IPS The Iowa Physiological Society

# 16th Annual Iowa Physiological Society Meeting

September 29, 2012

9 a.m. – 5 p.m.

Des Moines University

3200 Grand Avenue

Des Moines, IA 50312

IPS The Iowa Physiological Society

WELCOME!

Welcome to Des Moines University (DMU) for the 16th Annual Iowa Physiological Society Meeting! The mission of our society is to unite physiologists in networking and advancing physiology throughout the State of Iowa. This is at the molecular, cellular, organ and whole body levels. As such, this year's program includes a combination of oral and poster presentations on both research and education in physiology. The program also includes group activities that discuss current challenges in physiological research and education in different environments.

We have invited a panel of distinguished speakers and facilitators. The Keynote Address in Physiological Research will be delivered by **Dr. Michael Sturek**, Chair of Cellular & Integrative Physiology at Indiana University School of Medicine. Dr. Sturek has an outstanding career in cardiovascular research that encompasses experimental approaches at all levels – clinical, whole animal, organ, tissue, cell, and molecular. He is a very well-funded investigator and has received numerous honors, including Fellow of the Cardiovascular Section of the American Physiological Society.

**Dr. Dee Silverthorn**, Senior Lecturer, University of Texas at Austin, will deliver the Keynote Address in Physiological Education. In addition to being a successful physiologist, Dr. Silverthorn is a renowned author of physiology textbooks. She has received numerous teaching awards and honors, including the American Physiological Society's Claude Bernard Distinguished Lecturer and Arthur C. Guyton Physiology Educator of the Year. She is a former Editor in Chief of Advances in Physiology Education.

**Dr. Thomas Pressley** of Texas Tech University Health Sciences Center will join Drs. Matthew Henry (Des Moines University) and Dee Silverthorn in facilitating the Physiology Teaching discussion group. Dr. Pressley has been a successful bench investigator and educator. He is currently Distinguished Visiting Professor at the US Air Force Academy in Colorado and Chairman of the Education Committee of the American Physiological Society. The group discussions in Physiology Research will be facilitated by Dr. Michael Sturek and IPS Board Members Drs. Harald Stauss (University of Iowa) and Julia Moffitt (Des Moines University). These discussions are expected to bring everyone together in meeting the challenges we face in research and teaching today.

We have also invited researchers and educators from institutions in Iowa to present their research data and teaching approaches. Drs. Kim Huey (Drake University), Matt Henry (DMU), Mike Lyons (Grandview University) and Vanja Duric (DMU) will provide you with a variety of research and teaching presentations. In addition, undergraduate, graduate students, postdoctoral fellows, technicians and faculty have contributed posters to the program. I am positive that there will be something for everyone at this year's meeting.

This year's meeting is supported by the American Physiological Society, Des Moines University, Kent Scientific, DSITM, and Cyber-Anatomy Corp. We are glad that the poster competition for graduate and undergraduate students will have $200, $100, and $50 awards to first, second and third place winners in each category.

Thank you for your participation in the program and I wish you all a successful meeting!

Kim Tran
President, IPS

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## Agenda

**8:15 a.m.** **Breakfast, Registration, and Poster Set-Up**

**9 a.m.** **Opening Remarks**

**9:05 a.m.** **Research Presentation**
Skeletal Muscle Adaptation to Chronic Changes in Loading and Activation
*Kimberly Huey, Ph.D., Drake University*

**9:40 a.m.** **Teaching Presentation**
Peer Collaboration to Improve Understanding and Retention of Complex Concepts
*Mike Lyons, Grandview University*

**10:10 a.m.** **Teaching Presentation**
PBL, TBL, CBL – What the Hell? What can I do if I'm Trapped in a Lecture-Based Curriculum?
*Matthew Henry, Ph.D., Des Moines University*

**10:40 a.m.** **Break and Poster Sessions**

**11:30 a.m.** **Keynote Address in Physiological Research**
Mechanistic and Translational Research in Coronary Vascular Biology
*Michael Sturek, Ph.D., Indiana University School of Medicine*
Dr. Sturek's presentation is sponsored by DSI™.

**12:30 p.m.** **Lunch and Poster Viewing; Presentation of the IPS Seminar Series**

**1:30 p.m.** **Group Activities**

**Group A:**
*Location: Olson Medical Education Center*
Theme: Current challenges of doing research at the levels of community colleges, undergraduate environment, medical school and graduate school levels and how to adapt your research tool kit and network to meet those challenges.

- Short introduction presentation (10'):
  Productively Stupid … and Loving It!
  *Michael Sturek, Ph.D., Indiana University School of Medicine*
- Discussion Moderators:
  *Julia Moffitt, Ph.D., Des Moines University*
  *Harald Stauss, M.D., University of Iowa*
  *Michael Sturek, Ph.D., Indiana University School of Medicine*

**Group B:**
*Location: Student Education Center 115*
Theme: Current challenges of teaching physiology at the levels of community colleges, undergraduate environment, medical school and graduate school levels and how to adapt your teaching tool kits to meet those challenges.

- Discussion Moderators:
  *Matthew Henry, Ph.D., Des Moines University*
  *Thomas Pressley, Ph.D., Texas Tech University Health Sciences Center*
  *Dee Silverthorn, Ph.D., University of Texas at Austin*

**2:15 p.m.** **Highlights from Group Discussions**

**2:30 p.m.** **Research Presentation**
Role of MKP-1 in the Neurobiology of Depression
*Vanja Duric, Ph.D., Des Moines University*

**3 p.m.** **Break and Poster Viewing**

**4 p.m.** **Keynote Address in Physiology Teaching**
The Up-Side-Down Lecture: How to Get Students to Come to Class
*Dee Silverthorn, Ph.D., University of Texas at Austin*

**5 p.m.** **Poster Award Presentations and Closing Remarks**

**5:15 – 6 p.m.** **IPS board meeting**

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## Meeting Objectives

Upon completion of this meeting, participants will be able to:

1. Exchange the latest progresses in the field of physiological research and education.
2. Exchange best approaches to meet the current challenges in physiological education at different environments.
3. Discuss challenges to doing research at different environments in the current climate of funding and ways to meet those challenges.
4. Enhance communication, interaction and collaboration among scientists, educators and students via the above activities.

## Iowa Chapter of the American Physiological Society Board


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## Guest Facilitator and Speakers

**Michael Sturek, Ph.D.**

*Professor and Chair, Department of Cellular and Integrative Physiology, Indiana University School of Medicine*

Dr. Sturek received both a bachelor degree in Exercise Physiology and Psychology from Augustana College and a Masters degree in Exercise Physiology from Purdue University. He obtained his PhD in Pharmacology from the University of Iowa. Following postdoctoral training at the University of Chicago, he joined the Dalton Cardiovascular Research Center at the University of Missouri, where he advanced through the ranks to full Professor of Medical Pharmacology & Physiology and Internal Medicine in 2000. He came to Indiana University School of Medicine in 2004 as Professor and Chair of Cellular and Integrative Physiology. Dr. Sturek has an outstanding career in cardiovascular research that encompasses experimental approaches at all levels – clinical, whole animal organ, tissue, cell and molecular. His works have resulted in over 150 original articles. He is currently the principal investigator of 3 NIH grants and a co-investigator on 8 others. He has received numerous awards, including a Research Career Development Award from the NHLBI, American Diabetes Association Award of Outstanding Community Service in Reaching People and University of Missouri Order of Socrates Award for Teaching Excellence. Dr. Sturek was Fellow of the Council on High Blood Pressure Research, American Heart Association and Fellow of the Cardiovascular Section, American Physiological Society.

**Thomas Pressley, Ph.D.**

*Texas Tech University Health Sciences Center*

Tom Pressley's background is multidisciplinary, with undergraduate training in ecology and population biology within the Department of Earth and Planetary Sciences at Johns Hopkins University, doctoral training in biochemistry at the Medical University of South Carolina, and postdoctoral training in biochemistry and physiology at Columbia University College of Physicians and Surgeons. He has been a bench investigator and educator, first at the University of Texas Medical School in Houston and later at Texas Tech University Health Sciences Center (TTUHSC) in Lubbock. Tom was a Welcome Visiting Professor in the Basic Medical Sciences at the University of Northern Iowa and a professeur invité at the University of Poitiers in France. He is currently on sabbatical as a Distinguished Visiting Professor at the US Air Force Academy in Colorado. His recent position as Interim Dean of the Graduate School of Biomedical Sciences at TTUHSC, his chairmanship of the Education Committee of the American Physiological Society, and his long-standing membership on the admissions committees of medical and graduate schools have provided experience with curriculum development and the administrative side of science education.

**Dee Silverthorn, Ph.D.**

*Senior Lecturer, School of Biological Sciences, University of Texas at Austin*

Dr. Silverthorn studied biology as an undergraduate at Newcomb College of Tulane University, and received a Ph.D. in marine science from the Belle W. Baruch Institute for Marine and Coastal Sciences at the University of South Carolina. Her research interest is epithelial transport, and recent work in her laboratory has focused on transport properties of the chick allantoic membrane. Her teaching career started in the Physiology Department at the Medical University of South Carolina but over the years she has taught a wide range of students, from medical and college students to those still preparing for higher education. At the University of Texas-Austin she teaches physiology in both lecture and laboratory settings, and instructs graduate students on developing teaching skills in the life sciences. She has received numerous teaching awards and honors, including the American Physiological Society's Claude Bernard Distinguished Lecturer and Arthur C. Guyton Physiology Educator of the Year. Dee is a former editor-in-chief of Advances in Physiology Education and she works with members of the International Union of Physiological Sciences to improve physiology education in developing countries.

**1**

## Altered Expression of Glutamate and Synapse Related Genes in Postmortem Hippocampus of Depressed Subjects

**Vanja Duric**[1], Mounira Banasr[1], Craig A. Stockmeier[2,3], Arthur A. Simen[1], Samuel S. Newton[1], James C. Overholser[4], George J. Jurjus[3], Lesa Dieter[4], and Ronald S. Duman[1]

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[3]Department of Psychiatry, [4]Department of Psychology, Case Western Reserve University, Cleveland, OH 44106, USA

Major depressive disorder (MDD) has been linked to changes in function and activity of the hippocampus, one of the central limbic regions involved in regulation of emotions and mood. The exact cellular and molecular mechanisms underlying hippocampal plasticity in response to stress are yet to be fully characterized. In this study, we examined the genetic profile of microdissected subfields of postmortem hippocampus from subjects diagnosed with MDD and comparison subjects matched for sex, race and age. Gene expression profiles of the dentate gyrus and CA1 were assessed by 48K human HEEBO whole genome microarrays, and a subgroup of identified genes was confirmed by real-time polymerase chain reaction (qPCR). Pathway analysis revealed alterations in expression of genes with functional role in cytoskeletal and synaptic stability, as well as glutamatergic and serotonergic neurotransmission. Our findings demonstrate significant dysregulation of synaptic function/structure related genes *SNAP25, DLG2 (SAP93),* and *MAP1A*, and AMPA receptor subunit genes *GLUR1* and *GLUR3*. Altered expression of the serotonin (5-HT) receptor genes *HTR2C* and *HTR4* was also observed. Several of these human target genes were similarly dysregulated in a rat model of chronic unpredictable stress (CUS), and the effects reversed by antidepressant treatment. Together these studies provide new evidence that disruption of glutamatergic and serotonergic signaling pathways contribute to the pathophysiology underling MDD and provide interesting targets for novel therapeutic interventions.

*Supported by US Public Health Service grants MH45481 (R.S.D.), 2 P01 MH25642 (R.S.D.), MH67996 (C.A.S.) and P20 RR17701 (C.A.S.) and the Connecticut Mental Health Center (R.S.D.).*

**2**

## Probiotics in Yogurt Effect on Oral *Streptococcus Mutans*

**David R. Garner*** Ogden, UT, Mitchell W. Cooney Ogden, UT, Scott Wright Ogden, UT, Medical Laboratory

Science Department, College of Health Professions, Weber State University

Do probiotics found in yogurt affect the population of Streptococcus mutans in the oral cavity? S. mutans is the cause of common and extensive oral infections in 95% of the United States general population. One of the most common oral infections is dental caries, also known as cavities. In a double blinded study of 32 participants ages 18-40, participants were divided into two groups. Group 1 ate yogurt containing live probiotic organisms. Group 2 ate sterilized yogurt in which the bacteria were killed. The participants were evaluated over the course of nine weeks. They began the first three weeks by not eating yogurt. This was followed by three weeks of eating yogurt, and they finished with three weeks not eating yogurt. The S. mutans concentrations were evaluated from tooth scrapings using Dentocult® SM Strip Mutans kits over the nine week course of the experiment. While the results of previous published research completed in Finland suggest live cultures will reduce the population of S. mutans in the oral cavity, this is not what was observed in this study. No statistically significant difference was observed in the S. mutansconcentrations between the groups that ate yogurt with live culture vs. those that ate the sterilized yogurt.

## C-Reactive Protein and Alpha 1-Antitrypsin Levels in Tears of Extended-Wear Contact Lens Patients

**Derek Gnehm,** Aaron Barretta, Jordan Jonesa

Department of Clinical Laboratory Sciences, College of Health Professions

The purpose of this work is to evaluate tear levels of C-Reactive Protein (CRP) and α1-antitrypsin as indicators of ocular surface inflammation in the extended wear of silicone hydrogel contact lenses. Ten current wearers of lotrafilcon B and seven wearers of senofilcon A were used in a cross-over study. Tear samples were collected after one week of non-contact lens wear to establish a baseline value. A second sample was collected after a week of extended wear to establish the test value. High sensitivity ELISA screenings were used to quantify the tear samples.

α1-antitrypsin was significantly increased in tears of lotrafilcon B wearers. Senofilcon A wearers displayed a rise in α1-antitrypsin values that were not statistically significant. CRP did not show a significant increase in levels in either group. Clinical observations for inflammation were correlated with the quantified protein levels. Biochemical analysis of α1-antitrypsin in tears could be used as a reliable indicator of ocular surface inflammation. Further research could prove useful in determining the values of inflammation proteins in ophthalmic disease.



## Enhanced Interactions Among Molecular Switchers of Store-Operated Ca2+ Entry in the Vascular Endothelium by 17β-estradiol

**Rachel Firkins**, Jennifer Giles, Mark Ver Meer and Quang-Kim Tran

Department of Physiology & Pharmacology, Des Moines University

Estrogen exerts many cardiovascular effects via pre-genomic and genomic actions. Agonist-induced $Ca^{2+}$ signals play important roles in cellular functions. In the vascular endothelium, estrogen is known to have many $Ca^{2+}$-dependent actions. However, the chronic effects of estrogen on components of agonist-induced $Ca^{2+}$ signals and the underlying mechanisms are unknown. We have observed that chronic treatment with 17β-estradiol did not affect the endoplasmic store $Ca^{2+}$ content, but substantially increases the total Ca2+ signal produced in response to depletion of this $Ca^{2+}$ store in primary endothelial cells. Consistent with this, the rate of store-operated $Ca^{2+}$ entry is dose-dependently increased up to 3-fold in cells treated with 17β-estradiol, as determined by the $Mn^{2+}$ quenching method. No changes were found in the expression levels of the molecular switchers of store-operated $Ca^{2+}$ entry, including Stim1, Orai1, transient receptor potential $Ca^{2+}$ channels 1 and 4. However, coimmunoprecipitation experiments demonstrated that the interactions between Stim1 and these $Ca^{2+}$ channels are increased by up to 3-fold in cells treated chronically with 17β-estradiol. Thus 17β-estradiol increases store-operated $Ca^{2+}$ entry by enhancing the interactions among its molecular switchers. These mechanisms contribute to the effect of estrogen to increase total agonist-induced $Ca^{2+}$ signals in the vascular endothelium.

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## Multifaceted Regulation of $Ca^{2+}$ Efflux via the Plasma Membrane Ca2+-ATPase by 17β-estradiol in the Vascular Endothelium

**Jennifer Giles**, Rachel Firkins, Michelle Burgard and Quang-Kim Tran

Department of Physiology & Pharmacology, Des Moines University

Many of the beneficial effects of estrogen in the vasculature involve $Ca^{2+}$-dependent activities. $Ca^{2+}$ efflux via the plasma membrane $Ca^{2+}$-ATPase (PMCA) represents a major component of the $Ca^{2+}$ signaling machinery in cells. The acute and chronic effects of estrogen on PMCA activity and the mechanisms are not known. We observed in vascular endothelial cells that chronic exposure to physiological concentrations of 17β-estradiol causes a substantial increase in total agonist-induced $Ca^{2+}$ signal. Measurement of PMCA activity in living cells demonstrates that 17β-estradiol dose-dependently decreases PMCA activity without affecting PMCA expression levels. Inhibition of Src kinase activity not only restores PMCA activity in cells treated with 17β-estradiol, but increases it by 50%. Both acute and chronic 17β-estradiol treatment promotes tyrosine phosphorylation at both the 135-kDa (PMCA) and 60-kDa levels, suggesting that PMCA interacts with a 60-kDa phosphotyrosine protein. We identified this 60-kDa protein to be a glycosylated form of the novel G protein-coupled estrogen receptor 1 (GPER). Tyrosine phosphorylation of GPER is increased by estrogen treatment and is prevented by inhibition of Src kinase activity. Consistent with the 50% increase in PMCA activity upon Src inhibition in estrogen-treated cells, the interaction between PMCA and calmodulin is increased by 50%, an effect that occurs independently of Src kinase activity. This increase is consistent with a 2-fold upregulation in total calmodulin expression level. These results indicate that estrogen inhibits PMCA activity by promoting Src-dependent tyrosine phosphorylation and interaction with GPER, effects that mask the stimulatory effect of enhanced CaM binding.

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## Endothelial Regulation of Calmodulin Expression in Vascular Smooth Muscle Cells

**Michael Stencel**, Jennifer Giles, Mark Ver Meer and Quang-Kim Tran

Department of Physiology & Pharmacology, Des Moines University

Interactions between vascular endothelial cells and the underlying smooth muscle cells (VSMCs) are of paramount importance in maintaining vascular functions. Calmodulin (CaM) is involved in a wide variety of cellular functions and is a limiting factor in both cell types, with free cytoplasmic CaM constituting only a small fraction of the total cellular CaM. Currently nothing is known about potential interactions between ECs and VSMCs as it involves CaM. We have begun to investigate the possibility that vascular endothelial cells impact VSMC functions via CaM-dependent activities, using a co-culture model of primary culture vascular endothelial cells and smooth muscle cells isolated from the same vessels. DNA microarray analysis, RTPCR and Western blotting demonstrated VSMCs in co-culture with proliferating ECs express on average ~2-fold more CaM than monocultured VSMCs. Media fractionation and eNOS inhibition experiments indicate that the CaM-elevating effect was exerted by a soluble factor that is not nitric oxide. The CaM-elevating effect exerted by ECs is strongly dependent on endothelial density, such that at a starting 50% confluency of VSMCs, a starting 20% endothelial confluency triggers the greatest CaM increase in VSMCs, whereas a starting 70% endothelial confluency yields no visible effect on VSMC CaM expression after 48 hrs. Pharmacological inhibition of cyclooxygenase-1 and endothelin-1 receptors ($ET_A$ and $ET_B$) does not affect the observed increase in CaM. The data suggest that proliferating endothelial cells produce a soluble factor that can regulate CaM-dependent signaling in VSMCs via alterations in total cellular CaM expression. We have subsequently developed a novel approach for simultaneous measurement of free $Ca^{2+}$ and $Ca^{2+}$-CaM signals in VSMCs in co-culture with endothelial cells. This approach is expected to provide valuable information regarding endothelium-dependent changes in $Ca^{2+}$ and $Ca^{2+}$-CaM -dependent signaling in VSMCs.

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## GPER Interacts with Stim1 and Regulates Store-Operated Ca2+ Entry in Vascular Endothelial Cells

**Lara Terry**[1], Jennifer Giles[2], Mark Ver Meer[2], and Quang-Kim Tran[2]

[1]Biochemistry, Cellular and Molecular Biology Program, College of Arts and Sciences, Drake University,
[2]Department of Physiology and Pharmacology, Des Moines University

The novel G protein-coupled estrogen receptor (GPER) has been found to participate in numerous cardiovascular functions. Store-operated $Ca^{2+}$ entry (SOCE) is an essential mechanism that is required for many cellular functions. The stromal interaction molecule 1 (Stim1) was recently been found to function as a $Ca^{2+}$ sensor in the ER lumen that oligomerize upon depletion of the store $Ca^{2+}$ content and interact with PM $Ca^{2+}$ channels to activate them, triggering SOCE. We have observed that activation of GPER using the GPER specific agonist G1 is associated with a dose-dependent inhibition of SOCE in primary vascular endothelial cells. Interestingly, the GPER specific antagonist G15 increases SOCE in cells unstimulated by GPER intrinsic or exogenous ligand. In addition, preliminary experiments using GPER antisense oligomer suggest that GPER knockdown can increase SOCE by up to 80% compared to scrambled oligomer treatment. Coimmunoprecipitation revealed that GPER exists in endothelial cells as a glycosylated protein and forms a complex with Stim1 even in the absence of ER store depletion or $Ca^{2+}$ entry. These data suggest that GPER may be an important regulatory input of store-operated $Ca^{2+}$ entry via its interaction with Stim1.

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## Ovariectomy Induces Increased Arrhythmogenesis and Expression of pS368-connexin 43 in Response to Acute Myocardial Ischemia

**Erica G. Jarrett**, Rachel M. Firkins, Matthew K. Henry and Julia A. Moffitt

Department of Physiology and Pharmacology at Des Moines

Previous studies have shown that estrogen-deficient animals have a greater predisposition to cardiac arrhythmias, but the mechanism for this remains unknown. Connexin 43 (Cx43) expression and phosphorylation plays an important role in regulating the normal propagation of electrical stimuli within the heart, allowing for a normal rhythm to be maintained. Previous studies indicate that increased phosphorylation at S368 of Cx43 is induced with acute myocardial ischemia (AMI), suggesting that phosphorylation at this site plays a role in arrhythmogenesis. The link between estrogen loss and an increase in arrhythmias indicates that changes in Cx43 expression and phosphorylation may play a role in the mechanism of this increased predisposition to arrhythmias. Our goal in the current study was to simultaneously examine the arrhythmic response and changes in Cx43 expression and phosphorylation at S368 in order to gain a better understanding of ischemic induction of cardiac arrhythmias following the loss of estrogen. We hypothesized that ovariectomized animals would have an increased incidence of cardiac arrhythmias in addition to corresponding shifts in Cx43 expression and increased phosphorylation of S368 in response to acute AMI. Anesthetized, ventilated female ovariectomized (n=3) and intact (n=2) Sprague-Dawley rats were subjected to 20-minutes of AMI produced through ligation of the left coronary artery. Cardiac arrhythmias that occurred in response to AMI were measured using a standard lead II electrocardiogram, which were then analyzed and quantified. Blood pressure was also monitored for the duration of the experiment. Immediately following the 20 minutes of AMI, the heart was removed and the left ventricle flash frozen. Western blot analysis was then performed on the left ventricle to examine total Cx43 expression as well as pS368-Cx43 expression. In comparison to intact animals that had undergone a similar

level of AMI, ovariectomized animals exhibited an increase in arrhythmic burden. Correspondingly, the ovariectomized animals also have an increase in the pS368/Total Cx43 ratio. These data establish that the AMI is effective for producing cardiac arrhythmias in a manner that allows for simultaneous arrhythmic induction and examination of shifts in Cx43 expression and phosphorylation. Preliminary results indicate that ovariectomized animals appear to be more sensitive to the AMI induced-arrhythmias and phosphorylation of S368.

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## Cardiovascular Conditioning Status Impacts the Phosphorylation Pattern of Connexin-43

**Jillane Mulvey,** Rachel Firkins, Kevin Ehlers, Julia Moffitt, Matthew Henry

Department of Physiology and Pharmacology at Des Moines

Gap junctions exist at the cell membrane to connect adjacent cells and allow intracellular communication. The functional gap junction is composed of individual protein subunits called connexins. Connexin-43 (Cx43) makes up gap junctions in several tissues and has been found to have a crucial impact on cardiovascular health. Regulation of Cx43 protein is achieved by post-translational modifications, which can enhance gap junction assembly or down-regulate cell-cell communication. Down-regulation of gap junctions often correlates with a pathological cardiovascular state; including ischemia, hypoxia, and increased susceptibility to arrhythmias. Cardiovascular conditioning via exercise training has been well-documented to prevent the incidence of arrhythmias, while cardiovascular deconditioning, through hindlimb unloading (HU), shows definite increases in vulnerability for arrhythmias. Previous studies have demonstrated that altering the conditioned state of the heart will cause changes in the expression and/or phosphorylation of Cx43, however the precise impact of the phosphorylation status of Cx43 during various states of cardiovascular conditioning is not well understood. We hypothesize that cardiovascular conditioning will cause a decrease in phosphorylation at sites known to decrease Cx43 function (S255, S262, S279/282, and S368), while cardiovascular deconditioning will cause phosphorylation at these sites to increase. To stimulate a conditioned and deconditioned state, we have utilized two models. Animals underwent 9 weeks of daily spontaneous wheel running (DSR) or 14 days of HU to induce a conditioned and deconditioned state, respectively, and compared to cage controls. To assess the impact of the conditioned state on Cx43 expression and phosphorylation, Western blot analysis was conducted using antibodies for total and phosphorylated forms of Cx43. Our findings show that the conditioned state did not impact the phosphorylation status at S279/282. Both conditioning models altered the phosphorylation status of S255 and S368. Serine 255 phosphorylation appears to be increased in both deconditioning and conditioning models. However, each model impacted Cx43-pS368 differently. HU increased the amount of hyperphosphorylated Cx43-pS368 and decreased the hypophosphorylated Cx43-pS368. In contrast, DSR appears to increase both the hyper- and hypophosphorylated Cx43-pS368. Thus, it appears the type of cardiovascular conditioning induces a more complex pattern of Cx43 phosphorylation than would be predicted based on the known roles of S255, S262, S279/282, and S368.

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## Cx43 mRNA Extraction and RT-PCR from Rat Left Ventricle

**Jon Senkler**, Rachel Firkins, Matthew Henry, Julia Moffitt

Department of Physiology and Pharmacology at Des Moines

Cardiovascular deconditioning in humans is the exposure to prolonged bedrest or microgravity environments and results in orthostatic intolerance. Hindlimb unloading (HU) in rats, a model of cardiovascular deconditioning, results in increased resting heart rate and cardiac autonomic imbalance. Previous data indicate that cardiac autonomic imbalance increases the risk of cardiac arrhythmias in humans and animals. Connexin43 (Cx43) is the gap junction protein found mainly in the left ventricle and allows conduction of electrical impulses. Changes in the expression and/or phosphorylation of Cx43 results in myocardial uncoupling and an increased likelihood of reentrant arrhythmias. Previously we found that HU results in changes in the expression of phosphorylated Cx43. In the current study we wanted to determine if HU resulted in changes in Cx43 mRNA levels and protein levels. Rats underwent 10-14 days of HU or CC (casted control) deconditioning. Animals were sacrificed and the left ventricle (LV) was then collected. The LV was sectioned in half and part was used for RT-PCR and the other for Western blotting. Results show that Cx43 mRNA levels were not different between the HU and CC. However, Cx43 protein expression was increased in HU vs. CC, consistent with previous findings. These preliminary findings suggest that changes in Cx43 following HU are occurring following gene transcription. Therefore, it appears that increases in arrhythmias due to HU may be a result of a post-transcriptional process such as an increase in translation or post-translational modification.

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## The Effects of Exercise Training on Cardiac Arrythmogenesis and Left Ventricular Connexin 43 Expression in Aged and Young Rats

**Amanda J. Jepson[1],** Emily R. Garnett[2], Ashley N. Davenport[1], Zachary Kadow[3], Matthew K. Henry[1], and Julia A. Moffitt[1]

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The occurrence of cardiac arrhythmias increases with age, although the mechanisms responsible for this effect are poorly understood. Exercise training (ET) has been shown to reduce the incidence of cardiac arrhythmias likely through improved cardiac sympathovagal balance. We hypothesized that aging would result in increased arrhythmogenesis at rest and during an acute stressor, while ET would help reverse these effects in young (4-6 mo) and aged (24-25 mo) F344 rats. Rats underwent 10-11 weeks of treadmill training (11-14 m/min, 60 min/day, 5 day/week) or equivalent sedentary handling protocol. Subcutaneous electrocardiographic (ECG) leads were implanted aseptically at the end of the young sedentary (YS, n=9), young exercise (YEx, n=7), aged sedentary (AS, n=6), and aged exercise (AEx, n=6) training protocols to allow for ECG data acquisition via the Actiwave telemetry system. The arrhythmic index was calculated using a modified scoring system at baseline (BL), followed by an acute stressor consisting of sympathetic stimulation (isoproterenol, (ISO), 0.15mg/kg, s.c.), and brief restraint (BR). Data indicate that physiological adaptations were consistent with ET in both young and aged rats as evidenced by an increase in the left ventricular to body weight ratio, a decrease in resting heart rate, and an increase in soleus citrate synthase activity. The total arrhythmic index (BL+ISO+BR) was significantly higher in aged verses young rats while

ET showed a strong trend toward reversing this effect in both groups. Connexin43 (Cx43), the predominant myocardial gap junction protein, is essential for normal ventricular rhythmicity. Reduced left ventricular expression and change in phosphorylation status of Cx43 is associated with increased arrhythmogenesis. Therefore, we were interested in the expression and phosphorylation status of Cx43 following sedentary and ET conditions young and aged rats. Interestingly, ET resulted in an increase in Cx43 expression and a strong trend toward decreased arrhythmogenisis in aged rats. Young rats displayed no difference in Cx43 expression. These preliminary data support the hypothesis that ET provides a protective benefit against sympathetically induced arrhythmogenesis in young and aged rats. Future studies will further investigate the role of Cx43 in mediating the increased arrhythmogenesis observed with aging and changes associated with ET.

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## Cellular Mechanisms for the Protective Effects of Prior Exercise on Statin-Associated Muscle Dysfunction

**Michelle Godar**, Carissa Chamney, and Kimberly Huey

Drake University, College of Pharmacy & Health Sciences, Des Moines, IA

The most common side effect of statins, skeletal muscle myopathy, is more likely in exercisers. We previously reported that as little as 3 days of prior exercise prevents statin-associated losses in muscle force, and statin treatment did not prevent exercise associated up-regulation of heat shock protein (Hsp). Within the Hsp family, Hsp25 and αB-crystallin are associated with maintaining muscle integrity and reducing muscle oxidative stress, both of which may preserve muscle force output. Thus, current experiments tested the hypothesis that the protective effects of prior exercise against statin-induced muscle dysfunction were associated with higher levels of contractile proteins and reduced oxidative stress compared to untrained muscle. Mice received daily atorvastatin (15 mg/kg) or saline for 2 wks, with/without voluntary wheel running (RW) (Novel & Sedentary groups), and prior exercise groups completed 1 or 3 days of RW before saline or statin treatment with RW (n= 6-7/group). In vivo plantarflexor isometric force was measured with a dual mode lever system, and total muscle contractile proteins and protein carbonyls were measured with myofibrillar and colormetric plate assays, respectively. Statin treatment significantly reduced RW activity days 1-3 in novel compared to 1 or 3 day prior exercise groups. Statin treament reduced force in sedentary novel, and 1d-exercise groups compared to saline (21, 35, and 21%, respectively, $p<0.05$), while 3d of prior exercise prevented statin-associated force loss. Statin treatment was associated with lower levels of contractile proteins in sedentary and novel groups only compared to saline (18 and 26%, respectively, $p<0.05$). In sedendary groups, statin treatment significantly decreased total protein carbonyls compared to saline (8.6±2.1 vs. 2.1±0.8, $p<0.05$). In novel, 1d, and 3d RW groups, total protein carbonyls were lower than sedentary with either saline or statin treatment ($p<0.05$). These results suggest that 3, but not 1 day of exercise, prior to statin treatment can protect against losses in muscle force, and maintenance of contractile proteins may contribute to this protective effect.

13

## Hypoxia-Induced Changes in sFLT-1 and JMJD6 mRNA Expression in Human Trophoblast

**Rebecca Dieter*, Humayun Ahmed*,** Ronald Torry (Mentor)

Ellis Pharmacogenomics Laboratory, College Of Pharmacy And Health Sciences,Drake University

Placenta growth factor (PlGF) is needed for normal placentation. PlGF binds to membrane bound Flt-1 (mFlt-1) and soluble Flt-1 (sFlt-1) receptors on cells. Binding to mFlt-1 results in intracellular signaling, but binding to sFlt-1, a splice variant of mFlt-1 lacking a trans-membrane domain, does not. The expression of sFlt-1 in trophoblast increases during hypoxia but the mechanism for this is unknown. Recent studies show that Jumonji domain-containing protein 6 (Jmjd6) regulates expression of s-Flt1 in endothelial cells. However, it is unknown if trophoblast express Jmjd6, if hypoxia alters its expression, or if hypoxia-induced increase in s-Flt-1 expression is associated with decreased Jmjd6 mRNA expression in these cells. Trophoblast were isolated from normal placentae and cultured under normoxic (21% $O_2$) or hypoxic (1-2% $O_2$) conditions for 24 hours. Total RNA was reversed transcribed into cDNA and fold changes in sFlt-1, mFlt-1, and Jmjd6 mRNA expression was analyzed by real-time PCR. Hypoxia increased sFlt-1 mRNA expression in 3/5 cultures, but the average increase was not significant (1.25$\pm$0.42-fold; P>0.05). Hypoxia did not alter mFlt-1 expression (0.97$\pm$0.02 fold; P>0.05). Hypoxia tended to decrease Jmjd6 mRNA expression in 3/4 cultures, but variability limited statistical significance. Interestingly, the association between changes in sFlt-1 and Jmjd6 mRNA expression during hypoxia was negatively correlated (r = -0.95; p = 0.046). There was no correlation between mFlt-1 and Jmjd6 expression. In conclusion, Jmjd6 mRNA is consistently expressed in trophoblast and changes in sFlt-1 expression in this small sample appear to be inversely related to Jmjd6 expression.

*These authors contributed equally to the work.

14

## A Quercetin Enriched Diet Slows Disease Progression in Dystrophic Skeletal Muscle

**Katrin Hollinger**, Elizabeth Snella, R. Andrew Shanely, Joshua T. Selsby

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Appalachian State University, Human Performance Lab; North Carolina Research Campus, Kannapolis, NC 28081

Duchenne muscular dystrophy (DMD) is an X-linked muscle disease caused by a mutation in the dystrophin gene resulting in production of a non-functional dystrophin protein. Dystrophin-deficiency results in progressive muscle necrosis and fibrosis leading to loss of muscle function and ultimately death. Quercetin (QCN) is an orally available sirtuin 1 (SIRT1) activator, which in turn, can increase Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) activity by deacetylation. Importantly, transgenic up-regulation and gene delivery of PGC-1α have been independently shown to alleviate some aspects of dystrophic pathology in dystrophin-deficient mice (mdx mice). Our hypothesis is that animals fed a QCN enriched diet will have less muscle injury than animals receiving a control diet. To test our hypothesis 3 mo old mdx mice were fed a diet containing 0% or 0.2% QCN for 6 mo, and the diaphragms removed at 9 mo of age. Control and QCN treated mice ate similar amounts of food and grew at a similar rate during the study period. Dietary QCN reduced the number of extracellular nuclei by 37% ($p<0.05$), increased the number of muscle cells by 20% ($p<0.05$) and reduced central nucleation by 33% ($p<0.05$) in diaphragms from treated animals compared to control. Fibrosis was similar between groups. Next, we measured expression of select genes in order to make initial inroads into the mechanism underlying these histological improvements. We found that gene expression of mitochondrial transcription factor A was increased 46% ($p<0.05$), Cox II was

increased 68% ($p<0.05$) and ferritin heavy chain was increased 69% ($p<0.05$) in diaphragms from QCN fed animals compared to control. These data suggest strongly that a QCN enriched diet slows disease progression, at least in part, by driving expression of oxidative genes. Partially supported by the Martin Fund.

**15**

## Increased Plasma Leptin Levels as a Result of Disrupted Light-Dark Cycles

**Ashley Urra**

St. Ambrose University

The effects of disruption in light-dark cycles on leptin levels and growth are not fully understood. There is, however, evidence suggesting that an association exists between disrupted light-dark cycles and obesity. This is caused by decreased plasma leptin levels, allowing for increased appetite due to the inactivation of the JAK/STAT Pathway. The increase in appetite therefore causes increased weight gain; this hypothesis was tested by exposing mice to an all-light environment. Though slight trends were found supporting the hypothesis in body weight, there were no statistical differences between groups for any measurement. By performing this study with a larger population size and for an extended period of time, it is possible that statistical differences could be found.

**16**

## Clonidine Treatment Improves Thermoregulatory and Autonomic Responses to Exertional Heat Stress and May Contribute to Reduced Fatigue and Faster Recovery

**Navita Choudhary**, Anne M. Moss, Kevin C. Kregel and Harald M. Stauss

Department of Health & Human Physiology, The University of Iowa, Iowa City IA

In previous studies we demonstrated that repeated exposure to heat stress (HS, 45$^{o}$C ambient temperature) or exercise (7-11 m/min on running wheel) in the heat (ExHS, 39$^{o}$C ambient temperature) elicits characteristic hemodynamic, thermoregulatory, and autonomic responses in rats. In the current study we investigated the effect of the sympatholytic drug clonidine on these responses to exertional (ExHS) or non-exertional (HS) heat stress.

Blood pressure (BP), heart rate (HR) and core temperature ($T_c$) were continuously recorded telemetrically for 5 days before (baseline), during, and 3 days after (recovery) a 5-day ExHS, HS, or sham (thermoneutral conditions, no exercise) protocol. Exploratory behavior that is affected by factors such as anxiety and fatigue was assessed using an open field test. Clonidine (33 µg/kg/day) or placebo was continuously administered via osmotic minipumps starting 3 days before the first ExHS, HS, or sham protocol day. On the protocol days, rats were exposed to ExHS, HS, or sham conditions twice a day. Experimental conditions were terminated once $T_c$ reached 41.8$^{o}$C (ExHS and HS) or after 90 min (sham, average ExHS duration).

In the nights following each of the five protocol days, $T_c$ and HR were significantly elevated in placebo-treated rats exposed to ExHS and HS compared to sham. Clonidine reduced these increases in $T_c$ and HR, suggesting improved thermoregulation. Compared to sham, nocturnal BP values in placebo-treated rats were more elevated in the ExHS than in the HS group. Clonidine reduced nocturnal BP in ExHS but not in HS, suggesting that the nocturnal hypertension in placebo-treated rats exposed to ExHS is related to increased sympathetic tone. In line with this observation, in placebo-treated rats, low frequency (LF) systolic

BP variability, reflecting sympathetic modulation of vascular tone, was more elevated in the ExHS than in the HS group. Furthermore, clonidine reduced the elevated LF systolic BP variability in the ExHS but not in the HS group. Compared to baseline, exploratory behavior declined during the 5 protocol days in ExHS and HS, which was likely related to fatigue. In ExHS, this decline in exploratory behavior was blunted by clonidine treatment. Furthermore, recovery of exploratory behavior in ExHS was more pronounced and faster in clonidine- compared to placebo-treated rats.

We conclude that clonidine treatment improves thermoregulation and reduces sympathetic responses to chronic exposure to exertional heat stress. These beneficial effects of clonidine may reduce fatigue and contribute to faster recovery following chronic exposure to exertional heat stress as indicated by the improved exploratory behavior in the open field test.

Supported by funding from DoD/USAMRMC #W81XWH-10-2-0115.

**17**

## Modifying a High Fat Diet with Omega-3 Mono-Unsaturated and Poly-Unsaturated Fats Improves Coronary Vascular Dysfunction

**Oltman CL,** Dake B, Sivitz WI, Yorek MA, Lamping KL

Iowa City Veterans Affairs Health Care System, Iowa City, IA
Department of Internal Medicine, Roy J and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA

Our hypothesis is that partial replacement of dietary saturated fats with mono- or poly-unsaturated fats improves coronary vascular dysfunction. We fed C57BL6 male mice a high saturated fat diet (60% lard) for 12 weeks before substituting half of the fat with poly- [n-3 menhaden oil: MO or n-6 safflower oil: SO] or mono-unsaturated fat [olive oil: OO] for 4 weeks. After 16 weeks weight and basal glucose were elevated and glucose utilization was reduced in saturated fat fed compared to normal mice (4% fat). Substitution with MO restored basal glucose and glucose utilization to normal whereas neither SO nor OO diets were different from saturated fat diet. Responses to insulin, acetylcholine and sodium nitroprusside (SNP) were evaluated in isolated pressurized coronary arteries from each group. Insulin responses were attenuated in coronary arteries from mice fed a high saturated fat diet and improved when diets were modified with MO, OO or SO. There was a modest reduction in relaxation to acetylcholine in mice fed high fat diet, which was not improved when the diets were modified with MO, OO or SO. SNP responses were not altered by high saturated fat diet. We conclude that enrichment of high fat diet with mono- and poly-unsaturated fats improves glucose utilization and insulin reactivity, but not acetylcholine reactivity in coronary arteries.

Support: Dept Veterans Affairs

## Angiotensin-[1-7] Infusion Reduces Skeletal Muscle Fibrosis and Restores Locomotor Activity and Sympathovagal Balance in a Mouse Model of Muscular Dystrophy

**Rasna Sabharwal**, and Mark W. Chapleau

University of Iowa, and Veterans Affairs Medical Center, Iowa City, IA

Muscular dystrophy is a catastrophic, fatal neuromuscular disease in need of new therapies. Angiotensin II binding to AT1 receptors (AT1R) contributes to fibrosis in dystrophic skeletal muscle and AT1R blockers are currently being tested in clinical trials. Recently, we reported that autonomic dysregulation precedes and predicts development of cardiomyopathy in sarcoglycan delta deficient (Sgcd-/-) mice with muscular dystrophy (*Clin Auton Res, 2010*). We hypothesized that infusion of the angiotensin peptide Ang-[1-7] will rescue skeletal muscle, locomotor, and autonomic nervous system phenotypes in young Sgcd-/- mice. Control and Sgcd-/- mice were infused with Ang-[1-7] (300 ng/kg/min) for 8 wks beginning at 3 wks of age. Blood pressure (BP), heart rate (HR) and activity were recorded by telemetry in treated and untreated mice. Baroreflex sensitivity (BRS, sequence technique) and resting cardiac vagal and sympathetic tone (HR responses to atropine and propranolol) were measured. BP, activity, BRS and vagal tone were lower in Sgcd-/- vs. control mice, whereas sympathetic tone was higher (Table). Ang-[1-7] normalized activity, BRS, and sympathovagal balance in Sgcd-/- mice without affecting BP, and did not influence any variable in control mice. Skeletal muscle fibrosis present in Sgcd-/- mice (18±1%, n=9) was markedly reduced by Ang-[1-7] (3±1%, n=5). We conclude that Ang-[1-7] reduces skeletal muscle fibrosis and restores locomotor activity and sympathovagal balance in Sgcd-/- mice, without lowering BP. Ang-[1-7] and/or enhancement of its endogenous production may provide a novel therapeutic approach to muscular dystrophy.

| *P<0.05 *vs.* Control; †P<0.05 *vs.* Untreated | Control Mice | | Sgcd-/- Mice | |
|---|---|---|---|---|
| | Untreated (n=5) | Treated (n=5) | Untreated (n=5) | Treated (n=5) |
| Mean 24h BP (mmHg) | 120±4 | 116±4 | 100±2* | 100±3 |
| Mean Activity (c/min) | 11±1 | 12±1 | 4±1* | 10±1† |
| BRS (ms/mmHg) | 2.2±0.2 | 2.0±0.1 | 1.0±0.2* | 1.9±0.1† |
| Vagal tone (Δbpm) | 111±19 | 103±20 | 51±11* | 96±14† |
| Symp tone (Δbpm) | -99±6 | -75±11 | -136±19 | -73±8† |



## Hydrogen Peroxide Mediates the pH Conditioned Chloride Conductance in Nodose Ganglia Neurons

**Runping Wang**, Yongjun Lu, Carol A. Whiteis, Christopher J. Benson, Mark W. Chapleau, François M. Abboud

Cardiovascular Center and Department of Internal Medicine, University of Iowa, Veterans Affairs Medical Center, Iowa City, IA 52242, USA

pH sensitivity has been rarely studied in vagal afferents of nodose ganglia (NG) neurons. Using whole-cell patch-clamp technique in isolated NG neurons, we recently identified a pH-conditioned Cl- current (pH-I) that was evoked following 2 or 3 brief (10s) exposures to low extracellular pH (7.0-6.0) in 16 of 22 (70%) cells

(FASEB J, 2012). The current is large (904.3±159.9pA) and prolonged, lasting 10~15 minutes, and causes significant depolarization (Δ35.2±4.4mV). In the present study, we tested the hypothesis that reactive oxygen species (ROS) mediates this pH-conditioned Cl- conductance. We found that the rate of increase in fluorescence [$(F-F_0)/F_0$] of NG neurons loaded with dihydroethidine (ROS dye) rose dramatically following the brief exposures to pH 6.0 from a control of 0.04±0.01 to 0.12±0.02 units/min over 10~15 minutes (n=31 neurons, $p<0.01$). Moreover superfusion of neurons with $H_2O_2$ induced currents that mimicked the pH conditioned currents. Because of similarities between the pH-conditioned Cl- conductance and the previously described "swell-activated" Cl- current induced with hypoosmotic solutions, we superfused the NG neurons with the $H_2O_2$ scavenger PEG-catalase (1000 units/ml). PEG-catalase blocked significantly ($p<0.01$) the "swell" response to 210 mOsm from 23.3±6.3pA/pF to 0.57±0.39 pA/pF (n=4) as well as the pH-conditioned response from 25.7±6.5pA/pF (n=6) to 6.9±1.4 pA/pF (n=12). The superoxide scavenger PEG-SOD did not affect the current. These results indicate that both pH-conditioned and swell-induced responses are mediated by $H_2O_2$. Opening of these outward Cl- conductances that cause sustained depolarization of vagal afferents may induce a beneficial reflex sympathoinhibition during myocardial ischemia/acidosis or initiate a gastro-intestinal post-prandial satiety reflex.

**20**

## Estimation of Effective Reflecting Distance and Aortic Pulse Wave Velocity from Peripheral Blood Pressure Waveforms in Young and Older Humans

**Gary Pierce**[1], Darren Casey[2], Jess Fiedorowicz[1], Douglas Seals[3], Timothy Curry[2], Jill Barnes[2], Harald Stauss[1]

[1]Univeristy of Iowa, [2]Mayo Clinic, [3]University of Colorado Boulder

It has been argued that aortic pulse wave velocity (APWV) cannot be determined from the reflected wave transit time (Δt) because the effective reflecting distance (EfRD, aortic valve to distal reflecting site) is not defined anatomically. We hypothesized that EfRD can be estimated from demographic/anthropometric data and used to indirectly determine APWV from peripheral blood pressure (BP) waveforms in humans. Invasive (n=25, brachial artery) and non-invasive (n=15, EndoPAT) BP waveforms were converted into aortic BP waveforms (transfer function) and Δt computed from decomposed forward and reflected waves. True EfRD was determined from measured carotid-femoral pulse wave velocity (CF-PWV) (SphygmoCor) and Δt. Stepwise regression analysis resulted in the equation: EfRD= 0.173*age+0.661*BMI +34.548 cm, used to indirectly estimate EfRD and APWV in the original 40 healthy adults, and in a separate cohort of young sedentary (YS, n=6; 22±2 years; $VO_{2max}$ 39±2 ml/kg/min), older sedentary (OS, n=24; 62±1 years; VO2max 27±1 ml/kg/min), and older endurance-trained (OT, n=14; 61±2 years; $VO_{2max}$ 46±2 ml/kg/min) subjects. CF-PWV and indirectly determined APWV were highly correlated (n=40, Pearson's R=0.65, P<0.01; interclass correlation coefficient ICC=0.64, P<0.01). In YS, OS and OT, EfRD and APWV were 52.0±0.5, 61.8±0.4 and 60.6±0.5 cm (all P<0.05) and 6.4±0.3, 9.6±0.2, and 8.1±0.2 m/s (all P<0.05), respectively. In healthy adults, APWV can be reliably derived from invasive and non-invasive peripheral BP waveforms using age and BMI to determine EfRD. This method can detect the distal shift of the reflecting site with age and the increase in APWV with sedentary aging that is attenuated with endurance exercise.

## Regulation of Toll-Like Receptors in the Heart

**Madhu V. Singh**

Cardiovascular Center, Carver College of Medicine, University of Iowa, Iowa City, IA 52242

Toll-like receptors (TLR) are pathogen and injury sensing receptors that are components of the innate immune system. We have shown that myocardial infarction induces complement factor B (*Cfb*), a pro-inflammatory component of the innate immune system through activation of TLRs. TLRs have been shown to play a role in the pathology of hypertension and myocardial infarction. Our published studies showed that inhibition of the calcium and calmodulin-dependent protein kinase II (CaMKII) attenuates toll-like receptor (TLR) mediated nuclear factor kappa B (NF-κB) activation and resulting pro-inflammatory gene expression. Myeloid differentiation primary response gene 88 (MyD88) is an adapter protein that is required for NF-κB activation by TLR and interleukin receptor 1 (IL-1R) signaling. In this study we demonstrate that expression of CaMKII promotes MyD88-mediated NF-κB activation. Moreover, CaMKII and MyD88 exist together in a complex in the cells. CaMKII is capable of directly binding MyD88 and MyD88 is substrate for CaMKII-catalyzed phosphorylation. Thus, CaMKII-mediated MyD88 binding and phosphorylation may contribute to CaMKII-mediated NF-κB activation. To our knowledge, this is the first demonstration of a CaMKII target protein in the innate immune system.

## MECHANISTIC AND TRANSLATIONAL RESEARCH IN CORONARY VASCULAR BIOLOGY

Indiana University School of Medicine

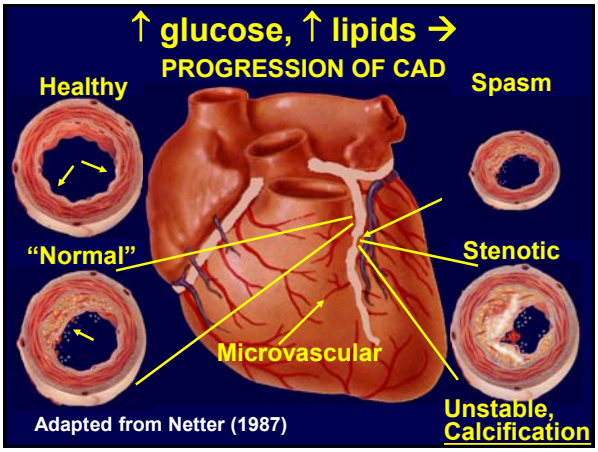
Purdue University

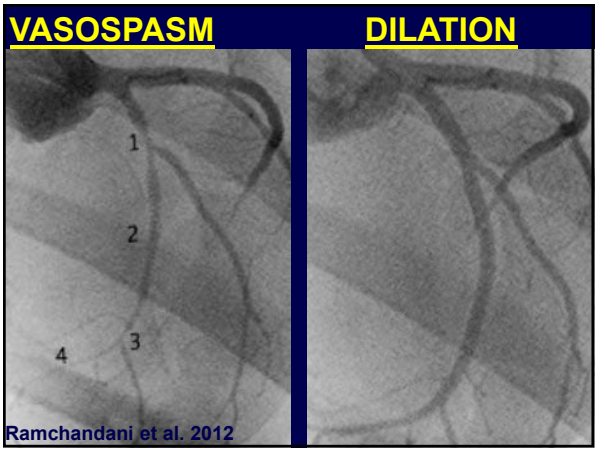
IndianaCTSI | ACCELERATING CLINICAL AND TRANSLATIONAL RESEARCH

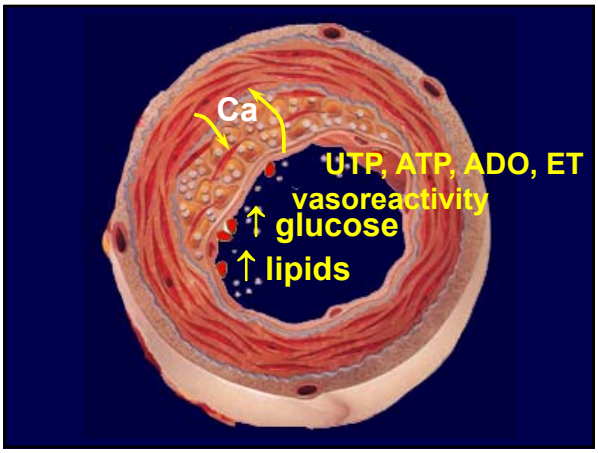
Dr. Michael Sturek, Professor and Chair
Cellular & Integrative Physiology, IUSM; Biomed. Engin.,Purdue
msturek@iupui.edu

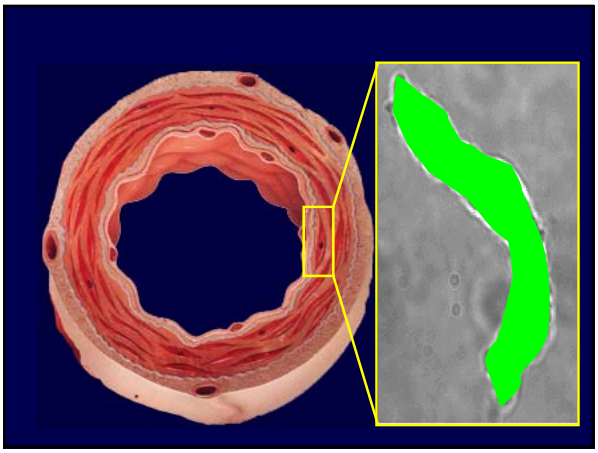
May 27, 2011

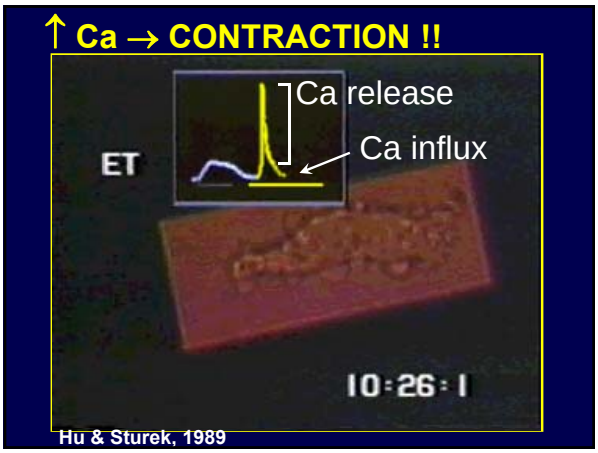
1. Overview CAD,calcification,specifcity
2. Define translational, swine models
3. Yucatan: diabetic dyslipidemia
   SR Ca release, Ca influx, Ca localiz.
4. Ossabaw: obesity → pre-diabetes
   SR Ca release, Ca influx
5. Contractile→proliferative→osteogenic
6. Targeting, not "just association"
7. Conclusions

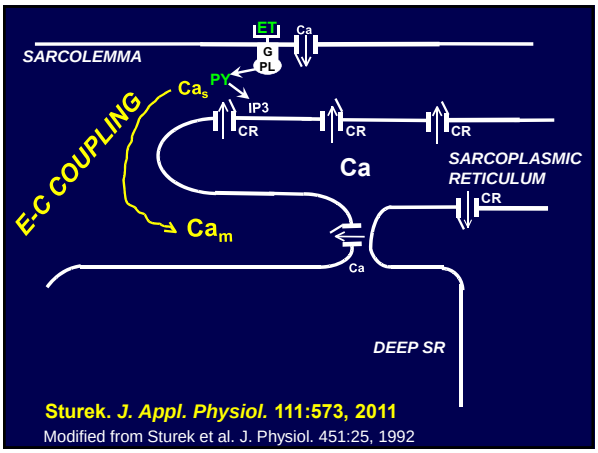


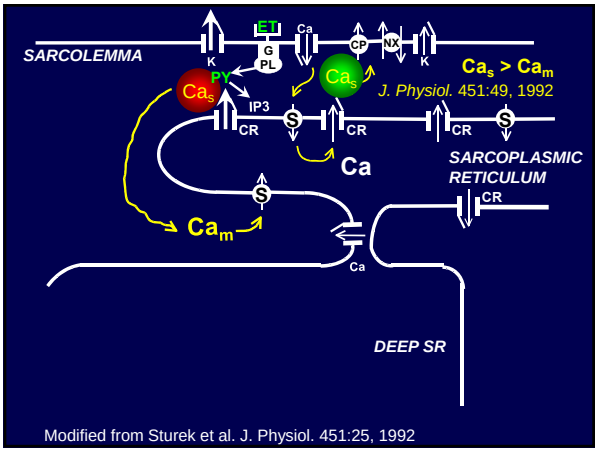



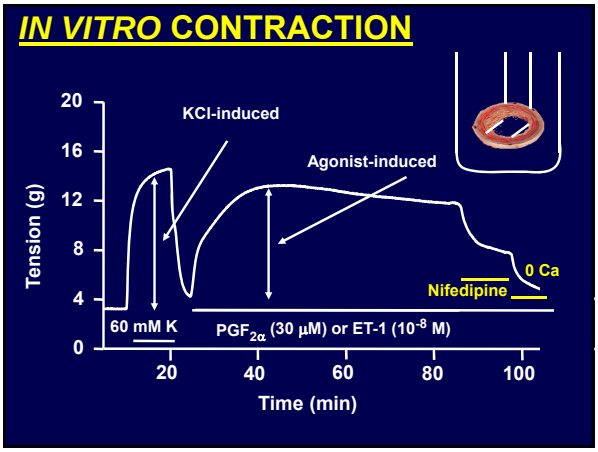



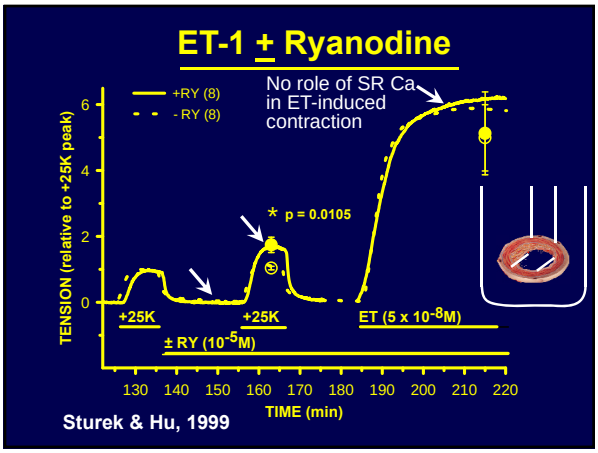



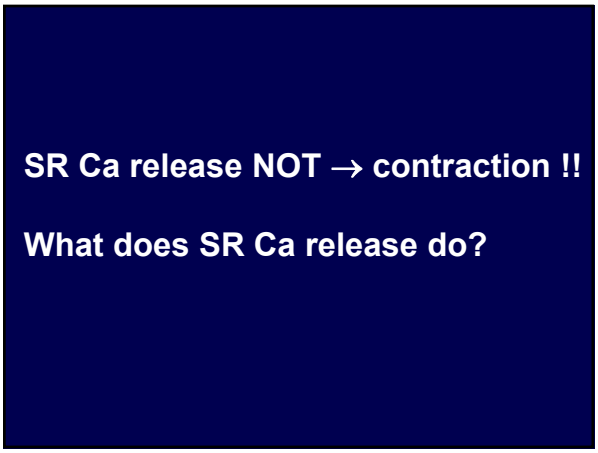

↑ Ca → CONTRACTION !!
Ca release
Ca influx
ET
10:26:1
Hu & Sturek, 1989


SARCOLEMMA
ET
Ca
G
PL
PY
IP3
CR
E-C COUPLING
Ca
SARCOPLASMIC RETICULUM
DEEP SR
Sturek. J. Appl. Physiol. 111:573, 2011
Modified from Sturek et al. J. Physiol. 451:25, 1992


SARCOLEMMA
ET
Ca
CP
NX
K
G
PL
PY
IP3
CR
Cas > Cam
J. Physiol. 451:49, 1992
Ca
SARCOPLASMIC RETICULUM
DEEP SR
Modified from Sturek et al. J. Physiol. 451:25, 1992


IN VITRO CONTRACTION
KCl-induced
Agonist-induced
Tension (g)
20
16
12
8
4
0
60 mM K
PGF2α (30 μM) or ET-1 (10-8 M)
Nifedipine
0 Ca
20
40
60
80
100
Time (min)

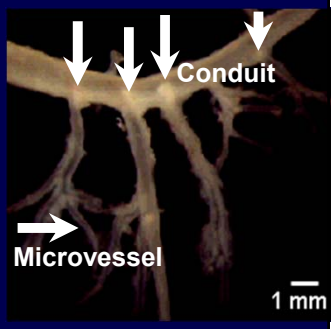

ET-1 ± Ryanodine
+RY (8)
- RY (8)
No role of SR Ca in ET-induced contraction
* p = 0.0105
TENSION (relative to +25K peak)
6
4
2
0
+25K
+25K
ET (5 x 10-8M)
± RY (10-5M)
130
140
150
160
170
180
190
200
210
220
TIME (min)
Sturek & Hu, 1999


SR Ca release NOT → contraction !!
What does SR Ca release do?

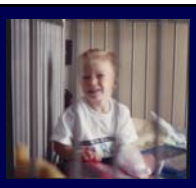
## ARTERIAL SPECIFICITY

**MACROVASCULAR**
Conduit
Atherosclerosis

**MICROVASCULAR**
Flow regulation
Dysfunction
Remodeling



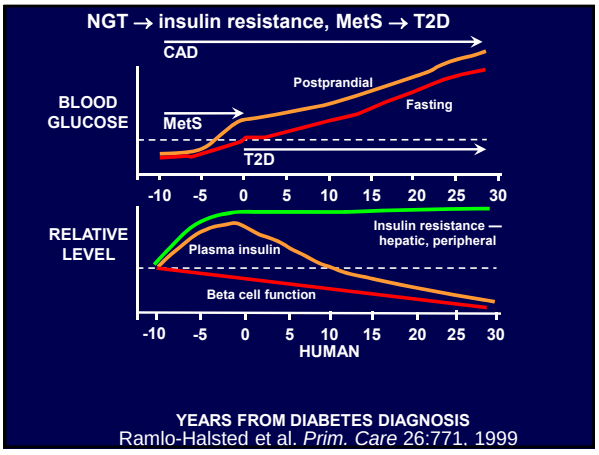

## TYPE 1 DIABETES



## DIABETIC COMPLICATIONS

- blindness
- lower limb amputation
- kidney failure
- 4-fold ↑ coronary artery disease
- 80% die of atherosclerotic event
- all vascular complications !
- cardiomyopathy


*Indianapolis Star*, 3/20/05




Ramlo-Halsted et al. *Prim. Care* 26:771. 1999

## METABOLIC SYNDROME (MetS), PRE-DIABETES → TYPE 2

1. Central obesity
2. Insulin resistance
3. Glucose intolerance
4. Dyslipidemia (↑LDL/HDL)
5. Dyslipidemia (↑TG)
6. Hypertension

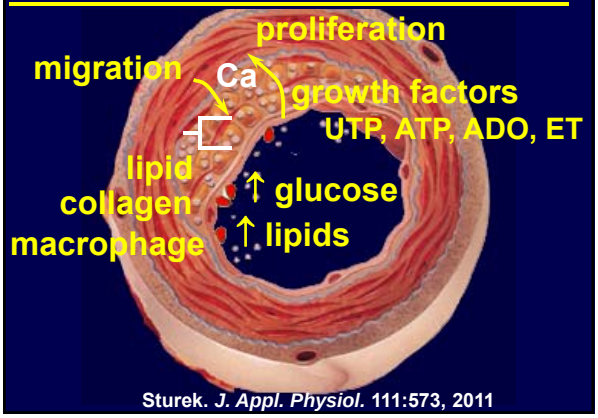
Eckel et al. *Lancet* 365:1415, 2005
WHO, 1999; European Group, 1999; ATP III, 2001
Kahn et al. *Diabetes Care* 28:2289, 2005
Kahn et al. *Circulation* 113:2943, 2006

## LONG-TERM GOALS

- Understand underlying mechanisms of CAD in obese, metabolic syndrome, diabetic patients.
- Develop adjunct therapies to minimize cardiovascular complications.
- Rapid translation to the clinic

Libby, Ridker, Hansson. *Nature* 473:317, 2011

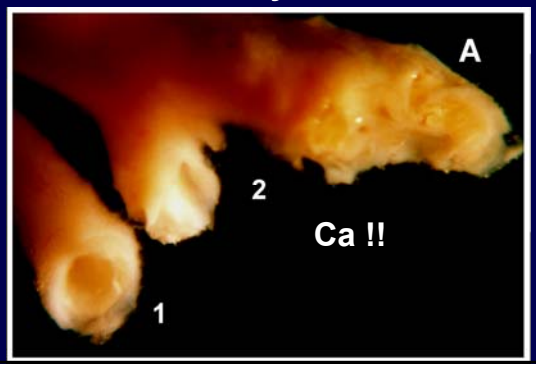
## ATHEROSCLEROSIS MECHANISMS



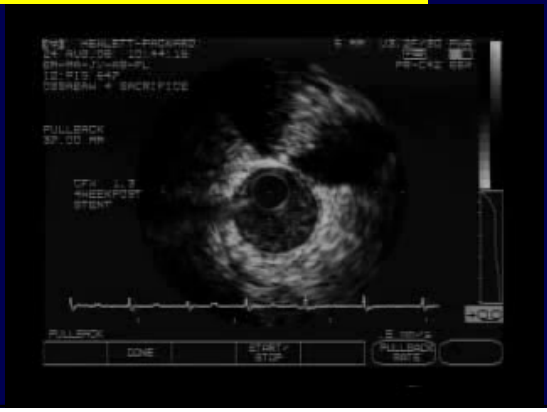

Sturek. *J. Appl. Physiol.* 111:573, 2011

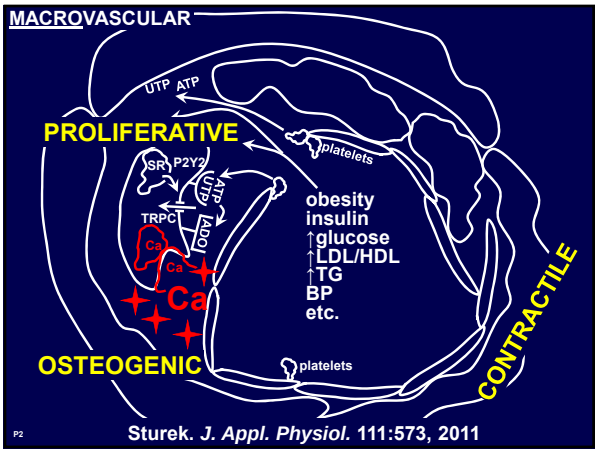
## ATHEROSCLEROSIS MECHANISMS

Human, sedentary




## VASCULAR CALCIFICATION






Sturek. *J. Appl. Physiol.* 111:573, 2011

1. Overview CAD,calcification,specifcity
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   SR Ca release, Ca influx
5. Contractile→proliferative→osteogenic
6. Targeting, not "just association"
7. Conclusions

## DEFINE "TRANSLATIONAL"

BENCH → BASIC SCIENCE

"TRANSLATIONAL"

BEDSIDE → CLINICAL RELEVANCE IN ACADEMIC MED CTR

## DEFINE "TRANSLATIONAL"

Indiana University School of Medicine CTSI

"... applying discoveries made during research (in the lab, through animal studies, etc.) to the development of clinical trials and studies in humans ..."

→ degrees of "translational"

## DEFINE "TRANSLATIONAL"

BENCH → BASIC SCIENCE

T1

"TRANSLATIONAL"
molecular
cell culture
organ culture
c. elegans
mouse
rat
rabbit
dog, sheep
pig, primate

FAILURES X

Most "translational"?

BEDSIDE → CLINICAL RELEVANCE IN ACADEMIC MED CTR

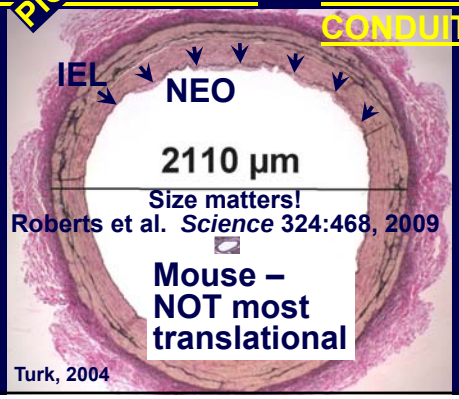
## INTACT ANIMAL - WHY THE PIG?

- Heart, coronary circulation human-like
  - few native collaterals
  - pharmacology
  - heart rate, metabolism
  - atherosclerotic lesions
  - coronary interventions

Johnson et al. *Thromb. Haemost.* 81:835, 1999
Touchard, Schwartz. *Toxicol. Pathol.* 34:11, 2006

## OF MICE AND MEN PIGS

## CORONARY CONDUIT




Size matters!
Roberts et al. *Science* 324:468, 2009

Mouse – NOT most translational

Turk, 2004

## PIG-HEADED !?

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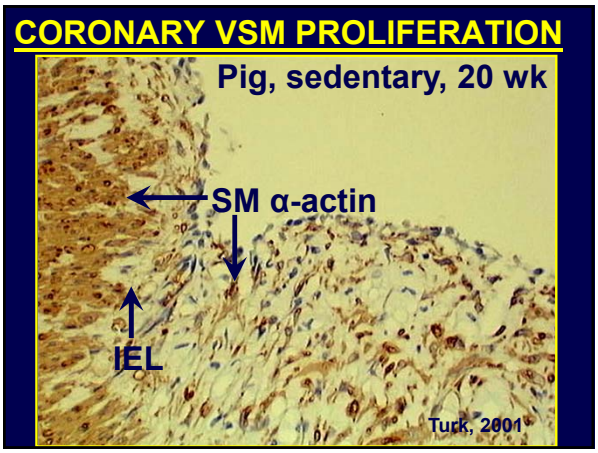


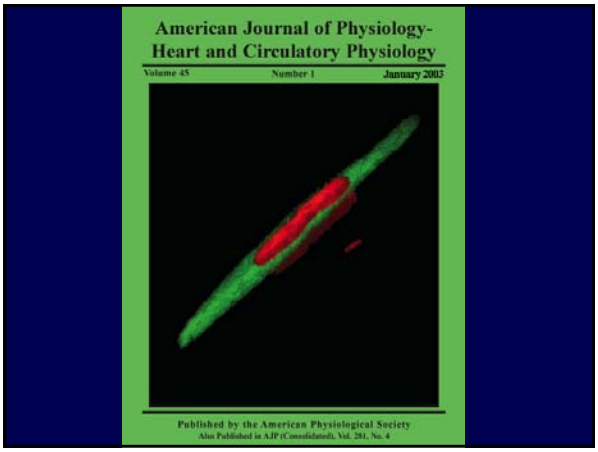


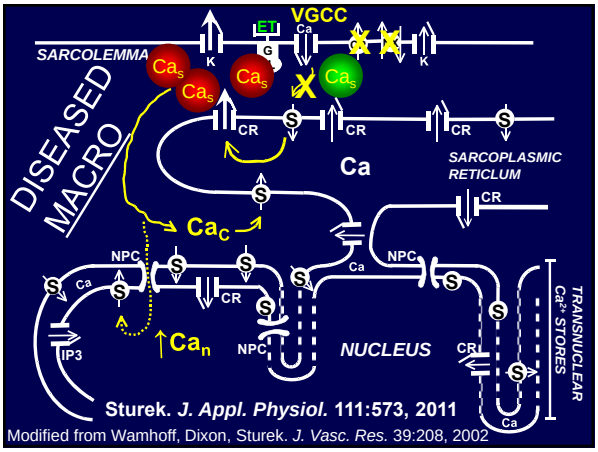


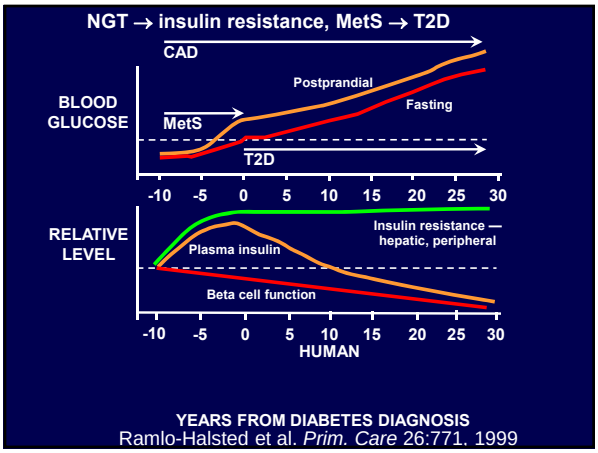


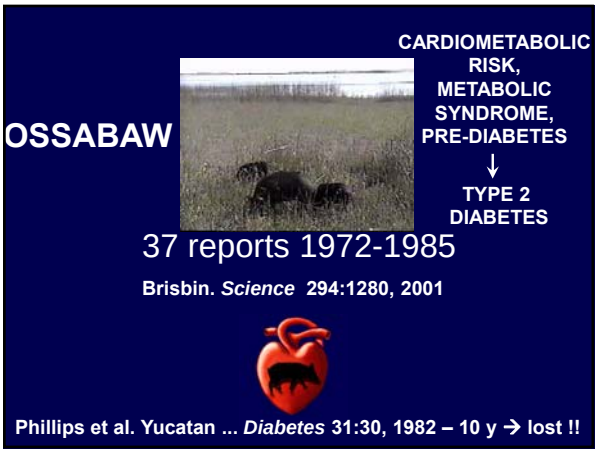

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## "THRIFTY GENOTYPE" (OBESITY)

OSSABAW

ACTIVE LEAN | INACTIVE OBESE

~8 years development model at IUSM, Purdue
Only research and breeding colony in world !!

Kreutz et al. *Diabetes Metab Syndr Obes* 4: 99, 2011

## MORBID OBESITY

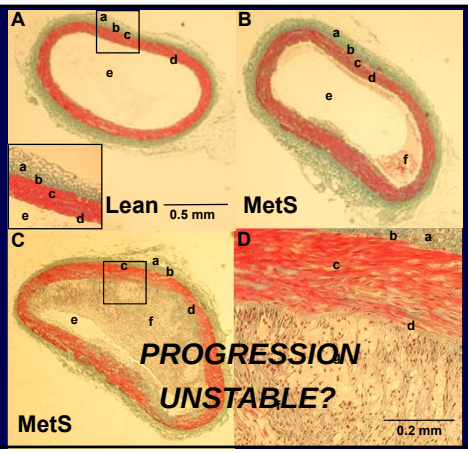
## EXERCISING LEAN YUCATAN PIG

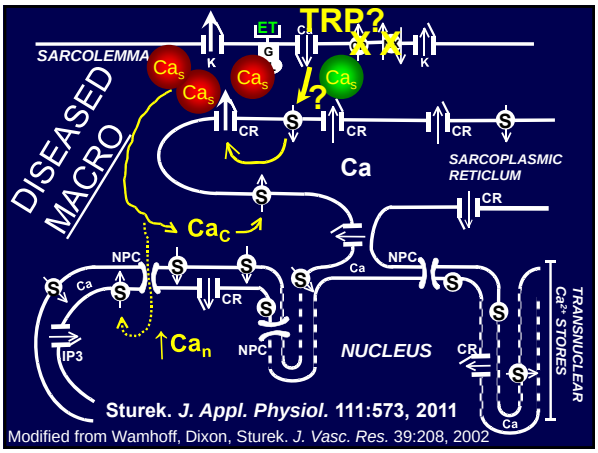
**4 d / wk, 30 min / d, 75% max HR, 16 wk**

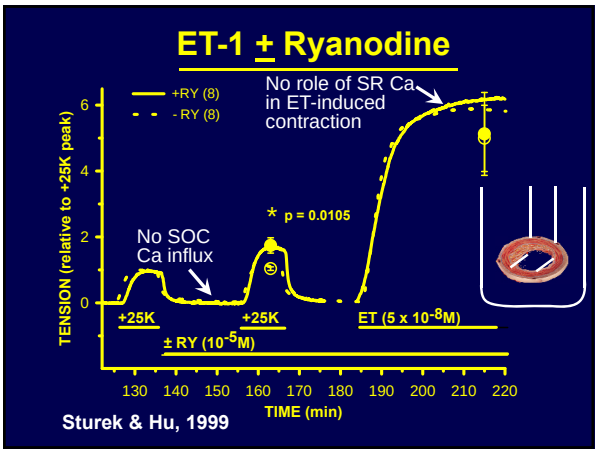
## LARGE ANIMAL MODEL MetS →T2D

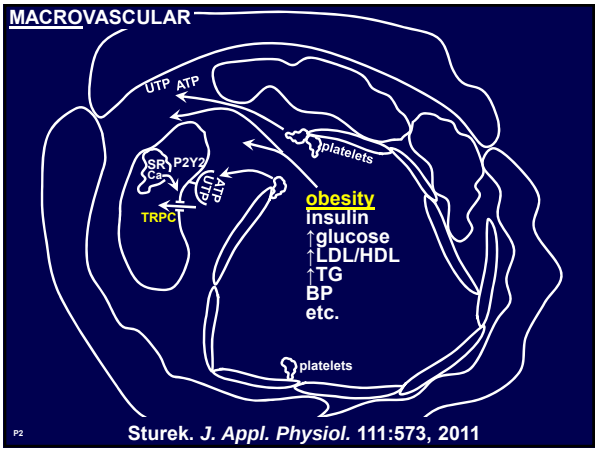
| | Yucatan | Ossabaw |
|---|---|---|
| 1. Obesity | No | Oss>Yuc |
| 2. Insulin resistance | No | Yes |
| 3. Glucose intolerance | No | Yes |
| 4. Dyslipidemia (↑LDL/HDL) | Yes | Yes |
| 5. Dyslipidemia (↑TG) | No | Yes |
| 6. Hypertension | No | Yes |

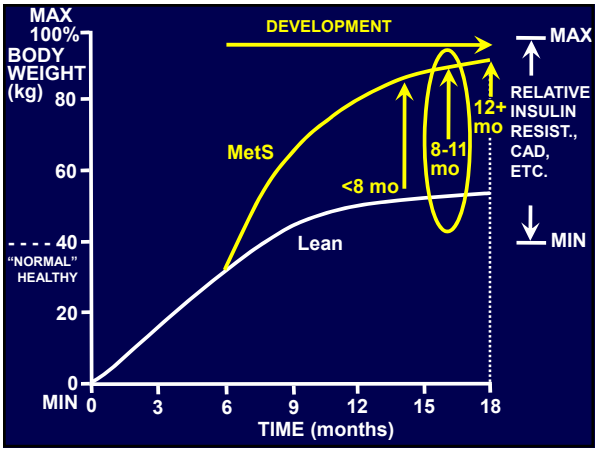
Sturek et al. In *Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques*, 2nd Ed. M. Swindle (Ed.). p. 397, 2007
Lee et al. *Hepatology* 50:56, 2009
Edwards, Neeb et al. *Cardiovasc. Res.* 85:631, 2010
Neeb et al. *Comp. Med.* 60:300, 2010

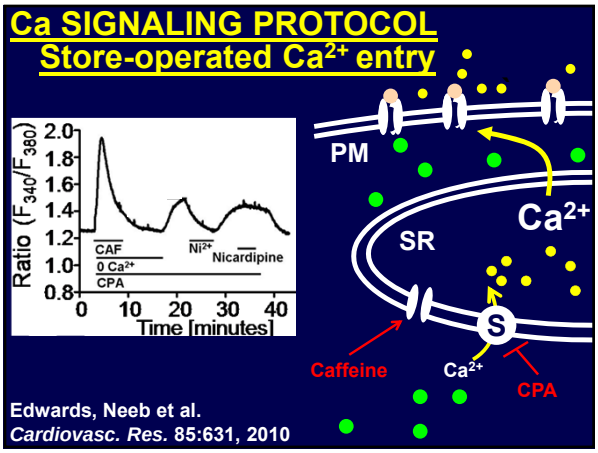


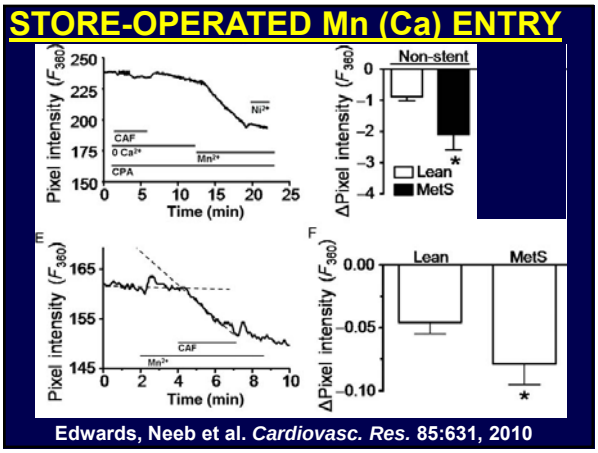

TRP?
SARCOLEMMA
DISEASED MACRO
ET
K
G
CR
Ca
SARCOPLASMIC RETICULUM
NPC
NUCLEUS
IP3
TRANSNUCLEAR Ca2+ STORES
Sturek. J. Appl. Physiol. 111:573, 2011
Modified from Wamhoff, Dixon, Sturek. J. Vasc. Res. 39:208, 2002


ET-1 ± Ryanodine
+RY (8)
- RY (8)
No role of SR Ca in ET-induced contraction
* p = 0.0105
No SOC Ca influx
TENSION (relative to +25K peak)
+25K
+25K
ET (5 x 10-8M)
± RY (10-5M)
TIME (min)
Sturek & Hu, 1999


MACROVASCULAR
UTP ATP
platelets
SR
P2Y2
TRPC
obesity
insulin
↑glucose
↑LDL/HDL
↑TG
BP
etc.
Sturek. J. Appl. Physiol. 111:573, 2011


MAX
100%
BODY WEIGHT (kg)
DEVELOPMENT
MetS
<8 mo
8-11 mo
12+ mo
Lean
"NORMAL" HEALTHY
RELATIVE INSULIN RESIST., CAD, ETC.
MIN
TIME (months)


Ca SIGNALING PROTOCOL
Store-operated Ca2+ entry
Ratio (F340/F380)
CAF
0 Ca2+
CPA
Ni2+
Nicardipine
Time [minutes]
PM
SR
Ca2+
Caffeine
CPA
Edwards, Neeb et al.
Cardiovasc. Res. 85:631, 2010


STORE-OPERATED Mn (Ca) ENTRY
Pixel intensity (F360)
Time (min)
Non-stent
Lean
MetS
ΔPixel intensity (F360)
Edwards, Neeb et al. Cardiovasc. Res. 85:631, 2010

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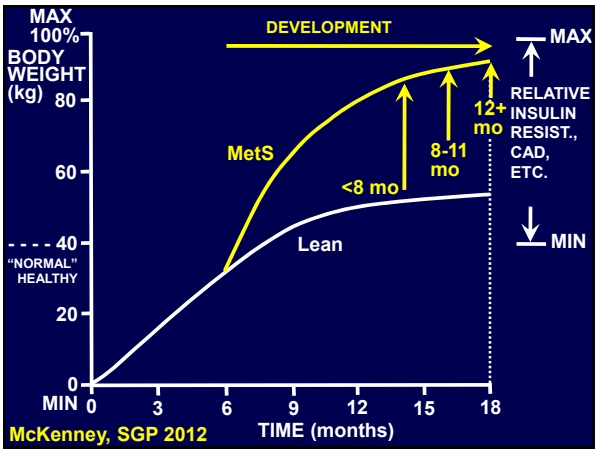
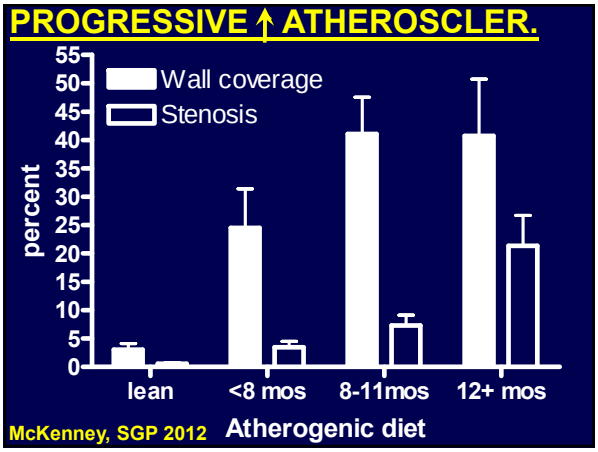
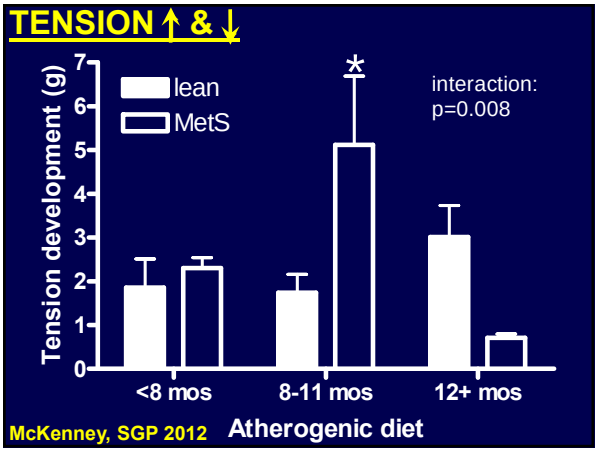
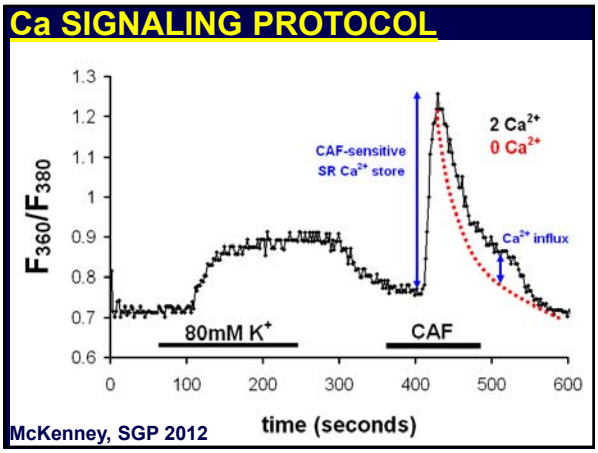
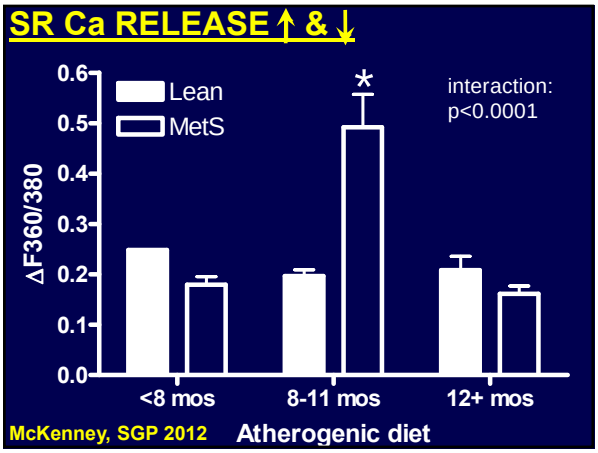


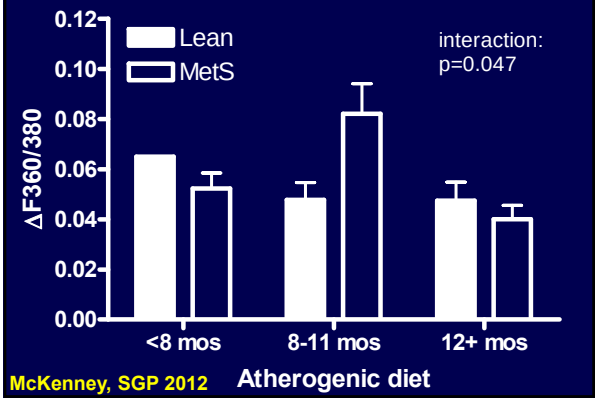



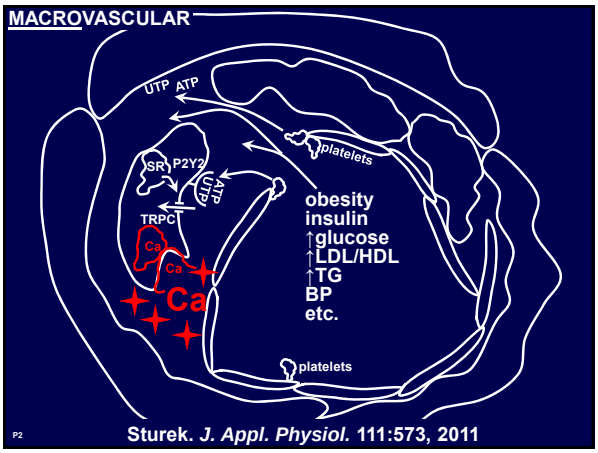



## STORE-OPERATED Ca INFLUX ↑ & ↓



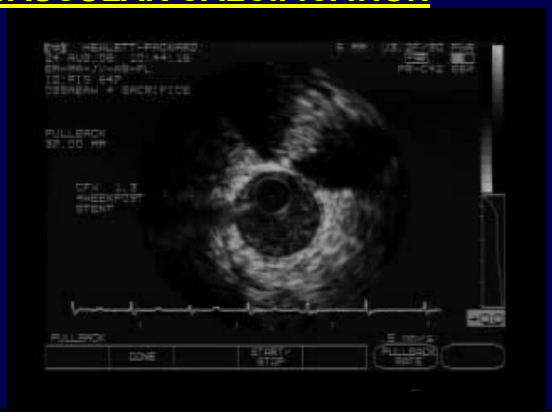

McKenney, SGP 2012

## MACROVASCULAR



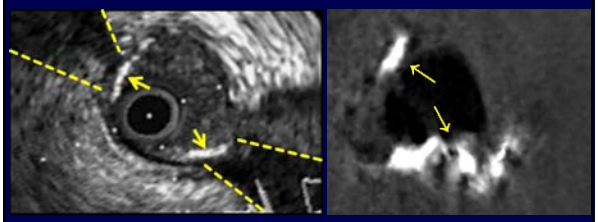

Sturek. *J. Appl. Physiol.* 111:573, 2011

## VASCULAR CALCIFICATION



## VASCULAR CALCIFICATION

**IVUS** **MICRO-CT**



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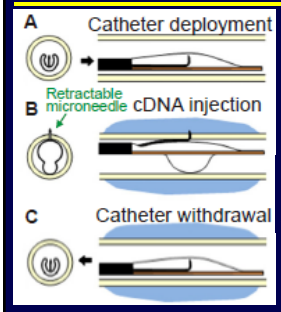
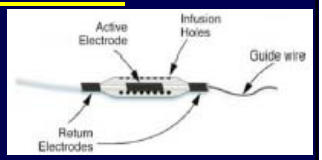
## LOCAL GENE DELIVERY

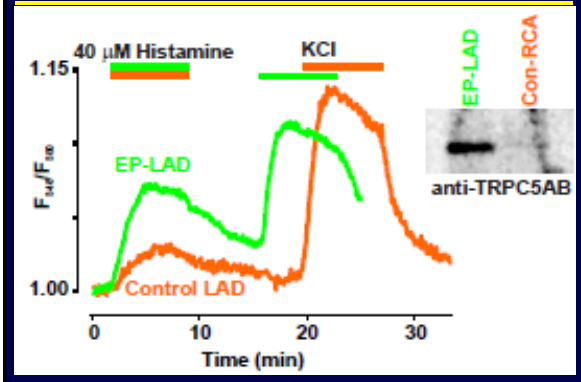



**Microinfusion catheter,**
Mercator MedSystems
(Karanian et al. J. Vasc. Interv. Radiol. 21:1555, 2010)




**Electroporation catheter,**
Cardigant Medical, Inc. (Seidler et al. Pharm. Res. 22:1685, 2005)

## LOCAL GENE DELIVERY OF TRPC5




Obukhov, Bryd, Alloosh, Sturek, 2011

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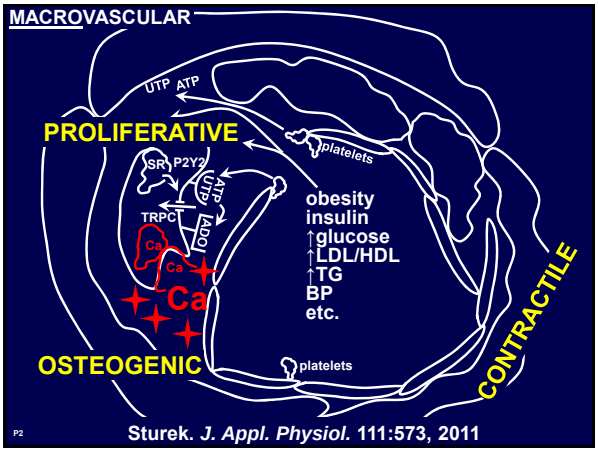
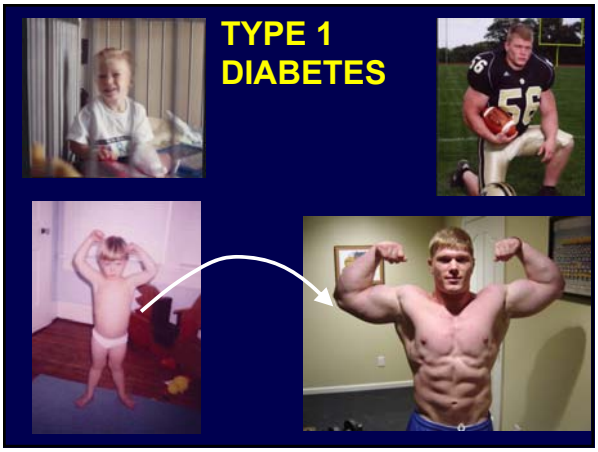



Sturek. *J. Appl. Physiol.* 111:573, 2011




## CONCLUSION

Superb mimickry of human Mets, T2D?, etc.
→ *TRANSLATIONAL RESEARCH, MOLECULAR MECHANISMS VASCULAR CALCIFICATION*

THANK YOU

# The importance of stupidity in scientific research

**Martin A. Schwartz**

Department of Microbiology, UVA Health System, University of Virginia, Charlottesville, VA 22908, USA
e-mail: maschwartz@virginia.edu

*Accepted 9 April 2008*
*Journal of Cell Science 121, 1771 Published by The Company of Biologists 2008*
*doi:10.1242/jcs.033340*



I recently saw an old friend for the first time in many years. We had been Ph.D. students at the same time, both studying science, although in different areas. She later dropped out of graduate school, went to Harvard Law School and is now a senior lawyer for a major environmental organization. At some point, the conversation turned to why she had left graduate school. To my utter astonishment, she said it was because it made her feel stupid. After a couple of years of feeling stupid every day, she was ready to do something else.

I had thought of her as one of the brightest people I knew and her subsequent career supports that view. What she said bothered me. I kept thinking about it; sometime the next day, it hit me. Science makes me feel stupid too. It's just that I've gotten used to it. So used to it, in fact, that I actively seek out new opportunities to feel stupid. I wouldn't know what to do without that feeling. I even think it's supposed to be this way. Let me explain.

For almost all of us, one of the reasons that we liked science in high school and college is that we were good at it. That can't be the only reason – fascination with understanding the physical world and an emotional need to discover new things has to enter into it too. But high-school and college science means taking courses, and doing well in courses means getting the right answers on tests. If you know those answers, you do well and get to feel smart.

A Ph.D., in which you have to do a research project, is a whole different thing. For me, it was a daunting task. How could I possibly frame the questions that would lead to significant discoveries; design and interpret an experiment so that the conclusions were absolutely convincing; foresee difficulties and see ways around them, or, failing that, solve them when they occurred? My Ph.D. project was somewhat interdisciplinary and, for a while, whenever I ran into a problem, I pestered the faculty in my department who were experts in the various disciplines that I needed. I remember the day when Henry Taube (who won the Nobel Prize two years later) told me he didn't know how to solve the problem I was having in his area. I was a third-year graduate student and I figured that Taube knew about 1000 times more than I did (conservative estimate). If he didn't have the answer, nobody did.

That's when it hit me: nobody did. That's why it was a research problem. And being *my* research problem, it was up to me to solve. Once I faced that fact, I solved the problem in a couple of days. (It wasn't really very hard; I just had to try a few things.) The crucial lesson was that the scope of things I didn't know wasn't merely vast; it was, for all practical purposes, infinite. That realization, instead of being discouraging, was liberating. If our ignorance is infinite, the only possible course of action is to muddle through as best we can.

I'd like to suggest that our Ph.D. programs often do students a disservice in two ways. First, I don't think students are made to understand how hard it is to do research. And how very, very hard it is to do important research. It's a lot harder than taking even very demanding courses. What makes it difficult is that research is immersion in the unknown. We just don't know what we're doing. We can't be sure whether we're asking the right question or doing the right experiment until we get the answer or the result. Admittedly, science is made harder by competition for grants and space in top journals. But apart from all of that, doing significant research is intrinsically hard and changing departmental, institutional or national policies will not succeed in lessening its intrinsic difficulty.

Second, we don't do a good enough job of teaching our students how to be productively stupid – that is, if we don't feel stupid it means we're not really trying. I'm not talking about 'relative stupidity', in which the other students in the class actually read the material, think about it and ace the exam, whereas you don't. I'm also not talking about bright people who might be working in areas that don't match their talents. Science involves confronting our 'absolute stupidity'. That kind of stupidity is an existential fact, inherent in our efforts to push our way into the unknown. Preliminary and thesis exams have the right idea when the faculty committee pushes until the student starts getting the answers wrong or gives up and says, 'I don't know'. The point of the exam isn't to see if the student gets all the answers right. If they do, it's the faculty who failed the exam. The point is to identify the student's weaknesses, partly to see where they need to invest some effort and partly to see whether the student's knowledge fails at a sufficiently high level that they are ready to take on a research project.

Productive stupidity means being ignorant by choice. Focusing on important questions puts us in the awkward position of being ignorant. One of the beautiful things about science is that it allows us to bumble along, getting it wrong time after time, and feel perfectly fine as long as we learn something each time. No doubt, this can be difficult for students who are accustomed to getting the answers right. No doubt, reasonable levels of confidence and emotional resilience help, but I think scientific education might do more to ease what is a very big transition: from learning what other people once discovered to making your own discoveries. The more comfortable we become with being stupid, the deeper we will wade into the unknown and the more likely we are to make big discoveries.

The Upside Down Lecture:

How to get students to come to class

Dee U. Silverthorn

University of Texas at Austin

## Overview

- What is the upside-down lecture?
- Why change how we teach?
- Changing the class structure
- Student and faculty reactions

| TRADITIONAL CLASS | UPSIDE DOWN CLASS |
| --- | --- |
| Students take notes on content in lecture (or get it from the note-taking service…) | |
| ⇩ | ⇩ |
| Students go home to learn content and work problems. | |

## Why should we change the way we teach?

- Knowledge
- Technology

## How People Learn

- National Research Council Committee on Developments in the Science of Learning
- www.nap.edu

## *HOW PEOPLE LEARN*

People learn best when

- they are responsible for their own learning.
- they can practice applying knowledge.
- they receive ungraded feedback (formative assessment).

## The instructor learns

## Two models of the upside-down lecture

- The interactive classroom
- The team-based learning approach

## Learning content before class

HOW DO YOU CONVINCE STUDENTS TO READ BEFORE CLASS?

## The answer?

## Model 1 – UT Austin: What happens in the classroom?

- Brief overview of the material
- Informal dialogue – any questions?
- Ungraded assessment -- Clickers!

## Types of questions

- Structure using Bloom's taxonomy
  - -- Lower level: remember / understand
  - -- Higher level: apply / analyze / evaluate
- "On the fly" – requires instructor confidence
- Opinion / feedback
- Case studies

Testing for memorization…

or

"How can I talk about this if they don't know what it is?"

UTMB

Testing for conceptual understanding …

Ask something that's not in their reading

Opinion/feedback questions

- Find out what students are thinking
- Introduce humor

Model 2 – UTHSC San Antonio
Team-based learning
(Charles Levinson)

- Class divided into permanent teams
- For each unit:
  - Introductory overview
  - Reading assignments and questions
  - Faculty available in office hours
  - Friday morning group session

UTHSC-SA Fridays

How do you get student "buy-in"?

1. Make it worth points.
   - Model 1: not for correct answers
   - TBL model: for correct answers
2. Make it relevant.
3. Talk to the students.

CAUTION!

Student resistance

**The biggest impediment to faculty change?**

Unsuccessful faculty believe:

Successful faculty believe:

Successful implementation

1. Define your goals/objectives.
2. Start small.
3. Talk to the students.
4. Give students tools for adapting.
5. Match assessment to method.

ACKNOWLEDGMENTS:

- Patti Thorn, Ph.D.
- Graduate teaching assistants at U.T. Austin
- Marilla Svinicki, Ph.D. and staff of the UT Center for Teaching Excellence

Colleagues from:

- Teaching Section, American Physiological Society
- International Union of Physiological Sciences
- HAPS and the Integrative Themes in Physiology project

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IPS The Iowa Physiological Society