IPS The Iowa Physiological Society

17th Annual Iowa Physiological Society Meeting

September 21, 2013

9 a.m. – 5 p.m.

Des Moines University

3200 Grand Avenue

Des Moines, IA 50312

Agenda

8 a.m.	**Breakfast, Registration, and Poster Set-Up**
8:50 a.m.	**Opening Remarks**
9 a.m.	**Keynote Address in Physiological Research** Rationally Designing Novel Therapeutics for Duchenne Muscular Dystrophy: From Basic Science Discoveries to Pre-Clinical and Clinical Studies *Bernard J. Jasmin, Ph.D., University of Ottawa*
10 a.m.	**Research Presentation** Altered DNA Repair in Ovaries of Obese Mice Following Chronic Exposure to 7,12-Dimethylbenz[a]anthracene *Shanthi Ganesan, Ph.D. Graduate Student, Iowa State University*
10:15 a.m.	**Research Presentation** Alpha-Dystroglycan Glycosylation in Cancer *Daniel Beltran, Ph.D., University of Iowa*
10:30 a.m.	**American Physiological Society Address in Research Advocacy** Advocacy for Physiological Research *T. Richard Nichols, Ph.D., Georgia Technical Institute*
11:30 a.m.	**Continuing Discussions and Poster Viewing**
12 p.m.	**Lunch and Poster Viewing**
1:30 p.m.	**Research Presentation** The G Protein-Coupled Estrogen Receptor 1 in Ca^{2+}/Calmodulin-Dependent Signaling in the Vasculature *Kim Tran, M.D., Ph.D., Des Moines University*
2 p.m.	**Research Presentation** Autonomic and Angiotensinergic Mechanisms in Muscular Dystrophy and Associated Cardiomyopathy *Rasna Sabharwal, Ph.D., University of Iowa*
2:15 p.m.	**Research Presentation** Decreased Arrhythmic Burden Following Exercise in Associated with a Decrease in Cx43 Phosphorylation Specifically at Serines 255 and 279/282 *Erica Thomas, MSBS, Des Moines University*
2:30 p.m.	**Research Presentation** Targeting Inflammation in Arterial Aging in Humans: Translational Physiology *Gary Pierce, Ph.D., University of Iowa*
3 p.m.	**Research Presentation** Autophagy and the Metabolic Phenotype of Skeletal Muscle *Vitor Lira, Ph.D., University of Iowa*
3:30 p.m.	**Teaching Presentation** Outcomes of a Research-Based Approach to Undergraduate Human Physiology Laboratory *Jackie Brittingham, Ph.D., Simpson College*
3:45 p.m.	**Teaching Presentation** Repeated Testing Increases Lab Performance and Preparedness *Justin Brown, Ph.D., Simpson College*
4 p.m.	**Keynote Address in Physiology Education** How to Get Your Students Attention Without Wearing a Clown Suit *Bryon Wiegand, Ph.D., University of Missouri*
5 p.m.	**Poster Award Presentations and Closing Remarks**
5:15 – 6 p.m.	**IPS Board Meeting**

Welcome

Welcome to Des Moines University for the *17th Annual Iowa Physiological Society Meeting*! The mission of our society is to unite physiologists in networking and advancing physiology throughout the State of Iowa. As such, the IPS is devoted to fostering education, scientific research, and dissemination of information in the physiological sciences across the State and region. This year's meeting will feature talks and discussions covering a range of physiological topics from the molecular to the organismal and include basic and applied research.

The keynote address in Physiological Research will be delivered by **Bernard Jasmin, Ph.D.** from the University of Ottawa. The APS is also sponsoring **T. Richard Nichols, Ph.D.** from the Georgia Institute of Technology who will lead a discussion regarding research advocacy. The Harman Endowment has sponsored **Bryon Wiegand, Ph.D.** from the University of Missouri to deliver our keynote address in Physiology Education. The meeting will also feature a number of additional speakers from across the state, some selected from submitted abstracts. These talks will include an astounding breadth of Physiology as well as emphasize novel findings and thoughts regarding instruction. Finally, the program is completed by a poster session featuring work done by promising graduate and undergraduate students, post docs and faculty members.

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

Sincerely,

Joshua Selsby, Ph.D.
President, Iowa Physiological Society

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Meeting Objectives

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

1. Identify physiological changes caused by dystrophin deficiency.
2. Engage in a meaningful discussion regarding the importance of animals for research.
3. Describe cardiovascular function in health and disease.

Iowa Chapter of the American Physiological Society Board

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Exhibitors

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Guest Speakers

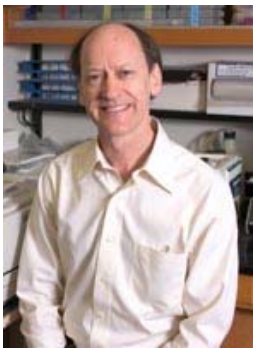
Bernard J. Jasmin, Ph.D.
Professor and Vice-Dean of Research, University of Ottawa

Dr. Jasmin obtained his Ph.D. in 1988 from the Université de Montréal. Following postdoctoral work in Paris, France, and at the University of Miami School of Medicine, Dr. Jasmin was recruited as an Assistant Professor in 1992 by the Department of Physiology at the University of Ottawa. He quickly moved through the ranks and was promoted to Full Professor in 2000. In 2002, he became Chair of the Department of Cellular and Molecular Medicine (CMM). His vision in this role was to enhance the quality and stability of academic teaching programs, while also increasing the research intensity of the Department. In the Spring of 2009, he accepted the position of Vice-Dean, Research, in the Faculty of Medicine, and he now provides direction to broad-based and interdisciplinary strategic research initiatives while also optimizing resource allocations.

While assuming varied administrative responsibilities and being recognized as an excellent teacher, Dr. Jasmin has been able to maintain a vigorous research program that focuses mainly on deciphering the regulatory cascades and signalling pathways involved in controlling expression of synaptic proteins in both neuronal and skeletal muscle cells. In this work, the emphasis is placed on studying questions that are relevant for our understanding of the physiopathology and eventual treatment of various neuromuscular and neurological diseases and conditions such as Duchenne muscular dystrophy, myotonic dystrophy, skeletal muscle atrophy and axonal regeneration.

Over the years, the excellence of Dr. Jasmin's research has been recognized by invitations to present at prestigious scientific meetings and institutions, and by several awards including a Scholarship Award from the Medical Research Council of Canada in 1994, a Young Investigator Award from the University of Ottawa in 1997, an Investigator Award from the Canadian Institutes of Health Research in 1999 and, more recently, a Scientist of the Year Award from the Ontario Chapter of Muscular Dystrophy Canada and a Quality of Life Award from the Canadian Institutes of Health Research via its Institute of Musculoskeletal Health and Arthritis.

T. Richard Nichols, Ph.D.
Professor, Chair of Applied Physiology at Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology



Nichols received a Ph.D. in Physiology from Harvard University and did postdoctoral work in motor systems research at the University of Alberta. His first faculty position was the in the Department of Kinesiology at the University of Washington. He then accepted a faculty position in the Department of Physiology at Emory University in 1983 and rose through the ranks to Professor and Interim Chair. He then took the position of Chair of the School of Applied Physiology at Georgia Tech in 2007. His research concerns the manner in which the musculoskeletal system and spinal cord interact to contribute to the control of balance and locomotion, and currently features investigations of the functions of force feedback from Golgi tendon organs. The research is also being applied to the understanding of the consequences of peripheral nerve injury, spinal cord injury and tendon transfer for the organization of spinal circuits and for motor coordination.

Bryon Wiegand, Ph.D.
Associate Professor, Animal Sciences, University of Missouri

Bryon Wiegand is Associate Professor of Animal Science at the University of Missouri, specializing in meat science. He received his B.S. degree in Animal Science from the University of Missouri, the M.S. degree in Animal Breeding from Auburn University, and the Ph.D. degree in Animal Science from Iowa State University. He joined the Illinois State Animal Science faculty in 2000, where he had both research and teaching responsibilities. He taught seven upper level undergraduate and two graduate level courses in the food animal and research methods curriculum. In 2007, he joined the faculty at the University of Missouri where he teaches Live Animal and Meat Evaluation and Physiology and Biochemistry of Muscle. He also Co-teaches Introductory Animal Science Lab Practicum and Meat Investigations, a graduate laboratory methods course.

Dr. Wiegand's research efforts are focused on nutritional interventions to improve meat quality. His specific area of expertise is the lipid profile of fresh meat products. He has garnered $1.1 million in research funding support since 2000. He has published 25 refereed journal articles, 58 abstracts, 13 extension publications, one book chapter, and one meat science lab textbook. He has mentored 26 graduate students. Bryon supervises the University of Missouri Meat Sciences Processing Facility, serves as Block and Bridle Club Faculty Advisor, and is the Technical Advisor to the Missouri Association of Meat Processors.

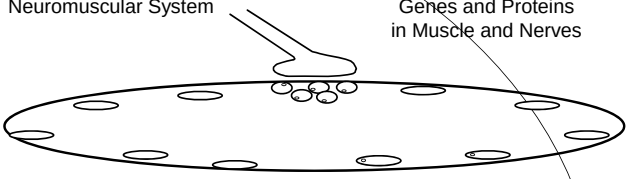
Dr. Wiegand has served as a member of AMSA on the Graduate Research Poster Competition Committee and was nominated for the Early Career Achievement Award in 2008 and 2009. Wiegand has served multiple years on the Midwest ASAS Academic Quadrathlon Committee and on the Meat Science and Muscle Biology Committee. He chaired Meat Science and Muscle Biology in 2004. Bryon is in his third year as the Secretary and Program Chair of the Midwest Section of the American Society of Animal Science and his second, three year term as a member of the Journal of Animal Science Editorial Board. Dr. Wiegand has been honored with six teaching awards at the collegiate level. The most current of these awards being the Outstanding Teacher Award given by the Midwest Section of ASAS and the University of Missouri Provost's Outstanding Teaching Award.

Guest Speaker Presentations

The Neuromuscular Junction

Plasticity of the Neuromuscular System

Regulation of Synaptic Genes and Proteins in Muscle and Nerves



Duchenne Muscular Dystrophy
Myotonic Dystrophy Type 1
Skeletal Muscle Atrophy

Rationally Designing Novel Therapeutics for Duchenne Muscular Dystrophy:

From Basic Science Discoveries to Pre-clinical and Clinical Studies

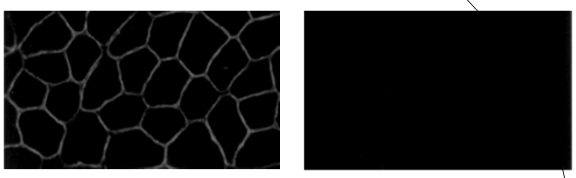
Duchenne Muscular Dystrophy

- Prevalent and severe X-linked disease affecting approximately 1 in ~ 3,500 males
- Defect caused by mutations/deletions in the DMD gene called dystrophin
- Progressive muscle loss due to exhaustive cycles of degeneration and regeneration
- Death in 2nd or 3rd decade of life due to respiratory or cardiac failure

Mutations/Deletions Result in Absence of Functional Dystrophin at the Sarcolemma

Normal

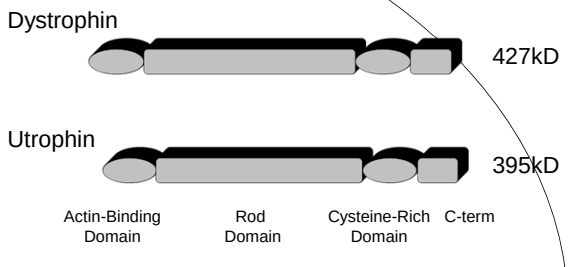
DMD



Potential Treatments for Duchenne Muscular Dystrophy

- Pharmacological Interventions (GC)
- Dystrophin Gene Therapy
- Myoblast & Stem Cell Therapy
- Up-Regulation of Related Genes

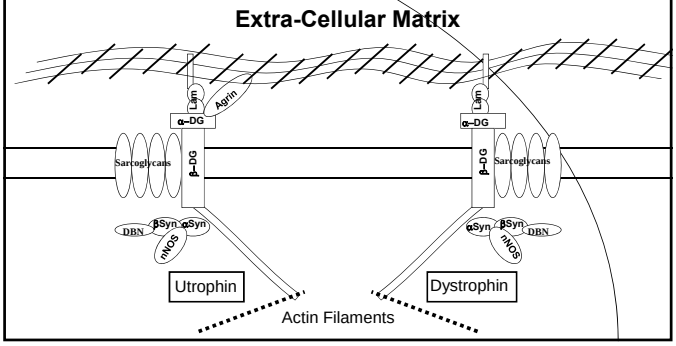
Utrophin: A Good Candidate



Characteristics of Utrophin

- 1 Mb gene located on chromosome 6q24
- High sequence identity with dystrophin
- Large cytoskeletal protein (395 kDa)
- Ubiquitously distributed

Dystrophin/Utrophin-Associated Proteins



Up-Regulation of Utrophin All Along the Sarcolemma as a Therapy for Duchenne Muscular Dystrophy

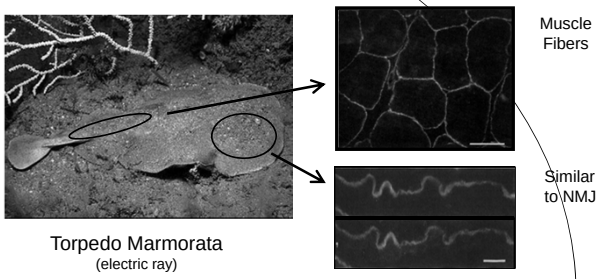
Identify the Molecular Mechanisms Regulating Utrophin A to Increase Its Expression via Pharmacological Intervention

Models to Study the Mechanisms Regulating Utrophin A Expression

- Neuromuscular Junction
- Fast versus Slow Muscles

Basic Science Discovery 1:

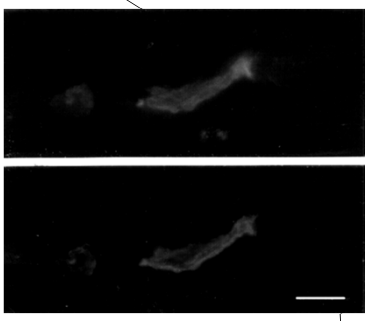
Utrophin A is a Synaptic Isoform of Dystrophin



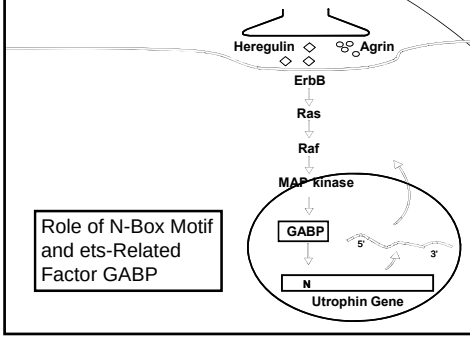
Torpedo Marmorata
(electric ray)

Synaptic Localization of Utrophin A

Role in the Complete Differentiation of the Postsynaptic Membrane



Transcriptional Control of Utrophin A by Nerve -Derived Factors



Role of N-Box Motif and ets-Related Factor GABP

AChR Genes
AChE

Models to Study the Mechanisms Regulating Utrophin A Expression

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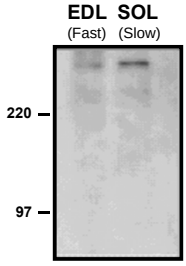
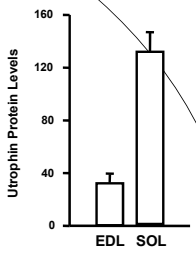
- Fast versus Slow Muscles

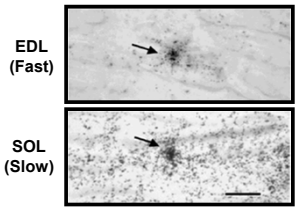
Basic Science Discovery 2:

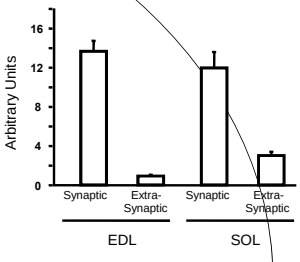
Utrophin A Levels in Fast versus Slow Muscles





Extrasynaptic Utrophin A mRNA in Fast vs Slow Muscles





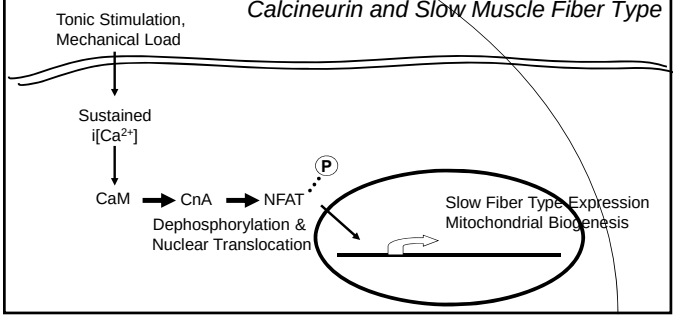
Clinical Implications

Fast Fibers are Preferentially Affected in DMD

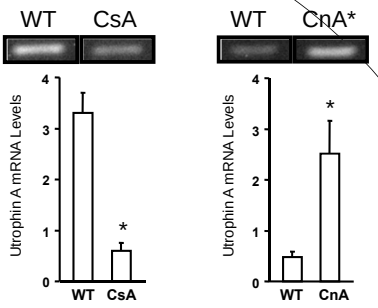
Without Utrophin A, DMD Would Be More Severe

Factors Involved in the Differential Expression Between Fast and Slow Muscles?

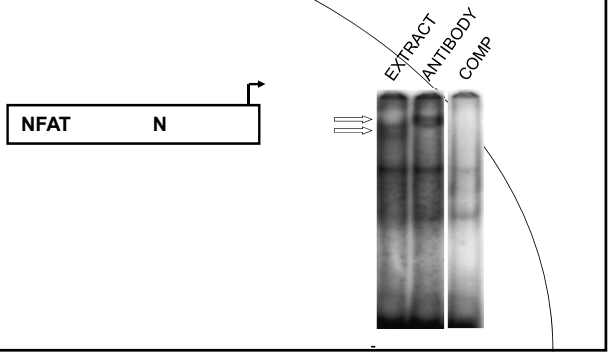
Calcineurin and Slow Muscle Fiber Type



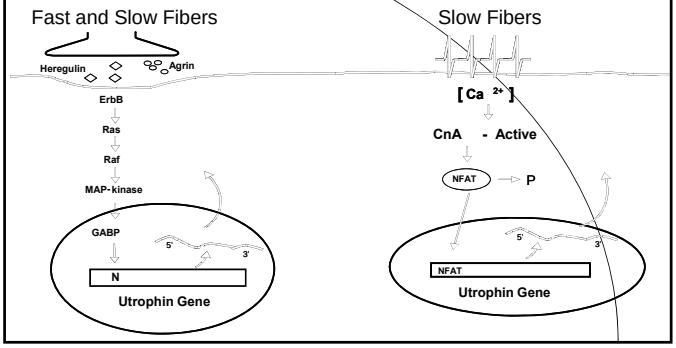
Calcineurin Activity Regulates Utrophin A Expression



NFAT Binding Activity in Promoter A

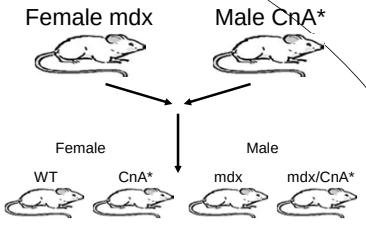


Calcineurin and Utrophin A Regulation



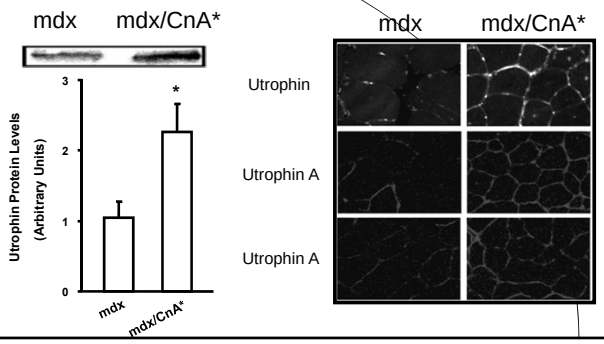
Stimulation of Calcineurin Activity in Dystrophic Muscle Fibers Should Be Beneficial Due to Increased Expression of Utrophin A

Generation of mdx - CnA* Mice
(Proof of Principle)



PCR for MCK-CnA* transgene
and
Immunolabeling for Dystrophin

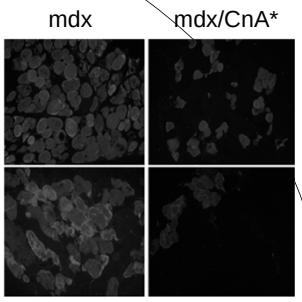
Increased Utrophin A Expression in mdx/CnA* Muscles



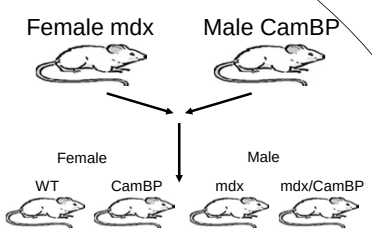
Improved Membrane Integrity in mdx/CnA* Muscles

Evans Blue Uptake and Staining

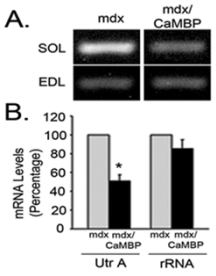
(Improvement Also Seen With Several Other Markers)



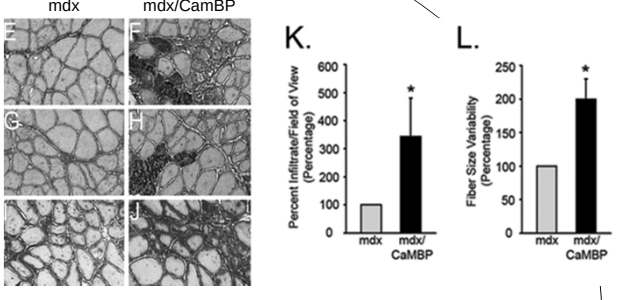
Generation of mdx/CamBP Mice



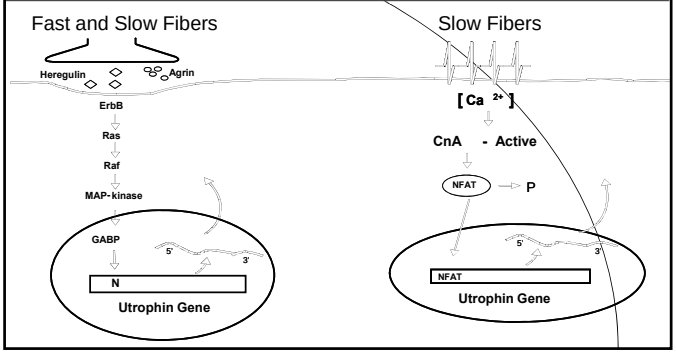
Decreased Utrophin A mRNA Expression in Slow Muscles



Increased Pathology in Slow Muscle



Calcineurin and Utrophin A Regulation



Clinical Implications for DMD

- Use of immunosupressants that inhibit calcineurin activity, i.e. CsA and FK506
- Fast fibers are preferentially affected in DMD
- Specific target for pharmacological manipulation
- Mechanism for beneficial effects of glucocorticoids

Role of the Co-Activator PGC-1α

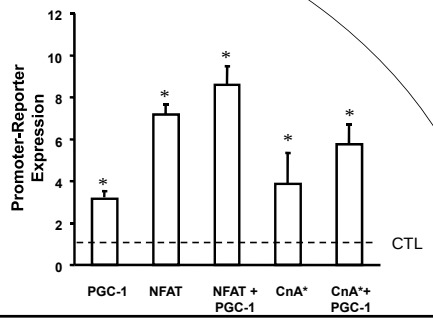
(Peroxisome Proliferator Activated Receptor γ Coactivator 1α)

Master Regulator of Mitochondrial Biogenesis

Stimulates Expression of Oxidative Enzymes

Involved in Slow Muscle Fiber Transition

Additive Effects of CnA - NFAT + PGC-1α on Utrophin A Promoter Activity



GABP (NRF2) + Calcineurin/NFAT + PGC-1α

↓

Co-Regulation of Utrophin A Gene Expression with Genes Encoding the Slow Oxidative Muscle Phenotype

↓

Promotion of the Slow Myogenic Phenotype as a Therapeutic Approach to DMD?

Increasing Amount of Slow Muscle as a Therapy for DMD

↓

Potential Role of "*Exercise Mimetics*"

Compounds that Induce Phenotypic Plasticity Toward the Slow, Oxidative Myogenic Program

Potential Role of "*Exercise Mimetics*"

Compounds that Induce Phenotypic Plasticity Toward the Slow, Oxidative Myogenic Program

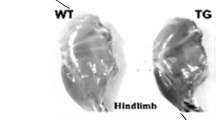
AGONISTS

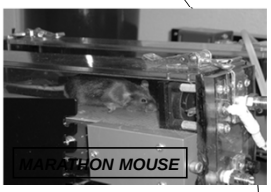
PPARδ/β **AMPK**

PPARδ/β and Skeletal Muscle

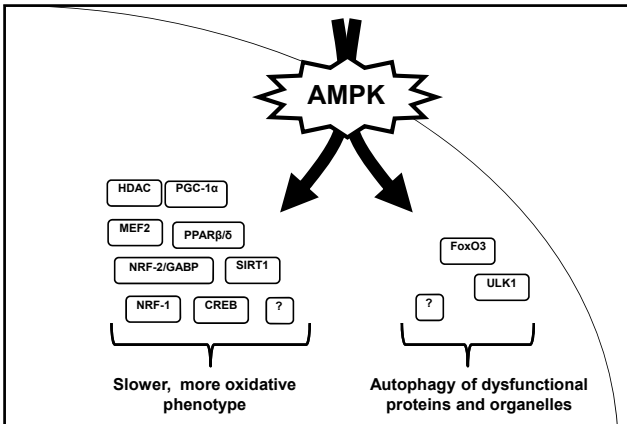
PPARδ/β is Highly Expressed in Muscle

Overexpression Promotes Slow Oxidative Myofiber Program



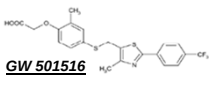


(Grimaldi, 2003 – Evans, 2004)



From Target Identification to Testing Active Molecules in Pre-Clinical Studies

1)



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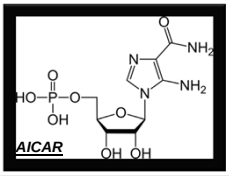
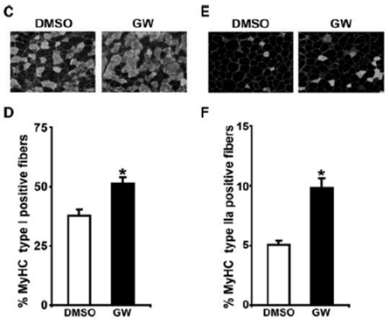


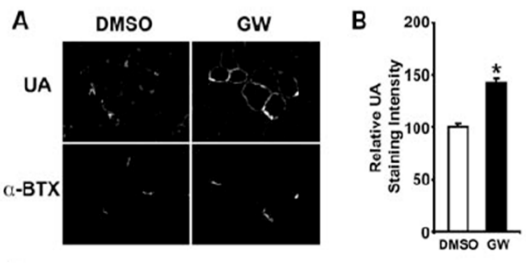
AICAR

+

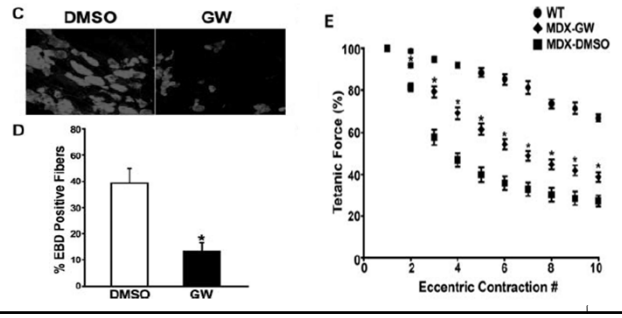
1) Activation of PPARδ/β with GW Increases Expression of Slower MyHC in mdx Mice



1) Increased Expression of Utrophin A in GW-treated mdx Mice



1) Increased Sarcolemmal Stability in GW-treated mdx Mice



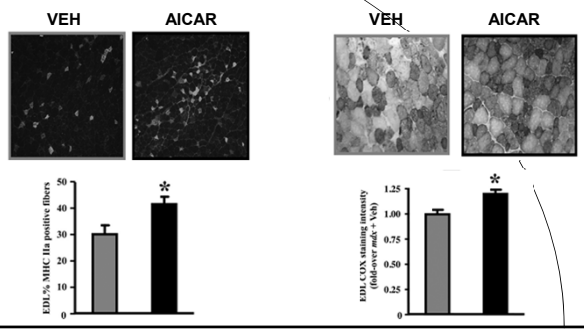
Some Parameters Not Changed

Central Nucleation
Fibrosis

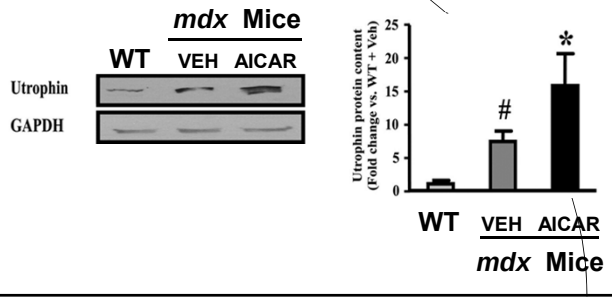
↓

Age for Treatment Initiation !

2) Activation of AMPK with AICAR Stimulates the Slow, Oxidative Phenotype in mdx Mice



2) Increased Expression of Utrophin A in AICAR-treated mdx Mice



From Basic Science Discoveries to Pre-clinical/Clinical Studies

↓

Increasing Amount of Slow Muscle as a Therapy for DMD

↓

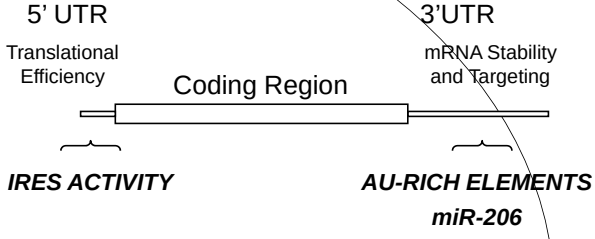
Role of "*Exercise Mimetics*"

Other Possible Targets for Utrophin Up-Regulation

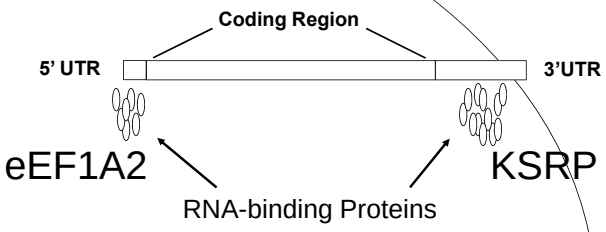
Everything is Not Only Transcriptional !!!

Identification of Additional Key Regulatory Steps: From Post-Transcriptional Mechanisms to Translational Control

Basic Science Discovery 3:
Post-transcriptional Regulation of Utrophin A

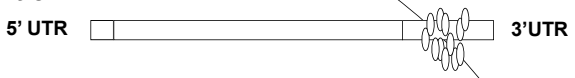


RNA-protein Interactions

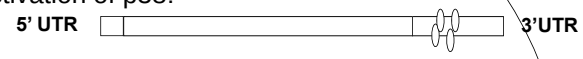


p38 Regulates the Interaction Between Utrophin A mRNA and KSRP

Control:



Activation of p38:



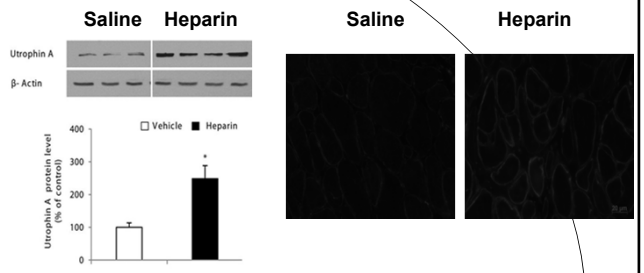
1 - Increased Sequestration of KSRP with 14-3-3

2 - Decreased Expression of KSRP

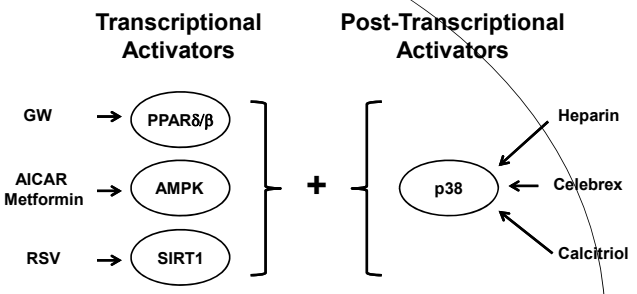
↓KSRP

↑*mRNA Stability*

p38 Activation Increases Utrophin A in mdx Mice

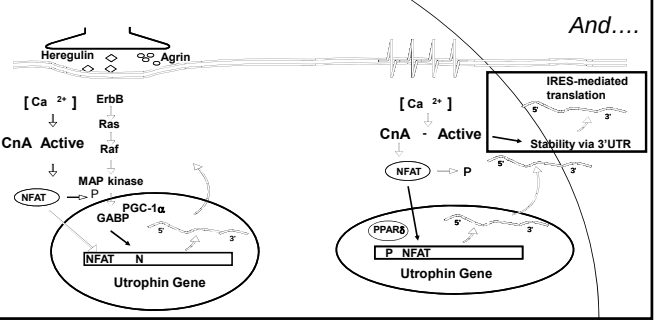


Clinical Trials & Combinatorial Therapy



Multiple Pathways Regulate Utrophin A in Skeletal Muscle Fibers

And….



From Basic Science Discoveries to Pre-clinical and Clinical Studies With Re-purposed Molecules and Drugs

Acknowledgements

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Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

MDA Muscular Dystrophy Association
Fighting Muscle Disease

The Rachel Fund for Myotonic Dystrophy

AFM
Association Française contre les Myopathies

CSA ASC

Muscular Dystrophy Canada
let's make muscles move

Jesse's Journey

The American Physiological Society

Advocacy for Physiological Research

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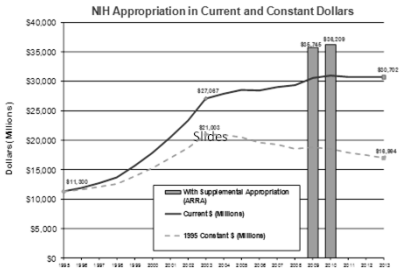
Why be a science advocate?

- NIH's buying power has been eroding for a decade because the budget has been flat in the face of inflation – and now Congress is trying to tackle the deficit
- Meanwhile, animal rights groups have stepped up efforts to undermine the support of Congress and the public for animal research
- The public can help only if it understands the importance of biomedical research.

Research Funding Advocacy

NIH funding



Slide courtesy of FASEB Office of Public Affairs

Research funding objective

- Our research enterprise needs predictable, sustainable funding growth
  - Although competition promotes creativity, boom and bust cycles of funding drive talented people out of the system and waste our prior investments

Research funding misconceptions

1. "All the money is spent in Bethesda"
2. "Our tax dollars are being wasted on useless projects"
3. "Even if the federal government doesn't fund the research, industry will"

Be prepared to challenge statements like these!

The spectrum of basic to translational research

- Basic research lays the groundwork for solving larger problems
  - NIH includes fundamental research as an important goal for funding
- Breakthroughs often come in surprising ways
- Example: the discovery of pattern-generating networks in the spinal cord.

For more on the process of research, see:

➢**FASEB's *Breakthroughs in Bioscience***
http://www.faseb.org/Policy-and-Government-Affairs/Publications/Breakthroughs-and-Horizons-in-Bioscience.aspx

➢***Science Fortune: How unpredictable research advances have saved millions of lives***
http://www.faseb.org/portals/0/pdfs/opa/ScienceFortuneBrochure.pdf

Integrating the Life Sciences from Molecule to Organism

Biomedical research should be a national priority

- To provide patients with hope for better outcomes
- To generate economic activity in communities across the U.S.
- To fuel economic competitiveness through innovation, including biologically inspired design.

Integrating the Life Sciences from Molecule to Organism

Stability

Our research enterprise needs funding growth that is:

- Predictable
- Sustainable
  - Boom and bust cycles of funding drive talented people out of the system and waste prior investment

Integrating the Life Sciences from Molecule to Organism

Animal Research Advocacy

Integrating the Life Sciences from Molecule to Organism

Key Points

- Researchers use alternatives as much as possible
- Animal studies are essential to curing diseases affecting humans and animals, and understanding the impacts of environmental changes.
- Animal research is heavily regulated, and scientists are committed to treating animals humanely

Integrating the Life Sciences from Molecule to Organism

Misconceptions

1. "We don't need animal research"
2. "Research is animal abuse"
3. "No one looks out for the animals"
4. "Treatments developed in animals don't work on people"

Animal research remains necessary

- Experimental research and modeling at multiple levels of organization are essential to understand homeostasis, adaptation, and behavior, which in turn are necessary to understand the response of organisms to injury, disease and changes in the environment.
- Research is much more than "animal testing"

Animal research remains necessary

- Animal research is an integral part of the search for cures.
- We have to understand basic biology, *including systems physiology*, to learn how to treat/cure disease
- Animals are used along with computer models, cell cultures and human studies
- Comparative studies inspire engineering design.

Animal research is humane

- People want the benefits of biomedical research, but they will support research *if and only if* they are confident that animals are treated humanely
- Accusations of mistreatment undermine public confidence

Researchers care about animal welfare

- An animal care and use committee at each institution rigorously reviews all proposed studies
- Veterinarians help design protocols
- Animals get care from skilled technicians and veterinarians

Researchers care about animal welfare

- Most research is not painful
- If an animal is in pain, pain medication must be given unless this would interfere with the research
- Studies must have defined endpoints. In an animal is suffering, it will be removed from the study or euthanized

Public support hangs in the balance

- The mistreatment of animals undermines public confidence in research
- The impact is doubly damning when we are not forthright about what happened
  - If the claim is false, explain why
  - If it is true, explain what happened
  - If it was at your facility, make sure something is done about it

"Treatments developed in animals don't work on people"

- There are many similarities in how cells and organs work in warm-blooded animals
  - For that reason, many of the same drugs (antibiotics, pain relievers, etc.) are prescribed to humans and for animals
  - If a species has a different response to a drug, that can provide clues about how the drug works
- Animal species respond in similar ways to injury and to subsequent rehabilitation.

Be a research advocate

- Be a game changer: Tell people what you do!
  - The role that animals play in research
  - Why they are needed
  - The protections in place to ensure that they are treated humanely

Advocacy Guides

- Animal Research Cures (APS) http://www.animalresearchcures.org/Advocacy.htm
- Americans for Medical Progress http://www.amprogress.org/advocacy

What is our goal?

- Don't expect others to see the world your way
- Seek common ground on a few key points
  - Animals studies remain essential to the search for cures
  - Animal studies are done selectively when no alternative is available

Actions you can take

Contact Congress

- Arrange to meet with your Representatives and Senators. Contact information is available on their websites.
- Respond to legislative alerts
  - Your representatives won't know you care about issues unless you tell them!
  - Easy to do—sample message is there
  - Greater impact if you personalize it
  - Share alerts with your colleagues

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Prepare what you will say

- APS has general talking points that address research funding and animals in research
  - See http://www.the-aps.org/mm/SciencePolicy/Advocacy/Advocacy-Resources/Talking-Points.htm
- Ask APS whether there is any pending legislation you should support or oppose
  - Contact SciencePolicy@the-aps.org

Integrating the Life Sciences from Molecule to Organism

Reach out to your local community

- Speakers' bureaus
  - Schools
  - Civic associations
- Invite the public to the university
  - public lectures
  - Laboratory tours
  - Research experiences for high school students and teachers

Integrating the Life Sciences from Molecule to Organism

Encourage the next generation

- Go to career day at a local school
- Participate in APS's PhUn Week

http://www.the-aps.org/mm/Education/K-12/EducationProjects/PhUn-Week

Integrating the Life Sciences from Molecule to Organism

Encourage the next generation

- Lesson plans from the Foundation for Biomedical Research http://www.fbresearch.org/TwoColumnWireframe.aspx?pageid=187
- Discussion-based classroom activity from Speaking Honestly–Animal Research Education (SHARE) http://sharehappens.org/

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Speak up for science

- Send a letter to the editor or comment online if an article misrepresents research
- Attend a Science Café event – or organize one http://www.sciencecafes.org

Integrating the Life Sciences from Molecule to Organism

The American Physiological Society

Want more information?

Visit the APS Science Policy Website
http://www.the-aps.org/mm/SciencePolicy
and sign up for the APS Science Policy News
sciencepolicy@the-aps.org

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Philosophical presuppositions of the antivivisection movement

- Activism and medicine 100 years ago
- Vance RP (1992) An introduction to the philosophical presuppositions of the animal liberation/rights movement. JAMA 268: 1715 – 1719.

Integrating the Life Sciences from Molecule to Organism

Current events

- Negociationisover.com
- Lynn Fairbanks, J. David Jentsch, Dario Ringach
- Advance Directive: Researchsaves.org
- PETAkillsanimals.com
- Animal Enterprise Terrorism Act

Integrating the Life Sciences from Molecule to Organism

Research Presentations

Altered DNA Repair in Ovaries of Obese Mice Following Chronic Exposure to 7,12-Dimethylbenz[a]anthracene

Ganesan, S*., Nteeba, J., Keating, A.F.

Department of Animal Science, Iowa State University, Ames, IA

Increased incidence of miscarriage and birth defects in offspring are observed in obese women. Exposure to 7,12-dimethylbenz[a]anthracene (DMBA) can destroy or damage oocytes. Double-strand DNA breaks (DSB) are a DMBA-induced cytotoxic lesion in extra-ovarian tissues. Ataxia telangiectasia mutated (ATM) is a phosphatidylinositol-3 kinase (PI3K) family member that initiates the DNA repair response (DRR). Since DMBA can induce DNA damage, and PI3K signaling is critical for the DRR, we hypothesized that DMBA-induced DNA damage response would be altered in the ovaries of obese mice. Wild type (lean) non agouti *(a/a)* and KK.Cg-A^y/J heterozygote (obese) mice (18 weeks of age) were dosed with sesame oil or DMBA (1mg/kg) for 14 days and mice were sacrificed 3 days thereafter. qRT-PCR was used to measure mRNA levels of *Atm, Xrcc6, BrcaI, Rad 51, Parp1 and Prkdc.* There were lower ($P < 0.05$) basal ovarian levels of *Atm, Xrcc6, and Brca1* and greater ($P < 0.05$) levels of *Parp1*, in obese mice relative to lean controls. In lean mice, DMBA decreased ($P < 0.05$) *Atm* mRNA levels, but increased ($P < 0.05$) *Xrcc1, Rad51, Brca1, Parp1* and *Prkdc* levels, relative to vehicle controls. Obesity partially blunted the DMBA-induced increased ($P < 0.05$) *Rad51* mRNA, increased ($P < 0.05$) *Atm* and *Brca1* mRNA and caused a greater ($P < 0.05$) response in *Prkdc* and *Parp1* mRNA expression, compared to lean mice treated with DMBA. Thus, DMBA exposure induces the ovarian DRR, which is altered during obesity.

Supported by ES016818 to AFK and AAUW fellowship to SG.

Alpha-Dystroglycan Glycosylation in Cancer

Daniel Beltrán Valero de Bernabé*, Tobias Willer, Kevin P. Campbell

Howard Hughes Medical Institute, Department of Molecular Physiology and Biophysics, Department of Neurology and Internal Medicine, University of Iowa, Roy J. and Lucille A. Carver College of Medicine, Iowa City, IA

Alpha-dystroglycan mediated cell anchorage to the extracellular matrix has been shown to be compromised in several types of cancer, including pediatric cancers, glioblastoma, and breast, prostate, ovarian and lung cancers. The alpha-dystroglycan receptor function is mediated by a laminin binding sugar moiety which is O-mannosyl linked. Post-translational modification of alpha-dystroglycan is carried out by a battery of known and putative glycosyltransferases that include POMT1/2, POMGNT1/2, LARGE1/2, FKTN, FKRP, B3GNT1, POMK, B3GAINT2, ISPD and TMEM5. LARGE1 is as bifunctional glycosyltransferase with both xylosyltransferase (Xyl-T) and glucuronyltransferase (GlcA-T) activities synthesizing a novel heteropolysaccharide structure on alpha-dystroglycan which is essential for dystroglycan receptor function. We recently demonstrated that dystroglycan functional glycosylation and binding to its main ligand laminin is lost in epithelial derived cancer cell lines as a result of the silencing of LARGE1. To investigate if other genes may underlie alpha-dystroglycan hypoglycosylation in cancer we analyzed the alpha-dystroglycan glycosylation status and the expression levels of all known dystroglycan modifying glycosyltransferases in a large collection of commercially available cancer cell lines. We discovered a number of cancer cell lines that express low levels of LARGE1 but present a functionally glycosylated alpha-dystroglycan. Analyzing the glycosyltransferase expression profile of these cells we detected high expression levels of LARGE2, a LARGE1 paralog. This data suggests that LARGE2 can compensate for the loss of LARGE1 expression and that the expression of both genes should be silenced for alpha-dystroglycan hypoglycosylation to occur. Additionally, we have identified cancer derived cell lines that expressing high levels of LARGE1/2 present a non-functional alpha-dystroglycan form, indicating that there are additional genes and/or factors involved in cancer-associated dystroglycan loss of function. We provide genetic and biochemical evidence to define the genetic mechanisms involved in alpha-dystroglycan hypoglycosylation during cancer progression.

The G Protein-Coupled Estrogen Receptor 1 in Ca2+/Calmodulin-Dependent Signaling in the Vasculature

Kim Tran, M.D., Ph.D.

Des Moines University, Des Moines, IA

The novel G protein-coupled estrogen receptor 1 (GPER/GPR30) has increasing been identified as an important modulator of estrogen-mediated effects. Data presented will include a novel approach to identify calmodulin-binding domains in GPER/GPR30 and its roles in signaling via Ca2+ and calmodulin in the vasculature.

Autonomic and Angiotensinergic Mechanisms in Muscular Dystrophy and Associated Cardiomyopathy

Rasna Sabharwal

Department of Internal Medicine, University of Iowa, Iowa City, IA

Sarcoglycan-δ (Sgcd) is a subunit of dystrophin glycoprotein complex that is involved in maintaining integrity of sarcolemma during muscle contraction. Mutations in Sgcd cause muscular dystrophy characterized by muscle weakness, atrophy and dilated cardiomyopathy in animals and humans. Renin angiotensin system (RAS) is involved in maintaining cardiovascular homeostasis and comprises of two axis - Angiotensin II (AngII) acting *via* type 1 receptors and Ang(1-7) acting *via* Mas receptors. We hypothesized that activation of RAS contributes to skeletal muscle and autonomic dysfunction in Sgcd deficient (Sgcd-/-) mice at a young age before development of left ventricular (LV) dysfunction. LV function (echocardiography); blood pressure (BP), heart rate (HR), locomotor activity, and indices of autonomic function (radiotelemetry) were measured in conscious mice. Young Sgcd-/- mice exhibited increased fibrosis and oxidative stress in skeletal muscle, decreased locomotor activity, increased AngII/AT_1R and reduced Ang(1-7)/Mas expression, and severe autonomic dysfunction; but normal LV function. Autonomic function continued to deteriorate in Sgcd-/- mice with age and was accompanied by LV dysfunction at older ages. Subgroups of control and Sgcd-/- mice were treated with Ang(1-7) chronically for 8-10 wks. Ang(1-7) decreased oxidative stress and fibrosis in skeletal muscle, increased locomotor activity, prevented autonomic dysfunction and restored AngII/AT_1R vs. Ang(1-7)/Mas balance in young Sgcd-/- mice.

Conclusions: In muscular dystrophy (i) AngII-induced skeletal muscle and autonomic dysfunction is evident at a young age; (ii) autonomic dysfunction worsens with age; (iii) correcting the early autonomic dysfunction by Ang(1-7) or enhancing its endogenous production may provide a novel therapeutic approach.

Targeting Inflammation for Arterial Aging in Humans: Translational Physiology

Gary L. Pierce, Ph.D.

University of Iowa, Iowa City, IA

Cardiovascular disease (CVD) remains a major cause of morbidity and mortality in the U.S. of which older age is a primary risk factor. The increased risk of developing CVD with advancing age in humans has been attributed in large part to two fundamental alterations in arterial structure and function associated with advancing age: 1) impaired vascular endothelial function of conduit and resistance arteries, and; 2) stiffening of the large elastic thoracic central arteries (e.g., aorta, carotid). The mechanisms responsible for these physiological changes with aging are not completely understood, but strong epidemiological and experimental evidence suggests that inflammation and oxidative stress may be a common mechanistic link contributing to both. Recent evidence suggests that chronic activation of nuclear factor kappa B (NFκB), a key proinflammatory transcription factor that regulates expression of

hundreds of inflammatory genes, contributes to arterial endothelial dysfunction with aging in part related through the development of vascular oxidative stress. We will advance the idea that arterial NFκB may be a novel therapeutic target in the treatment of endothelial dysfunction with aging in humans. We will describe recent translational evidence in humans and rodents that select pharmacological (e.g., salsalate) and lifestyle (e.g., habitual aerobic exercise) strategies prevent or reverse arterial aging in part through inhibition of endothelial NFκB signaling and suppression of oxidative stress.

Autophagy and the Metabolic Phenotype of Skeletal Muscle

Vitor Lira, Ph.D.

Department of Health and Human Physiology, Obesity Research and Education Initiative, Fraternity Order of Eagles Diabetes Research Center, The University of Iowa

Macroautophagy, hereafter referred to as autophagy, is a catabolic process required for the degradation of long-lived proteins and protein aggregates as well as dysfunctional organelles (e.g., mitochondria). A robust induction of autophagy in response to energy stress has been documented; however, very little is known about the regulation and functional role of basal autophagy levels in skeletal muscle phenotype determination. Using both physiological and genetic approaches in vivo (i.e. exercise training and muscle-specific PGC-1α transgenic mice, respectively) we have observed that basal autophagy is regulated in parallel to mitochondrial content. To ascertain the functional role of basal autophagy in muscle adaptation to training we studied heterozygous mice for the critical autophagy protein Atg6/Beclin1 (Atg6 het). Despite having normal basal autophagy when sedentary, Atg6 het mice exhibited an attenuated increase in basal autophagy when exercise trained along with a blunted increase in mitochondrial content and capillary density in skeletal muscle. In addition, Atg6 het mice were unable to improve endurance performance with training. Our results reveal that basal autophagy levels are higher in oxidative versus glycolytic muscles and that exercise training-induced elevation of basal autophagy is required for normal oxidative adaptations in muscle.

Outcomes of a Research-Based Approach to Undergraduate Human Physiology Laboratory

Jackie Brittingham, Ph.D.

Professor of Biology, Simpson College, Indianola, IA

Simpson College initiated an innovative human physiology laboratory experience in 2009, leading efforts to transform the undergraduate biology curriculum to meet the needs of the next generation of biomedical researchers. The BIOL225 Human Physiology laboratory experience equips students to collaborate in small research teams on open-ended, investigative projects in order to enhance their understanding of key concepts, their critical thinking skills and their practice of the scientific method, while gaining valuable hands-on familiarity with essential equipment. This project was designed to evaluate the research-based approach to teaching an undergraduate human physiology laboratory. Analysis of several direct and indirect measures was performed to measure the impacts on student outcomes. A test of data analysis and interpretation skills was administered before and after the course (pre vs. posttest) and correlated to student grades in the course. Rubrics were designed to directly measure student performance on specific components of the scientific method including hypothesis development, experimental design (including protocols for the use of human subjects) data collection and analysis as well as written and oral communication of results. Additionally, formal and informal feedback was compiled to determine student perceptions and attitudes related to their experience in this laboratory course. Assessment of these measures will serve to inform further curriculum reform in the department and serve as a model for other programs seeking similar transformation in undergraduate science laboratories.

Repeated Testing Increases Lab Performance and Preparedness

Justin Brown, Ph.D.

Simpson College, Indianola, IA

To effectively participate in laboratory courses, students must be able to apply both new and previously learned course materials, such as vocabulary research techniques. Therefore, students must retain information over time and common study habits such as cramming can greatly reduce laboratory performance. To better understand practices that promote knowledge retention, previous studies have compared the effectiveness of repeated testing to the effectiveness of repeated studying. These reports suggest that repeated testing (even in the absence of studying) promotes greater retention than repeated review. However, few studies have translated this finding from controlled laboratory settings to the classroom. This study aimed to address repeated testing in the context of a laboratory class. 36 students in a human anatomy class were quizzed weekly. Quiz questions were randomly drawn from a question bank that included material from that day's lab and all previous labs. Subjectively, lab preparedness was greatly increased compared to students who completed standard pre-lab reports. 100% of students reported that repeated testing was helpful and the most consistent feedback was that students requested a greater number of quizzes that cover more topics. Overall, findings suggest that low-stakes repeated testing could improve laboratory courses.

Poster Abstracts

1 (G)

Decreased Arrhythmic Burden Following Exercise in Associated with a Decrease in Cx43 Phosphorylation Specifically at Serines 255 and 279/282

Jon G. Senkler*[1], Erica G. Thomas[1], Rachel M. Firkins[1], Matthew K. Henry[1], Julia A. Moffitt[2]

[1] *Department of Physiology and Pharmacology, Des Moines University, Des Moines, IA*
[2] *Department of Kinesiology, Saint Ambrose University, Davenport, IA*

Cardiovascular diseases, the leading cause of death for the last decade, lead to serious cardiac arrhythmias which can be caused by gap junction dysfunction. Gap junction proteins (GJP) are located between cardiac myocytes and allow for coordinated propagation of electrical signals. Changes in left ventricular expression and phosphorylation of Connexin 43 (Cx43) may be a primary mechanism responsible for altering the propagation of cardiac action potentials and lead to cardiac arrhythmias. Exercise training (ET) has well-documented antiarrhythmic benefits. Several studies have demonstrated alterations in Cx43 expression and phosphorylation following chronic exercise training. In the current study, we examined the relationship between Cx43 phosphorylation and the arrhythmic response to acute myocardial ischemia, and determined if chronic exercise training modulated this effect. Rats underwent 8 weeks of ET through spontaneous wheel running or served as sedentary controls. Under Inactin anesthesia, rats were subjected to 20 minutes of myocardial ischemia through ligation of the left coronary artery and electrocardiographic data were recorded. Arrhythmias detected during this period were identified according to the Lambeth Convention guidelines and quantified by an established scoring method. Following the ischemic period the heart and hindlimb muscles were dissected and flash frozen. Cx43 expression and phosphorylation in the left ventricle was examined by Western Blot analysis. There was a significant decrease in phosphorylation of Cx43 specifically at S368 in the exercised animals when compared to sedentary controls, but not at S255 or S279/282. ET decreases phosphorylation of S368 but not S255 or S279/282 in response to an ischemic event.

2 (UG)

The Effects of Moderate Intensity Exercise Training on the Incidence of Supraventricular Arrhythmias and Atrial Connexin40 and Connexin43 Expression in Young and Aged Rats

Zachary A. Kadow*[1], Amanda J. Jepson[2], Rachel M. Firkins[2], Ashley N. Davenport[2], Matthew K. Henry[2], Julia A. Moffitt[3]

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Supraventricular arrhythmias are the most prevalent of all arrhythmias, with the elderly being at the greatest risk. Exercise training has been repeatedly shown to reduce supraventricular arrhythmic susceptibility. Connexin 40 (Cx40) is known to be the primary regulator of conduction between atrial cardiomyocytes and alteration of its expression has been associated with atrial arrhythmia development. Recent evidence also suggests that Connexin 43 (Cx43) assists controlling atrial electrical conduction. We hypothesized exercise reduces atrial arrhythmic susceptibility in young and aged animals and would be accompanied by increased atrial Cx40 and Cx43 expression. Groups of young and aged F344 rats underwent treadmill exercise training or sedentary handling. Subcutaneous electrocardiographic leads were then implanted following the respective exercise or sedentary protocols. The arrhythmic index (AI) was calculated using a modified scoring system totaling supraventricular arrhythmias during a baseline period (BL), sympathoexcitation (ISO), and psychological stressor (BR). AI was significantly reduced during BL and ISO periods in young exercise animals compared to sedentary counterparts. Western blot analysis showed significantly greater atrial Cx40 expression in young exercise compared to young sedentary rats, while there was no significant change in the aged animals. Interestingly, a significant increase was observed in atrial Cx43 expression in aged exercise rats compared to sedentary counterparts. These preliminary results indicate moderate exercise is cardioprotective in a young animal model by reducing supraventricular arrhythmias and increasing atrial Cx40 expression while a similar aged model resulted in no changes in arrhythmic susceptibility but did increase in atrial Cx43 expression.

3 (G)

Characterization of Calmodulin-Binding Domains in the Angiotensin II Receptor Type 1A

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Angiotensin II receptor type 1A (AT1R) is a G protein-coupled receptor responsible for the many important effects of angiotensin II (AngII). In vascular smooth muscle cells, AngII induces contraction via a Ca^{2+}-calmodulin dependent process. We have begun to test the idea that signaling via AT1R involves CaM also at the receptor level. In vascular smooth muscle cells isolated from porcine aortas, AT1R coimmunoprecipitates with CaM upon stimulation by AngII or thapsigargin, a specific SERCA inhibitor that triggers classic store-operated Ca^{2+} entry. In an attempt to identify all CaM-binding domains in AT1R, we have generated a series of FRET-based biosensors that consist of each of the four sub-membrane domains (SMDs) of AT1R flanked by an enhanced yellow fluorescent protein (EYFP) and an enhanced cyan fluorescence protein (ECFP), named BSAT1R-SMDs. In response to purified Ca^{2+}-saturated CaM, purified BSAT1R–SMD2 and BSAT1R-SMD3 display drastic disruption of FRET formed between ECFP and EYFP, characteristic of conformational changes caused by direct binding of CaM to sub-membrane domains 2 and 3 of AT1R. These changes are reversed upon chelation of Ca^{2+}. BSAT1R-SMD1 does not response to Ca^{2+}-CaM, while BSAT1R-SMD4 displays a weak change in FRET upon CaM addition, due likely to a long SMD4 linker. Titrations of purified Ca^{2+}-saturated CaM yielded dissociation constants of ~ 47 and 0.3 μM, respectively for SMD2 and 3. The Ca^{2+} sensitivities of these interactions were ~ 4.3 and 0.1 μM, determined by monitoring concurrent responses of the respective BSAT1R and a suitable Ca2+ indicator (indo-1) or XRhod-5F. These data strongly suggest that CaM is involved in AngII signaling via direct interactions with multiple domains in AT1R. Given the drastic differences in affinity and Ca^{2+} sensitivities of the interactions, it is likely that these domains interact with CaM in distinct physiological scenarios. On-going efforts are being made to narrow down the precise locations of the CaM-binding domain on SMD4 and the functional impact of these individual interactions.

4 (G)

Regulation of Store-Operated Ca^{2+} Entry in the Vascular Endothelium by the G Protein-Coupled Estrogen Receptor 1

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The novel G protein-coupled estrogen receptor 1 (GPER/GPR30) has been found to participate in numerous cardiovascular functions. Store-operated Ca^{2+} entry (SOCE) is an essential mechanism that is required for many endothelial cell functions. We found 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. Overexpression of GPER in HEK 293 cells is associated with a 40% decrease in the rate of SOCE, while knockdown of GPER in endothelial cells using antisense oligomer directed against GPER increases SOCE by approximately 50%. GPER coimmunoprecipitates with the stromal interaction molecule 1 (Stim1), an essential molecular switcher of SOCE in cells. Overexpression of GPER in HEK 293 cells is associated with substantial decreases in total Ca^{2+} signal triggered by thapsigargin. Interestingly, SOCE measured in cells stably expressing fusions between EYFP and individual sub-membrane domains (SMDs) 1 – 4 of GPER does not differ from internal controls. These data indicate that GPER may be an important regulatory input of store-operated Ca^{2+} entry via its interaction with Stim1. Since the EYFP-SMD fusions are all cytoplasmically expressed, the data may also suggest that proper membrane docking (PM or ER) of full-length GPER is required for it to exert its effect on SOCE.

5 (G)

The G Protein-Coupled Estrogen Receptor 1 Regulates Endothelial Ca^{2+} Efflux

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The novel G protein-coupled estrogen receptor 1 (GPER/GPR30) has been demonstrated to have a vast array of cardiovascular effects. We have tested the hypothesis that the G protein-coupled estrogen receptor 1 (GPER/GPR30) is involved in the regulation of calcium efflux in endothelial cells (ECs). In primary vascular endothelium, the specific GPER/GPR30 agonist G1 dose-dependently inhibits the activity of the plasma membrane calcium-ATPase (PMCA) measured dynamically in living cells. Overexpression of human GPER in HEK 293 cells results in reduction of PMCA activity. Knockdown of GPER/GPR30 using antisense oligomer is associated with an enhancement of PMCA activity. Coimmunoprecipitation shows complex formation between PMCA and GPER/GPR30 upon treatment with SERCA pump inhibitor thapsigargin in cells pretreated chronically with or without E2. In HEK 293 cells expressing fluorescent reporters constructed based on individual sub-membrane domains (SMDs) of GPER/GPR30, PMCA coimmunoprecipitates with SMDs 1, 3, and 4 of GPER/GPR30, but not with SMD2. These data suggest that GPER/GPR30 regulates calcium efflux in endothelial cells by interacting with PMCA through the receptor's sub-membrane domains 1, 3, and 4 and inhibiting the pump's activity partly via this mechanism.

6 (G)

Fusions Between Nuclear Estrogen Receptors and Red Fluorescent Protein

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The reduction in circulating estrogen concentration in post-menopausal women is associated with a substantial increase in risk and incidence of cardiovascular disease. Hormone replacement therapy is expected to restore the cardiovascular protection conferred by estrogen but has not shown to provide the desired effects. Our central hypothesis is that specific targeting of estrogen receptor subtypes could produce better outcomes and prevent undesirable effects. We have previously observed that chronic estrogen treatment substantially upregulates in the vasculature the expression of total cellular calmodulin, a ubiquitous yet limiting signaling molecule. Data presented in this poster is part of the studies identifying the estrogen receptor(s) (ERα, ERβ, or the novel G protein-coupled estrogen receptor (GPER)), responsible for the effect of 17β-estradiol to upregulate cellular CaM. ER-α and ER-β were PCR amplified from existing plasmids encoding the respective human sequences. The red fluorescent protein DsRed2 was then fused to the C-terminus of each receptor. The fusion DNAs were then introduced into a mammalian plasmid. The plasmids were then transiently transfected into human embryonic kidney HEK 293 cells. Intracellular imaging contrasting $DsRed_2$ and loaded fura-2/AM fluorescence revealed nuclear expression for ERα -$DsRed_2$ and ERβ -$DsRed_2$ fusions, consistent with the functional role of ERα and ERβ as nuclear receptors. The successful heterologous expression of these fusion proteins allows subsequent for evaluation of total CaM expression following chronic treatment with 17β-estradiol.

7 (G)

Novel Fluorescent Reporters for the Stromal Interaction Molecule 1

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The stromal interaction molecule 1 (Stim1) is a Ca^{2+} sensor in the endoplasmic/sarcoplasmic reticulum whose Ca^{2+} sensing triggers its oligomerization and subsequent activation of store-operated Ca^{2+} entry via direct interactions with Ca^{2+} channels at the plasma membrane. We have generated a set of fluorescent reporters based on the intraluminal domain Stim1 where dimerization and Ca^{2+} binding take place. These fluorescent reporters consist of a FRET pair (ECFP-EYFPc) flanking the Ca^{2+}-binding loop of the canonical EF hand, the entire canonical EF hand, the hidden EF hand, and the sterile α motif (SAM), or a combination of these fragments. All reporters display enhanced FRET upon Ca^{2+} binding and FRET disruption upon Ca^{2+} chelation from a Ca^{2+}-saturated state. Reporters generated from the unmodified canonical EF domain and the Ca^{2+} binding loop of this domain display the largest dynamic range in Ca^{2+}-dependent responses. However, double-loop or triple loop chimeras of the linker yield significant improvement in the dynamic range of the biosensors upon interaction with Ca^{2+}. Analyses of spectrofluorometric Ca^{2+} titrations yielded apparent dissociation constants in the millimolar range for these reporters. To investigate the role of the negative charges in the Ca^{2+}-binding loop of Stim1, we generated D to K substitutions at position 2 & 3, or 2, 3, 5, and 7 in the Ca^{2+}-binding loop and introduced these into the triple loop biosensor configuration. The D76/77K substitutions reduces affinity for Ca^{2+} binding, whereas D76/77/81/83K quadruple substitutions completely abolished Ca^{2+} binding. These reporters allow highly quantitative assessment of factors controlling the Ca^{2+}-sensing capability of Stim1. The data indicate that negative charges in the Ca^{2+}-binding loop play an essential role in the interaction between Stim1 and Ca^{2+}.

8

Stress Dynamically Alters Neural Activity Through Bi-Directional Modulation of NMDA Receptors in the Prefrontal Cortex

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Repeated exposure to stress results in progressively divergent effects on cognitive behaviors that are dependent on the integrity of networks in the medial prefrontal cortex (mPFC). To investigate molecular mechanisms responsive to variable repetition of mild stress, we measured persistent neural activity, *in vitro*, from mPFC slices in mice that had been exposed to 10 minutes of forced swim for 1, 3 or 10 days. Acute, short-term stress facilitated persistent activity by increasing event duration while 10 days suppressed event duration and amplitude. These dynamic changes were accompanied by a similar bi-directional modulation of the NMDA/AMPA receptor current ratio, and important synaptic mechanism for sustaining the persistency of neural activity. Specifically, short-term stress led to potentiated NMDA currents with slower decay kinetics, and extended stress produced smaller currents with faster decay. The inhibitory action of ifenprodil, a specific blocker of NMDA receptors containing NR2B subunits, was more effective in current suppression following light stress and less effective after longer stress compared to naïve controls. Persistent activity and glutamate receptor balance in the neocortex have been linked to working memory and impulse control. Therefore, these results could provide insight for generating therapeutic strategies to prevent or reverse stress-induced cognitive deficits.

9

Identification of Three Distinct Calmodulin-Binding Domains in the G Protein-Coupled Estrogen Receptor 1

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The novel G protein-coupled estrogen receptor (GPER/GPR30) is being increasingly identified as an important GPCR in many organ systems. Calmodulin (CaM) is a ubiquitous Ca^{2+} transducer that is required for the activities of numerous cellular proteins. We have begun to test the idea that CaM is involved in GPER-dependent signaling at both the receptor and downstream levels. We observed that CaM and GPER reciprocally coimmunoprecipitates in vascular smooth muscle cells upon stimulation with 17β-estradiol, GPER agonist G1 or thapsigargin, an inhibitor of the sarcoplasmic reticulum Ca2+-ATPase. To identify the CaM-binding sites in GPER/GPR30, we have developed a series of novel FRET-based biosensors whose responses allow identification and highly quantitative characterization of CaM-binding properties of CaM-binding domains in GPER/GPR30. Three separate CaM-binding domains were found in sub-membrane domains 2, 3 and 4 of GPER, with distinct dissociation constants in their Ca^{2+}-dependent interactions with CaM. The three CaM-binding domains also display separate Ca^{2+} sensitivities for complex formation with CaM. These differences suggest separate roles of the three intracellular loops of GPER in their Ca^{2+}-dependent activity. The data also suggest that GPER-CaM association takes place upon induction of store-operated Ca^{2+} entry and without stimulation by specific ligands.

10 (UG)

The Amplitude of the Rat P300 Event Related Potential (ERP) in Behavioral Chains, Suggests the ERP is a Brain Response to the Recognition of Conditioned Reinforcing Stimuli

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The P300 Event Related Potential (ERP) is a time locked, averaged EEG to task relevant stimuli. Previous research in our laboratory has shown that the robust P300 ERP in rats is a correlate of the brains recognition of a conditioned reinforcer. More recently, we found that the P300 ERP to a tone that predicted reinforcement was indistinguishable from a one that predicted non-reinforcement, linking the P300 ERP to the information in the cue even when the information was predicting the absence of a reinforcer. Here, we extended that research by manipulating the delay of reinforcement to separate the larger amplitude ERP to the primary reinforcer (food) from the smaller amplitude ERP to the secondary reinforcer (tone). By analyzing the ERP amplitude to a tone that predicted lever insertion and lever insertion itself, we demonstrated that ERP amplitude increased as a function of proximity to the primary reinforcer in a behavioral chain, further supporting the conditioned reinforcement hypothesis. Normally, the click of the food dispenser and food delivery are practically simultaneous. By manipulating the probability of food delivery given the tone, and click of the food dispenser we demonstrated extinction of the ERP to both stimuli, and increased in attention to extraneous stimuli following non-reinforcement. In humans, alterations in the P300 ERP are trait markers for schizophrenia and early onset Alzheimer's disease. Since rat models of schizophrenia and Alzheimer's disease exist, the rat P300 ERP may be a useful tool for research on these diseases.

11 (UG)

Cellular and Behavioral Differences in Transient Receptor Potential Channel 4 Knockout and Wild Type Rats

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The canonical transient receptor potential (TRPC) family of Ca^{2+} permeable, non-selective cation channels is abundantly expressed throughout the brain and plays a pivotal role in modulating cellular excitability. The TRPC4 subtype expression in the adult rodent brain is restricted to a network of structures that receive dopaminergic innervation, suggesting an association with motivation and reward-related behaviors. Here, we have shown selective expression of TRPC4 channels in a subpopulation of dopamine (DA) neurons in the ventral tegmental area (VTA) and the substantia nigra. Additionally, we have found that while there are no differences between TRPC4 knockout (KO) and normal wild type (WT) rats in simple or complex learning paradigms using food or water rewards, TRPC4 KO compared to WT rats showed reduced self-administration of cocaine and reduced cell firing rates in VTA dopamine neurons. Finally, we investigated the effect of cocaine on impulsivity in TRPC4 KO and WT rats using a differential reinforcement of low rate (DRL) reinforcement schedule. We found that acute cocaine administration significantly increased early responding (impulsiveness) in WT but not KO rats. Since deletion of the trpc4 gene, does not impair learning involving natural rewards, but does affect behavior controlled by cocaine reward, these data demonstrate a novel role for the TRPC4 channel in cocaine reinforcement and suggest that functional TRPC4 expression may be important model for investigating cocaine addiction and cocaine-induced synaptic plasticity in DA neurons.

12 (UG)

There's Something in the Water: Effects of Endocrine Disrupting Chemicals (EDCs) on Offspring Viability in Adult Zebra Finches, *Taeniopygia guttata*

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Endocrine disrupting chemicals (EDCs), including bisphenol-A (BPA), are found in nearly all plastics and many naturally-occurring plant-derived substances. EDCs act by mimicking the effects of estrogen or testosterone, and even plastic containers that are considered food safe have been shown to release estrogen-like chemicals into their contents. In mammals, fetal exposure to BPA leads to lower birth weight, changes in accessory reproductive gland development, alterations in sexual differentiation in the brain, and altered social behaviors. Elevated EDC levels have also been found in obese children and obesity itself can result in earlier onset of puberty. Our study sought to investigate the complex relationship between exposure to EDCs, body weight, onset of puberty, and reproductive success in adulthood. In order to study this, we administered drinking water to breeding adult zebra finches via BPA-positive and BPA-negative plastic bottles. Animals in our negative control received water from a glass bottle and animals in our positive control received estrogen-supplemented (0.25 nmol/g body weight/animal) water from a glass bottle. Preliminary results show no effect on overall egg production, but decreased egg viability ($p < 0.0001$), low chick hatch weight ($p < 0.0001$), and reduced survivability in animals exposed to estrogen and estrogenic compounds through daily water consumption. Despite no correlation between individual body weight at hatch and weight at fledge, offspring from the negative control treatment weighed more at fledge ($p = 0.0046$), even though they tended to fledge at a slightly younger age.

13 (UG)

Number of Metal Spikes in the Baseball Cleat Forefoot May not Effect Baseball Drill Performance or Perception of Cleat Traction

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Baseball shoes (cleats) with metal spikes differ in the number and arrangement of spikes in the forefoot region. The purpose of this study was to compare the performance of young adult males wearing 3-spike, 5-spike, and 6-spike mid-price baseball cleats during a 5-10-5 drill in the grass, a 5-10-5 drill in the gravel, and a baserunning drill. Each subject performed all three drills thrice, once in each type of cleat. A counterbalanced design was used for both cleat and drill presentation orders. No significant differences in performance were found as a function of the baseball cleat itself, although it took subjects significantly longer to complete the 5-10-5 in the gravel compared to the grass ($p=0.031$) and subjects trended to have more ball pick-up fumbles on the gravel compared to the grass ($p=0.076$). After each drill, subjects indicated their perception of the cleats' traction using a 10-cm visual analogue scale. No significant differences in traction were found for any comparison. There were no significant differences in the subjects' ranking of cleats based on general preference. These results suggest that the number of metal spikes in the forefoot region of baseball cleats does not influence subjects' performance during common baseball field drills.

14 (UG)

Effects of Energy Gel Composition on Blood Glucose and Fuel Utilization in Males and Females During Submaximal Exercise

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Carbohydrate ingestion immediately before and during submaximal exercise raises blood glucose which tends to promote greater carbohydrate oxidation we compared 2 energy gels with different carbohydrate compositions, all simple sugars (Honeystinger, HS) or a 60:40 ratio of complex to simple sugars (PowerBar, PB). We hypothesized the simple sugar gel would be absorbed and oxidized more rapidly than the 60:40 ratio gel and thus HS will be associated with higher blood glucose and RER (i.e. higher carbohydrate oxidation) than PB. A ParvoMedics metabolic system measured oxygen consumption (VO_2), heart rate (HR) and respiratory exchange ratio (RER) during 60 min of submaximal (~ 50-60% VO_2 max) cycling in 4 males and 4 females in a repeated measures design. Energy gels were consumed before exercise and at 30 min. Blood glucose was measured at 0, 30, and 60 min and lactate at 60 min with glucose and lactate meters. Repeated measures ANOVA compared effects of gender and gel at 60 min. There was a significant effect of gender and interaction of gender and gel on glucose and a significant interaction on HR. HS and PB blood glucose were 95 and 86 g/dl, respectively, in males and 101 and 97 g/dl, in females. HS and PB HR were 143 and 132 bpm, respectively, in males and 142 and 149 bpm, in females. RER and lactate were not different between genders or gels. In summary, glucose levels were higher with HS than PB in males and females, but did not affect fuel utilization based upon RER.

15 (UG)

Somatosensory Perception of Running Shoe Mass

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Running shoes are often marketed to consumers based on mass. Fifty young adult males participated across two separate experiments to determine how well they could perceive running shoe masses using hands versus feet. Foot perception was conducted identically in both experiments: 25 subjects were blindly fitted with five different test shoes one-at-a-time and asked to rank the heaviness of each individually using visual analogue scales (VAS) and then comparatively using verbal rankings. Hand perception was conducted differently in the two experiments. In Experiment A, subjects were allowed to heft all shoes simultaneously, whereas in Experiment B shoes were presented to subjects individually as they were in the foot portion.

Foot Portion: There were no pass effects so the two data sets were combined. Residuals analysis indicated verbal accuracy in mass perception was 30%. One-way ANOVA with Tukey HSD indicated significant effects such that subjects were able to differentiate the heaviest and second-heaviest shoe both from the lightest in both verbal and VAS. Verbal and VAS data correlated (R2=0.64).

Hand Portion: Verbal accuracy was 92% in Experiment A and 63% in Experiment B. ANOVA on verbal scores indicated that subjects could differentiate all shoe pairings in Experiment A and all but two in Experiment B. VAS data for Experiment B indicated only a third of pairings were significantly differentiated, and verbal and VAS data correlated (R2=0.67). Altogether, these results strongly indicate the foot perceives mass poorly compared to the hand, and may suggest that consumers' perceptions of shoe mass may come more from handling shoes than wearing them.

16

Effects of Hsp27 Overexpression and Phosphorylation State on Muscle Hypertrophy and Contractile Function Induced by Functional Overload in Mice

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Functional overload (FO, removal of major synergists) induces muscle growth and increased strength and fatigue resistance. FO is associated with significant increases in Hsp25 (rodent form of Hsp27) expression and phosphorylation in hypertrophying muscle suggesting its role in muscle adaptation to loading stressors. Since phosphorylation can modify stress-related Hsp25 functions, we tested the hypothesis that overexpression of human Hsp27 (TG) or a nonphosphorylatable Hsp27 mutant (MUT) will respectively increase and decrease FO-induced muscle growth and function vs. wild type (WT) mice. Plantaris isometric force and fatigue were measured *in vivo* with a dual mode lever system 14 or 30 d after FO or sham surgery. Plantaris mass increased ~ 2 fold with FO in all groups, but was lower in MUT than WT at 14 and 30d ($p<0.05$). Plantaris maximal isometric force was 30% lower in MUT than WT at 30d and 32% lower in TG than WT at 30d ($p<0.05$). Fatigue resistance was significantly lower in MUT than WT at 30d, but was not different between TG and WT groups ($p<0.05$). Results suggest that elevated Hsp27 levels may alter long-term force adaptation to FO while preventing Hsp27 phosphorylation attenuates adaptations in both force and fatigability compared to WT with 30d of FO. This suggests Hsp27 phosphorylation dependent functions (e.g. reducing apoptosis) may be involved in processes that increase muscle strength and fatigue resistance.

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17 (G)

Dystrophin Insufficiency Causes a Becker Muscular Dystrophy-Like Phenotype in Swine

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Duchenne muscular dystrophy (DMD) is caused by a dystrophin deficiency while Becker muscular dystrophy (BMD) is caused by a dystrophin insufficiency or expression of a partially functional dystrophin protein. Deficiencies in existing mouse and dog models necessitate the development of a novel large animal model. Our purpose in this investigation is to characterize diaphragm and heart muscles taken from 8 wk male pigs with a dystrophin insufficiency and healthy male littermates (HML). Dystrophin protein expression was decreased 63% in diaphragms and 70% in hearts from BMD pigs compared to HML by Western blot. These findings were supported by immunohistochemical analysis. Quantitative RT-PCR using primers directed against the 3' end of the dystrophin transcript showed a 61% and 34% reduction in mRNA abundance in the diaphragm and heart, respectively, in BMD pigs compared to HML. Consistent with the human disease, dystrophin insufficiency was associated with decreased expression of α-sarcoglycan and a 5-fold increase in serum creatine kinase activity compared to HML. Further, focal necrosis with fibrosis and fatty infiltration was evident in histologically stained diaphragms but not the hearts from BMD pigs at the 8wk time point. We conclude that these pigs exhibit a phenotype consistent with BMD and should be considered as a translational and preclinical model for this disease.

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18 (UG)

Quercetin Supplementation Preserves Muscle Fibers in Dystrophic Diaphragm

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Duchenne muscular dystrophy (DMD) is the most common, fatal, X-linked disorder world-wide. It is characterized by the loss of muscle function and results in premature death due to respiratory complications and cardiomyopathy. Several studies have shown that quercetin, a compound found in plant pigments, increases muscle mitochondrial biogenesis by increasing SIRT1 and PGC-1α. In this investigation the extent to which a quercetin enriched diet can diminish the progression of Duchenne muscular dystrophy through the activation of the SIRT1/PGC-1α pathway was explored. The pathway leads to the expression of utrophin and increased mitochondrial biogenesis. Our hypothesis is that a 0.2% quercetin enriched diet will protect dystrophic muscle from disease related injury. To test this hypothesis, 3 month old mdx mice were placed on quercetin enriched diets for 6 months. The diaphragms were then assessed using immunohistochemistry for signs of homogeneous cell size and fiber type distribution. The number of cells per area increased 30% in the animals treated with the quercetin enriched diet but all other measures were similar to the control. These results indicate quercetin is preventing disease related muscle injury.

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Putting the Math into Biology and the Bio into Mathematics: Creating an Interdisciplinary Undergraduate Concentration in Mathematical Biology

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In spring 2013, St. Olaf College added a Mathematical Biology concentration to the curriculum in order to address the critical need for quantitatively literate professionals in biological fields including physiology. The core course is Mathematics of Biology taught by a mathematician with a wet-lab taught by a biologist. The interdisciplinary field of mathematical biology combines experiment, mathematical theory, statistics and computation. This summer, we worked with a small team of students to create four laboratories that will engage students in all of these areas by requiring them to collect data and practice the essential modeling techniques of formulation, implementation, validation, and analysis. The labs use discrete and continuous models to study population growth, enzyme kinetics, random walks and glucose homeostasis. We also began the work of infusing the two disciplines strategically into additional courses including Calculus I, introductory biology and Animal Physiology. For example, an introductory lab was developed to measure exponential and logistic growth in bacterial cultures and use numerical solutions to alter parameters as well as algebra to explore cell size and material fluxes. Furthermore, heat exchange and allometric model exercises were revised to be more mathematically explicit for Animal Physiology. We found the problems engaged the introductory students (to their surprise). Importantly, we learned that the student-development team was tenacious and comfortable crossing disciplinary boundaries showing how a student-faculty working group can accomplish interdisciplinary curricular development. The success of the curriculum for biology and mathematics majors will be assessed as it is implemented.

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Abnormal Immune Cell Populations in SHR Hypertension

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We have found that autonomic modulator of the immune system is abnormally proinflammatory in the spontaneously hypertensive rats (SHR) and may contribute to hypertension. Nicotine activates cholinergic receptors on splenocytes and suppresses IL-6 release in response to TLR ligands in Wistar-Kyoto (WKY) controls, but enhances it in pre-hypertensive SHR. The objective of this study was to test (1) whether immune cell populations change as SHR age and (2) whether nicotine influences immune cell populations in SHR. We performed immuno-phenotyping of splenocytes from WKY and SHR at ages ranging from 0 days to 38 weeks. In 0-day-old SHR splenocytes, a specific subset of cells (CD161+) was significantly higher than in WKY. The CD161+ cell population increased with age in SHR reaching 30% of the lymphocyte population. Infusion of nicotine increased the of CD161+ splenocytes in SHR but not in WKY. CD161+ cells produce IL-17, a pro-inflammatory cytokine involved in hypertension. Upon induction by phorbol myristate acetate and ionomycin, expression of IL-17 mRNA was increased to a greater extent in SHR vs. WKY splenocytes. We conclude: 1) SHR exhibit a developmentally high abundance of CD161+ immune cells that increases with age as hypertension develops, and (2) nicotine selectively increases the number of potentially IL-17-producing CD161+ splenocytes in SHR. To our knowledge, this is the first demonstration of an age-related increase in a specific immune cell population (CD161+) in genetic hypertension.

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Mechanisms Involved in an Acidic pH-conditioned NOX-mediated Chloride Conductance in Nodose Sensory Neurons

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We recently reported a novel prolonged low pH-conditioned current in nodose sensory neurons following brief exposures to acidic extracellular pH. This current is similar to the Swell-Activated Chloride current, and mediated by NADPH oxidase (NOX) dependent hydrogen peroxide. Since NOX is known to be activated by intracellular (IC) alkaline pH, we hypothesized that low extracellular pH conditioning activates NOX by inducing IC alkalinity. We first buffered the IC pipette solution with 40mM HEPES, and found that the pH-conditioned current was inhibited from 11.8±2.0 pA/pF to 3.4±0.7 pA/pF (n=6, $p<0.01$), indicating that an IC pH change is required for the current activation. The pH sensitive fluorescence dye 2'-7'-bis(carboxyethyl)-5(6)-carboxyfluorescein showed significant but transient decreases in IC pH during the brief exposures to extracellular low pH (n=12). However, sustained reductions of IC pH to 7.0 and 6.0 did not elicit the current. Instead, increases in IC pH to 7.5, 7.6 and 8.0 dose-dependently induced a similar current averaging 9.2±1.8, 16.3±4.1 and 30.6±9.7 pA/pF. Finally, inhibition of proton extrusion by blocking the Na+-H+ exchanger with amiloride and proton channels with Zn2+ inhibited the low pH-conditioned current (n=6). We therefore conclude that transient extracellular pH reductions can be transmitted intracellularly and activate Na+-H+ exchanger and proton channels. Our data suggest that the resulting activation of NOX and increase in chloride conductance involve IC alkalinization.

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