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Abstracts

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MiP*school* and MiP*conference* 2023

Editors
Erich Gnaiger, Alba Timon-Gomez, Lisa Tindle-Solomon, Paolo Cocco

Organizers
Carolina Gnaiger, Verena Laner

Summary

The Mitochondrial Physiology Society MiP*s* was founded at the 3rd MiP*conference* in Schröcken, Austria, 2003-Sep-12 to 16 [1], and the 1st MiP*summer school* took place in Schröcken in 2007-Jul-12 to 18 [2]. At this occasion, the first edition of the 'Blue Book' was introduced [3] which is available complementary to the MitoEAGLE 'Mitochondrial Physiology' communication [4] as the 5th edition published in 2020 in BEC [5]. In 2023 we continue the tradition of 'Mitochondrial Physiology: The Many Faces and Functions of an Organelle' [6] with MiP*events* held in various countries in Europe and USA, joining at the 13th MiP*school* [7] and 15th MiP*conference* [8] in Obergurgl, Austria.

1. MiP2003
2. MiPschool Schroecken AT 2007
3. Gnaiger E ed (2007) Mitochondrial pathways and respiratory control. 1st ed:96 pp.
4. Gnaiger E et al — MitoEAGLE Task Group (2020) Mitochondrial physiology. https://doi.org/10.26124/bec:2020-0001.v1
5. Gnaiger E (2020) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 5th ed. https://doi.org/10.26124/bec:2020-0002
6. MiP2005
7. MiPschool Obergurgl 2023
8. MiP2023 Obergurgl AT

Keywords – abstracts; mitochondria; MiP*school*; MiP*conference*; Bioenergetics Communications

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Mitochondrial structure and function, respiratory supercomplexes, and respiratory control

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MiP*school 2023*

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19:00 Dinner
20:30 **Opening**

2023-Jul-24 Monday

MiP*school 2023*

08:30 **Erich Gnaiger** *(AT)* Structural bioenergetics – mitochondrial membrane potential and protonmotive force.

10:00 Coffee / tea break

10:30 **Leonid Sazanov** *(AT)* The molecular structure of respiratory supercomplexes and their bioenergetic function.

12:00 Lunch / Walk & Talk

15:30 **Karin Busch** *(DE)* Complex I and ATP synthase - Regulation of large OXPHOS complexes by small peptide subunits.
16:15 **Christopher Axelrod** *(US)* Fission, fusion, and mitochondrial function.

17:00 Coffee / tea break

17:30 Roundtable and discussion

19:00 Dinner

20:30 **Open session**

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MiP*school 2023*

08:30 **Anthony Moore** *(UK)* Mitochondrial respiratory control and Q redox states - which Q pools are detected?
09:15 **Erich Gnaiger** *(AT)* Additivity of flux in pathways converging at the Q junction: supercomplex channeling and excess capacity of downstream respiratory complexes.

10:00 Coffee / tea break

10:30 **Luiza Cardoso** *(AT)* Electron push and pull in respiratory control and Q redox state.
11:15 **Erika Fernandez-Vizarra** *(IT)* Q redox state, respiratory control, and mitochondrial disease.

12:00 Lunch / Walk & Talk

15:30 **Roundtable and discussion: Q**

17:00 Coffee / tea break

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PS-2 Joanna Jasinska *(PL)* Modulation of mitochondrial large conductance potassium channels activity by infrared light.
PS-3 Murilo Othonicar *(BR)* Mitochondrial alternative enzymes and supercomplexes: functional and evolutionary insights.
PS-4 Patricia Owesny *(DE)* Impairment of cardiac function and mitochondrial energy metabolism through diet induced obesity in aging.
PS-5 Howard Phang *(US)* Screening marine natural products for bioenergetic effects in human cell models.
PS-6 Karolina Pytlak *(PL)* Mitochondrial alternative enzymes and supercomplexes: functional and evolutionary insights.
PS-7 Sara Stanic *(CZ)* Cold-induced non-shivering thermogenesis in skeletal muscle of obesity resistant mice.
PS-8 Sophie Strich *(AT)* Bioenergetic profiling of kinase inhibitors reveals drug off-target effects in colon cancer cell models.

19:00 *Dinner*

20:30 **Open session**

2023-Jul-26 Wednesday MiP*school 2023*

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10:00 *Coffee / tea break*

10:30 **Luiza Cardoso** *(AT)* A demo experiment on measurement of Q redox states simultaneously with high-resolution respirometry.

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15:30 **AS-1 Volker Ullrich** *(DE)* Cytochrome c as a cardiolipin dioxygenase.
15:45 **AS-2 Ranin Saleem** *(CA)* Mitochondrial physiology in cardiac muscle of deer mice native to high altitude.
16:00 **AS-3 Howard Phang** *(US)* Screening marine natural products for bioenergetic effects in human cell models.
16:15 **AS-4 Pushpalata Kayastha** *(PL)* Mitochondrial changes in eutardigrade Paramacrobiotus experimentalis to heat related stress.
16:30 **AS-5 Amit Kumar Nagwani** *(PL)* Mitochondria functionality in tardigrades under the hypomagnetic field.

17:00 *Coffee / tea break*

17:30 **Meet the expert**

19:00 *Dinner*

20:30 **Conclusions, awards and feedback followed by Raphael Raich and his accordion performing traditional Tyrolean songs at their best**

2023-Jul-27 Thursday - Departure

MiP*school 2023*

09:00 Departure/Relaxing day for participants of MiPconference 2023

15th MiP*conference*: Bioenergetics Communications on mitObesity and Healthy Aging.

PROGRAM

2023-Jul-27 Thursday - Arrival

MiP*conference 2023*

17:30 Welcome reception
19:00 *Dinner*
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2023-Jul-28 Friday

MiP*conference 2023*

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Chairs: Verena Laner and Orian Shirihai

08:30 A1-1 **Anthony Molina** *(US)* Targeting exercise intolerance in older, obese, heart failure patients.
08:55 A1-2 **Heather Petrick** *(CA)* Age- and sex-related differences in mitochondrial ADP sensitivity in human skeletal muscle.
09:10 A1-3 **Ana Valencia** *(US)* T-cell mitochondria exhibit a functional decline with obesity that is aggravated by weight loss.
09:25 A1-4 **Tal Yardeni** *(IL)* The host immune system and the gut microbiota are regulated by the mitochondrial DNA**.**

09:50 Coffee/tea break

Session A2. Exercise, training and hypoxia

Chairs: Dominique-Marie Votion and Alexander Karabatsiakis

10:25 A2-1 **Steen Larsen** *(DK)* Mitochondrial adaptations to weight loss – lifestyle, surgery or medication.
10:50 A2-2 **Henver Brunetta** *(BR)* Chronic aerobic training reverts the negative effects of high-fat high-sucrose diet on the left ventricle of adipocyte-specific DICER knockout mice in association with improved mitochondrial function.
11:05 A2-3 **Daniel Sadler** *(US)* Early life exercise training counters metabolic perturbations imparted by low parental cardiorespiratory fitness.
11:20 A2-4 **Bengt Kayser** *(CH)* Obesity and hypoxia: from etiology to therapy.
11:45 A2-5 **Steven Hand** *(US)* Oxidative stress and repetitive metabolic transitions: Challenges due to lifestyle of invertebrate extremophiles.

12:00 Lunch / Walk & Talk

Session A3. Nutrition and lifestyle in mitObesity

Chairs: Alba Timon-Gomez and Adam Chicco

15:30 A3-1 **Atan Gross** *(IL)* M(a)TCH2-ing up metabolism and apoptosis at the MOMbrane.
15:55 A3-2 **Graham Holloway** *(CA)* Mitochondrial bioenergetics as a nexus-point for the beneficial effects of nitrate in diverse tissues.
16:20 A3-3 **Patricia Owesny** *(DE)* Impairment of cardiac function and mitochondrial energy metabolism through diet induced obesity in aging.

16:35 **A3-4 Pavla Stankova** *(CZ)* Effect of telmisartan on nutritionally induced nonalcoholic steatohepatitis in mice.

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Chairs: Luiza Cardoso and Pablo Garcia-Roves

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17:50 **A4-2 Lukas Alan** *(CZ)* Dynamic complexomic analysis reveals a role for Von Willebrand Domain-containing Protein 8 (Vwa8) in mitochondrial morphology and substrate preference.

18:05 **A4-3 Lisa Guerrier** *(FR)* Human white adipose tissue mitochondrial respiration: effect of body composition.

18:20 **A4-4 Peter Schoenfeld** *(DE)* A view on brain's problem with fatty acid burning.

19:00 Dinner

20:30 **Poster session**

P-1 Lukas Alan *(CZ)* Dynamic complexomic analysis reveals a role for Von Willebrand Domain-containing Protein 8 (Vwa8) in mitochondrial morphology and substrate preference.

P-2 Daria Barkova *(SK)* Effects of short term and long term aerobic-strength training on muscle metabolism in the elderly.

P-3 Attila Kolonics *(HU)* Accelerated epigenetic changes may contribute to the development of metabolic syndrome revealed by NADH FLIM.

P-4 Murilo Othonicar *(BR)* Mitochondrial alternative enzymes and supercomplexes: functional and evolutionary insights.

P-5 Patricia Owesny *(DE)* Impairment of cardiac function and mitochondrial energy metabolism through diet induced obesity in aging.

P-6 Howard Phang *(US)* Screening marine natural products for bioenergetic effects in human cell models.

P-7 Sarah Piel *(DE)* Effect of dimethyl fumarate on cerebral mitochondrial metabolism in a porcine model of pediatric in-hospital cardiac arrest.

P-8 Karolina Pytlak *(PL)* Mitochondrial defect in human bronchial epithelial cells lacking the BKCa channel.

P-9 Linsey Stiles *(US)* Mitochondrial respirometry and ATP hydrolysis measurements in previously frozen tissue samples.

2023-Jul-29 Saturday

MiP*conference 2023*

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Chairs: Tal Yardeni and Anthony Molina

08:30 **B1-1** **Adam Chicco** *(US)* Delta-6 desaturase inhibition reverses aberrant cardiolipin remodeling and mitochondrial dysfunction in the obese mouse heart.

08:55 **B1-2** **Cesare Granata** *(DE)* Novel mitochondrial respiration protocols reveal organ-specific reliance on ketone body metabolism in mice.

09:10 **B1-3** **Markus Keller** *(AT)* Exploring the influence of the lipid environment on mitochondrial membrane structure and function.

09:25 **B1-4** **Yvonne Wohlfarter** *(AT)* The Janus-faced nature of HSD10 in cardiolipin biosynthesis and mitochondrial function.

09:40 **B1-5** **Luke Whitcomb** *(US)* Polyunsaturated fatty acid metabolism contributes to age-related impairment of cardiac mitochondrial calcium tolerance.

09:55 **B1-6** **Luiza Cardoso** *(AT)* Electron transfer from beta-oxidation and TCA cycle and impact of OXPHOS coupling on NADH and coenzyme Q redox states.

10:10 Coffee/tea break

Session B2. Type 2 Diabetes

Chairs: Sarah Piel and Markus Keller

10:30 **B2-1** **Christopher Axelrod** *(US)* Skeletal muscle mitochondrial dynamics in obesity and type 2 diabetes.

10:55 **B2-2** **Pablo Garcia-Roves** *(ES)* Remission of obesity and insulin resistance. Is that enough?

11:20 **B2-3** **ez** *(ES)* Impact of obesity on white adipose tissue plasticity: addressing depot-specific responses.

11:35 **B2-4** **Dominik Pesta** *(DE)* Respiratory capacity of skeletal muscle and peripheral blood mononuclear cells of male and female individuals with type 2 diabetes.

11:50 **B2-5** **Lubos Sobotka** *(CZ)* Macronutrients energy metabolism and obesity.

12:05 Lunch / Walk & Talk

Session B3. Respiratory complexes

Chairs: Tatyana Court and Atan Gross

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Session B4. Diagnostic approaches

Chairs: Alejandra Romero and Lubos Sobotka

16:30 B4-1 **Alexander Karabatsiakis** *(AT)* Combined metabolite and lipid fingerprinting of blood serum reveals biomarker candidates of altered mitochondrial bioenergetics in peripheral blood mononuclear cells of female patients with depression.

16:55 B4-2 **Dominique-Marie Votion** *(BE)* Serum acylcarnitines profile for diagnosis, prognosis and monitoring therapeutic intervention in equine atypical myopathy.

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17:35 B4-4 **Alba Timon-Gomez** *(AT)* Substrate-uncoupler-inhibitor titration protocol for analyzing carbohydrate and fatty acid metabolism.

17:50 **Conclusions**

18:00 **MiPs General Assembly**

19:00 Dinner

20:30 – 21:30 **Social evening with Trachtengruppe Umhausen. Award ceremony.**

2023-Jul-30 Sunday – Departure

MiP*conference 2023*

09:00 Bus departure

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- NAD(P)H redox state
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- Hypoxia and O_2 kinetics
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- ATP production
- pH, Ca^{2+}, $NO^{\cdot}$
- Photosynthesis

»mitochondria, cells and bioenergetics in

- Isolated mitochondria
- Tissue homogenate
- Permeabilized muscle fibers
- Permeabilized cells
- Living cells
- Chloroplasts

High-Resolution Respirometry and Redox Biology

13th MiP*school*:
Mitochondrial structure and function, respiratory supercomplexes, and respiratory control

ABSTRACTS

AS-1

Cytochrome c as a cardiolipin dioxygenase.

Ullrich Volker[1], Heidler J[2], Schildknecht S[1], Daiber A[3], Frensch M[1], Wittig I[2], Brüne B[2]

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2. Med. Fac. Dept. Biochem. II, Frankfurt, DE
3. Univ Med Center Dept Cardiol Univ Mainz, DE

Introduction: Micelles containing cardiolipin (CL) and phosphatidylcholine in presence of cytochrome *c* (Cytc) and H_2O_2 were reported to catalyze peroxidationes of typical peroxidase substrates but also of CL itself (Kagan et. al, Biochem. 45,4998, 2006). This can be explained by complex formation of CL with Cytc under removal of the Met80 sixth ligand of the heme.

Methods: O_2 consumption was measured polarographically (Orobos Instruments) and diene formation spectrally at 237 nm.

Results and Discussion: Cytc addition to CL micelles caused a burst of O_2 uuptake that could be repeated until CL or O_2 were depleted. About 4.5 mol of O_2/mol CL were taken up forming products with mainly 2,4,6 or 8 additional O-atoms. Diene formation initially followed the same kinetics but stopped or was reversed before O_2 uptake was completed. In presence of 2 M KCl Cytc acted catalytically with slower kinetics in three phases and showed oxidative modifications of the protein. The required peroxide tone originated from autoxidized CL and was upregulated during progress of the reaction. Significance for the process of opening of the permeability pore is suggested.

Keywords: cardiolipin; cytochrome *c*; oxygen uptake; diene formation

Cite: Ullrich V, Heidler J, Schildknecht S, Daiber A, Frensch M, Wittig I, Brüne B (2023) Cytochrome *c* as a cardiolipin dioxygenase. In: https://doi.org/10.26124/bec:2023-0002

AS-2

Mitochondrial physiology in cardiac muscle of deer mice native to high altitude.

Saleem Ranim, Scott GR
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Introduction: High-altitude environments are characterized by cold temperatures and low O_2 levels (hypoxia). Small mammals at high altitude thus face the metabolic challenge of maintaining thermogenesis to cope with cold in a hypoxic environment that can constrain aerobic ATP supply. Circulatory O_2 delivery by the heart is essential for supporting tissue O_2 demands, but it is unclear whether evolved or plastic changes in cardiac mitochondria help overcome constraints on thermogenesis in high-altitude environments.
Method: We examined this issue in deer mice (Peromyscus maniculatus). Mice from populations native to high altitude and low altitude were born and raised in captivity, and adults were acclimated to warm (25 °C) normoxia or cold (5 °C) hypoxia (~12 kPa O_2 for 5-6 weeks) in a full-factorial design. Mitochondrial function was studied by high-resolution respirometry and fluorometry in permeabilized tissue from left ventricles and was complemented by assays of several metabolic and antioxidant enzymes.
Results and discussion: Mitochondrial capacities for oxidative phosphorylation and electron transport were similar between populations and were unaffected by acclimation to cold hypoxia, as were activities of citrate synthase and cytochrome oxidase. However, exposure to cold hypoxia increased activities of lactate dehydrogenase, which were also greater in highlanders than in lowlanders, likely to augment capacities for lactate oxidation. Furthermore, mitochondrial emission of reactive oxygen species was lower in highlanders than in lowlanders across environments, associated with lower levels of lipid peroxidation and protein carbonyls. Therefore, phenotypic plasticity and evolved changes in cardiac mitochondria help deer mice cope with metabolic challenges at high altitude.

Keywords: High-altitude hypoxia; deer mice; cardiac mitochondria; reactive oxygen species

Cite: Saleem R, Scott GR (2023) Mitochondrial physiology in cardiac muscle of deer mice native to high altitude. In: https://doi.org/10.26124/bec:2023-0002

AS-3 / P-6*poster* / PS-5*poster*

Screening marine natural products for bioenergetic effects in human cell models.

Phang Howard J[1], Gerwick W[1,2], Molina AJA[3]

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2. Scripps Inst of Oceanography, Univ of California, San Diego, La Jolla, CA, US
3. Dept of Medicine, Univ of California, San Diego, La Jolla, CA, US

Mitochondrial bioenergetic decline is a well known biological hallmark of aging, suggesting that mitochondria-targeting therapeutics have great potential in treating age-related diseases and conditions [1]. Despite this, their efficacy within the context of human aging remains largely unknown. We sought to develop a phenotypic screening platform to identify agents that directly modulate mitochondrial function in human cells.

Figure 1. Workflow of respirometry screen

Marine natural products (MNP) represent a large, under-explored chemical space with immense therapeutic potential [2]. We screened a MNP library of 125 pure compounds at 10, 1, and 0.1 µg/mL incubated for 24 hours with with primary human dermal fibroblasts (pHDF) as summarized in Figure 1. We leveraged the San Diego Nathan Shock Center which houses 50+ pHDF lines derived from healthy donors across a spectrum of adult age. Cultured pHDF retain age-related phenotypes including mitochondrial bioenergetic decline, which presents a robust opportunity to identify bioenergetic effects within the context of human aging [3]. Thus, we used pHDF from a donor representative of an "older" phenotype (74 years of age) to ensure aging relevance.

We identified numerous compounds that modulate mitochondrial function in a dose-dependent manner. Our primary outcomes were change in basal or maximal respiration using high throughput respirometry (Agilent Seahorse XFe96). This screening platform successfully identified compounds with stimulatory as well as inhibitory effects on respiratory capacity. Future steps include further validation of hit compounds using high-resolution respirometry on the Oroboros O2k. These studies will elucidate mechanistic effects on the electron transfer system as well as effects on cells of different donor ages.

1. Murphy MP, Hartley RC (2018) Mitochondria as a therapeutic target for common pathologies. https://doi.org/10.1038/nrd.2018.174.
2. Liang X, Luo D, Luesch H (2018) Advances in exploring the therapeutic potential of marine natural products. https://doi.org/10.1016/j.phrs.2019.104373.
3. Auburger G, Klinkenberg M, Drost J, Marcus K, Morales-Gordo B, Kunz WS, Brandt U, Broccoli V, Reichmann H, Gispert S, Jendrach M (2012). Primary Skin Fibroblasts as a Model of Parkinson's Disease. https://doi.org/10.1007/s12035-012-8245-1.

Keywords: marine natural products; drug discovery; screening

Cite: Phang HJ, Gerwick W, Molina AJA (2023) Screening marine natural products for bioenergetic effects in human cell models. In: https://doi.org/10.26124/bec:2023-0002

AS-4

Mitochondrial changes in eutardigrade *Paramacrobiotus experimentalis* to heat related stress.

Kayastha Pushpalata[1], Wieczorkiewicz F[2], Kaczmarek L[1], Izabela P[2]

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Tardigrada (water bears) are well known for their ability to undergo cryptobiosis and survival in extreme conditions. The best-known type of cryptobiosis for their survival is anhydrobiosis i.e. response to lack of water. In this state tardigrades are able to tolerate high pressure, very high and low temperatures, space vacuum, and high levels of UV, and ionizing radiation. These results in various ultrastructural changes in tardigrades, including in mitochondria. We analyzed the effect of different temperatures (20 °C, 35 °C, 37 °C, 40 °C and 42 °C) on the ultrastructure of mitochondria in the tardigrade *Paramacrobiotus experimentalis* Kaczmarek et al. 2020. Analyzes were conducted in active specimens, specimens in anhydrobiosis (tun), and rehydrated specimens. The analysis will provide knowledge about changes in the ultrastructure of tardigrades caused by different temperatures. Our results will also determine whether anhydrobiosis protects against temperature-induced ultrastructural changes.

Keywords: water bears; Tardigrada; Eutardigrada; heat stress; mitochondria

Cite: Kayastha P, Wieczorkiewicz F, Kaczmarek L, Izabela P (2023) Mitochondrial changes in eutardigrade *Paramacrobiotus experimentalis* to heat related stress. In: https://doi.org/10.26124/bec:2023-0002

AS-5

Mitochondria functionality in tardigrades under the hypomagnetic field.

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2. Dept. of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University, Poznań, PL

Introduction: Tardigrades are considered as one of the toughest animals on Earth due to their remarkable ability to withstand extreme condition. An example of these conditions is hypomagnetic field (HMF, static magnetic field with an intensity <5 μT), which is known to influence the metabolic processes including mitochondria functioning. However, very few studies considering HMF impact were performed for organisms able to survive under extreme conditions and considered as suitable for outer space colonization. Therefore, we decided to check the impact of HMF on the tardigrade *Paramacrobiotus experimentalis* focusing on mitochondria functionality reflected by the mitochondrial inner membrane potential ($\Delta\Psi$) having regard to age and sex.

Methods: Females and males from 3 different age classes (i.e., 30-60, 150-180 and >300 days) were extracted from laboratory culture and divided into experimental and control groups exposed to HMF and standard magnetic field (SMF), respectively, for three different durations i.e., 7 days, 15 days and 30 days. The HMF treatment was performed in a special anti-magnetic chamber whereas SMF treatment was performed in a climate chamber. TMRM staining of intact animals was used to estimate $\Delta\Psi$.

Results and discussion: The calculated FITMRM index indicated HMF-related changes in $\Delta\Psi$ dependent on age and sex. Accordingly, HMF effect was most pronounced for the oldest animals and males appeared to be more sensitive to HMF than females that correlated with the survival rate. The results provide an insight into mechanisms of HMF effect that could be useful for organization of space travels and living outside the Earth.

1. Mo W, Liu Y, He R. (2014) Hypomagnetic field, an ignorable environmental factor in space? https://doi.org/10.1007/s11427-014-4662-x
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4. W, Idzikowski B, Kowalski W, Kosicki J, Kaczmarek Ł (2021) Tolerance of two anhydrobiotic tardigrades Echiniscus testudo and Milnesium inceptum to hypomagnetic conditions. https://doi.org/10.7717/peerj.10630

Cite: Nagwani AK, Kaczmarek L, Kmita H (2023) Mitochondria functionality in tardigrades under the hypomagnetic field. In: https://doi.org/10.26124/bec:2023-0002

13th MiP*school*:
Mitochondrial structure and function, respiratory supercomplexes, and respiratory control

POSTERS

PS-1*poster*

Mitochondrial cytochrome c oxidase subunit 4 isoform 2 (Cox4i2) promotes the hypoxia-induced reduction of the electron transfer system and fosters superoxide production.

Giordano Luca[1], Nolte A[1], Wittig I[2], Pak O[1], Knoepp F[1], Ramser K[3], Wahl J[3], Cabrera A[2], Hüttemann M[4], Grossman LI[4], Pecina P[5], Ghofrani HA[1], Seeger W[1,6], Weissmann N[1], Giehl K[7], Sommer N[1]

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Introduction: Hypoxia in the lung alveoli triggers the contraction of the small precapillary pulmonary arteries, i.e., hypoxic pulmonary vasoconstriction (HPV), avoiding life-threatening hypoxemia. Pulmonary arterial smooth muscle cells (PASMCs) are involved in HPV, with the mitochondrial cytochrome c oxidase (COX) subunit 4 isoform 2 (Cox4i2) playing an essential role in the acute oxygen sensing1. Nonetheless, the molecular mechanism by which Cox4i2 sensitizes the whole COX remains unclear. **Methods:** We analysed superoxide production by MitoSOX, oxygen consumption by high-resolution respirometry, redox changes of the electron transport system (ETS) by RAMAN spectroscopy, and supercomplex formation by blue native gel analysis of PASMCs isolated from wild type (WT) and Cox4i2 knockout mice (Cox4i2 KO) exposed to normoxia or hypoxia. To figure out the role of Cox4i2-specific cysteine residues we generated mouse epithelial (CMT167) cells overexpressing either Cox4i1, or WT Cox4i2, or Cox4i2 mutants (C41S, C55A, C109S), and we tested their superoxide production and oxygen affinity. **Results:** Respiration, abundance, and COX assembly were similar in WT and Cox4i2 KO PASMCs. On the contrary, hypoxia-induced production of superoxide and the reduction of ETS components (NADH, ubiquinol, cytochrome c) was prevented in Cox4i2 KO PASMCs. CMT167 cells expressing either Cox4i1, or Cox4i2 mutants lacked hypoxia-induced superoxide production, which was detected only in cells expressing WT Cox4i2. Overexpression of Cox4i1, or Cox4i2, or Cox4i2 mutants did not affect oxygen affinity. Our findings suggests that Cox4i2 does not alter superoxide production by rearrangement of supercomplexes, but by the reduction of the ETS, likely mediated by the cysteine residues.

1. Sommer N, Hüttemann M, et al (2017) Mitochondrial Complex IV Subunit 4 Isoform 2 Is Essential for Acute Pulmonary Oxygen Sensing. https:// doi/10.1161/CIRCRESAHA.116.310482

Keywords: cytochrome *c* oxidase; hypoxia; oxygen sensing; Cox4i2

Cite: Giordano L, Nolte A, Wittig I, Pak O, Knoepp F, Ramser K, Wahl J, Cabrera A, Hüttemann M, Grossman LI, Pecina P, Ghofrani HA, Seeger W, Weissmann N, Giehl K, Sommer N (2023) Mitochondrial cytochrome c oxidase subunit 4 isoform 2 (Cox4i2) promotes the hypoxia-induced reduction of the electron transfer system and fosters superoxide production. In: https://doi.org/10.26124/bec:2023-0002

PS-2*poster*

Modulation of mitochondrial large conductance potassium channels activity by infrared light.

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Recent studies point out that mitochondria are not only a source of ATP in the cell, but more and more data indicate their role related to Ca2+ buffering, production of reactive oxygen species (ROS) and activation of intracellular signaling pathways of necrosis and apoptosis. Recent studies clearly indicate that mitochondrial potassium channels (mitoK) present in the inner mitochondrial membrane play an important protective role in the ischemia-reperfusion processes of myocardial cell damage. These results were obtained using low molecular weight chemicals. Due to the lack of selective modulators of potassium channels, we opted for an alternative approach to modulate the activity of mitoK channels by changing the redox state of the respiratory chain, which we have demonstrated in previous studies. Some respiratory chain proteins are thought to absorb infrared (IR) light. Cytochrome c oxidase (COX) may be important in these mechanisms because it has four metal redox centers: binuclear CuA, CuB, heme a, and heme a3. All these metal centers are able to absorb light waves in the IR region. Data obtained in our laboratory indicate that COX may be functionally linked to mitochondrial high-conductance Ca2+-activated potassium channels (mitoBKCa) in the U87 cell line1. Using the patch-clamp technique with the illumination system, we exposed the mitoBKCa channel. We observed that in the presence of ferricyanide, channel activity was inhibited and that mitoBK channel activity could be restored by 820 nm illumination, suggesting that COX is involved in the modulation of mitoBK channel activity.

1. Szewczyk A and Bednarczyk P (2018) Modulation of the Mitochondrial Potassium Channel Activity by Infrared Light. https://doi.org/10.1016/j.bpj.2017.11.288

Cite: Jasińska J, Bednarczyk P, Kalenik B, Kulawiak B, Wrzosek A, Szewczyk A (2023) Modulation of mitochondrial large conductance potassium channels activity by infrared light. In: https://doi.org/10.26124/bec:2023-0002

PS-3*poster* / P-4*poster*

Mitochondrial alternative enzymes and supercomplexes: functional and evolutionary insights.

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Funding: FAPESP(grant 2021/06711-2, 2022/01509-3) & CNPQ(grant 141001/2019-4)

Oxidative phosphorylation (OXPHOS) dysfunction can lead to decreased ATP levels and excessive reactive oxygen species (ROS) formation. Alternative enzymes (AEs) have been successfully used in model organisms to bypass OXPHOS defects and prevent high ROS levels, despite vertebrates and insects having lost their coding genes throughout evolution [1,2,3]. To get a deeper insight into the possible differences between AE-bearing and -lacking animals, we compared the genes coding for subunits of the OXPHOS complexes in tunicates of the genus *Ciona* with orthologs in *Drosophila* and humans. We found that *Ciona* species lack subunits necessary for the formation of respiratory supercomplexes (SCs), which are supramolecular organizations of the invidual OXPHOS complexes able to streamline electron transfer and prevent excessive ROS formation[4]. This suggests that *Ciona* species do not form SCs, or do so differently. In agreement, we also found that the *Ciona intestinalis* AE alternative oxidase (AOX), when transgenically expressed in *Drosophila melanogaster*, preferentially receives electrons from the mitochondrial glycerol-3-phosphate dehydrogenase, which is not known to be involved in SCs. Only when *Drosophila* SCs appear to be disrupted, AOX is able to receive all electrons from Complex I, a well known SC component. We are currently investigating SC formation in AOX-expressing flies and in *C. intestinalis*. Our findings could offer valuable insights for optimizing AOX expression in possible future therapeutic settings, and shed light on the evolutionary and functional variations between animal OXPHOS systems.

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Keywords: Mitochondria; alternative enzymes; supercomplex; *Ciona; Drosophila*

Cite: Othonicar MF, Garcia GS, Oliveira MT (2023) Mitochondrial alternative enzymes and supercomplexes: functional and evolutionary insights.. In: https://doi.org/10.26124/bec:2023-0002

PS-4*poster* / **A3-3** / P-5*poster*

Impairment of cardiac function and mitochondrial energy metabolism through diet induced obesity in aging.

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PS-5*poster* / **AS-3** / P-6*poster*

Screening marine natural products for bioenergetic effects in human cell models.

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PS-6*poster* / **P-8***poster*

Mitochondrial defect in human bronchial epithelial cells lacking the BK_{Ca} channel.

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Funding: This study was supported by a grant 2019/35/B/NZ1/02546 from the National Science Centre in Poland.

PS-7 *poster*

Mitochondria functionality in tardigrades under the hypomagnetic field.

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Introduction: Non-shivering thermogenesis (NST) is an energy-dissipating process that occurs in brown adipose tissue (BAT) and is activated by the adrenergic system. Earlier studies found that cold induces adrenergically activated NST in obesity-prone C57BL/6 (B6) mice, but not in obesity-resistant A/J mice. To investigate this difference, we studied the effect of cold acclimation on muscle NST.
Methods: Palmitoyl carnitine oxidation and cytochrome c oxidase (COX) activity (TMPD+ascorbate and KCN) was measured in muscle homogenates of A/J and B6 mice acclimated to 30 °C or to 6 °C using Oroboros Oxygraph. In parallel, amount of mitochondrial supercomplexes was assessed by Blue native electrophoresis.
Results and discussion: As expected, muscle of A/J mice exhibited higher amount of Scaf1 dependent supercomplex III2IV than muscle of B6 mice, and this amount was further increased by cold acclimation. Both palmitoyl carnitine oxidation and COX activity were induced by cold in A/J but not in B6 mice.
The higher oxidation capacity of muscle of cold acclimated A/J mice, possibly connected with supercomplex composition, may indicate that muscle represents the site of alternative NST instead of BAT in these mice. The distinct mechanism of NST could correspond to obesity resistance of this strain.

1. Janovska P. et al (2023) Impairment of adrenergically-regulated thermogenesis in brown fat of obesity-resistant mice is compensated by non-shivering thermogenesis in skeletal muscle. https://doi.org/10.1016/j.molmet.2023.101683

Keywords: Non-shivering thermogenesis, Supercomplexes, cold acclimation

Cite: Stanic S, Janovska P, Zouhar P, Bardova K, Otahal J, Vrbacky M, Mracek T, Adamcova K, Lenkova L, Funda J, Cajka T, Drahota Z, Rustan AC, Horakova O, Houstek J, Rosmeissl M, Kopecky J (2023) Cold-induced non-shivering thermogenesis in skeletal muscle of obesity resistant mice. In: https://doi.org/10.26124/bec:2023-0002

PS-8 *poster*

Bioenergetic profiling of kinase inhibitors reveals drug off-targer effects in colon cancer cell models.

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Protein kinases play an important role in numerous signaling pathways regulating cell proliferation, cell cycle, and metabolism. Deregulation of kinase functions have been connected to various human diseases, such as cancer [1]. In recent years, kinase inhibitors have gained recognition by aiming to block single or multiple oncogenic kinase pathways [2]. In these lines, blockade of kinase activities has been shown to converge on the central energetic organelle, the mitochondria [3, 4]. Furthermore, colon cancer cells rely on mitochondrial OXPHOS as major source of energy, contradicting the Warburg effect [5]. To increase the understanding of small molecule-based kinase blockers and their cell-type-specific adverse effects, we set out to record the impact of kinase drugs on mitochondrial respiration using High-resolution FluoRespirometry in several colon cancer cell models. We observed that the impact of kinase inhibitors depends on the mutational background of the tested cancer cell lines as well as on cell culture medium formulations [6]. First, we detected off-target effects of sunitinib, an FDA-approved multikinase blocker, only in a more physiological cell culture medium as compared with classical formulations. Second, mitochondrial profiling of the glycolytic kinase inhibitor PFK158 revealed off-target mitochondrial dysfunction. Third, we were able to show that inhibition of kinase signaling is connected to mitochondrial reactive oxygen species (ROS), which can be influenced by protein kinase modulators. In summary, cell-based mitochondrial bioenergetic profiles have the power to identify off-target effects of kinase inhibitors and allow a detailed mechanistic insight on drug-induced perturbations in cancer cell metabolism.

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Keywords: kinase signaling; mitochondria; kinase inhibitors; cancer; drug off-target effects

Cite: Torres-Quesada O, Strich S, Feichtner A, Schwaighofer S, Doerrier C, Schmitt S, Gnaiger E, Stefan E (2023) Bioenergetic profiling of kinase inhibitors reveals drug off-targer effects in colon cancer cell models: In: https://doi.org/10.26124/bec:2023-0002

15th MiP*conference*: Bioenergetics Communications on mitObesity and Healthy Aging.

ABSTRACTS

Opening

From globesity to mitObesity.

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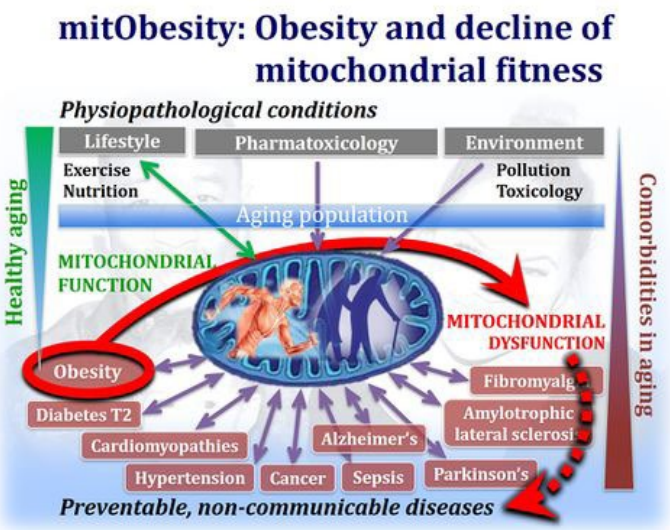
'Obesity is a complex condition, one with serious social and psychological dimensions, that affects virtually all age and socioeconomic groups and threatens to overwhelm both developed and developing countries' — the WHO perspective on 'globesity' (https://www.who.int/activities/controlling-the-global-obesity-epidemic). Obesity defined as BMI≥30 (WHO) is biased, overestimating obesity thresholds in taller persons (men) but underestimating it in smaller groups (women) — a gender data gap. Here obesity is defined as accumulation of excess fat-tissue mass, $M_{FE}=M_F-M_F°$. $M_F°$ is the fat mass per individual in the healthy reference population at any height and body mass $M°$ without overweight. Body fat excess, $BFE=M_{FE}/M°$, is related to body mass excess, $BME=M_E/M°$, where $M_E=M-M°$. A balanced BME is BME° = 0.0 with a band width of -0.1 towards underweight and +0.2 towards pre-obesity (overweight). The BME is linearly related to the body fat excess in women and men with statistical implications on mitochondrial functional fitness.



Aerobic spiroergometric capacity per body mass $V_{O2max/M}$ and mitochondrial respiratory capacity per muscle mass [1] decline as a function of BME. Compromised mitochondrial fitness across metabolically active organs provides a functional connection between obesity and comorbidities bound to redox imbalance, inflammation, oxidative stress, and insulin resistance: diabetes, cardiovascular and neurodegenerative diseases, various types of cancer (Figure 1). mitObesity is the leading cause of deaths and early aging, prevented by improved quality of life in active lifestyles with exercise and caloric balance. Obesity has reached the general news, without connection to mitochondria. How do we get from globesity to mitObesity to forge scientific results into knowledge impacting society, health system stakeholders, and politics?

Keywords: obesity; healthy reference population; body mass excess; body mass index; mitochondrial fitness; degenerative diseases

Cite: Gnaiger E (2023) From globesity to mitObesity. In: https://doi.org/10.26124/bec:2023-0002

A1 - Lifestyle and mitoalterations in obesity

A1-1

Targeting exercise intolerance in older, obese, heart failure patients.

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A1-2

Age- and sex-related differences in mitochondrial ADP sensitivity in human skeletal muscle.

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Introduction: Mitochondrial ADP sensitivity represents an important control point in oxidative phosphorylation. The sensitivity of mitochondria to ADP is lower in high-lipid environments, in aging males, and in young females compared to young males. However, the interaction between sex, age, and body composition (fat mass) in the regulation of mitochondrial ADP sensitivity remains unknown.

Methods: Vastus lateralis muscle biopsies were obtained from healthy, recreationally active, young males (n=21, 24±4 y, 22.7±2.2 kg/m^2 BMI), young females (n=20, 21±2 y, 21.7±2.2 kg/m^2), older males (n=13, 76±5 y, 25.8±2.5 kg/m^2), and older females (n=6, 70±6 y, 23.4±3.0 kg/m^2). Permeabilized fibers were prepared to measure mitochondrial ADP sensitivity. Whole-body DEXA scans were performed. Data (mean±SD) were analyzed using two-way ANOVAs.

Results and discussion: Body fat percentage was higher in females and older individuals (main effects). While maximal mitochondrial respiration did not differ between groups, mitochondrial ADP sensitivity was affected by sex and age. Specifically, in younger individuals mitochondrial ADP sensitivity was lower in females compared with males (~15 % higher apparent ADP Km, p=0.02). Older males also showed ~15% lower mitochondrial ADP sensitivity compared with young males (p=0.04). In contrast to young individuals, mitochondrial ADP sensitivity was numerically greater (~15 %) in older females when compared with older males (p=0.14) and younger females (p=0.12). However, there were no correlations between body fat percentage and mitochondrial apparent ADP Km in any group. We speculate that sex-based differences in mitochondrial ADP sensitivity are impacted by estrogen as opposed to body composition, as this response is lost with aging.

Cite: Petrick HL, Aussieker T, Fuchs CJ, Hermans WJ, Betz MW, Pinckaers PJM, Snijders T, van Loon LJC, Holloway GP (2023) Age- and sex-related differences in mitochondrial ADP sensitivity in human skeletal muscle. In https://doi.org/10.26124/bec:2023-0002

A1-3

T-cell mitochondria exhibit a functional decline with obesity that is aggravated by weight loss.

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Introduction: Weight loss (WL) promotes counterregulatory mechanisms that may involve mitochondrial (MITO) function to limit cardiometabolic benefit. This study compared T-cell MITO function in states of obesity (OB), active weight loss (OB-WL), weight loss plateau (OB-PL), regain (OB-RG), and healthy weight (HWC).

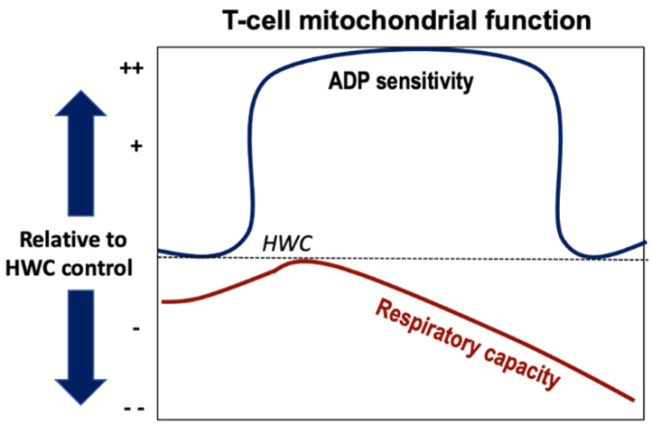

Figure 1. Differences in respiratory capacity and ADP sensitivity in T-cells in response to obesity and different phases of weight loss.

Methods: Participants with obesity (61.5%female, 39.5±10.8 yr, BMI 36.7±6.4) underwent a 24-week WL intervention and transmitted their daily weight for 18 months. T-cells (CD3+) were isolated from blood samples obtained at baseline, 6-month, or 12-months. We measured MITO respiratory capacity (MITO-RC) (XFe Analyzer) and sensitivity of membrane depolarization with ADP (IC50) (O2K Fluorespirometer).
Results: Compared to HWC, MITO-RC was lower in OB T-cells (4.1±1.7 vs. 3.3±1.0 pmol $O_2/10^6$ cells, $p<0.05$), and even lower in OB-PL (3.0±0.7, $p<0.05$) and OB-RG (2.7±0.3, $p<0.05$). Maximal membrane potential was also lower in the OB group and remained low in all phases of WL. IC50 did not differ in T-cells between HWC and OB but was lower in OB-WL and OB-PL (156±15 vs. 7±1 & 22±5, $p<0.05$).
Conclusions: T-cell MITO respiratory capacity is reduced in obesity and further aggravated in response to WL, particularly following a plateau. However, WL improved ADP sensitivity, suggesting a potential counterregulatory mechanism to meet energy demand. Findings suggest that the MITO function of T-cells is not restored by WL to resemble HWC and is rather altered in a way that could potentially limit cardiometabolic benefit of WL.

Keywords: ADP sensitivity; peripheral mononuclear cells; weight loss plateau; metabolic adaptation

Cite: Valencia AP, Melhorn SJ, Schur E, Marcinek DJ (2023) T-cell mitochondria exhibit a functional decline with obesity that is aggravated by weight loss. In: https://doi.org/10.26124/bec:2023-0002

A1-4

The host immune system and the gut microbiota are regulated by the mitochondrial DNA.

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Both mitochondrial DNA (mtDNA) lineages and the gut microbiota have been correlated with altered risk for a variety of human diseases including obesity. However, the mechanisms by which mtDNA variation and the gut microbiota modulate disease risk remains unknown. Our hypothesis is that both the gut microbiota and the immune system are modulated by the mitochondrial genome, in part through mitochondrial reactive oxygen species (mtROS) production, forming a critical link between the gut microbiota and disease initiation and progression. Our studies showed significant differences in gut microbiota in our conplastic mice which differ in their mtDNA lineages. Further, the transfer of the gut microbiota from a host of one mitochondrial genotype to a host of different mitochondrial genotypes shifted the gut microbiota composition toward that of the recipient animal. Moreover, we showed that host mtROS levels modulated the composition of the gut microbiota.

Those conplastic mice also exhibit markedly different capacities to sustain melanoma tumor growth. Relative to control mtDNA ($mtDNA^{B6}$) mice, the mice harboring NZB mtDNAs ($mtDNA^{NZB}$) have strong anti-tumor immune response while those with129 mtDNA ($mtDNA^{129}$) are the opposite. Reduction of mtROS by expression of mitochondrial catalase (mtCAT)Tg only in the hematopoietic cells changed the gut microbiota and obviated the anti-tumor effects on the $mtDNA^{NZB}$ and $mtDNA^{B6}$ mice. These observations suggest that disease severity (melanoma), and gut microbiota are regulated by the mtDNA's regulation of mtROS production in host immune cells, pointing to new potential pathways for understanding diseases etiology.

Cite: Yardeni T (2023) The host immune system and the gut microbiota are regulated by the mitochondrial DNA. In https://doi.org/10.26124/bec:2023-0002

A2 - Exercise, training and hypoxia

A2-1

Mitochondrial adaptations to weight loss – lifestyle, surgery or medication.

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Weight loss maintenance after lifestyle changes (diet and exercise) is challenging and unfortunately the success rate is low. This topic has received major attention and yet the explanation behind the apparent inability to maintain lifestyle changes remains unresolved. Combining increased physical activity and changes of the diet composition are typically first line of treatment to achieve weight loss, but gastric bypass surgery is also used for weight loss. Different drugs are on the market that induces a weight loss. The presentation will focus on mitochondrial adaptations after a weight loss induces by lifestyle changes or after gastric bypass surgery. We have conducted two clinical trials investigating weight loss after a lifestyle intervention or after gastric bypass surgery, with a focus on mitochondrial function in skeletal muscle and adipose tissue. Furthermore focus will also be on the effect of different weight loss medications and how these affects mitochondrial function.

Cite: Larsen S (2023) Mitochondrial adaptations to weight loss – lifestyle, surgery or medication. In https://doi.org/10.26124/bec:2023-0002

A2-2

Chronic aerobic training reverts the negative effects of high-fat high-sucrose diet on the left ventricle of adipocyte-specific DICER knockout mice in association with improved mitochondrial function.

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The negative effects of high-fat high-sucrose (HFHS) diet consumption on heart function are exacerbated in mice lacking DICER in adipocytes (AdicerKO). These findings suggest a protective role of adipocyte-derived microRNAs on heart physiology. Exercise training is known to have a protective role in cardiometabolic diseases. However, it is not known whether chronic aerobic training is able to rescue heart dysfunction in HFHS-fed AdicerKO mice. Here, we fed AdicerKO mice with a HFHS diet for 12 weeks, after confirming the deleterious effects of the diet on these mice, we submitted them to moderate aerobic training for 8 weeks, 5 days/week for 60 minutes each section while keeping them on HFHS-diet. Chronic aerobic training restored end-systolic volume and stroke volume in the hearts of HFHS-fed AdicerKO mice without changing ejection fraction. In addition, aerobic exercise increased left ventricle diameter in both, systolic and diastolic, phases. Notably, HFHS-fed AdicerKO-trained mice presented lower heart rate with no differences in systolic blood pressure compared to HFHS-fed AdicerKO sedentary mice. Mechanistically, chronic exercise training lowered mitochondrial H_2O_2 emission and oxidative stress alongside greater lipid- and succinate-supported mitochondrial respiration. Importantly, these effects were not followed by changes in triacylglycerol content within the left ventricle or fibrosis. In summary, chronic aerobic training is capable to rescue heart function of HFHS-fed AdicerKO mice in association with improvements in mitochondrial bioenergetics and redox balance.

Cite: Brunetta HS, Palermo Ruiz G, Ludwig R, Ruberti O, Bechara L, Consonni S, Rodrigues B, Ferreira JCB, Mori MAS (2023) Chronic aerobic training reverts the negative effects of high-fat high-sucrose diet on the left ventricle of adipocyte-specific DICER knockout mice in association with improved mitochondrial function. In https://doi.org/10.26124/bec:2023-0002

A2-3

Early life exercise training counters metabolic perturbations imparted by low parental cardiorespiratory fitness.

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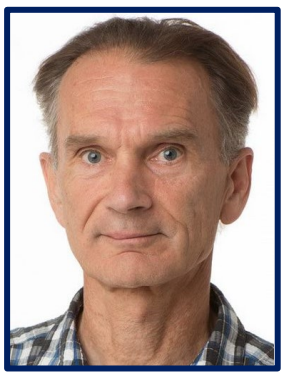
Introduction: Low cardiorespiratory fitness (CRF) is associated with a greater risk for metabolic disease. The potential for early life exercise training to overcome metabolic perturbations imparted by low intrinsic CRF remains unknown. We tested the hypothesis that early life exercise training would overcome whole-body and tissue metabolic defects imparted by low CRF.

Methods: At 26 days of age, rat low-capacity runners (LCR, *n*=20) and high-capacity runners (HCR, *n*=20) generated by artificial selection were assigned to either sedentary control (CTRL, n=10) or voluntary wheel running (VWR, *n*=10) for 6 weeks. Post-intervention, whole-body metabolic phenotyping was performed, and the respiratory function of isolated skeletal muscle and liver mitochondria assayed. Quantitative proteomics were performed on tissue samples.

Results and discussion: HCR-VWR performed 1.8-fold greater volume of wheel running than LCR-VWR (P<0.001). In LCR, VWR reduced body fat (*P*<0.001), increased total daily energy expenditure (+16 %, *P*=0.030), and enhanced glucose tolerance (*P*=0.040). Muscle mitochondrial respiratory function was unaffected by VWR in both strains, although VWR increased muscle mitochondrial protein content (both *P*<0.05). VWR enhanced the respiratory capacity of HCR hepatic mitochondria (+23 %, *P*=0.040). Proteomic analyses revealed lower capacity for fatty acid oxidation in muscle and liver of LCR-CTRL versus HCR-CTRL, which was not rescued by VWR. VWR reduced hepatic pyruvate kinase abundance in both strains (both *P*<0.013), indicating VWR may shift fuel preferences of hepatic mitochondria. These results reveal early life exercise training partially overcomes the metabolic phenotype imparted by low intrinsic CRF, although proteomic adaptations to early exercise training remain influenced by intrinsic CRF.

Cite: Sadler DG, Treas L, Ross T, Sikes JD, Britton SL, Koch LG, Børsheim E, Porter C (2023) Early life exercise training counters metabolic perturbations imparted by low parental cardiorespiratory fitness. In https://doi.org/10.26124/bec:2023-0002



A2-4

Obesity and hypoxia: from etiology to therapy.

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Author contributions: BK and SV contributed equally.

The increased prevalence of obesity worldwide has sparked interest in the potential therapeutic use of hypoxia for managing the disease, because lifelong exposure to altitude lowers the risk of obesity, while altitude sojourns are associated with weight loss. Exposure to hypoxia can lead to a negative energy balance by reducing energy expenditure through changes in resting metabolic rate and physical activity energy expenditure and by reducing appetite. On the other hand, obesity is frequently linked to sleep disorders that cause intermittent systemic hypoxia, which can result in cardiovascular and metabolic issues. Hypoxic regions within hypertrophic white adipose tissue can lead to chronic inflammation, and obesity is also a risk factor for acute mountain sickness. Despite these negative effects, intermittent hypoxia exposure has been shown to be beneficial in some cases and the potential therapeutic benefits of hypobaric or normobaric hypoxic exposure in individuals with obesity to lower body mass and improve health status are being studied. Various protocols have been developed, including actual altitude sojourns and intermittent normobaric hypoxic exposures, either at rest or in combination with physical activity. Several studies have shown benefits on body mass and cardiovascular and metabolic variables, but more research is needed before systematically integrating hypoxic exposure as part of obesity management programs. Further studies are also required to clarify the effects of hypoxia on appetite, the intestinal microbiota, and overall energy balance.

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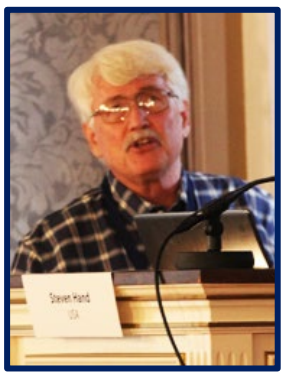
Cite: Kayser B, Verges S (2023) Obesity and hypoxia: from etiology to therapy. In https://doi.org/10.26124/bec:2023-0002



A2-5

Oxidative stress and repetitive metabolic transitions: Challenges due to lifestyle of invertebrate extremophiles.

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Funding: NSF grant IOS-1457061/IOS-1456809

Introduction: Invertebrate extremophiles experience metabolic transitions promoted by diapause, anoxia and extreme dehydration/rehydration [1-3]. For embryos of brine shrimp, *Artemia franciscana*, these reversible shifts are dramatic with respiration depressed below 1% of active states. Recovery from metabolic disruption in mammals is accompanied by generation of reactive oxygen species (ROS) that cause tissue damage during ischemia-reperfusion [4]. Yet embryos of *A. franciscana* survive frequent shifts in metabolism, which implies their mitochondria are poised to tolerate such reactivations without accumulation of damaging ROS.

Methods: Mitochondria were isolated [5] and subjected to anoxia for 30 min while controls received continuous normoxia [4]. Samples were pelleted and resuspended in oxygenated buffer containing fresh substrate, ADP and Amplex Red assay components [4]. Parallel samples included auranofin and dinitrochlorobenzene (DNCB) to inhibit thioredoxin reductase and glutathione peroxidase, respectively. Protein carbonyls, aconitase/citrate synthase activity ratios, and lipid hydroperoxides were quantified [4,6].

Results and Discussion: H_2O_2 accumulation did not increase significantly in mitochondria exposed to anoxia-reoxygenation compared to normoxic controls. By comparison, an 8-fold increase in H_2O_2 was reported for rat heart mitochondria given the same treatment [4]. As anticipated, inclusion of auranofin and DNCB statistically increased the H_2O_2 accumulation 2-3 fold in both control and experimental mitochondria. Consistent with the lack of elevated H_2O_2 after anoxia-reoxygenation, aconitase inactivation also was not detected compared to controls. Statistical increases were not observed in protein carbonyls or lipid hydroperoxides. Evidence suggests mitochondria from *A. franciscana* embryos are well protected against ROS accumulation and oxidative damage during severe metabolic transitions.

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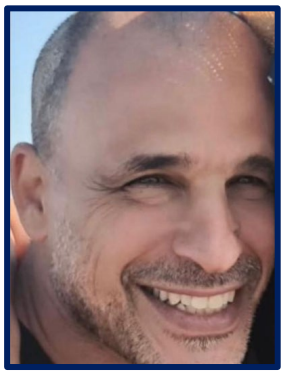
Cite: Arabie D, Hand SC (2023) Oxidative stress and repetitive metabolic transitions: Challenges due to lifestyle of invertebrate extremophiles. In https://doi.org/10.26124/bec:2023-0002

A3 - Nutrition and lifestyle in mitObesity



A3-1

M(a)TCH2-ing up metabolism and apoptosis at the MOMbrane.

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Mitochondria are highly dynamic organelles that play fundamental roles in pivotal cellular processes including energy production, metabolism, and apoptosis. We are interested in understanding how these different mitochondrial processes are coordinated to respond to cellular stress. Many of our studies are focused on a novel mitochondrial protein named mitochondrial carrier homolog 2 (MTCH2) that mediates the response of mitochondria to stress signals initiating at the plasma membrane or at the nucleus. In the TNF/Fas-death receptor pathway, MTCH2 acts as a receptor-like protein for BH3-only BID, important for cytochrome c release and for Fas-induced liver apoptosis in vivo. On the other hand, in the DNA damage pathway, MTCH2 acts as the down-stream effector of the ATM kinase/BID pathway in haematopoietic stem cells (HSCs), controlling HSC quiescence and survival via regulation of mitochondria metabolism. More recently, we revealed that MTCH2 also plays a role in regulating mitochondrial metabolism in skeletal muscle, protecting from diet-induced obesity, and a role in regulating mitochondrial fusion/elongation, which is important in driving the exit from naïve pluripotency in embryonic stem cells (ESCs). Thus, MTCH2 is an important regulator of mitochondria morphology and metabolism acting at the interface between homeostasis and apoptosis. Determining MTCH2's exact mechanism of action may lead to deciphering the mechanism by which BID, and perhaps other BCL-2 family members, regulate apoptosis.

Cite: Gross A (2023) M(a)TCH2-ing up metabolism and apoptosis at the MOMbrane. In https://doi.org/10.26124/bec:2023-0002

A3-2

Mitochondrial bioenergetics as a nexus-point for the beneficial effects of nitrate in diverse tissues.

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Mitochondria are highly dynamic organelles that play fundamental roles in pivotal cellular processes including energy production, metabolism, and apoptosis. We are interested in understanding how these different mitochondrial processes are coordinated to respond to cellular stress. Many of our studies are focused on a novel mitochondrial protein named mitochondrial carrier homolog 2 (MTCH2) that mediates the response of mitochondria to stress signals initiating at the plasma membrane or at the nucleus. In the TNF/Fas-death receptor pathway, MTCH2 acts as a receptor-like protein for BH3-only BID, important for cytochrome c release and for Fas-induced liver apoptosis in vivo. On the other hand, in the DNA damage pathway, MTCH2 acts as the down-stream effector of the ATM kinase/BID pathway in haematopoietic stem cells (HSCs), controlling HSC quiescence and survival via regulation of mitochondria metabolism. More recently, we revealed that MTCH2 also plays a role in regulating mitochondrial metabolism in skeletal muscle, protecting from diet-induced obesity, and a role in regulating mitochondrial fusion/elongation, which is important in driving the exit from naïve pluripotency in embryonic stem cells (ESCs). Thus, MTCH2 is an important regulator of mitochondria morphology and metabolism acting at the interface between homeostasis and apoptosis. Determining MTCH2's exact mechanism of action may lead to deciphering the mechanism by which BID, and perhaps other BCL-2 family members, regulate apoptosis.

Cite: Holloway GP, Petrick HL, van Loon LJC (2023) Mitochondrial bioenergetics as a nexus-point for the beneficial effects of nitrate in diverse tissues. In https://doi.org/10.26124/bec:2023-0002

A3-3 / P-5*poster* / PS-4*poster*

Impairment of cardiac function and mitochondrial energy metabolism through diet induced obesity in aging.

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Cardiac aging is a multifactorial process, which is associated with increased oxidative stress, cell death and mitochondrial abnormalities. These factors can lead to an overall impairment of cardiac function and substrate utilization [1,2]. With the increased prevalence of obesity and related comorbidities, especially coronary heart disease, it was proposed that obesity could present a condition of premature heart aging [3]. Therefore, our aim is to compare the impact of obesity and aging on heart function, as well as the cardiac energy metabolism, focusing on mitochondria.

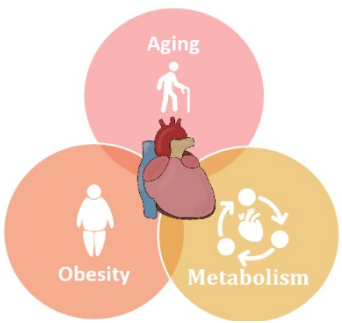

Figure 1. Interplay between aging, obesity and energy metabolism and the impact of heart.

Our experimental design of diet-induced obesity contains three different age groups (22, 76 and 106 weeks), where male C57BL/6J mice receive either a High fat/High-carb or a Standard diet for 8 weeks. After dietary intervention, mice underwent echocardiographic or metabolic treadmill analysis. Heart tissue was used for the Oroboros O2k measurement of mitochondrial bioenergetics. In further studies of cardiac energy metabolism Western blot and qPCR in heart tissue and isolated cardiomyocytes were performed.

Echocardiography revealed a decline in cardiac output in mice 76 and 106 weeks of age with a further decrease by High fat/High-carb diet. Interestingly, these effects were more pronounced in 76 weeks group. In the same group we investigated indications of an impaired mitochondrial energy metabolism, specifically associated with cardiomyocytes. Although, loss of cardiac function with age has been previously described, we demonstrate here a key role for mitochondrial energy metabolism in this loss of function.

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Cite: Owesny P, Hegemann N, Kuebler WM, Ost M, Grune T, Ott C (2023) Impairment of cardiac function and mitochondrial energy metabolism through diet induced obesity in aging. In https://doi.org/10.26124/bec:2023-0002

A3-4

Effect of telmisartan on nutritionally induced nonalcoholic steatohepatitis in mice.

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Introduction: In our previous study in a murine model of nonalcoholic steatohepatitis (NASH), we found reduced succinate-activated hepatic mitochondrial respiration and accumulation of succinate, a proinflammatory, profibrogenic, and oncogenic metabolite [1]. According to preliminary studies, telmisartan, an angiotensin II type 1 receptor blocker, positively affects insulin resistance and liver steatosis. This project aimed to investigate the effect of telmisartan on NASH in mice.

Methods: The NASH was induced in male mice fed a western-style diet (WD) for 36 weeks. During the last 6 weeks of the experiments, mice were administered daily telmisartan (oral gavage, 5 mg/kg b.w./day). Liver and epididymal fat histological changes were evaluated (Hematoxylin-eosin, Sirius red). Body parameters, plasma liver profile (VetScan), hepatic triglycerides, cholesterol, and the expression of selected proteins (WB/ELISA) and genes (qRT-PCR) were assessed. Mitochondrial respiration of liver homogenates was measured by high-resolution respirometry (OROBOROS Oxygraph-2k). Using Reporter Gene assay, telmisartan's activation of nuclear receptors was evaluated on HepG2 cells.

Results and discussion: Administration of telmisartan to mice fed a WD reduced absolute and relative liver weight and visceral adipose tissue weight, activities of ALT and AST, liver steatosis, and inflammation grade. These effects were accompanied by a significant increase in succinate-activated respiration in the ET state and the activity of succinate dehydrogenase. We confirmed that telmisartan is a PPAR-γ partial agonist and described the activating effect of telmisartan on the CAR receptor for the first time. Telmisartan appears to be a promising safety drug for treating NASH that affects metabolism at multiple levels.

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Keywords: NASH; Telmisartan; Succinate dehydrogenase; PPAR-γ; CAR

Cite: Stankova P, Peterova E, Dusek J, Elkalaf M, Cervinkova Z, Kucera O (2023) Effect of telmisartan on nutritionally induced nonalcoholic steatohepatitis in mice. In https://doi.org/10.26124/bec:2023-0002

A4 - Adipose tissue and fatty acid oxidation

A4-1

Major site of non-shivering thermogenesis: brown fat or skeletal muscle?

Kopecky J[1], Zouhar Petr[1], Janovska P[1], Bardova K[1], Otahal J[2], Vrbacky M[3], Mracek T[3], Adamcova K[1], Lenkova L[1], Funda J[1], Cajka T[4], Drahota Z[3], Stanic S[1], Rustan AC[5], Horakova O[1], Houstek J[3], Rosmeissl M[1]

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Funding: Supported by project no. LX22NPO5104 - funded by the European Union Next Generation EU

Heat production is essential for maintaining a constant body temperature, and is an important component of energy balance. Well-described mechanisms involved in heat generation include shivering of muscle and non-shivering thermogenesis (NST) in brown adipose tissue (BAT). Thermogenesis in BAT, which is dependent on the presence of the mitochondrial protein UCP1, is the focus of interest for its potential use in the treatment of obesity. Other mechanisms of NST and their significance are relatively poorly understood. We have shown [1] that obesity-resistant A/J mice acclimated to cold failed to increase adrenergically stimulated NST in BAT and activated NST in skeletal muscle instead. Heat generation in muscle involved increased calcium ion cycling in the endoplasmic reticulum associated with higher mitochondrial oxidative activity. The involvement of different thermogenic mechanisms could be related to the different susceptibility to obesity. The resistance of A/J mice to obesity may result, at least in part, from their ability to activate NST in muscle. Such mechanism may provide a more promising way to treat obesity than potential therapies based on increasing thermogenesis in BAT, as the capacity of skeletal muscle of adult human to burn fat energy stores is several fold greater than in BAT. Thus, only a relatively small increase in thermogenesis in muscle could significantly reduce adipose tissue deposition. How to achieve such an increase is a challenge for further research.

1. Janovska P et al (2023) Impairment of adrenergically-regulated thermogenesis in brown fat of obesity-resistant mice is compensated by non-shivering thermogenesis in skeletal muscle. https://doi.org/10.1016/j.molmet.2023.101683

Keywords: Non-shivering thermogenesis; Calcium cycling; cold acclimation

Cite: Kopecky J, Zouhar P, Janovska P, Bardova K, Otahal J, Vrbacky M, Mracek T, Adamcova K, Lenkova L, Funda J, Cajka T, Drahota Z, Stanic S, Rustan AC, Horakova O, Houstek J, Rosmeissl M (2023) Major site of non-shivering thermogenesis: brown fat or skeletal muscle? In https://doi.org/10.26124/bec:2023-0002

A4-2 / P-1*poster*

Dynamic complexomic analysis reveals a role for Von Willebrand Domain-containing Protein 8 (Vwa8) in mitochondrial morphology and substrate preference.

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Funding: The project is supported by the Grant Agency of the Czech Republic (23-06027S)

Introduction: Obesity is turning into a worldwide pandemic, with most patients also affected by other comorbidities such as type 2 diabetes, hypertension, or cardiovascular disease. With mitochondria being a major site for fatty acid oxidation, they represent an important target for obesity treatment. Mitochondria are dynamic organelles, and their morphology influences both the organization of membrane protein complexes as well as mitochondrial substrate preference1.

Methods: By combining 2-dimension blue native gel electrophoresis with proteomics and bioinformatics in heart mitochondria undergoing membrane remodelling we identified a strong correlation between the key cristae biogenesis protein Opa1 and Vwa8, a putative AAA+ ATPase with a dynein conformation. In order to study the role of Vwa8 protein in mitochondrial physiology, we developed the HEK293 Vwa8 knock-out cell line and Vwa8 KO mice.

Results and discussion: Vwa8 protein localized to the mitochondrial intermembrane space where it formed discrete spots. Deletion of Vwa8 led to an increase in mitochondrial respiration on fatty acids but not on glucose or glutamine. The Vwa8 KO mice showed decreased resting energy requirements as well as higher heat production, indicating a stronger preference for lipid oxidation. Moreover, the subcutaneous adipose tissue of Vwa8 KO mice showed increased markers of browning such as an increase in mitochondria content and lipid droplet multilocularity. The Vwa8 KO mice remained more insulin sensitive and with higher lean mass proportion upon a high-fat diet. In conclusion, Vwa8 affects mitochondrial substrate preference, induces browning of subcutaneous adipose tissue and represents a new target for obesity treatment.

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Cite: Alan L, Calvo E, Enríquez JA, Soriano ME, Bean C, Mracek T, Scorrano L (2023) Dynamic complexomic analysis reveals a role for Von Willebrand Domain-containing Protein 8 (Vwa8) in mitochondrial morphology and substrate preference. In https://doi.org/10.26124/bec:2023-0002

A4-3

Human white adipose tissue mitochondrial respiration: effect of body composition.

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Heat production is essential for maintaining a constant body temperature, and is an important component of energy balance. Well-described mechanisms involved in heat generation include shivering of muscle and non-shivering thermogenesis (NST) in brown adipose tissue (BAT). Thermogenesis in BAT, which is dependent on the presence of the mitochondrial protein UCP1, is the focus of interest for its potential use in the treatment of obesity. Other mechanisms of NST and their significance are relatively poorly understood. We have shown [1] that obesity-resistant A/J mice acclimated to cold failed to increase adrenergically stimulated NST in BAT and activated NST in skeletal muscle instead. Heat generation in muscle involved increased calcium ion cycling in the endoplasmic reticulum associated with higher mitochondrial oxidative activity. The involvement of different thermogenic mechanisms could be related to the different susceptibility to obesity. The resistance of A/J mice to obesity may result, at least in part, from their ability to activate NST in muscle. Such mechanism may provide a more promising way to treat obesity than potential therapies based on increasing thermogenesis in BAT, as the capacity of skeletal muscle of adult human to burn fat energy stores is several fold greater than in BAT. Thus, only a relatively small increase in thermogenesis in muscle could significantly reduce adipose tissue deposition. How to achieve such an increase is a challenge for further research.

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Keywords: Non-shivering thermogenesis; Calcium cycling; cold acclimation

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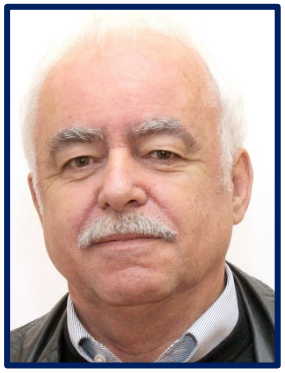

A4-4

A view on brain's problem with fatty acid burning.

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Distinct hypothalamic neurons sense blood levels of fatty acids (FA) and, thereby regulate caloric intake. Astrocytes have some capacity of β-oxidation. But, there are ongoing discussions on this question: Do neurons generally burn FA for energy generation?
Respiration and membrane potential of mitochondria of rat brain (RBM) and, for comparison, of liver (RLM) were measured without and with octanoate (l-octanoylcarnitine). In addition, H_2O_2 generation was measured with Amplex Red.
In line with previous studies, we found no evidence for a noteworthy β-oxidation of FA by RBM. This fits with theoretical considerations (1) and values obtained for capacities of enzymes of β-oxidation (2). But, these results contradict those of a previous study (3), reporting that RBM incubated with mixtures of FA (carnitine derivatives) plus other substrates (e.g. succinate) show substantial β-oxidation.
What could be possible reasons for disregarding FA as energy substrates by neurons? These are mainly: (a) Harmful activities of non-esterified long-chain FA on mitochondria. (b) Burning of FA costs more oxygen than glucose burning with respect to the energy yield. (c) FA oxidation by mitochondria is associated with more sites of superoxide generation. (d) Neurons are equipped with poor antioxidative capacity. In conclusion, burning of FA would expose neurons to intolerably high oxidative stress.

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Keywords: Non-shivering thermogenesis; Calcium cycling; cold acclimation

Cite: Schönfeld P, Reiser G (2023) A view on brain's problem with fatty acid burning. In https://doi.org/10.26124/bec:2023-0002

B1 - Fatty acid oxidation

B1-1

Delta-6 desaturase inhibition reverses aberrant cardiolipin remodeling and mitochondrial dysfunction in the obese mouse heart.

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Cardiolipin (CL) is a tetra-acyl mitochondrial phospholipid that supports the optimal function of several mitochondrial membrane proteins and processes. In the healthy mammalian heart, the majority of CL species contain four linoleate acyl chains (L4CL). A marked depletion of cardiac L4CL is paralleled by an increase in CL species containing docosahexaenoic acid (DHA) in hyperphagic obese (*Lep^{ob}*; OB) mice despite no change in dietary fat composition [1], but the mechanisms and functional relevance of these changes are unclear. We hypothesized that this shift in CL composition results from increased activity of delta-6 desaturase (D6D), the rate limiting enzyme in the biosynthesis of DHA and conversion of linoleate into highly unsaturated ω-6 fatty acids, by altering the distribution of fatty acids available for CL remodeling. To test this, we administered the selective D6D inhibitor SC-26196 (100 mg/kg/d in chow) to 4-5 month-old OB or lean (C57Bl/6) mice for 4 weeks. As hypothesized, D6D inhibition reversed obesity-related changes in cardiac CL composition, restoring L4CL and DHA-enriched CL species to within 5 % of levels in lean mice, which paralleled reciprocal shifts in the linoleate and DHA levels of total myocardial phospholipids. Obesity-related decreases in cardiac mitochondrial respiratory control by ADP (with NS pathway substrates) and greater mitochondrial H_2O_2 release during both LEAK and OXPHOS states were also abolished by D6D inhibition. These results corroborate accumulating evidence that cardiac CL composition is strongly influenced by the membrane fatty acids available for CL remodeling [2-3], and may impact the bioenergetic efficiency of mitochondrial respiration.

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Keywords: cardiolipin; polyunsatruated fatty acids; membrane composition; obesity; uncoupling

Cite: Chicco AJ, Le Catherine H, Mulligan Christopher M, Whitcomb LA, Evans AE, Routh Melissa A, Sparanga Genevieve C (2022) Delta-6 desaturase inhibition reverses aberrant cardiolipin remodeling and mitochondrial dysfunction in the obese mouse heart. In https://doi.org/10.26124/bec:2023-0002

B1-2

Novel mitochondrial respiration protocols reveal organ-specific reliance on ketone body metabolism in mice.

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Introduction: Ketone bodies (KB) are important substrates for the heart, particularly during heart failure [1], kidney [2], brain, skeletal muscle, and other organs [3]. Despite their significant role in health and disease [4], very limited research is available investigating KB-linked ATP production in mammalian tissues [5]; moreover, no optimized protocols exist to assess the interplay of key enzymes involved in ketolysis and their respective contribution to OXPHOS capacity.

Methods: β-hydroxybutyrate (HBA)- and acetoacetate (ACA)-linked mitochondrial respiration was assessed in the heart left ventricle (LV), kidney, liver, brain, and soleus of ~18-24-week-old C57BL/6J female mice (n=6-8). A novel protocol combining KB-linked and complex I (CI)+CII-linked mitochondrial respiration was also devised.

Results and discussion: The K_m for HBA was similar (~1 mM) in all tested organs. However, maximal HBA-linked respiration was different between organs ($p<0.001$), i.e., greater in the LV and liver (~32 pmol $O_2 \cdot s^{-1} \cdot mg^{-1}$), and lowest in the brain (5.2 pmol $O_2 \cdot s^{-1} \cdot mg^{-1}$). This protocol allows to determine β-hydroxybutyrate dehydrogenase activity in the liver. The Km for ACA and maximal ACA-linked respiration were greater in the kidney compared to the other tested organs (all $p<0.050$). Our novel KB+CI+CII combined respiration protocol indicated that the KB contribution to maximal respiration is 2- to 4-fold greater in the kidney (37.4 %) compared to all other organs (all $p<0.050$), confirming the kidney's reliance on KB metabolism [2]. Taken together, our novel protocols demonstrate an organ-specific response of mitochondrial respiration to different KBs.

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Keywords: ketone body; mitochondrial respiration; ketolysis; mitochondria; high-resolution respirometry

Cite: Zweck E, Piel S, Chadt A, Al-Hasani H, Kelm M, Szendrödi J, Roden M, Granata C (2023) Novel mitochondrial respiration protocols reveal organ-specific reliance on ketone body metabolism in mice. In https://doi.org/10.26124/bec:2023-0002

B1-3

Exploring the influence of the lipid environment on mitochondrial membrane structure and function.

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Mitochondrial membranes are dynamic organelles that contain a variety of lipids including the structurally unique, dimeric phospholipid class of cardiolipins. These lipids represent the substrate in which larger protein complexes are embedded, which in turn play an essential role in maintaining mitochondrial functionality. A pronounced tissue-specificity of mitochondrial membranes has been described, defined by the molecular composition of the fatty acyl side chains of the complex lipids from which they are formed. We recently could demonstrate that the lipid environment plays a pivotal role in controlling the side chain specificity of mitochondrial cardiolipins. Alterations in fatty acid availability or fatty acid metabolism, such as those occurring in response to nutrition and/or in a variety of human pathologies, can thus affect biochemical processes in and along mitochondrial membranes. This interplay between lipids and protein functions leads to a potentially strong influence on vital cellular processes such as mitochondrial respiration, the generation of reactive oxygen species, as well as signaling. However, not every lipid change that influences mitochondrial processes on a biochemical level must automatically have physiological effects in living cells. Compensatory mechanisms are at work that increase mitochondrial resilience to fluctuations in fatty acid metabolism, as long as these are within certain bounds. Thus, gaining an in-depth understanding of mitochondria requires an assessment of how their functions are regulated by the distinct lipid environments as found in different tissues and human pathologies.

Cite: Keller MA (2023) Exploring the influence of the lipid environment on mitochondrial membrane structure and function. In https://doi.org/10.26124/bec:2023-0002

B1-4

The Janus-faced nature of HSD10 in cardiolipin biosynthesis and mitochondrial function.

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Introduction: Human 17β-Hydroxysteroid dehydrogenase 10 (HSD10) is a crucial enzyme located in mitochondria that participates in isoleucine catabolism and is part of the mitochondrial RNase P complex [1,2]. Mutations in the *HSD10B17* gene have been linked to HSD10 disease, which causes progressive cardiomyopathy and cognitive function loss [3]. Recently, HSD10 has been reported to possess a phospholipase C-like activity towards cardiolipins, which are essential mitochondrial membrane lipids involved in various processes such as super-complex assembly, cristae formation, and apoptotic signaling cascades [4]. The transacylase tafazzin is remodeling cardiolipin side chains, and its deficiency leads to high levels of monolyso-cardiolipins and abnormal cardiolipin patterns [5].

Methods: To explore the role of HSD10 in cardiolipin homeostasis, we carried out a comprehensive analysis of cardiolipin profiles in different cellular contexts by means of LC-MS/MS [6]: We investigated the impact of HSD10 knockdown in wild-type cells, in a tafazzin-deficient background, and in fibroblasts derived from HSD10-deficient patients. Additionally, by supplementation with fatty acids such as linoleic acid and palmitic acid we simulated different lipid environments.

Results and Discussion: We found no evidence for the enzyme function of HSD10 to be involved in cardiolipin homeostasis in all conditions examined [6]. Thus, its previously reported cardiolipin cleaving function is likely to be regarded as an *in vitro* artefact. However, the HSD10's structural importance in the mitochondrial RNase P complex underscores its essential role in cellular function [7]. We show that the enzyme has evolved with significant evolutionary constraints to maintain this structure, possibly at the expense of achieving a high degree of substrate specificity and reaction rates [6].

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Cite: Wohlfarter Y, Eidelpes R, Yu RD, Sailer S, Koch J, Karall D, Scholl-Bürgi S, Amberger A, Hillen HS, Zschocke J, Keller MA (2023) The Janus-faced nature of HSD10 in cardiolipin biosynthesis and mitochondrial function. In https://doi.org/10.26124/bec:2023-0002

B1-5

Polyunsaturated fatty acid metabolism contributes to age-related impairment of cardiac mitochondrial calcium tolerance.

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Myocardial ischemia causes pathological increases in cardiomyocyte mitochondrial calcium (Ca^{++}), which trigger a series of events that contribute to cell death and myocardial necrosis. Previous studies in our lab and others indicate that metabolites of phosholipid-derived arachidonic acid (AA), an omega-6 polyunsaturated fatty acid (PUFA), contribute to mitochondrial permeability transition pore (mPTP) opening in response to Ca^{++} overload, leading to mitochondrial swelling, rupture, and release of reactive oxygen species (ROS) [1,2]. We hypothesized that age-related increases in these parameters result in part from greater mitochondrial production of AA from its abundant membrane PUFA precursor linoleic acid (LA) in response to Ca^{++} overload. To test this hypothesis, we evaluated effects of 50-400 μM Ca^{++} on O_2 consumption, ROS release and mPTP opening in cardiac mitochondria isolated from young (3 mo) and aged (24 mo) BALB/c mice in the presence or absence of an inhibitor of delta-6 desaturase (D6D), the rate-limiting enzyme in AA biosynthesis from LA. Results demonstrate that cardiac mitochondria from old mice release more ROS during oxidative phosphorylation and undergo more mPTP opening in response to Ca^{++} overload than mitochondria from young mice. D6D inhibition significantly attenuates these responses in both young and old mitochondria, but had greater impacts on old, largely abolishing the effect of aging on both ROS release and mPTP opening. Similar attenuation of mPTP opening was seen following inhibition of lipoxygenase enzymes (Baicalein), consistent with the hypothesized links between mitochondrial AA synthesis, eicosanoid production and mPTP in regulating responses of cardiac mitochondria to Ca^{++} overload.

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Keywords: mitochondrial calcium overload; permeability transition; polyunsaturated fatty acids

Cite: Whitcomb LA, Li Puma LC, Zilhaver PT, Izon CS, Chicco AJ (2023) Polyunsaturated fatty acid metabolism contributes to age-related impairment of cardiac mitochondrial calcium tolerance. In https://doi.org/10.26124/bec:2023-0002

B1-6

Electron transfer from beta-oxidation and TCA cycle and impact of OXPHOS coupling on NADH and coenzyme Q redox states.

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Author contributions: The experiments were designed by LHDC, CDoerrier, and EG; performed by LHDC, CDonnelly, CDoerrier, and TK; analyzed by LHDC, CDoerrier, and CDonnely. The abstract was prepared by LHDC and EG.

Introduction: Multiple mt-matrix dehydrogenases reduce NAD^+ to $NADH+H^+$, which is oxidized by CI (N-junction). Convergent electron flow through several mt-Complexes (CI, CII, CETF, etc) reduces electron transfer system (ETS)-reactive ubiquinone (UQ) to ubiquinol (UQH_2), which is oxidized by CIII (Q-junction). The aim of our study was to analyze the relationships between the N- and Q-redox states and electron transfer rates.
Methods: Respiration and N- or Q-redox fractions were measured simultaneously with the Oroboros NextGen-O2k. Multiple protocols were used with sequential titrations of substrates, inhibitors, and uncouplers [1, 2]: N-pool with pyruvate&glutamate&malate, mouse liver mitochondria; Q-pool with succinate&rotenone, octanoylcarnitine&malate or palmitoylcarnitine&malate, permeabilized HEK 293T. After substrates, ADP, CCCP and antimycin A were titrated.
Results and discussion: Varying energy supply upstream of the Q-junction by using combinations of substrates and ETS-inhibitors in the noncoupled state, the Q-pool became reduced in direct proportion to respiration. In contrast, varying downstream energy demand in the absence of ADP (LEAK), by ADP activation (OXPHOS), and by uncoupler titrations (ET capacity), the N- and Q-pools were reduced in indirect proportion to respiration. The opposite correlations between redox state and respiratory rate were explained by the contrasting effects of varying electron push from different fuel substrates of the ETS or electron pull modulated by coupling and corresponding energy demand. Special emphasis on the interaction between fatty acid oxidation, CI, and CII – all involving separate electron entries into the Q-junction [3] – is particularly relevant in the context of obesity and bioenergetics studies.

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Keywords: coenzyme Q; NADH; electron transfer system; OXPHOS; respirometry

Cite: Cardoso LHD, Donnelly C, Komlódi T, Doerrier C, Gnaiger E (2023) Electron transfer from beta-oxidation and TCA cycle and impact of OXPHOS coupling on NADH and coenzyme Q redox states. In https://doi.org/10.26124/bec:2023-0002

B2 - Type 2 Diabetes

B2-1

Skeletal muscle mitochondrial dynamics in obesity and type 2 diabetes.

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Obesity mediates the onset of lipid-induced insulin resistance, increasing the risk of type 2 diabetes. The inability of mitochondria to maintain core functions such as ATP synthesis, redox homeostasis, organelle quality control, and/or preservation of inheritance is proposed to link obesity-related insulin resistance to the onset and progression of type 2 diabetes, yet evidence remains elusive. To parse out the contributions of obesity versus peripheral insulin resistance, healthy weight adults were infused with an intralipid solution followed by evaluation of skeletal muscle mitochondrial function. The lipid infusion reduced insulin sensitivity and dampened mitochondrial membrane potential while increasing markers of mitochondrial fission and increasing the presence of autophagic vesicles, consistent with activation of the quality control machinery. Despite this, respiratory capacity and mitochondrial content were unaltered. From these studies, we concluded that activation of mitochondrial fission and quality control were early events in the onset of insulin resistance to defend cellular energy homeostasis. Subsequently, we conducted a cross-sectional analysis of individuals across the insulin sensitivity spectrum. We observed that markers of fission and quality control were markedly altered in patients with obesity and type 2 diabetes relative to obesity alone and healthy weight despite no apparent differences in respiratory capacity. Mitochondrial volume was incrementally lower in patients with obesity and type 2 diabetes relative to healthy weight. Collectively, we conclude that preservation of bioenergetic function in patients with obesity and type 2 diabetes is achieved by chronic activation of the quality control machinery which occurs at the expense of mitochondrial volume.

Cite: Axelrod CL, Kirwan JP (2023) Skeletal muscle mitochondrial dynamics in obesity and type 2 diabetes. In https://doi.org/10.26124/bec:2023-0002

B2-2

Remission of obesity and insulin resistance. Is that enough?

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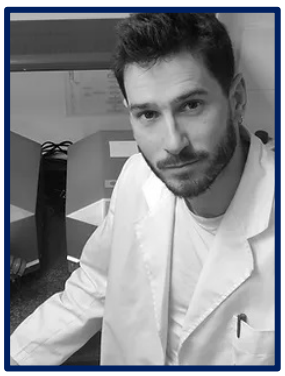
Effective preventive measures must be taken to address the obesity pandemic and reduce the risk of noncommunicable diseases. This includes health promotion initiatives such as promoting healthy lifestyles and increasing access to healthy foods and physical activity programs. Education is also key to ensuring that individuals understand the importance of staying fit and healthy.

Our "LiMa" (Lifestyle Matters) project uses a multidisciplinary approach aiming to examine the systemic and tissue-specific effects of obesity-related type 2 diabetes. This integrative approach has been in operation for more than a decade, providing novel insights about the underlying role played by each tissue in obesity-related pathophysiology and its progression. Significantly, we have found that obesity leaves a lasting 'metabolic fingerprint' in adipose tissue even after a successful restoration of a healthy state through a diet and exercise intervention. This metabolic memory was observed particularly in visceral fat, where mitochondrial performance was compromised as suggested by permanent alterations in mitochondrial transcripts and proteins, a functional decay, and a significant loss of mtDNA (1). Interestingly, no major impact was evidenced on the subcutaneous depot, which implies a further step towards shedding light on the complex relationship between adipose tissue and metabolic disease. We urge to validate the translational value of our preclinical model, since it could have an evident economic impact. It would enable earlier diagnosis and tailored interventions, reducing costs, improving patient outcomes, and providing new biomarkers to assess treatment success. This could be a game-changer for obesity and associated diseases.

1. Gonzalez-Franquesa et al (2022) Remission of obesity and insulin resistance is not sufficient to restore mitochondrial homeostasis in visceral adipose tissue. https://doi:10.1016/j.redox.2022.102353

Keywords: obesity; nutritional and exercise intervention; multidisciplinary approach; adipose tissue depots; mitochondrial dysfunction

Cite: Garcia-Roves PM (2023) Remission of obesity and insulin resistance. Is that enough? In https://doi.org/10.26124/bec:2023-0002



B2-3

Impact of obesity on white adipose tissue plasticity: addressing depot-specific responses.

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Chronic overfeeding has a profound metabolic impact on multiple tissues. Consequently, unraveling the differential adaptations in each of them is fundamental to understand the progression of obesity-related comorbidities. In our laboratory we have tackled this issue in a model of obesity and weight loss induced by a combined nutritional and exercise intervention. This model has enabled us to identify visceral adipose tissue as the most vulnerable organ to such stress, not only by the magnitude of changes observed in the obese state but most importantly, because of the permanent alterations we observe even after the restoration of adequate weight and metabolic health. Whether this fingerprint is a distinctive trait of the visceral fat or it is affecting other depots is still unsolved, although the recognized developmental, morphological as well as functional differences among fat depots might drive a differential response.

To this end, we aim to explore the subcutaneous adipose tissue behavior in our model, characterizing those significant indicators of vulnerability already identified in the visceral depot. These include linear regression models to correlate tissue mass and body weight, histological and immunohistochemical analysis to characterize the morphological remodeling of the tissue, the assessment of transcriptional changes in both tissues, as well as the impact on mitochondria through the evaluation of OXPHOS capacities and the quantification of mitochondrial DNA.

This comparative analysis suggests that unlike visceral fat, the detrimental impact of chronic overfeeding is blunted in subcutaneous adipose tissue, with no apparent consequences on its metabolic plasticity. Among the important points to consider, these findings could represent a relevant concern for the study of obesity-related pathophysiology in humans since, thus far, most longitudinal studies exploring adipose tissue responses to weight fluctuations have been addressed in subcutaneous biopsies due to ethical constrains.

Cite: Gama-Perez P (2023) Impact of obesity on white adipose tissue plasticity: addressing depot-specific responses. In https://doi.org/10.26124/bec:2023-0002

B2-4

Respiratory capacity of skeletal muscle and peripheral blood mononuclear cells of male and female individuals with type 2 diabetes.

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We wish to thank all patients and staff that participated in or helped with this study.

Introduction: Skeletal muscle mitochondrial function is altered in insulin resistant states. Its assessment, however, requires invasive muscle biopsies to obtain viable tissue for functional mitochondrial analysis. Blood cell-based bioenergetics potentially reflects systemic mitochondrial function. Here, we characterized respiratory capacity of skeletal muscle mitochondria and peripheral blood mononuclear cells (PBMCs) from patients with type 2 diabetes and assessed whether the latter reflect muscle mitochondrial respirometric measures.
Methods: For that purpose, 20 patients with type 2 diabetes (30 % female, 57±9 years, BMI 28±4 kg/m^2) participated in this study. We obtained muscle biopsies from the M. vastus lateralis and venous blood samples to isolate PBMCs. High-resolution respirometry was performed in duplicate to assess mitochondrial respiration from permeabilized muscle fibers and PBMCs using an established SUIT-protocol.
Results and Discussion: Combined NADH-linked (N) electron transfer and succinate-linked (S) OXPHOS capacity was 59.4±13.0 pmol/(s*mg) for muscle and 16.6±5.3 pmol/(s*10^6 cells) for PBMCs. NS-OXPHOS capacity was not different between females and males for muscle (66.5±9.5 vs 56.3±13.0 pmol/(s*mg), p=0.10) or PBMCs (19.5±5.3 vs 15.3±5.0 pmol/(s*106), p=0.10), respectively. While PBMC mitochondrial function was not correlated with skeletal muscle respiratory function across several respiratory states (all p>0.05), muscle NS-OXPHOS capacity correlated negatively with diabetes disease duration (r=-0.50, p=0.02). These results suggest that there are no sex-specific differences with regard to muscle and PBMC mitochondrial function in individuals with type 2 diabetes. While bioenergetic phenotypes in PBMCs do not reflect muscle mitochondrial function in this cohort, diabetes disease duration negatively associates with muscle mitochondrial function.

Keywords: mitochondrial function; type 2 diabetes; blood cells; skeletal muscle

Cite: Büscher FM, Schrage I, Bohmeier M, Kaiser-Stolz C, Kramme J, Rittweger J, Pesta D (2023) Respiratory capacity of skeletal muscle and peripheral blood mononuclear cells of male and female individuals with type 2 diabetes. In https://doi.org/10.26124/bec:2023-0002

B2-5

Macronutrients energy metabolism and obesity.

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Obesity is associated with insulin resistance, which is the cause of subsequent metabolic complications, including increased morbidity. Despite several decades of efforts to prevent the growth of obesity, its incidence continues to increase. We do not even know what ratio of nutrients is optimal for preventing obesity and insulin resistance, and the optimal ratio of carbohydrates to lipids has not been proven.
Some studies, including calorimetric measurements performed at our workplace, have shown that the oxidation of individual substrates does not correspond to their ratio in the given diet. However, this apparent paradox makes sense because food intake in humans is intermittent and usually does not occur during increased or even maximal physical activity. Energy and metabolic substrates are stored in the body during intake and are subsequently mobilized during periods of starvation and physical activity. As a result, the human body is never in true energy balance; storage and subsequent mobilization of energy is necessary for a functioning organism.
In addition, carbohydrates, fats and proteins are not only a source of energy, but also important substances with many functions [1]. After ingestion of a mixed meal, carbohydrates (especially glucose) are used for both oxidation and non-oxidative pathways (antioxidant, anaplerotic, cataplerotic processes). Only a relatively small fraction of glucose is a source for new lipid synthesis. Ingested fats are preferentially stored in adipose tissue and does not influence carbohydrate oxidation. The lack of glucose can explain more insulin resistance in whole organism than Randle cycle measured in vitro conditions [2].

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Keywords: Obesity; insulin resistance; glucose intake

Cite: Sobotka L, Sobotka O (2023) Macronutrients energy metabolism and obesity. In https://doi.org/10.26124/bec:2023-0002

B3 - Respiratory complexes

B3-1

Inhibition of ATP synthase hydrolytic activity restores cellular energy homeostasis in conditions of impaired respiration.

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B3-2

Effect of succinate dehydrogenase deficiency on mitochondrial function.

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Introduction: Succinate dehydrogenase (SDH) connects the TCA cycle by oxidizing succinate to fumarate and the respiratory chain by transferring electrons to ubiquinone. Mutations in SDH subunits have been associated with tumorigenesis as well as mitochondrial diseases. In this project, we focused on the flavoprotein subunit A of SDH (SDHA) which is primarily associated with inherited mitochondrial disease [1] and investigated the consequences of this subunit loss in HEK cells (SDHA KO).
Methods: We performed structural and functional characterizations of the SDHA KO model involving protein electrophoresis to study OXPHOS complexes and subcomplexes, label-free quantification of protein levels, measurement of cellular respiration using high-resolution respirometry and determination of NAD^+/NADH levels.
Results and discussion: Together with SDHA, other SDH subunits were downregulated as well, leading to the absence of assembled SDH. Moreover, a secondary downregulation of the majority of complex I and IV subunits was observed. The cellular respiratory capacity was severely decreased in the model, with SDH-dependent respiration completely abolished and complex I-dependent respiration attenuated reflecting the downregulation of respiratory chain complexes in general. Finally, the NAD^+/NADH ratio was increased in SDHA KO compared to the controls, indicating complex rearrangement of the TCA. The SDHA KO cells thus represent a suitable model to study metabolic rewiring and the effect of pathogenic SDHA mutations.

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B4 - Diagnostic approaches

B4-1

Combined metabolite and lipid fingerprinting of blood serum reveals biomarker candidates of altered mitochondrial bioenergetics in peripheral blood mononuclear cells of female patients with depression.

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Major depressive disorder (MDD) is characterized by impairments in mental and physical performance. Despite intensified hypothesis-driven research, applicable biomarkers for MDD are missing. Research showed that MD is associated with impaired mitochondrial bioenergetic functioning in peripheral blood mononuclear cells (PBMC). However, deeper biomolecular insights into bioenergetic and associated biochemical changes in blood underlying the pathophysiology of MDD are necessary to identify new biomarker candidates. Here, the biochemistry of PBMC-surrounding blood was analyzed using a hypothesis-free biomarker identification approach combining metabolite and lipid fingerprinting. Biochemical fingerprints of serum were compared between female individuals (N = 44) with and without MDD. Serum extracts were separated by liquid chromatography and detected with time-of-flight mass spectrometry. The data was analyzed by multiple group comparisons and correlations, as well as two multivariate classification procedures. Next, our previously identified alterations in mitochondrial bioenergetics in PBMC were co-considered as an outcome for our biomarker identification approach. Consequently, the most promising compound was tested for correlation with mitochondrial respiration. Nine biomarker candidates discriminated between MDD and non-MDD with high predictive accuracy (90.9 %). The detected compounds are involved in lipid and amino acid-metabolism. *9,10-dihydroxy-octadenedioic acid* was revealed as a robust biomarker candidate with a predictive accuracy of 81.8 % and significant mean positive correlation with parameters of mitochondrial respiration ($r = 0.31$-0.48, $p<0.01$). Our fingerprinting results highlight novel biomarker candidates and associated pathways for MDD research. The unraveled biochemical pathways indicate a modulated association of MDD with inflammation, oxidative stress, and mitochondrial bioenergetics. The biomarker candidates have to be replicated in independent cohorts of all ages & sexes.

Cite: Karabatsiakis A, Manrique JS, Stoll T, Hennessy T, Hill MM, Dietrich DE (2023) Combined metabolite and lipid fingerprinting of blood serum reveals biomarker candidates of altered mitochondrial bioenergetics in peripheral blood mononuclear cells of female patients with depression. In https://doi.org/10.26124/bec:2023-0002

B4-2

Serum acylcarnitines profile for diagnosis, prognosis and monitoring therapeutic intervention in equine atypical myopathy.

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Acer pseudoplatanus contains toxins responsible for poisoning in various species [1], including humans [2]. In equids, this intoxication induces an often fatal rhabdomyolysis syndrome known as atypical myopathy (AM); [3]. Blood analysis reveals a severe metabolic disturbance characterised by hyperglycaemia, high triglycerides, and lipid intermediates [4].

Toxins inhibit several steps of the fatty acid β-oxidation cycle that leads to the accumulation of acyl-CoAs in the mitochondria, which are scavenged into acylcarnitines. Also, competitive inhibition of long-chain fatty acid transport into mitochondria results into their accumulation conjugated with carnitine. In addition, inhibition of the catabolic pathway of branched-chain amino acids, particularly leucine, leads to the accumulation of branched acylcarnitines [2; 5].

Acylcarnitines in tissues may explain parts of the pathophysiological process, such as the cardiac myopathy occurring in AM. Also, acylcarnitines accumulation could promote muscle insulin resistance and contribute to the hyperglycaemia observed in AM horses [4]. The disease also results from severe impairment of mitochondrial bioenergetics [6; 7]. In AM, the serum acylcarnitines profile contributes to the diagnosis of the disease, its prognosis and is also a valuable aid in monitoring ongoing metabolic disturbances. In search of new therapeutic approaches for this environmental intoxication, we are currently designing toxicity assays with cultured cells [7] and zebrafish larvae. These models will help us to test different drugs by exploring their ability to prevent metabolic disturbances as indicated by the acylcarnitines profile. Indeed, in both models, the alteration of the acylcarnitine profile can be followed.

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Keywords: environment; myopathy; toxins; β-oxidation; acylcarnitines

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B4-3

Bioenergetics health index and parameters of mitochondrial respiration in relatively healthy individuals. Insight in mitochondrial respiration for diagnosis and future targeted treatments.

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The impairment of mitochondrial respiration, observed in neurodegenerative and cardiovascular disease, diabetes, cancer and migraine headaches, has emerged as a biomarker of mitochondrial dysfunctions [1]. Newer research are also trying to link conditions such as chronic fatigue, depression and other behaviour/mood disorders with mitochondrial malfunctioning [2].
In our study, we examined 88 (relatively) healthy volunteers, ages from 23 to 68, from which 36 individuals were taking some sort of medication (such as for asthma, high blood pressure, mood disorders), but they considered themselves fit and healthy. Volunteers were ask to follow their normal routines day prior the test. The blood was drawn 3 h before PBMCs isolation, followed by immediate Seahorse XF Cell Mito Stress Test (Agilent) on SeahorseXF96e instrument (Agilent). Our analysis consisted of carefully examining parameters of mitochondrial respiration: basal respiration, ATP-linked respiration, reserve capacity, maximal respiration, proton leak, non-mitochondrial respiration as well as bioenergetics health index (BHI) [3].
We observed difference between people who took some sort of medication for chronic but manageable comorbidities and completely healthy individuals. There was significant difference between BHI, reserve capacity, coupling efficiency and proton leak. We also observed that people who had regular sport activities (in the healthy group without any medication) seem to have lower proton leak. This difference was not significant but points out to the lifestyle impact to mitochondria [4].

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B4-4

Substrate-uncoupler-inhibitor titration protocol for analyzing carbohydrate and fatty acid metabolism.

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Introduction: Mitochondrial dysfunction in muscle tissue is associated with obesity (mitObesity) and its comorbidities. Many drugs and nutraceuticals used to treat these conditions target mitochondria. Early diagnosis of mitObesity is crucial for understanding the link between obesity, mitochondrial dysfunction, and its associated chronic comorbidities. Respirometry of mitochondrial preparations can assess electron transfer pathways and coupling in oxidative metabolism with high diagnostic resolution [1].
Methods: We developed a standardized protocol for functional diagnosis of mitochondrial defects using high-resolution respirometry [2]. This substrate-uncoupler-inhibitor titration (SUIT) protocol analyzes fatty acid oxidation (FAO) by adding 0.1 mM malate and octanoylcarnitine, with consideration of malate-linked anaplerosis to avoid overestimation of FAO [3-4]. The protocol is extended to stimulate the NADH-linked pathway by adding pyruvate and glutamate. Then succinate and glycerophosphate are titrated to investigate convergent CoQ-reducing pathways. A stepwise titration of uncoupler CCCP allows quantification of the electron transfer capacity. Residual oxygen consumption is assessed after inhibition by rotenone and antimycin A.
Results and discussion: To quantify FAO, malate was needed to avoid inhibition by accumulating acetyl-CoA. However, in the presence of mitochondrial malic enzyme, 2 mM malate stimulated respiration through the NADH-linked pathway in liver and brain mitochondria. Anaplerotic activity above endogenous respiration was minimized at a low (0.1 mM) malate concentration and subtracted from respiration obtained after addition of octanoylcarnitine. This SUIT reference protocol can be used as a general diagnostic tool for bioenergetic profiling in various sample preparations from different cell types, tissues, and organisms.

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Keywords: fatty acid oxidation; SUIT; reference protocol; mitObesity

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POSTERS

P-1*poster* / **A4-2**

Dynamic complexomic analysis reveals a role for Von Willebrand Domain-containing Protein 8 (Vwa8) in mitochondrial morphology and substrate preference.

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P-2*poster*

Effects of short term and long term aerobic-strength training on muscle metabolism in the elderly.

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Introduction: Regular exercise supports healthy ageing and reduces risk of elderly chronic diseases. Respirometry is an important tool in understanding the physiological adaptations in response to physical activity at cellular level. Previously, we showed that 3-month exercise training increases muscle metabolism in the elderly. Present study is aimed to assess the effects of long-term training on muscle oxidative capacity in the subset of individuals continuing regular training for 5 years.

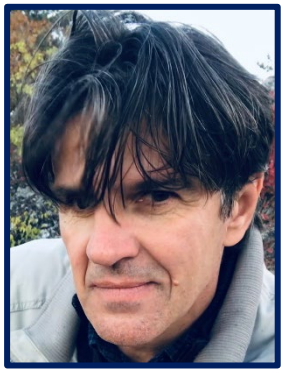
Methods: Volunteers (n=60, 66.9±1.2 years, 27.1±3.9 kg/m2) were recruited for 3-month intervention study: 36 of them underwent aerobic-strength training, 24 volunteers were active controls. A volunteer subpopulation continued aerobic-strength training for next 5 years (n=15), and is compared to non-exercising controls (n=15). Body composition, glucose tolerance, insulin sensitivity and other metabolic parameters were assessed. Samples of m. vastus lateralis obtained by biopsy were used for measurement of muscle mitochondria oxygen consumption by O2k high-resolution respirometry, applying RP1 SUIT protocol.

Results and discussion: Three-month exercise training enhanced muscle mitochondrial respiration rate in the elderly undergoing exercise training compared to controls. So far, two individuals completed follow up phenotyping after 5 years training. A slight deterioration in anthropometric (increased BMI by ~ 8 % and visceral fat content by ~ 36%) and metabolic parameters was observed, together with a reduction in muscle mitochondrial respiration (by ~ 15 %).

Short-term training improved the whole-body and muscle metabolism in the elderly. Obtaining data from exercising and non-exercising cohorts (currently ongoing) will allow us to assess the impact of a long-term intervention.

Keywords: exercise; physical activity; respiration; training; elderly metabolism

Cite: Barkova D, Ukropec J, Nemec M, Slobodová L, Schön M, Tirpáková V, Krumpolec P, Sumbalová Z, Vician M, Sedliak M, Ukropcova B (2023) Effects of short term and long term aerobic-strength training on muscle metabolism in the elderly. In: https://doi.org/10.26124/bec:2023-0002



P-3*poster*

Accelerated epigenetic changes may contribute to the development of metabolic syndrome revealed by NADH FLIM.

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Aging leads to a loss of muscle mass and a decline in skeletal muscle function (1) leading to imbalance between glucose and lipid metabolism (2). Low exercise capacity is highly correlated with skeletal muscle dysfunction and metabolic disorders (3). Age-associated factors intrinsic to the muscle, including defects in NAD^+ synthesis (4), reduced mitochondrial copy number (5), and epigenomic changes affecting the expression of metabolic genes (6) reported. We aimed to characterize mitochondrial fitness of liver in an inborn low- versus high-capacity runners (LCR/HCR) aged female rats to study the spread of metabolic dysfunction.

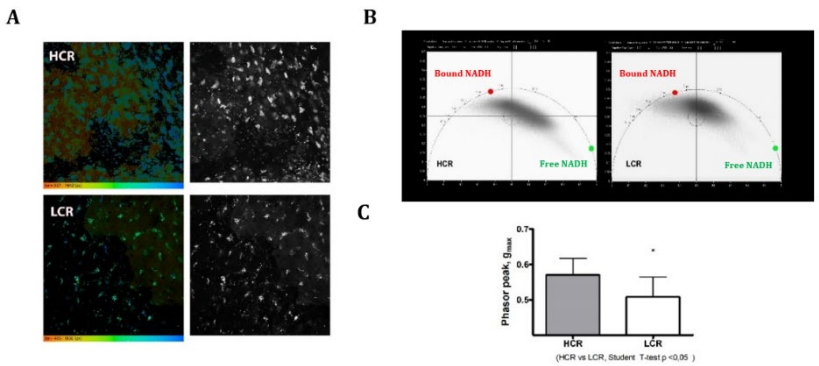

Figure 1. Low intrinsic running capacity promotes shifts toward more bound and less free NADH. TFLIM color maps of liver from HCR and LCR illustrate a significant depletion in free NADH level shown as the color shifts from more green (free NADH) to red (bound NADH) of NADH lifetime parallel with lower intensity of total NADH grayscale image (A). Summary of phasor plots from 5 animals from each groups shows the shift in distribution from free to bound NADH in liver cells of LCR comparing to HCR groups. Pure, free NADH with lifetime of 0.4 ns is shown by green circle and bound NADH with lifetime of 3.4 ns with a red circle on the semicircle. (B). Data are mean ± SD and were compared by Student T-test *$p < 0.05$ (C).

LCR/HCR rats (44th generation, 24 months old) used were artificially selected from genetically heterogeneous N:NIH stock (7). NAD(P)H lifetime imaging (FLIM) characterized liver metabolism in frozen tissues; basal and succinate induced ROS production was evaluated by Amplex Red in the presence of horseradish peroxidase, $\Delta\Psi_{mt}$ by TMRE in intact liver mitochondria.

HCR group was less vulnerable to metabolic disorder comparing to LCR group proofed by decreased body mass and increased VO_{2max}. It was further supported by mitochondrial analysis of intact liver mitochondria. Basal ROS production showed no difference between LCR and HCR groups although succinate induced ROS production was higher in LCR group at identical $\Delta\Psi_{mt}$. NAD(P)H FLIM uncovered subtle alterations: LCR groups had significantly less free NADH comparing to HCR groups (Fig.1).

In conclusion, epigenetic changes induced decline of metabolism correlated with deterioration of liver mitochondrial fitness. Succinate induced ROS-production at same membrane potential negatively correlated with free NADH-level.

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P-4*poster* / **PS-3***poster*

Mitochondrial alternative enzymes and supercomplexes: functional and evolutionary insights.

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P-5*poster* / **A3-3** / PS-4*poster*

Impairment of cardiac function and mitochondrial energy metabolism through diet induced obesity in aging.

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P-6*poster*/ **AS-3** / PS-5*poster*

Screening marine natural products for bioenergetic effects in human cell models.

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P-7 *poster*

Effect of dimethyl fumarate on cerebral mitochondrial metabolism in a porcine model of pediatric in-hospital cardiac arrest.

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Introduction: Despite advancements in cardiopulmonary resuscitation (CPR), secondary neurological injury remains the key determinant of successful recovery from cardiac arrest (CA) [1-3]. Currently, there are no established clinical therapies that preserve neurological function [4]. We previously found that acute decline in mitochondrial health up to 24 hours post-CA correlated with poor neurological outcome [5-6]. Here, we tested the potential of dimethyl fumarate (DMF), a derivative of the TCA-

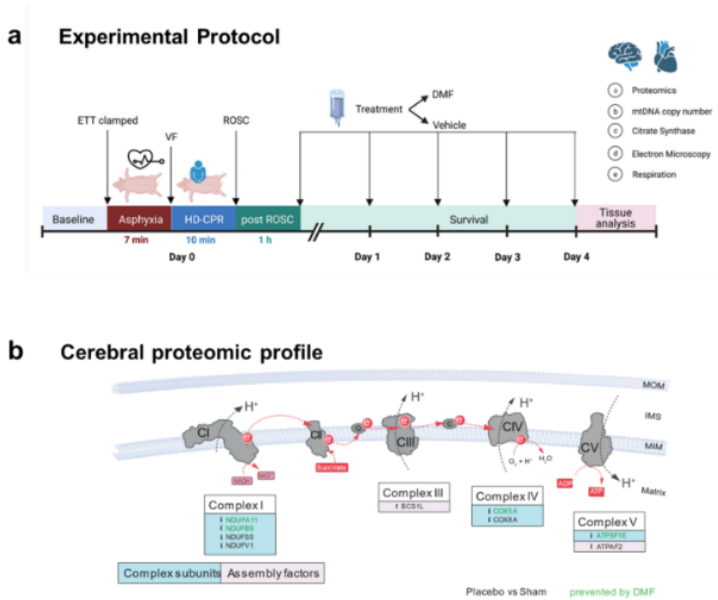

Figure 1. (a) Piglets underwent asphyxia by clamping of the endotracheal tube (ETT), followed by ventricular fibrillation (VF), cardiopulmonary resuscitation (CPR) and defibrillation until return of spontaneous circulation (ROSC). Next, animals received either DMF (30 mg/kg) or vehicle (Placebo) daily for four days. Sham animals underwent identical anesthesia protocols and instrumentation without CA. Tissues were collected for analysis of molecular markers. (b) Protein expression was measured in cortex and proteins with $p < 0.05$ were subjected to pathway-enrichment analysis (KEGG) and identification of mitochondrial proteins.

cycle intermediate fumaric acid shown to enhance mitochondrial bioenergetics [7], to improve mitochondrial injury in brain and heart following successful resuscitation after CA.

Methods: Female piglets representing toddler age underwent asphyxia, followed by ventricular fibrillation, cardiopulmonary resuscitation and defibrillation until return of spontaneous circulation. Subsequently, animals received daily treatment with DMF or vehicle. Sham animals underwent identical anesthesia protocols and instrumentation without CA. After 4 days, animals (n=5 of each group) were euthanized, tissues were harvested and their mitochondrial function, quantity and proteomic profile was analyzed.

Results and discussion: Mitochondrial content and function, as measured by citrate synthase activity and high-resolution respirometry, was reduced at 4 days following CA. In contrast, myocardial mitochondria demonstrated a complete restoration of mitochondrial content and function despite persistent changes in mitochondrial ultrastructure. DMF treatment prevented 25 % of the long-term proteomic changes in the brain, including proteins related to mitochondrial bioenergetics and oxidative stress. In addition, myocardial mitochondrial morphology was normalized by DMF. In this model of CA, mitochondria sustained persistent damage in an organ-specific manner. DMF partially prevents these long-term mitochondrial changes in myocardium and brain.

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Keywords: cardiac arrest; mitochondria; dimethyl fumarate; emergency medicine; metabolism

Cite: Piel S, McManus MJ, Heye K, Beaulieu F, Fazeliniae H, Janowska JI, McTurk B, Starr JP, Gaudio H, Patel N, Hefti MM, Smalley ME, Hook JF, Kohli NV, Bruton J, Hallowell T, Delso N, Roberts A, Lin Y, Ehinger JK, Karlsson M, Berg RA, Morgan RW, Kilbaugh TJ (2023) Effect of dimethyl fumarate on cerebral mitochondrial metabolism in a porcine model of pediatric in-hospital cardiac arrest. In: https://doi.org/10.26124/bec:2023-0002

P-8*poster* / PS-6*poster*

Mitochondrial defect in human bronchial epithelial cells lacking the BK_{Ca} channel.

Pytlak Karolina[1], Maliszewska – Olejniczak K[2], Sęk A[1], Szewczyk A[1], Bednarczyk P[2], Kulawiak B[1]

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Human bronchial epithelial (HBE) cells form an external barrier in the airways and are constantly exposed to factors such as urban dust.

Recently, the large conductance calcium-activated potassium (mitoBK_{Ca}) channel has been identified in the inner mitochondrial membrane of HBE cells. The pore-forming subunit of the channel is encoded by the *KCNMA1* gene, which also encodes plasma membrane BK_{Ca} channels. Mitochondrial potassium channels regulate mitochondrial membrane potential, oxygen consumption, mitochondrial volume and reactive oxygen species synthesis. Activation of mitoBK_{Ca} induces cytoprotection of cardiac and brain tissue.

In our project, we applied CRISPR/Cas9 technique to disrupt *KCNMA1* gene in the HBE cell line (16HBE14o- cells). The newly formed line showed no mitoBK_{Ca} channel activity. We also noticed changes related to the deregulation of the cell cycle. The loss of mitoBK_{Ca} significantly affected mitochondrial function. We observed a decrease in the rate of mitochondrial respiration. Furthermore, we analyzed the organization of respiratory chain complexes using Blue Native electrophoresis. In addition, analysis of the expression of selected genes encoding mitochondrial proteins showed changes in cells with disrupted *KCNMA1* gene. Nevertheless, a thorough understanding of the observed mitochondrial dysfunction requires further study. We conclude that the presence of the mitoBK_{Ca} channel in HBE cells is essential for the preservation of mitochondrial function and is important for the proper function of these cells as part of the human airways.

Cite: Pytlak K, Maliszewska–Olejniczak K, Sęk A, Szewczyk A, Bednarczyk P, Kulawiak B (2023) Mitochondrial defect in human bronchial epithelial cells lacking the BK_{Ca} channel. In: https://doi.org/10.26124/bec:2023-0002

P-9*poster*

Mitochondrial Respirometry and ATP Hydrolysis Measurements in Previously Frozen Tissue Samples.

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Impaired mitochondrial function has been shown to play a key role in diseases of metabolism and aging. Respirometry is the gold standard measurement of mitochondrial function, as it is an integrated metabolic readout of the final step of the electron transport chain (ETC). However, analysis of mitochondrial respiratory function in tissue requires processing and measurement of freshly isolated mitochondria. This requirement makes respirometry impracticable for standard clinical practice, clinical studies, retrospective studies, and higher throughput respirometry. We have validated a methodology to measure maximal mitochondrial oxygen consumption rates through Complex I, II, and IV of the ETC in previously frozen biological samples using Agilent XF Analyzers. Additionally, Complex V (CV) ATP hydrolytic activity can be measured with the pH channel. These measurements of Complex I-V activities are specific as demonstrated by inhibition with ETC inhibitors. Additionally, these approaches can be applied to tissue homogenates, which simplifies the sample preparation and reduces the required starting material compared with isolating mitochondria. We find that primary changes in the maximal respiratory capacity, detected in fresh tissue, are preserved in frozen samples. These techniques to measure mitochondrial maximal respiratory function and CV hydrolytic activity in frozen samples makes clinical mitochondrial assessment more feasible and adds a complementary approach to investigate the role of mitochondrial function in disease onset and progression.

Cite: Stanic S, Janovska P, Zouhar P, Bardova K, Otahal J, Vrbacky M, Mracek T, Adamcova K, Lenkova L, Funda J, Cajka T, Drahota Z, Rustan AC, Horakova O, Houstek J, Rosmeissl M, Kopecky J (2023) Mitochondrial Respirometry and ATP Hydrolysis Measurements in Previously Frozen Tissue Samples. In: https://doi.org/10.26124/bec:2023-0002

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7. Announcements

22nd European Bioenergetics Conference

August 26 to 31, 2024
Innsbruck, Austria
www.ebec2024.org

8. Becoming a MiP*member*

Operating as in informal group since MiP2003 in Schroecken, the MiP*society* was formally established at MiP2011 in Bordeaux as an international non-government organization with its legal base in Innsbruck, Austria. The MiP*society* organizes annual summer schools and biannual conferences, bringing together international leading scientists and young researchers in the rapidly expanding field of Mitochondrial Physiology. MiP*members* benefit from

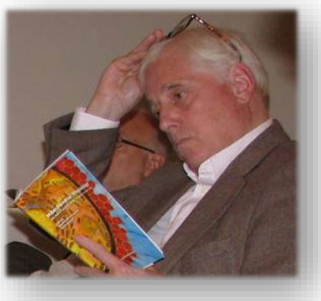
1. Earliest information on MiP*events*
2. MiP*circulars*
3. Reduced registration fees at MiP*events*

MiP in the **Mitochondrial Global Network: MitoGlobal** aims at providing a WorldWide information platform for scientific mitochondrial organizations and mitochondrial research consortia.

Application

Please, submit your application form (available for download at our website www.mitophysiology.org) to join as a MiP*member*. Your application will be confirmed by the Executive MiP*committee* by Email.

Honorary Gentle Science Member of the MiP*society*



Nobel laureate Professor Sir John Walker (Cambridge MBU, UK; Nobel prize 1997 in chemistry) has joined the MiP*society* as the first '**Honorary Gentle Science Member**' of the Mitochondrial Physiology Society, following his presentation of 'The ATP Synthase' at the MiP*summer* School 2012 on July 10 in Trinity Hall, Cambridge, UK.

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