences. Aortic BP and heart rate (HR) were measured in dams and their offspring by telemetry. When tested beginning at 10 weeks of age, male offspring of hypertensive dams showed an enhanced hypertensive response to sc ANG II (120 ng/kg/min, Δ41.6±2.6 mmHg) compared to male offspring of normotensive dams (Δ17.1±1.3 mmHg). In females, ANG II treatment produced only a slight, but significant increase in BP in offspring of either hypertensive (Δ7.5±2.5 mmHg) or normotensive dams (Δ11.6±2.5 mmHg). RT-PCR analysis of the lamina terminalis and the paraventricular nucleus tissues indicated upregulation of mRNA expression of renin-angiotensin-aldosterone system (RAAS) components and proinflammatory cytokines, including renin, angiotensinogen, mineralocorticoid receptor, interleukin (IL)-6 and IL-1β in male, but not female, offspring from hypertensive dams. The results suggest that maternal hypertension during pregnancy enhances pressor responses to ANG II through upregulation of the brain RAS and inflammatory cytokines in male offspring, and that female offspring are protected from these effects.

16.8
SEX DIFFERENCES IN CARDIOVASCULAR RESPONSES TO STRESS IN ADULT RATS PRENATALLY EXPOSED TO DEXAMETHASONE

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It is well known that even transient prenatal insults can impact cardiovascular function in adulthood. We have hypothesized that adult cardiovascular disease may have its origins *in utero* as a result of exposure to elevated levels of glucocorticoids. In support of this, we have shown that when pregnant rat dams are treated with the glucocorticoid, dexamethasone (DEX), for the last 4 days of gestation, female-specific changes in metabolism, core body temperature, autonomic function, depression-, and anxiety-like behaviors are detected in their adult offspring. The present study investigated the impact of prenatal DEX on arterial pressure and cardiovascular responses to stress in adult male and female offspring. Pregnant dams were administered DEX (0.4mg/kg per day, s.c.) or vehicle on gestation days 18-21. This resulted in a significant reduction in birthweight in DEX-exposed males and females. At 2-3 months of age, arterial pressure was assessed via radiotelemetry. Baseline pressures were collected for 3 days in males and for 7 days in females to evaluate blood pressure throughout the estrous cycle (determined by vaginal lavage). In order to assess whether prenatal DEX alters stress-induced hypertensive and tachycardic responses, rats were placed in a restraint tube for 20 minutes, followed by a 2 hour recovery period. Restraint-stress testing was performed on diestrus in females. In male rats, prenatal DEX had no impact on arterial pressure under basal conditions or in the elevations that occurred in response to restraint stress. In contrast, the systolic blood pressure in DEX-exposed females was ~10% below that of vehicle-exposed offspring throughout the estrous cycle. No treatment differences were observed in basal diastolic pressure or heart rate. However, prenatal DEX exposure resulted in an exaggerated hypertensive and tachycardic response to restraint stress (Peak percent increases over baseline: SBP: Veh 18% vs. DEX 35%, $p<0.05$; DBP: Veh 20% vs. DEX 50%, $p<0.05$; HR: Veh 20% vs. DEX 56%, $p<0.05$). Moreover, the time to return to baseline pressures and heart rate was longer in female rats prenatally-exposed to DEX. Taken together, these findings reveal sex-specific differences in the prenatal programming of stress-induced hypertension and further support a role for elevated glucocorticoids in development as an origin for cardiovascular disease states in females. Funding: NIH 5 P50 MH082679 and AZ Biomedical Research Commission ADHS14-082990.

17.0 AGING AND MENOPAUSE

17.1
PREHYPERTENSION AND ENDOTHELIAL FIBRINOLYTIC FUNCTION IN MIDDLE-AGED WOMEN

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Prehypertension (systolic blood pressure: 120-139 mmHg and/or diastolic blood pressure: 80-89 mmHg) is prevalent in ~30% of US adults and is associated with increased atherothrombotic vascular disease risk. We recently demonstrated that the capacity of the endothelium to release tissue-type plasminogen activator (t-PA) is markedly blunted in middle-aged men with prehypertension. Endothelial t-PA release is the primary endogenous defense mechanism against thrombus formation. Interestingly, the capacity of the endothelium to release t-PA has been shown to be significantly higher in middle-aged women compared with men, conferring greater cardiovascular protection. It is currently unknown whether prehypertension is associated with diminished endothelial t-PA release in women. Accordingly, we tested the hypothesis that, similar to men, blood pressure in the prehypertensive range is associated with reduced endothelial t-PA release in middle-aged women. Thirty-four sedentary, non-obese, post-menopausal, middle-aged women were studied: 17 normotensive (age: 57±1 yr; BMI: 26.1±0.8 kg/m^2; BP: 105/66±2/2 mmHg) and 17 prehypertensive (age: 56±1 yr; BMI: 26.6±1.0 kg/m^2; BP: 130/79±1/2 mmHg). All women were at least one year post menopause, not taking hormone replacement, and free of overt cardiometabolic disease. Net endothelial release of t-PA was determined, in vivo, in response to intrabrachial infusions of bradykinin (BK: 125-500 ng/min) and sodium nitroprusside (SNP: 2-8 μg/min). Basal and stimulated endothelial t-PA release was not significantly different between the groups. t-PA release increased simi-

larly in the normotensive (from 0.6±0.7 to 56.9±7.6 ng/100 mL tissue/min) and prehypertensive (from 0.6±1.1 to 54.9±8.5 ng/100 mL tissue/min) groups to incremental doses of BK. In fact, total t-PA release (area under the BK curve) was almost identical between the normotensive (284±46 ng/100 ml tissue) and prehypertensive (273±46 ng/100 ml tissue) groups. There was no effect of SNP on t-PA release in either group. In summary, contrary to our hypothesis, prehypertension does not adversely influence endothelial t-PA release in middle-aged women. Impaired fibrinolytic function does not appear to contribute to the increase in vascular risk with prehypertension in middle-aged women. Our findings suggest that mechanisms underlying prehypertension-related vascular risk may differ between women and men.

17.2
GENDER DIFFERENCES IN CIRCULATING MICROPARTICLES IN MIDDLE-AGED ADULTS

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The incidence of coronary heart disease (CHD) and stroke is ~50% higher in men compared with women between the ages of 45 and 65 years. The mechanisms responsible for the gender-related difference in cardiovascular disease risk are not completely understood. We and others have reported profound gender-related differences in vascular endothelial function in middle-aged adults. Clinical interest in circulating microparticles (MPs) has increased due to their putative role in inflammation, vascular health and cardiovascular disease (CVD). MPs are small vesicles (0.1-1μm) formed by the outward blebbing of the cellular plasma membrane and released into circulation by a variety of cell types. Circulating MPs originating from platelets (PMPs), endothelial cells (EMPs), monocytes (MMPs) and leukocytes (LMPs) are now recognized as biomarkers of vascular injury and are predictive of vascular events. There is currently a paucity of information regarding the influence of gender on circulating MPs. The aim of this study was to determine whether circulating PMPs, EMPs, MMPs and LMPs differ in middle-aged men compared with women. If so, this may contribute to gender-related disparity in CVD in middle-aged adults. Thirty healthy, sedentary, non-obese, middle-aged adults were studied: 16 males (age: 57±2 yr; BMI: 25.3±0.5 kg/m^2) and 14 females (age: 55±1 yr; BMI: 24.5±0.7 kg/m^2). All women were at least 1 year postmenopausal and not taking hormone replacement therapy. Circulating MPs were measured in platelet free plasma from peripheral blood samples. Cellular lineage was identified by flow cytometry utilizing cellular specific antibodies: PMPs (CD31$^+$/CD42b$^+$), EMPs (CD31$^+$/CD42b$^-$), MMPs (CD14$^+$) and LMPs (CD14$^-$). Circulating PMPs were ~200% higher (P<0.05) in females (111±28 MP/μL) compared with males (37±8 MP/μL). However, there were no significant gender-related differences in circulating EMP (393±66 vs. 388±41 MP/μL), MMP (237±53 vs. 266±50 MP/μL) or LMP (38±10 vs. 37±9 MP/μL) concentrations between the women and men. These results indicate that aside from PMPs, there is no influence of gender on circulating EMPs, MMPs or LMPs in middle-aged adults.

17.3
FOREARM VASCULAR CONDUCTANCE RESPONSES TO TERBUTALINE, A B_2-ADRENERGIC RECEPTOR AGONIST, DIFFER IN PREMENOPAUSAL VERSUS POSTMENOPAUSAL WOMEN

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Beta-adrenergic vasodilator responses are blunted in men at risk for developing hypertension; however, the role of β-adrenergic receptors in hypertension pathophysiology in women is unclear. It is possible that older, postmenopausal women, who have a greater hypertension risk in comparison to young, premenopausal women, have altered β_2-adrenergic receptor responsiveness. The goal of this study was to determine if forearm vascular responses to the β_2-selective, adrenergic-receptor agonist terbutaline are blunted in healthy older, postmenopausal women (59±2 years) compared to healthy young, premenopausal women (28±3 years). Forearm blood flow (FBF, venous occlusion plethysmography) and mean arterial pressure (MAP, intra-arterial brachial catheter) were measured at baseline and during intra-arterial infusions of terbutaline at 0.1, 0.5, 1.0, and 2.0 μg/100 ml tissue/min. These women did not differ in body mass index or blood pressure. Baseline FBF was similar in premenopausal and postmenopausal women (2.2±0.4 vs. 2.0±0.5 ml/100 ml tissue/min, respectively; p>0.05) and rose significantly within each group at the highest terbutaline dose (10.7±2.1 vs. 7.1±1.9 ml/100 ml tissue/min, respectively; p<0.05); however, there were no FBF differences between the groups. Baseline forearm vascular conductance (FVC=FBF/MAP*100) was not different between groups (2.4±0.4 vs. 1.8±0.4 ml/100 ml tissue/min/mmHg; premenopausal vs. postmenopausal, respectively; p>0.05). Terbutaline infusion at the highest dose resulted in a significant increase in FVC in both premenopausal and postmenopausal women (12.0±2.5 vs. 6.9±1.9 ml/100 ml tissue/min/mmHg; respectively; p<0.05). The increase in FVC was greater in premenopausal women when compared with postmenopausal women (interaction of group x dose, p<0.05). These data provide evidence to support that β_2-adrenergic receptor responsiveness is blunted with aging and menopause in healthy women. Funded by AHA 14PRE18040000, NIH HL HL83947 and HL118154, and NCATS UL1 TR000135 (CTSA).

17.4
ET_A RECEPTOR ANTAGONISM PREVENTS ANG II-INDUCED HYPERTENSION IN VCD-TREATED POST-MENOPAUSAL FEMALE MICE

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The VCD model of menopause (4-vinylcyclohexene diepoxide, VCD) preserves the "perimenopause" transitional period and the androgen secreting capacity of the residual ovarian tissue. Using this model of menopause, we recently demonstrated that perimenopausal mice are resistant to Ang II-induced hypertension and displayed minimal changes in blood pressure and cardiac remodeling. In contrast, postmenopausal mice develop a significant Ang II-induced hypertension sensitivity (significant increase in SBP and MAP), along with renal hypertrophy and cardiac fibrosis. Endothelin (ET-1), signaling through ET_A receptors, has been shown to promote renal damage and Ang II hypertension in male rodents, while premenopausal females were protected. To determine whether ET_A receptor signaling contributes to the increased sensitivity to Ang II hypertension in VCD-treated postmenopausal female mice (Meno), Ang II (800ng/kg/min, 14d) was infused with or without daily injections of the ET_A receptor antagonist ABT-627 (5mg/kg, i.p.) (ET_A). Premenopausal females received sesame oil vehicle with and without Ang (C, C/Ang II). Ang II infusion induced a significant increase in systolic blood pressure in VCD-treated postmenopausal mice compared to Ang II infusion in premenopausal mice (Con Δ2 ± 2 mmHg, C/Ang II Δ15 ± 2* mmHg, Meno/Ang II Δ37 ± 6$^{*\#}$ mmHg, *P<0.05 vs Con, #P<0.05 vs C/Ang II). ET_A receptor antagonism prevented this increase in blood pressure in postmenopausal females (ET_A Δ14 ± 3$^\$$ mmHg, $^\$$P<0.05 vs Meno/Ang II). Quantitative real-time PCR demonstrated that whole kidney mRNA expression of collagen type IV was significantly reduced with ET_A receptor antagonism (Meno/Ang II 1.0 ± 0.07 vs 0.68 ± 0.08 in ETA treated, P<0.05). Together, these data suggest that ET-1 signaling, via ET_A receptor activation, promotes Ang II induced hypertension and renal damage in postmenopausal females. Targeting this system may be an effective strategy to treat postmenopausal hypertension.

17.5
MYOGENIC TONE IS INCREASED IN RESISTANCE-SIZED ARTERIES ISOLATED FROM RAT MODELS OF POST-MENOPAUSAL PHYSIOLOGY

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Women are at increased risk of heart attack and stroke after menopause. Estrogen replacement therapy is the remedy for the symptoms of menopause (hot flashes, etc.); however, the mechanism for the cardio-protection is not clear. The myogenic behavior of resistance sized arteries is an index of the balance between vasodilatory and vasoconstrictory pathways. The increased risk of heart attack and stroke in postmenopausal women suggests an increase in vasoconstrictor pathways or a decrease in vasodilatory mediators. In order to evaluate this we chose to examine the myogenic tone of resistance-sized arteries isolated from two different rodent models of post-menopausal physiology. The rodent models include the aged female SHR and the ovariectomized pair-fed Long-Evans rat (OVX). Over a range of pressures (20-100mmHg), the myogenic tone was greater in the septal coronary arteries isolated from aged SHR rat compared to young SHR control (i.e. 12.2±3.2% vs 7.5±2.1% at 60mmHg). Greater myogenic tone was also displayed in the posterior cerebral arteries isolated from the aged SHR. In the OVX model, myogenic behavior was increased in coronary, cerebral, and mesenteric resistance sized arteries. For example, the septal coronary arteries tone at 80mmHg was 17.7±4.1% in the ovex compared to 7.4±2.5% in the sham controls. In these arteries, we examined the role for vasodilatory pathways involving nitric oxide and endothelin B receptor (ETB). Nitric oxide inhibition further increased myogenic tone in coronary arteries from OVX rats. Myogenic tone increased to 41±7.4% at 80mmHg during nitric oxide inhibition with L-NMA. However, aortic eNOS expression was not significantly altered in either of the models compared to controls. ETB inhibition also increased myogenic behavior in the resistance sized coronary and mesenteric arteries isolated from OVX rats. For

example, the coronary arteries percent tone was increased to 28.9±13.7% tone at 60mmHg. In conclusion, myogenic tone is increased in resistance-sized coronary and cerebral arteries isolated from both models of post-menopausal physiology. Furthermore, in the OVX model, vasodilatory pathways involving ETB and nitric oxide remain intact. This work is supported by NIH R15 HL09734.

17.6
CIRCULATING STEROID HORMONES HAVE NO INFLUENCE ON THE CARDIOVASCULAR BENEFICIAL EFFECT IN TRAINED HYPERTENSIVE POSTMENOPAUSAL WOMEN

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Introduction: It has been demonstrated that the prevalence of arterial hypertension increases in women after menopause that has been associated with estrogen deficiency. On the other hand, estrogen administration did not protect women from cardiovascular diseases (CVD). In addition, evidence has shown that high testosterone levels are associated with an adverse cardiovascular risk factor after menopause. However, most of these data are from experimental model of menopause. It is well known that cortisol plays an important role in CVD. Nonetheless, the effects of this steroid hormone are not fully understood in the development of CVD in women. **Aim:** Therefore, the goals of the study were: 1) to examine testosterone and cortisol concentrations in hypertensive (HT) postmenopausal women comparing with normotensive (NT) group, at baseline; 2) to examine the effects aerobic exercise training (AET) on BP and steroid hormones in both groups. **Methods:** In order to test the hypothesis, serum testosterone (fasting) and cortisol concentrations (fasting and postprandial state) were measured in 28 HT (57±1 yrs) and 33 NT (56±1 yrs) women at baseline and after AET. Supervised AET was performed in a treadmill, moderate intensity, 30-40 min, three times/week, 24 sessions. This study has been approved by UNESP Ethics Committee (4395/2010). **Results:** At baseline, no differences were found in both testosterone (NT: 0.8±0.1 and HT: 0.76±0.1 nmol/L) and cortisol (NT: 464.9±28.7 and HT: 453.6±24.6 nmol/L) between the two groups, in fasting state. Cortisol concentrations were also similar between the two groups (NT: 142.4±14.0 and HT: 137.5±16.6 nmol/L) measured at postprandial. After AET, there were no significant changes on steroid levels in both groups in fasting state. However, in postprandial we found a similar decrease in cortisol concentration from trained NT (-41%) and HT (-35%) postmenopausal women. AET was also effective in lowering diastolic BP (-5%) in HT group, but not in NT. **Conclusions:** Our data show that both steroids hormone have no influence on BP regulation in postmenopausal women. Moreover, both groups respond equally to AET in lowering cortisol concentrations, but differently to BP reduction. Thus, our findings suggest that another signaling pathway is involved in the cardiovascular beneficial effect in trained postmenopausal women. Financial Support: Fapesp.

17.7
RENAL FUNCTION IN AGING HYPERANDROGENEMIC FEMALE RATS

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Polycystic ovary syndrome (PCOS) is the most common reproductive disorder in premenopausal women (PMW), is characterized by hyperandrogenemia, metabolic syndrome and inflammation. Whether PMW who have had PCOS when young develop early cardiovascular disease (CVD) is controversial despite the fact that androgen levels remain elevated even after menopause. We have characterized a model of hyperandrogenemia in female rats and have aged them to 22 months to mimic hyperandrogenemia in PMW with PCOS. In the present study we tested the hypothesis that chronic exposure to hyperandrogenemia with aging in female rats has a deleterious effect on renal function. Female rats, implanted with dihydrotestosterone (DHT 7.5mg/90d) or placebo pellets (n=6/grp) beginning at 6 wks of age (pellets were changed every 85 d), were aged to 22-25 months. Renal function was measured by clearance studies in euvolemic, anesthetized rats (Inactin 110 mg/kg I.P.). Catheters were placed into femoral artery (continuous measurement of blood pressure (mean arterial pressure (MAP)), femoral vein (for infusion of 50% globulin/50% BSA in Ringer's at 10 mL/kg BW/hr for 45 min and then 1.25 mL/kg BW/hr throughout the study); jugular vein (for infusion of 3H-inulin 3 μCi/mL in saline at 1ml/hr). Tracheostomy was performed and a catheter was placed into the left ureter for urine collection. Two 30 min urine collections were performed with midpoint plasma samples taken. At the end of the study, a 23 g needle connected to PE10 tubing was inserted into the left renal vein to measure extraction of 3H-inulin across the kidney to calculate renal plasma flow (RPF). Aging DHT-treated females had significantly higher body weight (420±18 vs. 309±8 g, p=0.0001), MAP (130±5 vs 110±4 mmHg, p<0.05), left kidney weight (1.49±0.11 vs. 0.84±0.02 g, p<0.0001) than placebo controls. Placebo treated females had normal GFR whereas DHT-treated females had a 40% reduction in GFR (0.4217±0.07 vs 1.004±0.07 mL/min/g KW, p<0.01) and 40% reduction in RPF (2.311±0.41 vs 4.084±0.51 mL/min/g KW, p<0.05). Thus chronic hyperandrogenemia in aging females significantly reduces renal function, and likely contributes to hypertension. Studies must be done in PMW with PCOS that have elevated androgens after menopause to determine if their renal function is compromised. Our data would suggest that women who have had PCOS when younger do in fact have more CVD with age than non-PCOS women. Supported by NIH RO1HL66072 and PO1HL51971.

17.8
EFFECT OF ESTRADIOL REPLACEMENT IN HYPERTENSION IN THE AGING FEMALE DAHL SALT SENSITIVE RAT

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Menopause is associated with a higher prevalence of hypertension, obesity, and insulin resistance in women. The mechanisms underlying menopause-associated cardiometabolic comorbidities remain to be elucidated. Lack of estrogens had been proposed to be one of the main mechanisms. *In vivo* and *in vitro* studies suggest that estrogens decrease blood pressure (BP) acting as a vasodilator. However, randomized clinical trials have shown no effect of estradiol replacement on BP in postmenopausal women, suggesting that the time that estradiol replacement begins is a critical factor in the response to estradiol. Aging female Dahl Salt Sensitive (DS) rats develop spontaneous hypertension by 12 mos of age and are no longer estrous cycling. In the present study, we aimed to determine the impact of estradiol replacement on hypertension in the aging female DS rats, and hypothesized that chronic estradiol replacement would normalize BP in aging female DS. Female DS rats, aged 12 mos, were implanted subcutaneously with 17β-estradiol pellets of two increasing concentrations (1x and 5x) consecutively. Animals were maintained in standard rodent diet (0.3% NaCl) with free access to water. BP was measured by radiotelemetry throughout the study period. At the end of the experimental period, plasma estradiol, insulin, leptin and aldosterone were determined by radioimmunoassay and visceral fat weighted. The low estradiol dose (1x) increased plasma estradiol levels by about 3-fold compared to placebo (13.54±2.29 *vs*. 4.28±1.2 pg/ml; p<0.01). This dose of estradiol caused a transient 10 mm Hg reduction in BP that lasted only 4 days (164±2 *vs*. 154±5 mmHg; $p<0.05$) and then BP returned to baseline values (164±3 *vs*. 165±3 mm Hg). Subsequently, the higher dose (5x) of 17β-estradiol increased plasma estradiol by almost 40-fold compared to placebo (84.28±9.67 *vs*. 2.30±0.45 pg/ml, $p<0.001$), but caused only a transitory decrease in BP without reaching statistical significance. In contrast, high dose estradiol-treated rats had lower levels of plasma aldosterone (19.50± 1.66 *vs*. 44.62±8.36 ng/dl; $p<0.05$), leptin (4.32 ±0.62 *vs*. 8.20±1.45 ng/ml, $p<0.05$) and visceral fat (23±4 *vs*. 11±3 mg/gr body weight) at the end of the treatment. In summary, estradiol treatment caused a tachyphylactic effect on BP in aging female DS rats despite the sustained reduction in plasma aldosterone, leptin and visceral obesity. Our study suggests that the tachyphylactic effect of estradiol on BP with aging may contribute to the lack of cardio-protective effects of estradiol supplementation seen in postmenopausal women.

17.9
ROLE OF THE RENAL NERVES AND ANGIOTENSIN II IN A MODEL OF POSTMENOPAUSAL HYPERTENSION

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Hypertension in postmenopausal women is not as well controlled in men regardless of ethnicity of the cohort. In our model of postmenopausal hypertension, the aging female spontaneously hypertensive rats (PMR), we found that blood pressure remains 110 mm Hg despite concomitant treatment antagonists of angiotensin AT1 receptors, endothelin ETA receptors and 20-HETE synthesis inhibitors. We have also shown that the sympathetic nervous system and the renal nerves contribute to the hypertension in PMR. In the present study, we determined whether renal denervation in combination with AT1 receptor antagonists would reduce BP below that found with triple therapy. PMR (aged 18 mos, n=5-6/grp) underwent uninephrectomy, and two weeks

later, unilateral renal denervation (RD) or sham (S) surgery and telemetry transmitter implantation. After two weeks recovery, mean arterial pressure (MAP) and heart rate (HR) were recorded for 5 days. Then PMR were treated with losartan for 5 days, and kidneys were removed for measurement of norepinephrine (NE) content (D. Mattson, MCW). Renal NE content was higher in S-PMR than RD-PMR (S: 137.9±12.6 vs RD: 33.4±10.5). Renal denervation attenuated the hypertension in PMR by approximately 11% compared with shams (MAP: S: 188±6 vs RD: 167±6 mmHg; $p<0.01$). Losartan reduced MAP in both groups by similar percentages (9-11%) (S: 166±6 vs RD: 151±7 mmHg, $p<0.001$), but failed to normalize the BP. These results suggest that while renal denervation and AT1 receptor antagonism attenuation the hypertension in PMR, other mechanisms, likely endothelin and 20-HETE also contribute to their hypertension.These data also suggest that multiple interventions including pharmacotherapy may be required to control BP in postmenopausal women. Supported by NIH R01HL66072, P01HL05971 and AHA 14POST18640015.

17.10
ELDERLY WOMEN MAINTAIN BETTER CEREBRAL BLOOD FLOW REGULATION TO BOTH PRESSURE AND CARBON DIOXIDE THAN ELDERLY MEN

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Background: We have previously found that both male and female elderly individuals have intact cerebral autoregulation but impaired cerebrovascular reactivity, with women performing better on both. The goal of this work was to examine if there were differences in the cerebrovascular ability to dilate vs constrict with changing end-tidal CO_2 levels and if there were sex differences in this response. Previous data in the peripheral vasculature has demonstrated that populations with impaired endothelial function show a lack of dilation with intact constriction. **Methods:** We used transcranial Doppler to evaluate cerebrovascular reactivity in 419 (186 males) subjects over the age of 70 recruited as part of the MOBILIZE Boston study (MBS). The MBS is a prospective cohort study of a unique set of risk factors for falls in seniors in the Boston area. We assessed CO_2 vasoreactivity in cerebral vessels during both hypercapnia (8% inspired CO_2) and hypocapnia (mild hyperventilation) as well as cerebral autoregulation (sit to stand manuever). All procedures were approved by the local institutional review board. **Results:** Male subjects had significantly lower CO_2 vasoreactivity (Males: 2.8±0.7, Females: 3.1±0.8 %/mmHg CO_2, $p<0.001$) as we have previously reported. Examination of their response to reduced end tidal CO_2 (hypocapnia) found that there was no difference in the reduction of cerebral flow velocity or vasoconstrictor response (Males: 3.7±3.7, Females: 3.5±4.0 %/mmHg CO_2, $p=0.6$). In contrast, while both sexes had an impaired ability to vasodilate to CO_2, males demonstrated an even greater impairment than females (Males: 0.0±1.3, Females: 0.5±2.1 %/mmHg CO_2, $p<0.006$). Interestingly, there was no correlation between the vasodilatory or vasoconstrictor response and measures of cerebral autoregulation. In addition, controlling for diabetes, hyperlipidaemia or hypertension did not change the results. **Conclusion:** These data suggest that an impaired response to a dilatory cerebrovascular stimulus (hypercapnia) may indicate that cerebral endothelial dysfunction is present in aging. In contrast smooth muscle regulation of the vasculature remains intact since cerebral vessels were able to constrict during hypocapnia and dilate during a hypotensive stimulus while standing. Thus, improving endothelial function may result in improved dilation of vessels during stimuli that activate the endothelial pathways such as hypercapnia.

17.11
ESTROGENIC PHYTOCHEMICALS REDUCE BONE ADIPOSITY AND IMPROVES BONE QUALITY FOLLOWING OVARIECTOMY

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Menopause increases adiposity and the risk of osteoporosis. Partly as a result of the carcinogenic concerns of hormone replacement therapy, increasing numbers of postmenopausal women are taking botanical and dietary supplements to manage adverse body composition changes. Adipocytes and osteoblasts share a common progenitor cell, the mesenchymal stem cell, and thus botanical supplements may improve both adipose tissue and bone together. The efficacy of such supplements are often in question, which may be related to the "one molecule, one target" approach. Thus, the goal of the current research was to combine multiple natural products with synergistic activity as a result of actions on multiple molecular targets that impact the life cycle of adipocytes and bone precursor cells. Aged, ovariectomized (OVX) Fisher 344 rats from the National Institute of Aging colony were fed either a control diet or one containing various doses of phytochemicals (diet 1: 1000 mg/kg genistein, (G); diet 2: 500 mg/kg G, 200 mg/kg resveratrol (R), and 1000 mg/kg quercetin (Q); diet 3: 1000 mg/kg G, 400 mg/kg R, and 2000 mg/kg Q). Following 16 weeks, a dose-response in the number of adipocytes was found within femoral trabecular bone; diet 3 in particular caused a significant reduction compared to OVX controls ($p<0.01$). Bone adiposity was also found to be significantly correlated with the retroperitoneal fat depot, which was additionally reduced with dietary phytochemicals ($p<0.05$). Bone quality was determined using micro CT measures of the femoral bone. To be expected, OVX reduced bone quality compared to sham rats. Phytochemical supplementation improved trabecular bone quality compared to OVX, however did not completely restore it to levels of sham rats. Serum IGF-1, a bone-promoting hormone, was similarly reduced following OVX. Dietary phytochemicals (diets 1 and 3) improved IGF-1 levels compared to OVX-control rats. While we were unable to completely reverse the damage caused by surgical menopause, the phytochemicals used in our study improved trabecular bone quality and adiposity compared to OVX. Thus we conclude that synergistic, plant-derived compounds with estrogenic properties may be helpful as part of a combined effort to prevent maladaptive bone changes including adipocyte infiltration and structural loss. Further, we provide mounting evidence that dietary phytochemicals may reduce adiposity as a result of menopause. This abstract does not reflect US EPA policy.

17.12
EFFECTS OF MENOPAUSE AND ACUTE EXERCISE ON BRACHIAL ARTERY FLOW MEDIATED DILATION AND PLASMA ENDOTHELIAL MICROPARTICLES

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Menopause is associated with an increase in risk factors for cardiovascular disease. Some evidence suggests a decrease in endothelial function from the peri- to postmenopausal stages. As women move into later menopausal stages, they may not exhibit responses to exercise that are typical in most populations. **Objective:** To evaluate differences in markers of endothelial function in response to an acute bout of exercise in peri- and post-menopausal women. **Methods:** Perimenopausal (PERI: 47 ± 2.6 yr) and late postmenopausal (POST: 59 ± 2.0 yr) women, free of cardiovascular disease, completed an acute bout of exercise at 60-64% of VO_2 peak for 30 min. Prior to, and 30 min following exercise, flow mediated dilation (FMD) was measured and blood was collected to assess $CD62E^+$ and $CD31^+/CD42b^-$ endothelial microparticle (EMP) concentrations. FMD (PERI: n=4; POST: n=6) was assessed via imaging of the brachial artery at baseline and after reactive hyperemia. FMD (%Δ) was calculated as $(Diameter_{max} - Diameter_{rest})/Diameter_{rest} \times 100$. EMPs (PERI: n=3; POST: n=6) were quantified from plasma using fluorescence-activated cell sorting. **Results:** Values are presented at each time point ± SEM. Before exercise, PERI women had higher FMD (PERI: 8.4 ± 3.9 % vs. POST: 5.3 ± 0.9 %), lower $CD62E^+$ EMP concentration (PERI: 344 ± 19 EMPs/µl plasma vs. POST: 402 ± 88 EMPs/µl plasma), and higher $CD31^+/CD42b^-$ EMP concentration (PERI: 4533 ± 532 vs. POST: 3446 ± 901) compared to POST women. After exercise, PERI women had an improvement in FMD (10.0 ± 3.2%), a slight decrease in $CD62E^+$ EMP concentration (305 ± 35 EMPs/µl plasma), and a decrease in $CD31^+/CD42b^-$ EMP concentration (3870 ± 1223 EMPs/µl plasma). In POST women following exercise, FMD changed minimally (5.5 ± 1.0%), $CD62E^+$ EMP concentration increased (470 ± 99 EMPs/µl plasma), and $CD31^+/CD42b^-$ EMP concentration decreased (2986 ± 772 EMPs/µl plasma). Statistical significance was not achieved for any markers. **Conclusion:** These preliminary data indicate impaired endothelial function and enhanced endothelial activation in post- compared to peri-menopausal women at rest. However, perimenopausal women displayed more endothelial apoptosis. The lower FMD responsiveness and increase in $CD62E^+$ EMP concentration in response to exercise may indicate women in later menopausal stages are resistant to the beneficial vascular effects of acute exercise. Funding Source: Research Trust Fund (Witkowski), Start-up (Jenkins).

18.0 PLENARY LECTURE

18.1
STUDYING BOTH SEXES: A NEW FRONTIER FOR DISCOVERY

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The National Institutes of Health (NIH) funds basic, translational, and clinical research. From basic research to clinical care, studying both sexes is a guiding principle to aid in experimental design, hypothesis-generation and -testing, and expanding understanding and deriving knowledge toward turning discovery into health for both women and men. Numerous factors prompted the development of new NIH policy, announced in May 2014, to ensure that sex is considered a basic biological variable in NIH-funded preclinical research. These included scientific progress emerging from NIH-funded laboratories, congressional interest and support, and ongoing NIH efforts to enhance reproducibility and transparency in preclinical research. Starting with applications with receipt dates beginning January 25, 2016, NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies. Strong justification from the scientific literature, preliminary data, or other relevant considerations must be provided for applications proposing to study only one sex. Selecting an appropriate preclinical model that considers the role of sex in the context of a specific research question of interest, especially for studies that model human physiology and pathology, is central to the scientific inquiry process. Reference: Clayton, J.A. & Collins, F.S. 2014. NIH to balance sex in cell and animal studies. *Nature*. 509, 282-283. NIH Guide Notice NOT-OD-15-102: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html.

19.0 OBESITY, METABOLIC SYNDROME, GENDER AND SEX

19.1
IN UTERO CONSEQUENCES OF RODENT VERTICAL SLEEVE GASTRECTOMY ON MATERNAL HEALTH AND FETO-PLACENTAL DEVELOPMENT

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Despite many similar improvements between sexes in metabolic health following surgical weight loss, female reproductive health and transgenerational effects of surgery remain unclear. Our previous work in rodents suggests that following vertical sleeve gastrectomy (VSG, a surgery which resects 80% of the stomach), offspring born to VSG dams are small-for-gestational age. When challenged with a high fat diet (HFD) during adulthood, these animals are glucose intolerant and have levels of adiposity in excess to lean and obese control offspring. In the present studies, we sought to identify the key *in utero* insults that may be driving these defects. Female Long-Evans rats were placed on HFD for 3 weeks and then received either sham or VSG surgery. Females exhibited similar body weight and glucose and lipid improvements as previously reported. Females were then mated with males; during the first 2 weeks of gestation, VSG animals gained weight and consumed similar calories to control dams. During gestational days 12-18 (G12-18), VSG body weight gain precipitously dropped off. Blood pressure measurements taken at G19 showed significant reductions in mean arterial pressure in comparison to lean and obese controls. Animals were euthanized on G19 for analysis of uterine contents; the number of fetuses was reduced in VSG ($p < 0.05$) with reduction in total fetal mass in comparison to controls. Placental-to-fetal weight ratios were increased suggestive of placental insufficiency. Genes involved in inflammation (interleukin 1 receptor antagonist, metallopeptidase 9) and hypoxia (heme oxygenase 1) were up-regulated by Affymetix microarray analysis of harvested placental tissue. Taken together, these data indicate that gestational hypertension is not a cause of the reduced fetal growth and that hormonal and chemokine alterations induced by the surgery may be driving reduced fetal growth. These data further support that the beneficial effects of VSG surgery in adult females may have negative consequences on gestation and beyond to their offspring.

19.2
NUTRIENT SENSING MECHANISMS IN HYPOTHALAMIC CELL MODELS: NEUROPEPTIDE REGULATION AND NEUROINFLAMMATION

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Over-nutrition, through elevated saturated fatty acids, such as palmitate, and the ensuing hypothalamic inflammation is a major perpetuating factor in the development of metabolic diseases, such as obesity and diabetes. Inflamed neurons of the CNS fail to properly regulate energy homeostasis leading to pathogenic changes in feeding and weight. Hypothalamic neurons are particularly sensitive to pro-inflammatory signals, and it is these neurons that become inflamed first upon high fat feeding. Efforts are underway to identify therapeutic targets for this inflammatory state. Omega-3 fatty acids and their receptor, GPR120, have emerged as putative targets. We have generated a wide array of novel, immortalized cell models derived from the rodent hypothalamus to study activation of fatty acid receptors at the level of the individual neuron. Signal transduction pathways, as well as gene expression of pro-inflammatory cytokines and neuropeptides, were studied upon exposure to palmitate or tumor necrosis factor α (TNFα) in the presence of the omega-3 fatty acid docosahexaenoic acid (DHA). DHA pretreatment prevents the inflammatory state through endogenous GPR120. GPR120 activates both AKT and ERK; however, the anti-inflammatory action of this omega-3 FA receptor is AKT and ERK-independent and likely involves the GPR120-TAB1 interaction. These studies provide mechanistic insights into how fatty acids act at the level of the individual hypothalamic neuron, and potential avenues to resolve hypothalamic neuroinflammation. Refs: Belsham et al. FASEB J, 2010; Wellhauser and Belsham, J Neuroinflamm, 2014.

19.3
THE ROLE OF ESTROGENS AND ANDROGEN IN CONTROL OF GLUCOSE HOMEOSTASIS

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There are fundamental aspects of the control of glucose homeostasis that are regulated differently in males and females and may influence both the development of diabetes and the response to pharmacological intervention. There are gender differences in diabetes pathophysiology and prevalence and there are more diabetic men before puberty, while there are more diabetic women after menopause. The prevalence of pre-diabetic symptoms such as impaired fasting glucose and impaired glucose tolerance also differs by sex. Some result from the action of estrogens and androgens on glucose homeostasis after puberty and in adults. In females, estrogen favors glucose homeostasis via estrogen receptors (ERs) by ameliorating insulin secretion and sensitivity. In males, testosterone is converted to estrogen and maintains fuel homeostasis via ERs and the androgen receptor, which share related functions to improve insulin secretion and sensitivity.

20.0 PREGNANCY AND PRE-ECLAMPSIA

20.1
MECHANISMS OF MATERNAL UTERINE VASCULAR REMODELING DURING GESTATION

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The most accepted etiology of preeclampsia (PE) is that shallow trophoblast invasion of the maternal spiral arteries leads to placental underperfusion and triggers development of the maternal syndrome. This theory carries the hemodynamic prediction that, in normal pregnancy, reduced pre-placental flow resistance will also accelerate blood flow in proximal vessels and thereby increase shear stress and stimulate outward circumferential remodeling. We, and others, have shown that inhibition of endothelial nitric oxide synthase (eNOS) attenuates this process, implicating NO as the primary effector of arterial widening and supporting reduced NO signaling contributing to PE. Yet, uterine arteries and veins also *lengthen* considerably during pregnancy, and NOS inhibition has no effect on this axial elongation. Here, we hypothesized that axial growth may be triggered by myometrial distension secondary to fetoplacental growth. Using a rat model, myometrial distension was stimulated by infusing medical grade silicone into one uterine horn. The initial stretch was followed by continued myometrial expansion secondary to the accumulation of an exudate within the uterine lumen, and resulted in measurable arterial lengthening. Analysis of the exudate revealed significant increases in PDGF and VEGF, growth factors known to play a role in vascular remodeling. In summary: (1) Outward circumferential vascular remodeling is mediated primarily by endothelial NO, most likely secondary to increased shear stress due to placentation/spiral artery invasion. (2) Conversely, axial remodeling may be stimulated by placental and/or myometrial secretion of angiogenic factors such as VEGF into veins, followed by transfer into the periarterial space.

20.2
SPONTANEOUS SUPERIMPOSED PREECLAMPSIA IN DAHL SALT SENSITIVE RATS

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Preeclampsia is a leading cause of maternal morbidity and death worldwide, and our understanding of its pathogenesis and development of therapies for preeclamptic women have been hindered by a lack of spontaneous animal models of the disease. Our laboratory has characterized the Dahl salt sensitive (S) rat, a genetic model of hypertension and kidney disease, as a spontaneous model of superimposed preeclampsia. Blood pressure and urinary protein excretion are elevated in the Dahl S prior to pregnancy, but both are exacerbated during pregnancy. In addition, Dahl S rats exhibit glomerulomegaly, increased placental hypoxia, decreased placental vascularization, increased uterine artery resistance during late pregnancy, and increased soluble fms-like tyrosine kinase-1 (sFlt-1) and tumor necrosis factor-alpha (TNF-α). Furthermore, there is a greater incidence of fetal demise and intrauterine growth restriction in the Dahl S pregnancy when compared to pregnancy in the healthy Sprague Dawley strain. In summary, the Dahl S pregnancy phenotype is consistent with many of the characteristics observed in human superimposed preeclampsia; therefore this model could allow for the analysis of time-dependent changes throughout preeclamptic pregnancy, the discovery of new biomarkers for detection of preeclampsia, the identification of new therapeutic targets in populations with preexisting cardiorenal diseases, and determination of the long term cardiovascular outcomes for both mothers who have experienced preeclampsia and their offspring.

20.3
VASOPRESSIN: A NEW BEGINNING FOR THE END OF PREECLAMPSIA?

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Despite being in the medical literature for over 2000 years, the diagnosis and treatment for preeclampsia has essentially remained unchanged. To date, the only cure for this potentially devastating hypertensive disease in pregnancy is an often preterm delivery. It affects 5-7% of all pregnancies claiming the lives of 76,000 mothers and 500,000 children each year. The ability to predict, prevent, and treat preeclampsia is hampered by its unclear and multifactorial pathogenesis of which the initiating, first trimester mechanisms are uncertain. We have demonstrated that maternal plasma copeptin, a stable protein byproduct of arginine vasopressin (AVP) synthesis and release, is a robust predictor of the development of human preeclampsia *as early as the 6th week of gestation*. These data from our lab and others, suggest an early role of AVP in the pathogenesis of preeclampsia. Our group demonstrated that chronic infusion of AVP throughout mouse pregnancy phenocopies *all* the vascular, renal, obstetric, and immune phenotypes in human preeclampsia. Early immune dysregulation is an early, initiating mechanism of preeclampsia. AVP is a hormone active in many vascular, renal, growth, and immune mechanisms. Given its early dysregulation in human preeclamptic pregnancies and its ability to recapitulate all the phenotypes of human preeclampsia in mice, we contend AVP is a novel, mechanistic connection between the known early and mid-gestation molecular processes that cause preeclampsia.

21.0 POPULATION STUDIES-GENDER AND SEX IN CVD, RENAL DISEASE, AND METABOLIC SYNDROME

21.1
SEX DIFFERENCES IN RISK FACTORS FOR STROKE IN WOMEN

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Stroke is the third leading cause of death for women, and fourth-leading cause of death for men. Women account for a majority of stroke deaths (61%), and have a higher lifetime risk of stroke. Several risk factors for stroke are sex specific, such as pregnancy and pregnancy-related conditions (including preeclampsia, pregnancy-induced hypertension, gestational diabetes, premature birth, and birth of small size for gestational age). In addition, to the need for long-term data on the impact of pregnancy-related conditions and hormonal conditions, such as polycystic ovarian syndrome, intervention trials to reduce associated risk of stroke among these groups of women are needed. In addition, oral contraceptives and postmenopausal hormone therapy are associated with risk and used exclusively by women. Other risk factors have a higher prevalence or a higher associated risk of stroke in women, including diabetes mellitus, hypertension, atrial fibrillation, depression and psychosocial stress and trauma. Effective means of reducing risk of stroke among women with these conditions are needed. For example, among patients with atrial fibrillation, risk scores that take gender into account improve risk stratification; however, rates of anticoagulation have remained lower in women than men. Relatively similar risk reductions for both men and women have been observed in the primary prevention of stroke by lifestyle factors. A female-specific stroke score should be developed and evaluated to better reflect the risk of stroke in women across the lifespan. References: Bushnell C, et al. Guidelines for the Prevention of Stroke in Women: A Statement for Healthcare Professionals from the American Heart Association/American Stroke Association. *Stroke. 2014; 45: 1545-1588.*

21.2
GENDER DIFFERENCES IN HYPERTENSION AND HEALTH BEHAVIORS

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Hypertension is a key modifiable risk factor for cardiovascular disease in females and males. The presentation will highlight gender differences across the lifespan in cardiovascular disease, hypertension, and adherence to healthy lifestyle and medication-taking behaviors to improve hypertension control and reduce CVD risk. Efforts to overcome gender-specific barriers and tailor interventions that reduce risk for poor adherence and uncontrolled hypertension have the potential for substantive impact on reducing CVD across the lifespan and improving heart disease survival. The work was supported, in part, by the National Institutes of Health: Award R01 AG022536 from the National Institute on Aging, Award K12HD043451 from the Eunice Kennedy Shriver National Institute of Child Health & Human Development, and Award U54 GM104940 from the National Institute of General Medical Sciences for the Louisiana Clinical and Translational Science Center.

21.3
TOBACCO SMOKING EXPOSURE FROM CHILDHOOD TO ADULTHOOD AND ADULT SUBCLINICAL VASCULAR DISEASE

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Tobacco smoking has been well established as a major risk factor for cardiometabolic diseases. However, limited information is available regarding the effects of tobacco smoking exposure beginning in childhood on adult cardiometabolic conditions. The current study examined the adverse effects of tobacco smoking exposure beginning in childhood on body mass index (BMI), ankle-brachial pause wave velocity (abPWV) and carotid intima-media thickness (CIMT) in women and men from the Bogalusa Heart Study. Among non-smoking adults, exposure to secondhand smoking (SHS) either in childhood or in adulthood was associated with increased BMI only in women ($P<0.0001$), with women continuously exposed to SHS from childhood having the highest BMI compared to women with other SHS exposure statuses. Exposure to SHS either in childhood or in adulthood was associated with increased CIMT in both men and women, with individuals continuously exposed to SHS from childhood having the greatest CIMT compared to those with other exposure statuses. Despite having lower BMI, adult cigarette smokers had faster abPWV and greater CIMT in both men and women. Further, cigarette smoking significantly exacerbated the adverse effects of age and metabolic syndrome on CIMT and of blood pressure on abPWV. In conclusion, SHS exposure beginning in childhood is associated with increased BMI, arterial stiffness, and atherosclerosis; cigarette smoking in adult life increases arterial stiffness and atherosclerosis and exacerbates the adverse effects of other risk factors on arterial stiffness and atherosclerosis, in otherwise healthy adults. Support: NIH K12HD043451, 5R01ES021724, and 2R01AG016592; AHA 13SDG14650068. REFERENCES: Yun M, Li S, Ge S, Fernandez C, Chen W, Srinivasan SR, Berenson G (2015). Tobacco smoking strengthens the association between elevated blood pressure and arterial stiffness: The Bogalusa Heart Study. J Hypertens 33:266-274. Chen W, Yun M, Fernandez C, Li S, Sun D, Lai CC, Hua Y, Wang F, Zhang T, Srinivasan SR, Berenson GS (2015). Secondhand smoke exposure is associated with increased carotid artery intima-media thickness: The Bogalusa Heart Study. Atherosclerosis 240:374-379. Li S, Yun M, Fernandez CA, Xu J, Srinivasan SR, Chen W, Berenson GS (2014). Cigarette smoking exacerbates the adverse effects of age and metabolic syndrome on subclinical atherosclerosis: The Bogalusa Heart Study. PLoS One 9:e96368.

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