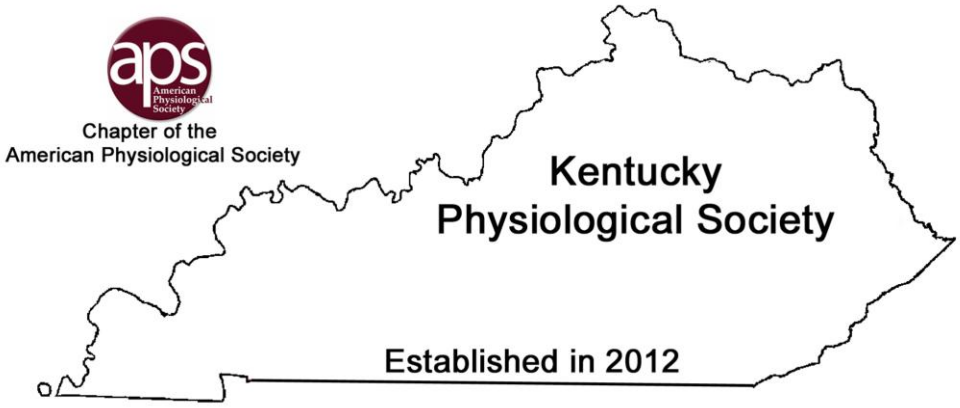


Kentucky Physiological Society
1st Inaugural Meeting
University of Kentucky
March 25, 2013

Introducing Physiology From Around KY

Sponsors:

Program for Monday March 25, 2013

Location: Univ. of KY. Student Center Grand Ballroom (3rd floor)

Parking and Info on web site

http://web.as.uky.edu/Biology/faculty/cooper/KYPhysiologicalSoc/default.htm

8:30 AM-3:30 PM: Registration table will be open

(Table at entrance to Grand Ball Room)

8:30 AM–Noon: Poster set up

(See registration table for assigned poster # and parking validation for free exit of parking garage for out of town guests)

10:00 AM-11:00 AM: Overview of Physiology and outreaching in KY

(10 minutes each) **Dr. Joshua** (U of L), **Dr. Reid** (UK), **Dr. Fultz** (Morehead), **Dr. Nakamura** (Murray State), **Dr. Park** (Univ. of Pikeville) and **Dr. Frazier** (UK outreach).

12:00-1:30PM: Box lunches provided (free) for people submitting abstracts or preregistering with a notice of attending (can pay when arriving).

12:30-1:20PM: Welcome to "KYPhys" by Dr. R.L. Cooper

Lunch Seminar by **Dr. Brian Delisle** (Dept of Physiology, Univ. of KY)

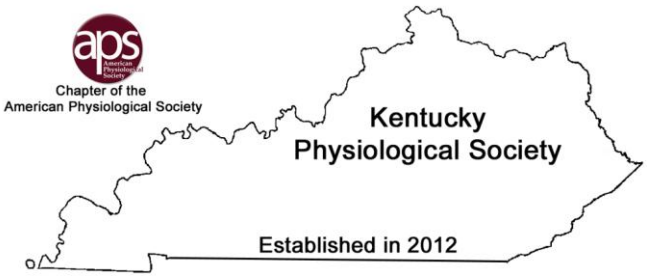
Title ***"The Physiology of Congenital Arrhythmia Syndromes: From Bedside to Bench and Back Again…"***

1:30-2:30: Even # posters being presented.

2:30-3:30: Odd # posters being presented.

3:45: Random drawings of poster cards (filled out information on registration cards for poster presenters). Prizes !!!!

4:00: KYPHYS- Business meeting in Small Ball Room 3rd floor.



KYPhys Officers and KYPhys Organizing Committee

The Executive Committee for 2012-2013

President: Dr. Robin L. Cooper, Univ. of KY. (1 yr. term)

President Elect: Dr. Michael B. Reid, Univ. of KY. (1 yr. term)

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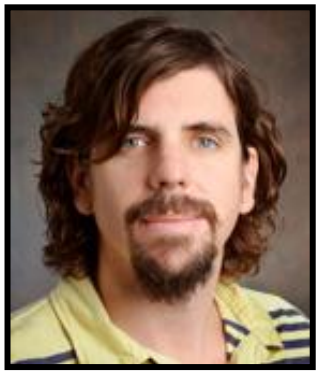
The $10 registration fee will give you a one-year membership to KY-PHYS, and will help defray meeting expenses.
Further details on the KY-PHYS inaugural meeting and program are available:
Hot link: http://web.as.uky.edu/Biology/faculty/cooper/KYPhysiologicalSoc/default.htm

KYPhys Plenary Lecture



BRIAN P. DELISLE, Ph.D.

Department of Physiology
University of Kentucky

The Physiology of Congenital Arrhythmia Syndromes: From Bedside to Bench and Back Again…

The ordered electrical excitation of the heart coordinates the efficient pumping of blood. Electrical impulses normally originate in the sinoatrial node and then propagate through the atria and into the ventricles. Arrhythmias are electrical disturbances that disrupt the normal initiation or propagation of the cardiac impulse. They can cause abnormally slow or fast heart rates, block impulse propagation, or initiate the impulse to circle in a "reentry" loop. Atrial arrhythmias can result in the formation of blood clots and increase the risk of stroke, and ventricular arrhythmias can cause inefficient pumping of blood, loss of consciousness, and sometimes death.

Long QT syndrome (LQT) is one of the most common congenital arrhythmia syndromes. LQT patients have a delay in the repolarization of their ventricles and are at increased risk for polymorphic ventricular tachycardia (torsades de pointes), which can cause a loss of cardiac output, syncope, and sudden death. LQT typically follows a dominant inheritance pattern and is linked to mutations in three different genes that encode cardiac potassium and sodium channels. Testing for genetic variants in LQT-susceptibility genes is helping to identify mutations that might cause the syndrome, but the considerable genetic variability among suspected LQT patients, as well as seemingly healthy individuals, makes accurately diagnosing and managing LQT difficult. A missed LQTS diagnosis can result in preventable sudden death, whereas a false LQT diagnosis can lead to unnecessary family stress, life-style restrictions, β-blocker therapy, and the expensive and sometimes painful implantation of cardioverter defibrillator. Our research program studies the mechanisms that underlie the dysfunction for LQT-linked mutations. Our goal is to identify strategies that improve the diagnostic, prognostic and therapeutic value of a genotype positive test for LQT, as well as identify novel strategies for the treatment of cardiac arrhythmias.

Dr. Delisle began his research career as an undergraduate at the University of Kentucky, where he was awarded a Howard Hughes Medical Institute Undergraduate Research Grant and an Oswald Research Creativity Award. He received his BS in Biology (1996) and his PhD from the University of Kentucky Department of Physiology and Biophysics (2001). He did his postdoctoral work at the University of Wisconsin-Madison and was awarded a National Heart Lung and Blood Kirschstein Fellowship. He was promoted to Assistant Scientist in 2005 and was awarded an American Heart Association Scientist Development Grant and Dave MaClain award. He has returned to the University of Kentucky Physiology Department as an Assistant Professor, and he is funded with an R01 from the National Heart Lung and Blood Institute. Since rejoining the Physiology Department, he has been awarded the Flexner Master Educator Award for Teaching/ Mentorship and the Department of Physiology Holsinger Teaching Award. He has over 25 peer-reviewed publications in the field of cardiac arrhythmias and electrophysiology.

Abstracts submitted for the 2012-2013 Annual meeting

Poster # is the number to match the number on the display panels

Instructions to Poster Presenters: From 1:30 to 2:30 even numbered posters will be presented by the authors and 2:30 to 3:30 the odd numbered posters will be presented. For this first year we decided not to have poster judging of presenters but instead the # poster will view the next higher # poster (#1 views #2 from 1:30-2:30 and #2 views #3 at 2:30-3:30). This ensures that every one has a chance to present their poster (note: The last poster # will view the first poster). This is good practice for students. Some are first-time presenters. You will have a short questionnaire to fill out of the poster/presenter you visited. These questioners will be collected in a box and around 3:30 a random drawing of door prizes will be given based on which papers are pulled out (must be present to win and information filled out). Of course after you finished viewing your next higher up numbered poster, please visit the other posters and mingle.

1. Changes in calcium cycling and twitch in isolated unloaded cardiomyocytes at different temperature and transmural region. C.S. Chung and K.S. Campbell

2. Epigenetic modification by miR-133a in the diabetic heart. C.Vishalakshi, Naira Metreveli, Suresh C. Tyagi, and Paras Kumar Mishra.

3. RNA Degradation is Elevated with Age-, but not Disuse-Associated Skeletal Muscle Atrophy. Aman Shah, Amy L. Confides, Jena L. Richards-White, and Esther E. Dupont-Versteegden
4. Vagus nerve promotes pulmonary fibrosis. N. Song, J. Liu, R. Perez, J. Yu

5. Abnormal QTc recovery following exercise identifies an LQT1 mutation that is insensitive to PKA. Daniel C. Bartos, John R. Guidicessi, Don E. Burgess, Michael J. Ackerman, and Brian P. Delisle

6. Satellite cells regulate the skeletal muscle environment by inhibiting fibroblast function. Christopher S. Fry, Jonah D. Lee, Janna R. Jackson, Tyler J. Kirby, Esther E. Dupont-Versteegden, John J. McCarthy, Charlotte A. Peterson.

7. Attenuation of conducted vasodilation in skeletal muscle arterioles during hyperhomocysteinemia. Givvimani S, Narayanan N, Armaghan F, Pushpakumar S and Tyagi SC.

8. RNA-binding motif protein 3 (RBM3) over-expression results in larger muscle fiber size in vivo and in vitro. Amy Confides, Andrew Judge, and Esther Dupont-Versteegden.

9. Parathyroid Hormone (PTH) decreases mRNA stability of the Type IIa Sodium-Phosphate Cotransporter (NpT2a). Rebecca D. Murray, Nina Lesousky, Syed J. Khundmiri, Barbara J. Clark, and Eleanor D. Lederer.

10. Satellite cell depletion negatively impacts voluntary wheel running performance in mice. J.R. Jackson, C.S. Fry, T.J. Kirby, J.D. Lee, J.H.Werker, C.S. Dean, J.J. McCarthy, E.E. Dupont-Versteegden, C.A. Peterson.

11. The effects of K^+ on skeletal muscle, synaptic transmission and the relationship with deep tissue injury of muscle. E. Burns, E.E. Dupont-Versteegden and R.L.Cooper

12. Educating the public on the association between COPD and oxygen therapy. Tania Boyechko and R.L. Cooper

13. The action of stimulating adenylyl cyclase within motor nerve terminals in the regulation of synaptic vesicles. Y. Zhu, W.-H. Wu and R.L. Cooper

14. Characterization of 5-HT receptor subtype in sensory-CNS-motor circuit in *Drosophila* larvae. A. Bankemper, Z. Majeed and R.L. Cooper

15. Analyzing expression pattern's and correlation's for HSET, Survivin & Ki67 genes in ER, PR & HER2 Negative Breast Cancer Patient's. Shrikant Pawar, Vaishali Phannu and Ritu Aneja

16. Confirmation of squalene in tears and sebum and its potential function. Douglas Borchman, Georgi Georgiev, Marta C. Yappert, and Norihiko Yokoi

17. Binding partners, motifs, and post-translational modifications of human Chmp1A protein. Maiyon Park

18. Cellular mechanism for the pharmacological correction of hERG mutations linked to the Long QT Syndrome. Jennifer L. Smith, Allison R. Reloj, Parvathi S. Nataraj, Daniel C. Bartos, Craig T. January, and Brian P. Delisle.

19. Investigation of potential relationship between epigenetic regulation of Chmp1A and its function in tumor suppression in pancreatic tumor cells. Maiyon Park, Sumanth Manohar, Matthew Harlow, Grace Nyiiro,

20. Sarcopenia is independent of lifelong muscle stem cell depletion.
Jonah D. Lee, Jyothi Mula, Christopher S. Fry, Janna R. Jackson, Tyler J. Kirby, Jake A. Beggs, Marilyn S. Campbell, Catherine P. Starnes, Esther E. Dupont-Versteegden, John J. McCarthy, Charlotte A. Peterson.

21. NFκB signaling and inducible nitric oxide synthase activity during pulmonary ischemia-reperfusion increase co-localization of fibrinogen/fibrin and platelets at sites of vascular leakage in rabbit lung. James T. Dixon, Evelyne Gozal, Leroy R. Sachleben, Jr., David Lominadze, Charla L. Juniel, Andrew M. Roberts.

22. Transmural variations in cellular level contractile function in the left ventricle of patients with endstage heart failure. Premi Haynes, Kristofer E. Nava, Benjamin A. Lawson, Charles S. Chung, Mihail I. Mitov, Stuart G. Campbell, Arnold J. Stromberg, Mark R. Bonnell, Charles W. Hoopes, Kenneth S. Campbell.

23. Right and left and vagus nerves regulate breathing synergistically. J. Liu, N. Song, M. Proctor, J. Yu

24. Effects of insulin on phenotypically-identified neurons of the nucleus tractus solitaries. C.B. Blake and B.N. Smith.

25. Renovascular remodeling in hypertensive dahl-ss rats: role of mmp inhibitor as a hypertension ameliorating agent. Sathnur Pushpakumar, Sourav Kundu, Naira Metreveli, Suresh C. Tyagi, Utpal Sen.

26. Maternally derived mitochondria in SHR exhibit significant reduction in oxidative phosphorylation gene expression. J.A. Collett, J.K. Paulose, V.M. Cassone and J.L. Osborn.

27. Hydrogen sulfide mitigates diabetic nephropathy through NMDA receptor mediated renal remodeling. S. Kundu, A. Tyagi, D. Coley, S. B. Pushpakumar, U. Sen.

28. A modified Huxley-type model that simulates activation-dependent rates of tension recovery. Kenneth S. Campbell

29. Ablation of MMP9 mitigates autophagy mediated contractile dysfunction of cardiomyocytes in Ins2+/- Akita. Paras K. Mishra, Vishalakshi Chavali, Naira Metreveli, Suresh C. Tyagi.

30. TRPA1-mediated damage sensing by the cochlear supporting cells. A.C. Vélez-Ortega, S. Maimaiti, R. Stepanyan, G.I. Frolenkov.

31. Epigenetic mechanism of atherosclerosis and hypertension in Hyperhomocysteinemia. Nithya Narayanan, Neetu Tyagi, Sebastian Pagni and Suresh C. Tyagi

32. Role of PLC-IP3-PKC pathway in 5-HT mediated heart rate modulation in *Drosophila* larvae. T. Crosthwaite, Z. Majeed and R.L. Cooper

33. Development of a new endocrine student laboratory exercise for physiology courses using invertebrates. A. Karic, J. A. Kennedy, and G. C. Nguyen and M. L. Danley.

34. The use of online review modules to prepare D.M.D. Students for Basic Science Courses. Cynthia J. Miller

35. STEM & Health: Stressors on the circulatory system. R.M. Krall, H. Cooper, S. Mayo, D. Johnson, K. Zeidler-Watters, S. Rose, R. Dixon and R.L. Cooper.

36. Teaching with leeches- An undergraduate neurophysiology module. J. Titlow, Z.R. Majeed, J.G. Nicholls and R.L. Cooper

37. Does Myosin-XVa deficiency result in constitutively open mechanotransduction channels? Diana Syam, Andrew J. Alexander, Catalina Velez-Ortega, Ghanshyam P. Sinha, and Gregory I. Frolenkov.

38. Teaching with crabs- An undergraduate physiology module. Z.R. Majeed, J. Titlow, H.B. Hartman and R.L. Cooper

39. Ouabain increase association between Na-K ATPase (Na-K) and NHE1 through N-terminal domain of Na-K . Syed J. Khundmiri.

40. Differential impacts of environmental salinity on *Procambarus clarkii* and *Orconectes rusticus.* M.K. Rhoads, C. Morrisey, L. Sunnenberg, and J.L. Osborn.

41. Inhibition of MMPs protects hypertensive cerebropathy in Dahl-salt sensitive rats. Anuradha Kalani, Sathnur Pushpakumar, Sourav Kundu, Pradip K Kamat Suresh C. Tyagi, Neetu Tyagi.

42. Pathology of two cypovirus variants in *Heliothis virescens* and *Campoletis sonorensis.* J.E. Noland, K. Ray, J. Deacutis, P. Houtz and B.A. Webb

43. Hydrogen sulfide mitigates cerebro-vascular remodeling during cerebral ischemia. Neetu Tyagi, Pradip K Kamat, Nithya Narayanan, Srikanth Givvimani, Suresh C. Tyagi.

44. Dopamine's influence on nervous system anatomy during juvenile development. D. Potts, J.S. Titlow and R.L. Cooper

45. A laboratory exercise in quantifying synaptic transmission: Quantal measures and analysis. S. Kenney and R.L. Cooper

46. Regulation of ERα gene expression by demethylation and increased Tet1 expression following MCAO in the female mouse brain. Jenne Westberry and Melinda Wilson

47. 5-HT modulates the heart rate through 5-HT2 receptor activation in *Drosophila* larvae. A. Stacy, Z. Majeed and R.L. Cooper

48. Sex differences of epigenetic changes following neonatal hyperoxia. T. Sengoku, J. Westberry, M. E. Wilson.

ABSTRACTS

Poster Presentations

1. Changes in calcium cycling and twitch in isolated unloaded cardiomyocytes at different temperature and transmural region.

C.S. Chung[1] and K.S. Campbell[1]

[1] Department of Physiology and Center for Muscle Biology, University of Kentucky, Lexington KY

Measurement and characterization of myocardial properties is typically taken at sub-physiologic temperatures and without regard to myocardial region (layer). We hypothesized that calcium transients and sarcomere length (SL) twitches in unloaded myocytes are different both in response to temperature and from different transmural region.

Methods: Myocytes were enzymatically digested and isolated from 3 mo female Sprague-Dawley rats and separated into three transmural (epi- [exterior], mid-, and endocardial [inner]) regions. Cells were loaded with Fura-2AM, a cell permeable fluorescent calcium indicator dye, paced at 0.5Hz and individual cells measured at three temperatures (25, 31, and 37oC) using an experimental design balancing temperatures and transmural region. Fluorescent calcium transients and sarcomere length during twitch (shortening and relengthening) were measured for 10 beats and averaged.

Results: Calcium transients were >50% shorter at physiologic vs room temperature. Calcium reuptake appears faster in epicardial vs endocardial myocytes. Timing of SL twitches were also >50% shorter at physiologic vs room temperature but were not dependent on transmural region. In contrast, twitch magnitude varies with transmural layer but not temperature, with the epicardial cells showing the largest magnitude twitch. Control experiments suggest that temperature dependent changes are not related to rundown or changes in pH that may occur at varying experimental conditions. A novel finding is that sarcomeric twitch properties are not strongly correlated with calcium timing. This suggests that sarcomeric (crossbridge) interactions, not calcium, control shortening and relaxation rates.

Summary: Increasing temperatures causes shorter duration transients and twitches, but does not change magnitudes of shortening. Transmural regions also exhibit different transients and twitches. These experiments also provide a novel perspective into the excitation twitch relationship.

2. Epigenetic modification by miR-133a in the diabetic heart.

C. Vishalakshi, Naira Metreveli, Suresh C. Tyagi, and Paras Kumar Mishra

Dept. of Physiology and Biophysics, University of Louisville, Louisville, KY-40202

Epigenetic modifications (changes in the gene expression without altering the primary genetic sequences) such as DNA methylation and miRNAs play crucial roles in modulating gene expression in pathological cardiac remodeling. MiR-133a (inhibits cardiac fibrosis and hypertrophy) is attenuated and DNA methylation is up regulated in diabetic hearts. However, the cross talk between miR-133a and DNA methylation is unclear. We hypothesized that miR-133a regulates DNA methylation by inhibiting Dnmt-1 (maintenance) and Dnmt-3a and -3b (de novo) methyl transferases in diabetic hearts. To test our hypothesis, we used the heart tissues of Ins2 +/- diabetic Akita and C57BL/6J (WT) mice, and two treatment groups of HL1 cardiomyocytes: (1) scrambled, miR-133a mimic and anti-miR-133a; and (2) 5mM glucose (CT), 25mM glucose (HG) and HG+miR-133a mimic. The levels of miR-133a, Dnmt-1,-3a and -3b were measured by RT-PCR, qPCR, Western blotting and fluorescence microscopy. The results revealed that miR-133a is inhibited but Dnmt-1 and -3b are induced in Akita. In cardiomyocytes, over expression of miR-133a inhibits but silencing of miR-133a induces Dnmt-1,-3a and -3b elucidating that miR-133a inhibits DNA methylation. In the HG group, Dnmt-1 but not Dnmt-3a and -3b is up regulated suggesting that acute hyperglycemia triggers maintenance methylation. The over expression of miR-133a mitigates glucose mediated induction of Dnmt-1 indicating that miR-133a attenuates the maintenance DNA methylation in diabetic cardiomyocytes. This is a novel mechanism of miR-133a mediated epigenetic modification in diabetic hearts.

3. RNA Degradation is Elevated with Age-, but not Disuse-Associated Skeletal Muscle Atrophy.

Aman Shah, Amy L. Confides, Jena L. Richards-White, and Esther E. Dupont-Versteegden

Department of Biology, College of Arts and Sciences, Department of Rehabilitation Sciences, College of Health Sciences, Division of Physical Therapy, Center for Muscle Biology, University of Kentucky

Aging and inactivity are both associated with decreased muscle size and protein content. The possible role of RNA degradation in the loss of protein has not yet been investigated. Therefore, we hypothesized that RNA degradation was elevated with muscle atrophy in aging and disuse. Brown Norway/Fisher344 male rats at 6 and 32 months were hindlimb suspended (HS) for 14 days to induce muscle atrophy or remained weight bearing (WB). Cytosolic extracts from gastrocnemius muscles were prepared for Western analysis of DCP-2 protein (marker of p-bodies) and RNA degradation assay. In vitro total RNA decay assay was performed using 30ug of total RNA (from tibialis anterior) incubated with 20ug of S15 extracts from gastrocnemius. RNA integrity was determined using the Agilent Technologies algorithm to calculate the RNA Integrity Number (RIN); decay rate and half-life were calculated for each sample. Results indicated an increase in DCP-2 protein at 32 months of age in both HS and WB groups. In addition, an almost 2-fold increase in decay rate and 48% decrease in half-life of total RNA was observed in muscle from 32 month old rats. However, no significant difference in decay rate and half-life was observed with disuse at either 6 or 32 months. We conclude that muscle atrophy associated with aging, but not disuse, may be due to a decrease in total RNA because of increased RNA degradation.
Supported by APS UGSRF and AG028925.

4. Vagus nerve promotes pulmonary fibrosis.

N. Song[1], J. Liu[2], R. Perez[2], J. Yu[2]

[1]University of Louisville - Louisville, KY/US, [2]Robley Rex VAMC - Louisville, KY/US

Pulmonary fibrosis is a devastating disease with no effective treatment because of our limited knowledge of its pathophysiological mechanisms. Recently, the vagus nerve has been recognized to play a significant role in a variety of pulmonary diseases. In the present studies, we hypothesize that activation of the vagus nerve promotes lung fibrosis through up regulation of transforming growth factor beta (TGF-β), a key fibrogenic factor. To test this hypothesis, we examined bleomycin-induced lung fibrosis (i.v.) in unilaterally vagotomized mice. We compared the extent and severity of fibrosis in the lung with and without vagal innervation. We found more severe fibrosis (subpleural, perivascular and peribronchiolar lesions) and destruction of alveolar architecture, as well as increased cellularity in the innervated lung. Collagen deposition was also higher in the vagally innervated lung. Collagen1A2 levels (by ELISA) were 0.71±0.22% and 0.48±0.17% ($n=10$, $p<0.01$) in innervated and denervated lungs, respectively. The percent area stained for collagen (blue color) with Masson Trichrome was also higher in the innervated lung (12.3±7%) lung (9.2±5%, $n=27$, $p<0.001$). Furthermore, inervated lungs had a higher protein level of TGF-β than denervated ones (0.75±0.18 v.s. 0.61±0.12%, $n=8$, $p<0.05$). Since vagotomy reduced TGF-β expression and alleviated fibrosis after bleomycin treatment, we conclude that the vagus nerve promotes lung fibrosis via activation of TGF-β pathway.

5. Abnormal QTc recovery following exercise identifies an LQT1 mutation that is insensitive to PKA.

Daniel C. Bartos, John R. Guidicessi, Don E. Burgess, Michael J. Ackerman, and Brian P. Delisle

Dept. of Physiology, Univ. of KY

KCNQ1 encodes the pore-forming alpha subunit that conducts the slowly activating delayed rectifier K^+ current (I_{Ks}) in the heart. The arrhythmogenic type 1 long QT syndrome (LQT1) is caused by loss of function *KCNQ1* mutations. Most LQT1-related cardiac events are triggered by exercise. During exercise, adrenergic signaling activates protein kinase A (PKA), which causes an increase of I_{Ks}. LQT1 patients harboring mutations resistant to PKA stimulation are at an increased risk for life threatening events. The use of treadmill stress testing can unmask LQT1 in patients that have normal resting QTc and may be predictive of PKA resistant mutations by determining the response of the QTc during recovery from exercise. Treadmill stress testing of LQT1 patients shows their QTc does not change or prolong during peak exercise when compared to their baseline or resting QTc. After cessation of exercise QTc prolongation occurs after 1 minute recovery and peaks at 3 minutes. At 5 minutes post exercise, the QTc recovers to similar values measured at the 1 minute recovery timepoint. The I235N-KCNQ1 missense mutation was identified in a large multi-generational LQT1 pedigree where 18/22 (82%) of the genotype positive members had a normal baseline QTc (<460ms), but several patients revealed latency in QTc recovery following treadmill stress testing. At 5 minutes post exercise, these patients' QTc did not recover to expected values. Interestingly, the abnormalities in QTc recovery were not observed in I235N patients receiving β-blocker therapy. Voltage-clamping experiments using HEK293 cells expressing wild-type (WT) or I235N with the I_{Ks} β-subunit KCNE1 showed that I235N decreased KCNQ1 current (I_{KCNQ1}) by 93% and caused a large positive shift in the midpoint potential for activation (V½). However, cells co-expressing WT & I235N (to mimic the patient's genotype) showed only a small decrease in I_{KCNQ1} (~30%) and shift in V½. Since the abnormalities in QTc recovery in I235N patients appear blunted by β-blocker therapy, we tested whether I235N prevented PKA activation of I_{KCNQ1}. Following extracellular perfusion of forskolin and IBMX to activate PKA, cells expressing WT increased I_{KCNQ1} by 64%, but I_{KCNQ1} did not increase in cells co-expressing WT & I235N. Computational simulations using a ventricular action potential (AP) model showed that reducing the I_{Ks} component by 30% increased the steady-state AP duration at 90% repolarization (APD90) by 1.6%. However, incorporating β-adrenergic signaling in the simulations showed that reducing the I_{Ks} component by 30% and rendering I_{Ks} insensitive to PKA activation increased the steady-state APD90 by 7.1%. We conclude I235N modestly affects resting QTc, I_{Ks} amplitude, and APD90, but it prevents PKA activation of I_{Ks} to cause a dangerous prolongation in the QTc/APD90 during β-adrenergic stimulation. These data demonstrate that treadmill testing may indicate *KCNQ1* mutations insensitive to PKA activation.

6. Satellite cells regulate the skeletal muscle environment by inhibiting fibroblast function.

Christopher S. Fry[1], Jonah D. Lee[1], Janna R. Jackson[1], Tyler J. Kirby[2], Esther E. Dupont-Versteegden[1], John J. McCarthy[2], Charlotte A. Peterson[1,2]

[1]College of Health Sciences and [2]Department of Physiology, University of Kentucky, Lexington, KY

Interaction between satellite cells and skeletal muscle extracellular matrix (ECM) has been demonstrated, but a role for satellite cells in regulating the ECM during hypertrophy has not been defined. We utilized mouse primary myoblasts and fibroblasts in co-culture in addition to a genetically modified mouse model (Pax7-DTA) to investigate the role of satellite cells in the regulation of muscle ECM. The Pax7-DTA mouse allows for the conditional depletion of satellite cells in adult muscle following tamoxifen administration. Vehicle/tamoxifen treated animals were randomized to sham or synergist ablation surgery, to overload the *plantaris* muscle. Microarray analysis 1 week after surgery showed loss of satellite cells was associated with increased expression of numerous collagens and ECM structural genes. Depletion of satellite cells led to attenuated hypertrophy and fibrosis of the *plantaris* with increases in both ECM deposition and fibroblast number following 8 weeks of overload. Co-culturing mouse myoblasts with fibroblasts led to decreased mRNA expression of *Col Ia2, IIIa1, VIa2* and *Fibronectin* compared to fibroblasts co-cultured with fibroblasts, suggesting a paracrine function of myoblasts that inhibits fibroblast ECM production. We conclude that a novel function of satellite cells both at rest and during hypertrophy is to regulate muscle fibroblasts and the ECM.

Funding: NIAMS R01AR060701, R21AG34453 and JB Kempner Postdoctoral Award.

7. Attenuation of conducted vasodilation in skeletal muscle arterioles during hyperhomocysteinemia.

Givvimani S, Narayanan N, Armaghan F, Pushpakumar S and Tyagi SC.

Department of Physiology and Biophysics,
University of Louisville School of Medicine
Louisville, KY-40202.

Vasomotor responses conducted from terminal arterioles to proximal vessels may contribute to match tissue demands and blood supply during skeletal muscle contraction. Conduction of vasodilatation (CVD) from distal resistance arterioles to the proximal arterioles and feeding arteries during metabolic demand is mediated by intercellular gap junctions in the vascular endothelium. The role of HHcy in musculoskeletal system during CVD is unclear. We hypothesize that during HHcy there is impaired CVD due to decreased expression of endothelial associated connexins and thus decreases tissue perfusion to the contracting skeletal muscles. ***Methods:*** CVD studies are performed in gluteus maximus muscle preparation of wild type (C57BL6/J) and CBS-/+ (HHcy) mice using intra vital microscopy. Expression of connexins and myostatin protein (an anti-skeletal muscle statin) is studied by western blot and Immunohistochemistry methods. Tissue perfusion to the acetylcholine (ACh) was assessed by Laser Doppler technique. ***Results:*** There is decreased CVD and tissue perfusion in response to acetylcholine in the CBS-/+ mice compared to wild type controls. There is decreased expression of connexins 37, 40 & 43 and increased expression of myostatin in CBS-/+ mice compared to wild type controls. ***Conclusion:*** Our findings suggest that CVD in skeletal muscle is decreased during hyperhomocysteinemia due to decreased expression of gap junction connexins.

8. RNA-binding motif protein 3 (RBM3) over-expression results in larger muscle fiber size in vivo and in vitro.

Amy Confides[1], Andrew Judge[2], and Esther Dupont-Versteegden[1]

[1] College of Health Sciences, Department of Rehabilitation Sciences, University of Kentucky, 40536

[2] Department of Physical Therapy, University of Florida, 32610

RNA-binding motif protein-3 (RBM3) is a cold-inducible protein that has been suggested to play a role in regulating muscle size because of its differential expression during atrophy and hypertrophy. RBM3 has been shown to enhance global protein synthesis and to inhibit apoptosis potentially owing to its modulatory effect on miRNA biogenesis. The purpose of this study was to determine if over-expression of RBM3 affects muscle size and we hypothesized that increased RBM3 abundance would be anabolic for skeletal muscle both under control or atrophy-inducing conditions. *In vitro*: C_2C_{12} myoblasts were transiently transfected with RBM3-YFP expressing plasmid and were differentiated into myotubes for 24 hours. In a subset of plates, dexamethasone (100 □M) was added for an additional 24 hours and myotubes were analyzed for size by measuring myotube diameter for RBM3-YFP transfected (green) and non-transfected cells. For *in vivo* studies, soleus muscles in the right legs of 15 month old rats were injected with RBM3 and GFP plasmid while the left leg received pCDNA and GFP plasmid after which the muscles were electroporated. Rats were either kept ambulatory (AMB, n=4) or were hind limb suspended (HS, n=4) for 14 days. Cross sectional area (CSA) of muscle fibers positive for RBM3 (green) was compared to non-transfected fibers. Results show that RBM3 positive myotubes were 30% larger in both control and dexamethasone treated myotubes compared to untransfected cells. Cells transfected with an empty YFP vector did not show a change in myotube size. Also, CSA of soleus muscle fibers which over-expressed RBM3 was 17% and 20% larger than non-transfected fibers in AMB and HS rats, respectively; CSA from the left leg showed no difference between GFP-positive and -negative fibers. We conclude that RBM3 exerts an anabolic effect on muscle fibers under control as well as atrophy-inducing conditions. This work was supported in part by a grant from the NIH/NIA (AG 028925).

9. Parathyroid Hormone (PTH) decreases mRNA stability of the Type IIa Sodium-Phosphate Cotransporter (NpT2a).

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In primary hyperparathyroidism, chronically elevated levels of PTH produce low serum phosphorus through inhibition of proximal tubule expression of sodium-phosphate cotransporters. We have previously shown that chronic PTH stimulation of proximal tubule cells is associated with decreased expression of NpT2a mRNA through destabilization, which is dependent on transcription. BLAST evaluation of the NpT2a promoter identified a CREB-binding region. We hypothesize that PTH decreases NpT2a mRNA stability through activation of the PKA pathway. Opossum kidney (OK) cells were transfected with luciferase reporter gene constructs containing either full-length or serially truncated NpT2a promoter regions. Luciferase reporter assays show no effect of PTH on the promoter function of the NpT2a gene. Direct activation of PKA with 8Br-cAMP and PKC with PMA for 2h produced a decrease in NpT2a mRNA of 35.6 ± 12.2% and 28.9 ± 4.3%, respectively. We conclude that PTH decreases NpT2a mRNA expression through destabilization of NpT2a mRNA, and that both PKA and PKC pathways may contribute to this effect. Support for this project is provided by VA to EDL.

10. Satellite cell depletion negatively impacts voluntary wheel running performance in mice.

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Satellite cells have long been thought to be responsible for muscle plasticity. Recent studies in the field using genetically-modified mouse models that allow for conditional satellite cell ablation have challenged this dogma. Although these studies confirmed that satellite cells are required for muscle regeneration, they surprisingly show that they are not required for muscle growth. While the role that muscle stem cells play in muscle growth and regeneration are being defined, their role in muscle response to aerobic exercise remains unexplored. **Therefore the purpose of the current study is to assess the involvement of satellite cells in response to voluntary wheel running**. Female Pax7-DTA mice were satellite cell depleted following tamoxifen administration. Mice were either ambulatory, or were wheel run for 8 weeks. Satellite cell depleted animals ran ~27% less km/day and 17% slower than non-depleted animals. Succinate dehydrogenase was significantly elevated in plantaris muscles with running, but staining intensity tended to be attenuated in satellite cell-depleted muscle. Myosin heavy chain isoforms were significantly altered with running, independent of satellite cell ablation. Similarly, fiber vascularization, quantified using the endothelial marker CD31, was elevated with running, but was unaffected by satellite cell depletion. **In conclusion, the presence of satellite cells appears to be beneficial to voluntary running performance. The metabolic processes that may be altered in the absence of satellite cells that contribute to decreased endurance are currently under investigation.** Funding: NIAMS R01AR060701

11. The effects of K^+ on skeletal muscle, synaptic transmission and the relationship with deep tissue injury of muscle.

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Currently, deep tissue injuries (DTI) of skeletal muscle and skin are being treated similarly to Stage III or IV pressure ulcers. Presently, this is the only accepted standard of treatment for DTI. The primary skeletal muscle damage can produce secondary effects which can increase the spread of the damage zone. This can come about by the additive effects of intracellular contents, particularly the ion K+, released from crushed muscle cells in the spreading of DTI. It has been known since the time. It is well known since the 1930's that fluid from damaged skin tissue would cause sensory neurons to stop responding (Feng, 1933). Also, it is well known that increasing the $[K+]_o$ in a saline Ringer solution 10 times the normal will result in cell (i.e., muscle) death. However, the consideration in the exposure time and effects of restoring normal $[K+]_o$ on the health of skeletal muscle has not been fully addressed. We are examining the effects of rapid rises of $[K+]_o$ over various periods of time before returning back to normal levels on the health of the muscle and the effects on synaptic properties at the neuromuscular junction. At present we are conducting investigations on the crayfish opener muscle as a model. We plan to gather information on treatment and assessment of DTIs in urgent care centers as well as establish rodent models for experimentation.

12. Educating the public on the association between COPD and oxygen therapy.

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Chronic Obstructive Pulmonary Disease is the most common lung disease, affecting over 12 million people in the United States. It is especially prevalent in the Mideastern portion of the United States, including states like Indiana, Ohio, Kentucky, West Virginia, and Tennessee. Many people who are in the advanced stages of the disease begin oxygen therapy with hopes of bettering their condition; however, they are not aware of the risk associated with taking oxygen in conjunction with having COPD, where breathing can be hypoxic-driven. Preliminary data from a pilot study, which focused on acid/base chemistry, showed that the general public, as well as people who work in the allied health field, are not aware of the basic physiological phenomena occurring within the body of a person with COPD. Knowing the basics of chemistry and biology is important when trying to understand basic physiological principles and are then important when prescribing a correct course of treatment. Because of the results of the pilot study, it was apparent that there was a need for the development of educational material that can better inform the public about oxygen and the regulation of body pH in people with healthy lungs and of those with COPD. To meet this need, a brochure was created to communicate this association. These types of educational tools can be used in a variety of locations in order to educate the public on the relationship between science and health-related issues. Education is a key step to having a healthier population.

13. The action of stimulating adenylyl cyclase within motor nerve terminals in the regulation of synaptic vesicles.

Y. Zhu, W.-H. Wu and R.L. Cooper

Department of Biology and Center for Muscle Biology, University of Kentucky

Past studies using the crayfish neuromuscular junction (NMJ) have shown that serotonin (5-HT) can enhance synaptic transmission even after synaptic depression is induced and that this is partially due to activation of the IP3 signaling. Recently it was shown that blocking PLC (which decreases in IP3 formation) dampens the 5-HT induced response; however, there was still a substantial action that was induced by 5-HT. cAMP has been suggested to be involved in 5-HT action in invertebrate neurons. To test the idea that 5-HT might stimulate cAMP to account for some of the enhanced responses forskolin (an adenylyl cyclase activator) was added to non-depressed and synaptically depressed NMJs. To examine for a commonality in altering synaptic transmission, NMJs of the larval *Drosophila* were also examined. What is interesting about the larval NMJs is that they are insensitive to application of 5-HT. The novel aspect in this study compared to previous studies is in examining if the reserve pool (RP) of vesicles can be recruited following depression of the readily releasable pool (RRP) of synaptic vesicles through activation of adenylyl cyclase.

14. Characterization of 5-HT receptor subtype in sensory-CNS-motor circuit in *Drosophila* larvae.

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Department of Biology and Center for Muscle Biology, University of Kentucky

It is known that serotonin (5-hydroxytryptamine, 5-HT) can modulate the sensory-CNS-motor activity in *Drosophila* larvae. But the 5-HT receptor subtypes underlying the circuit activity have yet to be investigated. We hypothesize that activation of 5-HT2 and/or 5-HT7 increases the circuit activity in *Drosophila* third instar larvae. It has been revealed that *Drosophila* genome encodes four 5HT receptor subtypes, 5-HT1ADro, 5-HT1BDro, 5-HT2Dro, and 5-HT7Dro. 5-HT receptors in *Drosophila* are G-protein coupled receptors (GPCR). 5-HT1A and 5-HT1B are coupled with Gα inhibitory (Gαi). 5-HT2 is coupled with Gαq heterotrimeric G-protein and 5-HT7 is coupled with Gα stimulatory (Gαs). To test the action of pharmacological agents on the 5-HT receptors, the segmental nerves leading to the CNS were stimulated at 40Hz, 10 pulses while the body wall muscle fibers on the contralateral side were monitored. The evoked EPSPs in muscles 6 or 7 were counted before and after adding various pharmacological agents. In this study, various 5-HT agonists (**5-HT1A agonist:** 8-Hydroxy-DPAT hydrobromide; **5-HT1B agonist:** CP 93129 HCl; **5-HT2 agonist:** DOI hydrochloride; and **5-HT7agonist:** AS 19) were used. We are now using a UAS-GAL4 system with specific UAS-RNAi-5-HT lines to knockdown specific 5-HT receptors in respective 5-HT receptor expressing neurons. Preliminary pharmacological results suggest 5-HT2 receptors heighten the activity within this circuit. In completing these studies we will be able to conclude which specific 5-HT receptor subtypes impact a sensory-CNS-motor circuit.

15. Analyzing expression pattern's and correlation's for HSET, Survivin & Ki67 genes in ER, PR & HER2 Negative Breast Cancer Patient's.

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Background:

Receptors like Estrogen (ER), Progesterone (PR) and Human Epidermal Growth Factor Receptor 2 (HER2) are important indicators in predicting the prognosis of human breast cancer. Human breast cancers with ER, PR and HER2 negative receptors have unidentified molecular targets, so are difficult to treat [2]. Moreover there is a high rate of recurrence and a poor prognosis in women with ER, PR and HER2 negative receptor breast cancer [3, 4]. The centrosome clustering protein, HSET, is up regulated in breast cancer tissues compared to normal breast epithelia, whereas Survivin gene is involved in tumorigenesis, including proliferation, migration, and invasion [5]. The immunohistochemical assessment of the proportion of cells staining for the nuclear antigen Ki67 in breast cancer tissue has become the most widely used method for comparing proliferation between tumor samples, thus making Ki67 an important biomarker for prognosis of breast cancer[6]. So we were interested in seeing the relations, interactions and gene expression's between HSET, Survivin and Ki67 genes in breast cancer patients with ER, PR and HER2 negative receptor's.

Materials:

R Environment, Affy Bio conductor package.

Methods:

2000 breast cancer samples were downloaded from GEO database, a bimodal filter on the gene expression data [1] was applied to identify Affymetrix probes 205225_at, 208305_at and 216836_s_at representing ER, PR & HER2 receptors. A Triple Negative Breast Cancer (TNBC) and Non Triple Negative Breast Cancer (Non TNBC) datasets were prepared. Along with these two major categories, a non TNBC dataset was divided in 7 sub categories as mentioned in Table no 1. A robust multiarray average (RMA) normalization was performed on all the chips for variance stabilization. We determined HSET, survivin and Ki67 gene expression in 2000 samples to see their gene expression patterns in progression of breast cancer. The correlation plots were plotted to see the relationships between different categories.

Results & Conclusions:

We found a significant Pearson correlation coefficient between Survivin and Ki67, where the two tailed P value was also found significant, indicating a moderate negative correlation. Similarly a significant Pearson correlation coefficient was found between HSET and Survivin with a moderate positive correlation. Both these values indicate a linear correlation ship between respective genes with a need to understand their functions and interactions in detail. We are further exploring cluster and network analysis for these genes and their involvement in TNBC's and Non TNBC's.

Cont… #15

Category	No of Chips (N = 2000)	Percentage (%)
ER+ PR+ Her2+	591	29
ER- PR+ Her2+	265	13
ER+ PR- Her2+	239	11
ER+ PR+ Her2-	190	9
ER- PR+ Her2-	57	2
ER+ PR- Her2-	68	3
ER- PR- Her2+	511	25
ER- PR- Her2-	64	3

Table 1: Categories with number of chips and respective percentages from the 2000 chips analyzed.

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16. Confirmation of squalene in tears and sebum and its potential function.

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Squalene is found on the skin in sebum and in meibum on the surface of the eye lid and tears. A ^{1}H NMR resonance at 5.2 ppm has been tentatively used to quantify squalene in human sebum and meibum. When the relative intensity of the resonance in the NMR spectra of human meibum is low as in dry eye associated with meibomian gland dysfunction, the tear film is unstable and patients have signs and symptoms of dry eye. When the intensity of the resonance is restored with azithromycin or doxycycline treatment, tear film stability is restored and patients no longer are afflicted by symptoms of dry eye. In this study, we confirmed that the 5.2 ppm resonance associated with human sebum and eye lid meibum lipid (ELML) is due to squalene.

Infrared, ^{1}H and ^{13}C NMR (heteronuclear single quantum correlation) spectroscopy, Langmuir trough and Brewster angle microscopy were used. Evaporation rates were measured in vitro by gravimetric analysis.

Due to the lack of squalene-squalene and squalene-wax interactions, squalene is likely to spread over the surface of skin or the tear film. It is possible that the thin layer of squalene could reduce the rate of evaporation on the surface of the skin and offer antioxidant, antibacterial, and anti-inflammatory protection to the skin and tears. At low surface pressures, squalene filled thinner regions of meibum films. It is this property of squalene that could potentially stabilize the tear film during break up by migrating to the areas without a tear film lipid layer offering protection to the cornea.

17. Binding partners, motifs, and post-translational modifications of human Chmp1A protein.

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Charged multivesicular body protein 1A/Chromatin modifying protein 1A (Chmp1A) is a member of Endosomal sorting complex required for transport (ESCRT)-III protein family, which is known to function in the formation and sorting of multivesicular bodies (MVBs) for lysosomal degradation of transmembrane proteins. Recently we have shown that Chmp1A functions as a novel tumor suppressor in human pancreatic tumor cells. To gain a better understanding of Chmp1A we identified binding partners by using co-immunoprecipitation and LC/MS/MS, and new motifs by searching database. In addition to a nuclear localization signal (NLS), coiled-coil and microtubule interacting and trafficking molecule domain (MIT)-interacting motif, we found that Chmp1A contains a highly conserved ubiquitin-interacting motif (UIM), and SUMOylation sites. Our proteomics data suggests that Chmp1A binds proteins in various cellular locations with diverse cellular processes. Moreover, LC/MS/MS analysis revealed Chmp1A with a molecular weight higher than predicted, indicating potential post-translational modifications with Ubiquitin (Ub) or Sumo. Our data indicates that Chmp1A interacted with ubiquitin, served as substrate for ubiquitin and sumo modifications. To confirm the specificity of binding, we generated a NLS deletion of Chmp1A, which removes two thirds of UIM. Full-length Chmp1A over-expression was detected in the nucleus and cytoplasm, but NLS deletion only in the cytoplasm. Ectopically induced Chmp1A co-localized with nuclear protein and showed significant binding with ubiquitin, which was abolished with over-expression of NLS-deletion. Collectively our study provides new insights into Chmp1A protein that would facilitate understanding its functions and mechanisms in health and disease.

18. Cellular mechanism for the pharmacological correction of hERG mutations linked to the Long QT Syndrome.

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The *human Ether-a-go-go Related Gene* (*hERG*) encodes Kv11.1 and underlies the rapidly activating delayed rectifier K^+ current in the heart, and loss-of-function *hERG* mutations cause the type 2 long QT syndrome (LQT2). The majority of LQT2-linked missense mutations decrease the trafficking of Kv11.1. An important finding is drugs that bind to Kv11.1 and block current ($I_{Kv11.1}$) can correct the trafficking for most of these mutations (pharmacological correction). We tested the hypothesis that pharmacological correction increases the trafficking of mutant LQT2 channels from the Endoplasmic Reticulum (ER). Voltage-clamping and Western blotting experiments of HEK293 cells expressing the trafficking-deficient LQT2 mutation G601S showed that pharmacological correction still occurred in cells treated with the protein synthesis inhibitor cycloheximide. Confocal analyses of HEK293 cells stably expressing wild type Kv11.1 or G601S showed that G601S is selectively stored in an intermediate ER compartment with BAP31. The intermediate BAP31 compartment does not overlap with the perinuclear ER compartment, transitional ER compartment, or the ER Golgi Intermediate Compartment. Treating cells in E-4031, a drug that corrects G601S trafficking, decreased G601S co-localization with intermediate BAP31 compartment and increased G601S immunostaining at the cell surface membrane. Additional experiments showed that treating cell in E-4031 for as little as 30 min was sufficient to caused the pharmacological correction of $I_{Kv11.1}$ for many hours. Together these data demonstrate that a steady-state subpopulation of LQT2 channels is stored separately in the BAP31 transitional ER compartment and their functional expression is readily corrected by E-4031 treatment.

19. Investigation of potential relationship between epigenetic regulation of Chmp1A and its function in tumor suppression in pancreatic tumor cells.

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Pancreatic cancer has one of the worst prognoses of any carcinoma and is the 4th major cause of cancer death. Thus identification of new factors regulating pancreatic cancer would facilitate the development of new therapeutics or early detection methods for pancreatic cancer. Chromatin modifying protein 1A / Charged multivesicular body protein 1A (Chmp1A) is a member of the ESCRT-III protein family. It has been shown that Chmp1A functions in chromatin condensation, and in gene silencing, by interacting with a transcriptional repressor Polycomb-group (PcG) protein, BMI1. We have shown that Chmp1A functions as a novel tumor suppressor through activation/phosphorylation of Ataxia Telangiectasias Mutated (ATM) and P53 in pancreatic ductal cancer cells (PanC-1). Although Chmp1A has been shown to function in chromatin condensation via modification of histone H3, the detailed effect of chmp1A on histone modification(s) or histone modifying enzyme(s) have not been investigated. In addition, it still needs to be examined if there is any correlation between the effects on chromatin modification of Chmp1A and its function in tumor suppression. Using serum starved HEK 293T cells, we performed Western blot and immunocytochemical analyses with various antibodies of histone modifications and modifying enzymes. Our data indicates that Chmp1A over-expression led to increases in tri-methyl H3 (27), methyltransferase, but decreases in di-methyl H3 (K4, K27 and K36) and Histones (2A and H3). Once the data is confirmed, similar experiments will be performed in pancreatic cancer cells and determined if there is correlation between chromatin modification(s) and growth suppression or promotion.

20. Sarcopenia is independent of lifelong muscle stem cell depletion.

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An unresolved question is whether the loss of muscle stem cells, known as satellite cells, with age contributes directly to sarcopenia. To address this question, we developed the Pax7-DTA mouse that conditionally and specifically ablates Pax7+ satellite cells following the administration of tamoxifen. Four month old mice were treated with tamoxifen or vehicle, and sacrificed at 5 or 24 months of age. With age, vehicle-treated plantaris muscles demonstrated a reduction (52%) in Pax7+ satellite cells. Tamoxifen-treated muscles showed greater than 90% ablation after 1 month and no recovery after 20 months, such that the aged mice lived the majority of their lives with a significantly reduced satellite cell pool. Despite satellite cell depletion with tamoxifen, muscle weight, myofiber cross-sectional area and function were all reduced with age independent of satellite cell number. The overall distribution of large myofibers was decreased with age, but not affected by the loss of satellite cells. However, the total number of small fibers (<600 um2), which were relatively more abundant in aged muscle, were reduced with tamoxifen in both groups. This drug effect suggests that myofiber regeneration is significantly blunted in muscle depleted of satellite cells. Myonuclear number did not change with age and was unaffected by satellite cell depletion. Furthermore, BrdU labeling in the 2 weeks prior to sacrifice showed little myonuclear accretion regardless of age or satellite cell status. These data provide convincing evidence that satellite cells do not play a role in maintenance of skeletal muscle mass across the lifespan. Thus, the aging satellite cell niche does not contribute to sarcopenia, suggesting that regeneration and lifelong muscle maintenance are distinct processes. Strategies to combat sarcopenia should focus on anabolic processes within muscle fibers independent of preserving the satellite cell pool.
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21. NFκB signaling and inducible nitric oxide synthase activity during pulmonary ischemia-reperfusion increase co-localization of fibrinogen/fibrin and platelets at sites of vascular leakage in rabbit lung.

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Platelet adhesion and fibrinogen/fibrin (Fg/Fb) deposition cause endothelial dysfunction during acute lung injury. We examined the hypothesis that during pulmonary IR, platelet activation, recruitment of Fg/Fb to the vascular wall and subsequent leakage are associated with NFκB and inducible nitric oxide synthase (iNOS) activation pathways. Subpleural microvessels and alveoli were examined by video microscopy of the right lung in anesthetized rabbits with open chest and ventilated lungs. IR was caused by occluding the right pulmonary artery for 2 h. Injection of fluorescently labeled albumin and a polyclonal antibody to Fg/Fb into the pulmonary circulation via the right atrium during early reperfusion revealed areas of microvascular leakage and interstitial edema associated with Fg/Fb deposition. Platelets were detected by immunohistochemistry of tissue samples at sites of vascular leakage in conjunction with Fg/Fb. On average, IR (n=3) caused a 6-7 fold increase in albumin leakage, platelet adhesion, and albumin-Fg/Fb-platelet co-localization compared to control. Additionally, IR increased NF-κB activation in lung lysates as detected by phosphorylation of IκB. These effects were attenuated by treatment with an iNOS activity inhibitor (1400W) before IR. Our data suggest that NFκB and iNOS activation may play a critical role in the regulation of platelet Fg/Fb–induced microvascular leakage during pulmonary IR. Supported in part by American Lung Association-Kentucky to AMR and NIH grant #HL080394 to DL.

22. Transmural variations in cellular level contractile function in the left ventricle of patients with endstage heart failure.

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As heart failure becomes more progressive, remodeling of the left ventricle occurs. Little is known about the cellular level changes across the ventricular wall during this remodeling. Our hypothesis was that the contractile properties at the cellular level become homogenous across the ventricular wall in failing hearts as compared to nonfailing hearts. To investigate this hypothesis, we procured through wall left ventricular samples from patients (n=10) undergoing heart transplants at the University of Kentucky and from nonfailing organ donors (n=5). Mechanical assays were performed on chemically permeabilized preparations that were obtained from the sub epicardial, midwall and sub endocardial regions of the ventricular wall. Maximum power output- a cellular level measure of the ability of the heart to eject blood during systole was assessed. The results showed that in the nonfailing samples, the power output was significantly higher in the midwall as compared to sub endocardium and sub epicardium (p-value < 0.05), suggesting heterogeneity in power across the wall. In contrast, transmural samples from failing hearts were not significantly different from each other indicating homogeneity across the wall. Furthermore, power decreased by ~ 30% (p-value = 0.01) in samples from patients with endstage heart failure as compared to nonfailing hearts. This decrease in power may reflect the replacement of myocyte by fibrotic tissue confirmed by increase in collagen to tissue ratio (p=0.03) by picrosirius red staining in failing midwall samples. This study suggests that there is a cellular level contractile dysfunction across the ventricular wall in endstage heart failure.

23. Right and left and vagus nerves regulate breathing synergistically.

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Pulmonary fibrosis is a devastating disease with no effective treatment because of our limited knowledge of its pathophysiological mechanisms. Recently, the vagus nerve has been recognized to play a significant role in a variety of pulmonary diseases. In the present studies, we hypothesize that activation of the vagus nerve promotes lung fibrosis through up regulation of transforming growth factor beta (TGF-β), a key fibrogenic factor. To test this hypothesis, we examined bleomycin-induced lung fibrosis (i.v.) in unilaterally vagotomized mice. We compared the extent and severity of fibrosis in the lung with and without vagal innervation. We found more severe fibrosis (subpleural, perivascular and peribronchiolar lesions) and destruction of alveolar architecture, as well as increased cellularity in the innervated lung. Collagen deposition was also higher in the vagally innervated lung. Collagen1A2 levels (by ELISA) were 0.71±0.22% and 0.48±0.17% ($n=10$, $p<0.01$) in innervated and denervated lungs, respectively. The percent area stained for collagen (blue color) with Masson Trichrome was also higher in the innervated lung (12.3±7%) lung (9.2±5%, $n=27$, $p<0.001$). Furthermore, inervated lungs had a higher protein level of TGF-β than denervated ones (0.75±0.18 v.s. 0.61±0.12%, $n=8$, $p<0.05$). Since vagotomy reduced TGF-β expression and alleviated fibrosis after bleomycin treatment, we conclude that the vagus nerve promotes lung fibrosis via activation of TGF-β pathway.

24. Effects of insulin on phenotypically-identified neurons of the nucleus tractus solitaries.

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In addition to regulating glucose utilization peripherally, insulin crosses the blood brain barrier, is transported into the brainstem, and affects whole-body metabolism through central mechanisms. Brainstem nuclei, specifically the dorsal motor nucleus of the vagus (DMV) and nucleus tractus solitarius (NTS), critically regulate visceral function. The NTS receives viscerosensory vagal input, and projects heavily to the DMV, which supplies parasympathetic vagal motor output to the abdominal viscera. Pathologies in which insulin is dysregulated, including diabetes, can disrupt this circuit, leading to gastric and other autonomic dysfunction. In the DMV, insulin significantly reduces glutamate release and neuronal excitability, with no effect on inhibition, suggesting a presynaptic effect on glutamatergic input, likely from NTS neurons. We used whole-cell patch-clamp recordings in brainstem slices of transgenic mice (FVB-TgN, GAD-GFP) to identify effects of insulin on synaptic properties of putative glutamatergic NTS neurons. Preliminary data suggests that insulin inhibits sEPSCs and action potentials in non-GFP neurons, but not in GFP+ cells. Neuronal phenotype of the recorded neurons will be verified using single-cell PCR. These experiments are currently being conducted in diabetic (streptozotocin-treated) mice. Such regulation may influence glucose utilization in the dorsal vagal complex and thus, autonomic visceral regulation.

25. Renovascular remodeling in hypertensive dahl-ss rats: role of mmp inhibitor as a hypertension ameliorating agent.

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High salt diet has long been associated with chronic hypertension. The development of renal injury in Dahl salt-sensitive (SS) hypertensive rats is characterized by structural and functional changes involving vascular remodeling. Increased activity of matrix metalloproteinases (MMPs) leading to alteration in the extracellular matrix (ECM) is one of the main mechanism contributing to increased peripheral vascular resistance. We hypothesized that MMP inhibition will modulate ECM remodeling by decreasing its activity and thus reduce mean arterial blood pressure. Dahl-salt sensitive (Dahl-SS) and Lewis rats were fed on high salt diet. The groups were 1) Dahl-SS, 2) Dahl-SS+GM6001 (non-specific MMP inhibitor), 3) Lewis, and 4) Lewis+GM6001. GM6001 was given at 0.5mg/mL by intra-peritoneal injection on alternate days for 3 weeks. Blood pressure, laser doppler flowmetry for renal cortical blood flow and barium angiography for renal vascular density were measured. Results: Mean arterial blood pressure was 172.10 ± 0.57 mm Hg in hypertensive Dahl-SS rats compared to 136.12 ± 1.22 mm Hg in Dahl-SS+GM6001 rats. The mean arterial pressure in Lewis and Lewis+GM6001 groups were 97.08 ± 0.56 and 87.63 ± 2.93 mm Hg respectively. Laser doppler flowmetry showed reduced renal cortical blood flow (1333.33 flux units) in Dahl-SS rats compared to Dahl-SS rats treated with GM6001 (1605 flux units). Barium angiography demonstrated increased renal vascular density with patent branches in the renal cortex of animals treated with MMP inhibitor, GM6001. Conclusion: Our results suggest that in hypertensive Dahl-SS rats, inhibition of MMP attenuates high blood pressure, maintains patency of renal cortical vessels thus improving cortical blood flow.

26. Maternally derived mitochondria in SHR exhibit significant reduction in oxidative phosphorylation gene expression.

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Mitochondrial (Mt) dysfunction contributes to the pathophysiology of renal function and promotes cardiovascular disease including hypertension. We hypothesize that renal Mt-genes derived from female SHR are linked to high blood pressure in offspring of SHR and normotensive (NT) Brown Norway (BN) rat crosses. After breeding a female Okamoto-Aoki SHR (MAP=160mmHg) with a BN male (MAP=91mmHg), hypertensive female progeny were backcrossed with the founder BN for 5 consecutive generations. Mt-protein coding genes (13 total) and nuclear transcription factors mediating Mt-transcription were evaluated in kidney, heart and liver of NT(n=20) vs. HT(n=20) SHR/BN using quantitative real-time PCR. GAPDH was used as reference. Renal but not liver or heart Mt-gene expression was decreased ~2-5 fold in 12 of 13 protein coding genes of HT SHR/BN. Renal but not liver mRNA expression of nuclear transcription factors for Tfam, NRF1, NRF2 and Pgc1α were decreased in HT SHR/BN. This renal specific reduction of nuclear and Mt-gene expression may down-regulate oxidative metabolism and increase renal reactive oxygen species production in SHR leading to hypertension and cardiovascular disease.

27. Hydrogen sulfide mitigates diabetic nephropathy through NMDA receptor mediated renal remodeling.

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Diabetic nephropathy is a leading cause of vascular morbidity and mortality. Recently, the involvement of N-methyl-D-aspartate receptor (NMDAR) and hydrogen sulfide (H2S) in diabetes associated complications has been implicated. Our aim in this study was to determine whether H2S mitigates diabetic nephropathy by modulating gap junction and matrix proteins by antagonizing NMDAR. We used a diabetic model (Akita, C57BL/6J-Ins1Akita), matrix metalloproteinase-9 knockout (KO) [(MMP-9-/-)] and double KO of Akita/MMP-9-/- mice and in vitro cell culture. Results: Diabetic kidneys showed decreased levels of H2S and its enzymes CBS and CSE in both mRNA and protein level and increased expression of NMDA-R1, connexin-40, -43 (Cx-40,-43) and MMP-9. Treatment with H2S reversed these effects. In double KO mice, NMDA-R1 was high but Cx-40, -43 was normal. Additionally, treatment with NMDA-R1 blocker dizicilpine (MK801) failed to disrupt connexin. Glomerular endothelial cells treated with high glucose indicated dysregulated Cx-40, -43. Inhibition of NMDA-R1 by MK801, silencing MMP-9 by siRNA or H2S treatment preserved gap junction proteins. We conclude that in diabetic nephropathy, remodeling is mediated by NMDA-R1 associated MMP-9 activation and H2S plays a significant role in mitigating nephropathy.

28. A modified Huxley-type model that simulates activation-dependent rates of tension recovery.

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Many laboratories now estimate how quickly cross-bridges cycle in chemically permeabilized muscle preparations by measuring how quickly tension recovers to its steady-state after a rapid-shortening/re-stretch perturbation. Brenner, who developed this technique (PNAS, 85:3265-3269, 1988), showed that the rate of force development (k_{tr}) increased with the level of Ca^{2+} activation. Kenneth B. Campbell (no relation to the present author) developed an analytical model (Biophys J., 72:254-262, 1997) that attributed the activation-dependence of the rate constant to cooperative effects. The current work extends this approach by modifying a Huxley-type model (Prog Biophys Biophys Chem, 7:255-318, 1957) so that the proportion of binding sites that are available for cross-bridges to attach to (p_{on}) depends on the prevailing Ca^{2+} concentration and the muscle's history of movement. Specifically, $dp_{on}/dt = (k_{on}[Ca^{2+}] + \gamma p_{on})(1-p_{on}) - k_{off}(p_{on}-p_{bound})$, where k_{on} and k_{off} are rate constants, γ is a scaling factor, and p_{bound} is the proportion of binding sites that have myosin heads attached. During a rapid shortening/re-stretch maneuver, myosin heads are forcibly detached from binding sites because the Huxley-type f and g rate functions are strain-dependent. This increases the (p_{on}-p_{bound}) term above and the p_{on} population thus declines. At high Ca^{2+} concentrations, this effect is short-lived and p_{on} quickly returns to its steady-state value. k_{tr} under these conditions is thus limited by the f and g rate functions. At low Ca^{2+} concentrations, dp_{on}/dt is suppressed and k_{tr} is dictated by how quickly binding sites become available for myosin heads to attach to. Initial calculations have shown that when this model is driven by a realistic length perturbation, the simulated force records accurately reproduce those measured in real experiments using rat myocardial preparations.

29. Ablation of MMP9 mitigates autophagy mediated contractile dysfunction of cardiomyocytes in Ins2+/- Akita.

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Insulin2 gene of mice is orthologous to human Insulin and mutation in Insulin causes diabetic cardiomyopathy, which is associated with activation of matrix metalloproteinase-9 (MMP9). Autophagy is ubiquitous in all forms of heart failure. However, role of MMP9 in autophagy mediated contractile dysfunction is unclear. We hypothesize that ablation of MMP9 mitigates autophagy mediated contractile dysfunction in diabetes. To test the hypothesis, we created Ins2+/- /MMP9-/- mice, where MMP9 gene is deleted from diabetic Ins2+/-. We measured the levels of LC3B-II and Atg3 (autophagy markers) in C57BL/6J, Ins2+/- and Ins2+/- /MMP9-/- hearts. The results revealed induction of both LC3B-II and Atg3 in Ins2+/-, which is mitigated in Ins2+/-/MMP9-/-. The down regulation of autophagy in Ins2+/-/MMP9-/- suggests that targeted deletion of MMP9 attenuates autophagy in diabetes. To assess the specific role of autophagy in contractility, we administered Mdivi-1 (autophagy blocker) into C57BL/6J (control) and Ins2+/- mice (50mg/Kg, i.p) for 7 days and determined the improvement in contractility (±dL/dt) of cardiomyocytes by Ion-Optics device. We found no significant difference in the rate of contraction (dL/dt) and relaxation (-dL/dt) in C57BL/6J untreated and treated groups. However, there was significant improvement in ±dL/dt in Ins2+/- and treated. These findings elicit a novel regulatory role of MMP9 in autophagy in diabetes. It also suggests that inhibition of autophagy improves contractility in diabetic hearts. In conclusion, targeted deletion of MMP9 mitigates autophagy mediated contractile dysfunction in diabetes.

30. TRPA1-mediated damage sensing by the cochlear supporting cells.

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A single event of acoustic overstimulation leads to a fast release of extracellular ATP and an increase in oxidative stress in the cochlea, which can last for days or weeks. The transient receptor potential ankyrin 1 (TRPA1) channel is highly expressed in the inner ear epithelium and could be activated downstream of metabotropic ATP receptors or directly by the lipid peroxidation byproduct, 4-hydroxynonenal (4-HNE). However, the exact localization and physiological function of TRPA1 channels in the cochlea are still unknown.

Functional TRPA1 channels were assessed in mouse cochlear explants by the uptake of the small cationic dye FM1-43. After stimulation with a TRPA1 agonist, FM1-43 uptake was observed in wild type Hensen's cells (HeC), cells of the Kolliker's organ (CKO), epithelial cells near the spiral ligament, but not in Claudius's cells. Live cell ratiometric Ca^{2+} imaging revealed responses to the puff application of 4-HNE in the same cells that exhibited FM1-43 uptake. Ca^{2+} responses in HeCs were delayed, robust, long-lasting, and highly dependent on the baseline levels of intracellular Ca^{2+}. Occasionally, Ca^{2+} responses starting in the HeCs propagated into the CKO and triggered Ca^{2+} waves that required extracellular ATP. To study TRPA1 activation at the single cell level, whole cell patch-clamp recordings were performed in the presence of a gap junction blocker. Upon puff application of 4-HNE, inward currents were observed in wild type HeC, Deiters' and pillar cells.

TRPA1 channels have been involved in sensing cold, mechanical and chemical noxious stimuli by nociceptive neurons. Here we show that TRPA1 channels can sense chemical damage in cochlear supporting cells too. Supported by NIDCD/NIH (R01 DC009434)

31. Epigenetic mechanism of atherosclerosis and hypertension in Hyperhomocysteinemia.

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Although elevated levels of homocysteine (Hcy), known as Hyperhomocysteinemia (HHcy) is associated with aortic stenosis, dissection and hypertension, the mechanisms are not known. Aortic dissection tissue specimen from patients suffering from aortic aneurysm and stenosis were obtained and the mRNA levels of CBS, MTHFR, SAHH, PRX, NOX, Collagens, Elastin, Tissue Inhibitor of Metalloproteinases (TIMPs) and Matrix Metalloproteinases (MMPs) were measured. The levels of CBS, MTHFR, TIMP1, TIMP4, MMP1 and SAHH were high in aortic dissection samples compared to the controls. Since Hcy plays an important role in regulating the DNA methylation and to study the epigenetic control behind Hcy-mediated atherosclerosis and hypertension, the methylation levels of CBS and MBD2 were measured. Bisulfite treated DNA was amplified using CBS and MBD2 primers and the level of methylation was measured in cardiac tissue of wild type (WT), CBS (-/+), TIMP2 (-/-) and MMP9 (-/-) knockout mice. CBS was shown to be hypermethylated in CBS (-/+), TIMP2 (-/-) and MMP9 (-/-) when compared to WT samples. Also elevated mRNA levels of DNMT3a was observed in CBS (-/+) mouse. These findings suggest that differential expression and methylation levels of genes associated with Hcy metabolism are observed in human aortic stenosis and hypertension.

32. Role of PLC-IP3-PKC pathway in 5-HT mediated heart rate modulation in *Drosophila* larvae.

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The *Drosophila melanogaster* heart is proven to be a good model system for studying cardiac diseases that are related to human heart disorders. It has been shown that Ca^{2+} and K^{+} ions play important roles in cardiomyocyte action potential generation. Serotonin, 5-hydroxytrytamine (5-HT), application to the dissected larvae increases the heart rate; however, the underlying molecular signaling pathways have yet to be investigated. There are four 5-HT receptor genes in *Drosophila melanogaster* genome that encode 5-HT1ADro, 5-HT1BDro, 5-HT2Dro, and 5-HT7Dro. We hypothesize that 5-HT increases the heart rate through activation of PLC-IP3-PKC pathway. Here, third instar larvae were employed. The preparation was left for one minute inside saline after dissection and then the heartbeats were counted for the following minute. The dissected larvae are incubated inside inhibitors and activators for 10min then the heartbeats are counted for the next minute. The percentage change of heart rate before and after application of compounds is used as a measure to the effects of various chemicals. Various concentrations of Ca2+ are examined along with the action of 5-HT on the heart rate. A ryanodine receptor inhibitor was tested to determine if it blocks the positive chronotropic effect of 5-HT. Currently we are investigating a PLC inhibitor to disrupt the whole PLC-PKC pathway and a protein kinase C (PKC) inhibitor to disrupt one side of PLC-PKA signaling pathway. We expect that the various calcium concentrations might affect the action of 5-HT. Also, the inhibitor of ryanodine receptor might decrease the action of 5-HT.

33. Development of a new endocrine student laboratory exercise for physiology courses using invertebrates.

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The purpose of this project was to develop a new endocrine-based student lab using an invertebrate species, red swamp crayfish, or *Procambarus clarkii*. In crayfish, hemolymph glucose concentrations are maintained in part, by the action of crustacean hyperglycemic hormone (cHGH) produced in the sinus gland at the base of the eyestalk. Removal of the sinus gland renders the organism unable to elevate blood glucose concentrations in response to neural signaling. Three experiments were conducted to determine if this neuroendocrine pathway could be developed into a student laboratory exercise. During the first experiment, crayfish survival was monitored in a control (intact) group and a second group in which the eyestalks had been surgically removed. Results showed no significant difference of survival between the two treatment groups through 2-weeks post-surgery. During the second experiment, control and eyestalk removed crayfish were given subcutaneous injections of serotonin (final circulating dose 200 nM) at 0 (same day), 1, 2, 7, and 14 days post-surgery. The resulting hemolymph glucose concentrations were measured 1 hour after injection. Eyestalk removed vs. control crayfish showed significant differences ($p< 0.05$) when injections took place at 1-day and 14-days post-surgery. For the third experiment, eyestalk removed and control crayfish were injected with serotonin at 9 AM and 3 PM times of day (n= 10 per group), to determine if circadian rhythms might influence the glucose responses. Results showed no significant differences in responses between crayfish injected in the morning vs. afternoon. Overall, results show crayfish could be surgically modified up to two weeks in advance of the student laboratory without loss of the expected endocrine response or significant mortality. Results also show this endocrine pathway is robust enough that results of student experiments performed in the morning would not be significantly different from those performed during an afternoon laboratory session.

34. The use of online review modules to prepare D.M.D. Students for Basic Science Courses.

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There can be disconnect between the level or breadth of content covered in undergraduate coursework, and the expectations of professional-level faculty of their incoming students. Some Basic Science faculty members may assume that students have a good knowledge base in the material and neglect to appropriately review, while others may spend too much class time reviewing basic material. We hypothesized that the incorporation of online review modules into the Dental Physiology curriculum would allow students to review basic concepts, and would allow faculty members to analyze student preparedness and save valuable class time. Results indicated that the performance levels of incoming students was poor (57% average on a pre-test), and students often under-predicted their abilities (by 12% on average). Faculty expectations varied greatly between the different content areas, and did not appear to correlate with the actual student performance. Three review modules were created which produced a statistically significant increase in post-test scores (46% increase, $p<.0001$, $n=114-115$). The positive results of this study suggest a need for a restructuring of the Basic Sciences curriculum to incorporate online review units and revise introductory course material tailored to students' strengths and needs.

35. STEM & Health: Stressors on the circulatory system.

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The goal of these exercises is to understand the circulatory system while integrating STEM and health-related issues. For high and middle school students the ability to assembly a model from a provided kit to represent the human circulatory system and relate the physics of how pressures are altered due to health related issues (obesity, arteriosclerosis) should promote the importance of why a healthy life style is advantageous. The hope is that providing cardiovascular kits to classrooms for teachers to use will bridge biology, physics and health concepts for an integrative approach to learning within STEM related around fluid dynamics and physiology. Four kits represent: (1) plaque formation and effect on flow, (2) elastic recoil and arteriosclerosis, (3) effects of blood viscosity, (4) differential blood pressure related to resistance. Since new national standards in teaching for secondary teachers in life sciences are to cover “modeling” as engineering and design, this activity is timely and very suited for a life science classroom for high & middle schools as well as college level. In the four activities (kits), the alteration in the extant of circulatory “tubing” to flow and the effect of resistance on blood pressure can be modeled and experimented with by inquiry learning. Funded by KY Department of Education Science Leadership Support Network.

36. Teaching with leeches- An undergraduate neurophysiology module.

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The freshwater leech has aided in the discovery of many basic neuroscience principles, especially in the fields of neurophysiology, ethology, and developmental biology. We have adapted four leech experimental protocols into a teaching module for upper level biology courses. Concepts demonstrated in these exercises include: action potentials and threshold, neural circuitry, synaptogenesis, muscle innervation, and sensory field mapping. The preparation is ideal for training because the neurons are large enough to be seen with inexpensive optics (40x magnification is sufficient), adult CNS tissue maintains activity in saline for several hours, and there are 21 relatively simple ganglia that permit trial and error and give multiple students an opportunity to collect intracellular recordings. With a single specimen students can 1) record from identified neurons in the ganglion, 2) dissect a patch of innervated skin and trace sensory receptors back to their respective cell bodies within the CNS, 3) isolate individual cells and culture them together to study synaptogenesis, and 4) inject dyes to visualize neural anatomy. Within the first lab session our students were able to inject current into sensory cells and observe the various action potential waveforms. At this poster we will be happy to discuss the laboratory setup and current research in leech neurobiology.

37. Does Myosin-XVa deficiency result in constitutively open mechanotransduction channels?.

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The organ of Corti contains mechanosensory cells forming one row of inner hair cells and three rows of outer hair cells (OHC). They have specialized mechanosensory microvilli-like protrusions on the apex of the cell, known as stereocilia. The molecular identity of the channels responsible for mechano-electrical transduction (MET) is yet unknown, but a number of evidence indicate that these channels are located at the tips of stereocilia. In wild type hair cells, the mechanical movement of stereocilia is transduced into an electrical signal when current flows through the open MET channels into the cell and depolarizes it. Stereocilia are supported by a core of parallel actin filaments that interact with other cytoskeletal proteins, including an unconventional myosin Myosin XVa (Myo15a). Myo15a is important in the maintenance of stereocilia structure but its role in the mechanotransduction process is not fully understood. In *Shaker-2* (*Sh2*) mice, a missense mutation, (260G>A), makes the motor domain of Myo15a dysfunctional and leads to profound deafness. Both inner and outer hair cells of homozygous *Sh2/Sh2* mice have abnormally short stereocilia. However, *Sh2/Sh2* OHCs start to degenerate after the first few days of postnatal development. We hypothesize that structural abnormalities due to the dysfunctional Myo15a result in redistribution of mechanical forces applied to the MET channels, which remain open, resulting in a standing current that continuously flows into the hair cell. This may result in a continuous influx of calcium that leads to disassembly and degeneration of stereocillia. Whole cell patch-clamp technique was used to record standing currents in OHCs of *Sh2/+* and *Sh2/Sh2* mice at different ages. MET channel-dependent component of the standing current was determined by bath application of dihydrostreptomycin (DHSM), a known blocker of the MET channels. Preliminary results show that MET-dependent component of the standing current is larger in *Sh2/Sh2* OHCs than in control, normally functioning *Sh2/+* OHCs. The patch-clamp recording data were supported by scanning Electron Microscopy (SEM) imaging that demonstrated concomitant degeneration of stereocilia with age. Supported by NIDCD/NIH (R01DC008861).

38. Teaching with crabs- An undergraduate physiology module.

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The blue crab *Callinectes sapidus* or the Dungeness crab *Cancer magister* are readily able to be used to teach physiology concepts of joint and tension reception. We have adapted three experimental protocols into a teaching module for upper level biology courses. Concepts demonstrated in these exercises include: recording the frequency of spikes that signal joint movement and position as well as correlation between the amount of muscle force generated with the activity of a tension nerve. Students also examine the anatomical arrangement of neurons within a chordotonal organ to associate the neurons' anatomy with their function. Vital as well as permanent staining techniques were taught which the students accomplished well and were able to obtain photos of the recorded neurons. Muscle force measurements were easily obtained with a force transducer and correlated with activity in the tension nerve. Students investigated two specific functional types of neurons in the chordotonal organs: movement or dynamic sensitive and those responding to static positions of the limb. Proprioception and tension reception was able to be investigated in multiple ways which allowed students to use inquiry based approaches to these exercises. The preparations are ideal for training because the nerves and muscles are readily dissected and recorded with common instrumentation within a teaching laboratory. In addition, these crab preparations remain viable in a minimal saline for several hours. At this poster we will be happy to discuss the laboratory setup and current research using these preparations.

39. Ouabain increase association between Na-K ATPase (Na-K) and NHE1 through N-terminal domain of Na-K .

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Low dose cardioglycoside increases Na-K activity in kidney basolateral membranes in an NHE1 dependent manner. TIRF and FRET microscopy suggested that 10 pM ouabain increases association between Na-K and NHE1. The aim of the study was to identify the molecular domains on Na-K and NHE1 required for association. Using mouse (MRPT) and human (HK11) kidney proximal tubule cells co-transfected with full-length GFP or RFP-NHE1, and full length mCherry-Na-K a-subunit or N-terminal deletion mutant of YFP-Na-K, FRET and TIRFM were performed before and after ouabain treatment. We also measured the effect of ouabain on Na-K activity in HK2 cells transfected with WT or scaffold domain mutated NHE1. Ouabain increased association between Na-K and NHE1. NHE1 and Na-K association was abolished in cells expressing N-terminal deletion mutant Na-K. Ouabain increased Na-K activity in HK2 cells expressing full length NHE1 but not in cells expressing scaffold domain mutated NHE1. These data suggest that low concentrations of ouabain increase Na-K association with NHE1 and this association involves N-terminal domain of Na-K and the scaffolding domain of NHE1.

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40. Differential impacts of environmental salinity on *Procambarus clarkii* and *Orconectes rusticus*

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Procambarus clarkii regulates internal hemolymph osmolality in response to changes in environmental salinity enhancing its ecological plasticity. *Orconectes rusticus* is an invasive species beyond the Ohio River Basin but its osmoregulatory processes are unstudied. Animals were acclimated to freshwater or brackish water medium (0.5% and 1.5%NaCl, respectively). Brackish water acclimation increased the hemolymph osmolality of *P. clarkii* by 92±10.6 mOsm/l and *O. rusticus* by 91±8.4 mOsm/l. After acclimation, gastrointestinal (GI) tracts were perfused with control (0.5% NaCl), 2.0% NaCl, and recovery (0.5% NaCl) solutions. Following brackish water acclimation, GI Na^+ excretion of *P. clarkii* increased in response to high NaCl by 35.9±3.7µmol/min while that of *O. rusticus* increased by only 28.4±2.73 µmol/min ($p<0.05$). After fresh water acclimation, changes in Na^+ absorption after 2.0% NaCl perfusion were not observed. The improved osmoregulation of *P. clarkii* after brackish water exposure may enhance the ability of the species to survive environmental osmotic challenges while *O. rusticus* has yet to adapt to altered environmental salinity.

41. Inhibition of MMPs protects hypertensive cerebropathy in Dahl-salt sensitive rats.

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Introduction: Hypertensive cerebropathy is a pathological condition associated with cerebral edema and breakdown of the blood-brain barrier (BBB). However, the molecular pathways leading to this condition remains obscure. We hypothesize that inhibition of MMPs modulates BBB disruption by decreasing MMP that ameliorate hypertensive cerebropathy. **Methods:** Dahl-salt sensitive and Lewis rats were fed high salt diet. The groups were 1) Dahl-salt sensitive (DC), 2) DC+GM6001 (non-specific MMP inhibitor), 3) Lewis (LC), and 4) LC+GM6001(LT). GM6001 was given at 0.5mg/mL by IP route, alternate days for 3 weeks. Blood pressure was measured by tail-cuff. The brain tissues were analyzed for oxidative stress, MMP-9 activity and tight junction proteins (TJP) by Western blot and Zymography. **Results:** Mean arterial blood pressure was 169.10 ± 0.57 mm Hg in hypertensive Dahl-SS rats as compared to 134.12 ± 1.2 mm Hg in Dahl-SS+GM6001 rats. The mean arterial pressures in Lewis and Lewis+GM6001 groups were 96.66 ± 0.54 and 87.69 ± 2.9 mm Hg respectively. The p47 and nitrotyrosine protein expression were increased but super oxide dismutase-1(SOD-1) and TJP levels (Z0-1, occludin) were decreased in hypertensive Dahl-SS and Lewis rats compared to GM6001 treated rats. MMP-9 protein expression and activity was increased in hypertensive Dahl-SS and Lewis rats compared to GM6001 treated rats. Brain section were stained for collagen and hypertensive Dahl-SS and Lewis rats showed increase in collagen as compared to GM6001 treated rats. **Conclusion:** Our results suggest that in hypertensive Dahl-SS rats, pharmacological inhibition of MMPs attenuates high blood pressure and prevents disruption of blood brain barrier.

42. Pathology of two cypovirus variants in *Heliothis virescens* and *Campoletis sonorensis.*

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Cypoviruses (Reoviridae) are segmented dsRNA viruses that infect primarily insects where they are known to cause minor, non-lethal infections in the guts of lepidopterans that result in delayed growth and some mortality in severe infections. However, cypoviruses have not been studied in most lepidopteran systems (the exception being Bombyx mori) due to the insignificant pathology and in particular has not been investigated in the context of the impact of CPV infection on parasitic wasps having infected lepidopteran hosts. We recently discovered a new cypovirus, the *Heliothis virescens* Cypovirus 5 (HvCPV5—wild type virus) in both the wasp and the parasitized lepidopteran host as well as a mutant of this virus (HvCPV5dm) which has provided a unique perspective on the differential pathologies of HvCPV5 in wasp and lepidopteran hosts. We have shown increased mortality of *Campoletis sonorensis*, the wasp parasitoid, when infected with HvCPV5. Unexpectedly, the wild type virus is more predominant in the parasitoid, whereas the mutant virus is preferentially associated with the caterpillar. To further study this system, we are investigating the pathology of each cypovirus variant with respect to *H. virescens* through feeding and microinjection. We are also investigating the transmission of the virus between *H. virescens* and *C. sonorensis* by the wasp larvae interacting with an infected host. Results from this study will provide an understanding of the interaction between the caterpillar, wasp and each cypovirus variant.

43. Hydrogen sulfide mitigates cerebro-vascular remodeling during cerebral ischemia.

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Cerebral stroke is a major cause of morbidity and mortality worldwide. Ischemia-reperfusion injury is thought to be mediated by free radical generation. Although there is robust increase in ROS, RNS, and active MMPs in ischemia/reperfusion (I/R) injury. Hydrogen sulfide (H2S), acts as a potent anti-oxidant, vasorelaxing and anti-hypertensive agent. However, the role of H2S in cerebrovascular remodeling during cerebral stroke is unclear yet. Therefore, we hypothesized that H2S ameliorated cerebral stroke by decreasing apoptosis, oxidative and proteolytic stresses. To test this hypothesis we employed 8-10 weeks old male wild type (WT) mice. An experimental group was: WT, WT+ NaHS (sodium hydrogen sulfide, precursor of H2S, 25 µmol/L in 0.9% normal saline dose); WT/I/R and WT/I/R+NAHS (258 µmol/L in 0.9% normal saline dose). Ischemia was performing for 30 min and reperfusion for 7days. NAHS was injected intra-peritoneally (I.P.) once daily for a period of 7 days after ischemia. The brain infarct area and permeability was measured using 2,3,5-triphenyltetrazolium chloride (TTC) staining and Evans Blue extravasation respectively. The brain tissues were analyzed for oxidative, proteolytic stresses and remodeling, by measuring super oxide dismutase-1 (SOD1), p47phox (NADPH oxidase subunit), nitrotyrosine, MMPs and TIMPs by *in-situ* immunolabeling and Western blot analyses. The levels of apoptosis were measured by TUNEL assay and caspase-3 levels. We found that there was increased in brain edema, Evans Blue leakage, brain infarct size, apoptosis, MMP-9 activation, oxidative, proteolytic stresses and remodeling in ischemia operated groups, which were ameliorated by NaHS treatments. Thus, our study suggested that H2S could be effective for the treatment of cerebral ischemia.

44. Dopamine's influence on nervous system anatomy during juvenile development.

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Changes in dopaminergic activity during embryogenesis have been shown to affect dendrite morphology in the peripheral nervous system of *Drosophila melanogaster* and in the mammalian striatum. Morphological differences then lead to abnormal behavior so we are curious about the role of dopamine homeostasis in maintaining synaptic connections. And because one of the most common insults to dopamine homeostasis in humans is prescription stimulant treatment for ADHD we are focused on the effects of methylphenidate (Ritalin®, dopamine transport blocker) on CNS structure during juvenile stages of development. To investigate this we have used the GAL-4/UAS system in *D. melanogaster* to drive expression of green fluorescent protein (GFP) to the cell membrane of specific neurons. These transgenic fly lines were then treated with methylphenidate (1mg/mL in standard fly food) for 24hr during the third instar larva stage, a treatment that has been shown to cause a slight reduction in mouth hook and body wall movements. The goal then has been to compare neural anatomy in these flies to their drug naive siblings. Fixed whole mount sections of the larval brains were visualized using confocal microscopy and GFP fluorescence was observed either in a subset of sensory neurons (sensory-GAL-4) or in dopaminergic neurons (ple-GAL-4). As expected there were no gross anatomical defects (e.g. cell death or abnormal wiring) in the treated flies from either line. But what we expected to see was differences in subcellular structures, e.g. numbers of synaptic boutons or secondary/tertiary branches. These details were not possible to discern in the sensory-GAL-4 line because of the density of cells expressing GFP in sensory neuropils and longitudinal tracts in the ventral nerve cord. This was also true of higher order branching in the ple-GAL-4 line but we were able to quantify synaptic boutons on identifiable dopaminergic neurons. We are in the process of analyzing these data and implementing a recombination strategy to label smaller subsets of neurons. These findings will reveal how the cell membrane dopamine transporter is involved in the maintenance of synaptic connections, which has functional implications for plasticity in brain regions receiving dopaminergic input.

45. A laboratory exercise in quantifying synaptic transmission: Quantal measures and analysis.

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The purpose of the presented laboratory exercise is for students to observe and measure quantal synaptic vesicular release of neurotransmitter. This exercise utilizes crayfish neuromuscular junctions (NMJs) because of the ease in dissection and viability of the preparation. Students electrically stimulate a crayfish motor nerve in order to measure evoked and spontaneous vesicular events through direct counts, amplitude measurements, and charge measurements to quantify synaptic transmission. The crayfish abdominal extensor muscles are in groups with some being tonic (slow) and others phasic (fast) in their synaptic phenotypes. The NMJs of the abdominal extensors are used to investigate quantal properties in synaptic transmission. Also, one can examine the influence of neuromodulators, pharmacological agents and various concentrations of Ca^{2+} in the extracellular fluid on synaptic transmission in these preparations. With a loose patch electrode (focal macropatch) place over NMJs one can record spontaneous and evoked quantal events. The methods taught in this lab procedure are direct counting of evoked quantal events, measuring the amplitude and area under the curve of both evoked and spontaneous responses and estimate a mean quantal content. Overall, using the three methods to index quantal transmission allows for comparison among the different approaches in order to determine which are the most useful for the type of synaptic properties. Discussions on the variation in the shapes of single quantal events, pre- and post-synaptic contributions to the quantal responses as well as effects on the mean quantal content measurements are tackled.

46. Regulation of ERα gene expression by demethylation and increased Tet1 expression following MCAO in the female mouse brain.

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Pre-treatment with 17-β estradiol (E2) protects the cortex from neuronal cell death caused by brain injuries such as middle cerebral artery occlusion (MCAO). This protection is largely dependent on the early presence of estrogen receptor alpha (ERα) in the cortex. ERα, however, is only transiently expressed in the cortex during neonatal development and is virtually absent in the uninjured adult brain. In our previous studies in female rats, we demonstrated that ERα mRNA expression is increased in the cortex on the injured side of the brain. There was also corresponding decrease in ERα promoter methylation after injury in female cortex. To extend these studies in mice, female mice underwent OVX and E2 or oil-vehicle replacement followed by permanent MCAO 1 week later. Brains were removed and snap-frozen at 12 or 24 hours following surgery. Two 2mm micro-punches were collected from both sides of the cortex. Punches were used for collection of mRNA or genomic DNA for real time PCR or methylation detection by methylation-specific PCR, respectively. In both groups of females, ERα mRNA was increased in the ischemic cortex, but the timing of the increase was dependent on the presence of E2, such that ERα mRNA was increased at 12 hours in the E2-treated group and not until 24 hours in the vehicle-treated group. In both groups, there was also a corresponding decrease in DNA methylation of the ERα promoter C by 12 hours. Although the enzymes involved in DNA methylation have been extensively studied, much less is known about demethylation in mammals. Recent studies have shown a role for Tet1, an enzyme that catalyses the conversion of 5-methylcytosine (5mc) of DNA to 5-hydroxyl-methylcytosine (5hmC) in demethylation of DNA in embryonic stem cells. Here we hypothesized that Tet1 mRNA would be increased when ERα mRNA is increased and presumably demethylated. Quantitative real time PCR for Tet1 mRNA showed Tet1 was significantly increased on the injured side of both groups. These data are the first to demonstrate a correlation between changes in methylation of the ERα promoter and gene expression in the mouse following MCAO and most importantly, a potential role for Tet1 in demethylation of target genes following injury.
Supported by NSF IOS0919944 and NSF IOS1121129.

47. **5-HT modulates the heart rate through 5-HT2 receptor activation in *Drosophila* larvae**.

A. Stacy, Z. Majeed and R.L. Cooper

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Serotonin (5-HT) accelerates the heart rate in the fruit fly *Drosophila melanogaster* larvae; however, the underlying mechanism has not yet investigated. In this study, we used pharmacological approach to get an insight into the type of 5-HT receptor that mediates the 5-HT action. For this purpose, various agonists and antagonists were applied on the semi-intact third instar larval preparations. Third instar larvae of CS flies were dissected in ventral side up position and the internal organs were removed. Then the dorsal cardiac vessel was exposed to count heartbeats. Various agonists for respective 5-HT receptor subtypes (5-HT1A, 5-HT1B, 5-HT2 or 5-HT7) were employed. The results have shown that α-methyl-5-HT maleate (5-HT2B agonist) significantly increased the heart rate at 100μM. However, 5-HT1A, 5-HT1B, 5-HT7 agonists did not have a marked effect on the heart rate. It is noteworthy that 5-HT has higher affinity than α-methyl-5-HT since 5-HT can accelerate the heart rate at lower concentration (100nM). Moreover, different antagonists were used. The results have shown that application of combination of 5-HT (100nM) and ketanserin (100μM), after incubation of the preparation inside ketanserin (100 μM) for 10 minutes, can inhibit the positive chronotropic effect of 5-HT on heart rate. Given these results, we came to a conclusion that the modulation action of serotonin on the larval heart is mediated by the activation of 5-HT2 receptor, which is coupled to Gαq protein. We will now use genetic approaches to corroborate the pharmacological approach results.

48. Sex differences of epigenetic changes following neonatal hyperoxia.

T . Sengoku, J. Westberry, M. E. Wilson

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Many sex differences in the brain are mediated by estrogen. In addition, estrogen protects against neural injury, is involved in the formation of synapses, and in learning and memory. Babies born prematurely are often placed on ventilators and/or are given supplemental oxygen for varying lengths of time. This increase in oxygen, while critical for survival, can cause long-term damage to the lungs, retinas and brains of these babies. In particular, hyperoxia causes apoptosis in neurons and alters glial activity. Given that survival rates for neonates is higher in females than males we hypothesized that hyperoxia can alter the regulation of genes, such as ER-alpha, BDNF, and GDNF all of which play a role in brain development, in a sexually dimorphic manner. Epigenetic modifications of genes play a critical role in neuronal differentiation and development. Conditions that disrupt the timing and regulation of these critical processes can lead to long-term detrimental effects. During early brain development estrogen, BDNF, and GDNF's effects on neurons leads to long-term differences in the male and female brain. Changes in the expression of these factors in turn could ultimately lead to deficits in cognition or conditions such as cerebral palsy and ADHD associated with neonatal hyperoxia. We predicted that male and female mice would have differences in BDNF and GDNF expression following hyperoxia. To test this hypothesis, newborn C57Bl/6 mice and their littermates were placed in hyperoxic or normoxic conditions from postnatal day 7-12. Hyperoxic conditions were maintained in plexi-glass chambers containing 75% oxygen. Normoxic mice were placed in room air. The mice were sacrificed and brains were removed at postnatal day 20-25. Brains were divided in half and one half frozen for mRNA and DNA analysis and the other half processed for histological examination. ER-alpha mRNA levels, as measured by RT-PCR, exhibited sex and brain region-specific changes following hyperoxia. These changes in mRNA expression were also associated with increased promoter methylation as measured by methylation-specific PCR. There were also significant differences in BDNF mRNA expression following hyperoxia. Together this data suggests that short-term exposure to hyperoxic conditions can have sexually dimorphic effects that could potentially persist and result in lasting changes in neuronal gene expression. Future studies will determine if these changes persist into adulthood and whether they result in behavioral or cognitive deficits.

Participant	**Location**	**Poster #**
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A. Bankemper	Univ of KY	14
Daniel C. Bartos	Univ of KY	5,18
C.B. Blake	Univ of KY	24
Douglas Borchman	Univ of Louisville	16
Tania Boyechko	Univ of KY	12
E. Burns	Univ of KY	11
K.S. Campbell	Univ of KY	1, 22,28
C.S. Chung	Univ of KY	1, 22
J.A. Collett	Univ of KY	26
Amy Confides	Univ of KY	8
Robin L. Cooper	Univ of KY	11-14,32,35, 38
T. Crosthwaite	Univ of KY	32
M. L. Danley	Univ of KY	33
Brian P. Delisle	Univ of KY	5, 18
James T. Dixon	Univ of Louisville	21
Esther E. Dupont-Versteegden	Univ of KY	3, 6, 8, 10,11, 20
Donald T. Frazier	Univ of KY	
Christopher S. Fry	Univ of KY	6,20
Michael Fultz	Morehead State Univ	
John C. Gensel	Univ of KY	
S. Givvimani	Univ of Louisville	7
Ming C. Gong	Univ of KY	
Premi Haynes	Univ of KY	22
J.R. Jackson	Univ of KY	10, 20
Irving Gilbert Joshua	Univ of Louisville	
Anuradha Kalani	Univ of Louisville	41
A. Karic	Univ of KY	33
S. Kenney	Univ of KY	45
Syed J. Khundmiri	Univ of Louisville	39
R.M. Krall	Univ of KY	35
S. Kundu	Univ of Louisville	27
Jonah D. Lee	Univ of KY	20
Z. Majeed	Univ of KY	14,32,36,38,47
Cynthia J. Miller	Univ of Louisville	34
Rebecca D. Murray	Univ of Louisville	9
Suguru Nakamura	Murray State University	
Nithya Narayanan	Univ of Louisville	31
J.E. Noland	Univ of KY	42
K. Paras	Univ of Louisville	29

Participant	**Location**	**Poster #**
Maiyon Park	The University of Pikeville	17,19
John C. Passmore	Univ of Louisville	
Shrikant Pawar	Western KY Univ	15
Charlotte A. Peterson	Univ of KY	6, 10, 20
D. Potts	Univ of KY	44
Sathnur Pushpakumar	Univ of Louisville	25
Michael B. Reid	Univ of KY	
M.K. Rhoads	Univ of KY	40
Andrew M. Roberts	Univ of Louisville	21
T. Sengoku	Univ of KY	47
Aman Shah	Univ of KY	3,
Jennifer L. Smith	Univ of KY	18
N. Song	Univ of Louisville	4, 23
A. Stacy	Univ of KY	47
Diana Syam	Univ of KY	37
Josh Titlow	Univ of KY	36,38,44
Neetu Tyagi	Univ of Louisville	43
Suresh C. Tyagi	Univ of Louisville	2, 7,25, 27,29,31,41,42
A.C. Vélez-Ortega	Univ of KY	30,37
Pedro L. Vera,	Univ of KY	
C. Vishalakshi	Univ of Louisville	2
Jenne Westberry	Univ of KY	46
Melinda Wilson	Univ of KY	46, 47
Y. Zhu	Univ of KY	13