Conference Program & Abstracts

2015 Physiological Bioenergetics

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#Bioenergetics15
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Russell Swerdlow
Univ. of Kansas
Yisang Yoon
Georgia Regents Univ.

Acknowledgements

The Meeting Organizers and The American Physiological Society gratefully recognize the generous financial support from the following:

National Institute of General Medical Sciences, NIH
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<td>9:00—11:30 AM Symposium II Mitochondrion on the Move: Networking in Health and Disease Yisang Yoon, Roberta Gottlieb, Gyorgy Hajnoczky</td>
<td>9:00—11:30 AM Symposium V Mitochondrial Adaptation and Susceptibility to Stress Paul Brookes Nika Danial</td>
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<td>12:30—1:30 PM Career Symposium: How to Succeed: A Research Scientist and Entrepreneur in Bioenergetics Brian Dranka</td>
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<td>5:00—5:10 PM Welcome and Opening Remarks</td>
<td>2:30—5:30 PM Symposium III Translational Bioenergetics Victor Darley-Usmar Sruti Shiva Russ Swerdlow Brian Dranka Anthony Molina</td>
<td>2:20—4:30 PM Symposium VI It’s Not Just the ATP! Signaling and Mitochondrial Function Ben van Houten Shannon Bailey Andreas Beyer</td>
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<td>5:10—6:30 PM Plenary Lecture I Doug Wallace John Lemasters</td>
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<td>6:30—8:30 PM Welcome and Opening Reception</td>
<td>5:30—7:30 PM Poster Session Social</td>
<td>5:00—7:00 PM Poster Session Social</td>
<td>7:00—9:30 PM Banquet and Awards Ceremony</td>
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Location:
The 2015 APS Conference: Physiological Bioenergetics: From Bench to Bedside will be held September 9—12, 2015 at the Westin Tampa Harbour Island Hotel, 725 South Harbour Island Blvd., Tampa, FL 33602, USA, telephone (813) 229-5000, FAX: (813) 229-5022.

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

On-Site Registration Fees:
APS Member……………………………………… $650
APS Retired Member…………………………… $450
Nonmember……………………………………… $800
Postdoctoral………………………………………. $500
Student………………………………………… $450

*The registration fee includes entry into all scientific sessions, poster socials, opening reception, and the closing conference banquet*. Must have a ticket for entry.

Payment Information:
Registrants may pay by institutional or personal check, traveler’s check, MasterCard, VISA or American Express or in United States Dollars. Checks must be payable to “The American Physiological Society” and drawn on a United States bank payable in US dollars.

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

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

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

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

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

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

Don’t forget to join us at the Welcome Reception directly after the Opening Plenary Session

Ballroom Foyer
6:30—8:30 PM
WEDNESDAY, SEPTEMBER 9, 2015

Plenary I

1.0  PLENARY I
Wedns., 5:00—6:30 PM, Harbour Island Ballroom.

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

5:10 PM

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

THURSDAY, SEPTEMBER 10, 2015

Symposia I

2.0  ENERGY SCHOOL I
Thurs., 8:00—9:00 AM, Harbour Island Ballroom.

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

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

8:30 AM

Symposia II

3.0  MITOCHONDRIA ON THE MOVE: NETWORKING IN HEALTH AND DISEASE
Thurs., 9:10—11:30 AM, Harbour Island Ballroom.


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

9:35 AM

10:00 AM  Break

10:30 AM

10:55 AM

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

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

Symposia III

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


2:30 PM

2:55 PM

3:20 PM

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

4:10 PM

4:35 PM

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

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

Career Session

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

Chairs:  Brian Dranka, Seahorse Bioscience.

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

Plenary II

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

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

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

### Poster Session

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<td>1</td>
<td>Transgenic Redox-indicator Mice Expressing Cytochrome c and Mitochondrial roGFP1. K. W. Wagner, B. K. Kolbrink, K. C. Can, B. K. Kempkes, and M. M. Müller. Univ. of Göttingen, Germany.</td>
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<td>Mitochondrial Reserve Capacity is Driven by Glutamine in Lung Cancer Cells with Mesenchymal Phenotype. Y. S. Si, D. B. Ulanet, J. B. Hurvitz, M. D. Dorsch, and K. M. Marks. Agios Pharma, Cambridge, MA.</td>
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<td>L-OPA1 Functions Independently of S-OPA1 by Forming Separate Structural Entities. H. L. Lee, and Y. Y. Yoon. Georgia Regents Univ.</td>
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<td>ATP Production and Oxygen Consumption in Isolated Mitochondria from H9c2 Cells. P. A. Albrecht, Warsaw Univ. of Life Sci., Poland.</td>
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<td>High Intensity Training Increases Mitochondrial Respiratory Capacity in Old Males but not Females. S. L. Larsen, T. D. Dohlmann, D. S. Sognaaard, F. D. Dela, and J. W. Helge. Univ. of Copenhagen, Denmark.</td>
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FRIDAY, SEPTEMBER 11, 2015

Symposia IV
8.0 ENERGY SCHOOL II
Fri., 8:00—9:00 AM, Harbour Island Ballroom.
Chair: Brian Dranka, Seahorse Bioscience.
8:00 AM 8.1 The Lactic Acidosis Consortium: A Multi-disciplinary Research Effort to Translate Gene Discovery into Better Management and Treatment for Patients with Mitochondrial Disorders. Yan Burelle, Univ. of Montreal, Canada.
8:30 AM 8.2 Mitochondrial DNA Content: Accurate Measurement and Evaluation as an Early Biomarker of Mitochondrial Dysfunction. Afshan Malik, King's Coll., London, UK.

Symposia V
9.0 MITOCHONDRIAL ADAPTATION AND SUSCEPTIBILITY TO STRESS
Fri., 9:10—11:30 AM, Harbour Island Ballroom.
9:10 AM 9.1 Withdrawn.
10:00 AM Break
11:10 AM 9.5 Increased Autophagy is Required for Mechanical Ventilation-induced Diaphragm Mitochondrial Dysfunction. Ashley Smuder. Univ. of Florida, Gainesville. (7.12).

DAILY SCHEDULE

PLENARY III
10.0 PLENARY III
Fri., 1:15—2:00 PM, Harbour Island Ballroom.
Chair: Victor Darley-Usmar, Univ. of Alabama at Birmingham.
1:15 PM 10.1 The Sugar Disconnection in Diabetic Mitochondrial Networks. Orian Shirihai, Boston Univ.
1:50 PM Break

Symposia VI
11.0 IT'S NOT JUST THE ATP! SIGNALING AND MITOCHONDRIAL FUNCTION
Fri., 2:20—4:30 PM, Harbour Island Ballroom.
2:45 PM 11.2 Tick, Tock: The Biological Clock Controls the Powerhouse. Shannon Bailey, Univ. of Alabama at Birmingham.
4:05 PM 11.6 Withdrawn.

POSTER SESSION II
12.0 POSTER SESSION II
Fri., 5:00—7:00 PM, Terrace.

Poster Board 27 12.1 Assessment of Peripheral Mitochondrial DNA Damage and Dysfunction as a Biomarker of Parkinson's Disease. C. C. Corey, N. J. Jensen, E. H. Howlett, A. W. Weinstein, K. E. Erickson, J.
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DAILY SCHEDULE

SATURDAY, SEPTEMBER 12, 2015

Symposia VII

13.0  MITOCHONDRIAL GENETIC AND METABOLIC PROGRAMS
Sat., 9:00—10:50 AM Harbour Island Ballroom.

Chairs:  Martin Brand, Buck Inst. for Res. on Aging.
Shannon Bailey, Univ. of Alabama at Birmingham.


Closing Remarks

14.0  CLOSING REMARKS
Sat., 10:50—11:00 AM Harbour Island Ballroom.

Chairs:  Victor Darley-Usmar, Univ. of Alabama at Birmingham.
Sruti Shiva, Univ. of Pittsburgh.


NOTES

Thank you! Thank you! Thank you! for the generous support from

National Institute of General Medical Sciences, NIH
Seahorse Bioscience
University of Pittsburgh, Ctr. for Metabolism and Mitochondrial Medicine
# 2015 APS Conference
**Physiological Bioenergetics: From Bench to Bedside**

### Abstracts of Invited and Contributed Presentations

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**Author Index**                                                                 | 30   |
Mitochondrial morphology changes dynamically mainly through fission and fusion. In recent years, the use of high-throughput respirometry has increased collective knowledge of the role of intermediary metabolism in numerous biological processes. In addition, measurements of glycolytic activity provide essential information on glucose utilization in cells. Such information, while useful, is not sufficient to understand how other metabolic pathways—such as ancillary glucose or mitochondrial pathways—are affected by pathologies or interventions. Integrating respirometry measurements with stable isotope-resolved metabolomics (SIRM) confers considerably more information regarding metabolism in general and allows for analysis of intracellular carbon flux in metabolic networks. The purpose of this talk is to present and discuss work flow plans and key data sets that integrate respirometry measurements with SIRM. Integration of these two techniques can divulge novel understanding of the metabolic underpinnings of cell growth, proliferation, adaptations to stress, and pathology.

HOW TO MEASURE AUTOPHAGY AND MITOPHAGY
Junhua Zhang1

1Pathology, Univ. of Alabama at Birmingham, 901 19th St. South, Birmingham, AL, 35204.

Autophagy and mitophagy are important cellular processes that are responsible for clearance of damaged biomolecules and organelles. These pathways are important for preserving organellar function and maintaining redox signaling. More than 30 proteins are involved in a highly regulated and multi-step mechanism. Perturbation of autophagy and mitophagy has been shown to contribute to many disease pathogenic mechanisms and therefore measurement of autophagy and mitophagy in different cell and tissue contexts and in response to physiological and pathological signals is essential to determine the roles of autophagy and mitophagy play in health and diseases. Bioc hemical, cell biological, histological and molecular methods are used for these measurements. These include using biochemical and microscopic methods to measure a lipid modified cytosolic protein LC3II to assess the size of autophagosomes in a given cell, association of LC3II with lysosomes and its entrance into the lysosomal compartment, and degradation of long lived proteins. This workshop will provide an overview of these methods and discuss their usage and interpretations. (NIHROI-NS064090) Zhang J (2013) Autophagy and mitophagy in cellular damage control. Redox Biology 1:19-23; Zhang J (2015) Teaching the basic of autophagy and mitophagy to redox biologists-mechanisms and experimental approaches. Redox Biology 4:242-259.
5.0 PLENARY II

5.1 SITES OF PRODUCTION OF MITOCHONDRIAL ROS: MECHANISMS AND PHYSIOLOGICAL FUNCTION
Martín Beard

1Brand Lab, Buck Inst. for Res. on Aging, 8001 Redwood Blvd, Novato, CA, 94945. Superoxide and H2O2 are generated at ten or more mitochondrial sites. Sites IIIα in complex III, I3 in complex I, and IIα in complex II have the greatest capacities in skeletal muscle mitochondria; site I3 in complex I has low capacity. The rate of superoxide/H2O2 production at any site depends on its redox state, so we can assess rates at different sites from measured redox states. Surprisingly, in a substrate mix mimicking resting muscle cytosol, the major contributors were IIα and IIIα, with contributions from I3 and IIIα. In medium mimicking contracting muscle, the total rate was fivefold less and site I3 was dominant, with contributions from Iα, IIα, and IIIα. These ex vivo results may mimic ROS production in muscle in vivo. By screening small molecule libraries against different sites, we identified novel suppressors of superoxide/H2O2 production at sites I3 and IIα, that do not affect oxidative phosphorylation. They suppress several physiological and pathological phenotypes, and provide new tools to identify the roles of mitochondrial ROS production in cells, and potential leads for pharmacological modifiers of ROS signaling and oxidative damage. References: Goncalves et al. (2015) Sites of superoxide and hydrogen peroxide production by muscle mitochondria assessed ex vivo under conditions mimicking rest and exercise. J Biol Chem 290, 209-227. Orr et al. (2013) Inhibitors of ROS production by the ubiquitine-binding site of mitochondrial complex I. Idenitified by chemical screening. Free Radic Biol Med 65, 1047-1059. Orr et al. (2015) Suppression of superoxide production from mitochondrial complex III. In revision.

6.0 TRANSLATIONAL BIOENERGETICS

6.1 MEASURING BIOENERGETIC HEALTH IN HUMAN POPULATIONS
Victor Darley-Usmar

1Mitochondrial Med. Lab., Univ. of Alabama at Birmingham, 901 19th St. S., Birmingham, AL, 35294. Bioenergetics is now at the forefront of our understanding of pathological mechanisms, new therapies and as a biomarker for the susceptibility of disease progression in metabolic diseases, neurodegeneration, cancer and cardiovascular disease. A key concept is that the mitochondrion can act as the “canary in the coal mine” by serving as an early warning of bioenergetic crisis in patient populations. Furthermore, cellular mitochondrial function is known to vary between populations due to differences in genetic background and in response to lifestyle changes including diet and exercise. It is clear that we urgently need new clinical tools to monitor changes in bioenergetics in patient populations. This is now possible due to the development of high-throughput assays to measure cellular energetic function in the small numbers of cells that can be isolated from human blood or from tissue biopsy samples. The sequential addition of well characterized inhibitors of oxidative phosphorylation allows a bioenergetic profile to be measured in cells isolated from normal or pathological samples. This profile can define the extent to which these cells utilize mitochondrial oxygen consumption to produce ATP, are using proton leaks for other processes or leak and the maximal respiration. Non-mitochondrial oxygen consuming pathways are also measured and are likely indicative of a pro-inflammatory state. Taken together we propose these parameters are a measure of bioenergetic health of a cell population. We therefore propose the development of the Bioenergetic Health Index (BHI), which is a single value that defines bioenergetic health based upon the analysis of cellular mitochondrial profiles in cells isolated from human subjects. Ultimately, BHI has the potential to be a new biomarker for assessing patient health of (or for) both prognostic and diagnostic value.

6.2 PLATELET MITOCNDRIA: FROM BIOMARKER TO BIOLOGICAL MECHANISM
Sruti Shiva

1Vascular Med. Inst., Dept. of Pharm. & Chem. Biol., Univ. of Pittsburgh, 200 Lothrop St., 1240E, Pittsburgh, PA, 15261. While it is well established that bioenergetic dysfunction plays a role in the pathogenesis of numerous diseases, mitochondrial dysfunction remains underappreciated in many patient populations because of the invisibility of obtaining tissue for mitochondrial studies. Platelets are easily accessible and have long been recognized to contain fully functional mitochondria. However, it remains unclear whether platelets harbor the bioenergetic dysfunction observed in other organ systems during pathology or whether mitochondrial dysfunction contributes to platelet pathology. We hypothesize that platelet bioenergetics can serve as a biomarker of specific diseases and that mitochondrial function regulates platelet thrombotic and inflammatory function. We have recently shown that patients with Sickle Cell Disease have altered platelet bioenergetics due to an inhibition of mitochondrial complex V, leading to increased membrane potential and augmented reactive oxygen species (ROS) production. We have shown that this augmented ROS directly leads to platelet activation. We now extend this study to determine whether platelet mitochondrial function is differentially altered in other disease cohorts including asthma, pulmonary hypertension, Parkinson's Disease and cardiac arrhythmias. We hypothesize, demonstrating differential bioenergetic profile in patients with each of these pathologies and discuss the role altered mitochondrial function in disease progression.

6.3 MITOCHONDRIAL BIOMARKERS FOR NEURODEGENERATIVE DISEASES
Russell Swerdlow

1Neurology, Univ. of Kansas, MS 6002, 4350 Shawnee Mission Pkwy., Fairway, KS, 66205. Mitochondrial dysfunction is observed across a spectrum of neurodegenerative diseases. This raises the question of whether mitochondrial-based biomarkers could be used to reveal the presence of disease or pre-disease, endophenotype states, and whether mitochondrial biomarkers could be used to guide the development of new therapies. Approaches with the ability to interrogate brain bioenergetics currently exist, although these approaches have limitations and more comprehensive and practical ways to assess brain mitochondrial function are needed. Interestingly, mitochondrial changes similar to those observed in the brains of patients with some neurodegenerative diseases are also detected in peripheral tissues, which suggests the possibility that mitochondrial function in peripheral tissues may be able to function as a surrogate for brain mitochondrial function. We have previously considered different options for the assessment of brain mitochondrial function and brain bioenergetics, as they relate to studies of diagnostics and biomarkers. We hypothesize, demonstrating differential bioenergetic profile in patients with each of these pathologies and discuss the role altered mitochondrial function in disease progression.

6.5 USING MACHINE LEARNING TO ADVANCE BLOOD BASED BIOENERGETIC PROFILING: A FOCUS ON GERIATRIC HEALTH
Anthony Molina

1Inst. Med., Section on Gerontology & Geriatric Med., Wake Forest Sch. of Med., Sticht Ctr. on Aging, Med. Ctr. Blvd., Winston Salem, NC, 27157. Blood based bioenergetic profiling is recognized to have potential diagnostic and prognostic applications. In primates, we have observed that the respirometric profile of blood cells can recapitulate the bioenergetic capacity of other tissues such as skeletal muscle. Our studies in older adults indicate that the respiratory capacity of PBMCs is associated with multiple measures of physical function, including: gait speed, Short Physical Performance Battery score, upper and lower body strength, and muscle quality. These physical function measures are recognized to be excellent proxies for morbidity and mortality in this age group. PBMCs are comprised of multiple cell types and do not encompass all cells accessible for blood based profiling. It is likely that different cell types and respiratory parameters will have variable utility with regard to prognostic and diagnostic applications. To address this, we are utilizing Machine Learning methods designed for high dimensional data analysis to identify respirometric signatures and patterns across multiple cell types that are most closely associated with clinical outcomes. This branch of artificial intelligence utilizes algorithms that can be trained by example to distinguish between groups or predict outcomes. Random Forests is an ensemble learning approach that can build powerful predictive models and detect subtle multivariate gait patterns. The strengths of this approach are: it does not suffer from overfit; it is robust to noise; it estimates error rate; it provides indices of variable importance; it works with mixes of continuous and categorical variables; it can be used for data imputation and cluster analysis; and it can deal with issues stemming from a large number of variables and a small sample size.
7.0 POSTER SESSION I

7.1 TRANSGENIC REDOX-INDICATOR MICE EXPRESSING CYTOSOLIC AND MITOCHONDRIAL ROGFP1

Kenneth Wagener1, Benedikt Kolbrin1, Karolina Can1, Belinda Kempe1, and Michael Müller1

Neuro/ Sinnepysilopologie, Univ. Göttingen, Humboldtsallee 23, Göttingen, D-37073, Germany.

Reactive oxygen species (ROS) and related redox changes contribute to cellular signaling and are linked to neuropathology and mitochondrial dysfunction. For long, redox imaging was limited by a lack of reliable optical probes. Genetically-encoded, fluorescent protein derived optical redox sensors bridge this gap. Demandng, however, the delivery of coding DNA to the tissue of interest. This requires transfection/transduction of cultured preparations or viral infections into each individual animal. To extend reliable redox imaging to adult and complex preparations while circumventing surgical procedures, we generated transgenic redox indicator mice. They express roGFP1 under the Thy1 promoter in the cytosol or the mitochondrial matrix almost throughout the brain. Neurolabeling confirmed neuronal expression of cytosolic and mitochondrial roGFP1, and Mitotracker staining verified its proper targeting. Mitochondrial roGFP1 is functional at all postnatal stages; any redox-modulating effects of the transgene can be ruled out. Detailed response calibrations of roGFP1 already detected regional differences in redox conditions among the hippocampal subfields. In conclusion, roGFP1 mice are valuable to analyze REDOX/redox signaling in various preparations during maturation and aging. Their crossbreeding with disease mouse models will unveil details on ROS formation and redox imbalance in the onset and progression of various neuronal disorders, degenerative conditions, and mitochondrialopathies. Supported by the Cluster of Excellence and the DFG Research Center Nanomicroscopy and Molecular Physiology of the Brain (CNMPB).

7.2 EFFECTS OF SKELETAL MUSCLE AGING ON MITOCHONDRIAL MORPHOLOGY AND DYNAMICS

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Muscle aging is now recognized that mitochondrial function, mitophagy and mitochondrial morphological changes, and although no difference in the content of proteins regulating mitochondrial fusion and fission, mitochondrial dysfunction remains unknown. Mitochondria and cytosol showed more oxidized redox baselines in aged muscles. Our results reveal that muscle aging is associated with complex changes in mitochondrial morphology that could interfere with mitochondrial function and mitochondrial fusion index (Mfn2-to-Drp1 ratio) was significantly increased in aged muscles. Our results reveal that muscle aging is associated with complex changes in mitochondrial morphology and could contribute to aging-related accumulation of mitochondrial dysfunction and sarcopenia.
Existing data suggest relationships exist between mitochondrial function, APP processing, and AD pathology. However, the relationship between mitochondrial function, cell bioenergetics, and APP could enhance our understanding of AD. To test the impact of bioenergetics on APP processing we measured APP mRNA, APP protein, and APP derivatives (soluble APPα, sAPPα, APP) in human neuronal SH-SY5Y cells with different bioenergetic manipulations. These manipulations include depletion of mitochondrial DNA (ρ0), glycolysis inhibition (2-deoxyglucose; 2DG), and varying medium glucose concentrations (0, 2.5, 25 mM). Endpoints were measured at 24 and 72 hours for the 2DG and variable glucose experiments. The effects of these manipulations on respiration and glycolysis were determined using a Seahorse XF24 analyzer. Relative to SH-SY5Y cells, SH-SY5Y ρ0 cells (which have a high glycolysis flux and negligible respiratory chain flux) had comparable full-length APP protein and mRNA levels, but lower medium sAPPα and APP levels. At both the 24 and 72 hour time points, 2DG treatment reduced glycolysis with no change in respiration. At 24 hours no changes were observed with any APP processing endpoints following 2DG treatment. At 72 hours, the 2DG treatment showed unchanged APP mRNA levels, reduced full length APP protein, medium sAPPα and APP levels. Relative to cells maintained at a high glucose level (25 mM), 0 mM glucose showed reduced glycolysis and increased respiration, while cells in 2.5 mM glucose showed increased respiration and comparable glycolysis. At 24 hours, cells maintained in 0 and 2.5 mM glucose had reduced medium sAPPα, but all other endpoints were unchanged. With 0 mM glucose, APP mRNA was unchanged, full length APP protein and medium sAPPα were reduced, while medium levels of APP were increased at 72 hours. Cells maintained in 2.5 mM glucose appeared to show intermediate changes to APP endpoints. Results suggest bioenergetically-stressed cells reduce APP translation, or alter processing, compartmentalization, or solubility of APP and its derivatives. Results from ρ0 cells are perhaps more consistent with this latter view. Experiments to resolve these questions are underway.

7.6 MODULATION OF MITOCHONDRIAL ADENINE NUCLEOTIDE TRANSLOCASE (ANT) REGULATION WITH AGEING
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By studying bioenergetic parameters (oxygenation and phosphorylation rates, membrane potential) in isolated mitochondria from aged rat muscle (gastronemius) we observed a decrease in mitochondrial affinity for ATP, and a change in ANT response to atracyloside. These age-induced modifications of ANT result in an increase in the ADP concentration required to sustain the same ATP turnover as compared to young muscle, and thus lower membrane potential and higher coupling efficiency under conditions of low ATP turnover, due to the down-regulation of basal proton leak caused by membrane potential decrease. The decrease in mitochondrial capacity driven by ANT alteration during ageing may also decrease reactive oxygen species (ROS) production as compared to young muscles for equivalent ATP turnover. ANT alteration with ageing may be the result of oxidative damage caused by ROS and may appear like a virtuous circle where ROS induce a mechanism that reduces their production. Because of the importance of mitochondrial ROS as therapeutic targets, we believe that this new mechanism deserves further studies. All experiments are in agreement with the European Guide for animal use. PD has a permanent license to conduct experiments on animals (03/17/1999, license 3308010). 1G. Gouspillou et al, Biochim Biophys Acta, 1797 (2010) 143-15. 2G. Gouspillou et al, Aging cell, 13 (2014) 39-48. 3M.D. Brand, L.F. Chien, P. Diolez, Biochem J, 297 (Pt 1) (1994) 27-29.

7.7 MITOCHONDRIAL RESERVE CAPACITY IS DRIVEN BY GLUTAMINE IN LUNG CANCER CELLS WITH MESENCHYAL PHENOTYPE
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Metastasis is the main cause of cancer mortality, and its initiation is enabled by a profound metabolic reprogramming referred to as the metabolic transition. It is desirable to identify specific drug targets for cancer cells with mesenchymal phenotype. Previously we have shown that lung cancers with mesenchymal phenotype are more sensitive to inhibition of glutaminase (GLS). As EMT can lead to changes in both the glycolytic and glutaminolysis pathways, we sought to investigate the importance of these fuels for mitochondrial respiration, and to understand the impact of GLS inhibition on mitochondrial function. We developed a cell-based assay to profile substrate preference and functional conditions. We first showed that transforming growth factor beta 3 (TGFβ3)-induced EMT was accompanied by the loss of glucose-driven reserve capacity. As a result, small molecule inhibition of GLS abolished reserve capacity and blocked proliferation in a TGFβ3-induced mesenchymal line without affecting the epithelial parental line. We further applied this assay to a lung cancer cell line panel, and demonstrated that cell lines with high sensitivity to GLS inhibitor were solely dependent on glutamine-driven reserve capacity. Taken together, our data demonstrate EMT is associated with a change in substrate utilization for mitochondrial reserve capacity in lung cancer cells, and reserve capacity may play a key mechanistic link between GLS inhibition and impaired cell proliferation.

7.8 L-OPA1 FUNCTIONS INDEPENDENTLY OF S-OPA1 BY FORMING SEPARATE STRUCTURAL ENTITIES
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Optic atrophy 1 (OPA1) is a dynamin-related membrane-remodeling protein that functions in mitochondrial fusion and cristae remodeling. Loss of OPA1 has been shown to cause defects in inner membrane fusion and oxidative phosphorylation (OXPHOS). OPA1 is expressed in multiple splice variants produced by alternative splicing at the N-terminal exons downstream of a transmembrane (TM) domain. These splice variants undergo partial or full proteolytic cleavage depending on exon combination after alternative splicing, resulting in small membrane-anchored non-cleavable L-OPA1, and cleavable L-OPA1 in OPA1-KO mouse embryonic fibroblasts (MEFs) and examined their capacities to restore OXPHOS function and mitochondrial fusion. We found that, while OPA1-KO cells failed to grow in galactose medium which forces cells to use OXPHOS to generate ATP, expression of L-OPA1 or S-OPA1 alone was sufficient to support cell growth in galactose medium. Similarly, L-OPA1 or S-OPA1 alone restored respiration in OPA1-KO MEFs. Analyses of respiratory complexes using blue-native gel electrophoresis (BNGE) indicated that OPA1-KO cells showed greatly diminished levels of complexes III, IV, and V, which was restored by L- or S-OPA1 alone indistinguishably. However, we observed that L-OPA1 was more effective than S-OPA1 in inducing mitochondrial elongation when fusion was inhibited, similar to previous observations in the conditions of nutrient starvation or cycloheximide treatment. Interestingly, analyses of oligomeric state of L- and S-OPA1 showed that, while non-cleavable L-OPA formed mostly hexamers, the majority of S-OPA1 was in dimers. In wild-type cells and cells expressing a cleavable L-OPA1 in OPA1 KO cells, L- and S-OPA1 also exhibited similar hexameric and dimeric patterns, respectively, as examined by 2-dimensional BNGE (BNGE followed by SDS-PAGE). These results suggest that although L-OPA1 is required for mitochondrial fusion, cristae maintenance for proper OXPHOS function can be supported by S-OPA1 or L-OPA1 alone.
7.11 REGULATION OF BIOENERGETICS AND ANGIGENIC RESPONSE IN VASA VASORUM ENDOTHELIAL CELLS BY EXTRACELLULAR PURINES AND HYPOXIA

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Cell proliferation is an energy taxing process, however, both the role of cellular metabolism in angiogenic endothelial cells and the regulation of cellular energy pathways by extracellular stimuli remain unexplored. Extracellular purines are wide accepted as important regulators of endothelial cell function. Our group has previously shown their autocrine/paracrine role in pulmonary artery vasa vasorum angiogenesis in a neonatal rat model of hypertension. Herein we investigated the role of mitochondrial and mitochondrial respiratory inhibitors, in this study we demonstrated that glycolysis and oxidative phosphorylation (OXPHOS) are both vital for ATP-stimulated vasa vasorum endothelial cell (VVEC) mitogenesis. We also showed that VVEC isolated from control animals exhibited higher rates of OXPHOS compared to those isolated from chronically hypoxic complements. Measurement of OXPHOS in digitonin-permeabilized VVEC showed that chronic hypoxia both in vivo and in vitro significantly decreased OXPHOS, Complex I and Complex II mitochondrial respiration. Additionally, F1F0 ATP-synthase β-subunit and Cytochrome C oxidase subunit IV expression levels were decreased, suggesting persistent hypoxia-induced phenotypical changes in VVEC bioenergetics. Cells cultured 7 days in Galactose [20mM] and Glucose [5mM] displayed augmented intracellular ATP production along with a significant increase in basal and maximal respiration rates. Furthermore, a one-hour nucleotide treatment [100uM] increased maximal respiration rates under said conditions. Interestingly, glycolysis experiments displayed a unique response to oligomycin [0.4;0.8;1.25uM] wherein a decrease in extracellular accumulation rate (ECAR) was observed despite exposure time [1.48 & 24h]. ATP stimulation increased ECAR while 2-deoxyglucose (2DG) also yielded an unorthodox response resulting in a marked decrease in ECAR followed by immediate recovery to pre-injection levels within 10 minutes. In parallel, lactate measurements showed an insignificant increase in response to oligomycin, while ATP concentrations spiked at the 4 hour mark in control and hypoxic VVEC not coinciding with OXPHOS changes. Finally, consistent with our previous observations in OXPHOS assays, ATP was shown to induce a transient increase in [Ca2+] in VVEC mitochondria. Therefore, perturbing and metabolic regulation of VVEC energy pathways may present a novel strategy for the treatment of vasa vasorum pathologic angiogenesis in hypoxic pulmonary hypertension.

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7.12 INCREASED AUTOPHAGY IS REQUIRED FOR MECHANICAL VENTILATION-INDUCED DIAPHRAGM MITOCHONDRIAL DYSFUNCTION

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Mechanical ventilation (MV) is a life-saving intervention for patients in respiratory failure. However, prolonged MV results in diaphragm weakness. While the mechanisms controlling MV-induced diaphragm atrophy are not fully elucidated, it has been demonstrated that mitochondrial function plays an important role in regulating skeletal muscle mass. Evidence in mechanically ventilated patients indicates that the autophagy/glycolytic proteolytic pathway is upregulated in the diaphragm. However, it is unknown if MV-induced increased autophagy occurs as a protective mechanism to degrade dysfunctional mitochondria or if increased autophagy exacerbates mitochondrial dysfunction. Therefore, these experiments were designed to determine the effects of accelerated autophagy on diaphragm mitochondrial function during MV. Cause and effect was determined by inhibiting MV-induced autophagy via adeno-associated virus overexpression of mutated autophagy-related protein 5 (ATG5) in the diaphragm of rats. Our results reveal that inhibiting autophagy prevented the MV-induced reduction in mitochondrial oxygen consumption. Further, transduction of mutated ATG5 prevented MV-induced increase in both mitochondrial ROS emission and caspase-3 activation. Finally, inhibiting autophagy prevented MV-induced increased expression of PINK1 and the fusion/fission proteins OPA1 and DLP1. Therefore, our data indicate inhibition of MV-induced autophagy is sufficient to protect against diaphragm mitochondrial dysfunction. Supported by NIH R21 AR064956 awarded to SKP.

7.13 MITOCHONDRIAL RESPIRATORY CAPACITY IS DECREASED IN RAT CARDIOMYOCYTES FOLLOWING EXPOSURE TO MATERNAL DIABETES AND HIGH FAT DIET

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Background: Offspring of diabetic mothers (ODM) are at risk of cardiovascular disease at birth and throughout life. Diabetic pregnancy is associated with increased circulating fuels that may cross the placenta to incite influences on the developing fetal heart. Emerging evidence suggests that bioenergetic reprogramming at the mitochondrial level plays a pivotal role in the pathophysiology of adult cardiac disease. However, the short and long-term consequences of exaggerated fuel exposure on mitochondrial function in the developing fetal heart are currently unknown. We determined the effects of maternal diabetes and high-fat (HF) diet on developmental programming of cardiac metabolism. Methods: Sprague Dawley rats received control (CD) or HF diet throughout the study. At gestational day 14 dams were injected with either citrate buffer (CB) placebo or streptozotocin (STZ) to induce diabetes producing offspring from the following groups: controls (CD-CB), diabetic exposed (CD-STZ), HF exposed (HF-CB), and double exposed (HF-STZ). Cardiomyocytes of primarily isolated cardiomyocytes (CM) from each litter were investigated using a Seahorse XF24 analyzer which measures oxygen consumption rate and extracellular acidification rate as markers of mitochondrial respiration and anaerobic glycolysis respectively. Pairwise analyses were performed and significance was set at p<0.05. Results: Basal respiration was significantly decreased in the HF-STZ suggesting changes in either ATP turnover, substrate oxidation or proton leak. Maximal respiratory capacity was significantly decreased in HF-STZ exposed CMs suggestive of mitochondrial substrate uptake and processing dysfunction. Moreover, a decrease in reserve capacity was also detected in the HF-STZ exposed CMs suggestive of inability to respond to an increase in energy demand. The diabetic and double exposed CMs also had limited glycolytic capacity. Conclusion: Late gestation diabetes reprograms fuel metabolism in the developing heart of offspring. These effects are exacerbated with a maternal HF diet. Additionally a reduced reserve capacity, especially with inability of fuel switch in diabetic exposed CMs suggests inability to produce sufficient ATP during elevated demands. Together, our findings have immediate implications in preventing developmentally programmed cardiac disease in ODMs. Funding: NIH K08HD078504, NICHD, SSOM-USD Faculty Grant, Sanford Health–SDSU Collaborative Research Seed Grant.

7.14 ATP PRODUCTION AND OXYGEN CONSUMPTION IN ISOLATED MITOCHONDRIA FROM H9C2 CELLS

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ATP in animal cells is first produced in cytoplasm in the process of glycolysis which is based on breaking glucose into two pyruvate molecules. Than pyruvate can be converted into lactic acid in the process of fermentation or can be involved in the respiration which takes place in the mitochondria. This study addresses the question whether the ATP generation is strictly correlated to oxygen consumption. For this purpose mitochondria were isolated from myocardial cells H9C2. Utilization of pure O2sm (isolated mitochondria) helps to avoid aberration of ATP measurements associated with the production of ATP in the glycolysis process, which impairs performance. ATP production was measured using the Luciferase-Luciferin method, which is based on the luminescence reaction between luciferin and ATP – catalyzed by luciferase. Oxygen consumption was measured with Oxygengraph-2. Preparation, storage, and sucrose solution were done as described. The background we used blockers such as rotenone and oligomycin. Two major findings were noted: After substrate are added either both ATP production and oxygen consumption increases significantly or only ATP production rises rapidly while oxygen consumption is not so much noticeable. This may be due to the process of iso-
lution, where the outer mitochondrial membrane may have been damaged losing its cation selectivity and cation leakage. Further investigations are required to elucidate possible underlying mechanisms of protection. Methods: Primary adult mouse cardiomyocytes were subjected to simulated IR injury using a modified Seahorse XF24 apparatus with drug addition at the onset of reperfusion. Cell death was estimated using LDH release. Drugs which protected cardiomyocytes in vitro were tested in a Langendorff model of IR injury, measuring functional recovery and infarct size. In separate experiments, metabolites extracted from perfused hearts were resolved by HPLC. Results: Nornicotine was identified as a cardioprotective agent in the in vivo model. It was observed that in perfused hearts, 10 nM nornicotine injected at the onset of reperfusion improved functional recovery and decreased in infarct size (31.3%±2.4 vs 49.2%±2.5 in non-treated hearts, p<0.05, n=16-20). Nornicotine also exhibited profound inhibitory effects on mitochondrial complex I activity. Succinate is known to accumulate in ischemia, and its rapid consumption during early reperfusion exacerbates reperfusion injury via ROS generation from electron backflow through complex I [PMID: 2538517]. In non-treated hearts, we confirmed that high post ischemic levels of succinate rapidly declined during the first 2 min of reperfusion. In contrast, nornicotine slowed post-ischemic succinate consumption, suggesting that electron backflow through complex I is the major pathway driving succinate consumption. Conclusions: Herein, we demonstrated that nornicotine was cardioprotective when delivered at early reperfusion in vitro and ex vivo. The mechanism of cardioprotection may be related to inhibition of rapid succinate consumption during early reperfusion via reverse electron flow back through complex I.

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7.18 MITOCHONDRIAL CHAPERONE GRP75 HAPLOINSUFFICIENCY PROMOTES LIVER TUMORIGENESIS BY ADAPTED METABOLISM

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1Molecular Med., Georgia Regents Univ., 1120 15th St., Augusta, GA, 30912, 2Cancer Ctr., Georgia Regents Univ., 1120 15th St., Augusta, GA, 30912. The reprogramming of energy metabolism is one of the hallmarks of cancer; however, underlying mechanism of mitochondria’s role in tumorogenesis remains unclarified. To determine whether and how mitochondria attributes to tumorigenesis, we investigated the effects of genetic inactivation of GRP75, a mitochondrial HSP70 chaperone, using a well-established cancer mouse model in which injection of precarcinogen diethylnitrosamine (DEN) at 15 days of age induces liver tumor within 7 months with a 100% prevalence. Our study revealed that hepatocytes of grp75+/+ mice, compared to grp75+/−/−, WT controls, exhibited distinct metabolic alterations associated with a lower respiratory capacity (oxygen consumption rate), higher glycolysis, decreased mitochondria membrane potential (MMP), and higher ROS production. Although both strains were sensitive to DEN-induced liver tumorogenesis, the grp75+/+ mice exhibited higher tumor burdens and accelerated tumor growth. Thus, partial inactivation of GRP75 promotes liver tumorigenesis by causing mitochondrial dysfunction, increasing ROS production, and engaging metabolic adaptive pathways promoting malignant transformation. Further detailed studies on the metabolic and molecular signaling pathways driving tumor progression in grp75+/−/− mice may provide potential targets for liver cancer treatment. Funding resources: The research was supported from the National Institutes of Health CA121951 (D.M.) and CA062130 (N.M.).

7.19 MITOCHONDRIAL ENERGY DEFICIENCY LEADS TO HYPERPROLIFERATION OF SKELETAL MUSCLE MITOCHONDRIA AND ENHANCED INSULIN SENSITIVITY

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nerrals muscle as determined by electron microscopy, mitochondrial DNA copy number and expression of citrate synthase (CS), succinate dehydrogenase and cytochrome c oxidase. This was accompanied by a greater proportion of oxidative-like myofibers showing increased staining for myosin heavy chain isoforms IIa and IIx in the Ant1 -/- mice. Next we measured oxygen consumption and reactive oxygen species (ROS) production in permeabilized myofibers from gastrocnemius muscle. The Ant1 -/- mice had a 80% and 90% decrease in state II and III respiration, respectively, when normalized to CS activity. However, because of mitochondrial hyperproliferation there is a 260% and 140% increase in state II and III respiration, respectively, when normalized to tissue mass. ROS production follows a similar trend where the amount of H2O2 produced is decreased in Ant1 -/- myofibers when normalized to CS activity, but increased when normalized to tissue mass. Finally, Ant1 -/- mice showed signs of improved insulin sensitivity. Ant1 -/- mice were found to be significantly more glucose tolerant and have an increased glucose sensitivity seems to happening independent of the PI3K-Akt signaling pathway as provided into 3 groups: control (C), 10-day hind limb unloading (H) and H with 3-days of reloading (R). Following treatments, mice were sacrificed by CO2 asphyxiation. Hind limb muscle groups were harvested, weighed and snap frozen. Quantification of CL and CER species in the left soleus was performed by mass spectrometry-based shotgun lipidomics. Permeabilized fiber bundles were prepared from the right soleus to measure mitochondrial respiration, H2O2 emission and CRC. Results: OC mice were sarcopenic, as evidenced by lower soleus, gastrocnemius and quadriceps mass, compared to YC. A similar degree of atrophy and mitochondrial dysfunction was evident in the soleus of both OH and YH groups following 10-days of unloading. However, a decrease in total mitochondrial content and distinct remodeling of CL and CER molecular species was only evident in the YH group. Moreover, C18:1 CRC content increased in the YH group, and C18:0, C23:0, and C24:1 CRC content was greater in YH, compared to the OH group. Following 3 days of reloading, the YR group recovered soleus mass. There was no recovery of soleus mass for OR. Mitochondrial respiration, H2O2 emission, CRC, and total mitochondrial content also recovered in the soleus of YR, but not OR. Conclusion: Alterations in profile of CL and CER species, recently identified as important mediators of mitochondophagy, occur in soleus from young mice during unloading. This in turn accompanies and perhaps facilitates effective recovery of muscle mass and mitochondrial function with reloading. This adaptive response was not evident in sarcopenic muscle. Grant Support: This research was supported by grants from National Institute of Aging (AG044437-PMC) and Diversified Translational Laboratory funding from Sanford-Burnham.

7.22 MITOCHONDRIAL DNA CHANGES AND DYSFUNCTION IN DIABETIC NEPHROPATHY

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The hypothesis underlying this work is that changes in circulating mitochondrial DNA (MtdNA) and subsequent mitochondrial dysfunction are key players in the pathophysiology of diabetic nephropathy (DN). To investigate this we examined the quantity and function of circulating MtdNA in patients with DN. In a cross-sectional study (N=168), the samples were studied as 3 groups: Healthy controls (HC, N=40), Diabetics without DN with more than 20 years duration of diabetes, no history of albuminuria (DN), and Diabetics with DN (N=88). A similar degree of atrophy and mitochondrial dysfunction was evident in the soleus of both OH and YH groups following 10-days of unloading. No differences were seen between male and female groups in terms of atrophy or mitochondrial dysfunction. In conclusion old males increase OXPHOS capacity in skeletal muscle after six weeks of HIT, whereas no improvements are seen in females. No difference was seen after training in adipose tissue. This training intervention did not change OXPHOS capacity in either of the genders. In conclusion old males increase OXPHOS capacity in skeletal muscle after six weeks of HIT, whereas no improvements are seen in females. No difference was seen after training in adipose tissue, but females have a higher OXPHOS capacity at baseline compared with males. Further analysis is needed to explain these gender differences. The study was supported by Oda and Hans Svenningsen Foundation.

7.23 COMBINED AMPK AND PPARA AGONISM IMPROVES EXERCISE PERFORMANCE IN TRAINED MICE

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Exercise training improves muscle function and fatigue resistance during endurance exercise. AMPK and PPARα agonists have been shown to mimic these effects in...
sedentary and mdx mouse model mouse however, their effects together with endurance exercise may have implications on mitochondrial function. Future studies will determine if PDM do not equilibrate their content with the rest of the mitochondrial population. This finding is also supported by their higher Tomm20 content. In conclusion, PDM represent a sub-population of mitochondria with unique protein composition, architecture and activity that is likely maintained due to their reduced level of interaction with the rest of the mitochondrial population. 7.25 MITOCHONDRIA DNA ISDamaged in MILITARY VETERANS WITH FATTIGING CONDITIONS Yang Chen1, Xihu Jiao1, Helena Hill1, Jacquesen Klein1, Danneel Ntimann1, and Michael Falvo,2 1Pharmacology/Physiology, New Jersey Med. Sch. Rutgers Univ., Med. Sci. Bldg., 185 South Orange Ave, Newark, NJ, 07101-1709, 2War Related Illness & Injury Study Ctr., VA New Jersey Hlth. Care Sys., 385 Tremont Ave, East Orange, NJ, 07018 Background: Gulf War illness (GWI) is the most prominent health issue affecting veterans of the 1990-1991 Gulf War, with 1 in 4 Gulf War Veterans suffering from GWI. GWI is characterized by multiple diverse symptoms of fatigue, muscle pain and cognitive dysfunction that are suggestive of mitochondrial impairment. Military exposures during deployment have been put forward in the etiology of GWI, many of which have been identified as genotoxic. To date, mitochondrial dysfunction in veterans with GWI has only been described indirectly. Objective: The goal of this study was to access more direct evidence of mitochondrial dysfunction in GWI, by measuring mitochondrial DNA (mtDNA) content and mtDNA damage in peripheral blood mononuclear cells (PBMCs) in veterans with GWI. Methods: Twenty-five veterans with GWI and six non-deployed healthy controls were recruited and provided PBMCs for total DNA extraction. Relative mtDNA copy number (i.e. mtDNA content) and mtDNA damage were determined by quantitative polymerase chain reaction. Results: Veterans with GWI had significantly higher mtDNA content (t=2.820, P=0.008) and mtDNA damage (t=2.037, P=0.051) than non-deployed controls. Conclusion: This is the first study to report direct evidence of higher mtDNA content and mtDNA damage in veterans with GWI, supporting prior indirect evidence of mitochondrial dysfunction in this group. Future studies are necessary to confirm these findings and determine their association with mitochondrial function. In addition, work in this area may guide new diagnostic testing and treatments for veterans suffering from GWI. 7.26 STATIN MALGYCIC PATIENTS HAVE IMPAIRED MITOCHONDRIAL RESPIRATORY FUNCTION IN SKELETAL MUSCLE Tine Dohlmann1, Jørn Wulff Helge1, Flemming Dela1, and Steen Larsen1 1Pharmacology/Physiology, New Jersey Med. Sch. Rutgers Univ., Med. Sci. Bldg., 185 South Orange Ave, Newark, NJ, 07101-1709, 2War Related Illness & Injury Study Ctr., VA New Jersey Hlth. Care Sys., 385 Tremont Ave, East Orange, NJ, 07018 Introduction: Statins reduce endogenous cholesterol synthesis, and is widely considered to decrease the risk of cardiovascular events. However, statin therapy is associated with development of side effects, such as muscle ache and pain (myalgia), but the mechanism is unknown. It has been shown that statins decrease the mitochondrial function in skeletal muscle, but the aim of this study was to investigate if statin induced myalgia is coupled with impaired mitochondrial respiratory function in human skeletal muscle and adipose tissue. Methods: Two groups of healthy adults in continuous simvastatin treatment (40 mg/day) were recruited for this study. One group (n=11, 5m/6f) experienced myalgia (SM) with a VAS score of 4.7±0.7, and the other group (n=13, 6m/7f) without side effects (VAS 0.3±0.2) served as controls (SC). The groups were matched for age, BMI, and VO2max (59±2 vs 61±2 yrs, 29±2 vs 29±1 kg/m2 and 265±5 vs 268±6 mL O2/min/kg, respectively). Mitochondrial respiration was measured in permeabilized muscle fibers and subcutaneous adipose tissue, using high-resolution respirometry. The protocols investigated maximal mitochondrial respiration with electron flow through complex (C) I, II, III, (OXPHOS capacity), and electron transport system (ETS) capacity. Results: In skeletal muscle, maximal mitochondrial CI respiration and ETS capacity were lower in SM compared to SC (49.2±5 vs 55±1 (P=0.004) and 72±3 vs 84±3 (P=0.004) pmol O2/mg, respectively), and OXPHOS capacity tended to be lower in SM (63±3 vs 70±3 (P=0.076) pmol O2/mg, respectively). In adipose tissue, ETS capacity was lower in SM compared to SC (2.1±0.1 vs 2.4±0.1 (P=0.036) pmol O2/mg, respectively), and females (n=13) had higher mitochondrial respiration through CI, and ETS, OXPHOS capacity, and ETS capacity, compared to men (n=11). The myalgic females (n=6) had a lower CI respiration, OXPHOS capacity and ETS capacity, compared to female controls (n=7). Discussion: We demonstrate, that statin induced myalgia is coupled to impaired mitochondrial function in skeletal muscle. Interestingly, mitochondrial respiration in adipose tissue was impaired in statin myalgic females, but not males. Since statins reduce synthesis of Ubiquinone (Q10) as well as cholesterol, reduced mito-
8.0 ENERGY SCHOOL II

8.1 THE LACTIC ACIDOSIS CONSORTIUM: A MULTI-DISCIPLINARY RESEARCH EFFORT TO TRANSLATE GENE DISCOVERY INTO BETTER MANAGEMENT AND TREATMENT FOR PATIENTS WITH MITOCHONDRIAL DISORDERS

Yan Burelle

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Mitochondrial dysfunction is involved in a large number of human pathologies, including a broad spectrum of rare but usually severe genetic mitochondrial diseases. While recent advances in next generation sequencing have led to considerable progress in the identification of the molecular and biochemical defects underlying a number of these genetic diseases, they have not translated into major improvements in patient management. This can be explained by the existence of numerous barriers including: i) limited information on the impact of the various mutations affecting the oxidative phosphorylation system (OXPHOS) on the mitochondrial, metabolic, and signaling phenotype; and ii) the underlying pathogenic mechanisms. Difficulties in choosing therapeutic strategies in absence of a detailed phenotypic signature, and lack of clinical tools or biomarkers, which are indicative of disease progression, clinically relevant outcomes, and impact of treatments. This presentation will provide an overview of the research effort made by our multidisciplinary research consortium to meet these challenges in patients with Leigh Syndrome French Canadian (LSFC), a severe disease prevalent in the Saguenay-Lac-St-Jean region in Quebec, which is caused by mutation of LRPPRC, a protein involved in the translation of mito-chondria-encoded polypeptides of the OXPHOS system. Funded by CIHR. References: Burelle et al. & LSFC consortium (2015). PLoS ONE, 10(3), e0120767; Sasuarm, et al. & LSFC consortium (2015). Human Molecular Genetics, 24(2).

8.2 MITOCHONDRIAL DNA CONTENT: ACCURATE MEASUREMENT AND EVALUATION AS AN EARLY BIOMARKER OF MITOCHONDRIAL DYSFUNCTION

Afshan Malik


The amount of mitochondrial DNA per cell is dependent on the cell’s bioenergetic requirements, under normal conditions there is a correlation between mitochondrial DNA content, mtmRNA and mitochondrial function. However we found that in conditions of disease, for example in diabetic cells, there is a disconnect between the amount of mtDNA and mitochondrial function. Increasingly greater numbers of studies are reporting alterations in mtDNA in disease conditions (2), however the methodology being employed is leading to confusion in the field because of two major issues, the presence of nuclear pseudogenes with high homology to the mitochondrial genome and dilution bias caused by the differing sizes of the nuclear and mitochondrial genomes. In this talk I will explain how to accurately measure mtDNA content and describe data showing that adaptive changes in mtDNA are an early event in disease suggesting that mtDNA content could be an early biomarker of mitochondrial dysfunction. References: 1. Czajka, Ajay, Grudni , and Malik (2015) Altered mitochondrial function, mitochondrial DNA and reduced metabolic flexibility in patients with diabetic nephropathy. EBInformedicine, in Press. 2. Malik and Czajka (2012) Is mitochondrial DNA content a biomarker of mitochondrial dysfunction. Mitochondron. 3. Malik et al., (2011) Mitochondrial DNA as a non-invasive biomarker: Accurate quantification using real time quantitative PCR without coinhibition of pseudogenes and dilution bias Biochemical and Biophysical Research Communications-412 (2011) 1-7.

9.0 MITOCHONDRIAL ADAPTATION AND SUSCEPTIBILITY TO STRESS

9.3 MITOCHONDRIAL FUEL SUBSTRATE SWITCHING AND THE EXCITATORY BRAIN

Niki Daniel

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Altered fuel utilization by the brain has profound effects on neuronal activity as evidenced by the effects of statin therapy on seizures and statin induced myalgia. Funding: This study is funded by the UCPH 2016 Fund.

11.0 IT'S NOT JUST THE ATP! SIGNALING AND MITOCHONDRIAL FUNCTION

11.1 MITOCHONDRIA MATTER: TARGETING MITOCHONDRIAL FUNCTION IN TUMOR CELLS

Bennett Van Houten1, Wei Qian2, Jing Ma2, Karen Hannon1, Molly Heft-Neal1, Nicholas Rhodie1, Charmane Law1, and Pradeep Wankhede1


The tumor cell Warburg effect is associated with increased glycolysis even in the presence of ample oxygen, and has dominated the cancer metabolism field for the last two decades. More recently it has been found that tumor cells our metabolically heterogeneous and can switch from glycolysis to oxidative phosphorylation depending on environmental conditions surrounding the tumor. Some forms of cancers, such as ovarian tumor cells, utilize fatty acids from adipocytes to fuel beta-oxidation in the mitochondria. We have therefore developed several strategies to attack mitochondrial function and inhibit tumor cell growth. We have found that CPT1 inhibitor, etomoxir, greatly decreases oxygen consumption in melanoma, breast and ovarian cancer cells, and given after cisplatin treatment acts synergistically to inhibit ovarian cancer cell growth. We have also found that mitochondrial division inhibitor-1 (midivi-1), which has been suggested to inhibit Drp1 and therefore block mitochondrial fission, causes G2/M arrest in tumor cells, but not normal cells. Furthermore we have found that midivi-1 synergistically increases cisplatin potency by causing both a Drp-1 and Bcl/Bak independent release of cytochrome C and subsequent cell death. Tumor cells also display increased reactive oxygen species (ROS) and are in a oxidant state and we have developed a proof of principle approach based on specific targeting of ROS to the mitochondria by fusing reactive oxygen generating fluorescent protein, KillerRed to TFAM. While this approach is effective, creating stable cell lines has been difficult. To overcome this problem we have developed a novel protein delivery system, that when activated by light can directly deliver singlet oxygen into the mitochondrial matrix. This approach greatly diminishes mitochondrial function within hours of damage. Finally we have developed a novel mitochondria-targeted lapachone using the alkene peptide isostere segment of the antibiotic graminicidin S (XJB-peptide). We have found that XJB-lapachone causes rapid loss of mitochondrial function within hours of damage. This study is funded by NIH grant R01 NS083844. 1. Lutas A, and Yellen G, Trends Neurosci, 2013, 36:32-40. 2. Gimenez-Cassina, A., et al., Neuron, 2012, 74:719-30.

11.2 TICK, TOCK – THE BIOLOGICAL CLOCK CONTROLS THE POWERHOUSE

Shannon Bailey
dominant-negative splice variant of TERT (b-del) is increased in most human to systolic hypertension concomitant with telomere shortening. The natural occurring drial oxidative phosphorylation. We have found an increase in b-del TERT in heart Erickson3, J.T. Greenamyre2, Samay Jain2, Sruti Shiva1, and Laurie Sanders2.

Catherine Corey1, Nicholas Jensen2, Evan Howlett2, Andrea Weinstein3, Kirk MARKER OF PARKINSON’S DISEASE DNA DAMAGE AND DYSFUNCTION AS A BIO- ASSESSMENT OF PERIPHERAL MITOCHONDRIAL 12.1 12.0 POSTER SESSION II 11.3 MITOCHONDRIAL TELOMERASE AND VASODILATION Andreas Beyer1 Medicine/Physiology, Med. Coll. of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI, 53226.

TERT, the catalytic subunit of telomerase elongates telomeres to prevent cellular aging. A potential role in the development of cardiovascular disease (CVD), especially the vascular endothelium has not been described. A mitochondrial role for TERT in regulating reactive oxygen species (ROS) has been shown positioning TERT as a key regulator of oxidative stress. Under physiological conditions blood flow stimulates endothelial release of nitric oxide (NO), mediating flow-mediated dilation (FMD) suppressing vascular smooth muscle proliferation and inflammation. In the in vivo setting the switch in mediator of FID to occur.

The sphingolipid ceramide has been shown to increase mitochondrial reactive oxygen species and is elevated in the plasma of patients at risk for heart disease. Previously we have shown that incubating human adipose arterioles with C2-ceramide inhibits nitric oxide (NO) as the mediator of flow induced dilation (FID), allowing mitochondrial-derived hydrogen peroxide (H2O2) to compensate. Ceramide also induces mitochondrial fission in cell culture by upregulating dynamin related protein 1 (DRP-1), however it is not known if ceramide-induced fission is critical for the switch in mediator of FID to occur.

Hypothesis: Inhibiting DRP-1 in human adipose microvessels prevents the compensatory H2O2-mediated dilation that occurs when NO is suppressed following ceramide treatment alone.

Methods: Discarded human adipose tissue was obtained at the time of surgery, microvessels (100-200 µm in diameter) were cannulated in an organ chamber, and the internal diameter was measured via video microscopy. FID was assessed during graded increases in intraluminal flow ± the NO synthase inhibitor L-NAME (100 µM) or the H2O2 scavenger polyethylene (PEG) catalase used alone or in combination. Vessels were exposed to flow of 15-37°C with C-2 ceramide (10 µM) ± Mdivi-1 (50 µM) MitoPYL, a fluorescent probe specific for mitochondrial H2O2, was used to quantify changes in mitochondrial H2O2 during flow. Results: Immunobiochemistry showed increased DRP-1 expression in microvessels treated with ceramide vs. vehicle. Mdivi-1 treatment alone had no effect on FID (max dilation 75 ± 5.6% vs. vehicle 76 ± 5.1%) which was mediated by NO since L-NAME abolished dilation (max dilation 4 ± 2.6%) and PEG-catalase had no effect on FID (max dilation 75 ± 5.6% vs. vehicle 76 ± 5.1%). Vessels treated with Mdivi-1 + ceramide did not dilate to flow (max dilation 6 ± 5.6%) and reduced generation of mitochondrial H2O2 was observed (the increase in Mito-PYL fluorescence with ceramide alone was 86 ± 31% compared to baseline, but with Mdivi-1 + ceramide only an 11 ± 14% increase was seen). Conclusion: Treating human adipose vessels with the DRP-1 inhibitor Mdivi-1 prevented compensatory, mitochondrial H2O2-mediated dilation from occurring in response to ceramide treatment, suggesting that mitochondrial fission may be necessary for this process to occur. This work was supported by the National Institute of Health HL113612-02 (DDG) and American Heart Association 14POST18780022 (MJ). 11.2 THE DRP-1 INHIBITOR MDIVI-1 PREVENTS COMPENSATORY MITOCHONDRIAL H2O2-MEDIATED VASODILATION INDUCED BY CERAMIDE TREATMENT IN HUMAN ADIPOSE ARTERIOLES Matthew Durand1, Julie Fejer2, Joseph Hockenberry3, and David Gutterman1.


Background: The sphingolipid ceramide has been shown to increase mitochondrial reactive oxygen species and is elevated in the plasma of patients at risk for heart disease. Previously we have shown that incubating human adipose arterioles with C2-ceramide inhibits nitric oxide (NO) as the mediator of flow induced dilation (FID), allowing mitochondrial-derived hydrogen peroxide (H2O2) to compensate. Ceramide also induces mitochondrial fission in cell culture by upregulating dynamin related protein 1 (DRP-1), however it is not known if ceramide-induced fission is critical for the switch in mediator of FID to occur.

Hypothesis: Inhibiting DRP-1 in human adipose microvessels prevents the compensatory H2O2-mediated dilation that occurs when NO is suppressed following ceramide treatment alone. Methods: Discarded human adipose tissue was obtained at the time of surgery, microvessels (100-200 µm in diameter) were cannulated in an organ chamber, and the internal diameter was measured via video microscopy. FID was assessed during graded increases in intraluminal flow ± the NO synthase inhibitor L-NAME (100 µM) or the H2O2 scavenger polyethylene (PEG) catalase used alone or in combination. Vessels were exposed to flow of 15-37°C with C-2 ceramide (10 µM) ± Mdivi-1 (50 µM) MitoPYL, a fluorescent probe specific for mitochondrial H2O2, was used to quantify changes in mitochondrial H2O2 during flow. Results: Immunobiochemistry showed increased DRP-1 expression in microvessels treated with ceramide vs. vehicle. Mdivi-1 treatment alone had no effect on FID (max dilation 75 ± 5.6% vs. vehicle 76 ± 5.1%) which was mediated by NO since L-NAME abolished dilation (max dilation 4 ± 2.6%) and PEG-catalase had no effect (max dilation 91 ± 2.2%). Vessels treated with Mdivi-1 + ceramide did not dilate to flow (max dilation 6 ± 5.6%) and reduced generation of mitochondrial H2O2 was observed (the increase in Mito-PYL fluorescence with ceramide alone was 86 ± 31% compared to baseline, but with Mdivi-1 + ceramide only an 11 ± 14% increase was seen). Conclusion: Treating human adipose vessels with the DRP-1 inhibitor Mdivi-1 prevented compensatory, mitochondrial H2O2-mediated dilation from occurring in response to ceramide treatment, suggesting that mitochondrial fission may be necessary for this process to occur. This work was supported by the National Institute of Health HL113612-02 (DDG) and American Heart Association 14POST18780022 (MJ).
Ischemic stroke is a leading cause of morbidity and mortality. Several studies have indicated that mitochondrial dysfunction plays an important role in the pathophysiology of stroke but the exact mechanisms are not clear. Our laboratory has made the novel and surprising finding that both mitochondrial protein mass and mitochondrial-derived vasodilation of the middle cerebral artery are intact on the side ipsilateral (Ipsi) to transient middle cerebral artery occlusion (MCAO) while severely reduced on the side contralateral (Contra) to MCAO. However, mitochondrial respiration following MCAO is unknown. We examined mitochondrial oxygen consumption rate (OCR) in MCAs following MCAO or sham operation and correlated it with mitochondrial DNA encoded proteins and MnSOD expression. Eight to ten week old, male, Sprague-Dawley rats were exposed to 90 min of ischemia and 48 h of reperfusion using the filament method, while the sham animals received anesthesia without filament insertion. The Seahorse Bioscience XF24 analyzer was used to measure OCR in isolated MCAs following experimental stroke or sham operations using oligomycin, FCCP, antimycin, and rotenone. Western blotting was used to determine protein expression in the arteries. The protein normalized OCR (pmol/min/mg protein) was significantly (p < 0.05) decreased in Contra MCAs compared with Ipsi and sham, with no statistical differences between Ipsi and sham. The basal respiration (128 ± 15), ATP production (49 ± 6), protein leak (68 ± 10), maximal respiration (238 ± 23), and the non-mitochondrial respiration (31 ± 3) were decreased in Contra compared with Ipsi and sham MCAs (196 ± 13; 83 ± 6; 114 ± 10; 333 ± 14; and 56 ± 3, respectively). All of the beta-actin normalized protein levels of Complex-II, III, IV, and MnSOD but not Complex-I were higher in Ipsi MCAs (136 ± 3; 104 ± 10; 35 ± 4; and 7 ± 1, respectively) compared with Contra (121 ± 6; 90 ± 3; 13 ± 2; and 82 ± 3, respectively). These results extend our previous findings that mitochondrial function in Ipsi MCAs is preserved while it is severely impaired in Contra MCAs. Furthermore, our results indicate specific therapies may be appropriate. This work was supported by NIH grants HL-077731 and HL093554, AHA grants 14SDG20490359 and 15POST23040005, and the Louisiana Board of Regents Support Fund Research Competitive Subprogram LEQSF(2014-17)-RD-A-11.

12.4 ROLE OF O-GlcNA CYLATION IN REGULATING MITOPHAGY AND MITOCHONDRIAL FUNCTION IN CARDIOMYOCYTES

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The post-translational modification of proteins by O-linked-N-acetylglucosamine (O-GlcNAc) has been implicated to play a role in cardiovascular disease and mitochondrial function. Acute increases in O-GlcNAcylation have been shown to protect cardiomyocytes against oxidative stress which has been associated with O-GlcNAcylation of mitochondrial proteins. We have previously shown that acute increases in O-GlcNAc attenuate loss in mitochondrial membrane potential. Autophagy, a highly conserved cell survival mechanism, is activated in the heart in response to stress. O-GlcNAc has also been reported to increase in response to cellular damage, is involved in regulating mitophagy. AC16 cells, an immortalized human cardiac cell line, were treated with 2,3-dimethoxy-1,4-napthoquinone (DMNQ, 20 µM), a redox cycling agent which increases mitochondrial reactive oxygen species (ROS) to induce mitophagy. Decreases in O-GlcNAc attenuate loss in mitochondrial membrane potential. Autophagy, a highly conserved cell survival mechanism, is activated in the heart in response to stress. O-GlcNAc has also been reported to increase in response to cellular damage. We have previously shown that acute increases in O-GlcNAc attenuate loss in mitochondrial membrane potential. Mitochondrial dysfunction has been implicated as a cause for energy deprivation in heart failure (HF). Male C57BL6/J mice (12 weeks old) were administered L-NG-Nitroarginine methyl ester (L-NAME, 0.3 mg/ml with 1% NaCl) in the drinking water, or Angiotensin II (AngII, 0.7 mg/kg/day) via subcutaneous osmotic pumps or a combination of both (L-NAME+AngII) for 5 weeks. Cardiac function, protein expression, mitochondrial reactive oxygen species production and mitochondrial function in isolated mitochondria and permeabilized fibers in response to pyruvate-malate (PM), palmitoylcarnitine-malate (PC), succinate and glutamate-malate (GM) substrates were measured. ADP-independent, substrate-dependent respiratory rates (state 2),
ADP-supported respiration rates (state 3) and respiratory control ratio (RCR) (state 3/state 4) were calculated for ETC supercomplexes. Name+Angel induced the most severe phenotype of HF characterized by edema, hypertrophy (increased heart weight/tibia length ratio), fibrosis, increased blood pressure and reduced ejection fractions to ~40%. L-NAME+Angel treated mice had robust deterioration of cardiac mitochondrial function, as observed by reduced RCR for PM, PC and GM but not for succinate in subcellular mitochondria. Interstitial mitochondria, only state 3 rates were significantly reduced in the L-NAME+Angel group versus controls for PM, PC and GM but not for succinate. However, mitochondrial membrane potential was not significantly different among the 4 groups. Cardiac myofibrils from L-NAME+Angel mice had increased ADP-supported oxygen consumption, uncoupled respiration and oligomycin rates for PM+succinate. Further, mitochondrial DNA content was reduced in AngII and L-NAME+AngII hearts. Production of reactive oxygen species (H2O2) was the highest in AngII and L-NAME+AngII groups. Phospho-AMPK and phospho-akt were reduced in hearts of L-NAME+AngII and L-NAME+AngII groups. We conclude that combination of L-NAME+Angel exacerbates cardiac contractile and mitochondrial functional deregulation compared with L-NAME and AngII alone, resulting in non-ischemic HF.

12.7 MITOCHONDRIAL FUNCTIONS IN THE REGULATION OF EFFECTOR MACРОPHAGE IN CORONARY ARTERY DISEASE

Rafal Nazerziewicz,1,2 Tsuyoshi Shirai,1 David Harrison1, and Cornelia Weyand

Macrophages play a pivotal role in coronary artery disease (CAD). Cytokine production is one of the main effectors functions of the macrophages. Cytokines trigger local inflammation in arteries and thus contribute to the progression of CAD. There is evidence that cytokine production plays a regulatory role in effector macrophages. We also investigated the mitochondrial reactive oxygen species (ROS)-dependent signaling regulates cytokine production in hyperinflammatory CAD macrophages. We used monocyte-derived macrophages from CAD patients and healthy subjects to characterize basic mitochondrial functions, including cellular respiration, mitochondrial membrane potential, ROS production and mitochondrial morphology. We found that CAD macrophages have distinctive fragmented mitochondria when compared to controls indicating functional alterations. In fact, we found that resting (M0) and activated (M1) macrophages show significant differences in mitochondrial and mitochondrial functions. CAD macrophages had a higher metabolic rate associated with a higher uptake of glucose. This resulted in higher basal and maximal mitochondrial respiration. In line with this observation, we found over 30% higher mitochondrial potential and mitochondrial ROS production in CAD. Increased mitochondrial ROS production in CAD macrophages depleted reduced glutathione by over 50%. Next, we analyzed detailed molecular mechanisms behind observed dysfunctions and identified a main regulatory mechanism in the mitochondria responsible for the ROS overproduction. We also investigated what are the molecular targets for ROS from the mitochondria and its functional consequences. Increased ROS production and mitochondrial metabolism induced a proinflammatory phenotype in CAD macrophages, including intensified B-6 and B-1b production. Manipulations of mitochondrial ROS by targeting hydrogen peroxide with mitoEhelen reversed changes in effector functions of macrophages. Correcting mitochondrial dysfunctions by pharmacological intervention also reversed the proinflammatory phenotype in CAD macrophages. Taken together our study indicates a critical role for mitochondria in the regulation of effector functions and a proinflammatory phenotype in CAD macrophages.

12.8 MITOCHONDRIAL PERMEABILITY TRANSITION DRIVES ROS GENERATION ASSOCIATED WITH DEGRADATION OF ELECTRON TRANSFER CHAIN SUPERCOMPLEXES IN HEART ISCHEMIA-REPERFUSION

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Background: Sustained ischemia-reperfusion (IR) induces Ca2+ overload and enhances generation of reactive oxygen species (ROS) predominantly by damaged mitochondrial electron transfer chain (ETC) complexes. These alterations and high [Pi] due to increased ATP depletion induce mitochondrial permeability transition (MPT) accompanied by the opening of non-specific MPT pores (MPTP) in the inner mitochondrial membrane. In addition, oxidative stress caused by IR increases oxidation of cardiac lipoprotein, a unique mitochondrial phospholipid, and leads to destabilization of supercomplex, as well as to decrease of supercomplexes (SCs). SCs are the large supramolecular assembly of ETC complexes that provide highly efficient flux of electrons through the ETC. Consequently, SCs increase ATP synthesis and significantly reduce electron leakage and ROS production due to short diffusion distances between ETC complexes. Both opening of MPTP and degradation of SCs are central players to initiate mitochondrial-mediated cell death, however the contribution of MPTP to SCs disassembly remains unclear. Hypothesis: MPT-induced ROS is associated with SCs dissociation in cardiac IR. Methods: The relationship between MPT, ROS and SCs were investigated using Langendorff rat hearts with or without 25 min of warm ischemia followed by 5 min or 60 min reperfusion in the presence or absence of the MPT inhibitor, sanglifehrin A (SA). Calcium-induced swelling of mitochondria was monitored to assess MPT opening. SCs were analyzed by Blue native electrophoresis followed by 2D SDS-PAGE was used to analyze individual ETC complexes and SCs. ROS levels were measured with Amplex Red. Results: Cardiac ischemia followed by 5 and 60 min reperfusion induced MPT opening and ROS generation. The production of ROS was inhibited by SA indicating that it is MPT-dependent. 2D SDS PAGE revealed that over 50% of complex I was involved in the SC I+III+IV while only ~10% of the complex remained unbound. Percent distributions of SCs were significantly affected by IR and the effects were dependent on the reperfusion time. We found a high SC I+IV and low SC I+II associated with increased complex 1 proportion at early (5-min) reperfusion. The changes remained after 60 min of reperfusion. Conclusion: Cardiac IR exerts various effects on mitochondrial SCs depending on reperfusion time. MPT-induced ROS presumably plays a key role in SCs disassembly. Funding sources: NHLBI NIH Grant SC1HL118669 (to SJ).

12.9 MITOCHONDRIAL RESPIRATION AND CALCIUM ACTIVATION ARE MAINTAINED IN THE PRESENCE OF HEART FAILURE LEVELS OF EXTRAMITOCHONDRIAL SODIUM

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An increase in cytosolic Na+ is a hallmark of heart failure. Elevated Na+ is thought to decrease mitochondrial matrix Ca2+ via the Na+–Ca2+ exchanger, inhibiting the Ca2+ activation of mitochondrial ATP production. However, the impact of elevated Na+ on mitochondrial respiration, matrix redox potential, and membrane potential (ΔΨm) during an acute increase in Ca2+ remains unknown. Isolated adult male rat ventricular mitochondria were exposed at maximal (State 3) and intermediate oxygen consumption rates (JL), achieved by modifying the Gibbs free energy of ATP (ΔGATP). ΔΨm was measured with a TPP+ electrode and matrix redox potential (NADH) was monitored using UV excitation and measuring emission with a spectrometer.

Mitochondria were incubated with normal (5 mM) or heart failure (15 mM) levels of NaCl, with and without added CaCl2 (940 mM free Ca2+). Maximal respiration rate was the rate at which whether mitochondria were incubated with 5 or 15 mM NaCl (215±28 vs 244±66 nmol O2/mg/min), with the addition of Ca2+ increasing respiration to the same level with either Na+ concentration (360±40 vs 342±10 nmol O2/mg/min). Additionally, the slope of ΔGATP vs JL relationship, a measure of whole mitochondrial conductance when fuel is saturating, did not differ between 5 and 15 mM Na+ (53±14 and 53±17). Ca2+ increased the slope of ΔGATP vs JL with either 5 or 15 mM Na+ (95±7 and 100±22). At either Na+ concentration, the matrix redox potential was constant across all values of JL with the NADH/NAD+ pool 20±5% reduced at a ΔGATP of -13.1 kcal/mol and 21±5% reduced at a ΔGATP of -14.4 kcal/mol. With Ca2+, the NADH/NAD+ pool was 24±3% reduced at a ΔGATP of -13.1 kcal/mol, but reduction increased to 41±1% at a ΔGATP of -14.4 kcal/mol at either Na+ level. The addition of Ca2+ to either Na+ concentration increased conductance (the effective activity) of the electron transport chain, shown by a 2.5-fold increase in the slope of the relationship between JL and the free energy difference between NADH and ΔΨm, as well as conductance of mitochondrial ATP production and transport (ΔΨm to ΔGATP). Healthy or failing heart [Ca2+]i activates respiration, and acute increases in Ca2+ activate respiration and increase the conductance of the oxidative phosphorylation pathway to the same level regardless of Na+ concentration. This implicates the importance of Ca2+ import via the mitochondrial Ca2+ unporter, potentially compensating for Na+-impairments. NIH (R01-HL095828A) to MWK; AHA (14POST20490181) to SKG.

12.10 AN ELECTRICALLY CONDUCTIVE MITOCHONDRIAL RETICULUM IN SKELETAL MUSCLE

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1MD, 20892.
ROLE OF MITOCHONDRIAL STRUCTURE, FUNCTION AND REDOX SIGNALING IN MEGAKARYOPOIESIS

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Platelets are circulating cellular fragments that play an important role in hemostasis, thrombosis and inflammation. Platelets contain low numbers of fully functional mitochondria and we have recently shown that these mitochondria play an important role in platelet thrombotic function. Specifically, we have shown that platelets from patients with Sickle Cell Disease (SCD) show inhibited mitochondrial complex V activity, which leads to increased mitochondrial reactive oxygen species production and subsequent thrombotic activation. Notably, while mitochondria contribute to mature platelet thrombotic function, it is unknown whether mitochondrial function determines platelet maturation and differentiation. Megakaryopoiesis is the complex process of producing mature megakaryocytes from hematopoietic stem cells. Platelets are shed from the sides of specialized projections of megakaryocyte cytoplasm called proplatelets. Importantly, the role of mitochondrial function in megakaryopoiesis is unknown. We hypothesize that early bioenergetic function is required for megakaryocyte differentiation into platelets and that mitochondrial function changes as megakaryocytes differentiate into platelets. Here, we measure mitochondrial function in human megakaryocytes as they differentiate into a platelet phenotype and find that there are distinct differences in oxidative phosphorylation over the differentiation process. We present preliminary results showing the bioenergetics profile of primary human megakaryocytes undergoing megakaryopoiesis. This work is significant since understanding the role of mitochondria in megakaryopoiesis will provide potential new targets and therapeutic approaches to modulate thrombopoiesis and thrombocytosis. In addition, these studies will help us further define the general applicability of platelet mitochondrial functional assays in SCD and other diseases that affect systemic or circulatory oxidant stress. Funding Source: Center for Metabolism and Mitochondrial Medicine.

12.12 EFFECTOR T CELLS UPRGULATE MITOCHONDRIAL METABOLISM DURING GRAFT-VERSUS-HOST DISEASE

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Hypothesis: During GVHD, effector T cells increase mitochondrial mass and oxidative metabolism. Methods: During GVHD, effector T cells increased mitochondrial mass as early as day 3 post-transplant compared to naive cells (MFI 3.0 vs. 0.9, p < 0.0001). This increase was substantiated by a concomitant elevation in mRNAs (2.7±0.52 vs. 1.0±0.07, p < 0.0001). All effector cells also increased protein levels of the mitochondrial protein VPDAC and the co-activator PGC-1α (1.78 vs. 0.36, relative density vs. naive cells, p < 0.0001). Notably, PGC-1α levels were minimally increased in T cells undergoing homoeostatic proliferation. GVHD T cells also enhanced their mitochondrial metabolism, with increased cellular and mitochondrial ROS levels versus naive/homeostatic cells (relative MFI 3.1 vs. 1.0, p < 0.0001). Increased ROS levels were directly tied to FAO, as ROS levels fell with etomoxir treatment. Finally, when PGC-1α T cells were stimulated in vitro in a mixed leukocyte reaction, there was a marked decrease in well-divided PGC-1α T cells, while undivided T cell numbers remained similar between wildtype and PGC-1α groups.

Conclusions: During GVHD, effector T cells up-regulate both mitochondrial mass...
and mitochondrial metabolism. In addition, PGC-1α, a driver of mitochondrial bio-
genesis, is required for survival of rapidly proliferating allogeneic T cells in vitro. Future studies will determine if modulation of PGC-1α, or its downstream targets, can mitigate GVHD and make blood and marrow transplantation a safer and more effective therapy.

12.14 EVIDENCE FOR INVOLVEMENT OF MITOCHONDRIAL MATRIX ROS AND HYPOXIA-INDUCIBLE FACTOR-1 IN THE GROWTH INHIBITORY EFFECT OF RESVERATROL
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Despite the well-known inhibitory effect of RES on cell growth, the molecular mechanism(s) behind it is not fully understood. We have shown that RES’s inhibition of cell growth is dependent upon Mn-superoxide dismutase (MnSOD) induction and an active mitochondrial respiratory chain (Robb, E.L. and Stuart, J.A. 2011; Robb E.L. and Stuart, J.A. 2014). These results suggest that mitochondrial matrix ROS is involved in the inhibition of cell growth caused by RES and similar molecules. A possible downstream target of mitochondrial ROS could link the modulation of mitochondrial matrix ROS to growth inhibition. This is the hypothesis inducible factor (HIF1). HIF1 is a heterodimeric transcription factor, which is redox-regulated via its HIF1α subunit. Mitochondrial ROS, including MnSOD levels specifically, have been implicated in HIF-1α stabilization (Kaewpila, S. et al., 2008), and HIF-1 stabilization has in turn been implicated in the growth of various cancer cells. We found that RES’s inhibition of PC3 (prostate cancer) cell growth was abolished when HIF-1α was stabilized by CoCl2 (a hypoxia mimetic) or IOX2 (a prolyl hydroxylase inhibitor). This may be linked to HIF-1’s induction of glycolytic machinery, as the expression of some HIF-1α gene targets was reduced in cells treated with RES. Interestingly, growth of PC3 cells in galactose media, which forces greater reliance on oxidative phosphorylation and prevents reliance on the glucose fermentation promoted by HIF-1, was not inhibited by RES. Also, the effects of RES on PC3 cell growth were substantially greater in cells grown under hypoxic conditions (as low as 0.4% O2). Together, these results are consistent with a role for mitochondrial matrix ROS, MnSOD, and HIF-1 in the cell growth inhibitory effects of RES. Research funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant.

12.15 MITOCHONDRIAL MOTILITY RESPONSE TO NUTRIENT ENVIRONMENT IN THE PANCREATIC Beta-CELL: ROLE OF MILTON1 NUTRIENT-SENSING THROUGH O-GlcNAc MODIFICATION

Kyle Trudell1, Gakuei Pekkarinen1, Samuel Sereda1, Thomas Schwarz1, and Oriam Shirihai1

Our previous work has shown that chronic exposure to high glucose and fatty acids, termed glucolipotoxicity (GLT), inhibits mitochondrial fusion and networking in the pancreatic beta-cell. However, it remains unclear how GLT affects mitochondrial motility in the beta-cell. Importantly, it is not fully understood what is the mechanism connecting nutrient availability to changes in mitochondrial motility and dynamics. Therefore we hypothesized that availability of glucose and fatty acids would modulate mitochondrial motility in the pancreatic beta-cell. We further hypothesized that changes to activity of Milton1, a key mitochondrial adaptor protein essential for motility, may be an essential mediator connecting nutrient availability to changes to activity of Milton1, a key mitochondrial adaptor protein essential for motility, and dynamics in the pancreatic beta-cell. To assess changes to mitochondrial motility in INS1 cells (beta-cell line), we performed time-lapse imaging and analyzed mitochondrial displacement. Exposure to GLT decreased mitochondrial motility, as assessed by reduction in average mitochondrial displacement. We found that O-GlcNAcylated, a regulatory mechanism which links protein activity to nutrient status, of Milton1 was increased by high glucose, fatty acids, or treatment with O-GlcNAcase inhibitor, PUGNAc. When wildtype Milton1 (Milt-WT) was over-expressed in INS1 or mouse islets, mitochondrial morphology was altered towards more elongated and aggregated architecture. Increasing cellular O-GlcNAc levels by high glucose or PUGNAc reversed this Milt-WT-induced phenotype, suggesting decreased Milton1 activity upon O-GlcNAcylated. Over-expressing a mutated form of Milton1 that is resistant to O-GlcNAcylated (Milt-Qmt) showed similar effect on mitochondrial elongation and aggregation, but was insensitive to high nutrient exposure. Accordingly, Milt-Qmt preserved mitochondrial connectivity and protected from GLT-induced cell death. However, Milt-Qmt expression in INS1 cells or mouse islets inhibited acute (~1 hour) glucose-induced stimulation of mitochondrial oxygen consumption and insulin secretion, suggesting O-GlcNAcylated of Milton1 may also play a regulatory role during glucose-stimulated insulin secretion in the beta-cell. Collectively, our findings suggest that changes to mitochondrial motility under a chronic high nutrient environment may contribute to mitochondrial and beta-cell dysfunction. Importantly, nutrient-sensing of Milton1 activity via O-GlcNAc modification is a key player connecting nutrient status to mitochondrial motility and dynamics in the pancreatic beta-cell. K.T. was supported by a National Science Foundation Graduate Research Fellowship under Grant No. DGE-0741448, and Levinsky Fellowship from Boston University School of Medicine. O.S. is funded by NIH grants RO1 DK35914, R01 DK56690, and R01 DK074778.

12.16 MITOCHONDRIAL FRAGMENTATION IN RESPONSE TO GLUCOLIPOTOXICITY REPRESENTS A COMPENSATORY ADAPTATION TO MAINTAIN BETA-CELL FUNCTION

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We have previously reported that high glucose and fatty acids, termed glucolipotoxicity (GLT), inhibits mitochondrial fragmentation and networking in cultured INS1 cells and mouse islets. However, it remains unclear if GLT causes mitochondrial fragmentation in human islets and whether this fragmentation plays a compensatory or pathological role during GLT-induced beta-cell dysfunction. To address if GLT alters mitochondrial shape in human beta-cells, islets were cultured in media with normal nutrients (5mM glucose, 10% BSA) or media with high glucose (11.2 mM) and fatty acids (1mM oleate and palmitate) for 4 days. Assessing mitochondrial morphology and membrane potential (MMP) by confocal imaging revealed that GLT significantly fragments mitochondria and increases MMP heterogeneity in human beta-cells. Concomitantly, glucose-stimulated insulin secretion (GSIS) was inhibited. Genetically inducing mitochondrial fragmentation by decreasing levels of mitochondrial fusion protein, Mfn2, in INS1 cells protected from GLT-induced cell death. Conversely, preventing GLT-induced fragmentation by expression dominant-negative construct of mitochondrial fusion protein, DRP1 (DRP1-DN), further disrupted MMP and causes accumulation of depolarized mitochondria in human beta-cells. Moreover, GLT-induced inhibition of GSIS is further decreased in human islets expressing DRP1-DN. In conclusion, mitochondrial fragmentation in response to GLT represents a compensatory adaptation to GLT insult, which works to preserve mitochondrial and beta-cell function. K.T. was supported by a National Science Foundation Graduate Research Fellowship under Grant No. DGE-0741448, and Levinsky Fellowship from the department of Medicine at Boston University School of Medicine. O.S. is funded by NIH grants RO1 DK35914, R01 DK56690, and R01 DK074778.
12.18 **KNOCKDOWN OF VOLTAGE-DEPENDENT ANION CHANNELS 1 AND 2 INHIBITS MITOCHONDRIAL FISSION BY DECREASING BINDING OF DYNAMIN-RELATED PROTEIN 1 TO MITOCHONDRIA**

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**Background:** In cancer cells, mitochondria continuously undergo fission and fusion. Mitochondrial fission involves the binding of dynamin-related protein-1 (Drp1) with mitochondrial fission factor (Mff) and fusion 1 protein (Fis1) located in the mitochondrial outer membrane (MOM). Voltage dependent anion channels (VDAC), the most abundant proteins in MOM, comprise 3 isoforms - VDAC1, VDAC2 and VDAC3. Previously in HepG2 cells, VDAC1/2 double knockdown promoted mitochondrial fusion. Here, we hypothesize that VDAC1 and VDAC2 are anchors for Drp1 and that VDAC1/2 double knockdown inhibits mitochondrial fission by preventing Drp1 binding to mitochondria. Our aim was to evaluate the effects of single and double VDAC knockdowns on mitochondrial Drp1 binding, mitochondrial membrane potential (ΔΨm) and morphology. **Methods:** HepG2 hepatoma cells were treated with siRNA to generate single and double knockdowns of VDAC1/2/3 in all combinations. As a positive control, mitochondrial fission was induced by the uncoupler CCCP (5 µM). Mitochondrial ΔΨm was assessed by confocal mi-croscopy of tetramethylrhodamine methylster (TMRR). Immunofluorescence using primary antibodies against Fis1, Drp1 and Tom20 assessed subcellular localization of Fis1 and Drp1. The Duolink proximity ligation assay was used to determine interactions of Drp1 with VDAC1 or VDAC2. **Results:** In wild type cells, mitochondria were short, branched and filamentous and indistinguishable from non-target siRNA and single knockdown of each VDAC isoform. After double VDAC1/2 knockdown but not VDAC2/3 or VDAC1/3 knockdown, mitochondrial filaments became longer and larger in diameter. Additionally, ΔΨm increased after VDAC1/2 double knockdown. Localization of Drp1 in hepatocytes by confocal microscopy revealed that VDAC1/2 double knockdown displaced Drp1 from the mitochondrial MOM. In HepG2 cells transfected with non-target siRNA but not in VDAC1/2 double knockdown cells, CCCP increased Drp1 binding to mitochondria. Knockdown of VDAC1/2 also decreased Duolink interaction of Drp1 with VDAC1 and VDAC2 both before and after treatment with CCCP. **Conclusion:** VDAC1/2 double knockdown promotes mitochondrial fusion by inhibiting Drp1-driven mitochondrial fission, and VDAC1 and VDAC2 appear to serve as anchors for Drp1 translocation to mitochondria. ACS 13-043-01 and CORRE Pilot GM103542 (ENM), T32DK083262 (DNB), AA022815, AA02191 and 14Z50.31.0028 (JJL).

12.19 **EFFECTS OF LOW LEVEL LASER THERAPY ON TENOCYTES IN HIGH GLUCOSE ENVIRONMENT**

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**Background:** High glucose (HG) environment acts as a potent inducer of cell dysfunction. In cancer cells, mitochondria continuously undergo fusion and fission. Assessing the expression of the mitochondrial fission protein Drp1 revealed that these cells display high levels of a lower molecular weight splice variant of Drp1 (~70Da). Current studies are underway to identify the molecular identity of the shorter Drp1 variant and its potential effects on compromised mitochondrial slicing observed in these cells. Further, larger scale population studies will reveal if mitochondrial dysfunction plays a significant role in HGSA. This may aid in the development of targeted glycolysis-based therapeutics for a sub-population of ovarian cancer cases.

12.20 **SCREENING ASCITES-DERIVED OVARIAN CANCER CELLS FOR HISTOLOGICAL SUBTYPE-SPECIFIC BIOENERGETIC SIGNATURES AND MITOCHONDRIAL DYSFUNCTION**

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We have previously established that ovarian cancer cell lines of different histological origins are characterized by distinct bioenergetics signatures (Dier et al. 2014 PlOS ONE). In particular, Ovarian Clear Cell Carcinomas (OCCC) display high oxygen consumption rate (OCR) and glycolytic flux compared to the more common high grade serous adenocarcinoma (HGSa) subtype. To assess if these findings are clinically relevant we used extracellular flux analysis to compare the bioenergetics profile of ovarian cancer patient ascites derived tumor cells. Similar to OCCC, high metabolic activity was associated with tumor cells isolated from a patient diagnosed with endometrioid ovarian cancer, a histological subtype that shares similarities in tissues of origin with OCCC. These data add to the notion that the term ovarian cancer comprises distinct diseases, with different gene expression, as well as metabolic signature. Further, using extracellular flux analysis a HGSa cell line (OVA420) was identified with compromised oxygen consumption. One of six ascites specimens similarly shared a lack of mitochondrial OCR and inability to respond to OCR stimulation by the mitochondrial uncoupler FCCP, suggesting that mitochondrial dysfunction may be associated with a sub-population of ovarian cancer adenocarcinomas. OVA420 cells and the HGSa specimen with compromised mitochondrial function displayed mitochondrial morphology changes, indicative of dysfunction in mitochondrial fusion/fission. Assessing the expression of the mitochondrial fission protein Drp1 revealed that these cells display high levels of a lower molecular weight splice variant of Drp1 (~70Da). Current studies are underway to identify the molecular identity of the shorter Drp1 variant and its potential effects on compromised mitochondrial slicing observed in these cells. Further, larger scale population studies will reveal if mitochondrial dysfunction plays a significant role in HGSa. This may aid in the development of targeted glycolysis-based therapeutics for a sub-population of ovarian cancer cases.
Mitochondria control cellular homeostasis through maintaining proper bioenergetic programs and signaling mechanisms. Mitochondrial dysfunction in chronic metabolic and inflammatory diseases such as obesity, diabetes, chronic kidney disease etc. underscores the significance of this organelle in maintaining normal health. Mitochon-
dria integrate the cellular bioenergetic program, a unique set of bioenergetic relationships between the individual parameters of the oxidative phosphorylation (basal, ATP-linked, proton-leak, maximal, reserve capacity and non-mitochondrial respi-
ration). These parameters demonstrate distinct aspects of mitochondrial function and their interdependence. Alteration in this program causes cellular stress and diseases, which suggests that identifying the defects associated with the bioenergetic program can be used to explain mechanisms of mitochondrial dysfunction in chronic in-
flammatory diseases. The objective of the study is to determine the bioenergetic health index (BHI, a single number that integrates the bioenergetic parameters) and relationships between the parameters that form the bioenergetic program in healthy subjects and chronic kidney disease patients. Methods: Using the mitochondrial stress test, bioenergetic parameters were determined in peripheral blood monocytes that are freshly isolated from healthy subjects (n=50) and chronic kidney disease patients (n=40) using the extracellular flux analyzer. The BHI was determined using the formula BHI = (Reserve Capacity x ATP-Linked Respiration)/(Proton Leak x Non-
Mitochondrial Respiration). The bioenergetic parameters were compared using multi-
variate analysis and linear regression methods. Results: Compared to the healthy sub-
jects, chronic kidney disease patients demonstrate a significantly lower BHI. Multi-
variate analysis of the bioenergetic parameters in healthy subjects show a high corre-
lation between basal mitochondrial respiration and ATP-linked respiration (R²=0.92, p<0.0001). Basal respiration is also correlated well with maximal (R²=0.428, p<0.00001) and with non-mitochondrial respiration (R²=0.437, p<0.00002). In chronic kidney disease, the correlation between basal respiration and ATP-linked respiration remains strong, but the correlation that existed between other bioenergetic parameters weakened considerably. Conclusion: These novel findings suggest that BHI can be used to determine the bioenergetic health of individual subjects. The distinct relationships between mitochondrial bioenergetic parameters suggest their potential utility in gaining insights into the mechanisms of diseases with bioenergetic dysfunction.

12.22 MITOCIONDIAL RESPIRATORY CAPACITY AND COUPLING CONTROL DECLINE WITH AGE IN HUMAN SKELETAL MUSCLE

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Mitochondrial health is critical to physiological function, particularly in tissues with high ATP turnover, such as striated muscle. It has been postulated that derangements in skeletal muscle mitochondrial function contribute to impaired physical function in older adults. Here, we determined mitochondrial respiratory capacity and coupling control in skeletal muscle biopsies obtained from young and older adults. Twenty four young (28±7 yrs) and thirty one older (62±8 yrs) adults were studied. Mitochondrial respiration was determined in permeabilized fibers from the m. vastus lateralis, either after the addition of substrates followed by either oligomycin or cyanide m-chloro-
phenyl hydrizide (CCCP). Thereafter, mitochondrial coupling control was calculated from the flux control ratios for CCCP and the coupling control ratio and factor for oligomycin. Maximal coupled respiration (respiration linked to ATP production) was lower in muscle from older vs. young subjects (53.3±3.2 vs. 40.9±3.1 pmol/sec;mg; P<0.01), as was maximal uncoupled respiration (61.5±4.6 vs. 50.7±3.4 pmol/sec;mg; P<0.05). Coupling control in response to the ATP synthase inhibitor oligomycin was lower in older adults (P<0.05), as was the mitochondrial flux control ratio, coupled respiration normalized to maximal uncoupled respiration (P<0.05). Calculation of respiratory function revealed lower respiration linked to ATP production (34.0±3.6 vs. 17.7±2.0 pmol/sec;mg; P<0.001) and greater reserve respiration (6.2±1.5 vs. 10.1±1.1 pmol/sec;mg; P<0.01), i.e. respiratory capacity not used for phosphorylation in muscle from older adults. We conclude that skeletal muscle mitochondrial respiratory capacity and coupling control decline with age. Lower respiratory capacity and coupling efficiency result in a reduced capacity for ATP production in skeletal muscle of older adults. This work was supported by the National Institutes of Health and Shriners of America.

12.23 INTERFERENCE WITH MITOCIONDIAL BIOENER-
GETICS BY TPP-IOA, A MITOCIONDRIA-TARGETED ANTI-APOPTOTIC INHIBITOR OF CYTOCHROME C PEROXIDASE ACTIVITY

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Recently, 3-hydroxypropyl-triethylphosphonium-conjugated imidazole-substituted oleic acid (TPP-IOA) was designed as an anti-apoptotic molecule targeting cyto-
chrome c’s redox-active catalytic site and thereby inhibiting a peroxidase activity that has been linked to apoptosis (Atkinson et al., Nature Comm. 2011; 2:497). TPP-IOA effectively mitigates radiation-induced death in both cell culture and animal models and thus may have therapeutic potential in pathological scenarios involving apoptotic cell death. However, many such possible scenarios require that TPP-IOA not be toxic to mitochondrial ATP production, and therefore it is essential to understand TPP-
IOA’s potential effects on oxidative phosphorylation. Using purified cytochrome c, isolated mitochondria, and cultured cells, we determined whether TPP-IOA can in-
hibit pro-apoptotic events and cell death without impairing mitochondrial bioenerget-
icity. Assessments with pure cytochrome c revealed that TPP-IOA inhibits cytochrome c peroxidase activity at doses marginally lower than those that interfered with cyto-
chrome c electron transfer rates. However, in isolated rat liver mitochondria TPP-IOA inhibited peroxidase activity at doses similar to those that perturbed electron transport and apparent proton leak. Since TPP-IOA affected oxidative phosphorylation at concentra-
tions similar to those inhibiting cytochrome c peroxidase activity, we compared the protection against cell death conferred to cells grown in galactose/glutamine media (promoting oxidative phosphorylation) versus those grown in glucose media (allowing glucose fermentation). Initial findings suggest that TPP-IOA is effective at in-
hibiting cell death in cells that are less reliant on oxidative phosphorylation. These findings suggest that therapeutic applications of TPP-IOA may be limited to patholo-
gies involving more glycolytic cell types (e.g. stem cells affected by radiation). Ad-
ditionally, this work highlights the general importance of evaluating the efficacy of mitochondria-targeted small molecules using cell culture models that are more reliant on mitochondrial respiration.

12.24 CHRONIC ALCOHOL EXPOSURE INCREASES SUS-
CEPTIBILITY TO OXIDATIVE STRESS IN HEPATO-
CYTES

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Chronic alcohol consumption decreases the expression and activity of the proteins in oxidative phosphorylation system resulting in differential sensitivity to mitochondrial specific modulators including nitric oxide. It is known that the lipid peroxidation product 4-Hydroxynonenal (4-HNE) is elevated in hepatocytes in response to chronic alcohol consumption and in other cell types and that it can cause mitochondrial dys-
function. We hypothesized that the decreased bioenergetic capacity in hepatocytes exposed to chronic alcohol will be more susceptible to 4-HNE and other hepatotoxins such as acetaminophen (APAP), an important over-the-counter pain reliever with high hepatotoxic potential. This was tested in a rat model of chronic alcohol con-
sumption using the Leber-Delmas pair fed control and ethanol feeding regimen. Hep-
tocytes were isolated from the control and alcohol-fed rats and the mitochondrial stress test was performed to establish the basal bioenergetic profile. Under control conditions we found that maximal respiration and reserve capacity was decreased in the hepatocytes from the chronic alcohol consuming animals. Next, we assessed the susceptibility to increasing concentrations of 4-HNE and APAP. In control hepato-
tocytes there was a concentration dependent decrease initially in reserve capacity but persistent and at 2 hr bioenergetic parameters were completely lost. APAP at 10 mM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed. At 100µM concentration was a profound change in bioenergetics observed.
and APAP. This work was supported by National Institute of Health grant R01 A1018841 (to SM Bailey) and A1013395 (to VM Darley-Usmar).

12.25 INCREASE MITOCHONDRIAL UNCOUPLING IN STORED PLATELETS
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Stored platelet concentrates have been shown to have decreased quality which could ultimately lead to worsening patient outcomes. This phenomenon termed the platelet storage lesion is characterized by the change in cell morphology, decreased aggregation, an increased glycolytic rate, and decreased mitochondrial function, the mechanisms of which are not clearly understood. In the present study, we measured the functional changes in mitochondrial and glycolytic function between freshly isolated and stored platelet concentrates. We used platelets between storage days 6-9 and measured mitochondrial and glycolytic bioenergetics using the Seahorse XF technology. Stored platelets showed decreased recovery after hypotonic stress compared to freshly isolated platelets although stored platelets did not show any differences in thrombin-mediated aggregation. The bioenergetic health index (BHI), an index of overall health of the platelets was decreased in stored platelets which were ascribed to a 10% decrease in basal oxygen rate, a 190% increase in proton leak and no change in maximal oxygen consumption compared to the freshly isolated cells. When mitochondrial ATP production was inhibited, the glycolytic rate was increased in stored platelets. In summary, stored platelet concentrates showed a decrease in oxidative phosphorylation that was predominately driven by an increase in mitochondrial proton leak.

12.26 CROSSTALK BETWEEN MITOCHONDRIAL ACETYL-COA METABOLISM, CYTOSKELETON MODIFICATIONS AND AUTOPHagy
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Lysine acetylation, a well characterized post-translational modification, is tightly coupled to the nutritional status of the cell. This connection occurs as the availability of the main substrate for acetylation, acetyl-CoA, fluctuates greatly with changing metabolic conditions. Recent studies have demonstrated that acetyl-CoA levels act as an indicator of cellular nutrient status, and increased abundance of this metabolite can serve as an indicator of cellular nutrient status, and increased abundance of this metabolite can block the induction of cellular recycling programs. Here we investigated the crosstalk between mitochondrial metabolic pathways and autophagy, using biochemical inducers of mitochondrial acetyl-CoA production. Treatment of cells with one compound, a co-factor of several mitochondrial metabolic protein complexes, led to the unexpected hyperacetylation of α-Tubulin in the cytosol. This acetylation was catalyzed by the α-Tubulin acetyltransferase, αTAT, and was dependent on a less function of the cytosolic deacetylase, HDAC6. Finally, we show that α-Tubulin hyperacetylation alters the flux of substrates through autophagy-related pathways, which may limit the ability of cells to remove dysfunctional mitochondria through autophagic mechanisms. Based on these results, we hypothesize that mitochondrial sources may act as a modulator of cellular recycling pathways, by regulating the cytoskeletal transport of substrates to the autophagy machinery. This work is funded in part by National Institutes of Health Award R01HL116729.

12.27 STUDY ON THE EFFECTS OF ALCOHOL AND CANNABINOL TREATMENT ON HYPOTHALAMIC PITUITARY GONADAL AXIS IN MALE WISTAR RATS
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This study investigated the effects of oral administration of alcohol and cannabinoil on the hypothalamic pituitary gonadal system in male adult rats. Twenty five male rats were purchased from NIPRD and were divided into five groups containing five rats each and were treated for a period of 48 days. Ethical approval was obtained from National Institute for Pharmaceutical Research and Development (NIPRD) and the experiment conducted was in conformance with guidelines for experimental procedures as set forth in the Declaration of Helsinki and the APS Guiding Principles in the care and Use of Animals. Group one serves as the control, group two was administered 5mg/kg body weight methanol, group three was given 3mg/kg body weight as 25%/v/v alcohol, group four was given 10mg/kg body weight cannabinoil and group five was treated with alcohol (3g/kg body weight as 20%/v/v) and cannabinoil (1mg/kg body weight). Drug administration was via oral gavage thrice daily throughout the experimental period. At the end of the experimental period, blood was collected via the retro-orbital sinus under anaesthesia and was allowed to clot for hormonal assay and the brain was dissected and immediately fixed. Semen analysis was carried out by exposing the testis together with the epididymis and the epididymis was carefully separated and caput was removed. The caput was then transferred unto a pre-warmed slide and lacerated to release some semen unto the slide surface. The animals were anesthetized and sacrificed for dissecting the testes and epididymides. The epididymides and testes were removed and weighed immediately. There was no significant change in the body weight, however, there was a significant change in the percentage weight difference in the experimental groups when compared with the control group. Serum level of testosterone of the groups treated with alcohol, cannabinoil, alcohol plus cannabinoil were significantly decreased (p<0.05) when compared with the control rats. However, there were reduction in sperm motility and sperm count of rats exposed to alcohol, cannabinoil alcohol plus cannabinoil. The results suggest that alcohol and cannabinoil administration have deleterious effect on male reproductive activities (system) in rats. Keyword: alcohol, cannabinoil, HPG-axis, histology, morpheme, sperm content, hormone profile.

12.28 REGULATION OF CARDIAC AUTOPHAGY BY ADIPONECtin UNDER HYPOXIC/ISCHEMIC STRESS
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Adiponectin is a hormone secreted from adipose tissue which confers anti-inflammato-y, anti-diabetic and cardioprotective effects. The role of autophagy in myocardial dysfunction and heart failure is now apparent, and recent work indicates regulation of autophagy by adiponectin is of functional significance. We used Adiponectin knock-out (Ad-KO) mice ± ischemia induced by coronary artery ligation (CAL) and H9A2 cardiomyocytes ± hypoxia to investigate the significance of adiponectin in regulating autophagy. We used Western blotting for LC3-II and H9C2 cells stably expressing tandem RFP/GFP-LC3 to show increased autophagic flux in response to adiponectin. This was confirmed by analysis of DQ-BSA degradation and transmission electron microscopy. Using the mouse model of cardiac ischemia, Western blotting analysis of LC3 and p62 indicated less autophagic clearance after CAL in Ad-KO versus wild type mice. Importantly, these changes in autophagy corresponded with enhanced CAL-induced necrosis and apoptosis in Ad-KO mice, as shown by HMGB-1 and cleaved caspase-3 levels, respectively. We also found higher bcl2/bcl2-2 ratio in ischemic Ad-KO hearts and levels of the pro-apoptotic protein Bax were induced to a greater extent by ischemia in Ad-KO versus wild type mice. Echocardiography analysis showed that CAL-induced cardiac dysfunction was exaggerated in Ad-KO mice. In conclusion, our data suggests that adiponectin is an important mediator of autophagic flux in cardiomyocytes and that lack of autophagic activity in hearts of Ad-KO mice after ischemia contributes to enhanced cell death and cardiac dysfunction.

12.29 LIPOCALIN-2 REGULATES CARDIOMYOCYTE AUTOPHAGY TO CONTROL APOTOSIS AND INSULIN SENSITIVITY
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Lipocalin-2 (Lcn2) also termed neutrophil gelatinase-associated lipocalin (NGAL) is a proinflammatory adipokine which has become established as an important biomarker for kidney disease. Here we propose that Lcn2 links the pathogenesis of heart failure and as a potential biomarker. Here we investigated its direct effects on autophagy in H9C2 cardiomyocytes and the functional consequences. After treating H9C2 cells with recombinant Lcn2 (1 μg/ml, 1 hour) we used transmission electron microscopy, Western blotting and immunofluorescence for LC3-I, stable overexpression of tandem fluorescent RFP/GFP-LC3, DQ-BSA degradation and MagicRed assay for lysosomal cathepsin activity to show that Lcn2 reduced autophagic flux. Lcn2 also reduced phosphoULK1 S555, increased phosphoULK1 S757. Importantly, this correlated with reduced insulin sensitivity. We then created an autophagy-deficient H9C2 cell model by overexpressing a dominant-negative Atg5 mutant and found that reduced autophagy levels also induced insulin resistance, and that adding rapamycin after Lcn2 could stimulate autophagy and recover insulin sensitivity. We also observed that long-term Lcn2 treatment contributed to hypoxia/reoxygenation-induced apoptosis in H9C2 cells via reducing autophagy. We have also shown that Lcn2 in-
increased intracellular iron levels, and reactive oxygen species production, to mediate pro-apoptotic effects. In conclusion, our study indicated that Lcn2 treatment caused insulin resistance and apoptosis and the use of gain and loss of function approaches elucidated a causative link between autophagy and these effects of Lcn2.

13.0 MITOCHONDRIAL GENETIC AND METABOLIC PROGRAMS

13.1 NOVEL SIGNALING PEPTIDES FROM THE MITOCHONDRIAL GENOME
Changhan David Lee1
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Mitochondria are known to be functional organelles, but their role as a signaling unit is increasingly being appreciated. We have recently identified a short open reading frame (sORF) within the mitochondrial 12S rRNA encoding a 16 amino acid peptide named MOTS-c (mitochondrial open-reading-frame of the twelve S rRNA -c) that regulates insulin sensitivity and metabolic homeostasis [1-3]. Its primary target organ appears to be the skeletal muscle and its cellular actions inhibit the folate cycle and its tethered de novo purine biosynthesis, causing a significant accumulation of AICAR levels concomitantly with AMPK activation. MOTS-c treatment in mice prevented age-dependent and high-fat diet-induced insulin resistance, as well as diet-induced obesity. These results suggest that mitochondria may be more actively engaged in regulating metabolic homeostasis than previously recognized, through the production of peptides encoded within its genome that act at the cellular and organismal level.


13.2 MITOCHONDRIAL NUCLEAR GENETIC CROSS TALK AND DISEASE: “MITO-MENDELIAN” GENETICS
Scott Ballinger1
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The genetic basis for common disease is generally thought to be “complex” involving both environmental – genetic interactions. Interestingly, the evolution and origins of the eukaryotic cell are thought to be the consequence of an estimated 1.5 billion years of endosymbiosis and adaptation to the environment involving mitochondrial-nuclear, or “Mito – Mendelian” genetic interactions. Herein, we suggest that Mito – Mendelian genetics, plays a major role in influencing cellular metabolism and response to disease risk factors and thus, susceptibility to disease development. These concepts will be discussed in the context of cardiovascular and metabolic disease. Funding was provided by the U.S. Army Medical Research & Material Command (W81XWH-07-1-0540d); National Institutes of Health (HL94518 and HL103859); and the Diabetes Research Center Bioanalytical Redox Biology Core (P30 DK079626).

13.3 THE CROSSTALK BETWEEN MITOCHONDRIAL FUNCTION, THE EPIGENOME AND GENE EXPRESSION
Janine Santos1
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Mitochondria are organelles known for their role in energy production through the process of oxidative phosphorylation (OXPHOS), a byproduct of which is reactive oxygen species (ROS). Mitochondrial function also gives rise to a diverse range of metabolic products that are known to function as co-factors of enzymes that epigenetically regulate the nuclear genome. We hypothesize that some of these metabolites may be rate-limiting for epigenetic reactions that regulate gene expression in the nucleus. The data obtained so far indicate that loss of OXPHOS function is accompanied by changes in some mitochondrial metabolites, decreases in histone acetylation, modulation of DNA methylation and changes in gene expression. Further experiments are ongoing to further tease out this crosstalk. This research was supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.
2015 APS Conference:
Physiological Bioenergetics—From Bench to Bedside

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APS Conference: Cardiovascular, Renal and Metabolic Diseases: Physiology and Gender
November 17-20, 2015 • Annapolis, Maryland

Experimental Biology 2016
April 2-6, 2016 • San Diego, California

APS Teaching Workshop: The APS Institute on Teaching and Learning
June 20-24, 2016 • Madison, Wisconsin

Physiology 2016
July 29-31, 2016 • Dublin, Ireland

APS Conference: Inflammation, Immunity and Cardiovascular Disease
August 24-27, 2016 • Westminster, Colorado

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