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Abstracts

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Editors

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Summary

MiP*school* 2025 Baton Rouge, US is the 14th installment of the Mitochondrial Physiology Society's flagship training school focused on the history, fundamentals, and conceptual advances in the field of cellular bioenergetics. Participants will gain and improve working knowledge in key areas of study within the field of mitochondrial physiology including substrate coupling control, mechanisms of oxidative phosphorylation and electron transfer, redox regulation, and structural organization of the respiratory system, and more. Lecturers will provide detailed presentations on the principles of bioenergetics, and keynote speakers will deliver talks on cutting edge applications in biochemistry, cell biology, physiology, and biomedicine spanning multiple levels of biological organization. The following book includes the compendium of invited and submitted abstracts to MiP*school* 2025, presented as oral and/or poster presentations from May 19th-22nd in Baton Rouge, LA, USA.

Contents

1. Submitted abstracts

Lipin-1 promotes macrophage mitochondrial fission for improved inflammation resolution

Oluwakemi O Igiehon, Temitayo T Bamgbose, Robert M Schilke, Chowdhury S Abdullah, Matthew D Woolard

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Chronic inflammation drives the pathophysiology of many cardiometabolic diseases. Pro-resolving macrophages clear up apoptotic cells and cellular debris by efferocytosis to resolve inflammation and restore tissue homeostasis. This imposes a metabolic burden on macrophages, necessitating the efficient metabolism of lipids derived from efferocytic cargoes for effective pro-resolving responses. Therefore, understanding the regulatory pathways that control macrophage lipid metabolic profiles is essential for uncovering mechanisms of inflammation resolution. Lipin-1 is a phosphatidic acid phosphatase with a transcriptional coregulatory (TC) activity that regulates lipid metabolism. We have shown that macrophage lipin-1 promotes inflammation resolution by enhancing efferocytosis and β-oxidation of fatty acids, while restraining lipid biosynthesis[1]. Our current study aims to define the mechanisms by which macrophage lipin-1 promotes β-oxidation/oxidative phosphorylation, efferocytosis and inflammation resolution. Using lipin-1 knockout (lipin-1 KO) and wildtype bone marrow-derived macrophages, we showed that macrophage lipin-1 facilitates mitochondrial fission, producing fragmented mitochondria with enhanced oxidative phosphorylation capacity compared to the elongated mitochondria[2]. Additionally, lentiviral transduction of lipin-1 KO macrophages with specific truncated forms of lipin-1 suggests that a novel non-canonical activity of lipin-1 is sufficient to restore and augment efferocytosis. Our findings uncover a novel role for lipin-1 in coordinating mitochondrial network and metabolic function in macrophages. This deeper understanding of lipin-1's influence on mitochondrial dynamics and macrophage function advances our knowledge of the cellular mechanisms underlying inflammation resolution and may inform future investigations into metabolic regulation in immune responses.

[1] Bamgbose TT, et al (2025) Lipin-1 restrains macrophage lipid synthesis to promote inflammation resolution. The Journal of Immunology 214, 85–103.
[2] Benador IY, et al (2018) Mitochondria Bound to Lipid Droplets Have Unique Bioenergetics, Composition, and Dynamics that Support Lipid Droplet Expansion. Cell Metabolism 27, 869-885.e6.

Cite: Igiehon OO, Bamgbose TT, Schilke RM, Abdullah CS, Woolard MD (2025) Lipin-1 promotes macrophage mitochondrial fission for improved inflammation resolution. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

Mitochondrial remodeling and energetics during FoxO1-mediated adipose transdifferentiation

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As the metabolic hub, mitochondria undergo dynamic remodeling in response to physiological or pathophysiological changes, such as obesity, inflammation, and ischemic diseases [1-3]. The high plasticity of adipose tissue (AT) allows the transdifferentiating from white AT to beige AT (adipose whitening), or vice versa (adipose browning or beiging). Our recent study underscored an important role of the transcription factor FoxO1 in regulation the plasticity of adipose tissue and systemic glucose metabolism [4]. Here we used high-resolution respirometry (HRR, Oxygraph-2k from Oroboros Instruments, Austria) and found that silencing FoxO1 in adipocytes stimulated mitochondrial energetics, including basal respiration (by 50%, $p<0.01$), maximal respiration (by 91%, $p<0.01$), and spare respiratory capacity (by 89%, $p<0.01$). HRR analysis adipose tissue explants indicated that silencing FoxO1 significantly increased mitochondrial fatty acid oxidation activity (FAO, $p<0.05$; FAO+D, $p<0.01$; FAO+CI+CII, $p<0.01$). The stimulation of mitochondrial energetics was associated with increased mitochondrial content in adipose tissue assessed by electron microscopy. Surprisingly, autophagic clearance of mitochondria appeared to be augmented in adipose lacking FoxO1. Pathway analysis indicated an upregulated mitochondrial biogenesis, suggesting that the higher mitochondrial content resulted from coordinated mitochondrial biogenesis and turnover via autophagy. Taken together, our data revealed a robust mitochondrial remodeling that is associated with augmented mitochondrial energetics during FoxO1-mediated adipose transdifferentiation.

[1] Wyant GA, Yu W, Doulamis IP, Nomoto RS, Saeed MY, Duignan T, McCully JD, Kaelin WG Jr (2022) Mitochondrial remodeling and ischemic protection by G protein-coupled receptor 35 agonists. Science 377(6606):621–629

[2] Lan A, Guerbette T, Andriamihaja M, Magnin B, Bordet M, Ferron PJ, Burel A, Viel R, Fromenty B, Corlu A, Blachier F, Bouguen G (2023) Mitochondrial remodeling and energy metabolism adaptations in colonic crypts during spontaneous epithelial repair after colitis induction in mice. Free Radic Biol Med 205:224–233.

[3] Wilson-Fritch L, Nicoloro S, Chouinard M, Lazar MA, Chui PC, Leszyk J, Straubhaar J, Czech MP, Corvera S (2004) Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. J Clin Invest 114(9):1281–1289.

[4] Shi L, Tao Z, Zheng L, Yang J, Hu X, Scott K, de Kloet A, Krause E, Collins JF, Cheng Z (2023) FoxO1 regulates adipose transdifferentiation and iron influx by mediating Tgfbeta1 signaling pathway. Redox Biol 63:102727.

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Hepatic ketogenic insufficiency exacerbates cognitive impairment and mitochondrial dysfunction in the 5xFamilial Alzheimer's disease mouse model

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Alzheimer's Disease (AD) has a devastating impact on affected individuals, their families, and the healthcare system accounting for nearly 7 million cases in the US alone. Glucose hypometabolism is a pivotal factor in AD progression, exacerbating cognitive decline and disrupting mitochondrial bioenergetics. Ketones are an alternative energy source produced by the liver that can ameliorate cognitive decline and disrupted mitochondrial bioenergetics, independent of glucose metabolism. To investigated whether loss of hepatic ketogenesis exacerbates AD pathology, 2-month-old female 5xFAD mice underwent knockdown of HMGCS2 using hepatocyte-specific adeno-associated virus AAV8-TBG-shRNA-EGFP-mHMGCS2 (vs AAV8-shRNA scramble control) followed by cognitive testing and brain mitochondrial assessment. Loss of HMGCS2, verified by western blot in whole liver and isolated hepatocytes ($p < 0.01$) significantly reduced serum ketone concentrations ($p < 0.01$) and significantly impaired spatial/recall memory (measured by Y-maze) at 3 and 4 months of age ($p < 0.05$). HMGCS2 knockdown also significantly reduced frontal cortex citrate synthase activity ($p < 0.001$), citrate synthase mRNA ($p < 0.01$), and mitochondrial transcription factor A mRNA ($p < 0.01$), without altering respiration or citrate synthase activity in brain mitochondrial isolates. Mitochondrial biogenesis is regulated by sirtuin 1 and sirtuin 3, which were significantly reduced at the transcript level with loss of hepatic HMGCS2 ($p < 0.01$). These data strongly support our hypothesis that reduced hepatic ketone production exacerbates AD-related metabolic and cognitive disturbances and establishes a mechanistic link between liver and brain health in AD progression.

Cite: Kelty TJ, Kerr NR, Abu-Salah MT, Taylor CL, Meers GM, Arnold WD, Rector RS (2025) Hepatic ketogenic insufficiency exacerbates cognitive impairment and mitochondrial dysfunction in the 5xFamilial Alzheimer's disease mouse model. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

Succinate does not increase reactive oxygen species generation in phosphorylating human mitochondria

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Reactive oxygen species (ROS) production is an integrated part of mitochondrial biology, serving a multitude of biological purposes. Metabolism of succinate, primarily through reverse electron transport (RET), have long been considered an important source of ROS generation. Here we show, that the previously documented succinateinduced ROS-production to a large degree can be attributed to the design of the experimental conditions, ranging from unphysiological downstream block of the electron transport chain, high proton motive force, absence of ADP or a highly reduced Q-pool. Using three types of preparations of human peripheral blood mononuclear cells (PBMCs), we demonstrate that in cells and mitochondria with functioning coupling between electron transport and ATP generation i.e. oxidative phosphorylation, ROS production from succinate is negligible or non-existing. Further, delivering succinate to intact cells using a prodrug strategy decreased generation of ROS during both CI-inhibited and non-inhibited conditions. We can conclude that in human blood cell mitochondria, no succinate-induced ROS-production is detected if the mitochondrial phosphorylating machinery is active. We suggest that care should be taken to not draw conclusions regarding ROS generation from the mitochondrial respiratory chain from experiments performed under conditions with unphysiological downstream blockage of the respiratory chain or ATP synthase.

Cite: Lenzer A, Yee I, Sekine S, Liu T, Chamkha I, Elmér E, Ehinger JK (2025) Succinate does not increase reactive oxygen species generation in phosphorylating human mitochondria. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

Exploring the impact of novel cardiolipin disease genes on mitochondrial function and bioenergetics

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Cardiolipin (CL) is a mitochondria-specific phospholipid essential for mitochondrial bioenergetics, oxidative phosphorylation (OXPHOS), mitochondrial membrane integrity, and apoptotic signaling. As a key component of the inner mitochondrial membrane (IMM), CL facilitates electron transport chain (ETC) supercomplex stabilisation, ATP synthesis, and mitochondrial dynamics. Disruptions in CL metabolism have been implicated in primary mitochondrial diseases (PMDs) and neurodegenerative disorders, highlighting the critical role of CL homeostasis in cellular energy metabolism and neuronal function.

Here, we report two novel, homozygous variants in the protein-tyrosine phosphatase mitochondrial 1 (*PTPMT1*) gene in three unrelated probands with multisystem PMD comprising mitochondrial myopathy and developmental regression. Loss-of-function mutations in *PTPMT1* lead to disrupted CL biosynthesis, compromising mitochondrial structure and function. Studies using patient-derived fibroblasts, skeletal muscle tissue, and *ptpmt1* knockout (KO) zebrafish models confirm that *ptpmt1* deficiency results in decreased CL levels, OXPHOS impairment, and mitochondrial dysfunction, highlighting the fundamental role of PTPMT1 in maintaining mitochondrial bioenergetics.

TRIAP1 (TP53 Regulated Inhibitor of Apoptosis 1) also plays a crucial role in CL metabolism by facilitating the transfer of phosphatidic acid (PA) from the endoplasmic reticulum to the IMM, a critical step in CL biosynthesis. The TRIAP1/PRELID1 complex is highly conserved and essential for mitochondrial lipid homeostasis. Pathogenic variants in *TRIAP1* have been identified in patients presenting with progressive skeletal myopathy, demonstrating both gain-of-function and loss-of-function effects on mitochondrial respiration. Functional analysis of patient-derived fibroblasts, skeletal muscle and CRISPR-Cas9 *TRIAP1* KO cells revealed that TRIAP1 dysfunction impacts mitochondrial energy metabolism, emphasizing the importance of TRIAP1 in maintaining mitochondrial homeostasis.

In summary, functional CL is essential for mitochondrial bioenergetics, and its dysregulation due to mutations in *PTPMT1* and *TRIAP1* leads to severe mitochondrial dysfunction. Understanding the mechanisms by which these proteins regulate CL

metabolism offers new insights into mitochondrial pathophysiology and provides a foundation for developing targeted therapies for mitochondrial disorders.

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[3] Falabella M, Vernon HJ, Hanna MG, Claypool SM, Pitceathly RDS (2021) Cardiolipin, Mitochondria, and Neurological Disease. Trends in Endocrinology & Metabolism 32, 224–237. https://doi.org/10.1016/j.tem.2021.01.006

Cite: Falabella M, Aref J, Gao J, Pizzamiglio C, Tabara LC, Munro B, Macken WL, Pope SAS, Heales SJR, Hanna MG, Horvath R, Prudent J, Taanman J-W, Pitceathly RDS (2025) Exploring the impact of novel cardiolipin disease genes on mitochondrial function and bioenergetics. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

Mitochondrial resilience mechanisms: structure-function studies of *Drosophila melanogaster* ATP synthase

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Mitochondria are complex organelles that regulate not only cellular metabolism but also cell death. In response to calcium and reactive oxygen species, they undergo mitochondrial permeability transition (mPT), caused by the opening of the mitochondrial permeability transition pore (mPTP). The mPTP is a non-selective 1.5 kDa channel that forms within the otherwise impermeable inner mitochondrial membrane. Under prolonged opening of the mPTP, mitochondria undergo an osmotic imbalance, leading to mitochondrial swelling and subsequent rupture, followed by the release of apoptotic factors and cell death. Although mPTP is crucial in regulating cell death, its identity, structure, and gating mechanism are poorly understood. To add to the complexity, different species of the animal kingdom have evolved to possess diverse properties of mitochondrial permeability transition. Species such as the brine shrimp, *Artemia franciscana*, and the fruit fly, *Drosophila melanogaster*, are resistant to mPT and mitochondrial swelling. Mitochondrial ATP synthase has been recently discovered to possess a channel within its c-ring with similar biophysical characteristics of the mPTP, such as conductance, probability of channel opening, and sensitivity to calcium. Therefore, using comparative structural and functional analysis of ATP synthase across species may help in understanding the gating mechanism and molecular identity of this channel. Using the CRISPR-Cas9 gene editing approach, cryo-electron microscopy, and electrophysiology experiments, we are investigating the gating mechanism of the ATP synthase leak channel in *Drosophila melanogaster* and mammals.

Cite: Betz LS, Kumar A, da Fonseca Rezende e Mello J, Mnatsakanyan N (2025) Mitochondrial resilience mechanisms: structure-function studies of *Drosophila melanogaster* ATP synthase. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

Anti-tuberculosis drug Bedaquiline ameliorates mitochondrial permeability transition by inhibiting the ATP synthase leak channel

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F_OF_1-ATP synthase is one of the most abundant proteins of the inner mitochondrial membrane and the primary enzyme responsible for ATP production in eukaryotic cells. Nevertheless, recently, it was also reported to play a prominent role in cell death by forming a large-conductance leak channel of mitochondrial permeability transition pore (mPTP), making it as an exceptional drug target. Bedaquiline (BDQ), a member of the diarylquinoline class of drugs, was shown to selectively target *Mycobacterium tuberculosis* but not mammalian ATP synthase and inhibit its catalytic activity. Here, we report the new role of the BDQ as a potent inhibitor of ATP synthase c-subunit leak channel in mammals. BDQ inhibited the single-channel activity of porcine heart ATP synthase in planar lipid bilayer recordings, significantly delayed the opening of the mPTP in calcium retention capacity assay, and ameliorated glutamate-induced cell death in primary hippocampal neurons. These findings reveal the potential new application of BDQ for treating mPTP-related diseases by targeting the ATP synthase c-subunit leak channel.

Cite: Kumar A, Smith EC, Mezghani I, Eedarapalli S, Wu Y, Amjad E, Park H-A, Mnatsakanyan N (2025) Anti-tuberculosis drug Bedaquiline ameliorates mitochondrial permeability transition by inhibiting the ATP synthase leak channel. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

α-Synuclein as a principal executor of ferroptosis via mPTP activation in erastin-induced neurodegeneration

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Ferroptosis, an iron dependent regulated form of cell death, has been increasingly associated with neurodegenerative disease, including Parkinson's disease (PD). This research investigates the role of α-synuclein as a significant inducer of ferroptosis by interacting with the mitochondrial permeability transition pore (mPTP) in an erastin-induced SH-SY5Y cell model. The characteristic ferroptosis indicators like accumulation of oxidative stress markers along with alteration of mitochondrial bioenergetic properties induced by erastin are reversed by ferrostatin-1 and liproxstatin-1. Additionally. the mPTP inhibitor cyclosporine A effectively prevented mitochondrial alterations and cell death induced by erastin implying the crucial role of mitochondrial permeability transition pore (mPTP) activation in ferroptotic death

Notably, extensive α-synuclein accumulation in erastin treated SHSY5Y cells was prevented by ferrostatin-1 and liproxstatin-1. Moreover, the knock-down of α-synuclein expression has markedly prevented mitochondrial impairment and ferroptotic death of the cells induced by erastin.

These results indicate that α-synuclein is not merely an oxidative stress byproduct but an active contributor to ferroptotic neurodegeneration. The association between α-synuclein and mPTP seems to be a key force behind mitochondrial impairment in PD, connecting ferroptotic mechanisms to neurodegeneration.

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Exercise training reverses skeletal muscle mitochondrial fragmentation and improves OXPHOS conductance in patients with obesity and type 2 diabetes

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Imbalanced skeletal muscle mitochondrial dynamics contribute to the onset and progression of type 2 diabetes (T2D). Skeletal muscle lipid accumulation directly contributes to the onset and progression of insulin resistance by early and sustained activation of mitochondrial fission mediated by dynamin-related protein 1 (DRP1). Consequently, chronic activation of DRP1 promotes mitochondrial network fragmentation and restricts skeletal muscle glucose uptake in patients with T2D. The purpose of this study was to determine if exercise training reverses hyperactivation of skeletal muscle mitochondrial fission in patients with T2D. 24 sedentary adults (49 ± 10 years) with obesity (BMI 36 ± 6 kg/m^2) and T2D (HbA1C 7.3 ± 1.3%; 1.3 ± 1.2 glucose lowering medications) were randomized to 12 wks of exercise training (Ex; n=11) or standard care (Ctrl; n=13). Participants randomized to Ex performed supervised aerobic exercise 5 d/wk for 60 min/session at 80-85% HR_{MAX}. Participants underwent a 3-day inpatient stay consisting of DXA, VO_{2peak}, and 2-stage hyperinsulinemic-euglycemic clamp at baseline and post intervention. Skeletal muscle biopsies were obtained for molecular analysis. Protein expression of mitochondrial fission markers was assessed via western blot. High-resolution respirometry was used to measure maximal mitochondrial respiration and OXPHOS conductance. Mitochondrial ultrastructure was assessed by ion beam scanning electron microscopy with machine learning segmentation. Relative changes from baseline were calculated, and comparisons were made with unpaired Student's *t*-tests. Age, sex, body weight, and diabetes status were similar at baseline. Ex increased VO_{2peak}, lean mass, and peripheral insulin sensitivity and reduced fat mass relative to Ctrl. Ex increased skeletal muscle myofibrillar connectivity as evidenced by the greater number of sarcomere branches and decreased lipid droplet volume per cell. Skeletal muscle pDRP1^{Ser616}, Mid49, and Mid51 were reduced in Ex after intervention compared to Ctrl. Additionally, Ex improved the sphericity and elongation of individual and networked mitochondria, resulting in improvements in fragmentation index compared to Ctrl. Ex improved maximal NADH- and succinate-linked OXPHOS and increased mitochondrial conductance. Overall, exercise training reversed hyperactivation of skeletal muscle mitochondrial fission, resulting in improvements in mitochondrial networking in patients with T2D. We also provide first-in-human evidence that exercise training increases skeletal muscle mitochondrial conductance, indicating physiologically relevant improvements in respiratory control. Collectively, these data indicate that exercise improves skeletal muscle insulin sensitivity in T2D, in part, by restoring mitochondrial dynamics and networking.

ClinicalTrials.gov Identifier: NCT02977442

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The role of mitochondrial coupling efficiency in MASH-hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is the most common primary liver cancer and a leading cause of cancer-related death. Obesity is the primary risk factor for developing metabolic dysfunction-associated steatotic liver disease (MASLD) and steatohepatitis (MASH), the fastest-growing cause of HCC. HCCs rewire mitochondrial bioenergetics to promote proliferation, shunting energetic precursors from oxidative phosphorylation (OXPHOS) in favor of protein, lipid, and nucleotides and control of cellular redox poise. However, the degree to which mitochondrial bioenergetic efficiency is required to initiate and sustain MASH-HCC's remains unclear. This study investigates the contribution of mitochondrial efficiency to HCC growth using BAM15, a pharmacological OXPHOS uncoupler, to reduce bioenergetic efficiency in both *in vitro* and *in vivo* models.

Methods: *In vitro* bioenergetic efficiency was evaluated by high-resolution respirometry and extracellular acidification monitoring in non-malignant hepatocytes (AML12) and a malignant HCC (HEPG2) line. Proliferation and apoptosis were evaluated with Sorafenib as a control. Cell viability was further analyzed across 900 human cancer cell lines exposed to BAM15 using DNA-barcode multiplexed profiling.

For *in vivo* studies, a combination of housing temperature, toxin, and diet were used for accelerated models of MASLD- and MASH-HCC in male and female wildtype C57BL/6J and PPARα deficient mice. At 2 weeks of age, pups were administered diethylnitrosamine, a hepatocarcinogen. In Model 1) mice were housed at 22 °C and fed a high-fat diet (HFD) rich in long chain saturated fatty acids (35%) derived from cocoa butter for 10 weeks to induce MASLD. In Model 2) mice were housed at 29 °C and fed a low-fat diet (LFD) or a high-fat, fructose, and cholesterol diet (HFFCD) for 16 weeks to induce MASH. After induction, mice were randomized to continue their diet or BAM15D (HFD or HFFCD + 0.1% w/w BAM15) for 12 weeks. Body weight and composition, food intake, and muscle function, tumor burden, liver outcomes, and mitochondrial function were measured.

Results: HCC cells displayed a Warburg-like energetic phenotype with downregulated OXPHOS and upregulated glycolysis. Loss of OXPHOS capacity in HCC was predominantly associated with impaired succinate oxidation, which serves as the primary coupling control pathway in hepatocytes. BAM15 reduced HEPG2 cell proliferation at micromolar and induced apoptosis more effectively than Sorafenib. Screening across >900 cancer cell lines revealed broad sensitivity to BAM15, with 85% of HCC lines exhibiting an IC50 < 4 μM. In mice, BAM15 reduced tumor burden, decreased steatosis, improved liver disease markers, and restored liver mitochondrial function in both wildtype and PPARα deficient male mice. Notably, female mice were largely protected from MASLD/MASH, loss of liver mitochondrial function, and HCC

compared to male mice. BAM15 prevented diet-induced weight gain and improved body composition and muscle function in both sexes independent of food intake.

Conclusion: HCC displayed loss of OXPHOS capacity particularly in succinate-linked ATP synthesis. Restricting mitochondrial bioenergetic efficiency with BAM15 impairs HCC proliferation and induces apoptosis, both *in vitro* and *in vivo*. These findings suggest that targeting bioenergetic efficiency could provide a novel therapeutic strategy for treating MASH-HCC, addressing both the cancer and the underlying metabolic disease.

Cite: Zunica ERM, Cole AL, Dantas WS, Townsend RL, Heintz EC, Yang S, Pederson KB, Axelrod CL, Ronis MJ, Kirwan JP (2025) The role of mitochondrial coupling efficiency in MASH-hepatocellular carcinoma. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

Mitochondrial hypermetabolism in a humanized APP Alzheimer's disease mouse model

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Alzheimer's disease (AD) is a devastating neurodegenerative disorder characterized by progressive cognitive decline and memory loss. Growing evidence implicates mitochondrial dysfunction as a central player in AD pathogenesis, contributing to bioenergetic failure, oxidative stress, and neuronal death. However, the precise mechanisms linking mitochondrial impairment to AD progression remain poorly understood. This study aims to investigate the role of mitochondrial dysfunction in AD-related neurodegeneration and explore potential therapeutic interventions.

The Alzheimer's disease mouse model used in this study expresses humanized amyloid-beta (Aβ) precursor protein (APP) and presenilin-1 (PSEN1) genes, recapitulating key features of familial AD, including Aβ plaque deposition. Using this model, we assessed mitochondrial function in mice in different age groups. Changes in mitochondrial morphology and bioenergetic function were measured through a combination of electron microscopy imaging and high-resolution respirometry (Oroboros). Our results reveal significant mitochondrial bioenergetic deficits, indicating the presence of a hypermetabolism phenotype in AD mice.

Our findings highlight mitochondrial dysfunction as a critical driver of neurodegeneration in Alzheimer's disease. Future studies will focus on translating these findings into clinically relevant strategies, including the development of mitochondria-targeted therapies for AD patients.

Cite: Mezghani I, Smith EC, Mnatsakanyan N (2025) Mitochondrial hypermetabolism in a humanized APP Alzheimer's disease mouse model. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

The effects of age and acclimation temperature on mitochondrial ROS production, respiration, and structure in western painted turtles (*Chrysemys picta bellii*)

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In mammalian hearts, reperfusion upsurges reactive oxygen species (ROS) production via succinate-driven reverse electron transport (RET). Yet, the adult painted turtle, which can survive ~170 days of anoxia at 3oC, shows an ability to avoid damage upon reoxygenation, making it an ideal model to study this clinically relevant insult. The hatchling is less anoxia-tolerant, surviving ~40 days at 3oC, and its handling of ROS production during reoxygenation may reflect this difference in anoxia tolerance. Our objective was to better understand the influence of development on mitochondrial ROS production and respiration in adult and hatchling painted turtles. Because reoxygenation naturally occurs during cold temperatures, when turtles emerge from overwintering in anoxic ponds, we also examined the effects of cold acclimation on mitochondrial function. To test the hypotheses that cold acclimation decreases mitochondrial respiration more in adults than hatchlings, and that hatchlings show a higher rate of maximal reactive oxygen species production, turtles of both age groups were acclimated to 20oC and 3oC, and ROS production of isolated cardiac ventricular mitochondria was measured during succinate-driven RET. Respiration rates were also measured with pyruvate/malate, palmitoylcarnitine, glutamate and succinate. Samples from the 20oC group were fixed in glutaraldehyde, embedded in epoxy, and sectioned onto grids for imaging using transmission electron microscopy (TEM). Our results showed that hatchlings had lower rates of succinate-induced ROS production via RET and State III respiration compared to adults. Cold acclimation reduced ROS production in both age groups. TEM images revealed structural differences in cristae membrane area between adults and hatchlings, providing a structural explanation for the observed differences in mitochondrial function. In conclusion, cold acclimation appears protective against oxidative stress from reoxygenation across developmental stages. Despite having a lower anoxia tolerance, hatchlings show lower rates of ROS production compared to adults, which may be due to a lower cristae membrane area in the mitochondria.

Cite: Hogue SM, McCommis KS, Warren DE (2025) The effects of age and acclimation temperature on mitochondrial ROS production, respiration, and structure in western painted turtles (*Chrysemys picta bellii*). In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

Fibroblasts exposed to serum collected after long-term calorie restriction have lower mitochondrial respiration

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Multiple lines of evidence suggest that calorie restriction may slow biological processes related to aging and extend lifespan. To determine whether calorie restriction affects mitochondrial function, we treated fibroblasts from adult donors with serum samples obtained from participants of the CALERIE (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy) study. The CALERIE study enrolled healthy, non-obese middle-aged (average 38y.o) participants to examine the effects of long-term calorie restriction. We studied the serum samples of 177 participants (calorie restriction CR=121, ad libitum AL=56) who had specimens available at two timepoints (12 and 24 months). We assessed the basal and maximal mitochondrial respiration of fibroblasts treated with dilute serum for 24 hours. Our results show that the baseline basal and maximal mitochondrial respiration of fibroblasts is positively correlated with the adiposity of the serum donor (R=0.112, P=0.137; R=0.183, P=0.015, respectively). Interestingly, treatment of fibroblasts with serum collected after 12 months of calorie restriction causes a decrease in both basal and maximal mitochondrial respiration (P=0.1854; P=0.0623, respectively). Fibroblasts treated with serum collected after 24 months of calorie restriction exhibited a further reduction in both basal and maximal mitochondrial respiration (P=0.0174; P=0.0092, respectively). These findings suggest that the serum of individuals on long term calorie restriction diet comprises factors that can reduce oxidative phosphorylation. This reduction in bioenergetic capacity may align with the theory that calorie restriction leads to a cellular energy-conserving state, which could have implications for cellular aging and metabolism.

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The role of kinase inhibitors in modulating mitochondrial function in cancer cells

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Protein kinases play key roles in regulating cell proliferation, cell cycle, and metabolism, with dysregulation linked to diseases like cancer and neurodegenerative disorders. Kinase inhibitors target oncogenic kinase pathways and have garnered significant attention for their therapeutic potential [1,2]. These inhibitors can significantly affect mitochondrial bioenergetics, often resulting in both on- and off-target effects on cellular function. To investigate the impact of kinase inhibitors and their cancer cell-type specificity, we evaluated their effects on mitochondrial respiration in various cancer cell models, including colon, breast, leukemia, and melanoma cell lines.In our study, we systematically profiled the impact of broad and specific kinase inhibitors on mitochondrial respiration in cancer cell models using high-resolution respirometry. Initially, we identified distinct bioenergetic profiles across different culture media [3], and further analysis revealed off-target effects of the FDA-approved multikinase inhibitor sunitinib in a more physiologically relevant medium. We also observed off-target effects of another multikinase inhibitor, sorafenib, which induced mitochondrial dyscoupling in all cell lines. Additionally, we profiled a leukemia cell line with CDK6 mutations [4] and explored the impact of the CDK4/6 inhibitor palbociclib. Furthermore, we uncovered off-target mitochondrial dysfunction from the non-FDA-approved glycolytic kinase inhibitor PFK158, highlighting its severe side effects. Finally, we examined mitochondrial ROS in melanoma and colon cancer cells, revealing cell type-specific profiles associated with aberrant kinase pathways.

In conclusion, our findings highlight that the effects of kinase inhibitors are influenced by factors such as cancer cell-specific phenotypes, mutational profiles, and cell culture medium formulations. We propose that cell-based mitochondrial bioenergetic profiling is a valuable tool for identifying off-target effects and providing mechanistic insights into drug-induced changes in cancer cell metabolism.

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Assessing human adipocyte bioenergetics as a tool for testing energy turnover enhancing drugs

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Mitochondrial function in white adipocytes plays a crucial role in regulating energy metabolism and maintaining overall systemic health. Reduced mitochondrial activity has been shown to impair adipocyte function and promote metabolic diseases such as obesity and type 2 diabetes. Thus, increasing energy metabolism in adipose tissue has great potential to improve human health. Lipophilic weak acids (i.e. N-acyl-amino acids (NAA)), which occur endogenously at low levels, could be instrumental to increase energy expenditure, as they catalyze proton leakage in mitochondria, thereby increasing uncoupled respiration in murine adipocytes [1]. Given the translational potential, a better understanding of the effects of NAAs on human adipocyte bioenergetics is required.

We tailored real-time monitoring of energy metabolism in intact and permeabilized human adipocytes using extracellular flux analysis of respiration and acidification, aiming to test and characterize the effects of a variety of NAA species. We measured the induction of proton leak respiration, maximum substrate oxidation, dose responses, and differences to niclosamide (NEN) and 2,4-dinitrophenol (DNP). Furthermore, we found new insights into the potential molecular mechanism.

Our results show that NAA with neutral amino acid residues induce uncoupled respiration in human adipocytes in a dose-dependent manner. Neither adenine nucleotide translocators, nor uncoupling protein 1 are required for the mechanism of uncoupling. Neutral NAAs, however, significantly reduce maximum oxidation rates, mitochondrial ATP-production, and coupling efficiency. The adverse effects on substrate oxidation occur sharply at a threshold concentration of > 25 µM, severely impacting adipocyte viability. The *in vitro* therapeutic index, based on induced proton leak and viability as determinants, is lower of NAAs than that of NEN and DNP [2]. These previously unrecognized side effects impair human adipocyte functionality, reduce the therapeutic index of NAAs *in vitro* and therefore, NAAs may require further chemical modification before they can be used as safe anti-obesity agents.

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Effects of lactate concentration on T-cell phenotype and mitochondrial respiration

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Lactate, once considered a byproduct of anaerobic metabolism, is now recognized as a key metabolite influencing cellular function and signaling pathways [1, 2]. Lactate plays a crucial role in regulating T-cell function and signaling pathways, particularly during exercise when blood lactate levels can exceed 4 mM [2]. Despite its importance, the impact of lactate on T-cell mitochondrial respiration remains poorly understood. This study investigates how exposure to physiologically relevant lactate concentrations (0.5 mM and 4.0 mM) affects mitochondrial respiration. Twelve healthy participants, with a mean age of 26.8 ± 3.5 years, a sex distribution of 5 males (42%) and 7 females (58%), and a mean BMI of 24.1 ± 3.4 kg/m², provided resting blood samples for T-cell isolation. T-cells were cultured in a plasma-like medium with either 0.5 mM (control) or 4 mM lactate for 1 hour. Phenotypes were assessed using flow cytometry, and mitochondrial respiration was measured using high-resolution respirometry in intact and α-chaconine permeabilized cells coupled with a SUIT protocol in MiR05 at 37°C. Compared to the control, the 4.0 mM lactate condition tended to have lower naïve CD4+ T-cells ($p = 0.077$), while naïve CD8+ T-cells were significantly lower ($p = 0.041$). In the routine (intact cell) state, oxygen flow (I_{O2}, pmols·s^{-1}·million T-cells^{-1}) was higher in the 4 mM lactate condition [2.25 (95% CI: 1.93, 2.57)] than in control 1.88 (95% CI: 1.57, 2.20; $p = 0.0009$). Likewise, in the fatty acid-linked (F-linked) LEAK state, the I_{O2} was higher in the 4 mM condition [0.46 (95% CI: 0.36, 0.55)] than the control [0.31 (95% CI: 0.21, 0.41); $p = 0.011$] in the presence of 10 µM palmitoyl-L-carnitine and 1.0 mM malate (F_L). After the addition of 2.5 mM ADP, F-linked OXPHOS state, the I_{O2} was higher in the 4 mM condition [2.26 (95% CI: 1.76, 2.75)] compared to control [1.84 (95% CI:1.35, 2.34; $p<0.01$)] (F_P). Furthermore, serial titrations of pyruvate (5 mM), glutamate (10 mM), succinate (10 mM), and rotenone (0.5 µM) revealed higher I_{O2} in the 4 mM lactate condition for all remaining OXPHOS conditions (FN_P, FNS_P, and S_P) compared to control (all $p<0.01$). Exposure to 4 mM, which is typically observed during high-intensity exercise, may stimulate the electron transport chain to enhance ATP production capacity in T-cells. This is supported by our findings that high lactate concentrations significantly increase oxygen consumption in various mitochondrial states, particularly in OXPHOS states, suggesting an enhanced OXPHOS capacity. A recent study also demonstrated that exposure to high levels of D-lactate also stimulated T-cells mitochondrial bioenergetics [3], which is consistent with our findings that used the more physiologically relevant L-lactate. This study shows the role of lactate in enhancing T-cell mitochondrial respiration, providing insights into immune cell metabolism under exercise-like conditions.

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Peroxisomes protect mitochondria and coordinate metabolic flexibility in skeletal muscle

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Mitochondria and peroxisomes are key organelles in cellular lipid catabolism. Limiting the mitochondrial lipid burden in skeletal muscle with tissue-specific deletion of carnitine palmitoyltransferase 1b ($Cpt1b^{M-/-}$) protects against metabolic dysfunction and glucose intolerance. Alternatively, the importance of peroxisomes in skeletal muscle biology is largely unknown. Here we report that skeletal muscle-specific peroxisome deletion ($Pex5^{M-/-}$) leads to mitochondrial dysfunction and mild glucose intolerance. More notably, functional peroxisomes are required for the improvements in glucose tolerance, insulin tolerance, mitochondrial biogenesis/function, and substantial genomic remodeling in $Cpt1b^{M-/-}$ mice as these beneficial adaptations do not occur in Cpt1b:$Pex5^{M-/-}$ double knockout mice. Overall, these results emphasize the importance of peroxisomes in skeletal muscle to maintain metabolic health, especially when the mitochondrial capacity is insufficient to handle the cellular lipid load alone.

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Differentiation of metabolic energy transfer by total AMPK and mitochondria-localized AMPK (mitoAMPK) in skeletal muscle

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AMP-activated protein kinase (AMPK) is a pleiotropic energy sensing bellwether molecule for metabolic integration within skeletal muscle. Our lab has recently discovered a pool of mitochondria-localized AMPK (mitoAMPK; OMM), and we are the first to demonstrate that mitoAMPK expresses distinct features of the trimeric holoenzyme composition (alpha1/2, beta2, gamma1) paralleled with (total) tAMPK (alpha1/2, beta1/2, gamma1/2/3). Further, we treated WT mice with the diabetes drug Metformin- classically activating AMPK by inhibiting ETC CI- and observed significant preferential activation of mitoAMPK over tAMPK. Further, we demonstrate that a C2C12 FRET-reporter specific for mitoAMPK signaling is activated by metformin, antimycin A, and oligomycin, but not AICAR, indicating an intra-mitochondrial source for primary mitoAMPK energy sensing. Pursuant to these preliminary data, we hypothesized that mitoAMPK is responsible for the functional capacity of the total AMPK pool in skeletal muscle. We firstly explore the role of tAMPK through a dysfunctional knock-in (KI) model with threonine-to-alanine mutation of AMPKa2-T172 by CRISPR/Cas9. Compared to WT, KI mice had severely diminished cardiorespiratory fitness (VO2max; $p<0.001$), suggesting dysfunctional metabolic response to increased bioenergetic demand. Global proteomics further elucidate a profound aberrant regulation of metabolic energy transfer centralized around mitochondrial form and fitness, demonstrating coordinated expression with clinical studies of the type II diabetes (T2D) skeletal muscle proteome. Functional creatine kinase clamp-mediated analysis in isolated mitochondria from the mixed fiber type plantaris showed significantly reduced maximal respiration (Oroboros O2k; *J*O2 ΔGATP-12.94; $p<0.001$) and ETC conductance (i.e., plasticity; $p<0.001$) with simultaneous increase in oxidant release (*J*H2O2 emission and leak; $p<0.001$). To discern the role of mitoAMPK in the tAMPK pool for these outcomes we generated an inducible muscle-specific transgenic (Tg) mouse for Inhibitor-Peptide for mitoAMPK (mitoAIP). Tg mice demonstrated diminished VO2max ($p=0.022$), *J*O2max ($p<0.008$), ETC conductance ($p=0.047$), and increased oxidant release from isolated mitochondria. Taken together, these data suggest that mitoAMPK plays *the* significant functional role in the total AMPK pool to regulate mitochondrial quality, providing a novel therapeutic target to promote metabolic function and prevent obesogenic diseases (i.e., T2D).

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ES1 plays a critical role in protecting the heart from metabolic carbonyl stress in mitochondria

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Metabolic carbonyl stress is derived from metabolic dysfunction characterized by the abnormal accumulation of reactive α-oxoaldehyde metabolites, such as methylglyoxal (MGO), that modify proteins and DNA, leading to cellular and tissue dysfunction. Elevated levels of MGO have been observed in aging, diabetes, and heart failure. Recently, a novel mitochondrial protein ES1 has been identified to play a critical role in detoxifying dicarbonyl and advanced glycation end products (AGE), potentially through deglycase and/or glyoxalase III enzymatic activity. However, ES1's role in metabolically intensive tissues, such as the heart, remains unknown. In this study, we investigate the metabolic role of ES1 in the heart, focusing on its function in managing metabolic carbonyl stress. Cardiomyocyte-restricted ES1 knockout (cES1-KO) mice were generated by crossing αMHC-*MerCreMer* mice with ES1*fl/fl* mice. Tamoxifen-induced cES1-KO was validated by western blot. Echocardiographic analysis of 3-month-old mice revealed a reduced ejection fraction and fractional shortening, indicating a reduced cardiac function. Consistently, cardiac expressions of hypertrophic markers, such as atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), were elevated in cES1-KO compared to control mice. Histological analysis showed increased collagen deposition, and transmission electron microscopy assessment revealed swollen mitochondria with marked loss of cristae density in the heart of cES1-KO compared to control mice. Moreover, mitochondrial respiration was impaired in cES1-KO hearts, particularly in Complex I and Complex II-dependent oxidative phosphorylation and electron transport chain. Proteomic analyses of the heart's mitochondria revealed a significant reduction of the translocase of inner mitochondrial membrane 13 (TIMM13), methylmalonyl-CoA mutase (MUT) levels, and a notable increase in growth hormone inducible transmembrane (GHITM) protein levels in cES1-KO compared to the control mice. Moreover, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis suggests the alteration of the tricarboxylic acid (TCA) cycle and glucose-derived metabolites processing. Moreover, we observed a significant increase in MGO and AGEs in cES1-KO hearts compared to controls. DJ-1, glyoxalase 1 (GLO1) expression, and GLO1 enzymatic activity were significantly elevated in the heart of cES1-KO mice, suggesting a compensatory mechanism to metabolize excess MGO. Our study suggests that mitochondrial ES1 in cardiomyocytes is critical in regulating mitochondrial morphology and function, at least partly by releasing carbonyl stress in the heart. (Support: NIH R01HL160969)

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MIRO1 regulates skeletal muscle insulin action, mitochondrial dynamics, and bioenergetic function

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Mitochondrial Rho-GTPase (MIRO1) is a critical regulator of mitochondrial trafficking and calcium homeostasis and by extension, mediates cellular energy balance and intracellular signaling. This study aimed to mechanistically decipher the role of MIRO1 in modulating skeletal muscle insulin action, which has been completely unexplored. In this study, skeletal muscle MIRO1 KO mice ($MIRO1^{MKO}$) and littermate controls were generated by crossing MIRO1-floxed mice with Acta1-Cre transgenic mice. 8-week-old animals were maintained at thermoneutrality and fed a high fat diet for 16 weeks to induce metabolic stress. Following dietary intervention, insulin sensitivity was assessed in vivo by insulin tolerance test (ITT) and skeletal muscle was insulin stimulated ex vivo to analyze insulin signaling protein targets. Additionally, C2C12 myoblasts with shRNA-mediated MIRO1 knockdown were utilized to evaluate glucose uptake and insulin signaling pathways. Bioenergetic capacity was evaluated by OXPHOS and ET Capacity measurements. $MIRO1^{MKO}$ mice exhibited increased insulin tolerance relative to littermate controls ($p<0.01$). Skeletal muscle sections from $MIRO1^{MKO}$ mice displayed increased GLUT4 localization at the cell surface under both basal ($p<0.001$) and insulin-stimulated conditions ($p<0.05$), accompanied by increased Akt phosphorylation at Thr308 ($pAkt^{Thr308}$) ($p<0.01$). Consistent with these findings, shRNA-mediated MIRO1 knockdown increased glucose uptake ($p<0.01$) and membrane-bound GLUT4 ($p<0.01$) in C2C12 myoblasts. $MIRO1^{MKO}$ mice and shMiro1 cells exhibited higher N-linked and S-linked OXPHOS capacity. Intriguingly, shMiro1 cells displayed reduced mitochondrial volume with increased elongation despite decreased branching as measured by MitoTracker Deep Red FM confocal imaging, along with reduced expression of mitochondrial fission and fusion proteins. Taken together, these results indicate that MIRO1 plays a critical role in mediating skeletal muscle insulin action via regulation of mitochondrial function and network morphology.

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Mus, Mitos, & Mammary Glands: Effects of heat stress during lactation on mitochondrial respiration

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Lactation is one of the most energetically demanding events in a female mammal's life. Female mammals can increase their sustained metabolic rate up to 7-fold, suggesting that mitochondrial performance is central to successful reproduction. However, the tissue-specific mechanisms supporting mammals' energetic capacity increase are currently understudied. This study focuses on how oxidative phosphorylation and the electron transport chain support increased energetic demand by measuring mitochondrial respiration in lab mice (Mus musculus) under standard conditions and a heat challenge. Furthermore, this study is among the first to utilize mammary tissue in mitochondrial respiration measurements. We hypothesized that exposure to high temperatures would inhibit mitochondrial performance, decreasing maximal respiration. Seven days post-gestation, experimental mice were moved to heat treatment (30C) or remained at standard temperature (22C). Processing occurred on the 12th day post-gestation. Mitochondrial respiration was measured in the maternal liver and mammary tissue due to their integral role in supporting lactation. Contrary to our hypothesis, preliminary data reveals no difference between the two groups in maximal (mammary: P= 0.95; liver: P= 0.41) or baseline respiration (mammary: P=0.84; liver: P= 0.19). Maternal and offspring body mass was significantly lower in 30C individuals (Maternal P = 2.22 x 10-5; Pup P= 1.69 x 10-7), indicating variation in nutrient allocation. Data on relative complex abundance and relative activity of the electron transport system complexes will be presented.

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The role of endogenous carbon monoxide (CO) in cellular respiration: Does the "silent killer" assist in tolerance to hypoxia and ischemia/reperfusion events?

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Northern elephant seals (*Mirounga angustirostris*) are premier diving marine mammals that routinely undertake 90-minute dives in which arterial O_2-hemoglobin saturation is reduced to less than 20%[1], making them one of the most hypoxia-tolerant mammals. During dives, their heart rates drop to 5-20bmp, and vasoconstriction occurs at peripheral tissues that leads to tissue ischemia. Upon surfacing from a dive to breath for 2-minute intervals, heart rates increase rapidly, and tissues are re-perfused. However, the molecular mechanisms by which elephant seals efficiently regulate cellular respiration at ischemic tissues following hypoxia/re-oxygenation events, without accumulating damage from oxidative stress, are poorly understood. Intriguingly, elephant seals possess high levels of endogenous carbon monoxide (CO)[2], which is naturally produced in the body through the degradation of heme via heme oxygenase (HO) enzymes. CO has been widely investigated as a cytoprotective gaseous molecule with a high affinity for hemoproteins, including cytochrome *c* oxidase (COX). CO can displace O_2 at the heme-binding site on COX and inhibit aerobic respiration, where affinity of CO for COX increases during hypoxia. Ironically, exogenous CO exposure was found to shift bioenergetics from glycolysis to oxidative phosphorylation in several cell culture models[3]. Given this background, we propose two mechanisms in which CO may influence elephant seal diving physiology at the molecular level: (1) endogenous CO displaces O_2 at COX in hypoxic tissues, limiting O_2 consumption during dives, thus conserving O_2 and prolonging dive duration; and (2) upon surface re-oxygenation, CO supports a shift in bioenergetics from glycolysis to oxidative phosphorylation, allowing for rapid O_2 consumption and ATP production during post-dive surface intervals. Notably, a recent study found that elephant seal vascular endothelial cells consume significantly more O_2 following hypoxia/re-oxygenation exposure compared to human endothelial cells[4], though it is uncertain if endogenously produced CO plays a role in this process. To investigate this, vascular endothelial cells collected from elephant seals and humans will be exposed to 6 conditions: (1) 21% O_2 (normoxia), (2) 21% O_2 + 100ppm CO (normoxia + exogenous CO), (3) 1% O_2 (hypoxia), (4) 1% O_2 + 100ppm CO (hypoxia + exogenous CO), (5) 21% O_2 + 10μM heme (normoxia + HO stimulation for endogenous CO production), and (6) 1% O_2 + 10μM heme (hypoxia + HO stimulation). O_2 consumption rates after each condition will be measured using the Mito Stress Test kit for Seahorse XFp Bioanalyzer following re-oxygenation. Findings from this work will help elucidate the potential role of endogenous CO in modifying O_2 consumption rates and ATP production in the face of hypoxia and re-oxygenation and may provide evidence for how certain hypoxia-tolerant species avoid hypoxia-related injuries.

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Kynurenic acid treatment enhances mitochondrial health and protects the heart from doxorubicin-cardiotoxicity

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Doxorubicin (DOX) is a potent chemotherapy drug that has been extensively used in cancer treatment. However, its major side effect, DOX-cardiomyopathy, results primarily from its impacts on mitochondrial energetics and oxidative stress in cardiomyocytes. Recent research demonstrates that kynurenic acid (KynA), a tryptophan metabolite, is cardioprotective against ischemia/reperfusion injury via G protein-coupled receptor 35 (GPR35)-induced ATP synthase inhibition by ATP synthase inhibitory subunit 1 (IF1) to prevent mitochondrial ATP depletion. We hypothesize that KynA is cardioprotective against DOX-cardiotoxicity via GPR35/IF1 signaling. Wild type (WT) and IF1 KO (IF1$^{-/-}$) (male,C57BL6J) received KynA (3mg/kg) or vehicle for one week, followed by weekly DOX (5mg/kg) or vehicle treatments for four weeks. The echocardiographic assessment confirmed DOX-induced cardiac dysfunction in all mice. However, KynA pretreatment significantly improved cardiac contraction with less declined ejection fraction (EF%) and LDH elevation in all DOX-treated mice. IF1$^{-/-}$ mice exhibited less reduced EF% and elevated LDH than WT mice, but KynA pretreatment showed no additional benefits. KynA pretreatment markedly attenuated DOX-induced histological (e.g.,cardiac dystrophy,fibrosis) and ultrastructural (e.g., sarcomere degeneration, mitochondrial abnormalities) changes in the heart of DOX-treated mice. IF1$^{-/-}$ mice exhibited less impaired cardiac pathologies than KynA pretreated WT mice. Western blot analysis revealed that DOX treatment increased cardiac IF1 expression in the vehicle but not KynA-treated mice, suggesting KynA directly or indirectly suppresses cardiac IF1 expression. In cultured neonatal cardiomyocytes (NCM) from WT, KynA significantly reduced DOX-induced cell death and mitochondrial and general reactive oxygen species (ROS) production. Cellular energetic analyses using the Seahorse Bioanalyzer revealed improved maximal and spare respiratory capacity in DOX-treated IF1$^{-/-}$ compared to WT NCMs. A similar effects was observed in KynA-treated cells following DOX exposure, relative to cells treated with DOX alone. However, KynA did not confer additional benefits when administered to IF1$^{-/-}$ NCMs. In summary, our study demonstrated that KynA and IF1 $^{-/-}$ enhanced mitochondrial function and preserved cardiac structure/function against DOX-induced cardiotoxicity. However, KynA treatment did not provide additive protection in IF1 $^{-/-}$ hearts. KynA's beneficial effects on mitochondria are at least partly by inhibiting DOX-induced cardiac IF1 overexpression. In conclusion, our findings support that KynA may serve as a novel therapy to mitigate doxorubicin-cardiomyopathy.

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Genetic screen in *C. elegans* uncovers non-canonical autophagy regulators

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Human lifespan has been on a steady increase over the past century, but human healthspan has not increased on the same trajectory, resulting in a larger population of humans that suffer from age-related diseases. Many age-related diseases result from increased protein aggregation or loss of proteostasis. Autophagy, which is one of the main protein degradation pathways, recycles cellular components via lysosomes; thus, lysosomes could be a major target for the treatment of age-related diseases. In recent work, we identified a class of non-canonical lysosomes that form tubular networks in the gut of starved or dietary restricted (DR) *C. elegans*. Significantly, induction of tubular lysosomes (TLs) increases autophagic potential and is a critical event in achieving the full beneficial effects of DR. In *Drosophila*, TLs can be stimulated ectopically by overexpressing the lysosomal gene *SVIP* (Small VCP Interacting protein). Although *C. elegans* do not have an *SVIP* ortholog, over-expression of either human or *Drosophila SVIP* in the gut of *C. elegans* resulted in many physiological improvements that mimic DR, including TL induction, increased autophagic cargo turnover, dramatic improvement of late-age mobility, and enhancement of late-age mitochondrial and muscle health. Thus, SVIP provides a route to stimulate TLs on demand and could be tapped for anti-aging interventions. To dissect the molecular mechanisms through which *SVIP* overexpression causes these effects, we performed mRNA sequencing from whole worms with and without *SVIP* overexpression, which resulted in 1375 upregulated genes compared to WT. Next, we employed an RNAi screening strategy to validate genes that disrupted autophagic cargo turnover in *SVIP* overexpression worms using the tandemly-tagged *SQST-1::mCherry::GFP* fluorescent marker. After screening 326 genes, we identified several genes whose inhibition resulted in defective autophagic cargo turnover, the most surprising of which were *ant-1.3/ant-1.4*, which encode ATP/ADP antiporter proteins that reside in the inner mitochondrial membrane and regulate ATP export and mitochondrial permeability. Using the loss-of-function mutant *ant-1.4(gk300)*, we validated that loss of *ant-1.4* caused a disruption of *SVIP*-dependent TL induction. The *ant-1.4(gk300)* mutant also reverted the thrashing rate of *SVIP* overexpression worms back to WT levels. We are now investigating how *ant-1.4* regulates lysosomes and autophagy and examining what effects *ant-1.4* has on the mitochondria. These studies could reveal a new connection between lysosomes and mitochondria.

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Mitochondrial hypoxia and reoxygenation result in the reorganization of ATP synthase: Could dynamic changes in mitochondria underlie exercise adaptations?

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Regular exercise induces hormesis, a physiological phenomenon in which mild exposures to stress trigger beneficial adaptations. Intermittent hypoxia (IH), an exercise mimetic, elicits similar metabolic benefits and is used to enhance athletic performance and promote health in clinical and aging populations. IH has shown to vastly reduce cardiac ischemia/reperfusion injury via inhibition of the mitochondrial permeability transition pore (mPTP)[1]. Structural alterations electron transport chain (ETC) induced by sublethal stress may precondition mitochondria to better withstand both acute and chronic stressful environments[2,3]. Increased myocardial workload in exercise and IH raises O_2 demand as mitochondria resynthesize ATP through ATP Synthase (Complex V, CV). While the heart avoids hypoxia during exercise through increased coronary blood flow, exercise and IH activate similar signaling pathways in response to oxidative stress that facilitate ETC structural adaptation, thus limiting mitochondrial sensitivity to Ca^{2+} overload and preserving bioenergetic function. Notably, in addition to shaping the inner mitochondrial membrane and mediating cristae formation, dimers of Complex V (CV_2) has been proposed to form the mPTP[4], which would predict the rapid ability of CV to reorganize into CV_2. However, whether this occurs on the acute time scale of mPTP opening remains unknown. Further, while both calcium overload and ischemia-reperfusion induce irreversible mPTP opening, whether a regulatory role for CV_2 exists during sublethal stress remains unclear. **PURPOSE**: To assess if 1) increases in Ca^{2+} or 2) hypoxia/reoxygenation (H/R) cause CV monomers to rapidly associate and form dimers in isolated cardiac mitochondria and affects calcium-induced mitochondrial swelling. **METHODS:** Cardiac mitochondria were isolated from C57BL/6 mice and added to a chamber with respiration media and fuels. Mitochondria were 1) exposed to zero, low, medium, or high Ca^{2+} or 2) stimulated to respire by adding ADP (100 µM) and Pi (10 mM); the chamber was capped to drive down pO_2, and sequential samples were aspirated during hypoxia (5', 15', and 35') and reoxygenation (5', 15', and 35'). Samples were immediately spun down, digitonized, and Blue Native Gel Electrophoresis was used to determine CV organization in CV_1 and CV_2. Ca^{2+}-induced swelling was assessed in control and hypoxia-reoxygenation samples by monitoring at absorbance at 525 nm. **RESULTS**: Acute presence of Ca^{2+} increased the presence of CV_2 and decreased the presence of CV_1, indicating CV dimers rapidly form in response to high Ca^{2+}. CV_2 decreased between baseline and 35' hypoxia; however, dimers increased following reoxygenation, indicating that these rearrangements likely occur during reperfusion, and are possible in the absence of Ca^{2+}. While higher Ca^{2+} induces mitochondrial swelling, consistent with irreversible mPTP, one cycle of 35' hypoxia/35' reoxygenation protected against mitochondrial swelling compared to control. **CONCLUSION:** Because we observed discrete increases in dimeric CV during one

cycle of H/R coupled with protection from calcium-induced swelling, we hypothesize that CV_2 may form to maintain mitochondrial integrity before irreversible mPTP occurs. This could indicate that CV_2 formation is a necessary component of transient mPTP to elicit cardioprotection developed during hormesis, specifically, maintaining sensitivity to $Ca^{2+}$and free radical release throughout sublethal stress exposures including exercise or exercise mimetics.

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Defense capacities against oxidative stress during mitochondrial transitions for embryos of *Artemia franciscana*

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Embryos of *Artemia franciscana* can survive harsh conditions in diapause and anoxia-induced quiescence for years by undergoing deep metabolic transitions [1]. These reversible states are survived for months to years. Because even short metabolic disruptions in mammals are accompanied by bursts of reactive oxygen species (ROS) that cause tissue damage during ischemia-reperfusion [2], we hypothesized that mitochondria from these embryos are mechanistically poised to avoid ROS bursts and the associated oxidative stress during metabolic recovery. H_2O_2 efflux was statistically identical between A/R versus normoxia groups ($p = 0.221$) [3]. The addition of auranofin and dinitrochlorobenzene, inhibitors of ROS scavenging pathways, promoted a five-fold increase in H_2O_2 release for the normoxic mitochondria, which confirmed that scavenging mechanisms substantially suppress routine ROS efflux. Yet when these same inhibitors were added to the A/R group, maximum H_2O_2 efflux was no greater than for normoxia. Treatment with rotenone, an inhibitor of Complex I and reverse electron transport (RET), produced only a modest decrease in H_2O_2 efflux. This result indicates that RET, a major contributor to ROS bursts in mammalian mitochondria, is not stimulated by A/R in *A. franciscana*. Lack of oxidative damage markers demonstrate that bouts of A/R do not cause significant oxidative damage in *A. franciscana* mitochondria. The capacity to downregulate Complex I activity through active-deactive conformations was tested and is not operative. Taken together, these data suggest that Complex I from *A. franciscana* simply may not possess the capability for RET and associated ROS surge. We analyzed antioxidant enzymes and small molecule antioxidants from diapause and post diapause embryos and found that alterations were fully explicable based on the large differences in metabolic rate between these states and the associated defense capacities against ROS required. A literature survey suggests that *A. francsiscana* embryos do not possess abnormally elevated defenses against oxidative stress; rather, avoiding ROS bursts during metabolic reactivation appears more important. [NSF grant IOS-1457061/IOS-1456809].

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Loss of adipocyte KAT8 results in lipoatrophy and oxidative stress in adipose tissue in mice

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KAT8 (lysine acetyltransferase 8), also known as MOF or MYST1, plays cell-specific roles in processes such as cell proliferation, differentiation, and survival/death by acetylating various protein substrates, including histones. Although KAT8 is expressed in adipose tissue (AT) and its gene contains single-nucleotide polymorphisms (SNPs) linked to body mass, its function in adipocytes had not been previously studied.

To investigate this, we created a novel mouse model with KAT8 depleted specifically from adiponectin-expressing cells ($KAT8^{AKO}$) and measured body weight, body composition, and insulin tolerance. We also examined gene and protein expression of antioxidant and mitochondrial targets in white AT (WAT), assessed mitochondrial copy number, and measured cellular and mitochondrial redox poise in AT explants and isolated mitochondria using electron paramagnetic resonance (EPR) spin trapping and simultaneous respirometry/fluorometry.

$KAT8^{AKO}$ mice developed lipoatrophy and insulin resistance, with reduced expression of antioxidant genes and mitochondrial oxidative phosphorylation protein complexes as well as decreased mitochondrial copy number. Gonadal WAT (gWAT) explants from both female and male $KAT8^{AKO}$ mice released more superoxide compared to controls, and isolated mitochondria from $KAT8^{AKO}$ gWAT showed impaired succinate oxidation and increased hydrogen peroxide production.

These findings suggest that adipocyte KAT8 is crucial for maintaining AT integrity and function, potentially through regulating oxidative stress and mitochondrial metabolism. The observed increase in reactive oxygen species (ROS) and defects in mitochondrial respiratory capacity in AT of $KAT8^{AKO}$ mice contribute to a deeper understanding of KAT8's role in AT biology and its implications for metabolic disorders associated with adipocyte dysfunction.

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eNOS -/- mice have impaired mitochondrial fatty acid utilization in response to energetic stress

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Endothelial nitric oxide synthase (eNOS) is essential for long-term skeletal muscle metabolic adaptation to exercise. Previous studies suggest that global eNOS-deficient ($eNOS^{-/-}$) mice exhibit impaired fatty acid transport and oxidation with age. However, the role of eNOS in acute exercise remains unclear. To investigate whether eNOS contributes to early metabolic responses to exercise, we studied young $eNOS^{-/-}$ mice that showed no overt phenotypic differences from their wild-type (WT) littermates (e.g., glucose tolerance, weight, plasma non-esterified fatty acid levels). Despite performing similarly to WT mice during an acute treadmill exercise bout, $eNOS^{-/-}$ mice displayed significantly reduced fatty acid oxidation 3h post-exercise, suggesting a blunted mitochondrial response to energetic stress. This impairment was further supported by elevated respiratory exchange ratios (RER) and gene expression (RNA sequencing) in the gastrocnemius, indicating a shift toward carbohydrate metabolism. To assess fatty acid oxidation independently of transport, we utilized the phosphocreatine–creatine kinase (PCr-CK) clamp method using pyruvate/malate ± octanoyl-carnitine in permeabilized plantaris muscle fibers. This is an *ex vivo* energetic stress test, conducted at physiologic ATP:ADP ratios. In WT mice, the presence of octanoyl-carnitine in the reaction chamber increased mitochondrial electron conductance, while in $eNOS^{-/-}$ mice, the addition had minimal effect, indicating impaired mitochondrial fatty acid utilization. Together, these findings reveal a novel role for eNOS in regulating acute skeletal muscle metabolic responses to exercise.

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Mitochondrial respiration is impaired in mouse models of NF1 patient mutations

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Purpose: Neurofibromatosis type 1 (NF1) is associated with altered metabolism, including reduced muscle function and low body mass/adiposity. Reduced mitochondrial function has been reported in several models of NF1 loss and may contribute to these metabolic features. NF1-null cells exhibit impaired NADH-linked respiration and succinate dehydrogenase activity. The purpose of this study is to characterize whole body and tissue metabolism in a mouse model of adult biallelic inactivation of *Nf1*. We hypothesized that loss of *Nf1* would result in reduced energy expenditure, decreased electron transport and oxidative phosphorylation.

Methods: To measure the metabolic effect of the nonsense allele c.2041C>T; p.Arg681*, $Nf1^{Arg681*/+}$ and $Nf1^{+/+}$ littermates were placed in Promethion indirect calorimetry cages for 4 days (n=15 per group). To measure mitochondrial function upon loss of heterozygosity, which drives NF1-related cancers, we used a tamoxifen-inducible systemic knockout model. $CAGGCre\text{-}ER^{TM};Nf1^{4F/Arg681*}$ (n=6) and $Nf1^{4F/+}$ (n=4) littermates were treated with tamoxifen for 5 days. 48 hours post tamoxifen, brain, heart, gastrocnemius, liver, and brown adipose samples were collected. Crude mitochondrial fractions were isolated for high-resolution respirometry. Using an Oroboros O2k machine, we measured NADH- and succinate-linked oxidative phosphorylation (OXPHOS) and electron transfer (ET) capacity and complex IV (CIV) activity. Citrate synthase activity was measured to confirm equal mitochondrial loading.

Results: $Nf1^{Arg681*/+}$ animals had significantly reduced energy expenditure ($p<0.05$), water consumption ($p<0.05$), and a profound reduction in pedestrian locomotion ($p<0.01$) compared to littermates. After loss of heterozygosity, $CAGGCre\text{-}ER^{TM};Nf1^{4F/Arg681*}$ mice exhibited wasting, with 10% reduction in body weight ($p<0.001$) compared to controls. In the brain and gastrocnemius, NADH-linked OXPHOS and ET, succinate-linked ET, and CIV activity were significantly lower in $CAGGCre\text{-}ER^{TM};Nf1^{4F/Arg681*}$ mice ($p<0.05$). A similar trend was observed in the heart, but there were no significant differences due to the study being underpowered. We found no significant differences in liver or brown adipose tissue. There was no difference in citrate synthase activity between groups.

Conclusions: In a heterozygous mouse model of the pathogenic NF1(p.Arg681*) nonsense allele, we confirmed that reduced NF1 leads to metabolic abnormalities. Our findings also demonstrate that NF1 supports complex I (NADH-linked) OXPHOS and ET in multiple tissues. Without NF1, the overall capacity for tissues to generate energy (ATP) is reduced. This is particularly true in the brain and skeletal muscle. These results suggest that mitochondrial respiration parallels NF1 function and that improving mitochondrial performance could be therapeutically beneficial, particularly for neurological and musculoskeletal manifestations of the condition.

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Mitochondrial dysfunction in the colon of hyperandrogenemic PCOS rats: Implications for IBS and metformin-induced side effects

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Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women with approximately 80% of PCOS women suffer from hyperandrogenemia. PCOS women often exhibited symptoms of irritable bowel syndrome (IBS) that is associated sub-acute gastrointestinal (GI) inflammation. Mitochondrial dysfunction is associated with the development of inflammation and hyperandrogenemia can alter mitochondrial function. We have shown increased inflammation and mitochondrial dysfunction are present in the colons in the hyperandrogenemic female (HAF) rats, which exhibit similar characteristics to PCOS women. Interestingly, the common treatment for PCOS, metformin, has been shown to cause GI side effects. Targeting mitochondria is a feasible treatment option to improve mitochondrial function and inflammation in the colon. This study aims to investigate if the mitochondrial-targeted therapy, mitoTEMPO, will improve mitochondrial function in the colons of HAF rats. At 4 weeks of age, female rats received dihydrotestosterone (DHT, 7.5mg/90 days) pellets. Rats received metformin (300 mg/kg/day in food) starting at 12 weeks of age. MitoTMEPO (1mg/kg i.p daily) is administered 10 days before tissue collection. At 15 weeks of age, colon tissues were collected for mitochondria isolation. Intact mitochondrial respiration was measured simultaneously with reactive oxygen species (mtROS) using the Oroboros O2k-FluoRespirometer. Complex I, II, III, IV, and aconitase activity were also measured. Data was normalized to mitochondrial content using citrate synthase (CS) activity. Mitochondria from the colon of HAF rats showed a significant decrease in complex I-driven respiration and complex II-driven respiration, respiratory control ratios, along with a significant decrease in complex I, II, III, IV, and aconitase activities. Colons from HAF rats also showed a significant increase in mtROS. In the HAF rats and control rats, metformin significantly decreased complex I-driven respiration, complex I activity and aconitase activity while increasing complex III activity. With the exception of the increase in complex III activity, mitoTEMPO significantly recovered mitochondrial function in the colons from HAF rats and those treated with metformin. The observed mitochondrial dysfunction in HAF model of PCOS suggests that mitochondrial dysfunction is a potential cause for the development of IBS symptoms in PCOS. The metformin-mediated mitochondrial dysfunction suggests that mitochondria play a role in the GI side effects associated with this medication. The improvement mitochondrial function by mitoTEMPO suggests that targeting mitochondria may provide a new avenue for treatment options for PCOS suffering from obsesity and IBS. This study provides a better understanding of the role of mitochondria in the development of IBS with PCOS and metformin treatment while providing an avenue for the development of strategies to re-establish normal mitochondrial function.

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Cardiac mitochondrial dysfunction in a hyperandrogenemia rat model of PCOS: Implications for cardiovascular comorbidities

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Hyperandrogenemia affects approximately 80% of women with PCOS. In this condition, the high expression of androgen receptors may impair cardiac mitochondrial function, potentially altering cardiac contractility. Cardiovascular comorbidities are commonly observed in PCOS patients. This study aims to investigate the mechanisms linking cardiovascular disease in PCOS patients to cardiac mitochondrial dysfunction. The hyperandrogenemia female (HAF) rat model exhibits characteristics like PCOS women including the cardiometabolic comorbidities. At 4 weeks of age, female rats received dihydrotestosterone (DHT, 7.5mg/90 days, s.c.) pellets. Echocardiography was measured at 4 months and 9 months of age to evaluate systolic and diastolic function of the heart. At 4 months and 9 months of age, heart tissues were collected for mitochondrial isolation. Intact mitochondrial respiration was measured simultaneously with reactive oxygen species (mtROS) using the Oroboros O2k-FluoRespirometer. Complex I, II, III, IV, and aconitase activity were also measured using Jasco V-750 spectrophotometer. Data was normalized to mitochondrial content using citrate synthase (CS) activity. At 4 months of age, PCOS rats showed a significant decrease in the respiratory control ratio (RCR) and CS activity, though complex I and III activities were not significantly reduced. Echocardiography revealed a decrease in systolic function (lower fractional shortening and ejection fraction) but no significant changes in diastolic function. At 9 months of age, PCOS rats showed a reduction in RCR, no change in CS activity, and decreased complex I and III activities. Echocardiography data indicated both systolic and diastolic dysfunction including increased isovolumetric relaxation time and elevated E/e' ratio in diastolic function. Additionally, we see the decreased in aconitase activity in both age groups. The cardiac mitochondrial dysfunction leading to oxidative stress accompanied by impaired cardiac function in the HAF model suggests the potential role of mitochondrial dysfunction in the development of cardiovascular comorbidities in PCOS women. This study enhances our understanding of mitochondrial dysfunction in the context of PCOS-related cardiovascular disease and provides insight into potential therapeutic strategies aimed at restoring normal mitochondrial function to treat these comorbidities.

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Investigating the role of Mei-P26 in metabolic regulation and behavior in *Drosophila*

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Obesity and metabolic disorders are growing public health concerns, with links to neurodegenerative diseases such as Alzheimer's and Parkinson's. Dysregulated mitochondrial function and metabolic signaling have emerged as central mechanisms driving these conditions. Our recent analysis of offspring from Western diet-fed fathers revealed proteomic dysregulation of mitochondrial and metabolic proteins, with concomitant changes in locomotor behavior and food consumption. Notably, we observed upregulation of MEI-P26 in offspring from Western diet-fed fathers, suggesting a potential role in diet-induced metabolic programming. However, its function in mitochondrial regulation and energy metabolism remains poorly understood.

To investigate potential role of MEI-P26 in metabolic homeostasis, and behavior, we used the GAL4-UAS system to manipulate *mei-P26* expression. We utilized GAL4 drivers for fat body a metabolically active organ equivalent to human adipose tissue. As a central hub for nutrient sensing, lipid metabolism, and systemic metabolic regulation, the fat body provides an optimal model for studying metabolic reprogramming.

Behavioral assays including FLIC (Food-Liquid Interaction Counter), locomotor analysis, respirometry, and Food consumption and excretion (Con-ex) were performed to assess changes in energy balance. MEI-P26 overexpression resulted in increased activity in males but decreased activity in females, while knockdown reversed these effects. Preliminary qPCR data from adult brains with altered *mei-P26* expression indicate dysregulation of mitochondrial dynamics genes and microRNAs, suggesting a potential role in metabolic regulation.

Ongoing experiments include lipid staining of brain and fat body tissues (BODIPY, MitoTracker, LysoTracker) to investigate lipid accumulation and mitochondrial health. Further validation through Western blotting and functional assays is underway to uncover the molecular mechanisms underlying MEI-P26's role in transgenerational metabolic reprogramming.

Based on our study we hypothesize that MEI-P26 might act as a metabolic regulator, linking mitochondrial dysfunction to diet-induced metabolic disorders and potential epigenetic inheritance of metabolic phenotypes. Given the parallels between *Drosophila* and mammalian metabolic pathways, these insights may inform future studies on the epigenetic regulation of metabolic diseases in humans.

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The contribution of tissue-specific mitochondrial respiration to individual variation in oxygen uptake during rest and exercise by the Gulf killifish, *Fundulus grandis*

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The energetic costs of maintenance and sustained activity by animals are largely met by mitochondrial oxidative phosphorylation. Here, we examined whether interindividual variation in rates of oxygen uptake in Gulf killifish, *Fundulus grandis*, at rest ($\dot{M}O_{2,\ standard}$) and during an incremental swim test (peak $\dot{M}O_{2,\ swim}$) is related to variation in mitochondrial oxygen uptake in five tissues: liver, brain, heart, oxidative skeletal muscle and glycolytic skeletal muscle. We hypothesized that interindividual variation in $\dot{M}O_{2,\ standard}$ correlates with the masses of visceral organs and/or with mitochondrial LEAK respiration (oxygen uptake required to offset the dissipation of the proton gradient in the absence of ATP synthesis) of liver, brain, or glycolytic skeletal muscle. We found that $\dot{M}O_{2,\ standard}$ was positively related to liver mass and its maximum capacity for oxygen uptake by the electron transport system (ETS) rather than LEAK. We also hypothesized that interindividual variation in peak $\dot{M}O_{2,\ swim}$ correlates with the capacities for mitochondrial oxidative phosphorylation (OXPHOS) or ETS of heart and oxidative skeletal muscle. We found that peak $\dot{M}O_{2,\ swim}$ was positively related to heart ETS. However, peak $\dot{M}O_{2,\ swim}$ was weakly, positively related to LEAK of glycolytic muscle, rather than to OXPHOS or ETS of oxidative muscle. Finally, we describe a novel negative relationship between the aerobic cost of transport and mitochondrial phosphorylation efficiency in glycolytic skeletal muscle. Individuals with a higher phosphorylation efficiency (i.e., lower LEAK relative to OXPHOS) achieve a performance benefit by consuming less oxygen to swim a given distance.

Cite: Brieske SD, Irving BA, Rees BB (2025) The contribution of tissue-specific mitochondrial respiration to individual variation in oxygen uptake during rest and exercise by the Gulf killifish*, Fundulus grandis*. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

Acylated urocortin 2 peptide therapy rescues muscle mass and fitness in a mouse model of sarcopenic obesity

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Sarcopenic obesity, or the loss of muscle mass and function associated with excess adiposity, is a largely untreatable medical condition associated with diminished quality of life and increased risk of mortality. To date, the treatment options for sarcopenic obesity remain extremely limited. Urocortin 2 (UCN2) is a member of the corticotrophin releasing factor family with high expression in skeletal muscle and putative functions in regulation muscle hypertrophy. Here, we investigated the effects of an acylated UCN2 peptide on reversal of muscle mass and function with intention to treat sarcopenic obesity. Aged male C57BL/6J mice (20 months) were randomized to a low-fat diet (LFD) or a high-fat diet (HFD) for 8 weeks. Following the dietary run-in period, mice on HFD were further randomized to a vehicle control (HFD+Veh; Tris-HCL) or a UCN2 treatment (HFD+UCN2; 0.15 mk/kg, 3X/week) group for 4 weeks. During the intervention period, mice were monitored for body weight and composition as well as food and water intake. Muscle function was assessed by wire hang and handgrip strength at baseline and 4 weeks post intervention. Energy expenditure was evaluated by whole body calorimetry over a one-week period prior to necropsy. Following treatment, organs were recovered, and mitochondria were isolated from gastrocnemius muscle to determine yield and bioenergetic capacity by high-resolution respirometry. HFD+UCN2 treatment decreased body weight and fat mass relative to HFD+Veh, while still being elevated relative to LFD. Loss of body weight was principally explained by decreased food intake relative to HFD+Veh and LFD, with no differences observed in daily energy expenditure. HFD+UCN2 restored muscle mass and function to the level of LFD, which was markedly decreased in HFD+Veh. Changes in muscle mass and function were not readibly explained by mitochondrial bioenergetics, as no differences were observed in respiratory capacity or efficiency. Taken together, these results demonstrate that acylated UCN2 effectively mitigates sarcopenic obesity in mice.

Cite: Baumgarten PAM, Zunica ERM, Dantas WS, Taylor AL, Heintz EC, Gibson NJ, Brozinick JT, Kirwan JP, Axelrod CL (2025) Acylated urocortin 2 peptide therapy rescues muscle mass and fitness in a mouse model of sarcopenic obesity. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

Heroin-seeking alters synaptic mitochondrial proteins

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Background: Chronic opioid use leads to long-lasting increases in drug-seeking behavior; however, the causal molecular and cellular mechanisms responsible are not fully understood. One mechanism may involve signaling through phospholipase Cgamma1 (PLCg1) in the nucleus accumbens (NAc). Since opioids increase NAc PLCg1 signaling, we hypothesized that reducing PLCg1 levels in the accumbens would increase heroin-seeking behavior. In addition, since both opioids and PLCg1 signaling regulate drug-induced dendritic spine density and morphology, we hypothesized that a reduction of NAc PLCg1 levels would modulate the synaptic proteasomal changes that occur following heroin self-administration.

Methods: We first infused a shRNA expression viral vector that reduces PLCg1 levels (AAV-shPLCg1) or a control virus bilaterally into the accumbens shell of rats using stereotaxic surgery. The rats then went through an established heroin self-administration, abstinence, and heroin-seeking protocol. In a separate experiment, we again infused AAV-shPLCg1 or a control virus into the accumbens, then we allowed rats to self-administer either heroin or saline for 12 days in a 2x2 design. Following seven days of abstinence, rats underwent a partial drug-seeking test, but were euthanized mid-session and accumbens tissue was harvested. Next, tissue was enriched for synaptosomes, and proteins were quantified using high resolution multiplexed liquid chromatography mass-spectrometry in an attempt to pinpoint the changes that may drive increased drug-seeking behavior.

Results: We first found that reducing PLCg1 in the accumbens led to an increase in context-associated heroin seeking during extinction conditions. We then examined accumbal synaptic proteasomal changes using mass-spectrometry. We found significant changes in the levels of 223 proteins in these enriched synaptosomal accumbal samples. Interestingly, many genes are involved in cellular respiration or oxidative stress, thus are involved in mitochondrial activity. Somes of these genes are related to the P-type ATPase or members of NADH:ubiquinone oxidoreductase families.

Conclusions: These results show that endogenous accumbal PLCg1 limits heroin-seeking behavior, suggesting that therapeutics targeting PLCg1 function might be helpful for treating opioid use disorder. In addition, PLCg1 either alone, or in combination with heroin, can alter the synaptic proteome of the accumbens, including proteins involved in mitochondrial activity. These results suggest that these accumbal synaptic mitochondrial changes could be causal to increased opioid-seeking behavior. Determining if these synaptic mitochondrial changes are causal to the behavior might reveal new therapeutic targets for treating substance use disorders and lead to a better understanding of the role of mitochondria in addictive disorders.

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2. Invited abstracts

Mitochondrial respiratory function in living cells

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Breathing happens subconsciously, but every breath sets off a vital journey. Oxygen (O2) enters through the nose and lungs, travels with the bloodstream, and reaches the brain, muscles, and every cell in the body. Deep within these tiny cells, oxygen kindles the fire of life in the mitochondria — microscopic structures comparable to bacteria. This pathway of oxygen links breathing (external respiration) to cell (internal) respiration. In the mitochondria, the energy of nutrients is converted into heat and a form of energy available for work. Mitochondria are electrochemical machines that consume oxygen and produce adenosine triphosphate (ATP), the cell's biochemical energy currency. Measuring cell respiration helps assess mitochondrial bioenergetic function to improve human performance, detect potential defects, and guide medical professionals in preserving their patient's aerobic capacity and vitality.

The following concepts on cell respiration are explained:

- Cellular routine respiration: controlled by the physiology of the living cell.
- Oxidative capacity: measured as maximal oxygen consumption decoupled from ATP production, in contrast to OXPHOS capacity — the capacity of oxidative phosphorylation.
- Leak respiration: idle respiration measured after inhibition of ATP production.
- Residual oxygen consumption: a small part of oxygen consumption that remains after fully inhibiting the oxidative capacity of the mitochondria.

Measurement of cell respiration in these experimentally controlled states and calculation of their relationships provides diagnostic information on mitochondrial fitness.

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Coenzyme Q junction and respiratory control

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In the electron transfer system (ETS), convergent electron flow through multiple mt-complexes (e.g.,CI, CII, CETFDH) reduce the Q-pool, reducing ubiquinone (UQ) to ubiquinol (UQH2) in the mt-inner membrane. UQH2 is oxidized downstream by CIII. Monitoring the redox state of ETS-reactive coenzyme Q (Q) is essential to understand the regulation of mitochondrial respiration. The Oroboros NextGen-O2k is equipped with the Q-Module to integrate high-resolution respirometry (HRR) with continuous amperometric monitoring of the redox state of a Q-mimetic (CoQ_2). CoQ_2 mimics the redox state of endogenous Q by equilibrating at the active sites of CI, CII, and CIII, enabling real-time analysis of the redox dynamics of Q in isolated mitochondria and permeabilized cells or tissues. The Q-Module allows calibration of the fully oxidized and reduced Q states, enhancing reproducibility and resolving chemical interferences via cyclic voltammetry. Steady-state control of O_2 concentration enables precise analysis of bioenergetic function from hyperoxia to deep hypoxia. Using Substrate-Uncoupler-Inhibitor-Titration (SUIT) protocols, the Q redox state and O_2 flux are simultaneously monitored in different coupling- and pathway-control states. Under coupling control, increasing mitochondrial energy demand (from leak to OXPHOS and electron transfer states) leads to partial oxidation of Q. Conversely, pathway control through substrate-driven stimulation of NADH- and succinate-linked pathways results in progressive Q reduction. These patterns emphasize that Q reduction is inversely related to respiratory load and directly proportional to electron push, showing pathway-specific differences in redox control. NS-pathway OXPHOS capacity is higher than either N- or S-pathways alone, yet showing incomplete additivity. Complete additivity would be achieved if the combined NS-pathway equals the arithmetic sum of the separate N and S-pathways. Incomplete additivity reflects interactions between supercomplex-bound and free Q-pools, supporting a plasticity model of Q-pool organization between solid- and liquid-state behavior. The complementary bioenergetic mechanisms of electron push by pathway control and electron pull by coupling control are clearly revealed by monitoring the Q redox state in combination with high-resolution respirometry. This approach advances our understanding of Q redox dynamics in mitochondrial physiology and pathophysiology and expands the diagnostic resolution of mitochondrial bioenergetics.

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Convergence and additivity of oxidative phosphorylation and electron transfer pathways; developing substrate, uncoupler, inhibitor, titration protocols

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High-resolution respirometry (HRR) enables stepwise evaluation of mitochondrial oxygen consumption rates (JO2) in response to titrations of substrates that support oxidative metabolism via distinct and overlapping enzyme pathways. Understanding these pathways and how they respond to saturating concentrations of substates *in vitro* is important for developing and interpretating HRR protocols. Fundamentally, substrates that are directly oxidized by NAD-dependent enzymes (e.g., pyruvate, glutamate, and malate) support JO2 through the oxidation of NADH by Complex I (CI-linked or “N-pathway” substrates), while succinate supports JO2 through its oxidation by succinate dehydrogenase (Complex II-linked or “S-pathway”), and fatty acid oxidation contributes electrons through the fatty electron transferring flavoprotein (ETF-linked or “F-pathway”). Upon entering the enter the electron transfer system (ETS), electrons from all these substrates reduce ubiquinone (Q) in the inner mitochondrial membrane, leading to a convergence of electron flow at the “Q-junction” that can influence the net flow of electrons to Complex III and ultimately JO2 by Complex IV. In addition, titrating millimolar concentrations of N and S pathway substrates can lead to allosteric and feedback inhibition of enzymes in the citric acid cycle, which can also impact net JO2 by altering rates of electron delivery to the ETS. In this presentation, I will provide an overview of the key concepts and common pitfalls in designing and interpreting multi-substrate HRR protocols to reveal variations in the control points of cellular bioenergetics relevant to basic biological and disease processes.

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Coupling efficiency: biological regulation and experimental states

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Cell respiration is studied in coupling control states which provide diagnostic information on mitochondrial function. Respiratory control ratios are calculated to analyze mitochondrial respiratory parameters independent of mitochondrial content. Respiratory protocols with living cells address the oxidative coupling efficiency based on measurement of oxidative capacity *E* and leak respiration *L*. Mitochondrial preparations, however, are required to asses OXPHOS capacity *P*. The concept of the respiratory acceptor control ratio (*RCR* = *P*/*L*) has a long tradition in bioenergetics. More recently, the 'classical' *RCR* has been replaced by the *L*/*P* coupling control ratio based on (*1*) conceptual grounds related to efficiencies ranging from 0 to 1 (*RCR* ranges from 1 to infinity), and (*2*) statistical arguments on normalization of distribution for proper estimation of the variability of results. The following concepts on cell respiration will be explained:

- Oxidative coupling efficiency = (*E*-*L*)/*E*
- OXPHOS coupling efficiency = (*P*-*L*)/*P*
- Routine ATP-efficiency = (*R*-*L*)/*R*
- Net routine control ratio = (*R*-*L*)/*E*
- Oxidative routine reserve = (*E*-*R*)/*E*

Normalization of cell respiration provides diagnostic information on mitochondrial performance which must be critically evaluated by consideration of mitochondrial density. In addition, lower efficiencies may not only be due to dyscoupling (increasing *L*). Decreasing *E* or *P* at constant *L* require different interpretations of 'efficiencies'.

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Mitochondrial role in the regulation of lactate shuttles

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Once thought of as a dead end metabolite, a fatigue agent, and marker of inadequate O_2 supply, in effect a metabolic poison, lactate was feared by athletes, physiologists, mountaineers, pulmonologists, cardiologists and ER and hospitalist physicians and others concerned about sepsis. However, we now need to take a second look at lactate and lactate metabolism. Consider that lactate production is a strain response to stress. Based on isotope tracer and other new technologies we now can appreciate the role of lactate in carbohydrate carbon flow that serves three purposes: 1) preferred energy substrate, 2) main gluconeogenic precursor, and 3) signaling molecule. The new vision is expressed within the within the Lactate Shuttle Concept. In fact, several Shuttles have been identified. In lab animals and human subjects as well as tissue and cell studies we observed postabsorptive Cell-Cell, Intracellular and Peroxisomal Lactate Shuttles. In this way, white muscle fibers feed red fibers in the same tissue, working muscle feeds the heart, brain and liver. Most recently we identified a Postprandial Lactate Shuttle for distribution of dietary carbohydrate. Given this context it emerges that the mitochondrial reticulum plays a defining role in governing energy substrate partitioning and carbon flow in vivo.

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Mitochondrial physiology: Fatty acid oxidation

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Fatty acid oxidation is favored in many tissues. The control of palmitoylcarnitine oxidation in normal rat liver mitochondria (mito) was investigated. The conversion of one equivalent of palmitate to carbon dioxide and water requires 46 equivalents of atomic oxygen. When citrate is the product, 22 units of atomic oxygen will be used per unit of palmitate. When acetoacetate is being formed, oxygen consumption is linked only to the dehydrogenations in β-oxidation and 14 oxygen atoms will be used. In the presence of rotenone and oxaloacetate, palmitate oxidation is limited to the acyl-CoA dehydrogenase step and only 7 equivalents of atomic oxygen are used. In rat liver mito, the rate of utilization of palmitoylcarnitine (36-37 pmoles/sec/mg mito protein; 4.3-4.4 nmoles/min/mg) was not affected by the kind of product to which it was converted. In contrast, heart mitoc oxidized palmitoylcarnitine past citrate, while skeletal muscle has complete oxidation using 46 equivalents of atomic oxygen.Long chain fatty acids are oxidized in the mitochondrial matrix via β-oxidation. They are activated on the cytosolic side of the mitochondrial outer membrane (MOM) by long-chain acyl-CoA synthetase (ACSL). The activated fatty acids as well as other substrates, ions, and nucleotides, cross the mitochondrial outer membrane (MOM) through the voltage-dependent anion channel (VDAC). In a study of the effects of exposure to the 22 kDa polyanion (PA22), rat liver mitochondrial ADP-stimulated glutamate, succinate (+rotenone), and palmitoylcarnitine (+malate) oxidation rates were not affected. However, oxidation of palmitate (+ATP, Mg^{2+}, CoASH, carnitine, and malate) and palmitoyl-CoA (+carnitine and malate) were inhibited at 1-2 nmol PA22 per mg mitochondrial protein. The next step is the carnitine-dependent transport of activated fatty acids, catalyzed by carnitine palmitoyltransferase 1a (CPT1a, an integral outer membrane protein), which converts fatty acyl-CoAs into acylcarnitines. Blue native electrophoresis of purified rat liver mitochondrial outer membrane (MOM) extracts yielded several high molecular weight bands containing CPT1a, ACSL, and VDAC; IP of MOM extracts with CPT1a antibodies or antisera against ACSL and VDAC revealed strong interactions between these 3 proteins. This strongly suggests that CPT1a forms hetero-oligomeric complexes with ACSL and VDAC to transfer fatty acids across the outer membrane. In similar studies using rat liver contact sites, in addition to the MOM proteins described above, mitochondrial inner membrane (MIM) proteins, the very-long chain acyl-CoA dehydrogenase, the phosphate carrier, and the adenine nucleotide translocase 2 were present in association with CPT1a, ACSL, and VDAC. The carnitine-acylcarnitine translocase was found only in lighter bands.

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Protonmotive force - from motive protons to membrane potential

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The protonmotive force (*pmF*) is central to oxidative phosphorylation (OXPHOS), coupling oxygen consumption (**OX**) in cell respiration to phosphorylation of ADP to ATP (**PHOS**). Defined as an electrochemical potential difference, the *pmF* consists of two components: the electric part pmF_{el}, linked to the transmembrane potential difference ($\Delta\Psi$), and the diffusive part pmF_{d}, related to the pH difference (ΔpH) across the mitochondrial inner membrane. Although pmF_{el} is dominant in animal mitochondria, pmF_{d} — often overlooked — contributes significantly under physiological conditions.

Peter Mitchell's chemiosmotic theory defines four integrated coupling modules. **Module 1:** The ATP synthase utilizes the *pmF* producing ATP (PHOS). **Module 2:** The electron transfer system generates the *pmF* by redox-driven proton transport (OX). **Module 3:** Coupling of proton translocation to electroneutral ion exchange modulates the balance from pmF_{d} to pmF_{el}. **Module 4:** The coupling membrane integrates these structural and functional coupling modules.

A ΔpH of only 0.5 units contributes approximately 15–20 % to the total *pmF*, emphasizing that pmF_{d} can provide a significant thermodynamic push. Oversimplified textbook conventions are challenged by rigorously incorporating stoichiometric numbers ν_{H^+} and the charge number z_{H^+} in the equations defining the advancement of proton translocation and the protonmotive force. A transparent theoretical framework bridges theory and experiment with an innovative conceptual drive.

Cite: Gnaiger E (2025) Protonmotive force - from motive protons to membrane potential. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

The gating mechanism of the mitochondrial ATP synthase leak channel

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Mitochondrial ATP synthase plays a key role in cell life and death by catalyzing ATP synthesis and housing a leak channel of mitochondrial permeability transition (mPT). mPT is a key cell death pathway in neurodegenerative disease and a promising target for potential therapeutic interventions. Prolonged opening of the mitochondrial permeability transition pore (mPTP) in mammals leads to the swelling and rupture of mitochondria, followed by cytochrome c release, which triggers apoptotic cell death. The mitochondrial ATP synthase c-ring was recently shown to form a large conductance channel with biophysical properties similar to the mPTP. Nevertheless, the conformational changes leading to the activation of ATP synthase c-subunit leak channel (ACLC) remain elusive. Unlike mammalian organisms, some crustacean and insect species survive anoxic conditions due to their resistance to Ca^{2+} and oxidative stress-induced mPTP activation. This unique ability could be related to distinct structural features and regulatory mechanisms governing ACLC activation in these organisms. A comparative structural and functional analysis of ATP synthases from different species will shed light on the gating mechanism of the ACLC and its contribution to mPT.

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Quality control and comparison of mitochondrial respiration media

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Quality control (QC) in mitochondrial respiration is essential to ensure accuracy and reproducibility at four levels: (*1*) chemicals and respiration medium, (*2*) sample preparation, (*3*) instrumental and technical reproducibility, and (*4*) data analysis**.** Specifically, the respiration medium plays a critical role in supporting mitochondrial function and its standardization is crucial for producing consistent results and facilitate inter-study comparison. This provides the rationale for developing a quality-controlled MiR05-Kit, which is a powder of seven components used to prepare MiR05. Mitochondrial respiration media are classically either KCl-based or sucrose-based. Sucrose-based solutions provide tighter coupling between respiration and ATP production. Mitochondrial respiration medium MiR05 was developed with reference to the intracellular formulation of organ storage solutions to avoid a high Cl^- concentration by using K-lactobionate and reducing the sucrose concentration. MiR05 is demonstrated to maximize ADP-stimulated respiration compared to KCl-based media and to stabilize membrane-bound cytochrome *c*. It supports higher mitochondrial respiratory capacities compared to medium Z in human skeletal muscle fibers. MiR05 is advantageous in fluorespirometric applications for the measurement of hydrogen peroxide production with Amplex UltraRed. QC of chemicals applied in mitochondrial bioenergetics, including components of respiration media, requires testing with mitochondrial preparations. Mitochondria from cultured cells, however, show variability of functional properties between batches or in a batch of cells cryopreserved over several years. To resolve this lack of a standardized mitochondrial sample, it was necessary to compare respiratory results simultaneously obtained using subsamples of the same batch of HEK 293T cells. In a study over four years, different lots and storage times of MiR05-Kit were tested to evaluate their consistency and long-term stability. Using six lots with storage times up to 51 months, we obtained consistent results in two complementary substrate-uncoupler-inhibitor titration (SUIT) protocols, covering twenty respiratory coupling and pathway states. Similarly, autoxidation of TMPD and ascorbate was not different between the lots and storage times of MiR05-Kit. The present strategy for QC of MiR05-Kit can be generalized for chemical QC, which is a major challenge to guarantee reliability and consistency of the results in mitochondrial respirometry.

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Mitochondria in brain ischemia reperfusion: More than just energy and ROS

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Mitochondrial dysfunction is central to ischemia/reperfusion (I/R) brain injury. While reactive oxygen species (ROS) imbalance and increased mitochondrial permeability have been implicated, we recently uncovered a distinct and previously unrecognized mechanism: mitochondrial Complex I inactivation via dissociation of its natural flavin cofactor, flavin mononucleotide (FMN). Using both *in vivo* and *in vitro* models of brain I/R, we examined mitochondrial function, flavoprotein content, performed proteomics/metabolomics analyses, and evaluated neurological deficits. During ischemia, Complex I activity declined at primary energy failure stage, while other respiratory chain complexes remained unaffected. Upon reoxygenation, Complex I activity partially recovered, but declined irreversibly after one hour, indicating secondary energy failure. We found that the irreversible inactivation of Complex I was due to the loss of enzyme-bound flavin mononucleotide (FMN), which impaired mitochondrial physiological activity as well as ROS generation. Metabolomics revealed dramatic increases in succinate and glycerol-3-phosphate during ischemia. Upon reoxygenation, their oxidation drives reverse electron transfer (RET) towards Complex I, resulting in pathological over-reduction of FMN and dissociation of the cofactor. The liberated reduced FMN *in situ* likely undergoes autoxidation and then is either re-incorporated back to the apo-Complex I or dephosphorylated to riboflavin. Riboflavin administration during I/R preserved mitochondrial respiration, reduced infarct size, and improved neurological outcomes *in vivo*. *In vitro*, oxidation of succinate/glycerol 3-phosphate induced rapid dissociation of Complex I FMN, but without affecting the subunit integrity of Complex I, respiratory supercomplex assembly, or post-translational modifications, as shown by proteomic analysis.This RET-induced flavin dissociation appears to be unique to the brain, due to the presence of a brain-specific long isoform of the NDUFV3 subunit located near the Complex I FMN-binding site. Unlike the canonical short version found in other tissues, the long isoform of NDUFV3 can reach toward the FMN-binding pocket and affect the flavin affinity to the apoenzyme. Our results establish Complex I as a critical driver of bioenergetic failure during the early phase of brain I/R injury. The RET-driven dissociation of FMN from mitochondrial Complex I represent a previously unrecognized targetable mechanism contributing to irreversible bioenergetic failure following cerebral ischemia.

Cite: Galkin A (2025) Mitochondria in brain ischemia reperfusion: more than just energy and ROS. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

Bioenergetic efficiency and regulation

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Mitochondrial respiration links substrate oxidation to ATP production through a tightly regulated process known as oxidative phosphorylation. However, the efficiency of this coupling can vary significantly, which in turn has profound effects on the cell's bioenergetic capacity and metabolic flexibility. Mitochondrial uncoupling refers to the disruption of the proton motive force across the inner mitochondrial membrane, resulting in the dissociation of electron transfer from ATP synthesis. While this process plays essential physiological roles such as thermogenesis and reactive oxygen species mitigation, excessive or dysregulated uncoupling can lead to depletion of the cellular energy charge and contribute to disease pathogenesis. This lecture will cover fundamental principles of the physiological mechanisms regulating bioenergetic efficiency. We will also explore how aberrant uncoupling may contribute to metabolic disorders and other pathophysiological states. Finally, we will examine emerging therapeutic strategies that manipulate mitochondrial efficiency, either by promoting or restricting uncoupling, to improve metabolic health and treat disease.

Cite: Axelrod CL (2025) Bioenergetic efficiency and regulation. In: Bioenerg Commun 2025.6. https://doi.org/10.26124/bec.2025-0006

Sensing, regulating, and therapeutically targeting mitochondrial bioenergetic balance

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Mitochondrial bioenergetic function reflects the interplay of three thermodynamic free energies that counterbalance one another and are therefore exquisitely poised to sense acute and/or chronic changes in cellular metabolic balance. The redox mechanisms that account for the ability to sense energy surplus, and how mitochondria invoke measures to counterbalance the reducing pressure and restore energy balance will be discussed. Therapeutic strategies to target mitochondrial bioenergetics to treat chronic energy surplus will also be discussed. Lastly, a new method to assess mitochondrial bioenergetic efficiency over the entire range of metabolic demand will be discussed.

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Bioenergetic adaptation in mitochondrial diseases

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Primary mitochondrial diseases (PMDs) of varying severity in children are linked to mitochondrial DNA (mtDNA) mutations or deletions impacting the electron transport chain (ETC). Developing better therapies require adequate benchtop models to evaluate the feasibility of assessing mitochondrial bioenergetics and mitochondrial dynamics in mitochondrial diseases. This study has focused efforts in patient fibroblasts and preclinical human induced pluripotent stem cells (hiPSCs) for mitochondrial diseases. We hypothesize that defective electron transfer system (ETS) caused by mitochondrial genome mutations contributes to aberrant mitochondrial dynamics and function. Results demonstrate that spare respiratory capacity or SRC was an important parameter of the cell's capacity to adapt to the defect. Mitochondrial fragmentation or hyperfusion was also associated with significant loss in mitochondrial membrane potential (MMP) in multiple patient fibroblasts and hiPSC models for PMDs. Our ongoing efforts in lineage-specific differentiation and metabolomic analyses will better aid in creating diagnostic and therapeutic platforms, thus benefiting the mitochondrial disease community.

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Immunoenergetics and exercise

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Acute exercise represents a robust physiological stressor that increases the number of circulating peripheral blood mononuclear cells (PMBCs). A phenomenon first highlighted by Schultz in 1893, where he observed that a 10-min bout of aerobic exercise led to an increase in circulating lymphocytes, which quickly returned to baseline levels following the cessation of exercise (1). Moreover, a subset of participants in the 1901 Boston Marathon experienced a similar exercise-induced leukocytosis (2). Fast forward 100 years, and researchers now have the tools necessary to comprehensively characterize exercise's acute impacts on PBMCs using flow cytometry and high-resolution respirometry. For example, we have shown that an acute bout of maximal exercise leads to a profound differential mobilization of different PBMCs subsets, an increase in routine (intact) cellular respiration, with minimal effect on cellular respiration under oxidative capacity (OXPHOS) conditions when normalized per million cells (cellular level) in collegiate swimmers (3). However, when cellular respiration under OXPHOS conditions was normalized per milliliter of blood (tissue-level), significant exercise-induced elevations in OXPHOS were consistent with the mobilization of more PBMCs. Consistent with the changes in OXPHOS, we observed changes in the T-cell compartment post-exercise, with reductions in naïve $CD8^+$ T-cells (highly oxidative) and a concomitant increase in more highly differentiated senescent $CD8^+$ T-cells (glycolytic) per unit of blood. This shift in cellular phenotype post-exercise could have contributed to the apparent observation that PBMCs did not enhance their OXPHOS capacity immediately post-exercise. Of interest, an hour-long session of low-intensity exercise has been shown to enhance fatty acid oxidation in isolated PBMC's (4). In a follow-up study (5), we examined the impact of 30 minutes of cycling at 10% above the lactate threshold on NK-cells respiration in young healthy women. One of the primary findings of this study was that the acute bout of exercise above the lactate threshold enhanced NK-cell respiration under OXPHOS and electron transfer (ET) capacity conditions, both at the cellular and tissue levels. These studies highlight the importance of performing respirometry experiments on distinct cellular subtypes in the peripheral immune compartment and the potential role that exercise intensity has on immune cell bioenergetics. Indeed, we also have preliminary data that incubating T-cells in plasma-like media with 4 mM L-lactate also stimulates their mitochondrial respiration compared to their matched controls at 0.5 mM L-lactate. Therefore, one of the purposes of this talk is to discuss the potential impact of the type of exercise (e.g., endurance, resistance, and anaerobic), duration of exercise, and likely the training status, age, and sex of the participants on circulating immune cells bioenergetics. A special emphasis will be placed on the importance of detailed phenotyping of immune cells via flow cytometry or cellular enrichment via negative selection to study the bioenergetic functions of unique immune cell subsets rather than mixed PBMCs.

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Mitochondrial function during extreme metabolic transitions: From bioenergetic control to oxidative stress

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Embryos of the brine shrimp *Artemia franciscana* exhibit severe metabolic arrest during their life cycle that is not normally survived by multicellular animals [1]. As they enter the hypometabolic state termed diapause, respiratory depression to less than 1% occurs as compared to active (post-diapause) embryos. Downregulation of trehalose catabolism during diapause restricts mitochondrial respiration, which is heightened by specific inhibitions within the mitochondria. The present work establishes the link between pyruvate dehydrogenase (PDH) phosphorylation and the inhibition of pyruvate dehydrogenase complex (PDC) catalytic activity in these embryos. The difference in catalytic activity of PDC in maximally phosphorylated and dephosphorylated samples was 16.5-fold. Western blot analysis of phospho-PDH content (site 1 of E1α-subunit, serine^{293}) confirmed the change in the phosphorylation state. These results show that PDH phosphorylation regulates PDC during diapause. In addition to PDC, other sites for blocking mitochondrial function are present during diapause. Compared with embryo lysates from post-diapause embryos, oxidative phosphorylation (OXPHOS) capacity *P* is depressed during diapause when either NADH-linked substrates for electron transfer (electron transfer capacity, *E*) through respiratory Complex I or the Complex II substrate succinate are used [2]. When pyruvate, malate and succinate were combined, respiratory inhibition by the phosphorylation system in diapause lysates was discovered as judged by *P/E* flux control ratios ($P<0.0001$). Inhibition was eliminated as the diapause extract was diluted ($P=0.0007$), consistent with the presence of a diffusible inhibitor. One candidate is long-chain acyl-CoA esters known to inhibit the adenine nucleotide translocator. Addition of oleoyl-CoA to post-diapause lysates markedly decreased the *P/E* ratio to 0.40±0.07 (mean±s.d.; $P=0.002$) compared with 0.79±0.11 without oleoyl-CoA. Oleoyl-CoA inhibits the phosphorylation system and may be responsible for the depressed *P/E* in diapause lysates. With isolated mitochondria, depression of *P/E* by oleoyl-CoA was fully reversed by addition of L-carnitine (control versus recovery, $P=0.338$), which facilitates oleoyl-CoA transport into the matrix and elimination by β-oxidation. In conclusion, severe metabolic arrest during diapause promoted by restricting glycolytic carbon to mitochondria is reinforced by depression of OXPHOS capacity and the phosphorylation system. Bouts of environmental anoxia are frequent for embryos of *A. franciscana.* For isolated mitochondria, H_2O_2 efflux does not increase significantly when exposed to anoxia followed by reoxygenation, nor do markers of oxidative damage as compared to normoxic controls. Evidence suggests mitochondria from *A. franciscana* embryos are well protected against oxidative stress during severe metabolic transitions. [NSF grant IOS-1457061/IOS-1456809].

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Mitochondrial bioenergetics and healthy aging

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In line with the principles of Geroscience, age-associated alterations in mitochondrial function have been shown to underlie a wide array of age-related diseases and conditions. This presentation will review mitochondrial bioenergetic profiling approaches that are being implemented in human/clinical studies. These include tissue and blood-based approaches that can serve as reliable biomarkers of mitochondrial health and have enabled researchers to examine the role of mitochondria in the physical and cognitive abilities of older adults. Importantly, we are also beginning to understand the factors that influence human mitochondrial bioenergetic capacity. These include intrinsic factors such as age and sex, as well as behavioral factors such as diet, exercise, and activity. We will also discuss ongoing efforts to identify circulating factors (e.g. proteins and metabolites) that drive differences in mitochondrial function associated with dementia and aging. Building upon our previous work demonstrating that blood-borne factors mediate age-related bioenergetic differences, our recent studies indicate that non-cellular factors present in serum can mediate bioenergetic differences associated with age and cognitive performance. Combining mitochondrial respirometry with multi-omic approaches, we are now able to identify circulating molecules that drive changes mitochondrial bioenergetics.

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Hepatocyte ATP homeostasis modulates peripheral control of food intake inhibition

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The capacity of the liver to serve as a peripheral energy sensor in the regulation of food intake has been debated for over half a century. Acute chemical inhibition of liver fatty acid oxidation or reduction in tissue ATP has been shown to stimulate food intake in a vagus nerve dependent manner. Further, maintenance of liver ATP levels and within-meal food intake inhibition is impaired in human subjects with obesity and obese pre-clinical models. Previously, we have shown decreased hepatic mitochondrial energy metabolism (i.e., oxidative metabolism & ADP-dependent respiration) in male liver-specific, heterozygous PGC1a mice results in increased short-term diet-induced weight gain with increased within meal food intake. We hypothesized that decreased liver mitochondrial energy metabolism in male LPGC1a+/- mice impairs meal termination following nutrient oral pre-loads. To test this hypothesis, liver mitochondrial respiratory response to changes in DG_{ATP} and adenine nucleotide concentration following fasting were examined in male liver-specific, heterozygous PGC1a mice. Further, food intake and feeding behavior during basal conditions, following nutrient oral pre-loads, and following fasting were investigated. We observed male LPGC1a+/- mice have reduced mitochondrial response to changes in DG_{ATP} and tissue ATP following fasting. These impairments in liver energy state are associated with larger and longer meals during chow feeding, impaired dose-dependent food intake inhibition in response to mixed and individual nutrient oral pre-loads, and greater acute fasting-induced food intake. These data support previous work proposing liver-mediated food intake regulation via ‘sensing’ of liver energy state.

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Adipose tissue thermogenesis and uncoupling proteins

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Non-shivering thermogenesis (NST) is one of the most fascinating evolutionary achievements, enabling high body temperatures and successful radiation of mammals and birds into cold environments. In many mammals, the mechanism of adaptive NST relies on the mitochondrial heater protein, uncoupling protein 1 (UCP1), in brown adipose tissue (BAT). In humans, dissipating excessive nutrient energy via thermogenesis in adipose tissue represents an attractive route to reduce obesity and its comorbidities. To unravel specific pathways that could be instrumental in therapeutically targeting human adipose tissue, we use unique animal models to receive evolutionary clues how thermogenesis was wired into adipose tissue. My laboratory has transformed our view on BAT evolution using comparative approaches, highlighting that some thermogenic pathways emerged late during mammalian evolution. Using bioenergetic approaches, we demonstrate that the thermogenic function of UCP1 has only sparked in the stem placental ancestor. Beyond the fundamental understanding of thermogenic evolution in adipose tissue, the goal is to enhance evolutionary-informed pathways in genetic mouse models to evaluate physiological significance, and to assess translational value in human adipocytes.

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Regulation of brown adipose tissue thermogenesis by PGC-1α isoforms

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Brown adipose tissue (BAT) is specialized for generating heat in both rodents and humans. When exposed to cold, non-shivering thermogenesis is rapidly activated by the sympathetic nervous system, which releases norepinephrine to stimulate β-adrenergic receptors (AR) on BAT. The transcriptional coactivator PGC-1α and its splice variant, NT-PGC-1α, are key regulators of cold-induced BAT thermogenesis. These coactivators are induced by β-adrenergic stimulation and orchestrate a transcription program by coactivating various transcription factors, such as PPARs and ERRs, resulting in enhanced mitochondrial biogenesis, fatty acid oxidation, respiration and thermogenesis. Our previous genome-wide DNA-binding (ChIP-seq) and gene expression (RNA-seq) analyses identified *Got1* as a novel target gene directly regulated by PGC-1α and NT-PGC-1α in BAT. Our recent findings reveal that cold-inducible GOT1 acts as a molecular switch that activates the malate-aspartate shuttle (MAS). MAS activation in BAT is crucial for maintaining mitochondrial fatty acid oxidation during cold-induced thermogenesis. Conversely, the loss of MAS activity due to *Got1* deletion reduces fatty acid oxidation, leading to increased glucose oxidation in mitochondria. Overall, our research uncovers a unique regulatory mechanism of MAS in BAT through the PGC-1α/NT-PGC-1α-GOT1 axis and highlights the role of MAS in mitochondrial fuel selection, enhancing our understanding of how BAT maintains fuel preference for fatty acids over glucose under cold conditions.

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Adaptation of energy metabolism to low oxygen availability

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Due to its role as the terminal electron acceptor in oxidative phosphorylation, oxygen is essential to mitochondrial electron transport and ATP synthesis. Therefore, decreased oxygen availability (hypoxia) threatens the bioenergetic homeostasis of cells, tissues, and organisms, and it is a feature of several human pathologies. However, certain physiological states (exercise) and natural habitats (high altitude or many aquatic habitats) are associated with low oxygen, demonstrating that animals possess a range of capacities to deal with hypoxia. This talk will review the direct and indirect effects of low oxygen on mitochondrial metabolism, the defenses against cellular energy imbalance, and the consequences of failure of these defense mechanisms. Humans and non-human model organisms, especially hypoxia-tolerant animals, will be discussed.

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Peroxisomes protect mitochondria and coordinate metabolic flexibility in skeletal muscle

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Mitochondria and peroxisomes are key organelles in cellular lipid catabolism. Limiting the mitochondrial lipid burden in skeletal muscle with tissue-specific deletion of carnitine palmitoyltransferase 1b ($Cpt1b^{M-/-}$) protects against metabolic dysfunction and glucose intolerance. Alternatively, the importance of peroxisomes in skeletal muscle biology is largely unknown. Here we report that skeletal muscle-specific peroxisome deletion ($Pex5^{M-/-}$) leads to mitochondrial dysfunction and mild glucose intolerance. More notably, functional peroxisomes are required for the improvements in glucose tolerance, insulin tolerance, mitochondrial biogenesis/function, and substantial genomic remodeling in $Cpt1b^{M-/-}$ mice as these beneficial adaptations do not occur in Cpt1b:$Pex5^{M-/-}$ double knockout mice. Overall, these results emphasize the importance of peroxisomes in skeletal muscle to maintain metabolic health, especially when the mitochondrial capacity is insufficient to handle the cellular lipid load alone.

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Precision targeting of mitochondrial bioenergetics in cancer

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Although all mitochondria produce ATP, emerging evidence from our laboratory and others reveals that the efficiency of this process varies significantly across the human body's more than 200 distinct cell types. This mitochondrial heterogeneity enables genetically identical mitochondrial populations to adapt their energy efficiency to the specific physiological demands of their host organs — a principle we refer to as mitochondrial specialization. Simply put, not all mitochondria are created equal; instead, they are bioenergetically customized to support their host cell's unique functions. Our laboratory is primarily focused on understanding how mitochondrial efficiency aligns with cellular physiology across tissues and how these relationships become disrupted in cancer. To address this, we have developed a state-of-the-art mitochondrial diagnostics platform that integrates comprehensive, discovery-driven bioenergetic phenotyping with large-scale mitochondrial proteomics using mass spectrometry. The overarching goal of our research is to create a biochemical 'blueprint' of the mitochondrial network in cancer, enabling the identification of actionable vulnerabilities intrinsic to malignant mitochondria. Currently, our group is particularly dedicated to leveraging these approaches to combat acute myeloid leukemia and colorectal cancer.

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