Physiological Bioenergetics: Mitochondria from Bench to Bedside
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Conference Program & Abstracts

2017 Physiological Bioenergetics

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2017 APS Conference
Physiological Bioenergetics
Mitochondria: From Bench to Bedside

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MMP from the large sub-sarcolemma mitochondrial pool to it has been proposed that these IBMS serve to distribute the mitochondria segments (IBMS) deep into the muscle cell. These embedded capillaries are surrounded by large pools of mitochondria near the plasma membrane that have large fraction of the mitochondrial volume is located in regions close to capillary indentations in the cell structure. A large portion of the MR connectivity is dependent on direct mitochondrial matrix continuity while in some regions of the muscle the connectivity is proposed to occur via poorly characterized electron dense regions (EDR) between adjacent mitochondria. Using a photo-activated mitochondrial uncoupler to regionally perturb the MMP, we have demonstrated that large regions of the MR are electrically coupled via a shared matrix as well as EDR structures and numerous EDR connections. These data are consistent with a mitochondria reticulum in muscle cells and the coupling is exclusively through large mitochondria structures and numerous EDR connections. These data are consistent with a mitochondria reticulum in muscle cells that couples large numbers of mitochondria together providing a rapid and uniform potential energy source throughout the cell to support ATP production. (NHLBI Division of Intramural Research).


2.0: ENERGY SCHOOL I

2.1 MITOCHONDRIA-TARGETED MOLECULES: TOOLS AND THERAPIES

David Brown

2.2 THE INS AND OUTS OF MITOPHAGY AND QUALITY CONTROL

Roberta Gottlieb

Mitochondria are born from pre-existing mitochondria like Athena from Zeus’s forehead. However, in a homeostatic system, before new mitochondria are formed, space must be made for them by clearing some of the old mitochondria through mitophagy. In this presentation, I will cover key mechanisms for mitochondrial quality control, as well as factors regulating mitochondrial biogenesis. Mitochondrial quality control involves multiple pathways including the ubiquitin-proteasome system - particularly for outer membrane proteins; intrinsic mitochondrial proteases - required for processing many imported mitochondrial proteins; mitochondrial-derived vesicles; the mitochondrial unfolded protein response; and mitophagy. Mitochondrial dysfunction is noted across numerous pathologies. Interventions that target cellular bioenergetics have enormous potential to mitigate disease burdens. This presentation has two major objectives. The first is to give an overview of various mitochondria-targeting strategies in development. The opportunities and challenges of targeting mitochondria will be presented. A selection of mitochondria-targeted compounds currently in clinical trials, as well as promising pre-clinical therapies in development, will be addressed. Emerging approaches that utilize peptide sequences as delivery vectors for mitochondrial cargo will also be discussed. The second objective is to describe ongoing work in my research laboratory using a series of cell-permeable peptides to treat heart disease. Several different peptides that improve cardiac mitochondrial function have been discovered, and their effects on various aspects of cellular bioenergetics will be presented. Funding support: NIH R01 HL123647.
Mitochondria from Bench to Bedside

3.1 NON-CANONICAL ROLE OF DYNAMIN-RELATED PROTEIN DRP1 IN REGULATING BIOENERGETICS OF CARDIAC MUSCLE CELLS

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Mitochondrial dynamics, including fission, fusion, and movement, is a fundamental mechanism in regulating mitochondrial function. Dynamin-related protein 1 (Drp1) is the major GTP hydrolizing protein that is responsible for fission. Studies have shown that Drp1 is abundantly expressed in adult cardiac myocytes. Paradoxically, compared to numerous cell types, adult cardiac myocytes exhibit very low frequency in mitochondrial fission. This dichotomy between the abundance of Drp1 and scarcity of mitochondrial fission has prompted us to investigate the non-canonical roles of Drp1 in cardiac muscle cells. Using multiple approaches encompass biochemical, genetic, and confocal, super-resolution, and electron microscopy, we have obtained results showing that mitochondrial Drp1 is preferentially localized in the mitochondria-associated sarcoplasmic reticulum (SR) membrane fraction (MAM), the region that bridges Ca2+ release site (SR) with energy producing site (mitochondria). Increased cytosolic Ca2+ transients promote translocation of Drp1 to mitochondria. Moreover, inhibition of Drp1 significantly decreases mitochondrial respiration with a modest increase in mitochondrial elongation. Drp1 inhibition also leads to a decrease in the frequency of transient and stochastic opening of the mitochondrial permeability transition pore (mPTP) which triggers mitochondrial flash. This leads us to propose an intriguing hypothesis that Drp1, through its induction of transient or subconductance flickering of mPTP openings, may cause physiological oscillations of mitochondrial membrane potential (ΔΨm). This in turn can trigger the oscillatory bursts of mitochondrial respiration and thus ATP generation, as shown previously. We have also obtained data further show that stressing the heart with chronic β-adrenergic receptor (β-AR) stimulation leads to a significant increase in CaMKII-dependent Drp1 phosphorylation and translocation to mitochondria, which causes excessive mPTP opening, reactive oxygen species (ROS) generation, cardiac hypertrophy, and ultimately, heart failure. In conclusion, our results show that Drp1 is strategically accumulated in the MAM in order to sense the localized high Ca2+ in the SR-mitochondria junctions during cardiac excitation-contraction coupling. The activation of Drp1 leads to enhanced mitochondrial respiration for ATP generation through mPTP transient openings as such the heart can maintain the balance between energy demands and supplies. However, excessive Drp1 activation leads to persistent mPTP opening and excessive ROS generation that causes cell injury and death. Support: NIH 2R01HL093671, 1R01HL122124, 1RO1114760. REFERENCES: Zhang H, Wang P, Bisetto S, Yoon Y, Chen Q, Sheu SS, Wang W. A novel fission-independent role of dynamin-related protein 1 in cardiac mitochondrial respiration. Cardiovasc Res. 113(2):160-170, 2017.

3.3 MITOCHONDRIAL DYNAMICS IN THE BRAIN

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Increasing evidence links dysregulation of mitochondrial dynamics and quality control to neurodegenerative diseases. In neurons, mitochondrial biogenesis, degradation through mitophagy, fission, fusion, and axonal transport are critical for maintaining mitochondrial function, protection of mitochondrial DNA, cell death mechanisms, and proper distribution of mitochondria to synapses and synaptic functioning. Neurons have specific features that likely contribute to the critical nature of proper function and regulation of mitochondrial dynamics: they do not replicate, and mitochondrial turnover is thought to be slower than in most other cell types; they have unique anatomy, with long, highly energy-requiring axons with specific compartmentalization of function; and they are highly dependent on adequate mitochondrial respiration. Evidence implicating dysregulation of mitochondrial dynamics and quality control is particularly strong in Parkinson’s disease (PD), directly linking both genetic and neurotoxic models of PD, and suggesting that this may be a common pathogenic mechanism of PD neurodegeneration. In PD, the degenerative process starts in distal axons, where mitochondrial dynamics are particularly important. In addition, the selectively vulnerable neurons that degenerate in PD throughout the brain share common features of being long, highly energy-requiring axons with sprouting terminals, and vulnerable neurons may contain fewer mitochondria. We study regulation of mitochondrial dynamics specifically in neurons and have found differences in dynamic processes such as mitophagy, compared to other cell types, in part due to bioenergetic differences. We also find early changes in neuronal mitochondrial dynamics in PD-relevant models, including early reversal of mitochondrial axonal transport in PD-vulnerable dopamine neurons in vivo. In addition, discovery of compartmentalized changes in mitochondrial density in PD-relevant neuronal models have led to studies to characterize neuroanatomical compartmentalization of mitochondrial biogenesis in cell bodies, axons, and dendrites. Better characterization of mitochondrial dynamics and quality control mechanisms in compartmentalized neurons will be important for understanding the role of dysregulation of these processes in neurodegenerative disease.

3.4 ELIMINATION OF PATERNAL MITOCHONDRIA IN MAMMALIAN EMBRYOS
Maternal inheritance is a signature feature of mitochondria. Sperm contribute mitochondria to the zygotes, but these paternal mitochondria are eliminated, so that inheritance of mitochondria and their DNA is uniparental. A role for mitophagy (the autophagic degradation of mitochondria) in the elimination of paternal mitochondria has been shown in early nematode embryos. It is not clear if a similar mechanism exists in mammalian embryos. To study the elimination of paternal mitochondria, we tracked the fate of sperm mitochondria in mouse embryos after fertilization. PhArm mice ubiquitously express mito-Dendra2, a photoconvertible fluorophore localized to the mitochondrial matrix. When PhArm males are mated to normal females, the resulting embryos contain fluorescently labeled mitochondria that can be tracked by fluorescence microscopy. We found that paternal mitochondria do not fuse with other mitochondria, eventually lose their membrane potential, and are selectively eliminated from the embryo by 80 hours after fertilization. In contrast, maternal mitochondria are stable and fusion-active. To test the role of specific genes in paternal mitochondria elimination, we used lentivirus encoding shRNA to disrupt the function of genes in the early embryo.

4.2 PLATELET BIOENERGETICS AS A BIOMARKER FOR MITOCHONDRIAL DYSFUNCTION IN HUMAN DISEASE

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It is well established that bioenergetic dysfunction plays a role in the pathogenesis of numerous “non-metabolic” diseases in all organ systems. However, this dysfunction remains poorly characterized in patient populations due to the invasiveness of obtaining tissue for mitochondrial studies. Here we hypothesize that measurements of bioenergetics in circulating blood platelets can be utilized as a biomarker of global bioenergetic dysfunction in human disease. We demonstrate that platelet bioenergetic profile remains stable over time in healthy humans and is uniquely altered in different pulmonary and vascular disease states. Additionally, we show data demonstrating bioenergetic variability among different natural cohorts including the aging population and differences in race and gender. Notably, we show that changes in mitochondrial function correlate with clinical parameters of disease and that platelet bioenergetic function is significantly correlated with bioenergetic parameters in other organs including the lung and skeletal muscle. Collectively, these data suggest that platelet bioenergetic profile is a robust and useful marker for assessing mitochondrial function in healthy humans and patients with disease. The implications of utilizing this method as well as of platelet bioenergetic dysfunction in the progression of pathology will be discussed.

**4.3 MITOCHONDRIAL DNA DAMAGE AS A BLOOD-BASED BIOMARKER FOR EARLY PARKINSON’S DISEASE**

**Evan Howlett**, **Nicholas Jensen**, **Catherine Corey**, **Andrea Weinstein**, **Kirk Erickson**, **Samuel Goldman**, **Caroline Tanner**, **Marie Armentero**, **Fabio Blandini**, **JT Greinamyre**, **Samay Jain**, **Sruti Shiva**, **Laurie Sanders**

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Parkinson’s disease (PD) is the most common neurodegenerative movement disorder and the diagnosis of living patients is primarily based on clinical criteria, which can lead to misdiagnoses. The validation of reliable biomarkers for PD remains a major unmet need and is critical towards therapeutic development. Here, for the first time we investigated mitochondrial DNA (mtDNA) damage as a systemic biomarker of PD. We used our novel PCR-based methodology to measure blood-based mtDNA damage in PD, Alzheimer’s disease (AD) and healthy subjects. Strikingly, mtDNA damage levels in idiopathic early PD were significantly increased in blood buffy-coat samples compared to healthy controls. Interestingly, we found a different cohort, that mtDNA damage is increased in the peripheral blood mononuclear cells (PBMCs) from PD subjects, indicating persistent mtDNA damage in circulating immune cells. In archived DNA samples from two additional cohorts, we were able to replicate these findings of increased mtDNA damage in buffy-coat and PBMCs in PD patients compared to healthy subjects. To address whether changes in mtDNA damage are found in other neurodegenerative diseases, we evaluated mtDNA damage between AD subjects and healthy controls in buffy-coat derived DNA – and found no differences, suggesting that mtDNA damage is not a peripheral broad-based neurodegenerative biomarker. Our data raises the possibility that mtDNA damage might form the basis of a blood-based biomarker of PD that could be used for patient stratification in clinical trials. Furthermore, we propose that levels of mtDNA damage can identify a subset of PD patients that are amenable to specific molecular targeting. Funding sources include: William N. and Bernice E. Bumpus Foundation Innovation Award, Mitochondria, Aging and Metabolism/Basic Biology Aging Pilot Project Program, Alzheimer’s Disease Research Center Seed Monies Grant Program and Pittsburgh Claude D. Pepper OAIC.

4.4 INTEGRATED APPROACHES TO TRANSLATIONAL REDOX BIOLOGY AND BIOENERGETICS

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Bioenergetic Health is a new concept which captures the experimental finding that translational research in metabolism must embrace the metabolic plasticity inherent in human populations to define what is normal. This is an essential first step in establishing the range of normal metabolic and bioenergetic parameters so that pathological indications can be clearly recognized. The data is now emerging from studies of bioenergetics and metabolomics in cells or platelets isolated from human blood. This is important since Bioenergetics is now at the forefront of our understanding of pathological mechanisms, new therapies and as a biomarker for the susceptibility of disease progression in metabolic diseases, neurodegeneration, cancer and cardiovascular disease. A key concept is that the mitochondrion can act as the “canary in the coal mine” by serving as an early warning of bioenergetic crisis in patient populations. Furthermore, cellular mitochondrial function is known to vary between populations due to differences in genetic background and in response to lifestyle changes including diet and exercise. It is clear that we urgently need new clinical tests to monitor changes in bioenergetics in patient populations. This is now possible due to the development of high-throughput assays to measure cellular energetic function in the small numbers of cells that can be isolated from human blood or from tissue biopsy samples. The sequential addition of well characterized inhibitors of oxidative phosphorylation allows a bioenergetic profile to be measured in cells isolated from normal or pathological samples. This profile can define the extent to which these cells utilize mitochondrial oxygen consumption to produce ATP, are using protons for other processes or leak and the maximal respiration. Non-mitochondrial oxygen consuming pathways are also measured and are likely indicative of a pro-inflammatory state. Taken together we propose these parameters are a measure of bioenergetic health of a cell population. We therefore propose the development of the Bioenergetic Health Index (BHI), which is a single value that defines bioenergetic health based upon the analysis of cellular mitochondrial profiles in cells isolated from human subjects. This is now being related to metabolomic and lipidomics analysis in platelets which has the potential to both define the molecular basis for individual variation in human populations and predict the response to stress. Ultimately, BHI has the potential to be a new biomarker for assessing patient health of (or for) both prognostic and diagnostic value.

5.0 POSTER SESSION I

5.1 METABOLIC REGULATION OF EXERCISE-INDUCED CARDIAC GROWTH

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Background: Exercise training promotes metabolic changes in the heart that are associated with physiologic cardiac growth; however, it is not known whether or how physical activity-induced changes in cardiac metabolism modulate myocardial remodeling. In this study, we tested whether exercise-mediated changes in cardiomyocyte glycolysis regulate the physiologic cardiac growth program.

Methods: We used radiometric, immunologic, metabolomic, and biochemical assays to measure changes in myocardial glucose metabolism in mice subjected to acute and chronic treadmill exercise. As tools to determine the relevance of changes in glycolytic activity, we determined how cardiac-specific expression of mutant forms of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK2) affect cardiac structure, function, metabolism, and gene programs relevant to cardiac remodeling. Metabolomic and transcriptomic screening were employed to identify metabolic pathways and gene sets regulated by glycolytic activity.

Results: Acutely, exercise reduced PFK2 phosphorylation, thereby decreasing myocardial glycolysis; however, after 4 weeks of exercise training and in the fully recovered state, myocardial PFK2 activity and glycolysis were increased compared with sedentary controls. Cardiac-specific expression of a kinase-deficient PFK2 transgene (GlycoLo mice) lowered glycolytic rate and regulated the expression of Cebp and Cited4, known to govern the physiologic cardiac-growth program. Correspondingly, GlycoLo hearts, in the absence of exercise, showed a form of cardiac growth similar to the exercise-adapted heart, replete with larger myocytes, enhanced cardiac function, and higher capillary-to-myocyte ratios. Expression of phosphatase-deficient PFK2 (GlycoHi mice) increased glucose utilization and promoted mild cardiac hypertrophy characterized by mild left ventricular dilatation, depressed cardiac function, and absence of activation of the physiologic growth program. Nevertheless, polarization of myocardial metabolism by both transgenes caused modest mitochondrial damage. Transcriptomic analyses indicated that myocardial metabolism coordinates key genes relevant to cardiac remodeling.

Conclusions: We conclude that PFK2-mediated decreases in glycolytic activity are a stimulus for physiologic cardiac growth and that metabolic flexibility is important to maintain mitochondrial health in the heart.

5.2 MITOCHONDRIAL FUNCTION AND TranScriptional Regulation during DERenervation-Induced SkeletAL Muscle ATROPHY.

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5.3 NITRO-OLEIC ACID PROTECTS MICE FROM DIET-INDUCED HEPATIC STEATOSIS AND INSULIN RESISTANCE.

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Age-related declines in muscle mass and function reduce quality of life. The mechanisms underlying these changes are still not defined, but loss of innervation and increased mitochondrial reactive oxygen species (ROS) generation are proposed to play a key role. The goal of this study was to measure the changes in mitochondrial function and gene expression that occur following loss of innervation. Mice were exposed to sciatic nerve transaction on one limb, sham surgery on the other, and then sacrificed at 0.5, 1, 2, 4, 7, and 14 days post-transection. The gastrocnemius muscle, innervated by the sciatic nerve, was collected for RNA isolation and measurement of ROS generation in isolated mitochondria (fluorometer) and permeabilized muscle fibers (Oroboros O2K). Gastrocnemius mass was significantly reduced at 4 days post-transection and was reduced 42% by 14 days. ROS production was significantly increased at 4 days post-trancection, and the elevated ROS production was correlated with the decrease in muscle mass (r²=0.3246, p=0.0008). RNAseq analysis showed that approximately 450 transcripts were differentially regulated post-transection with most changes occurring at 7 and 14 days. The biological processes of the differentially regulated transcripts at 12-24 hours and 2-4 days primarily involve stress responses, while those occurring at 7 and 14 days are characterized by a disruption in homeostasis of metabolic, cellular, and contractile functions. Four transcripts (RCS, Gadd45a, Gdf5, and Myog) were upregulated at all time points and are involved in calcium signaling, growth arrest, and stimulation of muscle reinnervation. The motifs for the transcription factors MeF2a-d were significantly enriched in the promoters of differentially regulated transcripts beginning at 4-7 days suggesting a fiber type shift to slow-twitch. Known age-related atrophy pathways (ATF4, UCP1) are induced by loss of innervation. Denervation causes muscle atrophy, increased ROS production, an acute stress response, a chronic dysregulation of metabolic, cellular, and contractile homeostasis, and induction of fiber type shift. Funding: 1R01AG050676-01A1 Defining the Relative Roles of Pre- and Post-Synaptic Events in the Initiation and Progression of Sarcopenia. Oklahoma Medical Research Foundation Pre-doctoral Scholarship
Obesity is the number one risk factor for the development of non-alcoholic fatty liver disease (NAFLD). Steatosis, the hallmark of NAFLD, is an accumulation of fat within the liver. Mitochondria play a central role in liver metabolism by tightly controlling the high rates of fatty acid oxidation (FAO). During the development of NAFLD, mitochondria are adapted to the increased lipid load in hepatocytes by increasing the rate of FAO. However, reactive oxygen species (ROS) generation is increased, which damages hepatocytes, and in turn induces inflammation. Evidence suggests that hepatic mitochondrial dysfunction lies at the core of the pathogenesis of NAFLD, although the underlying mechanisms responsible for this are still poorly understood. Current understanding supports that hepatic mitochondrial dysfunction and impaired cellular bioenergetics. This study aimed to determine the underlying mechanism.

Methods: Sprague-Dawley rats were fed either control or high-fat diet. On day 14 of pregnancy, dams were injected with citrate buffer or streptozotocin to induce diabetes then treated with twice daily insulin. Dams delivered offspring from four groups: controls, diabetes-exposed, diet-exposed, and combination-exposed. Hearts were harvested and analyzed by Western blot, mitochondrial imaging, and extracellular flux analyses which included fuel preference and respiratory complex function of permeabilized cells.

Results: Diabetes-exposed NRCM had fewer fusion and fission events, shorter mitochondria, lower mitochondrial copy number and decreased glycolytic and respiratory capacity. High-fat diet-exposed NRCMs have shorter mitochondria, higher mitochondrial copy number, increased expression of PGC1α and more oxidative stress. Mechanisms of altered dynamism appear to be sex specific. Exposed females have less OPA-1 protein expression while males have less MFN2 and MFF. Both sexes have lower DRP1 expression, especially following diet exposure. Diet-exposed females have a lower expression of Complex I (NDUFA2) and III (Cox5B) proteins. Permeabilized NRCMs from combination-exposed females also have a lower OCR with various complex I and III fuels including pyruvate, palmitoyl-carnitine and duroquinol.

Conclusions: Maternal diabetes and high-fat diet impair mitochondrial dynamics and respiratory complex function in cardiomyocytes from developing newborn offspring. Findings demonstrate the role of maternal conditions in mitochondrial health and cardiac disease in the developing fetus and uncover mechanisms of fuel-mediated cardiomyopathy.

Funding: NIH- NIGMS, P20GM103548, P20GM103620, NIH- NICHD K08HD078504, Sanford Research.
**Background:** Reports that skeletal muscle mitochondrial respiratory capacity is lower in older adults than in young adults implicates mitochondrial dysfunction in the aging process. Blood-cell bioenergetics has been suggested as an indicator of systemic mitochondrial health. We have shown that supplementation with essential amino acids (EAAs) plus arginine decreases plasma and liver triacylglycerols in older adults. The impact of such supplementation on mitochondrial respiration of circulating cells is unknown. Thus, we sought to assess the effect of eight weeks of EAAs + arginine supplementation on circulating cell bioenergetics in older adults with hypertriacylglycerolaemia.

**Methods:** Six (two men) older adults underwent testing before and after 8 weeks of supplementation with EAA + arginine (11g of EAAs ingested twice daily). A skeletal muscle biopsy as well as peripheral blood mononuclear cells (PBMC; n=4), and platelets (n=4) were collected pre- and post-supplementation. Mitochondrial respiration was determined in permeabilized myofibers from the m. vastus lateralis using Oroboros, and in intact PBMC and platelets using a Seahorse.

**Results:** Maximal coupled respiration (respiration linked to ATP production) in muscle was not significantly changed by 8 weeks of EAA supplementation. However, ATP-linked respiration, maximal respiration and reserve capacity were significantly decreased in PBMC following 8 weeks of EAA supplementation. While no significant differences in mitochondrial respiration were observed for platelets, the glycolytic capacity of platelets was decreased after EAA supplementation. No significant alterations in glycolysis were observed in PBMC.

**Conclusions:** These results indicate that EAA supplementation may lower respiration rates in PBMC. The mechanisms underlying these changes may include that by lowering lipids, as we previously observed, cellular stress is reduced and ATP demand is reduced, or that the substrate pool is altered and thus metabolism shifted. Future studies are needed in order to elucidate mechanisms and understand the implications of these novel findings.

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laboratory. Support: NIH R01 AG049762, P30 DK048520 and ULI TR001082, VA Merit, Denver Research Institute, and Eastern Colorado VA Geriatric Research, Education, and Clinical Center.

5.7 SKELETAL MUSCLE MITOCHONDRIAL DYSFUNCTION AND IL-33 RECEPTOR (ST2) GENE DELETION IN A MOUSE MODEL OF PULMONARY HYPERTENSION

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Pulmonary arterial hypertension (PAH) is a progressive, incurable disease of the pulmonary vasculature leading to right ventricular failure. Cytokines have been implicated in pulmonary resistance vessel remodeling, and an elevated serum IL-33-to-IL-33 receptor (ST2) ratio is a biomarker of PAH. Whether cytokines contribute to skeletal muscle pathology and disease progression is unknown. We hypothesized that muscle mitochondrial dysfunction occurs in PAH skeletal muscle through an IL-33-dependent cytokine pathway.

To test this hypothesis we used the Sugen/hypoxia (SuHx) model of pulmonary hypertension and adult WT and IL-33 receptor gene ablated (ST2-/-) mice. Diaphragm and soleus mitochondrial function were measured under maximal ADP-stimulated respiration (J_O2max), and uncoupled electron transport system capacity (ETS) conditions with glutamate, malate, and succinate as substrates. Regulators of mitochondrial fusion (Mfn2, Mfn1) and biogenesis (PGC1α) and muscle protein ubiquitination (MuRF1) were measured in gastrocnemius by Western blot.

Males: Diaphragm J_O2max was lower in WT-SuHx vs. WT-CON (112±33 vs 193±80 pmoLS.mg^-1; p<0.05), but not in ST2-/-SuHx (207±109 pmoLS.mg^-1). Soleus J_O2max was also lower in WT-SuHx vs WT-CON (89±38 vs 138±39 pmoLS.mg^-1; p<0.05), but not in ST2-/-SuHx (122±71 pmoLS.mg^-1). Gastrocnemius Mfn1/2 was lower (p<0.05) in WT-SuHx and ST2-/-SuHx vs WT-CON. MuRF1 was not different across treatment or genotype (p>0.05). PGC1α was lower in WT-SuHx vs WT-CON (p=0.057), but was not different when comparing ST2-/-SuHx vs ST2-/-CON. Females: Diaphragm J_O2max was unaffected by SuHx. However, ETS was lower in both WT-SuHx and ST2-/-SuHx vs. WT-CON (162±8 and 157±107 vs 256±41 pmoLS.mg^-1; p<0.05). Soleus J_O2max was unaffected by SuHx. Mfn1/2, MuRF1, and PGC1α were not affected in the gastrocnemius of SuHx females.

In male mice, SuHx conditions result in a profound reduction in skeletal muscle mitochondrial oxidative capacity associated with lower levels of fusion proteins. Global gene deletion of the IL-33 receptor (ST2) prevented a reduction in diaphragm/soleus mitochondrial respiration independent of fusion protein levels. Conversely, females exhibited a modest change in muscle mitochondrial function and no difference in the level of mitochondrial regulators. Inflammatory cytokines, such as IL-33, may play a role in skeletal muscle maladaptation to PAH and this appears to be sex-dependent. Support: US Department of Veterans Affairs and SDSU University Grants Program.

5.8 EFFECT OF LACTATE ON MITOCHONDRIAL RESPIRATORY FUNCTION IN SKELETAL MUSCLE

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Lactate is now recognized as not merely a metabolic substrate. We previously reported that single administration of lactate up-regulated genes related to oxidative metabolism including Pparc1a and Ucp3 in mouse skeletal muscle. To examine the role of lactate as a signal for improving mitochondrial function, we first investigated whether repeated lactate administration alters mitochondrial enzyme activity. C57BL/6J male mice daily received 1 g/kg of sodium lactate by i.p. injection for one week. We found that COX activity was increased in skeletal muscle after one week of lactate administration. There was a trend toward increased lactate transporter MCT4 protein content. The peak blood lactate concentration after the injection was declined on the last day (day 7) compared with that on the first day (day 1), which suggests that lactate is taken up by tissues and oxidized in mitochondria. Next, we measured mitochondrial respiration using a Seahorse extracellular flux analyzer. For this analysis, C2C12 myotubes were treated with 20 mM lactate for 24 hours. However, there were no effects of lactate on either basal or maximal oxygen consumption rates. Further studies using different concentration and/or duration of lactate treatment are required. In this study, we demonstrate that repeated lactate administration increases lactate clearance and mitochondrial enzyme activity in mouse skeletal muscle.
HETEROGENEITY OF ENERGY TRANSFORMATION IN PULMONARY ENDOTHELIAL CELLS: A LOOK AT THE MECHANICAL WORK

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Biological cells are no exception to the laws of physics. One such law is the law of energy conservation. Following this law, the cell transforms its energy currency to engage in three types of work: chemical, transport, and mechanical. While chemical and transport work is studied in great details, the mechanical work of the cells has never been quantified. Quantitative assessment of cellular mechanical work will be crucial to identify physical laws that govern processes such cell reorientation, barrier function, and migration. Using Monolayer Stress Microscopy, a novel in vitro platform to measure local mechanical forces around a cell, we have quantified mechanical work that each cell in an advancing monolayer does on its substrate as well as on its neighbors.

We measured mechanical work for three cellular systems: pulmonary artery endothelial cells (AECs), pulmonary microvascular endothelial cells (MECs), and pulmonary vein endothelial cells (VECs). These three cellular systems are known to exhibit remarkable functional and molecular heterogeneity. Here we found that over 940 minutes, although the cellular monolayers have an advancing front, the AECs with their uniform cobblestone morphology and negligible motion were most quiescent and VECs with their non-uniform mesenchymal morphology and non-coherent motion were least quiescent. The farther the cells were from being quiescent, the more strongly the cells were engaged in mechanical work. Across these cellular systems, the relative difference in the mechanical work on the substrate (WS) was not same as the relative difference in the mechanical work on the neighboring cells (WC). The cells that are known to have strongest barrier properties – the MECs – had the highest value for the ratio WS/WC, and the cells with weakest barrier properties – the AECs – had the lowest value for the ratio WS/WC. Moreover, the mechanical work of the fastest and most coherently moving cells – the MECs – was least sensitive to the cellular size, distance from the advancing front and their state of jamming. Taken together, this study presents a first quantitative assessment of cellular mechanical energies and opens new avenues for the mechanobiological assessment of pulmonary vascular diseases.

ROCK2 Modulates Skeletal Muscle Mitochondrial Function

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Background: Rho-associated coiled-coil containing kinase (ROCK) is a ubiquitous serine/threonine kinase with established clinical relevance in wide range of pathologies including metabolic diseases such as Type 2 Diabetes and muscular disorders. Previous studies have attempted to investigate ROCK’s role in skeletal muscle function but outstanding conflicts exist due to absence of isoform specific knockdowns and lack of tissue specificity with chemical inhibitor treatments. Similarly, while literature exists on ROCK’s effect on mitochondrial driven apoptosis, there is a gap in knowledge on isoform specific ROCK2 effect on skeletal muscle mitochondria.

Methods and Results: To study ROCK2 role in the skeletal muscle, we have generated a novel genetic mouse model with a skeletal muscle-specific deletion of ROCK2 (hereby known as Myo R2KO). To characterize in vitro phenotype, we first subjected mice to Forced Endurance Treadmill Test and discovered that Myo R2KO mice had decreased endurance performance compared to control. In addition, we discovered that after 6 week exercise stimulus, our Myo R2KO exhibited an increased heat production quantified by metabolic cages. Consequently, we wanted to measure energy utilization on a tissue level. We found no difference in glycolysis as measured by lactate readings, glucose uptake assay and Seahorse glycolysis stress test. However, we did delineate that primary myofibers isolated from Myo R2KO animals had poorer oxidative phosphorylation ability as measured by Seahorse Mitochondria Stress Test. Next we determined in vitro that Myo R2KO mitochondria had dysfunctional mitochondria function represented by increased ROS production, decreased ATP luminescence and decreased membrane potential measured through TMRE staining. We are currently validating potential molecular signaling mechanisms. Prelimina rily, we have found an increase in UCP3 levels in mRNA isolated from our Myo R2KO animals compared to control.

Conclusion: In conclusion, skeletal muscle ROCK2 is a critical regulator of mitochondria function. When skeletal muscle ROCK2 is knocked out mitochondria dysfunction occurs which leads to an in vivo phenotype of abnormal heat production and decreased motor performance. These
findings suggest that skeletal muscle ROCK2 may be a viable target to treat mitochondria dysfunction with long term implications for multiple diseases including Type 2 Diabetes and skeletal muscle atrophy.

5.11 SINGANLING MECHANISMS OF DRP1 TRANSLOCATION TO THE MITOCHONDRIA-SR ASSOCIATIONS IN ADULT MURINE CARDIOMYOCYTES

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Mitochondrial dynamics, including fusion, fission, and movement, is a fundamental mechanism in regulating mitochondrial function. The high level of Drp1 in the heart seems to be at odds with numerous studies showing that mitochondrial dynamics in adult ventricular myocytes occurs rather infrequently. Electron micrographs show that in adult mammalian cardiac myocytes, the majority of inter-myofibrillar mitochondria appear to be fragmented and tethered to the sarcoplasmic reticulum (SR) near the dyads. We tested the hypothesis that Drp1 is strategically accumulated at the mitochondria-SR associations (MAM) and that during continuous excitation-contraction coupling processes (e.g. heartbeats), the localized high Ca2+ in the SR-mitochondria junction further increases translocation of nearby cytosolic Drp1 to the MAM. **Methods and results:** Western blot analysis shows a Drp1 presence in the SR and crude mitochondria fractions. Furthermore, pure mitochondria and MAM fractions were obtained via Percoll purification of crude mitochondria. Proteomic results show that Drp1 is mostly presented and enriched in the purified MAM but not in the pure mitochondrial fraction. To confirm this biochemical data, the distance between SR (RyR2) and Drp1 (labelled with immunofluorescent antibodies) in adult cardiac myocytes was quantified in nanometer scale by using ZeissLSM880 confocal microscope-Airy-scan detector. Intriguingly, over 80% of Drp1 is located within 200 nm radius of RyR2. Similar results can be observed in preparations by using immunogold TEM. Moreover, Drp1 distribution along the dyads was studied by the localization of the Drp1 along the transversal side of the mitochondria (TOM20). A significantly increased translocation of Drp1 to the transversal sides was observed upon 15min-2Hz electric field stimulation in freshly isolated adult ventricular myocytes superfused with isoproterenol+Ca2+ (1mM) in comparison to myocytes incubated with quasi-Ca2+-free (2.5 μM) buffer. Finally, isolated hearts form rat retrogradely perfused (Langendorff) with Krebs buffer supplemented with isoproterenol+Ca2+ (1.8mM) showed a significantly elevated Drp1 accumulation at the MAM fraction vs. those perfused with quasi-Ca2+-free (2.5 μM) Krebs buffer. **Conclusion:** According to these results, we conclude that Drp1 is preferentially positioned at the mitochondria-SR associations and its recruitment likely involves Ca2+ signaling and/or beta-adrenergic activity.

5.12 THE MITOCHONDRIAL CIRCUIT BREAKER: NETWORK SECURITY IN THE SKELETAL MUSCLE MITOCHONDRIAL RETICULUM

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The skeletal muscle mitochondrial reticulum is a large, grid-like network of physically and electrically coupled mitochondria. While network connectivity allows for rapid communication and distribution of energy throughout the cell, this connectivity puts the entire network at risk as localized dysfunction can also quickly spread throughout the cell. We hypothesized there must be protective mechanisms in place to minimize spread of dysfunction throughout the skeletal muscle mitochondrial reticulum. To test this hypothesis, a photoactivatable mitochondrial uncoupler (MitoPhotoDNP) was loaded into isolated mouse soleus muscle fibers to enable precise control over mitochondrial depolarization within specific regions of the cell upon UV irradiation. The electrical response to localized mitochondrial depolarization was followed by monitoring the mitochondrial membrane potential (TMRM) through a confocal microscope. The structural response was evaluated by imaging muscle fibers containing a genetically encoded, photoswitchable (green-to-red) mitochondrial fluorescent protein (MitoDendra2). Immediately (~400 ms) upon irradiation of MitoPhotoDNP in the center of a muscle cell, there was a shared depolarization of the membrane potential in the irradiated and the surrounding regions consistent with electrical network connectivity. However, shortly thereafter, electrical separation between the irradiated and surrounding regions occurred where the irradiated region continued to depolarize while the non-irradiated region repolarized back to baseline levels with a time constant of 7.9±0.3 seconds. Mitochondria within the irradiated region began to physically disconnect from the network ~30 seconds after depolarization. Physical separation occurred through retraction of branched, elongated mitochondria into consolidated structures resulting in 32±4% fewer, but 33±8% larger mitochondria inconsistent with mitochondrial fission. Indeed, mitochondrial fission inhibitors, mdivi-1 or dynasore, did not prevent the physical separation mechanism. These data suggest dysfunctional mitochondria can be quickly electrically sequestered through a circuit breaker-like mechanism while damaged mitochondria are physically separated through a consolidation process that likely facilitates repair or replacement through mitophagy.
These rapid alterations in mitochondrial connectivity allow skeletal muscle fibers to quickly respond to local dysfunction within seconds and restore energy distribution to the remainder of the muscle cell.

5.13 EXERCISE COMBINED WITH CALORIE RESTRICTION-INDUCED WEIGHT LOSS, BUT NOT WEIGHT LOSS ALONE, IMPROVES MITOCHONDRIAL RESPIRATORY CAPACITY IN SKELETAL MUSCLE OF OLDER OBESE SUBJECTS

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Background: Skeletal muscle mitochondrial capacity has been linked to deranged energy metabolism in aging, obesity and type 2 diabetes. Calorie restriction has been shown to have “anti-aging” effects in animal models, and calorie restriction-induced weight loss (CRWL) and exercise can both improve several aspects of cardiometabolic health in human obesity. The effects of CRWL in older obese humans, however, are not known. The aim of this study was to investigate the effect of 6-month diet-induced weight loss intervention with or without the addition of exercise training on skeletal muscle mitochondrial respiratory capacity.

Methods: Twenty-six older adults with obesity were randomized to one of the following 6-month intervention: Health education (CON: n=9, 5M/3F, age=70±5yrs, BMI=35±5.8kg/m²), Calorie restriction-induced weight loss (CRWL: n=7, 2M/5F, age=71±5yrs, BMI=35.8±5.0kg/m²), or Weight-loss and exercise (WLEX: n=10, 5M/5F, age=68±3yrs, BMI=37.7±6.2kg/m²). CON subjects participated in biweekly health education sessions with no specific exercise/dietary advice. CRWL and WLEX participants had a goal of 10% weight-loss through calorie restriction. Subjects in the WLEX group completed a supervised exercise program. A percutaneous muscle biopsy of the vastus lateralis was collected pre and post intervention (p>0.05), subjects in the WLEX group presented an increased leak (PRE: 65±4±18.8 vs. POST: 79.1±29 pmol/s*mg; p=0.05), maximal OXPHOS (PRE: 258±4±40.6 vs. POST: 307±68.2 pmol/s*mg; p=0.04) and a trend to higher uncoupled respiration (PRE: 301.3±39.6 vs. POST: 346.8±79 pmol/s*mg; p=0.06) after the exercise training. Additionally, while Km was unchanged in the three groups after intervention, there was a trend for increased Vmax in the exercise group after training (PRE: 255±43 vs. POST: 304±66; p=0.08).

Conclusions: Exercise is required to improve mitochondrial respiratory capacity in skeletal muscle of older obese human subjects undergoing calorie restriction-induced weight loss.

5.14 WITHDRAWN

5.15 LONGITUDINAL FOLLOW-UP OF EQUINE MUSCLE MORPHOMETRICS AND ASSOCIATED METABOLIC PROPERTIES INDUCED BY 8 WEEKS OF TREADMILL TRAINING

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Reasons for performing the study: Aquatraining is increasingly incorporated into equine training and rehabilitation programs. However, little is known about the physiological responses to this type of training. Our research group previously reported the morphometric changes in 15 skeletal muscles of horses subjected to 8 weeks of aquatrainin. Objectives: (1) to compare muscle morphometric changes induced by dry treadmill training with those induced by aquatrainin, (2) to identify changes in muscle metabolic properties induced by treadmill training. Materials and Methods: seven healthy untrained horses completed an 8-week treadmill training program (20 min/session, 5 days/week, belt speed 1.25 m/sec). Morphometric assessment of 15 strategically chosen muscle groups was performed on 3 occasions (start, after 4 weeks and at finish) using transcutaneous ultrasound (B-mode Esaote, macroconvex, 5-7.5 MHz). Muscle biopsies were harvested at start and finish of the study, at rest, from the M. Pectoralis profundus (PP) and Vastus lateralis of the M. quadriceps femoris (QF). Principal Component Analysis was performed on the metabolomics results. Results: (1) there was a significant increase in muscle mass of the M. Trapezius cervical part (+57%), the M. PP (+29%), the M. Trapezius thoracic part (+26%), the M. Brachiocephalicus (+10%) and a significant decrease in muscle mass of the QF muscle (-18.8%), the M. Erector spinae lumbal part (-...
5.16 THE ROLE OF PARKIN IN AGE-DEPENDENT PLATELET MITOCHONDRIAL AND THROMBOTIC DYSFUNCTION

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Aging is one of the greatest risk factors for thrombosis and gives rise to conditions such as deep vein thrombosis, myocardial infarction and stroke. However, the mechanisms underlying age-related increased thrombosis remain unclear. Platelets are anucleate circulating cells that when activated, aggregate and catalyze thrombus formation. Prior studies have shown age-dependent increases in platelet activation, but the molecular mechanisms that lead to this activation remain unclear. Though platelets are anucleate, they contain functional mitochondria and an active ubiquitin-proteasome system (UPS). Given that age related declines in mitochondrial function and the UPS have been documented in other cell types, we hypothesized that age-dependent dysfunction in the UPS leads to increased mitochondrial reactive oxygen species (mtROS) production, which stimulates platelet activation leading to thrombosis. Herein, we demonstrate that platelets from healthy middle aged (36-70 years) and elderly (>75 years) human subjects show a decrease in UPS activity as well as decreased expression of the E3 ubiquitin ligase Parkin, a key regulator of mitochondrial proteins. This was accompanied by an increase in mitochondrial oxidant production compared to healthy young subjects (18-35 years). These results were recapitulated in a mouse model of healthy aging in which old (1.5 years) mice showed decreased time to occlusion in a ferric chloride thrombosis model, concomitant with increased production of platelet mitochondrial ROS and decreased UPS activity. Ongoing studies in wildtype and parkin knockout mice are testing whether decreased UPS activity directly leads to mitochondrial ROS production and whether these changes result in platelet activation.

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5.17 IMPACT OF EXERCISE TRAINING AND AGING ON THE SKELETAL METABOLISM IN RATS WITH LOW/HIGH INTRINSIC AEROBIC CAPACITY

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PURPOSE: Low exercise capacity is a strong and independent predictor of cardiovascular disease and early mortality. Exercise training is widely known to be beneficial to human health in different ways. Low intrinsic capacity runners (LCR) is a rat model which is especially useful to study the effect of altered energy metabolism and the effect of different interventions. Nuclear magnetic resonance (NMR) based metabolomics has been widely used to investigate metabolic change. The purpose of this study was to explore the metabolic profiles of LCR and high capacity runners (HCR) rats and to investigate the impact of exercise training in muscles metabolism by NMR based metabolomics.

METHODS: Animal model: LCR and HCR rat models were artificially selected by generations 29 and 30 as previously described. The maximal oxygen consumption (VO2max) was recorded to measure running capacity. Selected rat models were divided into two rounds according to the age difference (Round 1: 9 months age; Round 2: 18 months age) and performed training regimes. MR experiments: Rat soleus muscle were extracted with dual-phase extraction protocol. To perform NMR study. Raw MR spectra were pre-processed before multivariate analysis. Statistics: Partial least square discriminative analysis (PLS-DA) was used to the main metabolic differences between the groups. Three-way ANOVA was used to compare the mean difference of VO2max across the LCR and HCR groups with or without exercise training.

RESULTS: HCR rats showed a 54% and 30% higher VO2max compared with LCR rats without training, in Round 1 and Round 2 respectively. Exercise training did not change VO2max significantly in the LCR group, while it induced a significant increase (34%) in the HCR group in Round 1. PLS-DA discriminated LCR sedentary group from HCR sedentary group in both Round 1 and Round 2.
Exercise training also influenced metabolic profiling in both Round 1 and Round 2 of LCR groups. The loading plots demonstrate the metabolites (glutamine, glutamate, creatine, lactate, taurine) associated with discriminations.

CONCLUSION: Exercise training increased VO2max in HCR rats, but not in LCR. The results of this study suggest a strong interaction between the exercise training and the intrinsic exercise capacity, as evidenced by alteration in VO2max and metabolic profiles. Metabolomics analysis can potentially predict the impact of exercise training and aging on LCR rats. Some of the influential metabolites are detectable by clinical MRS examination.


5.19 MITOCHONDRIAL DYNAMICS IN SKELETAL MUSCLE IN PATIENTS ON MAINTENANCE HEMODIALYSIS

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Frailty and sarcopenia are commonly present in patients on maintenance hemodialysis (MHD) and increase the risk of morbidity and mortality. Mitochondria, as the principal source of energy, are important for proper muscle function. Mitochondria are dynamic organelles that are constantly undergoing either fusion or fission. Mitochondrial fusion leads to enlarged mitochondria and maximizes the oxidative capacity. Mitochondrial fission results in smaller mitochondria and is crucial for the segregation and elimination of damaged mitochondria. Oxidative stress, commonly present in patients on MHD, may damage the mitochondria. We have previously described reduced mitochondrial content and ultrastructural mitochondrial abnormalities in patients with chronic kidney disease on MHD. Thus, we now evaluate the hypothesis that mitochondrial fission is increased in patients on MHD, as a mechanism to remove damaged mitochondria. For this purpose, we evaluated mitochondrial dynamics in skeletal muscle biopsies from 10 patients on MHD and 15 controls with no history of CKD. The groups were matched by age, gender, and race. We measure mitochondrial size using transmission electron microscopy. We also measured OPA-1, Fis-1 and DRP-1, markers of mitochondrial dynamics, by western blot. The groups were also comparable in self-reported activity. Controls and patients on MHD were similar in terms of age (52.8±8.7 vs. 50.3±15.1), BMI (30.6±7.3 vs. 29.0±5.1), and gender. We found that mitochondria are smaller in patients with ESRD compared to individuals with no history of CKD (Figure 1A). We did not find any difference in the abundance of either OPA-1 or Fis-1 between the groups. We did find an increased content of DRP-1 in patients on MHD (Figure 1B). Smaller mitochondria may indicate a preferential mitochondrial fission in skeletal muscle from patients on MHD. Damaged mitochondria may be segregated and eliminated by mitophagy. This is consistent with our previous finding of increased mitophagy in patients on MHD. Future studies are required to evaluate how changes in mitochondrial dynamic and function may impact frailty and sarcopenia in patients on MHD.
5.20
LOCALIZED PROTON-COUPLING BIOENERGETICS IN MITOCHONDRIA
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Recently, we have preliminarily made a major breakthrough in understanding proton-coupling bioenergetics over the Nobel-prize work of Peter Mitchell’s chemiosmotic theory. The decades-longstanding energetic conundrum of alkalophilic bacteria as to how they are able to synthesize ATP has now, for the first time, been clearly elucidated using a newly modified proton motive force (pmf) equation based on the new proton electrostatic localization theory [Lee JW (2015), Bioenergetics 4: 121. doi:10.4172/2167-7662.1000121]. This work may have fundamental implications to better understand the bioenergetics in many other biological systems including mitochondria. For example, the pmf value reported previously with the Mitchellian equation in the last 50 years may need to be revisited for possible significant updates in mitochondria. Notably, with the conventional chemiosmotic theory, one would have to “adjust” the Delta pH values to get an energy efficiency <100% in mitochondria (Silverstein TP (2014) J Bioenerg Biomembr 46:229–241). This “elephant-in-room” scientific problem could now be addressed with the newly developed pmf equation. This presentation will show that the true total pmf value (including the local pmf) may be significantly larger than those previously reported in mitochondria, as they all used the classic Mitchellian pmf equation, which misses an important contribution from the localized protons. This finding may have far-reaching implications on many biological systems including human health and possibly also related to the fundamentals of human memory.

5.21
MITOCHONDRIAL REGULATION PROTECTS THE TURTLE HEART FROM OXIDATIVE DAMAGE AFTER REOXYGENATION
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Reintroducing oxygen after a period of anoxia causes oxidative damage to tissues by production of reactive oxygen species (ROS). Freshwater turtle hearts (Trachemys scripta) are remarkably resilient to oxidative damage and suffers no damage from reoxygenation even after months of anoxia during winter hibernation. Preventing ROS production from mitochondria might be key to this resilience. In the mouse, inhibition of mitochondrial complex I by the post-translational modification S-nitrosation has been shown to inhibit ROS production upon reoxygenation. In this study, we tested the hypothesis that turtles prevent oxidative damage after anoxia/reoxygenation by limiting ROS production via S-nitrosation of complex I. We acclimated turtles to low temperature and anoxia or normoxia and analysed isolated heart mitochondria for respiration rate, ROS production, enzyme activity and S-nitrosation of mitochondrial proteins. We also used the mitochondria specific S-nitrosating agent MitoSNO to analyse the effect of S-nitrosation on turtle heart mitochondria in vitro. We found that anoxia acclimation does reduce ROS production and respiration rate of purified mitochondria, and that S-nitrosation of complex I inhibits activity and ROS production after anoxia/reoxygenation in vitro. However, activity and S-nitrosation of turtle complex I was not affected by anoxia acclimation in vivo. Instead, lower activity of the mitochondrial marker enzyme citrate synthase and lower maximal respiration rate of anoxic turtle mitochondria indicates down-regulation of the content of mitochondria in the turtle heart during anoxia. Reducing the content of mitochondria in the turtle heart during prolonged anoxia may prevent ROS production upon reoxygenation and protect against oxidative damage.

5.22
12 WEEKS RESISTANCE TRAINING INCREASED SKELETAL MUSCLE MOTS-C, A MITOCHONDRIALLY ENCODED PEPTIDE, IN MEN WITH IMPAIRED GLUCOSE REGULATION
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Beyond all well-known retrograde signals, studies have identified mitochondria-linked factors that influence the cellular and extracellular environments, including mitochondria-derived peptides and mitochondria-localized proteins (1-3). The first described mitochondria-derived peptide was humanin (4), and recently another small peptide also encoded from the mtDNA called MOTS-c (mitochondrial open reading frame of the 12S rRNA-c) was discovered (5). MOTS-c has been shown to regulate glucose metabolism by activating AMPK and the glucose
transporter type 4 (GLUT 4) (5,6). Whether human skeletal muscle MOTS-c expression is influenced by exercise is not known.

**Purpose:** This study aimed to investigate whether endurance or resistance training over a 12-week period could induce changes in levels of MOTS-c protein in men with IGR both in skeletal muscle and in serum. The hypothesis was that MOTS-c is an exercise-responsive mitokine.

**Methods:** Male subjects (n=48) with impaired glucose regulation (IGR) were randomly assigned to resistance training (RT), Nordic walking (NW) or a control group (C). The training was performed 3 times/week during 12 weeks. Biopsies from the m. vastus lateralis and serum samples were obtained before and after the intervention. Skeletal muscle and serum protein and mRNA levels of MOTS-c were analyzed along with factors involved in metabolic regulation such as MEF2A, RIP140, PGC-1a and GLUT4.

**Results:** Skeletal muscle MOTS-c protein increased in the RT group (p<0.05, n=15), but did not change in the NW or C group. There were no significant changes of MOTS-c serum levels over time or between the groups. However, correlation analysis revealed a negative correlation between ∆MOTS-c protein in serum and ∆RIP140 protein in skeletal muscle (r=0.513, p<0.01). Interestingly, a positive but weak correlation between ∆MOTS-c protein in serum and ∆GLUT4 mRNA in skeletal muscle was detected (r=0.359, p=0.015).

**Conclusion:** In this study it is shown, for the first time, that MOTS-c protein levels increase in human skeletal muscle following 12 weeks of resistance training in men with IGR. This study also highlights the importance of studying mitochondria-derived peptides to further understand and improve mitochondrial and exercise related medicine.

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**5.23**

**ATF5 IS REQUIRED FOR MITOCHONDRIAL UNFOLDED PROTEIN RESPONSE-MEDIATED CARDIOPROTECTION DURING ISCHEMIA-REPERFUSION INJURY**

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**Introduction:** The mitochondrial unfolded protein response (mtUPR) is a compartment-specific, stress-induced retrograde signaling pathway. In *C. elegans*, the transcription factor ATF5-1 plays a central role in the mtUPR. Under mitochondrial proteotoxic stress, ATF5-1 enters the nucleus and upregulates a variety of genes including those for mitochondrial chaperones and proteases to reestablish mitochondrial proteostasis. ATF5 has been proposed to be the mammalian ortholog of ATFS-1, but only recently was it shown that ATF5 can rescue the mtUPR response in *C. elegans* lacking ATFS-1. Here, we use a mouse model to examine if induction of the mtUPR can convey cardioprotection in ischemia-reperfusion (IR) injury and if ATF5 is essential for this effect.

**Methods:** An ATF5 knockout mouse model on a C57BL/6J background was used with age- and gender-matched controls from the same colony, complying with NIH guidelines. Langendorff-perfused hearts were exposed to 30 min of ischemia followed by 60 min of reperfusion. IR injury was quantified by functional recovery of the rate × pressure product during reperfusion, and by infarct size measured by tetrazolium chloride staining. The mtUPR was induced using oligomycin, doxycycline, or chloramphenicol administered by intraperitoneal injection 6 h prior to Langendorff-perfusion.

**Results:** *Atf5*−/− mice had a 67% neonatal death rate, similar to previous reports, so mice of both genders were used in this study. In untreated mice, neither genotype (*Atf5*+/+, *Atf5*−/−, or *Atf5*−/−) nor gender had an effect on recovery from cardiac IR injury. However, when *Atf5*−/− or *Atf5*+/− mice were treated with oligomycin, doxycycline, or chloramphenicol, there was a significant increase in functional recovery and a significant reduction in infarct size. This cardioprotective effect of mtUPR induction prior to IR injury was absent in *Atf5*−/− mice.

**Conclusions:** This study shows that the in vivo pharmacologic induction of the mtUPR conveys cardioprotection after IR, with this effect requiring ATF5. Furthermore, the loss of ATF5 did not alter the susceptibility of untreated hearts to IR injury, as expected from the mtUPR signaling pathway being a transcriptional regulator. The results herein provide strong supporting evidence of the hypothesis that ATF5 is the mammalian ortholog of ATFS-1.

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**5.24**

**PROTEIN KINASE CΔ (PKCΔ) CONTROLS BASAL MITOCHONDRIAL BIOENERGETICS IN DOPAMINERGIC NEURONAL CELLS: RELEVANCE TO MITOCHONDRIAL DYSFUNCTION IN NEURODEGENERATIVE DISEASES**

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Mitochondria dictate both physiological and pathological processes in the nervous system. Mitochondrial dysfunction has been widely implicated in the pathogenesis of various neurodegenerative diseases, including Parkinson’s disease (PD). Previously, we demonstrated that protein kinase Cδ (PKCδ) is an oxidative stress-sensitive kinase that preferentially expresses in dopaminergic neurons and plays a key role in mediating dopaminergic neuronal apoptosis via caspase-3-mediated proteolytic activation of the kinase. We also demonstrated that dopaminergic neurotoxic insults trigger PKCδ translocation from the cytosol to...
Mitochondria from Bench to Bedside

5.25 BIOENERGETIC CHARACTERIZATION OF MULTIDRUG RESISTANT BREAST CANCER CELLS

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Multidrug resistance (MDR) is a common resistant mechanism of cancer cells to cytotoxic drugs in systemic therapy. MDR is characterized by increased expression of ATP-dependent drug exporting pumps (e.g. P-glycoprotein) which remove cytotoxic compounds from the cytosol. However, the mechanism whereby cancer cells rapidly respond to this increased ATP demand is not fully understood. There have been repeated reports suggesting highly glycolytic nature of MDR phenotype. However, recent studies have suggested that mitochondrial modulators have a significant role in preventing development of MDR phenotypes. It has been shown that MCF7Dox, a MDR variant of the MCF7 breast cancer cell line has highly glycolytic phenotype with increased glucose uptake/consumption rate in vitro and in vivo. To further analyze the glycolytic pathway, we developed a new approach to quantifying glycolytic rate using extracellular acidification. We confirmed that in MCF7Dox cells, glycolytic rate under basal conditions is significantly increased compared to MCF7 cells. Furthermore, MCF7Dox cells have a significant increase in compensatory glycolysis when mitochondrial ATP production is blocked. This metabolic profile switch is accompanied by a decreased dependency on glutamine to fuel mitochondrial respiration and increased tolerance of the MCF7Dox cells to glutamine deprivation as compared to the MCF7 cells. We performed calcein efflux studies in MCF7 and MCF7Dox cells to characterize ATP-dependent pump activity responsible for the MDR phenotype. MCF7Dox cells can export cytosolic calcine-AM fluorescent probe in contrast with MCF7 cells that retain it in the cytosol, confirming the difference in ATP-dependent pump activity of the cell variants. When MCF7Dox cells were pre-incubated with 2-deoxy-D-glucose to inhibit glycolytic ATP production, no effect on calcein efflux was observed. However, when cells were pretreated with the mitochondrial ATP-synthase inhibitor oligomycin, calcine efflux was completely blocked despite increased glycolytic activity observed under this condition, strongly suggesting that MDR mechanism relies on mitochondrial generated ATP for maintaining the chemoresistance phenotype. These findings have potential therapeutic relevance in the context of specific modulation of mitochondrial ATP production to prevent therapy resistance.

5.26 INACTIVATION OF MITOCHONDRIAL DEACETYLASE SIRT3 LEADS TO SOD2 HYPERACETYLATION, PROMOTES VASCULAR OXIDATIVE STRESS, INCREASES ENDOTHELIAL DYSFUNCTION, EXACERBATES HYPERTENSION AND TREATMENT WITH SOD2 MIMETICS RESCUE SIRT3 DEPLETED PHENOTYPE

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Mitochondria play an important role in redox cell signaling. Metabolic dysfunction and antioxidant depletion lead to dysregulation of redox signaling and oxidative stress which contribute to cardiovascular disease. Sirt3 is a key regulator of mitochondrial function and it activates major mitochondrial antioxidant enzyme SOD2 by deacetylation of specific lysine residues and Sirt3 depletion increases oxidative stress. We hypothesized that loss of vascular Sirt3 activity increases vascular oxidative stress and endothelial dysfunction promoting hypertension and end organ damage. The role of vascular Sirt3 was studied in human subjects with essential hypertension, wild-type C57Bl/6J mice, Sirt3−/−, tamoxifen-inducible endothelium specific Sirt3 knockout mice (EcSirt3KO) and tamoxifen-inducible smooth muscle specific Sirt3 knockout mice (SmcSirt3KO) using angiotensin II model of hypertension (0.7 mg/kg/day). Analysis of human subjects with essential hypertension showed 2.6-fold increase in SOD2 acetylation and 1.4-fold decrease in Sirt3 while SOD2 expression was not affected. Western blot analysis of mouse aorta showed 30% reduction of vascular Sirt3 and increased SOD2 acetylation by 2-fold after onset of angiotensin II-induced hypertension. Hypertension was markedly increased in Sirt3−/− mice in response to angiotensin II (0.7 mg/kg/day) compared with wild type mice and treatment with mitoTEMPO (1.5 mg/kg/day) normalized the blood
pressure and vascular relaxation in Sirt3−/− mice. Deletion of Sirt3 in smooth muscle exacerbated hypertension (165 mm Hg vs 155 mm Hg in wild-type) and significantly increased mortality in angiotensin II infused SmcSirt3KO mice (30% vs 3% in wild-type) which was associated with higher rate of aortic aneurysm formation (75% vs 10% in wild-type). EcSirt3KO mice had elevated basal blood pressure by 12 mm Hg and hypertension was exacerbated in EcSirt3KO mice. This was accompanied by impaired vascular relaxation, reduced production of endothelial nitric oxide supporting the pathological role of endothelial Sirt3 deficiency. Decrease in NO is a hallmark of endothelial dysfunction in hypertension due to vascular oxidative stress. Angiotensin II infusion increased vascular O2 by 2-fold in wild-type 3-fold in increase in O2 in SmcSirt3KO mice which exacerbated endothelial dysfunction (4-fold decrease in NO). Angiotensin II induced hypertension was associated with Sirt3 S-glutathionylation and scavenging of mitochondrial H2O2 in mCAT mice prevented Sirt3 inactivation, reduced SOD2 acetylation and diminished hypertension. These data indicate that hypertension is associated with reduced Sirt3 expression and redox inactivation of Sirt3. This leads to SOD2 hyperacetylation and SOD2 inactivation which promotes vascular oxidative stress, increases endothelial dysfunction, exacerbates hypertension, increases end-organ-damage and mortality. It is conceivable that Sirt3 agonists and SOD2 mimetics may have therapeutic potential in cardiovascular disease.

5.27 ESTROGEN TREATMENT AIDS MITOCHONDRIAL COMPLEX I KINETICS AND H2O2 EMITTING CAPACITY DIFFERENTLY IN LIVER VS. SKELETAL MUSCLE OF OVARIECTOMIZED MICE

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The use of estrogen (E2) therapies (ET) prevents many of the metabolic health challenges brought by surgical or natural menopause, including insulin resistance, metabolic syndrome and type 2 diabetes. However, the molecular mechanism(s) by which E2 affects metabolic tone across different tissues remains not well understood. We have previously shown ET protects cellular redox balance and insulin sensitivity in skeletal muscle (SM) of ovariectomized (OVX) mice, by modulating mitochondrial electron transfer efficiency. The liver comprises not only a key tissue for glucose homeostasis, but is also the main processing organ of exogenously-administered E2. Thus, the goals of this study were: 1) to further elucidate the mechanism(s) by which E2 protects mitochondrial function in SM, with an emphasis on the kinetics and H2O2 emitting capacity and topology (sites of O2/H2O2 leak) of complex I (C I), and 2) to compare the effects of OVX and ET on such parameters in liver mitochondria. 10 week-old C57BL/6N mice were OVX, and received +/- 2 weeks of ET via a subcutaneous mini-osmotic pump (1µg/day). In SM, ovarian E2 withdrawal decreased maximal NADH oxidation capacity (-43%, p<0.05), and increased ΔH2O2 emitting potential of C I at the Flavin site (I0) by three-fold (p<0.005). E2 treatment in vivo, as well as E2 exposure in vitro fully reversed these effects. In liver, OVX did not alter C I function or ΔH2O2 emitting capacity. However, E2 treatment in vivo was associated with a reduction in supercomplex levels (-33%, p<0.05) and a higher NADH oxidation capacity (+40%, p<0.05) without concomitant changes in the capacity of quinone reduction, supporting the hypothesis that C I may be prone to O2/H2O2 leak from the quinone binding site (I0). Accordingly, exposure of liver OVX mitochondria to E2 in vitro led to a two-fold higher ΔH2O2 emitting potential at site I0 (p<0.005). Given that C I decreased function and increased H2O2 production have been associated with the pathophysiology of non-alcoholic fatty liver disease, these results raise concerns on the safety of ET for women with, or at risk of, liver disease. The present findings provide novel insights for the development of alternative safer therapeutic strategies for naturally- and surgically-induced menopausal women. RO1 DK096907

5.28 PERMI (PGC-1 AND ERR-INDUCED REGULATOR, MUSCLE 1) IS REQUIRED FOR EXERCISE-INDUCED MITOCHONDRIAL BIOGENESIS AND ENHANCES OXIDATIVE CAPACITY IN SKELETAL MUSCLE

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Skeletal muscle mitochondrial content and oxidative capacity are important determinants of muscle function and whole body health. Mitochondrial content and function are enhanced by endurance exercise, and impaired in states or diseases where muscle function is compromised, such as myopathies, muscular dystrophies, neuromuscular diseases and age-related muscle atrophy. Hence, elucidating the mechanisms that control muscle mitochondrial content and oxidative function can provide new insights into states and diseases that affect muscle health. In past studies, we identified Pرم1 (PGC-1 and ERR-induced Regulator, Muscle 1) as a gene induced by endurance exercise in skeletal muscle, and regulating mitochondrial oxidative function in cultured myotubes. The capacity of Perm1 to regulate muscle mitochondrial content and function in vivo is not yet known. In this study, we use adenov-associated viral (AAV) vectors to increase or decrease Perm1 expression in skeletal muscles of adult mice. Compared to control vector, AAV1-Perm1 leads to significant increases in mitochondrial content and oxidative capacity. Moreover, AAV1-Perm1 transduced muscles show increased capillary density and resistance to fatigue (Cho et al., 2016). By
contrast, AAV1-shPerm1 decreased basal oxidative phosphorylation (OXPHOS) protein content and citrate synthase activity and attenuated mitochondrial biogenesis induced by exercise training. To gain insights into the pathways by which Perm1 remodels skeletal muscle, we next assessed the phosphorylation levels of kinases known to be important for mitochondrial biogenesis and other aspects of skeletal muscle oxidative function. We found that loss of Perm1 leads to decreases in phosphorylation of p38 and CaMKII. By contrast increased Perm1 expression enhances phosphorylation levels of p38 and CaMKII.

Conclusion: Taken together, our findings suggest that Perm1 is required for exercise-induced mitochondrial biogenesis and enhances oxidative capacity in skeletal muscle through modulation of p38 and CaMKII activity.

5.29
ATP PRODUCTION CAPACITY AND PARTITIONING BETWEEN GLYCOLYSIS AND OXIDATIVE PHOSPHORYLATION IN CELLS CATABOLIZING SINGLE SUBSTRATES
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The majority of cellular ATP production is shared between glycolysis and oxidative phosphorylation. This partitioning is highly dynamic, changing in response to substrates and to alterations in the activities of each production pathway; e.g., inhibition of electron transport will cause glycolytic ATP production to increase, given a suitable substrate. This dynamic relationship is driven not by transcriptional alteration or post-translational enzyme modification, but rather by the kinetic regulation that maintains a steady state intracellular ATP/ADP ratio. The sum of the bioenergetic capacities of each pathway defines a theoretical maximum rate at which the cell can generate ATP, defining the upper limit of ATP demand that can be placed on the cell before energetic collapse. We have previously described a method for calculating ATP production rates using extracellular flux measurements of oxygen consumption and acidification, and the resulting ATP production characteristics of C2C12 myoblasts using glucose as the sole exogenous substrate, including the total rate of production, its partitioning (the Glycolytic Index), the change in partitioning when glucose is added (the Crabtree Index) and when mitochondrial respiratory chain activity is altered (the Pasteur Index), and the theoretical range of ATP supply over which the cell can utilize either glycolysis or oxidative phosphorylation to meet ATP demand (the Supply Flexibility Index). Here, we demonstrate how these characteristics are influenced by different exogenous substrates. This research was funded in part by NIH R15 ES025917-01A1 to SM.

5.30
METABOLIC REGULATION OF NEUROINFLAMMATION
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Inflammation is an important physiological mechanism accompanying ischemic injury. The inflammatory response starts within minutes, and lasts weeks or months after ischemic injury. In stroke, the detrimental effects of pro-inflammatory activation of microglia, the resident macrophages of the brain, are underscored by the evidence that suppression of microglial activation limits cerebral injury. An increasing body of evidence suggests a link between cell metabolism, mitochondrial function and proinflammatory cytokine generation by immune cells. We previously demonstrated that that lipopolisaccharide (LPS)-induced activation promotes significant metabolic changes suppressing mitochondrial function and increasing glycolysis in microglial BV-2 cells. On the other hand, enhancement of mitochondrial function attenuated the LPS-induced oxidative and metabolic responses, and suppressed proinflammatory activation of microglia. In this study we investigated the modulation of the LPS-induced inflammatory response of microglial BV-2 cells by inhibition of miR-338. Several studies have demonstrated the potential of miR-338 inhibition to enhance and protect mitochondrial function through upregulation of mitochondrial proteins. Inhibition of miR-338 attenuated the LPS-induced oxidative and metabolic responses, and suppressed proinflammatory activation, which depended on HIF-1α mechanisms. Thus, miR-338 suppression provides a novel strategy to modulate proinflammatory cytokine production of relevance to inflammation-associated pathologies.

5.31
ACUTE MANEB EXPOSURE DISRUPTS BOTH GLYCOLYSIS AND MITOCHONDRIAL FUNCTION IN SK-N-AS NEUROBLASTOMA CELLS
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Maneb (MB), a manganese-containing fungicide, in combination with the herbicide paraquat (PQ) has been reported as an environmental risk factor for Parkinson’s disease (PD), as chronic MB exposure causes pathology similar to that of PD in mouse models. Although the mechanism of PQ toxicity through redox cycling and oxidative stress has been highly researched, MB’s contribution has not been fully described. MB has been
shown to preferentially inhibit complex III in vitro causing reduced mitochondrial function. More recently it has been shown that MB acts as a cysteine thiol modulator, acting on proteins with redox regulation through protein thiol adduction. Additionally, several proteins involved in cellular energy pathways have been shown to react through redox signaling and cysteine thiol regulation. Understanding the mechanisms of these compounds in neurons and their effect on mitochondrial function and energy pathways may aid in better characterization of PD pathology. In this study, the neuroblastoma SK-n-AS cell line was analyzed with acute maneb exposure using the Agilent Seahorse XFp Analyzer. Briefly, basal oxygen consumption and extracellular acidification rate were first measured, then 50µM MB was injected and a glycolysis stress test and mitochondrial stress test were conducted. The glycolysis stress test showed significantly decreased glycolysis rate and glycolytic capacity in cells exposed to 50 µM MB. Furthermore, the investigation of mitochondrial stress showed significantly decreased ATP production in the MB treated cells. These results support the hypothesis that enzymes involved in ATP production and glycolysis are disrupted through modification of protein thiols. Further investigation into these redox regulated proteins may lead to therapeutic targets and mechanistic understanding that can be applied to pathology and development. Funding: NIH/NIEHS (ES027593)

5.32 PRESYNAPTIC ENERGY UTILIZATION DURING HIGH-FREQUENCY SYNAPTIC TRANSMISSION AT THE CALYX OF HELD

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Maintenance of cellular energy as ATP is important in neurons, which are energetically expensive and consume ~20% of an organism’s energy at rest. While a considerable amount of energy is expended to regenerate the electrical polarization of neurons, efficient release and recycling of neurotransmitter consumes nearly half of the presynaptic neuronal energy budget. In general, mitochondria are the major suppliers of cellular energy in neurons, generating ATP via oxidative phosphorylation. However, the specific utilization of energy from cytosolic glycolysis and/or mitochondrial respiration at the presynaptic terminal, a spatially and functionally isolated compartment within neurons, remains unclear. We use a synapse amenable to physiological investigation and specialized for high frequency transmission in mice, the calyx of Held, to test the sources of energy utilized to support energy maintenance during activity-dependent neurotransmission. We show that acute inhibition of either glycolysis or mitochondrial respiration alters excitatory postsynaptic currents (EPSCs) during high frequency activity at this mammalian synapse before the onset of hearing. However, in mature calyx synapses tuned for sustained high frequency transmission, these differences are absent. Our data suggest a specific metabolic profile exists to support high-frequency information transmission, which changes over the course of postnatal synaptic maturation due to a greater reserve of ATP and enhanced ATP production following synaptic activity. As a result, the mature terminal is capable of firing for minutes in the absence of glycolysis or mitochondrial respiration.

6.0 ENERGY SCHOOL II

6.2 MTDNA STRUCTURE AND SIGNALING

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By virtue of its endosymbiotic origins, the eukaryotic cell represents a co-evolution between nuclear and mitochondrial genomes. The mitochondrion is a descendnet of an a-proteobacteria, and the majority of its genes have been transferred the to the host nucleus, leaving a remnant of the original a-proteobacterial genome residing inside the organelle. In mammals, this mitochondrial DNA (mtDNA) encodes 13 polypeptides, 22 tRNAs, and 2 rRNAs that are essential for proper cellular bioenergetic function and metabolism. Because changes in bioenergetics have become key features in several types of metabolic diseases, it has been suggested that mtDNA sequence variation can alter mitochondrial – nuclear interactions that influence cell function, and thus, disease susceptibility. To directly test this hypothesis, Mitochondrial – Nuclear Exchange (MNX) mouse models have been developed to determine the impact of different mtDNA – nuclear genome (nDNA) combinations upon common disease susceptibility, using cardiac disease and cancer models. Results from these studies are consistent with the hypothesis that different mtDNA – nDNA combinations change cellular bioenergetics and importantly, disease susceptibility. While these studies are focused upon basic factors germane to specific diseases, they also challenge current concepts concerning the basis for genetic susceptibility and thus, precision medicine. Further, they are relevant to mitochondrial gene therapies currently being pursued for pathogenic mtDNA mutation.

Mitochondrial Acetylation in Heart Failure

Iain Scott

Mitochondrial dysfunction has been implicated in several pathologies that culminate in heart failure. Under non-ischemic conditions the heart derives over 90% of its energy from oxidative phosphorylation, making mitochondrial fuel substrate metabolism a key node for potential disruption. As such, there is currently strong interest in characterizing mechanisms that regulate fuel metabolism in the heart. Lysine acetylation has been shown to regulate metabolic enzymes in a number of tissues, and recent research has suggested that cardiac mitochondrial energy metabolism pathways may also be regulated by this post-translational modification. Acetylation uses acetyl-CoA derived from fuel metabolism as a co-factor, thereby linking cellular nutrient availability to mitochondrial bioenergetic output. This aspect is of particular importance, as several forms of cardiac disease (e.g. heart failure, ischemia, diabetic cardiomyopathy) are characterized by alterations in the availability and utilization of fuel substrates. In most cases, protein acetylation status is controlled by the opposing activities of acetyltransferase and deacetylase enzymes. Using several methods to inhibit normal cardiac function, we are currently investigating the role of the acetyltransferase-related protein Gcn5l1 in regulating cardiac fuel metabolism. Our current data suggests that acetylation promotes fatty acid oxidation in the heart, and that this modification is regulated in part by Gcn5l1 activity. Driving fat oxidation under pathological conditions via this mechanism may reduce cellular bioenergetic efficiency, limiting overall cardiac output and promoting disease progression. (NIH K22HL116728 & R56HL132917).

Targeted Deletion of MNSod in Mouse Skeletal Muscle Leads to Elevated Superoxide and Increased Oxidative Stress, Mitochondrial Dysfunction and Reduced Force Generation but Does Not Initiate Muscle Atrophy

Bumsoo Ahn, Rizwan Qaisar, Shylesh Bhaskaran, Gavin Pharaoh, Rojina Ranjit, Kaitlyn Riddle, Holly Van Remmen

Mitochondrial dysfunction has been implicated in several pathologies that culminate in heart failure. Under non-ischemic conditions the heart derives over 90% of its energy from oxidative phosphorylation, making mitochondrial fuel substrate metabolism a key node for potential disruption. As such, there is currently strong interest in characterizing mechanisms that regulate fuel metabolism in the heart. Lysine acetylation has been shown to regulate metabolic enzymes in a number of tissues, and recent research has suggested that cardiac mitochondrial energy metabolism pathways may also be regulated by this post-translational modification. Acetylation uses acetyl-CoA derived from fuel metabolism as a co-factor, thereby linking cellular nutrient availability to mitochondrial bioenergetic output. This aspect is of particular importance, as several forms of cardiac disease (e.g. heart failure, ischemia, diabetic cardiomyopathy) are characterized by alterations in the availability and utilization of fuel substrates. In most cases, protein acetylation status is controlled by the opposing activities of acetyltransferase and deacetylase enzymes. Using several methods to inhibit normal cardiac function, we are currently investigating the role of the acetyltransferase-related protein Gcn5l1 in regulating cardiac fuel metabolism. Our current data suggests that acetylation promotes fatty acid oxidation in the heart, and that this modification is regulated in part by Gcn5l1 activity. Driving fat oxidation under pathological conditions via this mechanism may reduce cellular bioenergetic efficiency, limiting overall cardiac output and promoting disease progression. (NIH K22HL116728 & R56HL132917).

Adaptation to Loss of the Mitochondrial Phosphate Carrier in Skeletal Muscle

Erin Seifert, Lauren Anderson-Pulling, Yana Sharpadskaya, Oipei Li

The mitochondrial phosphate carrier (PiC), encoded by the nuclear gene SLC25A3, was purified more than 30 years ago. PiC is widely believed to serve as the primary means of inorganic phosphate (Pi) uptake into mitochondria for oxidative phosphorylation (oxphos) and for transporting Pi that buffers the vast amount of calcium that mitochondria can take up. However, it is only recently that the direct study of PiC in vivo has become feasible. This is due to the recent discovery of mutations in human PiC and the development of a PiC floxed mouse. Human SLC25A3...
mutations produce a severe clinical phenotype at birth and with striated muscle as a key affected tissue, and mice with cardiac-specific PiC loss eventually develop abnormal cardiac function. Yet, evidence of ample mitochondrial ATP and near normal organ function despite PiC depletion in humans and mice suggest that PiC expression far exceeds Pi needs and/or that functional compensation can be substantial, including the mitochondrial uptake of Pi through alternate pathways. To explore these possibilities we generated mice with Tamoxifen (Tam)-inducible skeletal muscle (Skm) PiC knockdown (Tam+Cre+); 3 weeks after Tam, PiC protein was <5% of control in Skm mitochondria. Despite minimal PiC, Tam+Cre+ mice gained weight normally and performed as control mice on a treadmill at slow speed. Though Tam+Cre+ mice fatigued faster during an incremental endurance treadmill test, they could still sustain some level of aerobic exercise. This provides in vivo evidence for compensatory Pi provisioning to PiC-depleted Skm mitochondria. Bioenergetics analysis in Skm mitochondria revealed that oxphos in PiC-depleted mitochondria ranges from zero to ~40% of control, and that this is robustly substrate-dependent. These observations along with other, more circumstantial, evidence such as lower weight of fat stores in Tam+Cre+ mice, suggest that PiC-depleted Skm mitochondria adopt a “work-around” in which compensatory Pi uptake occurs via the dicarboxylate carrier, at the expense of Krebs cycle cataplerosis, and with fatty acids as a preferred substrate. Yet, despite the ability to engage a “work-around” to supply ATP, endurance performance of Tam+Cre+ mice declines after several weeks of PiC loss, suggesting that the “work-around” is maladaptive or that it is offset by parallel stress signaling. Ongoing studies explore these possibilities. Funding: United Mitochondrial Disease Foundation; Thomas Jefferson University.

8.0 MITOCHONDRIAL SIGNALING: ATP AND BEYOND

8.1 MITOCHONDRIAL ROS IN NEURONAL SIGNALING

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Mitochondria are a main site of reactive oxygen species (ROS) production. The overproduction of mitochondrial ROS can cause oxidative damage to proteins, lipids and DNA, and is associated with a diverse range of disease pathologies. However, ROS also play a role in an organism’s physiology and mitochondrial ROS contributes to cell signaling pathways. Despite the dual role of ROS in health and disease, there are no means to simultaneously control the timing, quantity and site of ROS generation. To address this limitation, we use an optogenetic approach in C. elegans to investigate how ROS microdomains contribute to ROS damage or signaling. Novel genetically-encoded ROS generating proteins produce ROS in response to light and allow for the spatial and temporal control of ROS production. I will highlight ongoing studies from my laboratory that investigate ROS microdomains and their impact on physiologic outputs. Research supported by NIH R01 NS092558.

8.2 MITOCHONDRIA AS SIGNALING ORGANELLES

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For decades, the mitochondria have been primarily viewed as biosynthetic and bioenergetic organelles generating metabolites for the production of macromolecules and ATP, respectively. Our work has elucidated that mitochondria have a third distinct role whereby they release reactive oxygen species (ROS) and metabolites such as L-2HG to initiate physiological and pathological processes including hypoxic activation of HIFs, cellular differentiation, T cell activation and cancer cell proliferation. I will discuss our recent findings on how mitochondria as signaling organelles control cell fate and function.

8.3 MITOCHONDRIAL PROTEIN IMPORT AND SIGNALING

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Modulation of the mitochondrial protein import pathways can have regulatory effects on mitochondrial function. We have conducted chemical genetic screens to identify modulators for the TOM-TIM23, TIM22, and MIA protein import pathways. Neurodegenerative diseases such as Parkinson’s have been linked to a dysfunctional mitochondrial quality control system that is maintained by the proteins PINK1 and Parkin. Whereas mitophagic pathways are becoming well-characterized, little is known about the molecular mechanisms for PINK1 trafficking in mitochondria. We have used two probes to characterize PINK1 translocation. MB-12 promotes the accumulation of PINK1 on the mitochondrial surface without dissipating the mitochondrial membrane potential and Parkin is subsequently recruited. Conversely, MB-10 inhibits the accumulation of PINK1 on dysfunctional mitochondria. MB-10 likely targets TIMM44. Normal PINK1 import is not dependent on TIMM44, but the subsequent anchoring of PINK1 to the mitochondrial outer membrane upon insult requires TIMM44. Thus, we have new tools for dissecting PINK1/Parkin trafficking and studying the induction of PINK1-dependent mitophagy in cell and animal models. REFERENCES: Miyata, N., J. Steffen, M.E. Johnson, S. Fargue, C.J. Danpure, and C.M. Koehler. 2014. Pharmacologic rescue of an enzyme-trafficking defect in primary hyperoxaluria 1. Proc Natl Acad Sci U S A. 111:14406-14411; Miyata, N., Z. Tang, M.A. Conti, M.E. Johnson, C.J. Douglas, S.A. Hasson, R. Damoiseaux, C.A. Chang, and C.M. Koehler. 2017. Adaptation of a genetic

8.4 THE RELEASE OF MITOCHONDRI AS DAMAGE ASSOCIATED MOLECULAR PATTERN

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Mitochondria are organelles present in eukaryotic cells in charge of energy supply and control of cell death. Intriguingly, mitochondria share with bacteria multiple features, such as their outer and inner membrane content in cardiolipin, and a circular genome containing hypomethylated CpG DNA motifs (mtDNA). While damaged organs and activated cells can extrude their mitochondria, which are suggested to trigger innate immunity, we observed that blood platelets could also release their mitochondria, both as free organelles or encapsulated in plasma membrane vesicles, known as microparticles. Mitochondrial release by platelets is initiated by different triggers (e.g. thrombin, collagen, immune complexes), and by storage under blood bank conditions, suggesting that mitochondrial damage associated molecular patterns (DAMP) might contribute to inflammation that prevails in diseases implicating activated platelets and to transfusion adverse reactions. The monitoring of extracellular mitochondria in blood in chronic inflammation and in blood transfusion products may provide indications on mechanisms underlying systemic inflammation and may reduce transfusion-induced adverse reactions.

8.5 MITOCHONDRIAL SIGNALING IN MYOFIBROBLAST TRANSDIFFERENTIATION AND FIBROSIS

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When the heart is injured, fibroblasts transition from a structural role into contractile, synthetic myofibroblasts. This is crucial for scar formation after myocardial infarction to prevent ventricular rupture, but excessive fibrosis is maladaptive and leads to heart failure. Recent reports have identified an elevation in intracellular calcium ([Ca2+]i) as an important second messenger driving myofibroblast formation. While [Ca2+]i signaling appears to be necessary for both TGFranco’s-dependent and -independent fibrotic signaling pathways, other Ca2+ domains, such as mitochondrial calcium ([Ca2+]m), have not been explored. [Ca2+]m signaling is rapidly integrated into the mitochondrial matrix via the mitochondrial calcium uniporter channel, a mechanism theorized to integrate cellular energetic demand with metabolism and respiration. We’ve discovered that alterations in [Ca2+]m uptake are essential to myofibroblast transdifferentiation. I will discuss how genetic approaches revealed this novel signaling pathway and present data to support the notion that alterations in mitochondrial metabolism are crucial for the conversion of fibroblasts to myofibroblasts and the fibrotic response to injury.

9.0 POSTER SESSION II

9.1 CALCULATING MUSCLE OXIDATIVE ATP SYNTHESIS IN VIVO DURING HIGH INTENSITY CONTRACTIONS USING 31-PHOSPHOROUS MAGNETIC RESONANCE SPECTROSCOPY: ADJUSTING FOR CHANGES IN VMAX

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Noninvasive and continuous 31-phosphorus magnetic resonance spectroscopy (31P MRS) measures can be used to calculate the rate of oxidative ATP synthesis (ATPox) during skeletal muscle work under steady state conditions. However, the assumptions related to steady state energetics precludes the use of this approach during high-intensity contractions. Thus, the purpose of this study was to develop a method for calculating ATPox during non-steady state conditions. The vastus lateralis muscle of 16 young adults (23.5±2.3yr, 8 women) was examined during contractions in a Siemens 3T whole-body scanner using a dual-tuned surface coil (1H/31P, 6 x 8cm). Muscle oxidative capacity (rate of phosphocreatine (PCr) recovery, kPCR·s-1) following a brief, 24-s maximal contraction was determined and compared with kPCR·s-1 following an incremental contraction protocol to fatigue. The protocol consisted of five 2-min stages of maximal isokinetic knee extensions (120°·s-1, 30° range of motion), starting with a contraction frequency of 0.89±0.22 vs 1.02±0.22 mM·s-1, p<0.05). PCr recovery (tPCR) was also determined and used to calculate total ATP production for each stage. At the end of the ramp protocol to fatigue, ATPox calculated by our new method was compared with [Cr]·s-1 following an incremental contraction protocol to fatigue. We then modified this method by adjusting Vmax and Km to account for the observed declines in kPCR at fatigue, and compared ATPox calculated by both methods at the end of the ramp protocol to the initial rate of PCr recovery (tPCR). ATP production by glycolysis and the creatine kinase reaction were also determined and used to calculate total ATP production for each stage. At the end of the fatigue protocol, ATPox calculated by our new method was not different from tPCR (0.72 ±0.21 vs 0.70±0.14 mM·s-1, respectively, p=0.7), but ATPox calculated by the traditional method was faster than tPCR (0.85±0.17 vs 0.70±0.14 mM·s-1, respectively, p=0.01). Moreover, total ATP cost of contraction was lower when calculated by our new method compared to the traditional method (0.89±0.22 vs 1.02±0.22 mM·s-1, p<0.05). Collectively, these results suggest that steady state MRS methods overestimate ATPox during high intensity
contractions by not accounting for transient impairments in oxidative capacity, which subsequently inflates calculations of total ATP cost.

Funding: Institute for Applied Life Sciences and the Commonwealth Honors College

9.2 INTEGRATED MEASURES OF CELLULAR BIOENERGETICS AND MITOCHONDRIAL FUNCTION IN HUMAN PLATELETS IN THE DIAGNOSIS OF METABOLIC DISEASE

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Mitochondrial respiratory chain disorders comprehend a spectrum of mitochondrial genetic defects, which affect approximately 1 in 5000 individuals. The diagnosis is difficult due to the diverse clinical presentations that can be associated with a specific molecular defect in metabolism. The current approach relies on the detection of mitochondrial DNA (mtDNA) mutations or on the assessment of mitochondrial complex activities in tissue biopsies or cultured cells, which are time-consuming and lack standardized protocols suitable for clinical evaluation. In the present study we used specific inhibitors of mitochondrial complexes I, II, IV and V in platelets in order to validate the mitochondrial stress test (MST) in intact platelets and the complex activity assay in permeabilized platelets using the extracellular flux analyzer. For this purpose, platelets are pretreated with: rotenone (complex-I inhibitor), TTFA (complex-II inhibitor), oligomycin (ATP synthase inhibitor) or azide (complex-IV inhibitor) in a dose-response manner. Then, the activities of specific complexes were determined (CI, CII, CIV), as well as the MST. As expected the platelets pretreated with inhibitors (rotenone and TTFA) demonstrated a dose-response decrease in mitochondrial respiration with an associated increase in glycolytic flux. We present the results from two confirmed mitochondrial disease patients and compare them to healthy adults. Our results provide a framework for the diagnosis of mitochondrial respiratory chain disorders based on blood platelets mitochondrial complexes activities evaluation. This method requires a simple blood draw and is minimally invasive; the analysis can be automated, and takes less than four hours. This work was supported by a donation from Agilent-Seahorse Bioscience and the Foundation for Mitochondrial Medicine (to UAB).

9.3 TEMPERATURE-INDUCED CHANGES IN GLYCOLYTIC RESERVE AND MITOCHONDRIAL SPARE RESPIRATORY CAPACITY IN COLORECTAL CANCER AND HUMAN T-CELL LINES

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Therapy, as an approach for manipulating cellular bioenergetics, has recently attracted considerable attention from basic and clinical investigators working in the area of cancer, organ transplant and diabetes research. A number of studies and clinical trials have shown that therapy can be successfully used as a standalone or combined therapy for various metabolic conditions. However, the impact of temperature treatment on cancer or T-cell bioenergetics has not been studied in detail with a real time, microplate-based, label-free detection approach. The present study investigates how changes in temperature affect the bioenergetic characteristics (mitochondrial function and glycolysis) of three colorectal cancer (CRC) cell lines and primary human T-cells utilizing Seahorse XF96 technology. Experiments were performed at 32°C, 37°C and 42°C using assay medium conditions and equipment settings adjusted to produce equal oxygen and pH levels ubiquitously at the beginning of all experiments. The results suggest that temperature significantly changes multiple components of the glycolytic and mitochondrial function of all cell types tested. Under hypothermia conditions (32°C), the extracellular acidification rates (ECAR) of CRC cells were significantly lower compared to the same basal ECAR levels measured at 37°C. In primary human T cells, the ECAR/OCR rates increased at 42°C. Interestingly, the FCCP dose response at 37°C vs 42°C showed significant shifts in profiles, suggesting that single dose FCCP experiments might not be sufficient to characterize the mitochondrial metabolic potential when comparing groups, conditions or treatments. These findings provide valuable insights into the metabolic and bioenergetic changes of CRC and human T-cells under hypo- and hyperthermia conditions that could potentially lead to the development of better-targeted and personalized strategies for patients with cancer, metabolic disorders, diabetes or transplanted organs. Support: NCI and Markey Cancer Center, University of Kentucky.

9.4 GENETIC VARIATION IN MITOCHONDRIAL COMPLEX I ACTIVITY AMONG INBRED STRAINS OF MICE

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The present study focuses on the genetic variation in mitochondrial complex I activity among different inbred mouse strains to better understand the genetic basis of mitochondrial function.
Mitochondrial dysfunction has been shown to link to metabolic diseases, such as obesity, NAFLD, and diabetes. To dissect the genetic control of mitochondrial functions and its physiological role, we employed a panel of >100 strains of inbred mice, termed Hybrid Mouse Diversity Panel (HMDP), which allows for high resolution mapping. Previously, we have shown that when fed a high fat/high sucrose obesogenic diet, HMDP mice exhibited large variations in obesity, insulin resistance and hepatic steatosis which are dependent on their genetic background. We isolated mitochondria from 287 livers from 102 strains of male mice. Complex I activity was measured and normalized to citrate synthase activity. A 10-fold difference was observed between the extreme strains. We found that complex I activity was negatively correlated with obesity traits: body weight, body fat percentage, fat mass response to diet. It was also inversely correlated with subcutaneous fat mass but not fats from other depots. Parallel to the obesity connection, complex I activity was inversely correlated with food intake and HOMA-IR. These data show that complex I activity is tightly associated with body composition and insulin action. Metabolomic analysis revealed a negative correlation between complex I activity and plasma gamma-amino butyric acid (GABA). In the liver, complex I activity showed a negative correlation with 4-aminobutyrate aminotransferase (Abat), an enzyme catalyzing GABA catabolism and is involved in the mitochondrial nucleoside salvage pathway. Our finding is consistent with previous report that human subjects harboring homozygous ABAT missense mutation displayed increased GABA level and reduced mtDNA in the brain. These data suggest that GABA may be a useful plasma biomarker for mitochondrial function. Genome wide association mapping identified two significant loci on chromosomes 8 and 13. The chromosome 8 peak spans ~1.4Mb and contains 18 genes whereas the chromosome 13 peaks is ~1.7 Mb wide containing 4 genes. The identification and validation of causal genes in these loci will provide novel insight into the regulation of complex I function by nuclear genes.

9.5 COMMON AND CONTRASTING MECHANISMS OF MITCHONDRIAL QUALITY CONTROL AND OXIDATIVE STRESS IN PROLIFERATING AND POST-MITOTIC CELLS

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It has been long observed that oxidative stress, in particular mitochondrial damage, is associated with many chronic diseases, including atherosclerosis, cancer, diabetes, cardiovascular diseases, arthritis, hypertension and neurodegenerative disorders. The mechanisms of mitochondrial quality is control involve regulation of mitochondrial redox homeostasis, mitochondrial fission/fusion, mitochondrial biogenesis and degradation. 1) A few years ago, we have made the initial observation that differentiation of SH-SY5Y cells leads to increased mitochondrial membrane potential, increased mitochondrial reserve capacity, resistant to oxidative stress-induced cell death, and significant remodeling of mitochondrial electron transport chain proteins with increase of complex IV proteins without changes of other complexes or mitochondrial mass as assessed by citrate synthase activity and mtDNA/mtDNA ratio. 2) Following this study we have investigated the relationship of mitochondrial respiration, we have investigated the impact of Sirt3 knockout, which is associated with MnSOD inhibition and increase of mitochondrial reactive oxygen species, on mitochondrial respiration. Interestingly, Sirt3-/- cells exhibit normal mitochondrial respiration in the stress test. However, in response to starvation, Sirt3 knockout led to increased JNK activation, more severe decrease of maximal and reserve capacity oxygen consumption rate and more cell death. Despite that autophagic flux is upregulated in Sirt3 knockout cells, inhibition of autophagy exacerbates cell death, indicating that insufficient autophagy activation contributes to starvation-induced cell death. 3) In proliferation breast cancer cells, attenuated autophagic flux in response to GABARAPL1 knockout is associated with increased HNE-protein adducts and increased mtDNA damage. However, these oxidative stress phenotypes did not result in cell death, but an increase of GSH, increased mitochondrial number, increased mitochondrial protein VDAC, increase of mitochondrial basal OCR, membrane potential and total cellular ATP, and resistance to exogenous HNE-induced cell death. 4) In contrast to proliferating cells, we recently found that primary neuron mitochondria exhibit increased mtDNA damage when autophagy is inhibited, with associated attenuation of mitochondrial respiration and decreases of TCA cycle metabolites. Furthermore, mitochondrial fragmentation, increased mitochondrial peroxynitrite, and inhibition of mitochondrial respiration occur upon HNE exposure. Further inhibition of autophagy exacerbates mitochondrial respiratory deficits and initiates mitochondrial remodeling. In total, these studies demonstrate common and contrasting mechanisms of mitochondrial quality control and cellular responses to endogenous and exogenous oxidative stress in proliferating and post-mitotic cells.

9.6 NATURAL KILLER CELLS CONTRIBUTE TO PLACENTAL MITCHONDRIAL DYSFUNCTION IN RESPONSE TO PLACENTAL ISCHEMIA IN REDUCED UTERINE PERFUSION PRESSURE (RUPP) RAT MODEL OF PREECLAMPSIA
Introduction: Preeclampsia (PE), is characterized by new onset hypertension and is associated with immune activation and placental oxidative stress. Placental ischemia is believed to be the initial event in the development of PE. Cytolytic Natural Killer (NK) cells are elevated in the placentas of PE women. One mechanism of cytotoxicity of NK cells is release of proteins modulating mitochondrial dysfunction and oxidative stress which may play a role in the pathophysiology of PE. We have shown that placental ischemia induces NK cell activation in the reduced uterine perfusion pressure (RUPP) rat model of PE. Thus, we hypothesize that NK cell depletion could improve oxidative stress, mitochondrial function and blood pressure in RUPP rats.

Methods: Sprague Dawley rats were divided into three groups; normal pregnant (NP), RUPP and RUPP+NK cell depletion rats (RUPP+NKD). On gestational day (GD) 14, RUPP surgery was performed, and NK cells were depleted with Anti-asialo GM1 antibodies (7µg/100µL, i.p) on Gday 15 and Gday 17. On GD19 blood pressure (MAP), on Gday 15 and Gday 17. On GD19 blood pressure (MAP) was measured and placental mitochondria were isolated. Mitochondrial function was assessed by studying respiration and Complex I activity. Data are expressed as mean±SEM, statistical analysis included one way ANOVA and Bonferroni post hoc test.

Results: MAP was elevated in RUPP (n=9) compared to NP rats (n=10) (125±3 mmHg vs. 109±2 mmHg, p<0.05) which was normalized in RUPP+NKD (n=3; 106±6 mmHg), State 3 (313±16 vs. 423±15 pmol/sec/mg, p<0.05) and uncoupled (244±13 vs. 300±11 pmol/sec/mg, p<0.05) respiration rates were reduced in RUPP (n=7) vs. NP (n=8) but improved in RUPP+NKD (n=3; 398±28 pmol/sec/mg, p<0.05 vs RUPP). However, there was no change in uncoupled respiration. Respiratory control ratio (state 3/state 4) was significantly reduced in RUPP (n=7) vs. NP (n=8) (7±1 vs 11±1, p<0.05) but was improved in RUPP+NKD (n=3; 12±2, p<0.05 vs. RUPP). Complex I (12±3 vs. 23±2 mmol e-/min/mg, P<0.05) was drastically reduced in RUPP compared to NP but was increased in RUPP+NKD (n=3; 24±6, p=0.1349 vs RUPP) but did not reach statistical significance.

Conclusion: The reduction in mitochondrial respiration and complex I activity were improved with NK cell depletion in RUPP rats indicating the importance NK cells play in causing placental mitochondrial dysfunction and pathophysiology in response to placental ischemia of pregnancy. Funding: RO1HD067541(BL)/Office of Research, UMMC.
The relationship between insulin resistance (IR) and type 2 diabetes (T2DM) with mitochondrial dysfunction is controversial. Our laboratory has found mitochondrial dysfunction and impaired cerebrovascular function of young (10 - 12 w old) Zucker Obese (ZO) rats compared with their Zucker Lean (ZL) controls, when the ZO rats were IR but not hyperglycemic. However, mitochondrial respiration was normal in the arteries from ZO rats. The development of T2DM in 14 w old Zucker Diabetic Fatty Obese (ZDFS) rats resulted in a significantly decreased mitochondrial basal respiration rate and proton leak as well as altered protein expression of the voltage dependent anion channel (VDAC) and manganese superoxide dismutase (MnSOD) in large cerebral arteries of ZDF compared with Leans (ZDFL). The level of superoxide was significantly increased in cerebral blood vessels of ZDFS rats compared with ZDFL rats. In this study, we examined prolonged T2DM effects on cerebrovascular mitochondrial function.

Cerebral microvessels and large cerebral arteries, isolated from 21 w old, male ZDFS and ZDFL animals, maintained on high fat diet, were used to determine mitochondrial and non-mitochondrial protein expression using Western blot and mitochondrial oxygen consumption rate (OCR) measured by the Seahorse Bioscience Analyzer.

We found that levels of mitochondrial proteins for the Dynamin Related Protein-1, VDAC, Complexes II, III, and V, acetylated MnSOD, and for the non-mitochondrial, phosphorylated and total endothelial nitric oxide synthase (eNOS) were similar between the ZDFS and ZDFL rats. Surprisingly, the calculated components of mitochondrial respiration, ATP production, proton leak, basal respiration, maximal respiration, and spare capacity were similar between the ZDFS and ZDFL groups.

Our data suggest that the early stages of IR and T2DM results in selective mitochondrial dysfunction but that mitochondrial respiration and protein levels become normal with prolonged T2DM. More research is needed to determine and understand this compensatory mechanism and its implications in treatment of diabetic patients.

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Heart failure (HF) is the clinical syndrome causing the highest adult death rate in industrialized countries, with an annual death rate of 20-40% of the diagnosed cases, and a 50% survival chance 5 years from initial diagnosis. Among its complex etiology and pathophysiology, intracellular Ca\(^{2+}\) dysregulation has been recognized as one of the key manifestations of HF. Such intracellular Ca\(^{2+}\) dysregulation leads to a decrease in ATP and an increase of reactive oxygen species mediated by mitochondrial dysfunction. In patients with advanced HF it has been found an overexpression of the protein in charge of Ca\(^{2+}\) uptake into the mitochondria, the mitochondrial calcium uniporter (MCU). The modulation of MCU activity, like with the use of Ru360 could be a potential therapy against Ca\(^{2+}\) overload in HF. However Ru360 has a low cell membrane permeability. To overcome this limitation, the use of nanovehicles has been explored with the objective to increase the bioavailability of intracellular Ru360 with a controlled release.

In this work, Ru360 was encapsulated in a polymeric nanovehicle. This nanovehicle was characterized in terms of size distribution, surface charge, and loading capacity of Ru360. H9c2 cells were used to study the internalization of the nanovehicles by confocal microscopy and to study the modulation of [Ca\(^{2+}\)]\(_{m}\) transport. Results obtained show that the nanovehicle has an average diameter of 100 nm with a negative surface charge and a loading capacity of 40 nmole/mg of nanovehicle. Internalization into H9c2 cells is dose dependent and the nanovehicle can reduce the [Ca\(^{2+}\)]\(_{m}\) transport after 24 hours of incubation better than free Ru360.

**9.10 VASCULAR ENDOTHELIAL MITOCHONDRIAL FUNCTION PREDICTS DEATH OR PULMONARY OUTCOMES IN PRETERM INFANTS**

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**Rationale:** Vascular endothelial mitochondrial dysfunction contributes to the pathogenesis of several oxidant stress associated disorders. Oxidant stress is a major contributor to the pathogenesis of bronchopulmonary dysplasia (BPD), a chronic lung disease of prematurity that often leads to sequelae in adult survivors. **Objectives:** This study was conducted to identify whether differences in mitochondrial bioenergetic function and oxidant generation in HUVEC (human umbilical venous endothelial cells) obtained from extremely preterm infants were associated with risk for BPD or death before 36 weeks post-menstrual age.
Methods: HUVEC oxygen consumption and superoxide and hydrogen peroxide generation were measured in 69 infants. Results: When compared to HUVECs from infants who survived without BPD, HUVEC obtained from infants who developed BPD or died had lower maximal OCR (mean ± SEM in pmol/min/30,000 cells, 107 ± 8 vs. 235 ± 22, p < 0.001), produced more superoxide after exposure to hyperoxia (mean ± SEM in MitoSox Red fluorescence units: 89807 ± 16616 vs. 162706 ± 25321, p < 0.05) and released more hydrogen peroxide (H₂O₂) into their supernatant after hyperoxia exposure (mean ± SEM in resorufin arbitrary fluorescence units, 1879 ± 278 vs. 842 ± 119, p < 0.001). Conclusions: Our results indicating that endothelial cells of premature infants who later develop BPD or die have impaired mitochondrial bioenergetic capacity and produce more oxidants at birth suggest that the vascular endothelial mitochondrial dysfunction seen at birth in these infants persists through their postnatal life and contributes to adverse pulmonary outcomes and increased early mortality.

NANO-PARTICULATE EXPOSURE IMPACTS THE ADAPTIVE RESPONSE IN 6-MONTH AND 21-MONTH OLD FEMALE MICE
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Environmental toxicants, such as particulate matter, can act as an accelerator of protein damage, with excess accumulation of protein aggregates promoting the aging process. To counteract, cells rely upon a plethora of stress-responsive enzymes, including the mitochondrial Lon protease and the cytosolic 20S proteasome, both of which degrade damaged proteins. However, with age, their efficiency decreases. Our objective is to understand the age-related changes of the adaptive stress response associated with non-toxic amounts of nanoparticulate matter (nPM) in young (6 month) and middle-aged (21 month) female mice.

The adaptive stress response, or as recently coined ‘adaptive homeostasis,’ is a phenomenon, wherein exposure to non-damaging amounts of an oxidant triggers heightened expression of multiple stress-inducible enzymes, including the mitochondrial Lon protease and the 20S proteasome. Earlier invertebrate studies have shown a robust increase in the stress response in young organisms, which is lost with age. Here we explore a novel approach to assess the adaptive response in a mammalian model. Female mice were exposed to either ambient air or reaerosolized particulate matter (nPM) collected from the 110 Freeway (Southern California). 3 month and 18 month female mice were exposed for 5 hours a day, 3 days a week, for 10 weeks. Afterwards, heart, liver, and lung tissue was collected and protein expression, activity, and oxidation was assessed.

Our findings suggest nPM exposure and age impact the mitochondrial Lon protease and the proteasome expression and activity. More interestingly, is its impact upon indirect tissues (heart and liver). In all three tissue types, nPM exposure in young females, triggered a strong increase in Lon and the 20S proteasome. Yet, inducibility was lost in an age- and nPM-dependent manner. Interestingly, Nrf2, the transcriptional activator of the proteasome, showed age-related increases, accompanied by an age-related increase in its transcriptional suppressors, Bach1 and c-Myc. Thus our findings indicate the age- and nPM-related loss of the adaptive response. Moreover, this approach is first-of-its-kind in presenting a mammalian model of the adaptive stress response. Together, this work offers a clearer understanding of the effects of air pollution on aging and its impact on the adaptive stress response.

9.12 POTENTIAL LOCAL ADAPTATION OF MITOCHONDRIAL AND NUCLEAR PROTEIN DEGRADATION TO STRESS MEASURED IN LON PROTEASE AND PROTEASOME ACTIVITY IN A WIDELY DISTRIBUTED INTERTIDAL MARINE ORGANISM
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Evolution has crafted a diversity of phenotypes resulting in many organisms that are locally adapted to respond to the rigors of their native environment. In a widely distributed intertidal marine crustacean, Tigriopus californicus, previous studies have shown a longitudinal cline in thermal tolerance between separate populations. In this experiment, protein degradation patterns are compared between a southern population from San Diego with a higher thermal tolerance and a northern population from Santa Cruz with a lower heat tolerance. This study examines the how the major actors of protein degradation and by extension protein homeostasis, the Lon Protease in the mitochondria and the Proteasome in the cytoplasm respond to heat stress in locally adapted populations. This study aims to understand how evolutionary forces have shaped the patterns of protein regulation. Lon Protease activity was measured by the degradation of tritium labelled aconitase and Proteasome activity was measured by the degradation of a fluorogenic substrate and measured on a fluorometer. The impact of heat stress on protein degradation is different between the southern and northern population. Furthermore, the when these populations experience temperatures similar to the maximum temperature experienced in their environment, their protein degradation patterns are different than when they simply experience a sublethal heat stress, which indicates a potential local
adaptation of protein degradation to particular thermal regimes. Understanding how the pattern of protein degradation is modulated between these two populations could shed light on how this highly conserved process can be adjusted to a particular environment and how those adjustments can contribute to an organism’s greater fitness.

9.13 AGE-DEPENDENT ALTERATIONS IN MITOCHONDRIAL ENERGETICS IN HUMAN ATRIA

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Objective: Mitochondrial dysfunction in the senescent human heart has been documented; however, the molecular bases for the aging-associated decline in energy metabolism in the human heart are not fully understood. We examined transcription profiles of genes coding for mitochondrial proteins in atrial tissue from aged (≥65 years old) and comorbidities-matched adult (<60 years old) patients with preserved left ventricular function. We also correlated changes in functional activity of mitochondrial oxidative phosphorylation (OXPHOS) complexes with protein and gene expression changes.

Methods: Atrial appendage tissue from well-matched adult (50±8 years, n=23) and aged (73±6 years, n=25) patients undergoing elective coronary artery bypass graft surgery was used. Oxygen consumption rate (OCR) was measured in isolated mitochondria. Functional activity of individual OXPHOS complexes I-V was measured spectrophotometrically in tissue homogenates. Protein expression level of corresponding OXPHOS protein subunits was determined by Western blot. Gene expression profiling was performed using Affymetrix Human Genome U133 Plus 2.0 microarrays and RT-PCR.

Results: Isolated mitochondria from senescent hearts demonstrated a significant decrease in uncoupled OCR in the presence of carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (r=0.94, p=0.01) and state3/state 4 (r=0.84, p=0.04) when oxidized glutamate and malate. There was no age-dependent change in OCR in mitochondria oxidizing succinate or ascorbate with N,N,N',N'-tetramethyl-p-phenylenediamine. The functional activity of the individual OXPHOS complex I was significantly reduced with age (r=0.42, p=0.01). No age-dependent correlation was observed for functional activities of the remaining four OXPHOS complexes. This was associated with a significant reduction in complex I (NDUFB8 subunit) protein expression level (p=0.01). Of 78 genes that code for subunits of complexes I to V, the expression of 8 genes was significantly reduced in aged atria. These included 5 genes coding for subunits of complex I (NDUFA6, NDUFA9, NDUFB5, NDUFB8, NDUFS2, p<0.01) and one gene each for complexes II (SDHD, p=0.04), III (UQRC2, p=0.01), IV (COX7A2L, p=0.04), and V (ATPSG1, p=0.03).

Conclusion: Aging is associated with a selective decline in activity of OXPHOS within the broader transcriptional downregulation of genes regulating mitochondrial energetics, providing a substrate for reduced energetic efficiency in the senescent human atria.

9.14 MITOCHONDRIAL COUPLING AND OXIDATIVE PHOSPHORYLATION CAPACITY DIFFERS WITH LIFE HISTORY STRATEGIES IN THE WING DIMORPHIC CRICKET, GRYLLUS FIRMUS

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An individual’s life history strategy is defined by differential allocation of resources between growth, maintenance, activity, and reproduction, which results in variation in daily metabolic demands. Individuals also vary in their ability to efficiently produce ATP via oxidative phosphorylation (OXPHOS). Here, we sought to determine if variability in energy production and demand are correlated and test the hypothesis that differences in mitochondrial function occur concomitantly with variation in life history energy demands. Morphs of the wing dimorphic cricket (Gryllus firmus) specialize in either dispersal or reproduction. Compared to reproductive morphs, adult dispersal morph mitochondria were predicted to have higher OXPHOS capacities to support high metabolic demands of flight. Using an Oxygraph-2k high resolution respirometer (Oroboros Instruments, Innsbruck, Austria) we characterized rates of OXPHOS of isolated mitochondria from adult day five dispersal and reproductive morphs when stimulated by four separate substrate combinations: 1) pyruvate and malate, 2) glutamate and malate, 3) palmitoylcarnitine and malate, 4) succinate and rotenone. Regardless of substrate, average state 3 respiration rates were higher in dispersal morphs suggesting an increased oxidative phosphorylation capacity associated with this life history tactic. Notably, despite both supporting entry into complex I of the electron transport chain, OXPHOS differed markedly when stimulated by pyruvate and malate (dispersal=465.8 ± 63.6 pmolO2/s/mg; reproductive=223.2 ± 56.7 pmolO2/s/mg) compared to glutamate and malate (dispersal=97.6 ± 11.9 pmolO2/s/mg; reproductive=72.8 ± 8.4 pmolO2/s/mg). Morphs did not differ in state 4 respiration rates. Consequently, dispersal morphs have more coupled mitochondria with higher respiratory control ratios (RCR, state 3/state 4) and could be operating at a higher membrane potential. Reactive oxygen species (ROS) production increases exponentially with increases in membrane potential and when the redox
environment of the mitochondria is highly-reductive. The enhanced respiratory capacities may be necessary to produce sufficient ATP for dispersal, this may come with the associated cost of increased ROS. Future experiments will quantify both morph’s membrane potential and ROS generation. Overall, our findings provide evidence for a link between life history tactics and mitochondrial function. Funded by the Hellman Family Foundation

9.15
BUTYRATE ENHANCES MITOCHONDRIAL FUNCTION IN AUTISM LYMPHOBLASTOID CELLS UNDER PHYSIOLOGICAL STRESS
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Background: Butyrate (BUT) is a short-chain fatty acid derived from the enteric microbiome that positively modulates mitochondrial function, including enhancing oxidative phosphorylation and beta oxidation. BUT has been associated with autism spectrum disorder (ASD), a disorder that involves mitochondrial dysfunction. We have developed a lymphoblastoid cell line (LCL) model of ASD, with a subset of ASD LCLs demonstrating mitochondrial dysfunction (ASD-A) and others demonstrating normal mitochondrial function (ASD-N). Given the positive modulation of BUT on mitochondrial function, we hypothesized that BUT would have a preferential positive effect on ASD-A LCLs. To this end, we measured mitochondrial function and expression of key genes involved in mitochondrial responses to stress in ASD and age and gender-matched control LCLs following 24 and 48 h treatment with BUT (0-1mM) both with and without an acute increase in reactive oxygen species (ROS).

Results: In control LCLs, respiratory parameters linked to ATP production were attenuated by BUT at 1mM. In contrast, BUT significantly increased respiratory parameters linked to ATP production in ASD-A LCLs but not in ASD-N LCLs. In the presence of acute ROS, BUT increased respiratory parameters linked to ATP production for all groups. Analysis of individual LCL responses demonstrates that BUT modulated mitochondrial respiration to a final common set point. Gene expression analysis revealed that the highest concentration of BUT (1mM) increased expression of genes involved in mitochondrial fission (PINK1, DRP1, FIS1) and physiological stress (UCP2, mTOR, HIF1a, PGC1a) as well as genes thought to be linked to cognition and behavior (CREB1, CamKinase II).

Conclusions: These data show that the enteric microbiome derived short-chain fatty acid BUT can have beneficial effects on the mitochondria, which are dependent on dose and the intracellular redox state and vary depending on the underlying mitochondrial function of the cell. In general, these data suggest that BUT can enhance mitochondrial function in the context of physiological stress and/or mitochondrial dysfunction, and may be an important metabolite that can help rescue energy metabolism defects in ASD. This research was supported by the Arkansas Biosciences Institute (Little Rock, AR) to REF, and GoodLife Children’s Charities, Autism Canada and Autism Research Institute to DFM.

9.16
IN VIVO SKELETAL MUSCLE ENERGETICS AND FATIGUE ARE NOT DIFFERENT IN MEN AND WOMEN DURING INCREMENTAL DYNAMIC KNEE EXTENSION WORK
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Sex-based differences in human skeletal muscle energetics and fatigue have been shown during isometric contraction protocols, but less is known about energetic differences during fatiguing dynamic contractions. We hypothesized that men would fatigue more, accumulate more intracellular proton (H+) and inorganic phosphate (Pi), and rely more on glycolytic ATP production in vivo than women. Muscle oxidative capacity (rate of phosphocreatine recovery, kPCr·s⁻¹), and changes in cytosolic high-energy phosphates, pH and rates of ATP synthesis were measured in the vastus lateralis muscle of 8 women and 7 men (22.5±2.2 and 24.9±1.8 yrs, respectively) using 31-phosphorus magnetic resonance spectroscopy (MRS). Participants performed a 5-stage fatigue protocol consisting of maximal isokinetic contractions (120°·s⁻¹, 30° range of motion) inside the bore of a Siemens 3T MR system. MRS measurements were collected continuously (2-s temporal resolution, 6x8 cm³H¹⁸P dual tuned coil). Contraction frequency increased every 2-min from 0.1 to 0.125, 0.2, 0.25 and 0.5 Hz. Fatigue was quantified as the decline in peak power observed at the end of each stage and expressed as a percentage of baseline. MRS data were quantified in jMRSUI 6.0beta using AMARES. Relative concentrations of PCr, Pi, ATP, and phosphomonoesters were determined, and rates of ATP production by the 3 energy pathways (creatine kinase reaction, glycolysis, and oxidative phosphorylation) were calculated (Lanza et al., 2005, J Appl Physiol). No sex-based differences were observed for any study measures, including muscle oxidative capacity (p>0.05, all). Peak power at the end of stage 5 declined to 69.5±5.5 and 72.8±11.5 % of baseline for men and women, respectively. At the end of stage 5, [Pi] was 34.6±5.5 and 34.6±2.7mM, and pH was 6.76±0.16 and 6.78±0.12 in men and women, respectively. Fatigue at each stage was directly associated with [Pi] (r²=0.87) and [H⁺] (r²=0.98), and
indirectly related to oxidative capacity ($r^2=0.43$); $p<0.02$ for all. Notably, there were no sex-based differences in ATP flux through the 3 pathways. These results indicate that men and women experienced comparable metabolic and contractile perturbations during energetically-costly dynamic contractions, and, in both sexes, the development of fatigue was related to intracellular energetics, including oxidative capacity.

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9.17

**MITOCHONDRIA PROMOTE PROINFLAMMATORY MACROPHAGE FUNCTION IN CORONARY ARTERY DISEASE**

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There is evidence that mitochondria play a regulatory role in effector macrophages. We found that mitochondrial gene expression pattern in CAD macrophages is different from healthy control macrophages indicating changes in their functions. We hypothesized that mitochondria-dependent signaling regulates cytokine production in proinflammatory CAD macrophages. We used monocyte-derived macrophages from CAD patients and healthy subjects to characterize basic mitochondrial functions. In fact, we found that resting (M0) and activated (M1) macrophages show significant differences in metabolism and mitochondrial functions. CAD macrophages had a higher metabolic activity associated with a higher rate of glycolysis and mitochondria activity. We found that CAD macrophages had a higher rate of respiration, membrane potential, and mitochondrial ROS production. Increased mitochondrial ROS production in CAD macrophages depleted reduced glutathione. We found that CAD macrophages have distinctive fragmented mitochondria when compared to controls indicating functional alterations. Next, we analyzed detailed molecular mechanisms behind observed dysfunctions and identified a regulatory mechanism in the mitochondria responsible for the overproduction of ROS. Our data indicate that increased ROS production and mitochondrial metabolism induced a proinflammatory phenotype in CAD macrophages, including elevated IL-6 and IL-1β production. Targeting mitochondrial hydrogen peroxide reversed proinflammatory phenotype in CAD macrophages. Correcting mitochondrial hyperactivity by pharmacological intervention also reversed the proinflammatory phenotype in CAD macrophages. Taken together our study suggests a critical role for mitochondria in the regulation of effector functions and a proinflammatory phenotype in CAD macrophages.

9.18

**MITOCHONDRIAL GENETIC BACKGROUND INFLUENCES CELLULAR BIOENERGETICS AND MITOCHONDRIAL DNA DAMAGE IN HUMANS AT BIRTH**

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Cardiovascular disease (CVD) is the leading cause of death in the United States. African-Americans have a higher mortality rate from CVD than non-Hispanic whites; although the mechanisms underlying this disparity are not known. One factor often overlooked is the contribution of mitochondrial genetics to disease susceptibility. Mitochondria are both sources and targets of oxidative stress, which has been shown to contribute to endothelial dysfunction and CVD. Mitochondrial DNA (mtDNA) damage and changes in bioenergetics have also been associated with diseases of aging including CVD. We postulate that individuals with distinct mitochondrial haplogroups will have differing bioenergetic profiles and basal mtDNA damage levels which will result in differential susceptibility to CVD. Specifically, we hypothesize that individuals with African haplogroups will have different bioenergetics and higher basal mtDNA damage levels than Eurasian haplogroups.

To test this, bioenergetics and mtDNA damage were assessed in newborn cord blood and human umbilical vein endothelial cells (HUVECs) belonging to mtDNA haplogroups H and L, representing north Eurasian and African maternal ancestries, respectively. Newborn cord blood and endothelial cells were used in order to provide a “baseline” for mitochondrial function (from an individual with a relatively naive exposure history to CVD risk factors). Assessment of bioenergetics in cord blood cells is desirable as cells isolated from blood samples could serve as surrogates of systemic vascular health. Also, this approach enables assessment of mitochondrial function in a non-invasive manner in individuals throughout life for measuring various indices of mitochondrial health. This may be of potential utility in determining individual CVD risk and/or prognosis in CVD patients.

HUVECs from haplogroup L used less oxygen for ATP production and had increased levels of mtDNA damage compared with those in haplogroup H. HUVECs belonging to haplogroup L also had decreased basal and maximal bioenergetic capacities compared with haplogroup H. No significant difference was observed in bioenergetics assessed in cord blood cells, but experiments are ongoing in order to increase sample size. In conclusion, mitochondrial genetic background affects cellular bioenergetics and mtDNA damage in endothelial cells, and may contribute to CVD susceptibility.

*This research was conducted with IRB approval and in conformance with the Declaration of Helsinki.*
9.19 EFFECTS OF EPA INTAKE ON DENERVATION-INDUCED MITOCHONDRIAL ADAPTATION OF SKELETAL MUSCLE
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Eicosapentaenoic acid (EPA) is a kind of omega-3 polyunsaturated fatty acid that is abundantly contained in fish oil. EPA intake is effective for such as anti-inflammation and anti-obesity. We investigated the effects of EPA intake on denervation-induced mitochondrial adaptation in mice skeletal muscle. ICR mice (male, 8 weeks old) were daily administered Olive Oil (Control oil) or EPA at a dose of 300 mg/kg body weight by gavage for 4 weeks. After 2 weeks of oil intake, mice underwent unilateral sciatic nerve transection surgery. The hindlimb without denervation surgery served as the sham-operated control. Body and gastrocnemius muscle weight were not different between Olive Oil and EPA. PGC-1α and mitochondrial respiration proteins were decreased by denervation in both groups. Those proteins were higher in EPA group than those in Olive Oil group. In addition, EPA group contained higher levels of mRNA and protein related to mitochondrial fusion than Olive Oil group. Our results indicate that EPA intake rescues denervation-induced loss of mitochondria and enhances the expression of mitochondrial fusion molecules in mice skeletal muscle. This study was supported by a High Performance Project (Japan Sports Agency).

9.20 HEXOKINASE-II DISSOCIATION FROM MITOCHONDRIA TRIGGERS MITOPHAGY IN CARDIOMYOCYTES
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Introduction: There is emerging evidence that the metabolic pathway interplays with the survival pathway to preserve cellular homeostasis. Hexokinases (HKs) catalyze the first committed step of glucose metabolism and hexokinase-II (HK-II) is the predominant isoform in the heart. Our recent study revealed that HK-II positively regulates general autophagy in the absence of glucose. Mitochondrial HK-II (mitoHK-II) is regulated by Akt and provides mitochondrial protection against oxidative stress, while it is known to be decreased in the ischemic heart.
Hypothesis: We evaluated the hypothesis that mitoHK-II dissociation triggers mitochondria specific autophagy (mitophagy).
Results: mitoHK-II levels were significantly decreased in neonatal rat ventricular myocytes (NRVMs) subjected to simulated ischemia, in theperfused mouse heart subjected to global ischemia and in the heart subjected to myocardial infarction. To assess the role of mitoHK-II dissociation, mitoHK-II dissociating peptide (15NG) was expressed in NRVMs and in the adult heart using adenovirus or adenovirus-associated virus serotype 9 (AAV9). 15NG expression significantly decreased mitoHK-II levels in NRVMs and in the adult mouse heart. Remarkably 15NG expression induced Parkin translocation to mitochondria, robust ubiquitination of mitochondrial proteins and mitophagy assessed by Mito-Keima in NRVMs. These responses were reversed by the recovery of mitoHK-II by co-expression of HK-II but not by that of mitochondria binding deficient mutant of HK-II. Interestingly, 15NG expression did not induce mitochondrial membrane depolarization nor PARK1 stabilization at mitochondria, suggesting that the effects of mitoHK-II dissociation is not dependent on the well established mitochondria depolarization/PARK1 pathway. Modest dissociation of mitoHK-II did not induce mitophagic responses but enhanced ischemia-induced mitophagic responses and provided survival effects against ischemic stress.
Conclusions: These results suggest that mitoHK-II dissociation induced by ischemia can regulate Parkin dependent mitophagy, in conjunction with depolarization dependent mechanisms and that HK-II could confer cardioprotection by initiating mitophagy during ischemia.

9.21 REVISITING THE OPA1 FUNCTION FOR ENERGETIC MAINTENANCE
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The protein optic atrophy 1 (OPA1) is a dynamin-related large GTPase associated with the inner mitochondrial membrane. OPA1 is a critical factor connecting mitochondrial morphology and energetics by functioning in mitochondrial inner membrane fusion and cristae maintenance. Inner membrane-anchored long OPA1 (L-OPA1) undergoes proteolytic cleavage to form short soluble OPA1 (S-OPA1) lacking the transmembrane domain. Upon mitochondrial dysfunction associated with depolarization, apoptosis, or permeability transition, L-OPA1 is cleaved to form S-OPA1, which is suggested to prevent fusion of dysfunctional mitochondria. While the fusion activities of L- and S-OPA1 have been investigated, their roles in energetic maintenance are poorly understood. It is often thought that S-OPA1 is a functionally insignificant proteolytic product of L-OPA1 because OPA1 cleavage is observed with mitochondrial fragmentation and dysfunction. Nevertheless, cells contain a mixture of L- and S-OPA1 in normal conditions, suggesting the functional significance of maintaining both L- and S-OPA1. We used cells exclusively expressing L-, S-OPA1, or both and evaluated the differential functions of L- and S-OPA1. Our mitochondrial fusion assay elaborated that L-OPA1 has an intrinsic activity for mitochondrial fusion whereas the fusion activity of S-OPA1 is insignificant. Remarkably, we found that S-OPA1 alone without L-OPA1 is capable of maintaining OXPHOS function, as judged by growth in OXPHOS-requiring media, respiration measurements, and respiratory complex levels. Most
Nitrite is an established signaling molecule that regulates mitochondrial function in hypoxia by inhibiting respiration and mitochondrial oxidant production, but its effects on mitochondrial function in normoxia remain unclear. We previously showed that nitrite increases protein kinase A (PKA) activity in the heart during non-hypoxic conditions. However, the mechanism by which nitrite increases PKA activity and its consequences on mitochondrial function are unknown. Here, we demonstrate that in normoxic conditions nitrite (unlike nitric oxide) increases cAMP levels in HEK293 cell lines and in isolated mitochondria, leading to PKA activation. This increase in cAMP levels is due to nitrite-dependent inhibition of the mitochondrial localized phosphodiesterase 2A (PDE2A), which degrades cAMP. In addition to increasing PKA activation, we observed that nitrite increases the expression of A-kinase anchoring protein (AKAP1), which tethers PKA to the mitochondrial membrane. Consistent with the mitochondrial targeting of PKA, we show that nitrite induces the phosphorylation of Ser58 on mitochondrial complex IV-1 (a known PKA target) and increases its activity by 86%, leading to augmented basal and maximal respiration (48% and 29%, respectively compared to control). Pharmacological inhibition of PKA attenuates nitrite-dependent increase in respiration, complex IV activity and phosphorylation. These data demonstrate that nitrite is a unique signaling molecule able to increase cAMP levels in normoxia and show a novel mechanism by which nitrite selectively modulates mitochondrial PKA-dependent signaling through the inhibition of PDE2A. Further, these data show that nitrite is a versatile signaling molecule that not only modulates nitrogen dependent post-translational modification, but also modulates phosphorylation. Nitrite is a unique signaling molecule able to increase cAMP levels is due to nitrite-dependent inhibition of the phosphodiesterase and activates cAMP-PKA-AKAP1 signaling to modulate mitochondrial function in normoxia.
mitochondrial function or localization is disrupted, but the underlying mechanism is poorly understood. In this study, we investigate the role of mitochondria in synaptic vesicle (SV) recycling, by eliminating Dynamin-Related Protein-1 (DRP1) selectively in the presynaptic terminal at the calyx of Held synapse.

Floxed-DRP1 (DRP1fl/fl) mice were injected with AAV-cre-GFP at postnatal day 1 (P1) to inhibit DRP-1 expression presynaptically, and used for the study at P16 – P18. Infected (GFP-positive) cell soma in the ventral cochlear nucleus, and their respective calyx of Held presynapses in the contralateral medial nucleus of the trapezoid body, showed loss of DRP1 protein via antibody staining, thus generating a presynaptic-specific DRP1-KO (DRP1-preKO). Conditional DRP1-KO was confirmed by Western Blot of DRP1 from AAV-cre-GFP infected neuronal cultures. Volumetric reconstruction of the VCN cell body and calyx terminal showed significant increase in mitochondrial particle size in DRP1-KO somata and calyx presynaptic terminal.

Using postsynaptic voltage-clamp recording from calyx synapses, we find that DRP1-preKO exhibited enhanced basal evoked response (response to 0.1Hz stimulation) and a 3-fold increase in spontaneous synaptic activity (mEPSC). Standing readily-releasable pool (RRP) size was significantly reduced in DRP1-preKO, suggesting an important role for mitochondria in maintenance of SV modality at presynaptic terminal. Additionally, DRP1-preKO synapses have profoundly altered kinetics of the RRP: faster depression, increased initial release probability, and slower recovery after pool depletion were all observed. DRP1-preKO also showed a significant reduction in synaptic transmission delay. These results indicate that the proper functioning of mitochondria is essential for the regulation of synaptic vesicle release during activity, and selectively affect vesicle release during a train of stimuli. Ongoing experiments aim to determine the specific mechanism underlying the presynaptic defect in SV release.

9.25
MITOCHONDRIAL ALTERATIONS IN RIGHT VENTRICULAR DYSFUNCTION SECONDARY TO CHRONIC PRESSURE OVERLOAD

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Objective: The objective of this study is to characterize the effects of right ventricular dysfunction (RVD) on fatty acid oxidation (FAO) and mitochondrial biogenesis.

Methods: RVD was created in 8-week old male Wistar rats using pulmonary artery banding (PAB). Rats with sham surgery were used for comparison. Increases in ejection time (ET) and estimated pressure gradient across the constriction (AP) demonstrated RVD in PAB rats by serial echocardiography. 8 weeks post-surgery, invasive hemodynamics were measured and RV tissues were harvested for Western blot and RT-qPCR analysis of peroxisome proliferator-activated receptor alpha (PPARa), a marker for FAO, and PPAR-gamma coactivator 1-alpha (PGC-1α), a master regulator of mitochondrial biogenesis. All procedures were approved by the UW-Madison IACUC and conform with the APS "Guiding Principles in the Care and Use of Animals."

Results: Chronic pressure overload as well as diastolic and systolic RVD were confirmed in PAB rats by invasive measurements (Table1). Also, RV tissues from RVD showed a 3.3-fold increase in FAO and 0.5-fold decrease in mitochondrial biogenesis, suggesting a shift toward FAO metabolism and increased fatty acid uptake. Protein expression levels of PGC-1α and PPARα showed the same trends but did not reach statistical significance.

Table 1. Invasive hemodynamics and mitochondrial function-related gene expression in sham and RVD rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (n=8)</th>
<th>RVD (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;sub&gt;es&lt;/sub&gt; (mmHg)</td>
<td>27 ± 6</td>
<td>77 ± 9*</td>
</tr>
<tr>
<td>Diastolic indices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tau Weiss (ms)</td>
<td>5 ± 1</td>
<td>11 ± 2*</td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt; (mmHg/s)</td>
<td>-1816 ± 137</td>
<td>-2717 ± 348*</td>
</tr>
<tr>
<td>Systolic indices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;min&lt;/sub&gt; (mmHg/s)</td>
<td>2444 ± 124</td>
<td>4252 ± 546*</td>
</tr>
<tr>
<td>EF (%)</td>
<td>69 ± 5</td>
<td>40 ± 7*</td>
</tr>
<tr>
<td>CO (mL/min)</td>
<td>108 ± 22</td>
<td>37 ± 11*</td>
</tr>
<tr>
<td>Fold change in mitochondrial gene expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPARα</td>
<td>1.23 ± 0.02</td>
<td>3.37 ± 1.32*</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>0.71 ± 0.08</td>
<td>0.50 ± 0.03*</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM. P<sub>es</sub>, end-systolic pressure. dP/dt<sub>max</sub>, minimum rate of pressure rise. dP/dt<sub>min</sub>, maximum rate of pressure rise. EF, ejection fraction. CO, cardiac output. *p<0.05 vs Sham.

Conclusion: RVD due to chronic pressure overload alters the mitochondrial balance between FAO and glycolysis, which may implicate increased fatty acid accumulation in mitochondria (i.e., lipotoxicity) as a disease mechanism in RV failure secondary to chronic pressure overload. This study was supported by the NIH, under Ruth L. Kirschstein National Research Service Award T32 HL 007936 from the NHLBI to the UW-Madison Cardiovascular Research Center.

9.26
MICRORNA-338 REGULATES MITOCHONDRIAL FUNCTION FOLLOWING CEREBRAL ISCHEMIA BY TARGETING COX4I1 IN ASTROCYTES

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MicroRNAs (miRs) play a central role in regulating gene expression by inhibiting translation of target genes. Brain-enriched microRNA-338 (miR-338) is known to play an important role in mitochondrial and neuronal function,
however the role of miR-338 in stroke remains unknown. MiR-338 targets cytochrome-c oxidase subunit 4I1 (COX4I1), which plays an essential role in controlling ATP production and mitochondrial biogenesis. Astrocytes are critical regulators of neuronal homeostasis following stroke, and we have previously shown that interventions aimed at preserving astrocyte mitochondrial function following injury can be neuroprotective. This study investigated the effect of miR-338 inhibition on in vivo injury outcome following transient focal cerebral ischemia and on astrocyte function following in vitro ischemic injury. Pre-treatment of mice with intracerebroventricular injection of miR-338 antagonist 24 h prior to middle cerebral artery occlusion significantly reduced infarct size and improved neurological score after 24 h of reperfusion. As predicted, brain levels of COX4I1 were increased in miR-338 antagonist-treated mice. In vitro, primary astrocyte cell cultures subjected to glucose deprivation demonstrated decreased cell death when pre-treated with miR-338 inhibitor, and greater cell death when miR-338 levels were increased by mimic pre-treatment. Decreases in miR-338 were associated with increased ATP production, augmented cytochrome c oxidative activity and attenuated reduction of mitochondrial membrane potential, with preserved COX4I1 mRNA expression. Protection induced by inhibition of miR-338 was diminished by knockdown of COX4I1. In summary, miR-338 inhibition targets astrocyte mitochondrial function to improve outcome following stroke at least in part by regulating mitochondrial function and targeting COX4I1. MiR-338 therefore represents a potential therapeutic target for the treatment of ischemic stroke and other injury or disease states where mitochondrial function is impaired.

9.27 EFFECT OF AGE ON SKELETAL MUSCLE MITOCHONDRIAL FUNCTION: INSIGHT FROM NEAR-INFRARED SPECTROSCOPY
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Age is the greatest risk factor for chronic disease, and is associated with a marked decline in functional capacity and quality of life. A key factor contributing to loss of function in aging, is the decline in skeletal muscle function. While the exact mechanism remains incompletely understood, age-related mitochondrial dysfunction is thought to play a major role. To explore this question further, we recruited eight community-dwelling seniors (age: 74±4 years; m/f: 2/6; BMI: 24 ± 4) and eight young volunteers (age: 24±2 years; m/f: 5/3; BMI: 24 ± 2). Skeletal muscle oxidative capacity (i.e. mitochondrial function) was determined from the recovery kinetics of muscle oxygen consumption. Following a brief bout of handgrip exercise at 50% of one’s maximal voluntary contraction, a series of rapid, suprasystolic, arterial cuff occlusions were performed in conjunction with near-infrared spectroscopy placed over the flexor digitorum profundus (i.e. the primary muscle responsible for handgrip exercise). Muscle oxygen consumption was then calculated for each cuff occlusion, as the slope of change in oxygenated hemoglobin minus deoxygenated hemoglobin, and the maximal oxidative capacity was calculated from the recovery kinetics of muscle oxygen consumption. As expected, we observed a marked reduction in the initial rate of skeletal muscle oxygen consumption immediately post-exercise with age: -0.98±0.46 vs. -1.91±1.88 μM∙1∙s-1, P=0.02, old vs. young, respectively. Remarkably, this group difference persisted even when the workload was matched for absolute force (n = 5). In contrast to our hypothesis however, the rate constant of oxidative recovery following exercise, fit to a monoexponential recovery curve, was not found to be different with age: 56.5±7.4 sec vs. 50.6±7.5 sec, P=0.59, old vs. young, respectively. Taken together, we interpret these findings to reflect an overall reduction in mitochondrial content with age, rather than a frank impairment in mitochondrial function per se. However, a larger sample size is needed to confirm these results.
Funding: This work was supported by a UT Arlington Interdisciplinary Research Program grant.

9.28 PHOSPHOFRUCTOKINASE COORDINATES MITOCHONDRIAL AND ANCILLARY BIOSYNTHETIC PATHWAY ACTIVITIES IN THE CARDIOMYOCYTE
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Background: The heart is an omnivorous organ, using a myriad of substrates for both catabolism and anabolism. While the catabolism of glucose via glycolysis for energy provision is fairly well understood, it is less clear how the ancillary pathways of glucose metabolism, critical for synthesizing cellular building blocks and modulating stress responses, are regulated.
Methods: We used radiometric glycolytic assays, [13C6]-glucose isotope tracing, and extracellular flux analysis to understand how phosphofructokinase (PFK)-mediated changes regulate glucose carbon partitioning into catabolic and anabolic pathways. As tools to determine the relevance of PFK-mediated changes in glycolysis, we determined how transduction of mutant forms of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK2) affect cardiac myocyte catabolism and anabolism in vitro.
Results: Expression of kinase-deficient (Glyco16) or phosphatase-deficient (Glyco16)-PFK2 in neonatal rat cardiac myocytes coordinated regulated glycolytic rate and lactate production. Interestingly, in all groups, >40% of
glucose taken up by the cell was not catabolized to pyruvate, providing a substantial amount available for entry into the ancillary pathways (e.g. pentose phosphate, hexosamine biosynthetic, glycerolipids). Stable isotope resolved $^{13}$C isotopologue fractional enrichment patterns suggest that PFK activity regulates glucose carbon incorporation into the ribose and glycerol moieties of purines and phospholipids, respectively. However, under conditions of high PFK activity, low $^{13}$C incorporation into pyrimidines, UDP-N-acetylhexosamine, and fatty acyl chains of triglycerides suggest limitations in mitochondrial-derived oxaloacetate, acetyl-CoA, and fatty acids. Consistent with this idea, high glycolytic rates diminished mitochondrial activity and the coupling of glycolysis to glucose oxidation.

Conclusions: These findings suggest that PFK coordinates the activities of ancillary pathways of glucose metabolism by directly modulating glycolytic intermediate entry into such pathways and by indirectly regulating mitochondrial catabolism.

9.29 CORRELATION BETWEEN MITOCHONDRIAL FUNCTION AND MORPHOLOGY AFTER ISCHEMIA-REPERFUSION

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We have shown that mitochondria-derived vasodilation of middle cerebral arteries (MCAs), was retained 48 h after middle cerebral artery occlusion (MCAO) compared with arteries from the contralateral (contra) side or with MCAs from sham rats1. Although the magnitude of the mitochondria-derived vasodilation of ipsilateral (ipsi) MCAs was intact, the overall vasoreactivity, including the endothelium-dependent vasodilation1 and Ca$^{2+}$ sparks activity2, was significantly reduced when compared with contra and sham MCAs. In addition, ipsi mitochondria respired at an energetically inappropriate level compared with contra or sham MCAs. However, the correlation of these changes in mitochondrial function in relation to mitochondrial morphology in ipsi MCAs remains unclear. Therefore, we examined mitochondrial morphology in ipsi, contra, and sham MCAs, using transmission electron microscopy, from age-matched, male, Sprague Dawley rats, randomly exposed to MCAO or sham surgery.

MCAO had distinctive effects on mitochondrial morphology and density of ipsi but not contra MCAs. Mitochondria appeared damaged with areas of disrupted internal structures such as the sarcoplasmic reticulum in the vascular smooth muscle cells (VSM) of ipsi MCAs. Extensive mitochondrial fields, typically seen in naïve MCAs, were reduced in area. In contrast, mitochondria in endothelial cells (EC) in ipsi MCAs appeared more prevalent than in sham MCAs and lacked evidence of mitoptosis, seen in VSM cells. Mitochondria in EC cells of ipsi MCAs were in close approximation of adjacent mitochondria, which is suggestive of fission. Mitochondrial morphology, density, and relationship to other cellular structures in sham MCAs were similar to naïve arteries. Typical mitochondrial fields were present in VSM cells of contra MCAs and mitochondria in EC did not exhibit any characteristics distinct from sham or naïve MCAs.

Our data indicate that the altered mitochondrial function in ipsi MCAs corresponds to cell specific vulnerability of VSM and resiliency of EC to ischemic stress. Thus, given the critical role of mitochondria in promoting cell protection, mitochondria in cerebral vascular EC might represent an important therapeutic target in stroke patients. Funding: NIH (DWB: HL-077731; HL093554), LA Board of Regents (DWB; and PVK: LEQSF(2014-17)-RD-A-11), AHA (PVK: 14SDG20490359; IR: 15POST23040005; 17SDG33410366). 1I Rutkai et al. PMID25063798. 2I Rutkai, et al. FASEB J April 2017 31:1080.16.

9.30 ASPARATE IS NOT THE SOURCE OF SUCCINATE IN MYOCARDIAL ISCHEMIA.

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Background: Ischemia-reperfusion injury is the underlying pathology of acute myocardial infarction. The metabolite succinate accumulates during ischemia and is rapidly consumed at reperfusion, driving the generation of reactive oxygen species. Despite this detrimental role at reperfusion, ischemic succinate accumulation is evolutionarily conserved across diverse tissues and species, suggesting that it may serve a beneficial role in ischemia. Elucidating the pathway of succinate accumulation will provide insights into ischemic metabolism that could be leveraged for novel therapeutics.

Ischemic succinate is proposed to be derived from aspartate that is converted to fumarate via the malate/aspartate shuttle or purine nucleotide cycle. Fumarate is then reduced to succinate via a functional electron transport chain (ETC) of Complexes (Cx) I and II (Cx I-II ETC). Herein, we used a targeted metabolomics approach to test these hypotheses in a mouse cardiac model of ischemia.

Methods: Experiments complied with the NIH Guide for Care and Use of Laboratory Animals. Ischemic succinate accumulation was investigated in mouse heart mitochondria, cardiomyocytes, and perfused hearts. Metabolites were resolved using HPLC/photo-diode array. Metabolomic flux analyses were performed using heavy isotope tracking by LC-MS/MS.

Results: Hypoxic mitochondria generated succinate via a Cx I and II dependent mechanism. However, in both cardiomyocyte and perfused heart models, ischemic succinate accumulation was not sensitive to Cx I, Cx II, or Krebs cycle inhibitors. Appropriate inhibition of Cx I and
Mitochondria from Bench to Bedside

The human mitochondrial DNA (mtDNA) is present in multiple copies per cell and codes for 13 polypeptides, 22 tRNAs and 2 rRNAs, all of which required for oxidative phosphorylation (OXPHOS) activity. A large number of mutations (>200) in the mtDNA have been associated with clinical syndromes. However, only when their levels exceed a certain threshold, a bioenergetics defect ensues. Therefore, if the levels of mutant genomes could be reduced, the cell should recover its normal OXPHOS function.

We developed mitochondria-targeted TALEN (mitoTALEN) directed against a point mutation in the mtDNA of a heteroplasmic mouse model. This point mutation in the tRNA alanine gene (nt 5024 C>T) destabilizes the tRNA alanine, causing a mitochondrial protein synthesis defect in cells with high levels of the mutation. The mouse with approximately 70-80% mutant mtDNA develops a mild phenotype, developing a cardiomyopathy later in life (around 1 year of age). Experiments with cells from the mouse showed that the mitoTALEN designed was highly efficient against the mutant mtDNA. The two mitoTALEN monomers were expressed from AAV9 particles and injected in the Tibialis Anterior (TA) muscle of the right leg muscle. AAV9 coding for only one monomer was injected in the left TA. Mice were sacrificed 6, 12 and 24 weeks after injection. DNA analysis showed a reduction in the percentage of mutant mtDNA in the right TA at all time points. We did not detect a depletion in mtDNA nor significant levels of deletions, which were previously associated with mtDNA double-strand breaks. In addition, the decrease in mutant mtDNA load in the TA was accompanied by an increase in the levels of tRNA alanine, which is decreased by the 5024 C>T mutation.

In parallel, we are also designing smaller mitochondria-targeted DNA editing enzymes, with could facilitate in vivo gene therapy for mitochondrial diseases.

10.2 DNA DAMAGE DRIVES AGING VIA SUPPRESSION OF THE MITOCHONDRIAL UNFOLDED PROTEIN RESPONSE AND DAF-16/FOXO3A ACTIVITY

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DNA damage is implicated in the aging process, yet the mechanism by which it drives aging is not fully elucidated. To address this, we screened DNA repair-deficient ercc-1 worms, with increased endogenous nuclear DNA damage, for dysregulation of lifespan modulating pathways. Surprisingly, the mitochondrial unfolded protein response was suppressed, while DAF-16/FOXO3A was activated. The former was required for energy optimization and the latter to mitigate the accompanying increase in oxidative stress. However, the period of protection conferred by DAF-16/FOXO3A activation was limited and correlated with organismal dysfunction, in a CEP-1/P53 dependent manner. These DAF-16/FOXO dynamics were recapitulated in DNA repair-deficient primary cells and mice, as well as, wild-type mice, demonstrating that this is a conserved response. Collectively these data suggest that it is the mitochondrial reprogramming and failure to maintain the appropriate cellular stress responses in the face of chronic DNA damage that promotes age-related decline.

10.3 MITOCHONDRIAL FUNCTION AND THE EPIGENETIC LANDSCAPE

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Mitochondria are well-recognized for their role in ATP and reactive oxygen species generation, in addition to producing intermediate metabolites through the TCA (tricarboxylic acid) cycle. The crosstalk between metabolism and epigenetic modifications in the nucleus is becoming increasingly evident but the extent to which mitochondrial metabolites are required for these effects
remains largely unknown. We recently showed using a cell culture model of acute mitochondrial DNA (mtDNA) depletion that electron flow sustains a functional oxidative TCA cycle, which in turn is necessary to maintain histone acetylation in the nucleus. Following this work, we have interrogated genome-wide locus specific changes in histone acetylation and in DNA methylation, and the extent to which these regulate gene expression. The time-wise progression and the signals associated with these events will be discussed. Lozoya et al., submitted. This work was supported by the NIH intramural program.

10.4 MITOCHONDRIAL TARGETED OXIDANTS AND DNA DAMAGE

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Mitochondria dysfunction is associated with human pathophysiologies including: aging, cancer, cardiovascular disease and neurodegeneration. We sought to develop a targeted method for causing oxidative mitochondrial dysfunction in the absence of damage to other regions of the cell to test the controversial hypothesis that dysfunctional mitochondria generate sufficient flux of ROS to cause nuclear damage. A novel mitochondrial-targeted fluorogen-activating-peptide (mito-FAP) and a malachite green dye analog, which specifically targets singlet oxygen to Complex IV when irradiated with 660 nm light, induced rapid mitochondrial dysfunction in HEK293 cells. By controlling the amount of dye and light exposure we found conditions to reduce mitochondrial respiration to 10% of control within 4 hrs of damage, as measured by Seahorse Biosanalyzer. Within 4 hrs of damage, mitochondria displayed a fragmented appearance by STED microscopy. This singlet oxygen damage to the mitochondrial electron transport chain (ETC) caused a second wave of mitochondria-generated superoxide and loss of Complex I, III, and IV activities. Damage was abrogated by pretreatment with sodium azide, a singlet oxygen quencher or N-acetyl cysteine a ROS scavenger. Rho zero cells, lacking respiration, were refractory to the second wave of superoxide production. These data are consistent with mito-FAP + dye + light causing rapid singlet oxygen damage to the ETC, followed by dysfunctional ETC production of superoxide. This persistent mitochondria-induced ROS caused a loss of mitochondrial membrane potential and inhibition of cell growth, without apoptosis. Cell cycle arrest was accompanied by a loss in DNA replication and appearance of a replication stress marker, phospho-RPA32. Our experiment with nuclear-targeted HyPer, which is a fluorescent sensor specific for measuring hydrogen peroxide present in the nucleus, indicated appreciable hydrogen peroxide flux 24 hrs after the initial mitochondrial injury with singlet oxygen. Fragile telomeres were present 48 hr after mito-FAP damage supporting the concept that a flux of mitochondria-generated ROS can cause nuclear DNA damage. Finally, we have used this system to successfully ablate mitochondrial function in 5-day old zebrafish embryos expressing mito-FAP in the central nervous system. Together these results indicate a novel approach for studying the effects of mitochondrial damage in living cells and model organisms. Support: NIH grant R21ES025606.


LATE BREAKING ABSTRACT SUBMISSIONS

LB1 MITOCHONDRIAL ADAPTATIONS ASSOCIATED WITH THE LOSS OF HEMOGLOBIN AND MYOGLOBIN MAY CONSTRAIN CARDIAC PERFORMANCE OF ANTARCTIC FISHES

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The loss of expression of hemoglobin (Hb) and myoglobin (Mb) in Antarctic iceshies is associated with extensive mitochondrial remodeling in cardiac muscle. Mitochondrial density is 36.5 % in hearts of species that lack Hb and Mb, compared to 15.9 – 25.0 % in species that express the proteins, and mitochondria are enlarged due to a proliferation of phospholipids, but not proteins. We sought to determine how alterations in mitochondrial structure impact function, particularly in response to warming. The red-blooded species, Notothenia coriiceps and icefish, Chænocephalus aceratus, were held at ambient temperature and exposed to acute heat stress. The maximal activities of citrate synthase (CS) and lactate dehydrogenase (LDH), respiration rates of isolated mitochondria, adenyate levels, and changes in mitochondrial protein expression were quantified from hearts of animals held either at ambient temperature and following acute heat stress. Cardiac mitochondria of iceshies are extremely well coupled, but in response to warming, proton leak increases to a greater extent in iceshies compared to red-blooded species. The ratio of cytochrome c oxidase activity-to-state 3 respiration rate is significantly higher in mitochondria of +Hb/ +Mb species, suggesting a greater capacity to enhance flux through the respiratory chain when needed. Consistent with this, levels of ATP and energy charge are higher in hearts of +Hb/+Mb species at both ambient temperature and following acute heat stress. Maximal activity of CS is also higher in +Hb/+Mb species. Finally, proteomic analysis indicates that mitochondrial protein expression of aerobic metabolic enzymes and antioxidants increases in hearts red-blooded species but not the icefish in response to acute thermal stress. Together, these data suggest that cardiac performance at elevated temperature may be constrained by mitochondrial function in Antarctic iceshies. Funding was...
provided by a grant from the National Science Foundation (ANT 1341663 to KOB).

LB2

AGE-RELATED CHANGES IN MYOGENIC CAPACITY OF EQUINE SATELLITE CELLS
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Myogenic stem cells (satellite cells, SC) aid in muscle repair and growth during postnatal life. In response to injury or a growth stimulus, SC are activated, proliferate, and subsequently differentiate and fuse to form mature myofibers. As subtle myofiber injuries can occur routinely during daily muscle activity, continuous muscle repair is essential for muscle maintenance throughout life. However, the ability to regenerate muscle and replace damaged myofibers declines with age. The poor regenerative capacity of aged muscle is reportedly due to changes of the SC microenvironment, but intrinsic changes within an aged SC may also impair its function. Here, we examined differences in the intrinsic myogenic capacity and mitochondrial function between skeletal muscle SC from young and aged American Quarter Horses. Muscle biopsies were taken from the Gluteus medius (GLU) and the Triceps brachii (TRI) of young (2-4 yr; n=4) and aged (20-27 yr; n=4) horses. Satellite cells were isolated, proliferated in growth medium, and differentiated in serum-depleted medium. Satellite cells derived from aged compared to young TRI tended to display compromised proliferative capacity (P=0.062, day 4), and significantly lower differentiation (P=0.017) and fusion capacity (P=0.023). Satellite cells isolated from aged GLU exhibited a significant decline in proliferative rate (P=0.015, day 4) and a tendency for a decrease in differentiation capacity (P=0.079). During SC differentiation, gene expression of mitochondrial (mt) DNA encoded subunits of the electron transport system, ND1 (P=0.007, day 1; P=0.043, day 2) and COX1 (P=0.031, day 1; P=0.017, day 2), was lower in SC from aged compared to young TRI, despite a higher mt DNA copy number in aged SC (P=0.033, ND1 primer; P=0.021, COX1 primer). Interestingly, differentiating SC from aged compared to young TRI also showed decreased gene expression of mt biogenesis regulators PGC-1α (P=0.059), NRF1 (P=0.05) and TFAM (P=0.008); and differentiating SC from aged compared to young GLU displayed a decrease in expression of PGC-1α (P=0.024). Accumulation of autophagy cargo protein p62 (P=0.038) and autophagosome-bound protein LC3II (P=0.034) in differentiating SC from aged TRI suggested impaired autophagic activity. Collectively, our study suggest that age was associated with impaired mt function, biogenesis and possibly quality control in SC from equine skeletal muscle.

LB3

TARGETING MITOCHONDRIAL FISSION RESTORES INSULIN SIGNALING AND BIOENERGETICS IN SKELETAL MUSCLE INSULIN RESISTANCE

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Despite numerous investigations examining the effects of ischemia on the ability of cardiac mitochondria to produce ATP, it is still unclear the degree to which ischemia affects each component of mitochondrial energy transduction pathway. The present study examined mitochondrial function at respiration rates between resting and maximal and utilized force-flow analysis to determine the effect of ischemia on each step of the oxidative phosphorylation pathway. Mitochondria were isolated from rat hearts subjected to either 60 minutes of control coronary flow or 60 minutes of
Mitochondria from Bench to Bedside

SKELETAL MUSCLE MITOCHONDRIAL UNCOUPLING ACTIVATES THE INSULIN SIGNALING PATHWAY

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Obesity is an epidemic effecting nearly 100 million individuals in the United States. Upwards of $210 billion dollars are spent yearly treating acute and chronic conditions of obese patients. As such, therapeutic approaches aimed at reducing obesity and its related symptoms are highly warranted. Mitochondrial uncoupling agents are well known for their effects on enhancing metabolic rate and energy expenditure. By collapsing the proton gradient, energy is dissipated as heat, resulting in weight loss. Uncoupling agents have been used in the treatment of obesity since the 1930’s. However, the underlying mechanisms driving uncoupling-induced thermogenesis and its interactions with lipid metabolism remain poorly understood. To investigate the role of uncoupling in skeletal muscle metabolism and insulin resistance, fully differentiated C2C12 myotubes were treated with 20 μM of carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) for 4 hours. Furthermore, cells were differentially treated with 500 μM palmitic acid (PA) (18 hours), FCCP (4 hours), and insulin (10 minutes). Changes in mitochondrial dynamics and insulin signaling were assessed via Western blot. Treatment with FCCP significantly uncoupled (P<0.05) OXPHOS as demonstrated by OPA1 proteolytic processing. At 30 minutes and extending through the 4 hour time course, FCCP significantly increased AKT (t308 and s473) and AS160 phosphorylation (P<0.05). Treatment with PA significantly reduced OPA1 expression (P<0.05), as well as activation of the insulin signaling pathway (P<0.05). Insulin recovered proteolytic degradation of L-OPA1 in control and PA treated cells. The combined effects of FCCP and insulin significantly differed in PA-treated cells (P<0.05), suggesting an interaction-effect between FCCP and palmitate. Thus, skeletal muscle mitochondria act as energy sensors, acting through canonical signaling pathways in order to maintain homeostasis. Lipotoxicity appears to ablate this response, by both deteriorating communication within the mitochondria and intracellularly. These findings provide mechanistic insight into how lipids may contribute to mitochondrial dysfunction and insulin resistance in skeletal muscle. This study was funded by the NIH (R01-DK108089).
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*indicates invited speaker