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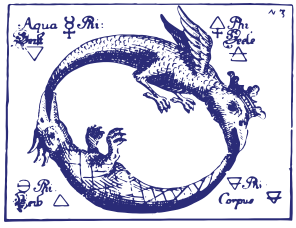
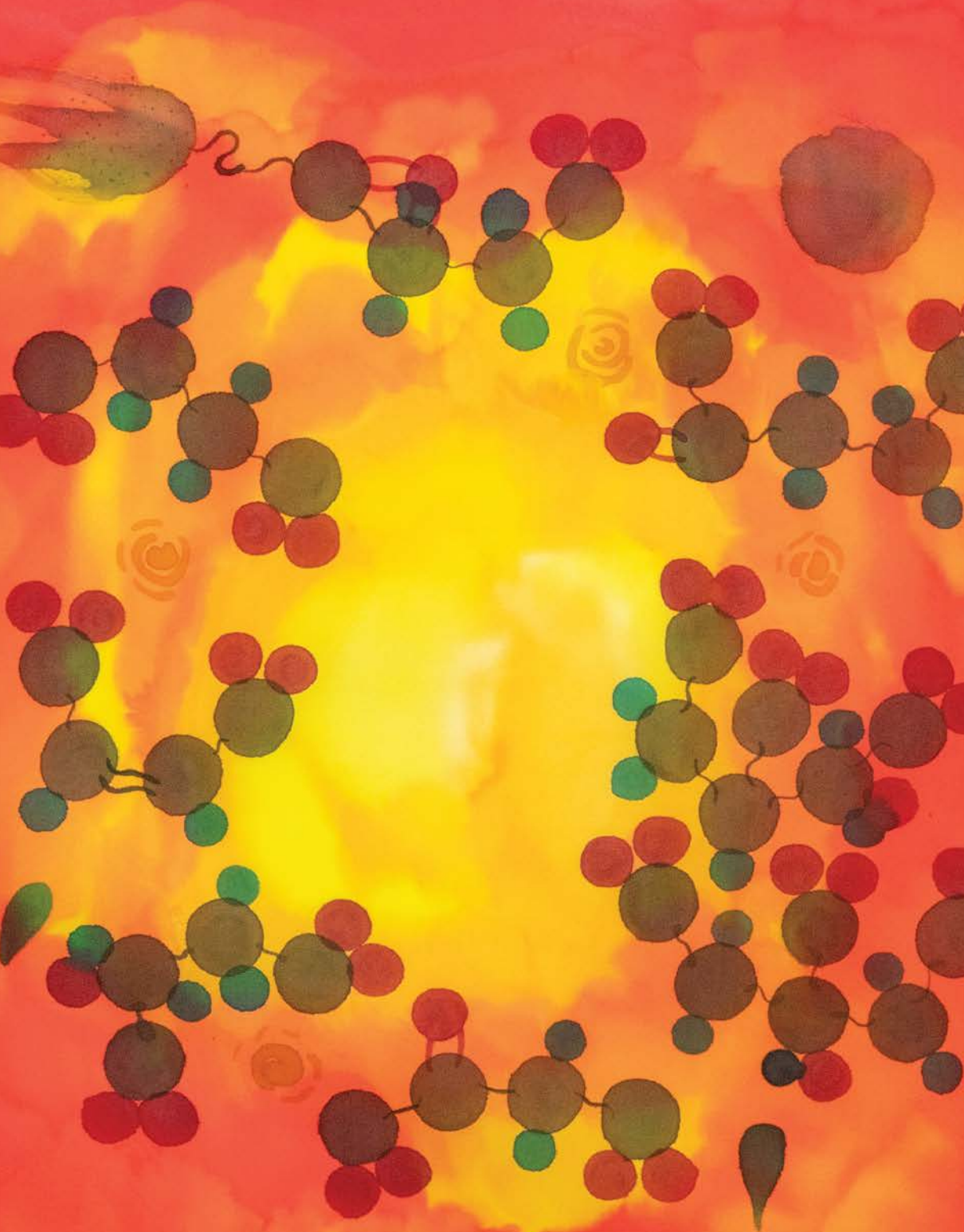


BIOBLAST2022

BEC Inaugural Conference

Book cover - Dragon reverse Krebs *by Odra Noel - www.odranoel.eu*



Krebs in red by Odra Noel

Abstracts

Cite
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Author contributions
EG scientific program, submission reviews, copyediting, author correspondence; LHDC scientific program, submission reviews, copyediting; LTS formatting, author correspondence; PC formatting, typesetting, templates.

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BIOENERGETICS COMMUNICATIONS

Conference organizers
Carolina Gnaiger
Verena Laner

Bioblast link

Bioblast 2022: BEC Inaugural Conference

Editors
Erich Gnaiger, Luiza HD Cardoso, Lisa Tindle-Solomon, Paolo Cocco

Summary

Bioblast 2022 is a follow-up of Bioblast 2012 [1] and the first life conference linked to the journal *Bioenergetics Communications* [2]. It can be seen in the line of MiP*conferences* of the Mitochondrial Physiology Society [3], which was founded at the 3rd MiP*conference* in 2003 [4]. The last one took place in 2019 in Belgrade, RS within the COST Action MitoEAGLE [5]. The MitoEAGLE Summit was prevented from happening by the pandemic lockdown. Instead, the MitoEAGLE Consortium of 666 coauthors completed the first publication in *Bioenergetics Communications* [6]. In the tradition of Bioblast and MiP [1,7], Bioblast 2022 is presented with the beauty of Odra Noel's *MiPArt* and is honored by her presence at the conference. We celebrate 30 years Oroboros Instruments. As a follow-up of the MitoEAGLE project [8], the MiP*society* and the Oroboros Ecosystem are the drivers of *Bioenergetics Communications*.

The endosymbiotic theories link the mitochondria and plastids to their free-living ancestors. Together, these are the bioblasts in spotlight of bioenergetics. Bioblasts and interactions with their hosts are the topics of *Bioenergetics Communications*, inaugurating the concept of *Living Communications*.

Keywords – abstracts; Bioenergetics Communications; Living Communications; bioblasts; mitochondria; plastids; chloroplasts

BEC BIOENERGETICS COMMUNICATIONS

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References

1. Gnaiger E, Meissner B, Laner V, eds (2012) Bioblast 2012: Mitochondrial Competence. https://bioblast.at/index.php/MiPNet17.12_Bioblast_2012
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1. Abstracts Bioblast 2022

Bioblast link

Bioblasts - the taxonomic unit of bioenergetics: mitochondria, aerobic bacteria, chloroplasts.

Gnaiger Erich
Oroboros Instruments GmbH, Innsbruck, Austria

Living Communication

Richard Altmann (1894) observed granula in prokaryotes and eukaryotes stained with osmium for light microscopic analysis of symbiotic and free-living bacteria and of 'elementary organisms' in metazoan cells (**Figure 1**). He viewed 'the protoplasm as a colony of bioblasts' [1]. One of the — either forgotten or famous — quotes from his book on the 'Elementarorganismen' (p 141) introduces a phenomenological link between bacteria and the mitochondria, as they were called later: 'Microorganisms and granula are at an equivalent level and represent elementary organisms, which are found wherever living forces are acting, thus we want to describe them by the common term bioblasts. In the bioblast, that morphological unit of living matter appears to be found'. Today, living matter is in the spotlight of environmentally concerned innovations [2].

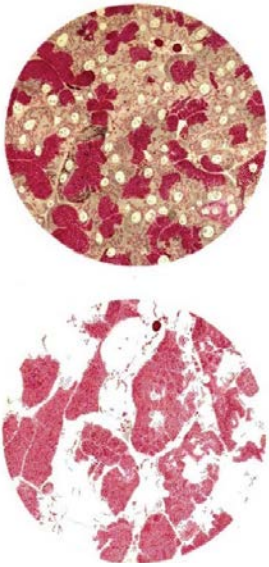

Figure 1. Altmann (1894): Panel VII 1: Pancreas of the mouse, osmium mixtures. Panel VII 2: Intracellular symbiotic bacteria in root nodules of *Coronilla glauca* (leguminous plant) [1].

In the signaling network of the cell, mitochondria are not merely passive receivers of centrally issued commands, but return messages to the cell including retrograde responses to the nucleus, death signals, regulation of the adenylate energy state, redox balance, ROS buffering, and ion homoeostasis [3]. Endosymbiotic theories link the mitochondria and plastids to their free-living ancestors. Consequently, the mitochondrial and chloroplast identity is organismic, distinct from organelles: bioblasts represent 'elementary organisms'. This controversial proposition has been presented at the launch of the Bioblast website when celebrating seven years of the Mitochondrial Physiology Society at MiP2010 [4]. The present summary is a *Living Communication* with updates aimed at continuing and extending the discussion on bioblasts as the taxonomic unit of bioenergetics.

The *Living Communication* with a focus on Open Science, quality rather than quantity, and references in BEC https-format are a testimony to signatories of DORA. As a scientific journal *Bioenergetics Communications* does not propagate yesterday's concepts in the arena of traditional journals. We can *do today's job* much better [5].

The life event *Bioblast 2022: BEC Inaugural Conference* brings together scientists from various fields of mitochondrial and algal research. There is a strong focus on the mitochondrion in health and disease. This other organism in our cells is an alien, whether it was once called an ozonophore, bioblast, or now a mitochondrion. The term mitochondrial organism may signal the transition from a science on ‚mitochondria in health and disease' to the study of ‚mitochondrial health'. Progress in our understanding of mitochondrial disease is tremendous, important, and staggering, with new dimensions emerging on successful therapies. Paradoxically, however, comparatively little has been achieved to provide quantitative and qualitative measures and functional criteria for defining mitochondrial health.

The term and concept of the *bioblast* looks at the parts or particles — microorganisms and granula, from free-living to symbiotic to intracellular endo-elementary organisms. The *holobiont* concept, in turn, focuses on the microbial-eukaryotic interactions and evolution, with symbiosis and the microbiome paving the way to endosymbiosis [6,7].

Odra Noel's *MiPArt*, from 'Hommage to pioneers' (**Figure 2**) to Mitchell's dream [8] is a study of life that transcends the limitations of science when science transcends the limitations of art. At Bioblast 2022, her focus is on the 'Krebs cycle that sits at the heart of metabolism' [9] and links mitochondrial pathways to respiratory control [10].

Figure 2. Odra Noel (2010): Hommage to pioneers I - Altmann's Bioblasts, *MiPArt Gallery* [4].

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A - Evolutionary perspectives of bioenergetics

A-01

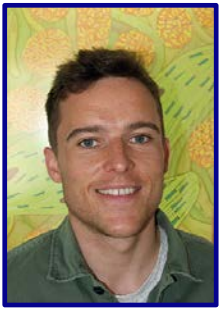
Bioblast link

The alpha and omega of metabolism: why the Krebs cycle brings the earth to life and our own lives to an end.

Lane Nick
Department of Genetics, Evolution and Environment, University College London - nick.lane@ucl.ac.uk

The Krebs cycle is usually perceived as the main source of NADH for oxidative phosphorylation, but it also provides carbon skeletons for the biosynthesis of amino acids, sugars, fatty acids, and by extension nucleotides. This biosynthetic role has become increasingly clear in cancer over the last decade, and Krebs cycle intermediates are now recognized as potent epigenetic signals controlling cellular growth phenotypes via transcription factors such as HIF1α. The biosynthetic basis of the Krebs cycle probably links to the earliest stages of cell evolution, where the longer reverse cycle is central to the structure of biochemistry. Recent experimental work shows that protometabolism probably arose from the reaction of H_2 and CO_2 in alkaline hydrothermal systems, in which steep proton gradients across Fe(Ni)S barriers drove the synthesis of Krebs-cycle intermediates and onwards flux into core protometabolism. I will show that the topology of pores and protocells (and even the Earth) has been preserved in the structure of prokaryotic cells, where reversible flux through the Krebs cycle is intimately associated with the proton-motive force and membrane potential. After the emergence of oxygenic photosynthesis and the accumulation of oxygen, Krebs-cycle flux became delicately poised as a mutual symbiosis between tissues in early animals, enabling them to adapt to persistent hypoxia and euxinia. At the Cambrian explosion, accelerated metabolic rates in the presence of oxygen forced evolutionary choices between repair and reproduction, hence ageing, with the consequence being diminished oxidative phosphorylation with age. I will show why it is not possible for some cells to alter their Krebs-cycle flux, notably pancreatic beta cells and neurons, which calibrate their membrane potential according to glucose availability. This link between Krebs-cycle flux and membrane potential might point to the emergence of elementary consciousness in single-celled organisms, including bacteria, in which fluctuations in membrane potential and the electrical fields generated amount to real-time integrated feedback on the homeostatic state of cells in relation to the outside world – the electrochemical basis of a 'feeling'.

Cite: Lane N (2022) The alpha and omega of metabolism: why the Krebs cycle brings the earth to life and our own lives to an end. In: https://doi.org/10.26124/bec:2022-0001



A-02

Bioblast link

The ABC of hypoxia – what is the norm.

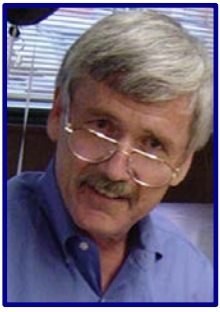
Donnelly Chris[1,2], Schmitt S[1], Cecatto C[1], Cardoso L[1], Komlodi T[1,3], Place N[2], Kayser B[2], Gnaiger E[1]

1. Oroboros Instruments, Innsbruck, Austria
2. Institute of Sport Sciences, Univ. Lausanne, Switzerland - chris.donnelly@unil.ch
3. Department of Medical Biochemistry, Semmelweis University, Budapest, HU

Hypoxia is a condition of oxygen levels below normoxia and opposite to hyperoxia. We here define the normoxic reference state by three complementary precepts: (**A**) **ambient** normoxia at sea level in the contemporary atmosphere and corresponding dissolved O_2 concentration at air saturation of aqueous environments; (**B**) **biological** compartmental O_2 levels at ambient normoxia under physiological activity of healthy organisms in the absence of environmental stress (e.g. in a diving human, a stranded whale, a thermally stressed animal); and (**C**) O_2 levels above the **control** region, i.e., where the capacity for oxygen consumption is not compromised by partial O_2 pressure as evaluated by its kinetics. Conversely, the **abc** of hypoxia is concerned with deviations from these reference points caused by different mechanisms: (**a**) **ambient** alterations of oxygen levels, (**b**) **biological** O_2 demand exceeding oxygen supply under pathological or experimental limitations of convective O_2 transport or O_2 diffusion, and (**c**) **critical** oxygen pressure in oxygen kinetics shifted by pathological and toxicological effects or environmental stress. The **ABC** of hypoxia may be of help in the design and interpretation of *in vitro* and *in vivo* experimental studies.

Keywords: ambient; anoxia; critical O_2 pressure p_c; functional hypoxia; hyperoxia; hypoxia; limiting O_2 pressure p_l; normoxia; oxygen O_2; O_2 concentration c_{O2} [µM]; O_2 pressure p_{O2} [kPa]

Cite: Donnelly C, Schmitt S, Cecatto C, Cardoso L, Komlodi T, Place N, Kayser B, Gnaiger E (2022) The ABC of hypoxia – what is the norm. In: https://doi.org/10.26124/bec:2022-0001



A-03

Bioblast link

Metabolic shutdown of bioenergetics: Protection of macromolecules and survivorship during stress.

Hand Steven C[1], LeBlanc BM[1], Anderson JA[1]
1. Dept Biological Sciences, Louisiana State Univ, Baton Rouge, USA – shand@LSU.edu

Supported by NSF grant IOS-1457061/IOS-1456809.

Metabolic depression in animals is positively correlated with survival under environmental stress. Selected invertebrates and certain fish enter diapause — a developmentally programed dormancy characterized by suppression of development and metabolism. In crustacean embryos the metabolic arrest of OXPHOS is profound, and accordingly these embryos survive years of anoxia and severe desiccation [1]. Mechanisms by which mitochondria and other cellular components tolerate such insults are multifaceted but include targeting of Late Embryogenesis Abundant (LEA) proteins to cellular compartments. LEA proteins are family of intrinsically disordered proteins (IDPs) reported to improve cellular tolerance to water stress. Open questions are whether gain of secondary structure by LEA proteins during drying is a prerequisite for this stabilizing function and whether their protective abilities as documented with isolated cells can be extended to a desiccation-sensitive, whole organism during water stress.

Proteins were characterized by catalytic activity, Western blotting and circular dichroism spectroscopy (CD). Recombinant AfrLEA2, a Group 3 LEA protein from *Artemia franciscana*, was expressed in bacterial cells and purified by HisTrap affinity chromatography and by anion exchange. Phosphofructokinase (PFK) was extracted from rabbit muscle and purified by ultracentrifugation, isopropanol precipitation, DEAE chromatography and heat treatment. We used incremental drying (equilibration to a series of relative humidities, RH) to test the ability of AfrLEA2 to protect desiccation-sensitive PFK. Fly lines of *Drosophila melanogaster* that expressed the PhiC31 integrase were injected with expression vectors containing the desired LEA transgenes. The non-parametric Kaplan–Meier method was used for analysis of survivorship curves.

After quantifying the ability of AfrLEA2 to protect PFK activity during incremental drying, parallel experiments used CD to measure gain of secondary structure in AfrLEA2. Protection of PFK by AfrLEA2 coincided with incremental gain of α-helix in AfrLEA2 as RH decreased [2]. To evaluate the impact of LEA proteins in whole organisms during water stress, embryos of *D. melanogaster* were dried to 80 % tissue water and then rehydrated [3]. Embryos from fly lines that expressed AfrLEA2 or AfrLEA3m eclosed 2 days earlier than wild-type embryos or embryos expressing green fluorescent protein (Gal4GFP control). With third instar larval stages, Kaplan–Meier survival curves indicated a significant improvement in survivorship in fly lines expressing AfrLEA proteins compared with Gal4GFP controls. The percent water lost at the LT50 (lethal time for 50 % mortality) for the AfrLEA lines was 78 % versus 52 % for Gal4GFP controls. Finally, offspring of fly lines that expressed AfrLEA2, AfrLEA3m or AfrLEA6 exhibited significantly greater success in reaching pupation, compared with wild-type flies, when

adults were challenged with hyperosmotic stress (NaCl-fortified medium) and progeny forced to develop under these conditions [3].

In conclusion, metabolic shutdown is a prerequisite for successful tolerance of severe stress in many species. Our experimental evidence links the acquisition of α-helix in a LEA protein with stabilization of a target protein (PFK) across a graded series of hydration states. Additionally, our gain of function studies with *D. melanogaster* show that LEA proteins improve tolerance to water stress in a desiccation sensitive-species that normally lacks these proteins.

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Keywords: metabolic arrest; Diapause; water stress; late embryogenesis abundant proteins; intrinsically disordered proteins

Cite: Hand Steven C, LeBlanc BM, Anderson JA (2022) Metabolic shutdown of bioenergetics: Protection of macromolecules and survivorship during stress. In: https://doi.org/10.26124/bec:2022-0001

A-04 *poster*

Bioblast link

Linking the mitonuclear genotype with mitochondrial function and organismal fitness.

Bettinazzi Stefano[1], Camus F[1], Dowling DK[2], Lane N[1]
1. University College London, UK - s.bettinazzi@ucl.ac.uk
2. Monash University, AU

Despite playing a key role in energy metabolism, mitochondria are uniquely exposed to perturbation because their functions depend on the correct interaction between two distinct genomes, the mitochondrial and the nuclear DNA. Even mild incompatibilities between the two genomes could impact mitochondrial functions with downstream repercussions on individual fitness. Climate change predictions estimate an increase in temperature and its variability, changes in food web structures, but also in the distribution of populations. Events that may generate mitonuclear mismatches (e.g. hybridization between separate populations) are therefore expected to increase in frequency following the shifts in thermal niches. In addition, temperature and dietary regimes are well-known metabolic stressors whose variation can potentially exacerbate mitonuclear incompatibilities. The aim of this research was to test how far mild mitonuclear variations, of the kind that could be found in natural populations following introgression in shifting niches, might affect organismal fitness.

I employed four experimental lines of the fruitfly *Drosophila melanogaster*, characterized by mitonuclear match or mismatch, to investigate the impact of

mitonuclear genotype over a wide array of phenotypic traits, including mitochondrial functions, reactive oxygen species metabolism and life-history traits.

Results indicate a general trade-off between bioenergetic capacity and fecundity in mitonuclear mismatched lines, most prominently in females than males. Cybrids tend to have a higher OXPHOS activity compared to the matched parental populations, as well as generally lower H_2O_2 efflux. The high mitochondrial respiration rate also links with a trend of higher locomotor activity, but with lower fecundity parameters. Finally, differences in thermal tolerance also exist, but link solely to the nuclear component, and not to the mitonuclear combination. Overall, results suggest that mitonuclear interactions might impact organismal fitness in an unpredictable way, potentially influencing local adaptation in a mutating world.

Keywords: mitochondria; mitonuclear coevolution; Drosophila; fitness; climate change

Cite: Bettinazzi S, Camus F, Dowling DK, Lane N (2022) Linking the mitonuclear genotype with mitochondrial function and organismal fitness. In: https://doi.org/10.26124/bec:2022-0001

A-05

Bioblast link

"Going south!" An Antarctic expedition to understand more about mitochondrial haemoglobin and ageing.

Ebanks B[1], Katyal G[1], Papetti C[2], Lucassen M[3], Marks FC[3], Chakrabarti Lisa[1]

1. Oroboros Instruments GmbH, Innsbruck, Austria
2. Facultad de Estudios Superiores Iztacala, UNAM, Tlanepantla, Estado de Mexico, Mexico
3. Dept of Botany, University of Innsbruck, Austria

Mitochondrial dysfunction is intimately associated with ageing and diseases that occur more frequently with advancing age. Work we published some years ago showed the presence of haemoglobin in mitochondria. We found variations in the quantity of mitochondrial haemoglobin in neurodegeneration and also in animals experimentally exposed to hypoxia. Non-erythrocyte haemoglobin was unexpected and its function is still not entirely clear. A standard approach to discover the function of a protein in a tissue is to remove it, knock it 'out' or 'down'. For essential proteins like haemoglobin in the vertebrate context, this is unlikely to be a successful route of investigation. However, there is an unusual group of fish known as the 'icefish' that do not express haemoglobin - these fish are only found in the cold waters of Antarctica. In collaboration with the German polar research institute (AWI) and other fish biologists, we started our investigations into how icefish maintain mitochondrial function without haemoglobin. This line of research led me to an expedition in Antarctica earlier this year, where we performed icefish respirometry on board RV Polarstern.

Keywords: haemoglobin; ageing; neurodegeneration; Icefish; Antarctica

Cite: Ebanks B, Katyal G, Papetti C, Lucassen M, Marks FC, Chakrabarti L (2022) "Going south!" An Antarctic expedition to understand more about mitochondrial haemoglobin and ageing In: https://doi.org/10.26124/bec:2022-0001

Pichia Pastoris red by Odra Noel

B - Methodological advancements in bioenergetics

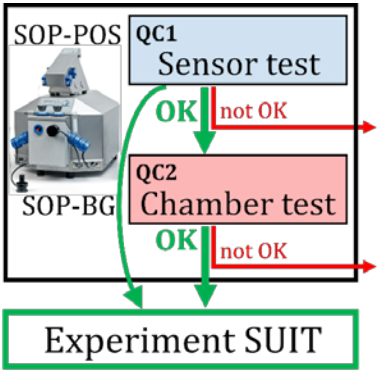
B-01

Bioblast link

Statistical analysis of instrumental reproducibility as internal quality control in high-resolution respirometry.

Baglivo Eleonora, Cardoso LHD, Cecatto C, Gnaiger E
Oroboros Instruments, Innsbruck, Austria

This work was part of the Oroboros NextGen-O2k project, with funding from the European Union's Horizon 2020 research and innovation programme under grant agreement nº 859770.



Evaluation of instrumental reproducibility is a primary component of quality control to quantify the precision and limit of detection of analytical procedures. A pre-analytical instrumental standard operating procedure (SOP) is implemented in high-resolution respirometry consisting of: (*1*) a daily SOP-POS for air calibration of the polarographic oxygen sensor (POS) in terms of oxygen concentration c_{O2} [µM]. This is part of the *sensor test* to evaluate POS performance; (*2*) a monthly SOP-BG starting with the SOP-POS followed by the *chamber test* quantifying the instrumental O_2 background. The chamber test focuses on the slope dc_{O2}/dt [pmol·s^{-1}·mL^{-1}] to determine O_2 consumption by the POS and O_2 backdiffusion into the chamber as a function of c_{O2} in the absence of sample. Finally, zero O_2 calibration completes the sensor test.

We applied this SOP in a 3-year study using 48 Oroboros O2k chambers. Stability of air and zero O_2 calibration signals was monitored throughout intervals of up to 8 months without sensor service. Maximum drift over 1 to 3 days was 0.06 pmol·s^{-1}·mL^{-1}, without persistence over time since drift was <0.004 pmol·s^{-1}·mL^{-1} for time intervals of one month, corresponding to a drift per day of 0.2 % of the signal at air saturation. Instrumental O_2 background $-dc_{O2}/dt$ was stable within ±1 pmol·s^{-1}·mL^{-1} when measured at monthly intervals. These results confirm the instrumental limit of detection of volume-specific O_2 flux at ±1 pmol·s^{-1}·mL^{-1}. The instrumental SOP applied in the present study contributes to the generally applicable internal quality control management ensuring the unique reproducibility in high-resolution respirometry [1].

1. Baglivo E, Cardoso LHD, Cecatto C, Gnaiger E (2022) Statistical analysis of instrumental reproducibility as internal quality control in high-resolution respirometry. https://doi.org/10.26124/mitofit:2022-0018.v2

Keywords: high-resolution respirometry HRR; polarographic oxygen sensor POS; air calibration; instrumental background; reproducibility; limit of detection; internal quality control IQC; standard operating procedure SOP

Cite: Baglivo E, Cardoso LHD, Cecatto C, Gnaiger E (2022) Statistical analysis of instrumental reproducibility as internal quality control in high-resolution respirometry. In: https://doi.org/10.26124/bec:2022-0001

B-02

Bioblast link

Elucidating the complexity of substrate-uncoupler-inhibitor titration protocols.

Chicco Adam J[1], Zilhaver PT[1], Whitcomb LA[1], Fresa KJ[1], Izon CS[1], Gonzalez-Franquesa A[2], Dometita C[3], Irving BA[3,4], Garcia-Roves PM[5]

1. Dept of Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA - adam.chicco@colostate.edu
2. NovoNordisk, Copenhagen, Denmark
3. Geisinger Obesity Institute, Danville, Pennsylvania, USA
4. Dept of Kinesiology, Louisiana State University, Baton Rouge, Louisiana, USA
5. Dept of Physiological Sciences, University of Barcelona, Barcelona, Spain

In mitochondria (mt) of most tissues and cells, NADH-linked substrates (N: pyruvate, glutamate, malate) in combination with succinate (S) support higher respiratory capacities in the OXPHOS or electron transfer (ET) state compared to the separate N- or S-pathway capacities supported by either N-substrates or succinate&rotenone. NS-substrate combinations are required for partial reconstitution of TCA cycle function in mt-preparations, to compensate for metabolite depletion into the incubation medium. NS in combination exerts an additive effect on OXPHOS or ET capacity (*P* or *E*, respectively) of convergent electron-input into the Q-junction. Substrate-uncoupler-inhibitor titration (SUIT) protocols are used to resolve the relative contributions of the N- and S-pathway to the NS-supported oxygen consumption rate ($J_{O_2,NS}$ = NS_P or NS_E). In SUIT protocols starting with N-pathway capacity, addition of succinate exerts a stimulatory effect (NS > N). Then the selective CI inhibitor rotenone is utilized to eliminate the contribution of the N-pathway to NS-pathway capacity, typically revealing an inhibitory effect on J_{O_2} and thus allowing quantification of S-pathway capacity (NS > S). However, in some types of mitochondria, rotenone added in the NS-state elicits a paradoxical increase in J_{O_2}, revealing a complex interaction of N- and S-pathway substrate oxidation on J_{O_2}.

We demonstrate an inhibitory effect of >1 mM malate or 4 µM malonate (a CII inhibitor) on $J_{O_2,N}$ supported by pyruvate (PM) and/or glutamate (PGM or GM). Collectively, our studies suggest that under these conditions, succinate formation is not completely prevented by the loss of TCA cycle intermediates. Thus, the S-pathway contributes to some extent to PM-, PGM-, or GM-supported respiration by oxidation of succinate formed endogenously in the TCA cycle and interacts with malate- and further fumarate- and oxaloacetate-concentrations to potently regulate J_{O_2} supported by exogenous N-substrates in a tissue-specific manner. Potential mechanisms are discussed to stimulate further experimentation aimed at elucidating the biological bases for

variations in NS-pathway flux in SUIT protocols used to study the additive effect of convergent electron flow at the Q-junction [1].

1. Chicco AJ, Zilhaver PT, Whitcomb LA, Fresa KJ, Izon CS, Gonzalez-Franquesa A, Izon CS, Dometita C, Irving BA, Garcia-Roves PM (2022) Resolving the Rotenone Paradox: elucidating the complexity of multi-substrate respirometry protocols. https://doi.org/10.26124/mitofit:2022-0017

Keywords: mitochondrial respiration; electron transfer system; succinate; glutamate; oxidative phosphorylation; high-resolution respirometry

Cite: Chicco AJ, Zilhaver PT, Whitcomb LA, Fresa KJ, Izon CS, Gonzalez-Franquesa A, Dometita C, Irving BA, Garcia-Roves PM (2022) Elucidating the complexity of substrate-uncoupler-inhibitor titration protocols. In: https://doi.org/10.26124/bec:2022-0001

B-03

Bioblast link

Using oxygen and hydrogen gas in studies of mitochondrial respiration and hydrogen peroxide production under hyperoxic, normoxic, and hypoxic conditions.

Schmitt Sabine, Gnaiger E
Oroboros Instruments GmbH, Innsbruck, Austria

Oxygen concentration c_{O_2} in tissues is tightly controlled by O_2 signaling to ensure sufficient O_2 supply for mitochondrial respiration and prevent oxidative stress at excessive O_2 levels. Similarly, c_{O_2} in the experimental medium must be controlled in studies with cultured cells and mitochondrial preparations to simulate extracellular and intracellular conditions in the tissues of origin.

Mitochondrial respiration is independent of c_{O_2} down to ~5 % air saturation [1]. In contrast, ROS production is a continuous function of c_{O_2} from hypoxia to hyperoxia [2]. Decreasing the c_{O_2} in the experimental chamber below air saturation is needed to (*1*) study mitochondrial function under physiologically relevant c_{O_2}, and (*2*) zoom into the low O_2 range to study O_2 kinetics under hypoxia. Increased c_{O_2} above air saturation is applied in experiments with permeabilized muscle fibers to prevent artificial O_2 diffusion limitation of respiration [3] or to induce hyperoxic stress.

Our newly developed Oxia (Oroboros Instruments) generates oxygen and hydrogen gas by electrolysis of H_2O [4]. O_2 or H_2 is injected into the gas phase of the open O2k-chamber which is closed when the desired c_{O_2} is reached. H_2 has been described to affect mitochondrial metabolism. Therefore, we evaluated the effect of lowering c_{O_2} on mitochondrial function in permeabilized HEK 293T cells by injecting either H_2 or the conventionally used N_2 gas. Simultaneous measurements of mitochondrial respiration and H_2O_2 production were performed in the LEAK and OXPHOS coupling states fueled by the NADH-linked substrates pyruvate & malate. Upon transition of c_{O_2} from ~160 to ~25 µM, O_2 and H_2O_2 flow of the permeabilized cells decreased even above the kinetic c_{O_2} range when cytochrome *c* oxidase becomes oxygen-limited [1]. However, the observed changes in O_2 and H_2O_2 flow were indistinguishable when either H_2 or N_2 were used for lowering c_{O_2}.

With Oxia H_2 and O_2 gases can be produced any time in experimentally required volumes directly at the bench besides the O2k. This is a safe, convenient, and economic alternative to the use of high-pressure gas tanks.

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Keywords: tissue normoxia; hypoxia; hyperoxia; high-resolution respirometry; mitochondrial H_2O_2 production

Cite: Schmitt S, Gnaiger E (2022) Using oxygen and hydrogen gas in studies of mitochondrial respiration and hydrogen peroxide production under hyperoxic, normoxic, and hypoxic conditions. In: https://doi.org/10.26124/bec:2022-0001

B-04

Bioblast link

Redox monitoring and respiration - a new horizon with the NextGen-O2k.

Cardoso Luiza HD[1], Doerrier C[1], Donnelly C[1], Komlódi T[2], Gnaiger E[1]

1. Oroboros Instruments GmbH, Innsbruck, Austria
2. Department of Biochemistry, Semmelweis University, Budapest, Hungary

This work was part of the Oroboros NextGen-O2k project, with funding from the European Union's Horizon 2020 research and innovation programme under grant agreement nº 859770.

NADH-linked substrates (N-substrates) of TCA-cycle and other mt-matrix dehydrogenases feed electrons through the N-junction into Complex I. The redox states of the NAD-pool — defined as the sum of $NAD(P)^+$ and NAD(P)H — and of the electron transfer system (ETS)-reactive coenzyme Q-pool [1] are linked in the N-pathway but are regulated independently by several convergent electron entries into the Q-junction [2]. Our results obtained with HRR and the NADH- and Q-Modules of the NextGen-O2k show that complementary to ET-pathway control of respiratory rate and redox state (redox push by electron input), coupling control exerts opposite effects on the metabolic parameters. Whereas redox push reduces the N- and Q-pool in the LEAK state at low O_2 flux and high protonomotive force *pmF*, stimulation of O_2 flux by ADP in the OXPHOS state [3] is accompanied by a redox pull to the oxidized state. Under these conditions, ET-pathways converging at the Q-junction yield partial additivity of O_2 flux, when ET-capacity downstream of Q and phosphorylation capacity exert flux control [2]. Monitoring of respiration together with NAD(P)H autofluorescence and Q-redox state [1] provides unique analytical and diagnostic power in the study of mitochondrial respiratory control at the N- and Q-junctions.

1. Komlódi T, Cardoso LHD, Doerrier C, Moore AL, Rich PR, Gnaiger E (2021) Coupling and pathway control of coenzyme Q redox state and respiration in isolated mitochondria. https://doi.org/10.26124/bec:2021-0003
2. Gnaiger E (2020) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 5th ed. https://doi.org/10.26124/bec:2020-0002
3. Gnaiger E et al — MitoEAGLE Task Group (2020) Mitochondrial physiology. https://doi.org/10.26124/bec:2020-0001.v1

Cite: Cardoso LHD, Doerrier C, Donnelly C, Komlódi T, Gnaiger E (2022) Redox monitoring and respiration - a new horizon with the NextGen-O2k. In: https://doi.org/10.26124/bec:2022-0001

B-05

Bioblast link

Tuning the assessment of coenzyme Q redox state and respiration in permeabilized skeletal muscle fibers.

Gama-Perez P[1], Cardoso L[2], Komlodi T[2,3], Bosch N[1], Gnaiger E[2], Garcia-Roves Pablo M[1,4]

1. Dept Physiological Sciences, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain - pgarciaroves@ub.edu
2. Oroboros Instruments, Innsbruck, Austria
3. Department of Biochemistry, Semmelweis University, Budapest, Hungary
4. Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain

This work was part of the Oroboros NextGen-O2k project, with funding from the European Union's Horizon 2020 research and innovation programme under grant agreement nº 859770.

Metabolic plasticity in skeletal muscle facilitates the response of this tissue to a myriad of physiological and pathological conditions. In this sense, mitochondria play a critical role and accordingly, the assessment of its content and functionality represents a breakthrough to understand this adaptive response. Mitochondrial function in skeletal muscle was initially explored by inferential combinations of oxygen kinetics and enzymatic assays in many different biopsy specimens. Nonetheless, these initial approaches have been replaced by more sensitive and reliable methods that allow direct measurements of oxygen consumption (e.g., high-resolution respirometry, HRR). Using polarographic oxygen sensors, HRR offers a detailed real-time assessment of respiration and, by the titration of different substrates and compounds, can provide relevant information about the function of specific components of the electron transfer system. In addition, the growing popularization of this methodology has been paralleled by mechanical and chemical permeabilization as the preferred choice for tissue preparation, since it offers critical advantages over commonly used alternatives (i.e., mitochondrial isolation). Essentially, the amount of tissue needed to perform HRR in permeabilized fiber (pfi) is significantly reduced, all mitochondrial populations (subsarcolemmal and intermyofibrilar) are equally represented, and mitochondrial network is preserved. Thus, HRR in pfi represents a suitable and increasingly widespread option to address skeletal muscle bioenergetics.

Beyond HRR, over the years additional instrumentation has been implemented to explore other complementary readouts that enhance our understanding of mitochondrial bioenergetics. Accordingly, HRR in combination with fluorescence or potentiometric

sensors allows reliable measurements of membrane potential, H2O2 and ATP production in a wide range of biological samples. Additionally, in an innovative effort to expand our exploratory capacity, the novel Oroboros NextGen-O2k Q-Module has been recently developed for a simultaneous real-time monitoring of oxygen consumption and Q-redox state using a three-electrode system, and a short-chain coenzyme Q mimetic (CoQ2) as a probe. This setup has already been successfully used to determine the Q-reduced fraction in isolated brain and heart mitochondria (Komlodi 2021). Hence, the main purpose of this study is to address the feasibility to simultaneously evaluate these complementary readouts in permeabilized muscle fibers.

Since we have demonstrated that reported CoQ2 concentrations in isolated mitochondria can negatively impact respirometry measurements in pfi, we first aimed to optimize the CoQ2 concentration for a proper sensitivity and permissibility. We observed that in the presence of pfi and absence of respiratory substrates, CoQ2 was fully oxidized, and could not be further oxidized upon addition of the CI inhibitor rotenone. This was followed by application of a substrate-uncoupler-inhibitor titration (SUIT) protocol, which permits to assess respiration and reduced Q-fraction in the NADH- and succinate-linked pathways in the LEAK, OXPHOS, and electron transfer (ET) coupling control states. We observed that the Q-pool became partially reduced upon addition of the respiratory substrates, pyruvate and malate, and even further reduced when succinate was employed. Addition of ADP in the presence of pyruvate and malate led to partial oxidation of the Q-pool, however, this was not pronounced. As expected for skeletal muscle, addition of uncoupler to reach ET-capacity did not affect either respiration or the reduced Q-fraction. In conclusion, these results demonstrate that it is possible to use the Q-Module to expand the evaluation of skeletal muscle mitochondrial physiology.

1. Komlódi T, Cardoso LHD, Doerrier C, Moore AL, Rich PR, Gnaiger E (2021) Coupling and pathway control of coenzyme Q redox state and respiration in isolated mitochondria. https://doi.org/10.26124/bec:2021-0003

Keywords: coenzyme Q; permeabilized muscle fibers; high-resolution respirometry; skeletal muscle; electron transfer system; Q-junction

Cite: Gama-Perez P, Cardoso L, Komlodi T, Bosch N, Gnaiger E, Garcia-Roves PM (2022) Tuning the assessment of coenzyme Q redox state and respiration in permeabilized skeletal muscle fibers. In: https://doi.org/10.26124/bec:2022-0001

B-06

Bioblast link

Mitochondrial calcium uptake capacity is lower than calcium retention capacity in the presence and absence of cyclosporin A.

Cecatto Cristiane, Cardoso LHD, Gnaiger E
Oroboros Instruments GmbH, Innsbruck, Austria - cristiane.cecatto@oroboros.at

This work was part of the Oroboros NextGen-O2k project, with funding from the European Union's Horizon 2020 research and innovation programme under grant agreement nº 859770.

Mitochondrial calcium homeostasis plays important roles in cell survival and cell death, and its dysfunction is involved in many diseases. Mitochondria contribute to the buffering of cytosolic free calcium levels and thereby to the regulation of calcium-dependent cellular processes by uptake and storage of calcium in the form of osmotically inactive calcium phosphates in the mitochondrial matrix. Calcium retention capacity is defined as the amount of calcium titrated up to a threshold where permeability transition triggers the release of calcium and several other substances such as cytochrome c. We measured calcium uptake and respiration in mitochondria isolated from mouse liver using the Oroboros O2k with Smart Fluo-Sensors and Calcium Green. Calcium Green did not inhibit respiration. Calcium concentrations increased sharply upon stepwise 5 µM calcium titrations and declined gradually during intervals of 6 min as a result of mitochondrial calcium uptake in the LEAK state. Calcium uptake was incomplete, however, upon initial and final titrations before the onset of permeability transition, as shown by the merely partial decline of calcium concentrations in the medium. Consequently, the actual calcium uptake capacity CaUC needs to be corrected for incomplete uptake. CaUC was 45 % of the conventionally defined calcium retention capacity in controls and 55 % in the presence of cyclosporin A. These results suggest that high calcium retention capacities reported in the literature require critical evaluation and should be replaced by calcium uptake capacities properly corrected for extramitochondrial calcium concentrations measured in the medium.

Keywords: calcium uptake; mitochondrial permeability transition pore; Calcium green; high-resolution respirometry; calcium retention capacity

Cite: Cecatto C, Cardoso LHD, Gnaiger E (2022) Mitochondrial calcium uptake capacity is lower than calcium retention capacity in the presence and absence of cyclosporin A. In: https://doi.org/10.26124/bec:2022-0001

B-07

Bioblast link

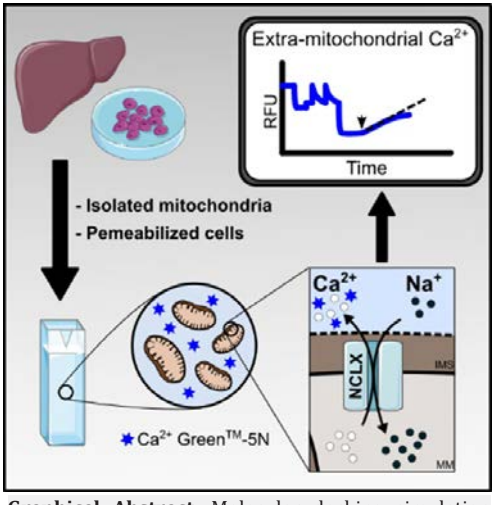
Measuring mitochondrial Ca^{2+} efflux in isolated mitochondria and permeabilized cells.

Serna Julian DC[1], de Miranda Ramos V[1], Cabral-Costa JV[1], Vilas-Boas EA[1], Amaral AG[2], Ohya G[1], Caldeira da Silva CC[1], Kowaltowski AJ[1]

1. Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, 05508-900, Brazil
2. Departamento de Fisiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, 05509-900, Brazil

Mitochondrial Ca^{2+} efflux is essential for mitochondrial and cell Ca^{2+} homeostasis. Mitochondrial inner membrane Ca^{2+}/H^{+} and $Na^{+}/Li^{+}/Ca^{2+}$ (NCLX) exchangers are known today to be plastic transporters [1], with important roles in physiological responses and pathological states. Until now, however, no consensus protocols were available to measure mitochondrial Ca^{2+} efflux, and we find that some published protocols may induce mitochondrial permeability transition pore (mtPTP) opening, underestimating the effects of these exchangers. In this work we describe a method to measure Na^{+}-sensitive and insensitive mitochondrial Ca^{2+} efflux activity in isolated mitochondria and permeabilized cells.

Mitochondria were isolated from mouse liver and rat heart. PLC/PRF/5 (hepatoma) and INS-1E (insulinoma) cells were permeabilized with digitonin. Digitonin concentrations were titrated using a high-resolution respirometer (OROBOROS). Outer mitochondrial membrane (OMM) integrity was assessed with exogenous cytochrome *c*. Calcium transport was measured following concentrations in the extramitochondrial medium using Ca^{2+}-Green 5N and an Hitashi F4500 Fluorimeter. The efflux protocol was performed as follows (figure 1): Mitochondria (isolated or in permeabilized cells), were loaded with a non-permeability transition inducing amount of Ca^{2+} and treated with ruthenium red to inhibit uptake. Ca^{2+} efflux was measured under non-stimulated conditions and also stimulated by 20 mM NaCl or LiCl. Ca^{2+}-Green 5N Kd values were determined. Fluorescence values were always transformed in $[Ca^{2+}]$ for quantifications.



Graphical Abstract. Molecular docking simulation result of the interaction between VDAC1 (in blue) and NHK1 peptide (in purple).

Widespread mtPTP formation is easily identifiable when measuring Ca^{2+} fluxes, as it leads to overt Ca^{2+} release, but we find it can often be overlooked when affecting a subset of the mitochondrial population. Total efflux rates increase with increasing Ca^{2+} loads due to a CsA-sensitive activity, which indicates that the mtPTP was responsible [2].

Our results demonstrate that using higher Ca^{2+} loads promote mtPTP opening even when the Ca^{2+} retention capacity has not been exceeded. Liver NCLX-mediated Ca^{2+} efflux activity had not been detected in many prior work [3,4]. In mice liver mitochondria, we were able to measure both Na^+- and Li^+-stimulated Ca^{2+} efflux, and to distinguish it from Ca^{2+}/H^+ exchange. Our protocol produces similar results in isolated rat heart mitochondria. Interestingly, NCLX activity was predominant in heart relative to Ca^{2+}/H^+ exchange. In digitonin-permeabilized PCL and INS-1E cells, we were also able to measure/characterize NCLX activity and to distinguish it from Ca^{2+}/H^+ exchange. The last approach provides an alternative path to measure mitochondrial efflux in cells when microscopy is not available, also leaving behind the restrains (and the complexity) present in intact cell models.

Overall, our approach allows us to dissect between Na^+-sensitive and insensitive Ca^{2+} efflux. We demonstrate that a vital point in obtaining consistent and reliable Ca^{2+} extrusion activity measurements through mitochondrial exchangers is to avoid mtPTP opening by either using low Ca^{2+} loads or adding cyclosporin A to all traces. In the absence of this step, at least part of the activity of NCLX and Ca^{2+}/H^+ exchange may be masked by mtPTP-promoted permeabilization. Using this method, we were able to demonstrate NCLX activity in mouse liver mitochondria and permeabilized liver hepatoma PLC/PRF/5 cells. Additionally, we validated our method in isolated rat heart mitochondria, as well as the insulinoma cell line INS-1E [5].

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Keywords: NCLX; mtPT; isolated mitochondria; permeabilized cells; calcium

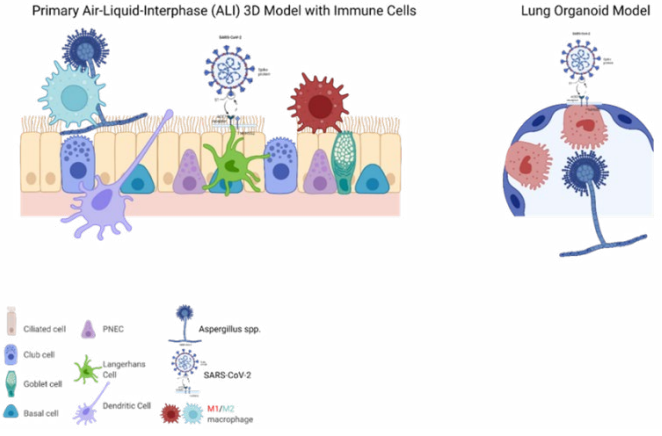
Cite: Serna JDC, de Miranda Ramos V, Cabral-Costa JV, Vilas-Boas EA, Amaral AG, Ohya G, Caldeira da Silva CC, Kowaltowski AJ (2022) Measuring mitochondrial Ca^{2+} efflux in isolated mitochondria and permeabilized cells. In: https://doi.org/10.26124/bec:2022-0001

B-08

Bioblast link

How to optimize respiratory models for SARS-CoV-2 research.

Posch W, Dichtl S, Zaderer V, Lass-Floerl C, Wilflingseder Doris
Inst of Hygiene and Medical Microbiology, Medical Univ of Innsbruck, Austria - doris.wilflingseder@i-med.ac.at



Sophisticated 3D cell culture tissue models experienced a boom in the last years and in particular human cell culture and 3D respiratory systems greatly supported the development of novel drugs and vaccines during the SARS-CoV-2 pandemic lately. These models provide multiple benefits in terms of similarities in differentiation, metabolism, receptor expression, polarity, infectivity compared to human tissues and thus provide excellent models to study very first interactions with the host during pathogen entry. Dependent on the experimental approach, the use of different 3D models is more beneficial – apical out lung organoids for e.g., high content screening (HCS) of treatment options, air-liquid interphase (ALI) models for e.g., easy incorporation of immune cells, screening of epithelial integrity or mucociliary clearance. This review will give an overview on the models established in our laboratory and on their applications [1].

1. Posch W, Dichtl S, Zaderer V, Lass-Flörl C, Wilflingseder D (2022) How to optimize respiratory models for SARS-CoV-2 research. https://doi.org/10.26124/mitofit:2022-0004

Keywords: respiratory models; air-liquid interphase; SARS-CoV-2

Cite: Posch W, Dichtl S, Zaderer V, Lass-Flörl C, Wilflingseder D (2022) How to optimize respiratory models for SARS-CoV-2 research. In: https://doi.org/10.26124/bec:2022-0001

B-09

Bioblast link

Physiometabolic RTCA on an automated platform for short and long term applications.

Heichler C, Nagy M, Wolf Peter
INCYTON GmbH, Am Klopferspitz 19A, 82152 Planegg/Martinsried, Germany - peter.wolf@incyton.com

The CYRIS® analysis platform is a multi-sensory approach to extract a large amount of information from a single cell-based assay automatically and in real-time. To demonstrate its capabilities, we performed an in-vitro hepatotoxicity assay with Acetaminophen and HepG2, with simultaneous monitoring of the key parameters oxygen consumption rate (OCR), extracellular acidification rate (ECAR), impedance and microscopic imaging. After 12 hours prior treatment measurement, different concentrations of Acetaminophen were tested over 24 hours, followed by 12 hours washout. The metabolic results showed a strong time- and dose-dependent change of OCR and ECAR through Acetaminophen. Morphologic changes monitored by impedance and microscopic imaging underpin these metabolic effects. The washout of Acetaminophen results in cellular regeneration in all parameters up to a concentration of 10 mM. The continuous measurement of OCR, ECAR, impedance and microscopic imaging enables multiparametric monitoring of cellular metabolic responses due to Acetaminophen in a single assay and provides an overall picture of its hepatotoxic effects [1].

1. Heichler C, Nagy M, Wolf P (2022) Evaluation of hepatotoxic effects of acetaminophen on HepG2 cells by parallel real-time monitoring in a multi-sensor analysis platform for automated cell-based assays. https://doi.org/10.26124/mitofit:2022-0006

Keywords: label-free cell-based assays; oxygen consumption; extracellular acidification; cellular impedance; imaging; automation; hepatotoxicity; acetaminophen

Cite: Heichler C, Nagy M, Wolf P (2022) Physiometabolic RTCA on an automated platform for short and long term applications. In: https://doi.org/10.26124/bec:2022-0001

B-10

Bioblast link

Contextual tissue cytometry with artificial intelligence–functional single cell analyses *in-situ*.

Ecker Rupert[1,2]
1. TissueGnostics GmbH, Vienna, Austria
2. School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia

In September 2021 the United States' Food and Drug Administration (US-FDA) has approved the first artificial intelligence- (AI)-based decision support system for prostate cancer diagnostics. This hallmark indicates a historic decision as it is the first time in the history of medicine that a regulatory body has accepted a software-only solution, which analyses microscopic images by using artificial intelligence! This indicates both, that technologies reach a performance and maturity level that makes diagnostic routine applications not only possible but also feasible and that the market demand for such solutions has reached a level where it has become viable for industry to invest in the development of commercial solutions as a return on investment can be expected.

Our research teams at TissueGnostics and Queensland University of Technology have joined forces to combine TissueGnostics' existing tissue cytometry technology platform and established knowhow with innovative AI solutions to establish The Virtual Histopathologist.

Tissue Cytometry permits to determine the *in situ* phenotype of cells as well as histological entities, like glands, vessels or tumor foci. Applications include but are not limited to the exploration of the cellular/tumor microenvironment and/or the spatial organization of cellular subpopulations, assessment of different bone structures, quantification of blood vessels and neovascularization as well as analysis of samples in multiplexing or multispectral mode.

Earlier attempts to analyse single cells in tissue have mostly been subject to visual estimation, or – at best – to manual counting for decades. Hence, experts usually had the choice of the "least of evils" between guessing and endless (manual) counting. In (tumor) immunology, infiltrating inflammatory cells need to be phenotypically characterized on a quantitative basis. To better understand the function of inflammatory cells in tumor development, type and number of inflammatory cells and their proximity to glandular/tumor structures have to be analyzed *in situ* and correlated with disease state. Using TissueFAXS™ Cytometry the time-consuming and error-prone human evaluation of stained histological sections can be approached with an observer-independent and reproducible technology platform, offering a high degree of automation, paired with user interaction at relevant points of the analytical workflow. This platform can be applied as

a means of tissue cytometry for both immunofluorescence and immunohistochemistry and thus constitutes the microscopic equivalent to flow cytometry (FACS).

The TissueFAXS Cytometry platform incorporates Machine & Deep Learning algorithms and can be used in clinical multi-center studies to determine the immune response to certain drugs *in situ*, measure proliferation, apoptosis, cytokine expression, signalling molecules, and others. It can do end-point assays as well as live-cell imaging and time-kinetic experiments. TissueFAXS Cytometry also promotes tissue cytometry to a new level of quality, where complex cellular interactions can be addressed on the single-cell level but still in histological context.

Cite: Ecker R (2022) Contextual tissue cytometry with artificial intelligence–functional single cell analyses *in-situ.* In https://doi.org/10.26124/bec:2022-0001

B-11 *poster*

Bioblast link

A versatile mitochondria isolation- and analysis-pipeline generates 3D nano-topographies and mechano-physical surface maps of single organelles.

Joshi Saurabh§[1], Hater F§[1], Eirich J[2], Palovaara J[1], Ellinghaus H[1], Heinkow P[2], Callenius H[1], Peter A[1], Schweser O[1], Kubitschke M[1], Madduri MK[1], Mathew AJ[1], Ciacchi LC[4], Kirstein J[1&], Maedler K[1&], Masseck OA[1&], Finkemeier I[2], Radmacher M[3*], Groß-Hardt R[*1]

1. University of Bremen, Centre for Biomolecular Interactions, 28359 Bremen, Germany
2. Plant Physiology, Institute of Plant Biology and Biotechnology, University of Muenster, Schlossplatz 7, DE-48149 Muenster, Germany
3. University of Bremen, Institute of Biophysics, Otto-Hahn Allee 1, D-28359 Bremen, Germany
4. University of Bremen, Hybrid Materials Interfaces Group, Faculty of Production Engineering and Centre for Environmental Research and Sustainable Technology (UFT), 28359 Bremen, Germany

§ These authors contributed equally to this work
& These authors contributed equally to this work
Correspondence to: gross-hardt@uni-bremen.de, radmacher@uni-bremen.de

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Living eukaryotic cells typically contain large quantities of highly dynamic mitochondria, which sustain the cells' energy and redox homeostasis. Growing evidence suggests that mitochondria can functionally differ among but also within cells. The extent and biological significance of mitochondrial diversity is still largely unexplored, due to technical limitations that hamper profiling of individual organelles. Previous measurements of the cell's interior have shown that membrane-bound compartments respond to metabolic manipulation by changes in their surface stiffness, suggesting that mechano-physical properties are a valuable readout of mitochondrial function. We here present the establishment of a robust multi-step analysis pipeline that allows one to profile mechano-physical properties of single mitochondria at the nanoscale using Atomic Force Microscopy (AFM) [1]. Firstly, we developed a rapid cell-type specific isolation

protocol (mRACE), which selectively functionalizes mitochondria with biotin, facilitating isolation by streptavidin decorated microbeads. We established the technique for human and rat cell cultures, the invertebrate Caenorhabditis elegans, and the model plant Arabidopsis thaliana. Based on this versatile tool, we detected diversity of mitochondrially associated proteins among different tissues, reflecting the trophic condition of the source material. Secondly, a rapid filtration-based mitochondria isolation protocol was established, which was combined with mRACE. Lastly, we established an AFM analysis platform, which generates 3D maps of the nano-topography and mechano-physical properties of individual mitochondria. The comparison of mitochondria with each other revealed an unprecedented diversity in their mechano-physical properties and suggests that shape is not the sole determining parameter for mitochondrial outer membrane stiffness. We expect our results to not only introduce a new dimension for basic mitochondrial research, but in addition to open the door for the exploitation of individual mitochondria for diagnostic characterization.

1. Saurabh J et al (2021) A versatile mitochondria isolation- and analysis-pipeline generates 3D nano-topographies and mechano-physical surface maps of single organelles. https://doi.org/10.1101/2021.10.31.466655 - The present abstract is a copy from the preprint.

Cite: Saurabh J, Hater F, Eirich J, Palovaara J, Ellinghaus H, Heinkow P, Callenius H, Peter A, Schweser O, Kubitschke M, Madduri MK, Mathew AJ, Ciacchi LC, Kirstein J, Maedler K, Masseck OA, Finkemeier I, Radmacher M, Groß-Hardt R (2021) A versatile mitochondria isolation- and analysis-pipeline generates 3D nano-topographies and mechano-physical surface maps of single organelles. In: https://doi.org/10.26124/bec:2022-0001

B-12

Bioblast link

Effect of isolation protocol of skeletal muscle mitochondrial subpopulations on bioenergetic function.

Lai Nicola[1,2], Kummitha CM[2], Hoppel CL[3,4]

1. Dept of Mechanical, Chemical and Materials Engineering, Univ of Cagliari Italy
2. Dept of Biomedical Engineering, School of Medicine, Case Western Reserve Univ Cleveland USA
3. Dept of Pharmacology, School of Medicine, Case Western Reserve University Cleveland USA
4. Dept of Medicine, School of Medicine, Case Western Reserve University Cleveland USA. - nicola.lai@unica.it

The cardiac and skeletal muscle subsarcolemmal (SSmt) and interfibrillar (IFmt) mitochondria have different biochemical and structural properties affecting energy metabolism in health and disease states. In both muscles [1, 2], the method to isolate mitochondria affects the quality and quantity of the SSmt and IFmt separated by subcellular fractionation techniques. An isolation protocol for skeletal muscle SSmt and IFmt was proposed by our group [2] in which the mitochondrial yield was increased with a recovery close to 80 % of the mitochondria present in the original skeletal muscle sample; SSmt oxidative capacity was 10 % lower than that of IFmt; minor damage of the mitochondrial inner and outer membranes. A human study on skeletal muscle ultrastructure and bioenergetics showed a reduced mitochondrial oxidative capacity in

patients with type 1 diabetes (T1D) [3]. Nevertheless, it was not investigated the effect of the disease on the bioenergetics of the two subpopulations of mitochondria. In this work, we compare the bioenergetic characteristics of SSmt and IFmt with those of the whole mitochondrial population. This comparison was obtained for both control (C) and T1D rats.

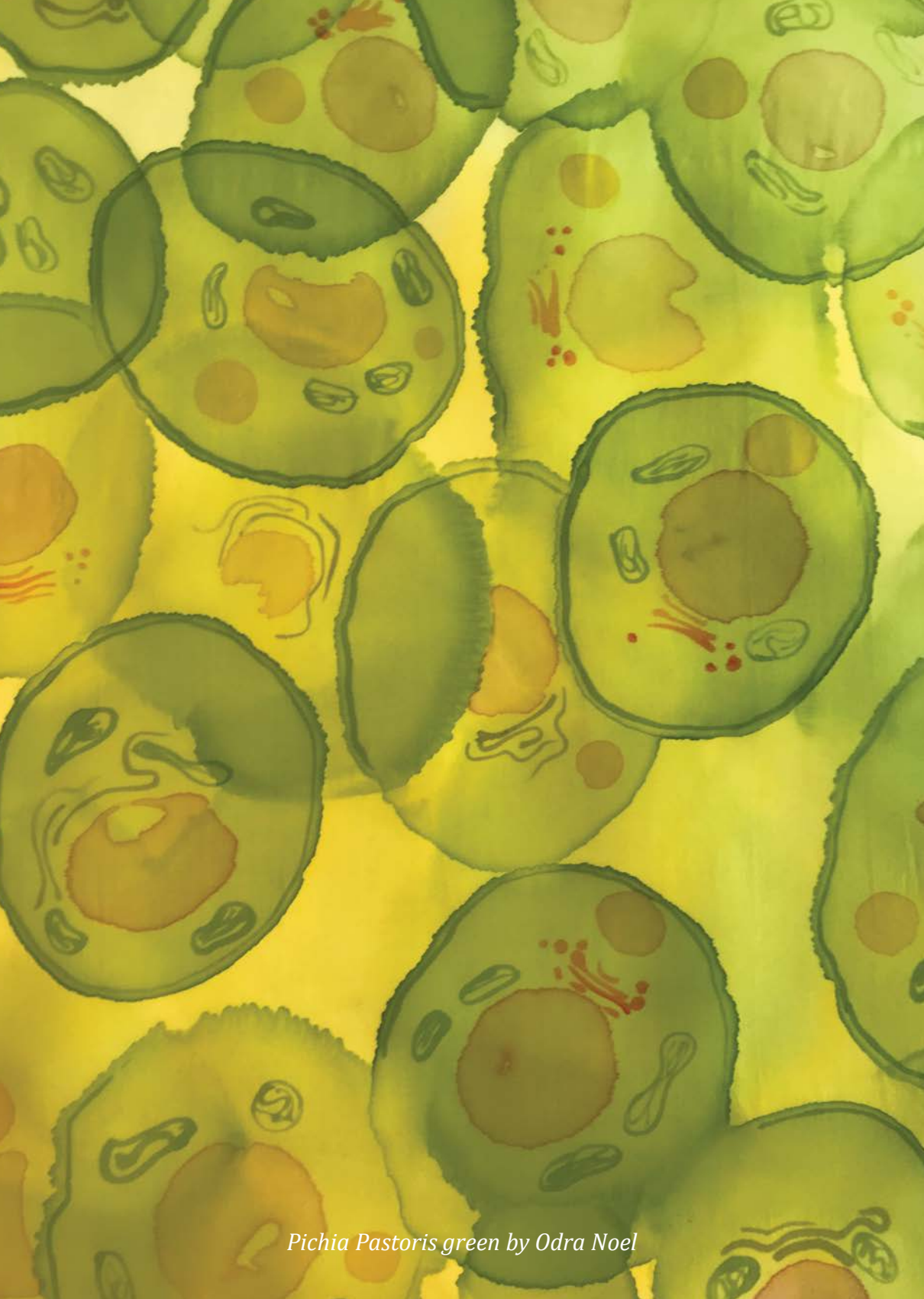
The T1D was obtained from Lewis rats treated with streptozotocin. We used our protocol [2] to isolate skeletal muscle SSmt and IFmt of C and T1D rats. The same protocol [2] was modified to isolate the whole population (Wmt) of skeletal muscle mitochondria of C and T1D rats. The oxidative phosphorylation rate was measured with a digitized polarographic system [4] with substrates of the electron transfer system: palmitoyl carnitine, palmitoyl-CoA, glutamate, and succinate.

The yields of SSmt and IFmt of T1D rats (1.5±0.4; 3.5±1 mg/g) from rat skeletal muscle were lower than those of the control group (2±0.5; 5.5±0.5 mg/g). In contrast, the yield of the whole population of mitochondria was similar in both groups of rats (C 7±0.9; 6.8±0.5 mg/g). OXPHOS capacity *P* was measured in presence of glutamate as substrate with kinetically saturating concentration (2 mM) of ADP and of the uncoupler dinitrophenol (0.2 mM, DNP). The oxidative phosphorylation assay showed that in the C group the OXPHOS capacity was lower in SSmt (Wmt 4200±250; *SSmt 3200±200; IFmt 3800±150 pmol·s^{-1}·mg^{-1}, P<0.01) and a similar adenylate acceptor control ratio P/L (Wmt 18±4; SSmt 21±3; IFmt 30±4); in T1D group the OXPHOS capacity was lower in SSmt and IFmt (Wmt 4050±260; *SSmt 2000±230; *,#IFmt 2600±250 pmol·s^{-1}·mg^{-1}, *P<0.01 different from Wmt group, # different from SSmt) and a similar P/L (Wmt 14±2; SSmt 14±3; IFmt 16±6). The electron transfer capacity upon collapsing of mitochondrial potential with the uncoupler DNP was for C rats (Wmt 5040±220; *SSmt 3950±190; IFmt 4600±180 pmol·s^{-1}·mg^{-1}, P<0.01) and for T1D rats (Wmt 5200±330; *SSmt 2400±300; *,#IFmt 3000±400 pmol·s^{-1}·mg^{-1}, P<0.01 different from Wmt group, # different from SSmt). For both C and T1D groups of rats, respiration rate difference between SSmt and IFmt were also observed in presence of palmitoyl carnitine and palmitoyl-CoA substrates.

The results of this study provide evidence of bioenergetic differences between the whole population and subpopulations of mitochondria. This work underlines that skeletal muscle bioenergetic differences may not be properly detected if both mitochondrial subpopulations are not isolated and biochemically characterized.

1. Palmer JW, Tandler B, Hoppel CL (1977) Biochemical properties of subsarcolemmal and interfibrillar mitochondria isolated from rat cardiac muscle. https://www.jbc.org/article/S0021-9258(19)75283-1/pdf
2. Lai N, M Kummitha C, Rosca MG, Fujioka H, Tandler B, Hoppel CL (2019) Isolation of mitochondrial subpopulations from skeletal muscle: optimizing recovery and preserving integrity. https://doi.org/10.1111/apha.13182
3. Monaco CMF, Hughes MC, Ramos SV, Varah NE, Lamberz C, Rahman FA, McGlory C, Tarnopolsky MA, Krause MP, Laham R, Hawke TJ, Perry CGR (2019) Altered mitochondrial bioenergetics and ultrastructure in the skeletal muscle of young adults with type 1 diabetes. https://doi.org/10.1007/s00125-018-4602-6
4. Potter L, Krusienski D, Kennedy J, Hoppel CL, Lai N (2020) Integrated approach for data acquisition, visualization and processing of analog polarographic systems for bioenergetics studies. https://doi.org/10.1016/j.ab.2019.113515

Cite: Lai N, Kummitha CM, Hoppel CL (2022) Effect of isolation protocol of skeletal muscle mitochondrial subpopulations on bioenergetic function. In: https://doi.org/10.26124/bec:2022-0001



Pichia Pastoris green by Odra Noel

C - Algal bioenergetics

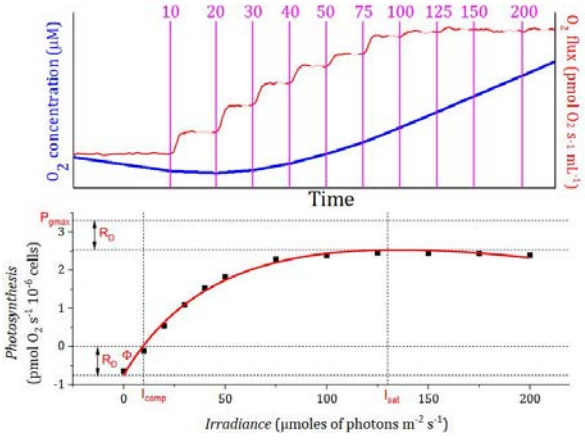
C-01

Bioblast link

Application of the NextGen-O2k for building photosynthesis-irradiance curves in microalgae.

Vera Vives AM, Perin G, Morosinotto Tomas
Department of Biology, University of Padova, Padova, Italy - tomas.morosinotto@unipd.it

This work was part of the Oroboros NextGen-O2k project, with partial funding from the European Union's Horizon 2020 research and innovation programme under grant agreement nº 859770.



The rate of oxygen evolution provides valuable information on the metabolic status and the photosynthetic performance of a cell, and it can be quantified by means of a photosynthesis-irradiance (PI) curve. Up to now, the construction of PI curves of unicellular organisms based on oxygen evolution has been difficult and time consuming due to the lack of sensitive instruments. Here we describe the setup of a reproducible method using the Oroboros NextGen-O2k equipped with the PhotoSynthesis-Module [1] for constructing PI curves based on oxygen evolution using low amounts of sample in the microalga *Nannochloropsis gaditana*, easily translatable to other algal species [2].

1. Went N, Di Marcello M, Gnaiger E (2021) Oxygen dependence of photosynthesis and light-enhanced dark respiration studied by High-Resolution PhotoRespirometry. https://doi.org/10.26124/mitofit:2021-0005
2. Vera-Vives AM, Perin G, Morosinotto T (2022) The robustness of NextGen-O2k for building PI curves in microalgae. https://doi.org/10.26124/mitofit:2022-0019

Keywords: photosynthesis; microalgae; oxygen evolution

Cite: Vera-Vives AM, Perin G, Morosinotto T (2022) Application of the NextGen-O2k for building photosynthesis-irradiance curves in microalgae. In: https://doi.org/10.26124/bec:2022-0001

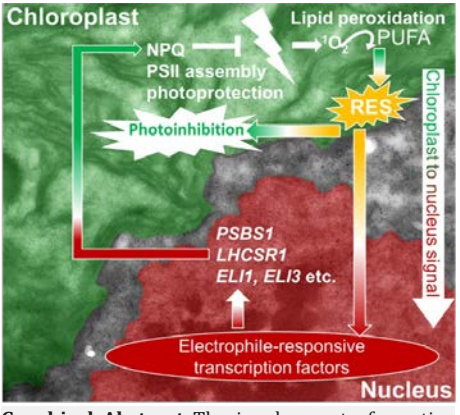
C-02

Bioblast link

Retrograde signalling during high light stress involves reactive carbonyl / electrophile species.

Roach Thomas
Department of Botany, University of Innsbruck, Austria - thomas.roach@uibk.ac.at

Singlet oxygen ($^1O_2$) is one of the most reactive of all reactive oxygen species (ROS), which is produced by chlorophyll during photosynthesis, and induces transcriptional changes in the nucleus, far from its reach. Here, using combined molecular, biochemical and physiological approaches, evidence is presented for how reactive carbonyl/electrophile species (RES) are involved in the acclimation of the model unicellular green alga *Chlamydomonas reinhardtii* to high light. Light stress led to an accumulation of RES, such as 2-propenal (acrolein) and 4-hydroxynonenal (HNE) [1,2], which are released when ($^1O_2$-derived lipid peroxides break down [3]. Treating cells with RES stimulated similar responses to high light, including higher tolerance to ($^1O_2$, similar patterns of protein carbonylation, and increased levels of glutathione [1,4], an important antioxidant involved in RES detoxification. A RNA seq. analysis revealed clear overlaps in gene regulation between RES-treated and high light-treated cells, which included an upregulation of many antioxidant enzymes and redox-related processes, as well as carotenoid and ubiquinone biosynthesis. However, most prominent was the overlap in down-regulated genes, whereby 70 % of the down-regulated genes under high light were also down-regulated by RES [1]. Moreover, the majority of these were shared when cells were treated with the photosenstizer Rose Bengal as an exogenous ($^1O_2$ source, confirming the specific role of



Graphical Abstract The involvement of reactive electrophile species (RES) in light stress responses of *Chlamydomonas reinhardtii*. Excess light increases the formation of singlet oxygen ($^1O_2$) from photosystem reaction centres in the chloroplast (green), which can induce lipid peroxidation of the thylakoid membrane lipids. Lipid peroxides decay to release RES (orange) that attack chloroplastic proteins, leading to protein carbonylation, but are also sensed by specific nuclear transcription factors (red), such as SOR1, whereby RES act as chloroplast-to-nucleus retrograde signals. Transcriptional alteration includes up-regulation of transcripts (white italics; black arrows) encoding mechanism that are involved in ROS and RES detoxification (*GSTS1, FSD1, NTR3*), including increasing glutathione (GSH1) and ascorbate (*VTC2*) contents, protecting proteins (*HSP22, GRX2, GSTS1*) and also mitigating excess light energy (LHCSR1, PSBS), thereby reducing light stress and RES formation. This pathway is superimposed over a false-coloured electron micrograph of an algal cell. The non-coloured region is the cytoplasm.

($^1O_2$ in RES signalling under high light stress. Finally, a comparison to differential gene expression in response to H_2O_2 revealed that half of the genes were also differentially expressed in the same direction after treatment with HNE, the only RES that increased upon treatment of cells with the same concentration of H_2O_2. Therefore, HNE is also a possible pathway for H_2O_2-mediated signalling.

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2. Roach T, Na CS, Stöggl W, Krieger-Liszkay A (2020) The non-photochemical quenching protein LHCSR3 prevents oxygen-dependent photoinhibition in Chlamydomonas reinhardtii. https://doi.org/10.1093/jxb/eraa022
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4. Roach T, Baur T, Stöggl W, Krieger-Liszkay A (2017) Chlamydomonas reinhardtii responding to high light: a role for 2-propenal (acrolein). https://doi.org/10.1111/ppl.12567

Keywords: photosynthesis; chloroplast; light stress; retrograde signalling; reactive oxygen species

***Cite*:** Roach T (2022) Retrograde signalling during high light stress involves reactive carbonyl / electrophile species. In: https://doi.org/10.26124/bec:2022-0001

C-03 *poster*

Bioblast link

Oxygen dependence of photosynthesis and light-enhanced dark respiration studied by high-resolution PhotoRespirometry.

Went N, Di Marcello M, Gnaiger Erich
Oroboros Instruments, Innsbruck, Austria – erich.gnaiger@oroboros.at

This work was part of the Oroboros NextGen-O2k project, with partial funding from the European Union's Horizon 2020 research and innovation programme under grant agreement nº 859770.

Algal biotechnology has emerged as a high-potential industry for efficient and CO_2-neutral production of biomass providing biofuels, food and feed, and a variety of carbon-based chemicals and pharmaceuticals. Algal metabolism is directly involved in the regulation of growth, cell concentration, and biosynthesis of biotechnologically-relevant phytochemicals such as vitamins, antioxidants, and immune response boosters. Photoautotrophic growth rates of algae are based on light-to-chemical energy conversion and CO_2 fixation, and any optimization of biomass production requires maximizing energy-use efficiency of photosynthesis and respiration, both of which vary as a function of light intensity. As such, the bioenergetic crosstalk between mitochondria and chloroplasts plays a key role in maintaining metabolic integrity and controlling intermediary metabolite production.

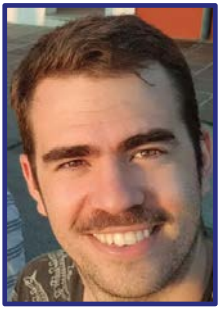
In the present study, we investigated how photosynthetic O_2 production and respiratory O_2 consumption was influenced as a function of light intensity, O_2 concentration, and culture density in the unicellular model green alga *Chlamydomonas reinhardtii*. Cultures were grown photoautotrophically in a modified Tris-Phosphate growth medium (TRIS, N- and P-nutrient replete) at 25 °C, pH 7.0, and light intensity of 100 µmol photons·s^{-1}·m^{-2} (16:8 h light:dark cycle). Kinetics of light-induced O_2 production

and dark respiration of these microalgae was measured under culture conditions and three cell concentrations, while varying O_2 concentrations in the Oroboros NextGen-O2k equipped with the PhotoBiology-Module [1] during stepwise increases of blue actinic light from from 10 to 350 µmol·s^{-1}·m^{-2}, followed by darkness, again at various controlled O_2 concentrations. Maximum net photosynthesis was inhibited by 40 % at hyperoxic O_2 concentrations of 550 to 650 µM, when ROS production is known to be increased [2,3]. Transient light-enhanced dark respiration [4] peaked within 30 to 60 s after light-dark transitions and was 3.5- to 4-fold higher than steady-state dark respiration independent of O_2 concentration in the range of 200 to 650 µM.

We conclude that high-resolution photorespiratory analysis provides a new method to investigate the oxygen kinetics of O_2 production and O_2 consumption that reveal interactions of chloroplasts and mitochondria under precisely regulated experimental light and oxygen regimes.

1. Went N, Di Marcello M, Gnaiger E (2021) Oxygen dependence of photosynthesis and light-enhanced dark respiration studied by High-Resolution PhotoRespirometry. https://doi.org/10.26124/mitofit:2021-0005
2. Komlódi T, Sobotka O, Gnaiger E (2021) Facts and artefacts on the oxygen dependence of hydrogen peroxide production using Amplex UltraRed. https://doi.org/10.26124/bec:2021-0004
3. Shimakawa G, Kohara A, Miyake C (2020) Characterization of light-enhanced respiration in cyanobacteria. https://doi.org/10.3390/ijms22010342

Cite: Went N, Di Marcello M, Gnaiger E (2022) Oxygen dependence of photosynthesis and light-enhanced dark respiration studied by high-resolution PhotoRespirometry. In: https://doi.org/10.26124/bec:2022-0001



C-04 *poster*

Bioblast link

Consequences of the inactivation of Complex I and Complex IV in the plant model *Physcomitrium patens*.

Vera-Vives Antoni M[1], Mellon M[1], Zheng K[2], Schwarzländer M[2], Alboresi A[1], Morosinotto T[1]

1. Department of Biology, University of Padova (Padova, Italy) – antonimateu.veravives@phd.unipd.it
2. Institute of Plant Biology and Biotechnology (IBBP), Westfälische Wilhelms-Universität Münster (Münster, Germany)

This work was part of the Oroboros NextGen-O2k project, with partial funding from the European Union's Horizon 2020 research and innovation programme under grant agreement nº 859770.

Photosynthetic organisms use light as the main source of energy to support their metabolism, but respiration is needed to support metabolism during the night (i.e. when light is absent) and in non-photosynthetic tissues such as roots and seeds. Respiration, however, is also active under illumination, tightly interconnected with photosynthesis.

Despite their biological relevance, the molecular pathways connecting photosynthetic and respiratory metabolism are far from clear. A major limitation for the advancement of knowledge in this field has been the lack of viable mutants with depleted respiration, as they are often lethal in plants.

To overcome this problem, the moss *Physcomitrium patens*, which can be vegetatively propagated from photosynthetic tissues, was used as a model to generate mutants with depleted respiration. We isolated lines without a functional Complex I (*ndufa5* KO) and without a functional Complex IV (*cox11* KO), Figure 1. We used the newly developed NextGen-O2k with the PhotoBiology-Module [1] to quantify O_2 consumption and evolution of intact plant tissues, exploiting the properties of *P. patens*.

The mutants show impaired growth, an unbalanced carbon metabolism and a rearrangement of the respiratory transfer system. The ultrastructure of mitochondria is altered, and the mutants have different cytosolic ATP dynamics. Despite not showing drastic differences in the composition of the photosynthetic apparatus, the respiratory mutants are photosynthetically less efficient, with reduced rates of both net CO_2 fixation and net O_2 evolution.

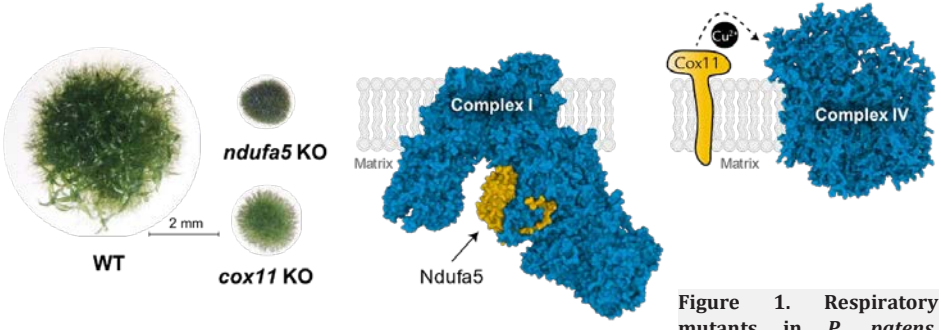


Figure 1. Respiratory mutants in *P. patens*. Ndufa5 is a structural subunit of CI, while Cox11 is an assembly factor of CIV. Both *ndufa5* KO and *cox11* KO show a strong growth impairment.

Our data confirm the importance of mitochondria and respiration in photosynthetic performance. Further studies on these mutants could led us to identify key players in the interaction between chloroplasts and mitochondria, and potential targets to optimize photosynthesis by genetic engineering.

1. Went N, Di Marcello M, Gnaiger E (2021) Oxygen dependence of photosynthesis and light-enhanced dark respiration studied by High-Resolution PhotoRespirometry. https://doi.org/10.26124/mitofit:2021-0005

Keywords: respiration; photosynthesis; plant mitochondria; photobiology

Cite: Vera-Vives AM, Mellon M, Zheng K, Schwarzländer M, Alboresi A, Morosinotto T (2022) Consequences of the inactivation of Complex I and Complex IV in the plant model *Physcomitrium patens*. In: https://doi.org/10.26124/bec:2022-0001

The ghost of Krebs by Odra Noel

D - Thermodynamic, kinetic, and molecular advances in bioenergetics

D-01

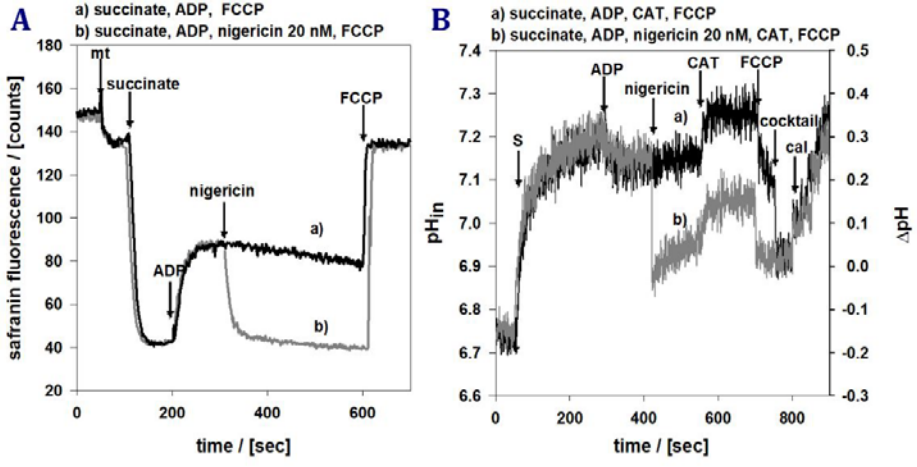
Bioblast link

The protonmotive force – not merely membrane potential.

Komlódi Timea, Tretter L
Department of Biochemistry, Semmelweis University, Budapest, Hungary

The protonmotive force *pmF* establishes the link between electrical and chemical components of energy transformation and coupling in oxidative phosphorylation in the mitochondrial electron transfer system. The electrical part is corresponding to the mitochondrial membrane potential $\Delta\Psi_{mt}$ and the chemical part is related to the transmembrane pH gradient ΔpH. Although the contribution of ΔpH to *pmF* is smaller than that of $\Delta\Psi_{mt}$, ΔpH plays an important role in mitochondrial transport processes and regulation of reactive oxygen species production. Separate measurement of $\Delta\Psi_{mt}$ and ΔpH allows for calculation of *pmF*. Methods for monitoring $\Delta\Psi_{mt}$ such as fluorescence dyes are generally available, while determination of ΔpH is more challenging.

In this review, we focus on the application of the fluorescence ratiometric method using the acetoxymethyl ester form of 2,7-biscarboxyethyl-5(6)-carboxyfluorescein



(BCECF/AM) for real-time monitoring of the intramitochondrial pH in isolated mitochondria. Knowing the intra- and extramitochondrial pH allows for calculating the ΔpH. Application of specific ionophores such as nigericin or valinomycin, exerts the possibility to dissect the two components of the *pmF* in different directions. Furthermore,

we tried to summarize those mitochondrial processes, such as production of reactive oxygen species, where the ΔpH has an important role [1].

1. Komlódi T, Tretter L (2022) The protonmotive force – not merely membrane potential. https://doi.org/10.26124/mitofit:2022-0012

Keywords: BCECF; intramitochondrial pH; matrix pH; ΔpH; mitochondria; mitochondrial membrane potential, $\Delta\Psi_{mt}$; nigericin; protonmotive force, *pmF*; reverse electron transfer, RET; safranin; triphenylphosphonium, TPP^+; valinomycin

Cite: Komlódi T, Tretter L (2022) The protonmotive force – not merely membrane potential. In: https://doi.org/10.26124/bec:2022-0001

D-02 *poster*

Bioblast link

Energy metabolism regulation by mitochondrial Ca^{2+} transport in the liver.

Vilas-Boas Eloísa A, Cabral-Costa JV, Ramos VM, Caldeira da Silva CC, Kowaltowski AJ

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brazil - elovilasboas@usp.br

Mitochondria are a major hub for Ca^{2+} handling and energy metabolism, however, the precise relationship between these two processes under physiologically relevant conditions in the liver is still not completely clear. Mitochondria can actively take up large amounts of Ca^{2+}, thereby acting as important intracellular Ca^{2+} buffers and affecting cytosolic Ca^{2+} transients [1]. Ca^{2+} uptake across the mitochondrial inner membrane into the matrix occurs through the mitochondrial Ca^{2+} uniporter complex (MCU), and Ca^{2+} efflux occurs through the Na^+/Ca^{2+} exchanger (NCLX). Mitochondria participate in crosstalk signals with the endoplasmic reticulum (ER), through microdomains formed between both organelles in which ions exchange mediates inter-organelle communication [2]. Excessive mitochondrial matrix Ca^{2+} is known to be deleterious due to opening of the mitochondrial permeability transition pore (mtPTP) and consequent membrane potential dissipation, leading to mitochondrial swelling, rupture and cell death [3]. But moderate Ca^{2+} within the organelle can directly or indirectly activate mitochondrial matrix enzymes, possibly impacting on ATP production [4]. However, direct studies so far are limited to uncovering the modulation of isolated enzyme activity, and only show increases in substrate affinity, not maximal velocity. The specific effects of changes in the affinity of these enzymes on overall oxidative phosphorylation in vivo have not been determined.

We explore this gap here, to determine if extra or intramitochondrial Ca^{2+} modulate oxidative phosphorylation in the liver. We used isolated mouse liver mitochondria and living AML12 hepatocytes, and measured oxygen consumption under different conditions using Oroboros O2k and Seahorse Extracellular Flux systems, respectively.

We found that isolated mitochondria present increased respiratory control ratios (1-*L*/*P*, a measure of oxidative phosphorylation efficiency) when incubated with low (2.4 ± 0.6 μM) and medium (22.0 ± 2.4 μM) Ca^{2+} concentrations in the presence of NADH-

linked substrates pyruvate & malate & α-ketoglutarate, respectively, but not Complex II-linked succinate. We investigated next if the increase in oxidative phosphorylation efficiency observed was dependent on mitochondrial Ca^{2+} uptake or cycling, using pharmacological inhibitors of the MCU (Ruthenium red, RuRed), which prevents Ca^{2+} uptake, and of the NCLX (CGP-37157, CGP), which prevents Ca^{2+} extrusion from mitochondria. Both RuRed and CGP reversed the increase in 1-L/P promoted by Ca^{2+} in the presence of pyruvate & malate & α-ketoglutarate, indicating that Ca^{2+} must enter the mitochondrial matrix to exert this effect. Interestingly, in living AML12 hepatocytes, both the decrease of cytosolic Ca^{2+} by chelation with BAPTA-AM and the increase of cytosolic Ca^{2+} due to thapsigargin (TG)-induced ER Ca^{2+} depletion led to decreased respiratory rates. In addition, we show that the decrease of mitochondrial respiration in the presence of high Ca^{2+} can be modulated by cytosolic Ca^{2+} chelation, inhibition of Ca^{2+} entry into the mitochondrial matrix with RuRed and MCU siRNA, or inhibition of mtPTP formation with cyclosporin A.

Overall, our results uncover a Goldilocks effect of Ca^{2+} on liver mitochondria, with specific "just right" concentrations that activate oxidative phosphorylation.

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4. Denton RM (2009) Regulation of mitochondrial dehydrogenases by calcium ions. https://doi.org/10.1016/j.bbabio.2009.01.005

Keywords: calcium transport; mitochondria; electron transfer system; oxidative phosphorylation; metabolic flux

Cite: Vilas-Boas EA, Cabral-Costa JV, Ramos VM, Caldeira da Silva CC, Kowaltowski AJ (2022) Energy metabolism regulation by mitochondrial Ca^{2+} transport in the liver. In: https://doi.org/10.26124/bec:2022-0001

D-03 *poster*

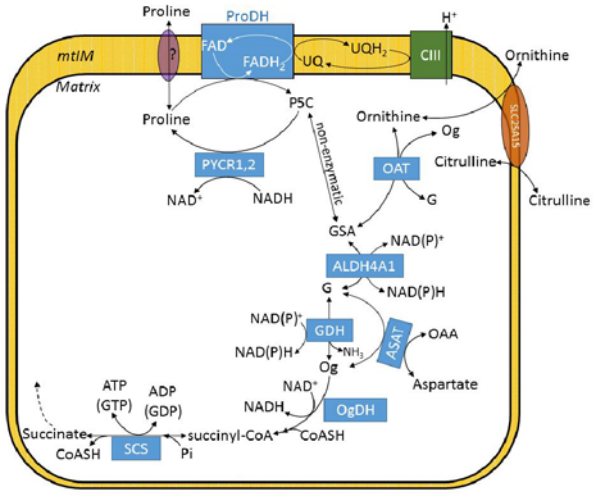
Bioblast link

Proline oxidation supports mitochondrial ATP production when Complex I is inhibited.

Pallag Gergely[1], Nazarian S[1], Ravasz D[1], Bui D[1], Komlódi T[1,2], Doerrier C[2], Gnaiger E[2], Seyfried TN[3], Chinopoulos C[1]

1. Dept of Biochemistry and Molecular Biology, Semmelweis Univ, 1094 Budapest, Hungary - pallag.gergely@semmelweis-univ.hu
2. Oroboros Instruments, 6020 Innsbruck, Austria
3. Biology Dept, Boston College, Chestnut Hill, MA 02467, USA

This work was supported by grants from NKFIH ([TKP2021-EGA-25], FIKP-61822-64888-EATV, VEKOP 2.3.3-15-2016-00012, 2017-2.3.4-TET-RU-2017-00003, KH129567, and K135027) to C.C. and from the project NextGen-O2k (Oroboros Instruments) which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 859770.



The oxidation of proline to pyrroline-5-carboxylate (P5C) leads to the transfer of electrons to ubiquinone in mitochondria that express proline dehydrogenase (ProDH). This electron transfer supports Complexes CIII and CIV, thus generating the protonmotive force. Further catabolism of P5C forms glutamate, which fuels the citric acid cycle that yields the reducing equivalents that sustain oxidative phosphorylation. However, P5C and glutamate catabolism depend on CI activity due to NAD^+ requirements.

NextGen-O2k (Oroboros Instruments) was used to measure proline oxidation in isolated mitochondria of various mouse tissues. Simultaneous measurements of oxygen consumption, membrane potential, NADH, and the ubiquinone redox state were correlated to ProDH activity and F_1F_O-ATPase directionality.

Proline catabolism generated a sufficiently high membrane potential that was able to maintain the F_1F_O-ATPase operation in the forward mode. This was observed in CI-inhibited mouse liver and kidney mitochondria that exhibited high levels of proline oxidation and ProDH activity. This action was not observed under anoxia or when either CIII or CIV were inhibited. The duroquinol fueling of CIII and CIV partially reproduced the effects of proline. Excess glutamate in the presence of malate, however, could not reproduce the proline effect, suggesting that processes upstream of the glutamate conversion from proline were involved. The ProDH inhibitors tetrahydro-2-furoic acid and, to a lesser extent, S-5-oxo-2-tetrahydrofurancarboxylic acid abolished all proline effects.

The data show that ProDH-directed proline catabolism could generate sufficient CIII and CIV proton pumping, thus supporting ATP production by the F_1F_O-ATPase even under CI inhibition [1].

1. Pallag G, Nazarian S, Ravasz D, Bui D, Komlódi T, Doerrier C, Gnaiger E, Seyfried TN, Chinopoulos C (2022) Proline oxidation supports mitochondrial ATP production when Complex I is inhibited. https://doi.org/10.3390/ijms23095111

Keywords: proline dehydrogenase; substrate-level phosphorylation; coenzyme Q; reducing equivalent

Cite: Pallag G, Nazarian S, Ravasz D, Bui D, Komlódi T, Doerrier C, Gnaiger E, Seyfried TN, Chinopoulos C (2022) Proline oxidation supports mitochondrial ATP production when Complex I is inhibited. In: https://doi.org/10.26124/bec:2022-0001

D-04 *poster*

Bioblast link

Mitonuclear interactions impact responses to metabolic and redox stress at different life stages in *Drosophila melanogaster*.

Rodriguez Enrique, Inwongwan S, Grover TF, Camus F, Lane N
Dept of Genetics, Evolution and Environment, University College London - enrique.rodriguez@ucl.ac.uk

Mitochondrial function depends on direct interactions between respiratory proteins encoded by genes in two genomes, mitochondrial and nuclear, which evolve in very different ways. Serious incompatibilities between these genomes can have severe effects on development, fitness and viability. The effect of subtle mitonuclear mismatches has received less attention, especially when subject to mild physiological stress. We investigate how various stressors affect phenotypic traits, mitochondrial function, metabolic pathways and gene expression in *Drosophila* larvae, and adults of both sexes. Flies fed either a high protein diet, the glutathione precursor N-acetyl cysteine (NAC), or the NADH precursor nicotinamide riboside (NR) and had sex, life-stage, and genotype-specific responses to these stressors. Metabolomic results point to changes in TCA cycle flux, while respirometry analysis shows changes in substrate use and complex I function. Our results support the notion that subtle mitonuclear mismatches can lead to diverging responses to mild physiological stress, undermining fitness in some cases, but surprisingly improving outcomes in other mismatched fly lines.

Keywords: mitonuclear interactions; *Drosophila melanogaster*; larvae; diet; stress

Cite: Rodriguez E, Inwongwan S, Grover TF, Camus F, Lane N (2022) Mitonuclear interactions impact responses to metabolic and redox stress at different life stages in *Drosophila melanogaster*. In: https://doi.org/10.26124/bec:2022-0001

D-05 *poster*

Bioblast link

$NAD(P)^+$/NAD(P)H pool and the art of mitochondrial survival.

Pallotta Maria Luigia
Dept Medicine and Health Sciences, Molise University, 86100 Campobasso, IT - pallottamarialuigia@gmail.com

Shift in energy metabolism offers immediate potential for intervention and thereby control of entire biochemical networks by identifying and targeting key control points both in physiological and pathological issues. Increasing evidence suggests that the pyridine nucleotide $NAD(P)^+$ has many more extensive biological functions than its classical role in energy metabolism. Hundreds of enzymes that catalyze substrate

oxidation use coenzyme NAD(P)$^+$. Thus it plays a key role in various biological processes such as aging, oxidative stress, epigenetics, immunological response, cell death, and much more.

In 2007 Sinclair' group [1] referred to the ability of mitochondria to dictate cell survival as "mitochondrial oasis effect" which states that the energetic and NAD(P)$^+$ content of mitochondria determines cell survival in face of genotoxic stress (i.e. DNA damage). Still not everything is clear about NAD(P)$^+$ biosynthesis in mitochondria. However, *de novo* and salvage pathways contribute to the biosynthesis of NAD(P)$^+$ in all organisms and both converge at the transfer of nicotinamide mononucleotide (NMN) or nicotinic acid mononucleotide (NaMN) on to the adenylyl group of ATP under diphosphate release (NMN+ATP↔NAD$^+$+PPi). Given that NMN is a potent inhibitor of the NAD-dependent DNA ligase, nicotinamide mononucleotide adenylyltransferase (NMNAT) activity would scavenge NMN, ensuring at the same time NAD$^+$ supply to the ligase reaction. Thus, NMN was added to different eukaryotic cells bioenergetically active (from yeast, plant, and mammalian cancer cells). Mitochondria were prepared starting from cells grown aerobically and analysed according to Pallotta et al (2004) [2]. NAD(P)$^+$ biosynthesis was tested by HPLC and spectroscopically [3].

Thus, our results suggest, that mitochondria can increase NAD(P)$^+$ content, probably via NMNAT and NADKinase and this "core" mitochondrial NAD(P)$^+$ pathway should be studied further — i.e. its regulation with a cocktail of ad hoc inhibitors — as a basis for future biotechnological applications and biomedicine studies in treating disorders with perturbed NAD(P)$^+$ supply or homeostasis (viz neurological, immunological and metabolic clinically oriented studies).

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Keywords: bioenergetics; NAD(P)$^+$/NAD(P)H+[H$^+$]; mitochondrial pool; cellular homeostasis; DNA damage; nicotinamide mononucleotide (NMN)

Cite: Pallotta ML (2022) NAD(P)$^+$/NAD(P)H pool and the art of mitochondrial survival. In: https://doi.org/10.26124/bec:2022-0001

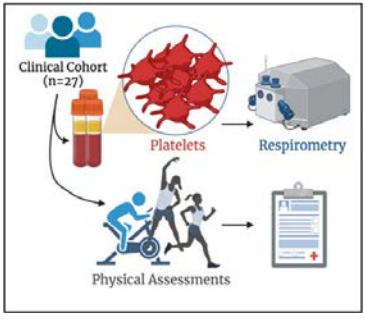
D-06

Bioblast link

Platelet bioenergetics are associated with resting metabolic rate and exercise capacity in older women.

Heimler SR[1], Phang HJ[1], Bergstrom J[1], Mahapatra G[2], Dozier S[1], Gnaiger E[3], Molina Anthony JA[1]

1. Division of Geriatrics, Gerontology, and Palliative Care; Department of Medicine, University of California San Diego School of Medicine, La Jolla, CA, USA - ajmolina@health.ucsd.edu
2. Section on Gerontology and Geriatrics, Sticht Center for Healthy Aging and Alzheimer's Prevention, Department of Internal Medicine, Wake Forest Baptist Medical Center, Winston-Salem, NC, USA
3. Oroboros Instruments, Innsbruck, Austria



This study investigates relationships between platelet mitochondrial bioenergetics and resting metabolic rate (RMR), body composition, and exercise fitness in women over 60 years of age. We report positive correlations between peak respiratory exchange ratio (RER) and RMR with five measures of platelet respiration, supporting the premise that blood cells can be utilized to report on mitochondrial function associated with physical health and fitness. Identifying mechanisms associated with physical performance among older adults supports the development of reliable biomarkers of healthy aging and can advance the development of efficacious interventions [1].

1. Heimler SR, Phang HJ, Bergstrom J, Mahapatra G, Dozier S, Gnaiger E, Molina AJA (2022) Platelet bioenergetics are associated with resting metabolic rate and exercise capacity in older women. https://doi.org/10.26124/mitofit:2022-0007

Keywords: platelets; resting metabolic rate RMR; cardiopulmonary exercise testing; OXPHOS capacity *P*; electron transfer capacity *E*; inverted regression analysis

Cite: Heimler SR, Phang HJ, Bergstrom J, Mahapatra G, Dozier S, Gnaiger E, Molina AJA (2022) Platelet bioenergetics are associated with resting metabolic rate and exercise capacity in older women. In: https://doi.org/10.26124/bec:2022-0001

D-07

Bioblast link

Exercise training-induced enhancement of electron flow in the OXPHOS system is more important than increasing the OXPHOS machinery content to improve ATP generation in human skeletal muscle.

Granata Cesare[1,2]

1. Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research, Heinrich Heine Univ, 40225 Düsseldorf, Germany
2. German Center for Diabetes Research, Partner Düsseldorf, München-Neuherberg, Germany. – cesare.granata@ddz.de

Mitochondrial health is implicated in multiple diseases and ageing, and is therefore an important determinant of an individual's quality of life [1]. Exercise training is an accessible and inexpensive therapeutic intervention that is extensively used to prevent, treat, and manage several lifestyle diseases [2], by enhancing mitochondrial biogenesis and improving mitochondrial bioenergetics. However, the intricacy of exercise training-induced mitochondrial adaptations remains, for the most part, unknown.

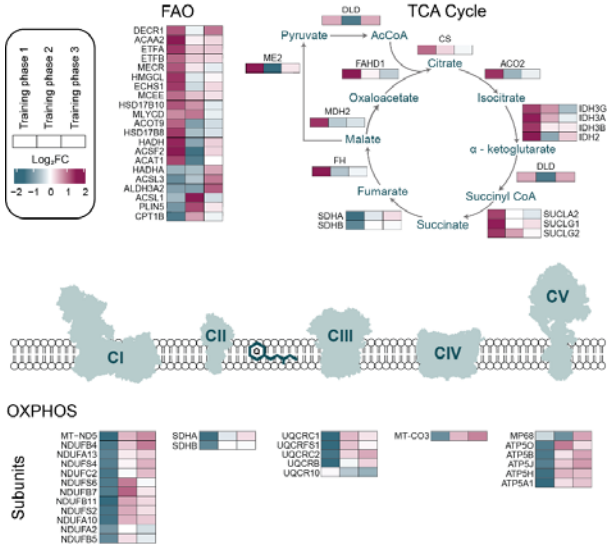


Figure 1: Overview of training-induced changes in mitochondrial protein functional classes related to ATP generation. Row clustering determined by unsupervised hierarchical cluster analysis. FAO fatty acid β-oxidation, TCA tricarboxylic acid cycle, OXPHOS oxidative phosphorylation.

By utilizing a multi-omics approach integrated with classic biological mitochondrial techniques, an in-depth investigation of the effects of three different and sequential volumes of high-intensity interval training on the mitochondrial transcriptome, proteome, and lipidome was performed in human skeletal muscle (*N*=10) [3].

Changes in mitochondrial respiration, enzymatic activity, supercomplex formation, and the content of selected subunits of the OXPHOS system mirrored, for the most part, changes in training volume, and were driven by the overall increase in mitochondrial content, as previously demonstrated [4]. Subsequently, by combining the power of 3 omics techniques with biochemical and in silico normalization, the bias arising from the training-induced increase in mitochondrial content was removed to unearth an intricate and previously undemonstrated network of differentially prioritized mitochondrial adaptations. These findings indicate that enhancing electron flow in the OXPHOS system is more important to improve ATP generation than increasing the abundance of the OXPHOS machinery, and do not support the hypothesis that training-induced supercomplex reorganization enhances mitochondrial bioenergetics [3].

This study provides an analytical approach allowing unbiased and in-depth investigation of training-induced mitochondrial adaptations that challenges our current understanding and calls for careful reinterpretation of previous findings.

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3. Granata C, Caruana NJ, Botella J, Jamnick NA, Huynh K, Kuang J, Janssen HA, Reljic B, Mellett NA, Laskowski A, Stait TL, Frazier AE, Coughlan MT, Meikle PJ, Thorburn DR, Stroud DA, Bishop DJ (2021)

High-intensity training induces non-stoichiometric changes in the mitochondrial proteome of human skeletal muscle without reorganisation of respiratory chain content. http://hdl.handle.net/11343/296256
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Keywords: OXPHOS; mitochondria; exercise training; proteomics; lipidomics

Cite: Granata C (2022) Exercise training-induced enhancement of electron flow in the OXPHOS system is more important than increasing the OXPHOS machinery content to improve ATP generation in human skeletal muscle. In: https://doi.org/10.26124/bec:2022-0001

D-08

Bioblast link

Ca^{2+}-induced mitochondrial adaptations in response to a single session of sprint interval training.

Zanou N, Donnelly C, Kayser B, Place Nicolas
Institute of Sport Sciences, Univ. Lausanne, Switzerland - nicolas.place@unil.ch

Sprint interval training (SIT) has been shown to induce similar or greater mitochondrial adaptions as compared to moderate intensity continuous training (MICT) despite a much lower training volume. Previous data suggests that intramuscular Ca^{2+} handling is altered in response to a single session of SIT, as ryanodine receptors (RyR1, the intracellular Ca^{2+} release channels) can be markedly affected [1]. Ca^{2+} leak through RyR1 has previously been associated with pathological conditions such as skeletal muscle weakness [2], although it was recently suggested that an acute transient SR Ca^{2+} leak via RyR1 channels paradoxically might have a beneficial role [3]. We therefore assessed whether a single session of SIT resulted in leaky RyR1 and whether this leak of Ca^{2+} could contribute to mitochondrial remodeling. Muscle biopsies collected after a single session of SIT or MICT in recreationally-trained participants revealed dissociation of calstabin1 from RyR1, a signature of leaky RyR1, in response to SIT only. To assess the underlying mechanisms, cellular models based on SIT- and MICT-mimicking stimulation were developed and they also revealed a leaky RyR1 signature after the SIT stimulation pattern only. Using various pharmacological interventions, we could establish that the Ca^{2+} leaking through RyR1 entered the mitochondria and enhanced mitochondrial content / function, as attested by increased levels of mitochondrial oxidative phosphorylation proteins and enhanced NADH-linked mitochondrial respiratory capacity [4]. Our data thus show that acute Ca^{2+} leak through RyR1 can induce beneficial mitochondrial adaptations and thus contributes to the multiple health promoting benefits of exercise.

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Keywords: ryanodine receptor; mitochondria; sprint interval training; human; calcium

Cite: Zanou N, Donnelly C, Kayser B, Place N (2022) Ca^{2+}-induced mitochondrial adaptations in response to a single session of sprint interval training. In https://doi.org/10.26124/bec:2022-0001

D-09 *poster*

Bioblast link

Exercise alters mitochondrial physiology and has age-specific impacts on the fitness and lifespan of *D. melanogaster*.

Ebanks Brad, Wang Y, Katyal G, Sargent C, Ingram TL, Bowman A, Moisoi N, Chakrabarti L
University of Nottingham, UK - mbybdpo@nottingham.ac.uk

Biological ageing is one of the biggest risk factors for a range of diseases, including cancers, neurodegenerative disease, and heart disease. Mitochondrial dysfunction is associated with both ageing and diseases of ageing. As exercise is increasingly being viewed as a potential anti-ageing therapy, we sought to assess the impact of exercise on the fitness and lifespan of *D. melanogaster*[1]. In addition, we assessed the exercise-induced changes to mitochondrial physiology through high-resolution respirometry and label-free mass spectrometry. Exercise in late life extends the lifespan of male and female *D. melanogaster* compared with those exercised throughout their entire lifetime. Exercise also increases Complex-II-linked respiration and upregulates the expression of proteins from the electron transfer Complexes I, III, IV.

1. Ebanks B, Wang Y, Katyal G, Sargent C, Ingram TL, Bowman A, Moisoi N, Chakrabarti L (2021) Exercising *D. melanogaster* modulates the mitochondrial proteome and physiology. The effect on lifespan depends upon age and sex. https://doi.org/10.3390/ijms222111606

Keywords: Drosophila; ageing; exercise; lifespan; mitochondria; proteomics; respirometry

Cite: Ebanks B, Wang Y, Katyal G, Sargent C, Ingram TL, Bowman A, Moisoi N, Chakrabarti L (2022) Exercise alters mitochondrial physiology and has age-specific impacts on the fitness and lifespan of *D. melanogaster*. In https://doi.org/10.26124/bec:2022-0001

D-10

Bioblast link

Hacd1 and *Hacd2* genes control mitochondrial energetic efficiency through the modulation of mitochondrial membranes phospholipid composition.

Prola A, Khadhraoui N, Vandestienne A, Blondelle J, Wintrebert M, Tiret L, Pilot-Storck Fanny
Univ Paris Est Creteil, EnvA, IMRB, Team Relaix, F-94700 Maisons-Alfort, France - fanny.storck@vet-alfort.fr

Mitochondrial energy efficiency involves optimal coupling between oxidative processes and ADP phosphorylation, which implies an advanced structure-function relationship of the mitochondrial inner membrane (mtIM). Alteration of this oxidative phosphorylation process can lead to mitochondrial disease, as in the case of mutations of the *HACD1* gene which cause a congenital myopathy characterized by muscle weakness and exercise intolerance [1].

We generated mutant mice for *Hacd1* gene as well as its close paralog, *Hacd2* gene that shares high structural and functional similarity with *Hacd1*.

We identified that *Hacd1*-deficient mice present a muscle-specific twofold reduction in OXPHOS coupling. This alteration of mitochondrial efficiency blunts ATP production during exercise and leads to increased lactate accumulation. However, it also increases energy expenditure at rest and as such, confers a protection to *Hacd1*-deficient mice from diet-induced obesity [2]. HACD1 (3-hydroxyacyl-coenzyme A dehydratase, member 1) is part of the synthetic pathway of very long chain fatty acids, i.e., fatty acids comprising 18 carbons or more [3]. In *Hacd1*-deficient mice, mitochondrial phospholipid content is halved, with the mtIM-specific cardiolipin being the most affected. This defect is associated with cristae dilation, and we demonstrated that cardiolipin enrichment of isolated mitochondria from *Hacd1*-deficient mice was sufficient to restore OXPHOS coupling. *Hacd1* deficiency thus reveals that cardiolipin content controls mitochondrial coupling and energetic efficiency in muscle [2].

In parallel, the *Hacd2* gene exhibits an early and broad expression and we identified that a reduction of its expression leads to cachexia and ultimately death of mice during their first month of life. This devastating condition is associated with elevated lactate levels and impaired mitochondrial coupling in the kidney and liver [4]. We further show that complete loss of *Hacd2* expression is associated with major cardiovascular malformations and lethality at mid-embryonic development. In mutant embryos, mitochondria show compartmentalization and high amounts of oxidized cardiolipin, as well as a strong reduction in OXPHOS coupling efficiency (Khadhraoui et al, in prep).

In conclusion, our studies reveal that mutations in *Hacd1* and *Hacd2* genes lead to muscle-specific or systemic mitochondrial diseases, respectively, prompting the screening of their mutation for patients suffering from orphan mitochondrial diseases. Our work further reveals that *Hacd1* and *Hacd2* genes are new actors in the genetic control of mitochondrial membrane phospholipid composition and hence, mitochondrial energetic efficiency.

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Keywords: OXPHOS; cardiolipin; myopathy; mitochondrial disease; very long chain fatty acids

Cite: Prola A, Khadhraoui N, Vandestienne A, Blondelle J, Wintrebert M, Tiret L, Pilot-Storck F (2022) *Hacd1* and *Hacd2* genes control mitochondrial energetic efficiency through the modulation of mitochondrial membranes phospholipid composition. In https://doi.org/10.26124/bec:2022-0001

D-11

Bioblast link

Segmental regulation of intestinal mitochondrial function.

Axelrod Christopher L, Heintz EC, Albaugh VL, Kirwan JP
Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA - christopher.axelrod@pbrc.edu

The intestinal mucosa (IM) comprises the inner lining of the intestinal tract, largely consisting of enterocytes with smaller sub-populations of enteroendocrine, immune, goblet, and stem cells. Respiration in IM cells supports nutrient absorption, barrier function, production of mucus, antimicrobial molecules, and growth factors, as well as cellular regeneration. Despite this, little is known of the integrated respiratory functions of mitochondria along the gastrointestinal (GI) tract. The purpose of this study was to develop a procedure to analytically determine respiratory flux of IM derived from varying segments of the GI tract.

Whole, intact intestine was harvested from C57BL/6J mice euthanized by CO_2. The GI tract was flushed with ice cold saline, segmented by the jejunum, duodenum, and ileum, and fileted to expose IM. IM were collected directly into MiR05 by scraping the brush border membrane vesicles from the tissue, and mitochondria were subsequently prepared using a glass tissue homogenizer. Prior to assay, total protein was determined by BCA assay and uniformly normalized. Mitochondrial function was determined using a SUIT protocol to determine NADH- and succinate-linked oxidative phosphorylation and electron transfer capacity as well as Complex IV activity. The enzymatic activity of citrate synthase was determined on cryopreserved specimens using spectrophotometry.

Respiration across segments was highly coupled and limited predominantly by OXPHOS, not electron transfer. Substrate coupling was similar between duodenum and jejunum, with depressed NADH- and increased succinate-linked flux in the ileum. Respiratory activity was highest in the duodenum and decreased in a stepwise fashion

distally along the GI tract. Conversely, citrate synthase activity was highest in the ileum and decreased in a stepwise fashion proximally along the GI tract.

Mitochondria contained within the IM of the GI tract are energetically robust with differential activity and volume based upon proximity of the segment.

Keywords: gastrointestinal tract; intestine; mucosa; mitochondria; OXPHOS

Cite: Axelrod CL, Heintz EC, Albaugh VL, Kirwan JP (2022) Segmental regulation of intestinal mitochondrial function. In: https://doi.org/10.26124/bec:2022-0001

D-12 *not presented*

Bioblast link

The extraordinary energy metabolism of the bloodstream *Trypanosoma brucei* forms: a critical review and a hypothesis.

Alencar MB[1], Ramos EV[1], Silber AM[1], Zíková A[2,3], Oliveira Marcus F[4,5]

1. Lab Biochem Trypanosomatids - LaBTryps, Dept Parasitol, Inst Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil
2. Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Branišovská 31, 37005, České Budějovice, Czech Republic
3. Faculty of Science, University of South Bohemia, Branišovská 31, 37005, České Budějovice, Czech Republic
4. Lab Bioquím Resposta Estresse, Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil
5. Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (INCT-EM), Rio de Janeiro, RJ, Brazil - maroli@bioqmed.ufrj.br

The parasite *Trypanosoma brucei* is the causative agent of sleeping sickness and involves an insect vector and a mammalian host through its complex life-cycle. *T. brucei* mammalian bloodstream forms (BSF) exhibit unique metabolic features including: (*1*) reduced expression and activity of mitochondrial enzymes; (*2*) respiration mediated by the glycerol phosphate shuttle (GPSh) and the *Trypanosome* alternative oxidase (AOX) that is intrinsically uncoupled from generation of mitochondrial membrane potential; (*3*) maintenance of mitochondrial membrane potential by ATP hydrolysis through the reversal of F_1F_O ATP synthase activity; (*4*) strong reliance on glycolysis to meet their energy demands; (*5*) high susceptibility to oxidants. Here, we critically review the main metabolic features of BSF and provide a hypothesis to explain the unusual metabolic network and its biological significance for this parasite form. We postulate that intrinsically uncoupled respiration provided by GPSh-TAO system would act as a preventive antioxidant defense by limiting mitochondrial superoxide production and complementing the NADPH-dependent scavenging antioxidant defenses to maintain parasite redox balance. Given the uncoupled nature of the GPSh-TAO system, BSF would avoid apoptosis-like processes by maintaining mitochondrial membrane potential through the reversal of ATP synthase activity using the ATP generated by glycolysis. This unique "metabolic design" in BSF has no biological parallel outside of Trypanosomatids and highlights the enormous diversity of the parasites mitochondrial processes to adapt to distinct environments [1].

1. Alencar MB, Ramos EV, Silber AM, Oliveira MF (2022) A unifying hypothesis for the extraordinary energy metabolism of bloodstream *Trypanosoma brucei.* https://doi.org/10.26124/mitofit:2022-0009

Keywords: alternative oxidase; glycerol phosphate; reactive oxygen species; cell death; *Trypanosoma brucei*; mitophagy; antioxidant

Cite: Alencar MB, Ramos EV, Silber AM, Zíková A, Oliveira MF (2022) The extraordinary energy metabolism of the bloodstream *Trypanosoma brucei* forms: a critical review and a hypothesis. In: https://doi.org/10.26124/bec:2022-0001

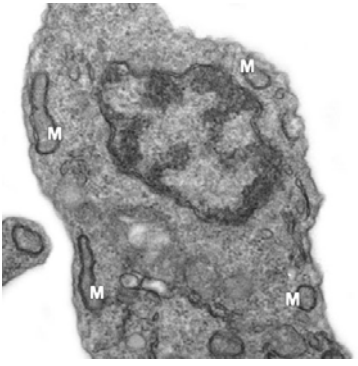
D-13 *not presented*

Bioblast link

Mitochondrial plasticity in trypanosomatids as a stress adaptation mechanism.

Bombaça ACS[1,2], Menna-Barreto Rubem FS [2]

1. Laboratório de Estudos Integrados em Protozoologia, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil - rubemb@ioc.fiocruz.br
2. Laboratório de Biologia Celular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil



Neglected tropical diseases impact more than a billion people globally, with millions of them at risk of infection by parasites of the Trypanosomatidae family. The need to colonize different environments in their hosts means that trypanosomatids are constantly subjected to stress situations, among which the presence of reactive oxygen (ROS) and nitrogen (RNS) species, requiring intense metabolic remodeling to ensure the parasites survival in hostile environments. Additionaly to the classical role in bioenergetics, mitochondrion has a decisive contribution to the oxidative stress, due to the electron leakage from the electron transfer system (ETS). The presence of several functional peculiarities made the mitochondrion of trypanosomatids an unique organelle, considered an excellent target for drug intervention. Some trypanosomatids such as *Leishmania* spp. can avoid the microbicidal mechanisms of the host cells, exhibiting a profile of natural resistance to oxidative and nitrosative stresses. Here, we discussed data about mitochondrial susceptibility and adaptative processes obtained by our group in the last 17 years. Mechanistic proposals of preclinical drugs was reviewed, as well as different pathways associated with metabolic and mitochondrial remodeling during the life cycle of trypanosomatids, including the possible biological role of ROS and RNS resistance and its impact on the interaction with vertebrate and invertebrate hosts [1].

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Keywords: trypanosomatids; mitochondrion; bioenergetics; oxidative stress; chemotherapy

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E - Perspectives of bioenergetics in health and disease

E-01

Bioblast link

The Crabtree effect and clinical nutrition.

Sobotka Lubos, Sobotka O
3rd Department of Internal Medicine, Gerontology and Metabolism, University hospital and Faculty of Medicine in Hradec Kralove, Charles University, CZ - pustik@lfhk.cuni.cz

Glucose (Glc) and its polymers are the most abundant organic molecules in nature. It is produced by green plants out of CO_2, water and energy from the sun. Glc is the foundation to many other molecules (e.g., nucleic acids, amino acids, lipids, mucopolysaccharides) and plays a crucial role in the formation of reducing equivalents necessary for anabolic, antioxidative, immune, and other processes. Our cells can also oxidize Glc to gain energy in the form of ATP but due to limited supplies in the human body it is used preferably for non-oxidative metabolism.

Anabolic pathways such as the pentose phosphate pathway require fuel in the form of Glc. During anabolism, an incomplete oxidation and continuous turnover of Glc/lactate occurs on the level of the whole organism. This turnover is higher during growth, pregnancy and regeneration but also during inflammation, oxidative stress and other stressful situations [1].

Almost one hundred years ago, Crabtree showed that various dividing cells metabolise Glc to lactate despite functional mitochondria and sufficient oxygen. This pathway is preferred over complete Glc oxidation despite full enzymatic equipment for oxidative phosphorylation. This phenomenon was called the Crabtree effect.

In our organism, part of Glc is completely oxidized if the dietary intake is higher than the requirements for non-oxidative anabolic pathways. Low Glu oxidation and insulin resistance indicate that intake is lower than requirements for non-oxidative pathways. This assumption was apparent in our recent observation that increased Glc intake improved insulin sensitivity in diabetic and nondiabetic ICU patients [2]. Therefore, aerobic glycolysis due to Crabtree effect may indicate higher needs of carbohydrates during nutritional support [3].

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Keywords: glucose; insulin resistance; metabolism; nutrition; Crabtree effect

Cite: Sobotka L, Sobotka O (2022) The Crabtree effect and clinical nutrition. In: https://doi.org/10.26124/bec:2022-0001

E-02

Bioblast link

Estimation of energy pathway fluxes in cancer cells - beyond the Warburg effect.

Moreno-Sánchez Rafael[1,2], Robledo-Cadena DX[3], Pacheco-Velázquez SC[3], Rodríguez-Enríquez S[4]

1. Facultad de Estudios Superiores Iztacala, UNAM, Tlanepantla, Estado de Mexico, Mexico
2. Oroboros Instruments GmbH, Innsbruck, Austria
3. Departamento de Bioquímica, Instituto Nacional de Cardiología, Ciudad de México 14080. Mexico
4. Laboratorio de Control Metabólico, Carrera de Medicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México; Los Reyes Ixtacala, Hab. Los Reyes Ixtacala Barrio de los Árboles/Barrio de los Héroes, Tlalnepantla 54090, Mexico

Glycolytic and respiratory fluxes were analyzed in cancer and non-cancer cells. The steady-state fluxes in energy metabolism were used to estimate the aerobic glycolytic and mitochondrial (oxidative phosphorylation OXPHOS) contributions to the cellular ATP supply. The rate of lactate production, corrected for the fraction generated by glutaminolysis, is proposed as the appropriate way to estimate glycolytic flux. In general, the glycolytic rates estimated for cancer cells are higher than those found in non-cancer cells, as originally observed by Otto Warburg. The rate of ROUTINE *R* cellular O_2 consumption corrected for LEAK respiration *L* measured after inhibition by oligomycin (a specific, potent and permeable ATP synthase inhibitor) becomes the respiratory *R-L* net ROUTINE capacity, which is proposed as the appropriate way to estimate mitochondrial ATP synthesis-linked O_2 flux or net OXPHOS flux in living cells. Detecting non-negligible O_2 consumption rates in cancer cells has revealed that the mitochondrial function is not impaired, as claimed by the Warburg effect. Furthermore, when calculating the relative contributions to cellular ATP supply, under a variety of environmental conditions and for several different types of cancer cells, it was found that OXPHOS was the main ATP provider over glycolysis. Hence, OXPHOS inhibitors can be successfully used to block ATP-dependent processes such as cellular migration in cancer cells. These observations may guide the re-design of novel targeted therapies.

Keywords: oxidative phosphorylation; oxygen uptake; glycolysis; cell ATP supply; cancer cell migration

Cite: Moreno-Sánchez R, Robledo-Cadena DX, Pacheco-Velázquez SC, Rodríguez-Enríquez S (2022) Estimation of energy pathway fluxes in cancer cells - beyond the Warburg effect. In: https://doi.org/10.26124/bec:2022-0001

E-03

Bioblast link

A comparison of bioenergetics in human tongue pre-cancerous dysplastic oral keratinocytes and squamous cancer cells.

Karavyraki M[1], Gnaiger E[2], Porter Richard K[1]

1. School of Biochemistry, Trinity Biomedical Science Institute, Trinity College Dublin, Ireland - rkporter@tcd.ie
2. Oroboros Instruments, Innsbruck, Austria

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In an endeavour to understand the metabolic phenotype behind oral squamous cell carcinomas [1], we characterised the bioenergetic profile of a human tongue derived cancer cell line (SCC-4 cells) and compared this profile to a pre-cancerous dysplastic oral keratinocyte (DOK) cell line also derived from human tongue. The human SCC-4 cancer cells had greater mitochondrial density but lower mitochondrial O_2 flow per cell J_{O_2} than DOK cells. The lower cell J_{O_2} in SCC-4 cells can be partially explained by lower NADH-related enzymatic activity when compared to pre-cancerous DOK cells. In addition, SCC-4 cells have greater extracellular acidification rate (an index of glycolytic flux) when compared to DOK cells. In addition, treatment with recombinant human IL-6 (rhIL-6), known to drive anoikis resistance in SCC-4 cells but not DOK cells, impairs oxygen consumption in SCC-4 but not DOK cells, without affecting mitochondrial density. We conclude that SCC-4 cells have a less oxidative phenotype compared to DOK cells and that IL-6 attenuates mitochondrial function in SCC-4 cells while increasing glycolytic flux.

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Keywords: oral squamous cancer cells; mitochondria; Interleukin 6; dysplastic oral keratinocytes; oxygen consumption

Cite: Karavyraki M, Gnaiger E, Porter RK (2022) A comparison of bioenergetics in human tongue pre-cancerous dysplastic oral keratinocytes and squamous cancer cells. In: https://doi.org/10.26124/bec:2022-0001

E-04

Bioblast link

Metabolic switch supports proliferation in regenerating liver.

Endaya BB[1], Le TDD[1], Kucera L[2], Lopes Oliveira G[1,3], Neuzil Jiri [1,4]
1. Inst of Biotechnology, Czech Academy of Sciences, Prague, Czech Republic
2. Inst of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic
3. University of Coimbra, Portugal
4. Griffith University, Southport, Qld, Australia

Our research has documented horizontal transfer of mitochondria in a mouse model of cancer from stroma to cancer cells with compromised oxidative phosphorylation (OXPHOS) in order to restore respiration. Functionally, the restoration of the ability to respire was attributed to the OXPHOS-linked activity of dihydroorotate dehydrogenase (DHODH), a mitochondrial enzyme catalysing the 4th reaction of the *de novo* pyrimidine synthesis. One biological event epitomized by high proliferation is liver regeneration after partial hepatectomy (PHx), with liver re-growth after 60 % PHx in mouse in 5-6 days. Consistent with the notion that this process requires highly robust liver cell proliferation, we found that blocking the *de novo* pyrimidine synthesis prevents the regeneration process. Additionally, to support the massive requirement for proliferation, the liver switches from ammonia detoxification to diversion of this toxic metabolite into pathways supporting formation of high levels of glutamate and glutamine, substrates of amino acids and nucleotides. Thus, to sustain high proliferation rates in regenerating liver, metabolism switches to anabolic pathways, also making use of ammonia that would otherwise be converted into the waste product urea.

Cite: Endaya BB, Le TDD, Kucera L, Lopes Oliveira G, Neuzil J (2022) Metabolic switch supports proliferation in regenerating liver. In: https://doi.org/10.26124/bec:2022-0001

E-05 *poster*

Bioblast link

Hypoxia-induced lesions to different age Wistar rat brain mitochondria.

Arandarcikaite Odeta, Umbrasas D, Borutaite V
Lithuanian University of Health Science, Neuroscience Institute, Eiveniu 4, Kaunas, Lithuania - odeta.arandarcikaite2@lsmuni.lt

Ischemic stroke is one of the leading causes of disability and mortality worldwide, but therapeutic approach are limited. Ischemia causes inhibition of mitochondrial respiration, mitochondrial permeability transition pore (MPTP) opening and subsequent cell death processes. The risk for ischemic stroke is increasing with aging, but there is very little information about aging-related changes in mitochondrial functions and proteomics.

In this study, we investigated ischemic lesions to 7 days, 2-3, 7-10 and 24-26 months-old rats brain mitochondria respiration and MPTP sensitivity to Ca^{2+} with particular focus on mitochondrial Complex I. Results have shown that hypoxia inhibited cortical mitochondrial respiration rate of animals from all age groups and reduced mitochondrial calcium retention capacity (CRC) in 2-3, 7-10 and 24-26 months animals groups. Hypoxia induced inhibition of cerebellar mitochondrial respiration in 7 days, 2-3 and 24 month-old groups, but in the 7-10 month-old group there were no statistically significant effect compared to control. CRC after hypoxia were reduced in 10 and 24-26 months - old rats cerebellum.

Further injury investigation revealed that hypoxia inhibits the activity of Complex I in 2-3, 7-10 and 24-26 month-animals. Mitochondrial protein expression study showed an age-related decrease of Complex I protein NDUFS2 levels and subsequent increase in mitochondrial respiration in aged animals. Altogether, we demonstrated that hypoxia induces MPTP opening and inhibits mitochondrial Complex I activity in adult and aged animals groups.

Keywords: ischemic stroke; mitochondrial permeability transition pore; aging; mitochondrial Complex I

Cite: Arandarcikaite O, Umbrasas D, Borutaite V (2022) Hypoxia-induced lesions to different age Wistar rat brain mitochondria. In: https://doi.org/10.26124/bec:2022-0001

E-06 *poster*

Bioblast link

The role of enkephalin in hypoxic preconditioning.

Bergmeister Lisa[1], Doerrier C[2], Gnaiger E[2], Schwarzer C[1]

1. Dept of Pharmacology, Medical University of Innsbruck, Innsbruck, Austria
2. Oroboros Instruments GmbH, Innsbruck, Austria

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Hypoxic preconditioning (HPC) is the application of mild transient hypoxia which protects the brain against a following more severe hypoxic insult as it occurs during epileptic seizures. HPC decreases seizure susceptibility and severity as well as neuronal damage in the hippocampus. The delta opioid receptor (DOR) and its primary endogenous ligands, the neuropeptides met- and leu-enkephalin (Enk), are thought to be involved in the neuroprotective actions of HPC. Recently, we showed that Enk influences mitochondrial respiration that may contribute to the neuroprotective effects of the Enk/DOR system. The present study aims at investigating the effects of the Enk/DOR system on structural and functional alterations of mitochondria in HPC.

Wild type (WT) and met-Enk-knockout (met-Enk-KO) mice were exposed to hypoxia. Subsequently, we determined the seizure threshold, analyzed mitochondrial function and dynamics.

In WT mice after HPC we observed an elevated seizure threshold, improved mitochondrial reserve capacity and increased mitochondrial fusion. In addition, our results suggest mitochondrial biogenesis after HPC in WT mice. Naïve met-Enk-KO mice had an increased seizure threshold and increased mitochondrial fusion but no changes upon HPC.

The observed mitochondrial alterations after HPC in WT mice could explain improved neuronal survival and increased seizure threshold. Enhanced mitochondrial reserve capacity improves energy supply in stress situations and increased mitochondrial fusion is associated with neuronal survival and elevated Ca^{2+} storage capacities. However, the precise role of met-Enk in HPC is unclear but we observed adaptive mechanisms in WT mice upon hypoxia which are absent in met-Enk-KO mice.

Keywords: hypoxic preconditioning; Enkephalin/delta opioid receptor system; mitochondria; epilepsy

Cite: Bergmeister L, Doerrier C, Gnaiger, Schwarzer C (2022) The role of enkephalin in hypoxic preconditioning. In: https://doi.org/10.26124/bec:2022-0001

E-07 *poster*

Bioblast link

In rat brain and heart acute hypoxia induced mild changes in OXPHOS in both sexes.

Isola Raffaella[1], Noli R[1], Isola M[1], Crisafulli A[2], Vargiu R[1], Loy F[1], Lai Y[1]

1. Dept Biomedical Sciences, Univ of Cagliari, IT
2. Dept Medical Sciences Public Health, Univ of Cagliari, IT - isola@unica.it

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Acutely exposure to low oxygen concentrations, impairs the capability to perform muscular workout. On the other hand, repeated and prolonged exposure to low PO2 may improve physical performance due to a progressive adaptation of the body. This is mainly due to enhanced hemoglobin and red blood cells content, and decreased sympathetic autonomic nervous system input. These changes were evaluated in a number of chronic hypoxia studies [1-2], whereas acute hypoxia outcomes are still unknown.

This study investigates on acute hypoxia and normoxia impact on cardiac and brain mitochondrial bioenergetics and the possible occurrence of different gender responses.

We used male and female Wistar rats that had been trained for 5 weeks, 1h/day, on a treadmill set at 35 cm/s. The day of the experiment they were allowed to run on the treadmill for 30 minutes in hypoxia (at the same oxygen concentration of an altitude of 4000 mt.) or in normoxia. After euthanasia, we removed the brain and the heart and isolated brain mitochondria, subsarcolemmal (SSM) and interfibrillar (IFM) heart mitochondria [3]. Mitochondrial bioenergetics was assessed by Clark-type electrode, testing for oxidative phosphorylation (OXPHOS): complex I (glutamate plus malate), complex II (rotenone plus succinate), complex III (rotenone plus durohydroquinone), complex IV (rotenone plus tetramethyl-p-phenylenediamine and ascorbate), Palmitoyl CoA as lipid substrate and adding at the end of the assay dinitrophenol (DNP) to test uncoupled respiration with these substrates.

After acute hypoxia, brain male mitochondria showed an increase of uncoupled respiration at complex II and IV, whereas female mitochondria displayed no significant difference compared to controls.

In heart male IFM mitochondria, following acute hypoxia, ADP/O decreased at complex I and II, compared with controls. Furthermore, in the same complexes data showed an increase of respiratory control ratio, but only complex I resulted statistically significant. These data suggest that hypoxia induced a mild uncoupling of IFM.

Among female heart mitochondria, SSM only showed a decrease in state 3 of complex II after acute hypoxia.

In conclusion, in both genders cardiac and brain mitochondrial bioenergetics change after athletic training in acute hypoxia.

It seems that in female cardiac mitochondria hypoxia induced an impairment of complex II activity, while in male heart mitochondria the result need further investigation as it could be linked to a reported increased activity of ATPase under hypoxia [4] or a defective OXPHOS with a possible enhanced ROS production.

In male brain mitochondria the increased of uncoupled respiration might be linked to a better efficiency of electron transfer system (ETS). Future studies will need to verify these results.

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Keywords: hypoxia; heart mitochondria; brain mitochondria; Clark-type electrode; trained rats

Cite: Isola R, Noli R, Isola M, Crisafulli A, Vargiu R, Loy F, Lai Y (2022) In rat brain and heart acute hypoxia induced mild changes in OXPHOS in both sexes. In: https://doi.org/10.26124/bec:2022-0001

E-08

Bioblast link

Renal respiratory conductance: a complex matter.

Lerink Lente JS[1,2], Lindeman JHN[1]

1. Dept of Surgery, Leiden Univ Medical Centre - l.j.s.lerink@lumc.nl
2. Nuffield Dept of Surgical Sciences, Univ of Oxford

The kidney's energy requirements equal that of the heart. In line with this, it has recently been shown that metabolic derailment during transplantation or major surgery underlies delayed graft function [1] or acute kidney injury [2], respectively. Hence, strategies to prevent or minimize the post-ischemic injury should focus on prevention of metabolic injury or optimal use of residual metabolic capacity.

Recently, normothermic machine perfusion (NMP) has been introduced as an ex-situ means to preserve, assess and remodel, and to test interventions on an organ under

relatively physiological circumstances. Although this technique proved successful for shorter periods of perfusion, longer periods unveil profound (metabolic) challenges, such as excessive lactate production, indicative of failing renal metabolism. This implies that the basic physiologic metabolic requirements, let alone the conditions required to test metabolic interventions, are not fulfilled in the prevailing NMP protocols. Therefore, better tailored strategies, such as optimal substrate composition to drive ATP synthesis, are of major importance.

McLaughlin et al previously used the creatine kinase (CK) clamp to measure respiratory conductance, a measure for the tissue's mitochondrial metabolic competence in the presence of the supplied substrates [3]. The authors exposed murine mitochondria from different tissues to a physiologically relevant change in ATP/ADP ratio to monitor tissue-specific metabolic responses to an energy challenge. This technique could provide insight in optimal substrate compositions to drive ATP synthesis in renal cells. It was reported that renal mitochondria showed a peculiar phenotype, by uniquely responding to the clamp in the presence of succinate in combination with Complex I-inhibitor rotenone [3].

Since our previous work indicated profound interspecies differences in mitochondrial function and susceptibility [4], and because the use of isolated mitochondria ignores the critical cytoplasm-mitochondrial axis, we aimed to validate their findings in permeabilized porcine renal biopsies.

Kidneys from abattoir pigs were retrieved after a period of brief warm ischemia. Kidneys were subsequently transported on ice, and cortex biopsies were taken. Cellular plasma membranes were permeabilized before assessment. The biopsies were subsequently transferred to an O2k-respirometer and exposed to the CK clamp in the presence of different substrate combinations. Maximal respiration and respiratory conductance were calculated, and mitochondrial membrane integrity was assessed to monitor physical mitochondrial damage.

In line with the sparsely available preclinical data, renal cells did not respond to a change in energy availability in terms of respiratory activity when pyruvate, glutamate, malate or a combination of the three were provided. Only when Complex I-inhibitor rotenone was supplied before adding succinate, the biopsies showed a response to a change in ATP/ADP ratio. Moreover, maximal respiration is higher in the presence of succinate and rotenone compared to other substrate combinations.

In conclusion, the CK clamp provides in-detail information on metabolic responses to a change in ATP/ADP ratio in the presence of specific substrate combinations, which may help to define an optimal perfusate composition to stimulate renal metabolism during NMP. Previous findings regarding the need for Complex I inhibition to observe a response to changing ATP/ADP ratios have been validated, but are still poorly understood.

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Keywords: substrate preference; metabolic conductance; kidney; Complex I; respirometry

Cite: Lerink LJS, Lindeman JHN (2022) Renal respiratory conductance: a complex matter. In: https://doi.org/10.26124/bec:2022-0001

E-09

Bioblast link

Pre-transplant mitochondrial respiration as a clinical prognostic marker during static cold storage and machine perfusion of the liver.

Meszaros Andras T[1], Hofmann J[1], Gnaiger E[2], Oberhuber R[1], Hautz T[1], Öfner D[1], Schneeberger S[1]

1. organLife, Organ Regeneration Center of Excellence and Daniel Swarovski Research Laboratory, Dept Visceral, Transplant and Thoracic Surgery, Medical Univ Innsbruck, Innsbruck, Austria - andras.meszaros@i-med.ac.at
2. Oroboros Instruments, Innsbruck, Austria

The growing demand for liver grafts promotes the use of extended criteria organs, but donor and organ biomarkers with strong predictive value in liver transplantation are lacking. To assure optimal clinical outcome, pre-transplantation evaluation of organ quality is needed. Bioenergetic performance during static cold storage (SCS) or machine perfusion (MP) may correlate with organ function after liver transplantation (LT) [1]. Martins et al. described a relationship between mitochondrial respiration, membrane potential and postoperative aminotransferase values and outcome of LT [2]. Flavin mononucleotide (FMN) in the perfusate as a damage marker during hypothermic MP has been proposed as biomarker by other groups [3]. We aimed at using a high-resolution approach to assess mitochondrial respiratory capacities during SCS and normothermic MP (NMP) and test for their predictive value towards the outcome in clinical liver transplantation.

High-resolution respirometry (HRR, O2k, Oroboros Instruments) was chosen to maximize resolution with minimal amount of tissue sample required (< 20 mg wet weight liver wedge biopsy) [4]. Mitochondrial respiration was characterized in tissue homogenates by assessing the succinate-linked coupling control. Mitochondrial parameters were then correlated with clinical outcome ("early allograft dysfunction", EAD and "liver graft assessment following transplantation", L-GrAFT scores). 43 liver allografts were enrolled in the SCS cohort, and 71 liver allografts were enrolled in the NMP cohort of prospective clinical studies. In the latter cohort, livers underwent NMP (OrganOx Metra) for up to 24 h, of which 47 livers were transplanted. Biopsy samples were collected at the end of SCS, at 1 h, 6 h and end of NMP.

HRR allowed the assessment of mitochondrial respiration within 2 h after sample collection. We observed a considerable variability in mitochondrial respiration between grafts after SCS and during NMP. In the cohort of SCS livers without NMP, *P-L* control efficiency correlated with EAD (0.8 in the group of initial function compared to 0.7 in EAD-

livers; p = 0.02). For the NMP cohort, in the multivariate analysis, area-under-the-curve (AUC) values of LEAK respiration, cytochrome *c* and *P-L* control efficiencies during the first 6 h of NMP correlated with L-GrAFT.

There is a clear relationship between mitochondrial function/damage of the liver and clinical outcome upon transplantation, as was shown previously. FMN can be readily assessed in the perfusate during MP but not in SCS organs. Based on our results, in SCS livers, *P-L* control efficiency measured before transplantation correlates with EAD. During NMP, AUC values for markers of outer mitochondrial membrane damage, ATP synthesis efficiency and dissipative respiration during the first 6 h of NMP predict clinical outcome (L-GrAFT). Assessment of mitochondrial respiratory capacities by HRR is therefore a promising tool to select optimal grafts with or without machine perfusion.

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Keywords: transplantation; liver; mitochondria; respiration; outcome

Cite: Meszaros AT, Hofmann J, Gnaiger E, Oberhuber R, Hautz T, Öfner D, Schneeberger S (2022) Pre-transplant mitochondrial respiration as a clinical prognostic marker during static cold storage and machine perfusion of the liver. In: https://doi.org/10.26124/bec:2022-0001

E-10

Bioblast link

Nutrient overload and insulin resistance precede reductions in mitochondrial respiration in skeletal muscle after bed rest.

Eggelbusch Moritz [1,2,3], Hendrickse P[4], Schoonen R[1], Giakoumaki I[4], Kerkhoff TJ[1], Grootemaat AE[5], Rittweger J[6], Ganse B[4], Weeghel van M[7], Mulder ER[6], Degens H[4], Wüst RCI[1]

1. Lab for Myology, Dept of Human MVMT Sci, Fac of Behav and MVMT Sci, Vrije Univ Amsterdam, Amsterdam MVMT Sci, NL
2. Dept of Nutrition and Dietetics, Amsterdam UMC, Loc VUmc, Amsterdam MVMT Sciences, NL
3. Fac of Sports and Nutrition, Ctr of Expertise Urban Vitality, Amsterdam Univ of Appl Sci, NL
4. Manchester Metropolitan Univ, Res Ctr for MSK Sci & Sports Med, Fac of Sci and Engrg, UK
5. Electron Microsc Ctr Amsterdam, Dept of Med Biol, Acad Med Ctr, NL
6. Inst of Aerospace Med, German Aerospace Ctr (DLR), DE
7. Amsterdam UMC, Univ of Amsterdam, Lab Genetic Metab Diseases, Amsterdam Cardiovasc Sci, NL - m.eggelbusch@amsterdamumc.nl

Physical inactivity contributes to insulin resistance and the development of type 2 diabetes [1]. Metabolic flexibility, the ability to efficiently adapt substrate oxidation depending on demand and supply, decreases in response to bed rest [1,2]. A mismatch between nutrient uptake and utilization rates likely underlies these metabolic alterations [3], but it is unknown if glycogen and lipid accumulation, or mitochondrial dysfunction occur before insulin resistance develops. Here, we determined nutrient load, insulin sensitivity, metabolite concentrations and mitochondrial function in skeletal muscle in humans undergoing short- and long-term bed rest, and assess the order of occurrence of events.

24 healthy individuals (23-54 years, 8 women) participated in the 60-days artificial gravity bed rest study by ESA (AGBRESA). Oroboros respirometry, metabolomics and lipidomics (on a subset) were performed on *vastus lateralis* muscle biopsies before, after 6 (short-), and 55 (long-term) days of bed rest. Glycogen and intramyocellular lipid deposits were assessed in electron microscopy images, and fasted blood glucose and insulin levels determined for the calculation of the HOMA-IR score.

Blood glucose remained constant throughout bed rest, but HOMA-IR scores were higher after short- and did not further change after long-term bed rest. Short-term bed rest did not cause significant changes in mitochondrial respiration, whilst long-term bed rest significantly reduced NADH- and oxidative phosphorylation capacity. Glycogen content and lipid droplets significantly increased after 6 days but only lipid droplets further accumulated after day 55. Metabolomics and lipidomics revealed reduced fatty acid oxidation and increased glucose metabolism after short-term bed rest, which manifested throughout the bed rest duration, suggestive of continuous alterations in skeletal muscle metabolism.

Short-term bed rest caused nutrient overload, a shift from fatty acid metabolism to glycolysis, and insulin insensitivity before a reduction in oxidative phosphorylation capacity was observed after long-term bed rest.

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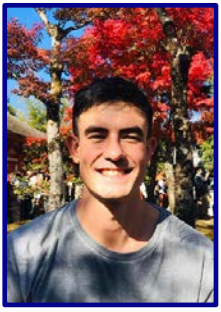
Keywords: bed rest; skeletal muscle; nutrient overload; insulin resistance; mitochondrial respiration

Cite: Eggelbusch M, Hendrickse P, Schoonen R, Giakoumaki I, Kerkhoff TJ, Grootemaat AE, Rittweger J, Ganse B, Weeghel van M, Mulder ER, Degens H, Wüst RCI (2022) Nutrient overload and insulin resistance precede reductions in mitochondrial respiration in skeletal muscle after bed rest. In: https://doi.org/10.26124/bec:2022-0001



E-11

Bioblast link

Mitochondrial function is not impaired in type 1 diabetes but does not respond to 4-weeks endurance training.

Goulding Richie P[1], Strating S[1], van der Laan M[1], Noort W[1], Kolodyazhna A[1], Bloemers FW[2], Wüst RCI[1]

1. Laboratory for Myology, Faculty of Behavioural and Movement Sciences, Amsterdam Movement Sciences, Vrije Univ Amsterdam, Amsterdam, NL
2. Dept of Trauma Surgery, Amsterdam Movement Sciences, Amsterdam UMC, Vrije Universiteit Amsterdam, De Boelelaan 1117, Amsterdam, NL

Type 1 diabetes mellitus (T1DM) is a disease characterized by the destruction of the insulin-producing, pancreatic β-cells, where an inability to maintain glucose homeostasis causes multi-organ problems. It has been reported that young, otherwise healthy individuals with T1DM display significant skeletal muscle mitochondrial dysfunction when compared to physical activity-matched healthy controls. This suggests that skeletal muscle mitochondria possess poorer plasticity with respect to the beneficial effects of exercise training, however, this hypothesis has never been tested.

Here, we tested the hypothesis that 1) individuals with T1DM would evince a lower maximal oxygen uptake (V_{O2max}) and mitochondrial oxidative phosphorylation (OXPHOS) capacity compared to healthy controls matched for age, sex and physical activity, and 2) the magnitude of increase in OXPHOS capacity following 4-weeks endurance training would be lesser in individuals with T1DM compared to controls.

Vastus lateralis muscle biopsies were taken and cardiopulmonary exercise testing was performed on patients with T1DM and healthy controls, both before and after 4-weeks of moderate intensity endurance training. Mitochondrial respiration was assessed in permeabilized fibers using high-resolution respirometry (Oroboros O2k, Innsbruck, Austria).

Data collection is still ongoing. Preliminary results suggest a lower V_{O2max} in T1DM compared to controls (T1DM: 38.8±11.5 mL·kg^{-1}·min^{-1}, N=18, controls: 44.7±8.0 mL·kg^{-1}·min^{-1}, N=9) and OXPHOS capacity appears similar between groups (T1DM: 99.5±38.2 pmol·s^{-1}·mg^{-1}, N=15, controls: 100.3±20.0 pmol·s^{-1}·mg^{-1}, N=8), however, uneven group sizes preclude statistical comparison at present. In the subset of eight T1DM patients that have currently undergone the training intervention, exercise training improved whole-body exercise capacity in T1DM patients (peak power output, pre: 272±77 W, post: 281±81 W, p=0.04), however, OXPHOS capacity did not respond to exercise training (pre: 94.5±39.2 pmol·s^{-1}·mg^{-1}, post: 94.2±33.1 pmol·s^{-1}·mg^{-1}, p=0.98).

Inconsistent with our first hypothesis, our preliminary data indicate no deficit in mitochondrial function when compared to matched healthy controls. Consistent with our second hypothesis, however, mitochondrial oxidative capacity did not respond to four weeks of endurance training despite improvements in whole-body exercise capacity. Hence, these data provide the first empirical support for the notion that individuals with T1DM possess inherently poor mitochondrial plasticity. It is expected that data collection and analysis will be completed by August 2022, and the full data set will illuminate the mechanistic underpinnings of these initial observations.

Keywords: type 1 diabetes; exercise; mitochondrial function; oxidative capacity

Cite: Goulding RP, Strating S, van der Laan M, Noort W, Kolodyazhna A, Bloemers FW, Wüst RCI (2022) Mitochondrial function is not impaired in type 1 diabetes but does not respond to 4-weeks endurance training. In: https://doi.org/10.26124/bec:2022-0001

E-12 *poster*

Bioblast link

Relationship of insulin resistance and mitochondria-associated endoplasmic reticulum membranes MAM in an *in vitro* lipotoxicity model.

Ivanovic Tijana[1], Krako Jakovljevic N[1], Pavlovic K[1], Ciric D[2], Kravic-Stevovic T[2], Markovic I[3], Lalic NM[1]

1. Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Centre of Serbia, Faculty of Medicine, Univ Belgrade - zujovictijana@gmail.com
2. Inst of Histology and Embryology, Faculty of Medicine, Univ Belgrade
3. Inst of Medical and Clinical Biochemistry, Faculty of Medicine, Univ Belgrade

Excessive lipid accumulation in hepatic cells is the hallmark of several metabolic disorders, particularly hepatic steatosis, insulin resistance (IR) and diabetes mellitus. Recent studies have shown that phosphorylation of Akt, a key kinase in the insulin signaling pathway, may occur in ER – mitochondria contact sites, known as MAMs (Mitochondria-Associated endoplasmic reticulum Membranes) [1]. One of the key MAM proteins is Glucose–regulated protein 75 (Grp75) which enables calcium transport from endoplasmic reticulum to mitochondria. Grp75 levels were found to be significantly reduced in IR *in vitro*, along with Akt phosphorylation levels and MAM concentration, implying that this protein could be the link between IR and MAM. Furthermore, mitochondrial dysfunction has widely been associated with diabetes and IR, which highlights the importance of MAM exploration. In order to investigate the effects of lipotoxicity on MAM and mitochondrial function, we treated hepatocytes with palmitate, a common unsaturated fatty acid used for studying *in vitro* lipotoxicity.

Human hepatocellular carcinoma (Huh7) cell line was used for all experiments. Cells were treated with 0.4 mM palmitate for 24 h. Cell viability was estimated by using acid phosphatase assay. For microscopy analysis, cells were transfected with Split - GFP pasmid that targets MAM region, and stained with MitoTracker Red. Samples were examined using confocal microscopy. For investigating insulin signalling and MAM protein levels, immunoblotting was performed. Mitochondrial respiration was measured with Oroboros O2k high-resolution respirometry.

Viability of Huh7 cells was not affected by palmitate. Preliminary data showed that palmitate treated cells had a lower number of MAM and lower mitochondrial content with reduced levels of GRP75, compared to untreated cells. Palmitate treatment impaired insulin response, observed as lower phosphorylated Akt levels compared to untreated cells. Mitochondrial function was altered in PA treated cells, which was observed by lower respiration rate in ROUTINE and OXPHOS state and *P-L* control efficiency (1-*L*/*P*) compared to control cells, as characterized previously in detail [1].

To conclude, palmitate treatment induced IR, which was confirmed by impaired insulin signaling. Furthermore, the adverse effects were found in mitochondrial function and content and number of MAM, which may imply the potential of MAM in insulin

signaling. Taking into account that mitochondria in our model had decreased respiration rate and impaired coupling efficiency, it is important to additionally clarify the relationship between these processes and their importance for mitochondrial function and energy metabolism.

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Keywords: lipotoxicity; diabetes; hepatocytes; MAM; mitochondria

Cite: Ivanovic T, Krako Jakovljevic N, Pavlovic K, Ciric D, Kravic-Stevovic T, Markovic I, Lalic NM (2022) Relationship of insulin resistance and mitochondria-associated endoplasmic reticulum membranes MAM in an *in vitro* lipotoxicity model. In: https://doi.org/10.26124/bec:2022-0001

E-13

Bioblast link

Coordination between mitochondrial-associated membranes MAMs, mitochondrial function and protein synthesis in exercised and myopathic muscles.

Colomb M, Tuifua L, Bertrand-Gaday C, Pessemesse L, Vernus B, Asikan E, Py G, Ollendorff V, Chabi Beatrice
DMEM, Université de Montpellier, INRAE, Montpellier, France - beatrice.chabi@inrae.fr

Skeletal muscles mitochondria are key organelles that control not only ATP production but also fulfill important short term and/or long-term metabolic adaptations to various stimuli and constraints including exercise. However, mitochondria do not function independently of the cell context, as they form dynamic and privileged contact sites with the endoplasmic reticulum (ER), called mitochondrial-associated membranes (MAMs). MAMs play important roles in calcium transfer as well as phospholipids synthesis, exchange and transfer between ER and mitochondria and in the control of glucose metabolism.

Recent reports, including our own publications indicate that MAMs stand as a crucial hub in skeletal muscles that integrate and coordinate ATP production by mitochondria and protein synthesis, a major energy-consuming process. This important checkpoint allows to respond to energetic challenging conditions like exercise, fasting or hypoxia by blunting Akt/mTOR dependent protein synthesis, which spares ATP and limits energetic stress [1]. Moreover, we have also shown recently that a single bout of physical exercise can induce an immediate decrease of MAMs contacts sites in skeletal muscles and a simultaneous reduction of protein synthesis [2].

Neuromuscular myopathies are often associated with mitochondrial defects that can affect various functions or content of this organelle, and have important impact in the phenotype of these pathologies. Moreover, alterations in the muscle protein balance (synthesis versus degradation) are frequently observed in neuromuscular dystrophies, sometimes associated with MAMs dysfunction. However, we do not know yet whether MAMs defects could be a cause or a consequence in these muscular pathologies as MAMs dynamics remains poorly characterized in skeletal muscles in relation with mitochondrial function and protein synthesis notably following exercise.

In this study, we first analyzed the coordination between protein synthesis and mitochondrial respiration as a first step to describe the functional relationship of the hub made of MAMs dynamics, mitochondrial respiration and protein synthesis in skeletal muscle. Therefore, mitochondrial respiration and protein synthesis as well as AMPK and AKT/mTOR signaling were compared in skeletal muscle homogenates from 6 months old (mo) C57BL/6J at rest, following acute exercise (45 min treadmill exercise) and after a recovery period (3 h). These analyses were also performed in skeletal muscle homogenates of 12 mo control (C57BL/10) and mdx mice, model of Duchenne Muscular Dystrophy (DMD).

Preliminary results showed no significant change in mitochondrial respiration while AMPK activation is rapidly induced following acute exercise and goes back to rest level during recovery period. In contrast, protein synthesis tended to be blunted following acute exercise and during the recovery period. Data from mdx mice are currently under investigation.

This study will shed light on the functional relationship between mitochondrial respiration, protein synthesis and MAMs dynamics in resting, exercised and pathologic skeletal muscles. This could pave the way for future therapeutic routes to treat muscular disorders.

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Keywords: Duchenne muscular dystrophy; skeletal muscle; exercise; mitochondria; protein synthesis;

Cite: Colomb M, Tuifua L, Bertrand-Gaday C, Pessemesse L, Vernus B, Asikan E, Py G, Ollendorff V, Chabi B (2022) Coordination between mitochondrial-associated membranes MAMs, mitochondrial function and protein synthesis in exercised and myopathic muscles. In: https://doi.org/10.26124/bec:2022-0001

E-14

Bioblast link

Impact of nitric oxide promotors on mitochondrial bioenergetics in a murine model of Alzheimer's disease.

Irving Brian A[1,2], Stampley J[1], Quiriarte H[2], Wigger Z[2], Stephens J[1,2], Soto P[1,2], Allerton TA[2]

1. Louisiana State University
2. Pennington Biomedical Research Center - brianairving@lsu.edu

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Although Alzheimer's disease (AD)'s underlying pathophysiology is incompletely understood, reductions in mitochondrial bioenergetics are observed during AD development. Reductions in nitric oxide (NO) bioavailability can reduce cerebral blood

flow, promote the deposition of β-amyloid (Aβ), and contribute to mitochondrial dysfunction. However, pathological elevations in NO can also inhibit mitochondrial respiration and mitochondrial quality control. High-Fat Diets (HFD) are associated with reductions in NO bioavailability and AD development. Therefore, we sought to investigate the effects of dietary NO donors (Na^+-nitrite and citrulline) on mitochondrial bioenergetics in female APPswe/PS1dE9 (APP/PS1) fed a HFD.

We fed 10-week-old APP/PS1 transgenic mice, and their littermate controls (wild-type, WT) either a normal chow diet, HFD, or HFD supplemented with a NO promoter (Na^+-nitrite or L-citrulline) for six months. Specifically, 100 mg/L Na^+-nitrite or 2.5 mM L-citrulline was provided in their drinking water. The mice were euthanized, and the hypothalami were carefully dissected out and placed in ice-cold BIOPS. The hypothalami were homogenized in a mitochondrial respiration media (MiR05-Kit, pH 7.1).

We used high-resolution respirometry (HRR, Oroboros O2k) coupled with a standardized substrate-uncoupler-inhibitor-titration (SUIT) protocol to measure respiration rates in duplicate during LEAK (State 4), OXPHOS capacity (State 3), and electron transfer capacity (ET) states in permeabilized hypothalami homogenates at 37 °C and O_2 concentrations between ~450 µM and ~150 µM. We supplement the MiR05 with α-chaconine (40 µM) to chemically permeabilize the plasma membranes and synaptosomes. First, we measured NADH-linked LEAK respiration (N_L) in the presence of pyruvate (5 mM), malate (2 mM), and glutamate (10 mM) in the absence of ADP. We measured NADH-Linked OXPHOS (N_P) following the addition of a saturating concentration of ADP-Mg^{++} (5 mM). Next, we assessed the mitochondrial membrane integrity using cytochrome *c* (10 µM). We measured NS-linked OXPHOS (NS_P) after the addition of succinate (10 mM). Next, we titrated in carbonyl cyanide m-chlorophenyl hydrazine (CCCP) (0.5 µM/step) to achieve NS-linked ET capacity (NS_E). Next, we titrated rotenone (0.5 µM) to measure succinate-linked ET capacity (S_E), followed by the titration of glycerol-3-phosphate (15 mM) to measure SGp-linked ET capacity (SGp_E). Finally, we added antimycin A (2.5 µM) to measure residual oxygen consumption (*Rox*). The respiration rates were normalized per mg mass [$pmol \cdot s^{-1} \cdot mg^{-1}$], referred to as oxygen flux (J_{O_2}).

The final body and fat masses of HFD-fed APP/PS1 mice (48.2 g & 17.7 g) were significantly higher than those of HFD-fed WT mice (42.4 g & 14.3 g). NO donors (Na^+-nitrite or citrulline) had no effect on body mass or fat mass. There was a significant group effect ($p<0.05$) effect on OXPHOS and ET capacity. Specifically, the APS/PS1 mice had significantly lower OXPHOS and ET capacity while on the HFD compared to WT. The NO donors (Na^+-nitrite or citrulline) could rescue the OXPHOS and ET capacity in the APS/PS1 mice fed a HFD.

In summary, the APS/PS1 mice had lower OXPHOS and ET capacity than their WT controls while on an HFD. Physiologically relevant NO donors may provide an opportunity to mitigate some of the negative consequences of AD pathology.

Keywords: Alzheimer's disease; hypothalamus; bioenergetics; diet; nitric oxide

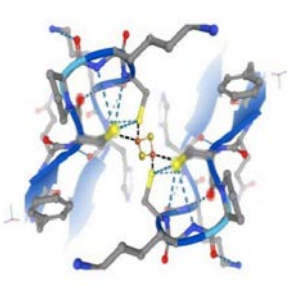
Cite: Irving BA, Stampley J, Quiriarte H, Wigger Z, Stephens J, Soto P, Allerton TA (2022) Impact of nitric oxide promotors on mitochondrial bioenergetics in a murine model of Alzheimer's disease. In: https://doi.org/10.26124/bec:2022-0001

E-15

Bioblast link

NUBPL: a mitochondrial Complex I deficiency disorder.

Abed Rabbo M[1], Stiban Johnny[2]
1. Dept of Medical Genetics, University of British Columbia, Canada
2. Dept of Biology and Biochemistry, Birzeit University, Palestine - jstiban@birzeit.edu



Mitochondrial ailments are diverse and devastating. Defects in mitochondrial DNA or its products lead to wide range of deficiencies in the mitochondrial electron transfer system and its ensuing energy production. Accessory proteins required for the assembly and function of the respiratory complexes are also required for healthy, coupled, and energy-producing mitochondria. Recently, the protein nucleotide binding protein like (NUBPL or IND1) was identified as an iron-sulfur cluster transfer protein specifically for Complex I. Since the presence of multiple iron-sulfur clusters in Complex I is necessary for its activity, deficiency in NUBPL leads to severely dysfunctional mitochondria, with upregulated compensatory Complex II activity. Here we present a short review of the debilitating disease related to NUBPL deficiency [1].

1. Abed Rabbo M, Stiban J (2022) NUBPL: a mitochondrial Complex I deficiency disorder. https://doi.org/10.26124/mitofit:2022-0005

Keywords: Complex I; NUBPL; IND1; iron-sulfur clusters; mtDNA helicase; bioenergetics

Cite: Abed Rabbo M, Stiban J (2022) NUBPL: a mitochondrial Complex I deficiency disorder. In: https://doi.org/10.26124/bec:2022-0001

E-16 *not presented*

Bioblast link

Bioenergetics health index ratio in Leigh Syndrome patient fibroblasts as a measure of disease severity.

Iyer Shilpa[1], Bakare A[1], Dean J[2], Chen Q[3], Thorat V[4], Huang Y[4], LaFramboise T[4], Rao RR[5], Lesnefsky EJ[2,6]
1. Dept of Biological Sciences, J. William Fulbright College of Arts and Sciences, University of Arkansas, Fayetteville, Arkansas, USA - si012@uark.edu
2. Cardiology Section Medical Service, McGuire Veterans Affairs Medical Center, Richmond, Virginia, USA
3. Pauley Heart Center, Division of Cardiology, Dept of Internal Medicine, Virginia Commonwealth University, Richmond, Virginia, USA
4. Dept of Genetics and Genome Sciences, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA
5. Dept of Biomedical Engineering, College of Engineering, University of Arkansas, Fayetteville, Arkansas, USA

6. Dept of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, Virginia, USA

Leigh Syndrome (LS), is a severe neuro-metabolic disorder and has no current cure or adequate cellular models to understand the rapid fatality associated with the disease [1,2]. Other symptoms are widespread tissue malfunction in brain stem and muscle in LS patients. We hypothesize that altered bioenergetic function caused by mitochondrial genome mutations in the electron transfer system (ETS) may lead to rapid fatality in LS. The extent to which pathogenic mtDNA variants regulate disease severity in LS is currently not well understood. To better understand this relationship, we computed the mitochondrial bioenergetics health index (mtBHI) and glycolytic bioenergetics health index (glycoBHI) for measuring overall mitochondrial dysfunction in LS patient fibroblast cells harboring varying percentages of pathogenic mutant mtDNA (T8993G, T9185C) exhibiting deficiency in ATP synthase or Complex I (T10158C, T12706C). The mtBHI was based on four key aspects of mitochondrial respiration: ET capacity minus ROUTINE respiration (*E-R*), net ROUTINE respiration (*R-L*), residual oxygen consumption *Rox* after inhibition by rotenone and antimycin A (ROX state), and LEAK respiration *L*. The glycoBHI was based on four key aspects of cellular proton efflux rate linked to glycolysis [3].

Our results indicated that (*1*) high heteroplasmy was detected in disease lines affecting ATP synthase and low heteroplasmy was detected in disease lines affecting NADH dehydrogenase; (*2*) levels of defective enzyme activities of the ETS correlated with the percentage of pathogenic mtDNA; (*3*) mitochondrial respiration was disrupted in diseased lines with variable *E-R*; (*4*) mitochondrial ATP synthesis rate was decreased while glycolytic ATP synthesis rate was elevated in diseased cell lines.

Based on the overall analysis of the five diseased patient-specific fibroblasts, the glycoBHI emerged as a sensitive indicator of mitochondrial defects because the cells had switched 'on' the glycolytic pathway. GlycoBHI was significantly increased in all cell lines compared to control BJ-FB and was indeed sensitive to mitochondrial dysfunction. We also computed the 'composite BHI ratio' (OXPHOS/Glycolysis) by dividing mtBHI/glycoBHI values because the cell lines were utilizing both OXPHOS (although highly defective) and glycolysis pathways to maintain the energy requirements in the individual cell line.

Overall, these results suggest that as long as the precise mechanism of LS has not been elucidated, a multi-pronged approach that takes into consideration the specific pathogenic mtDNA variant, along with a composite BHI ratio, can aid in better diagnosis and understanding the factors influencing disease severity and rapid fatality in LS.

Future experiments will determine whether mitochondrial morphology depend on mtDNA mutation load and whether they influence bioenergetics within a cell. Our ongoing studies are focused on evaluating mutation burden in human induced pluripotent stem cells (hiPSCs) reprogrammed from these patient fibroblast cells, followed by bioenergetic analyses in differentiated neurons and muscle cells derived from hiPSCs. Results from these studies will address the knowledge gaps that exist in the understanding of relationships among mtDNA mutations, morphology, function, and cell fate that may ultimately contribute to devastating mitochondrial disorders.

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Keywords: mitochondrial disorders; Leigh syndrome; glycolysis; mitochondrial respiration; bioenergetics health index

Cite: Iyer S, Bakare A, Dean J, Chen Q, Thorat V, Huang Y, LaFramboise T, Rao RR, Lesnefsky EJ (2022) Bioenergetics health index ratio in Leigh Syndrome patient fibroblasts as a measure of disease severity. In: https://doi.org/10.26124/bec:2022-0001

E-17

Bioblast link

Effects of eye-movement desensitization and reprocessing (EMDR) therapy on mitochondrial bioenergetics in immune cells from patients with post-traumatic stress disorder (PTSD): a pilot study.

Karabatsiakis Alexander[1,2], Kolassa I-T[2], Tumani V[2]

1. Dept of Clinical Psychology II, Institute of Psychology, University of Innsbruck, Innsbruck, Austria - alexander.karabatsiakis@uibk.ac.at
2. Dept of Clinical & Biological Psychology, Institute of Psychology and Education, University of Ulm, Ulm, Germany
3. Deptt of Psychiatry, Ulm University, Ulm, Germany

Post-traumatic stress disorder (PTSD) is a mental disorder associated with the exposure to chronic and/or traumatic psychological stress. PTSD causes severe individual suffering, impairments in everyday-life functioning and psychosomatic complaints. Using cross-sectional study designs, we [1] and others [2,3] provided evidence for impaired mitochondrial function in different biological samples including intact peripheral blood mononuclear cells (PBMC) collected from patients with major depressive disorder (MDD), one highly prominent psychiatric comorbidity of PTSD. However, data on the possible reversibility effects following psychiatric treatments are sparse but represent an aspect of highest interest for translational biomarker research in the fields of clinical psychology and psychiatry. Here, we present initial data from a pilot study reporting treatment effects of eye-movement desensitization and reprocessing (EMDR) therapy, a clinically approved and specialized treatment option for PTSD, on mental functioning in combination with mitochondrial bioenergetics and biogenesis.

Three female patients diagnosed with PTSD and comorbid MDD were recruited in the Psychiatry Unit of University Hospital in Ulm (Germany), which also provided blood samples for an initial cross-sectional investigation of mitochondrial function in PBMC. One patient agreed to be also followed longitudinally and blood samples were collected at two additional time points across treatment with EMDR. All participants provided written informed consent before participation in the pilot study. Psychiatric impairments related to depression were measured with the Beck Depression Inventory (BDI) [4] as previously reported [1]. To characterize mitochondrial function, PBMC were isolated from non-fasting EDTA-buffered whole blood using density gradient centrifugation procedures. Isolated intact PBMC were cryopreserved using standardized procedures

and thawed samples were used for high-resolution respirometry using the O2k (Oroboros Instruments, Austria). Following the measurement of oxygen consumption rates, mitochondrial density was measured in shock-frozen samples using spectrophotometrical assessment of citrate synthase activity (CSA). Respiration data and CSA results were compared to a group of previously characterized individuals free of any history of mental disorders (control group, *n*=38).

While respiration levels and mitochondrial mass were highly stable over a five-weeks interval in the control group, the samples from the three patients with PTSD showed a significant reduction in these mitochondrial parameters before EMDR treatment. A significant improvement in the clinical severity of depressive symptoms was found with EMDR treatment. In addition, mitochondrial oxygen consumption and mitochondrial mass normalized to the level of the mentally-stable control subjects.

Effects of EMDR treatment on patients with PTSD and comorbid MDD seem to include not only an improvement in the level of mental functioning but can also be associated with a normalization of mitochondrial respiration and mitochondrial density in PBMC. The underlying biomolecular processes that lead to these normalization processes associated with EMDR treatment need further investigation. Here, changes in inflammation and bioenergetic metabolism are of special interest for future studies. Towards a clinically-applicable biomarker in the field of clinical psychology and psychiatry, the robustness of our first observation requires another full study cohort with a longitudinal design to demonstrate and confirm mitochondrial bioenergetics and biogenesis as two interrelated biomarker candidates to be used in psychotherapy and psychopharmacological research.

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Keywords: post-traumatic stress disorder; depression; PBMC; mitochondria; EMDR

Cite: Karabatsiakis A, Kolassa I-T, Tumani V (2022) Effects of eye-movement desensitization and reprocessing (EMDR) therapy on mitochondrial bioenergetics in immune cells from patients with post-traumatic stress disorder (PTSD): a pilot study. In: https://doi.org/10.26124/bec:2022-0001

E-18 *poster*

Bioblast link

Early life stress and perceived chronic stress are associated with increased immune cell mitochondrial DNA copy number in healthy individuals.

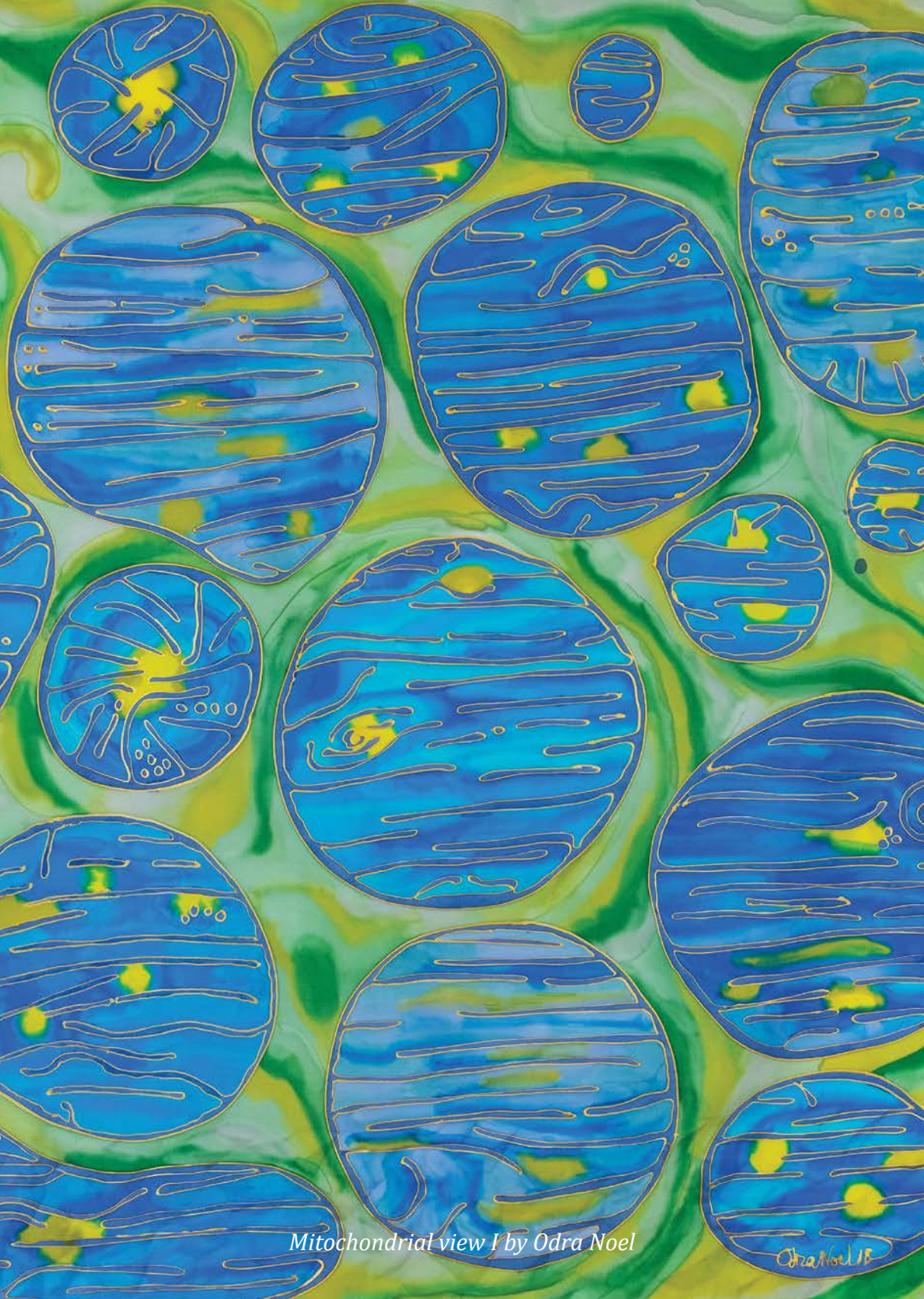
de Punder Karin[1,2], Karabatsiakis A[2], Martens DS[3], Heim C[1,4], Entringer S[1,5,6]

1. Charité – Universitätsmedizin Berlin, Freie Univ Berlin Humboldt-Univ Berlin, Berlin Inst Health (BIH), Inst Med Psychol, Berlin, Germany
2. Inst Psychol, Dept Clin Psychol-II, Univ Innsbruck, Innsbruck, Austria
3. Centre Environ Sci, Hasselt Univ, Diepenbeek, Belgium
4. Dept Biobehav Health, Coll Health Human Develop, Pennsylvania State Univ, Pennsylvania, USA
5. Dept of Pediatrics and Develop, Health Disease Res Program, School Med, Univ California, Irvine, CA, USA
6. Develop, Health and Disease Research Program, School of Medicine, University of California, Irvine, CA, USA

Exposure to various forms of psychological stress represents a potent risk factor for the development and progression of a wide range of age-related physical and mental disorders. Evidence suggests that alterations in mitochondrial function might underly this risk since mitochondria represent a key site of sensing biological stress signals and have been associated with a number of age-related diseases. Mitochondrial DNA copy number (mtDNAcn) is considered a biomarker of mitochondrial biogenesis and function, however, only limited empirical data is available on the effect of psychological stress conditions, like early life stress and chronic stress, on mtDNAcn variation. In the present study, blood samples were drawn from 24 healthy men and women. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood and relative mtDNA content was estimated using a quantitative real-time PCR (qPCR) method. Early life stress exposure was measured using the Adverse Childhood Experiences questionnaire (ACE) and chronic perceived stress was assessed using the Perceived Stress Scale. Our results indicated that individuals that were exposed to childhood adversity displayed higher PBMC mtDNAcn compared to non-exposed individuals (t (22) = -2.2, p = 0.04). In addition, we observed that individuals that perceived high stress levels showed increased PBMC mtDNAcn compared to individuals perceiving low stress levels (F = 4.37, p = 0.03). It might be concluded that the observed changes in PBMC mtDNAcn reflect an adaptation of mitochondria to psychological stress.

Keywords: mitochondrial DNA copy number; early life stress; chronic stress

Cite: de Punder K, Karabatsiakis A, Martens DS, Heim C, Entringer S (2022) Early life stress and perceived chronic stress are associated with increased immune cell mitochondrial DNA copy number in healthy individuals. In: https://doi.org/10.26124/bec:2022-0001



Mitochondrial view I by Odra Noel

F - Mitochondrial pharmacology

F-01

Bioblast link

Towards a treatment for mitochondrial disease: current compounds in clinical development.

Åsander Frostner Eleonor[1,2], Simón Serrano S[1,2], Chamkha I[1,2], Donnelly E[1], Elmér E[1,2], Hansson MJ[1,2]

1. Abliva AB, Medicon Village, 223 81 Lund, Sweden
2. Mitochondrial Medicine, Department of Clinical Sciences Lund, Lund University, Lund, Sweden

Primary mitochondrial diseases are a heterogeneous group of rare genetic disorders affecting approximately 125 persons per million. Mutations underlying these diseases give rise to biological changes (including decrease in cellular energy production and increase in reactive oxygen species), leading to organ failure, and commonly early morbidity. Mitochondrial diseases often present in early childhood and lead to the development of severe symptoms, with severe fatigue and myopathy being some of the most prevalent and debilitating clinical signs.

There are currently no cures for mitochondrial diseases, nor any approved pharmaceutical treatments for multisystemic disorders.

Current drug development in mitochondrial diseases focuses mainly on modulation of oxidative stress, regulation of the expression of genes involved in metabolic pathways, modulation of coenzymes, induction of mitochondrial biogenesis, and energy replacement.

In this short review, we present the current landscape of mitochondrial disease drug development, focusing on small molecules in clinical trials conducted by industrial sponsors [1].

1. Åsander Frostner E, Simón Serrano S, Chamkha I, Donnelly E, Elmér E, Hansson MJ (2022) Towards a treatment for mitochondrial disease: current compounds in clinical development. https://doi.org/10.26124/mitofit:2022-0014

Keywords: mitochondria; primary mitochondrial disease; genetic disorders; MELAS; myopathy

Cite: Åsander Frostner E, Simón Serrano S, Chamkha I, Donnelly E, Elmér E, Hansson MJ (2022) Towards a treatment for mitochondrial disease: current compounds in clinical development. In: https://doi.org/10.26124/bec:2022-0001

F-02

Bioblast link

The decylTPP mitochondria-targeting moiety lowers electron transport system supercomplex levels in primary human skin fibroblasts.

Bulthuis EP[1], Einer C[2], Distelmaier F[1], Groh L[3], van Emst-de Vries SE[1], van de Westerlo E[1], van de Wal M[4], Wagenaars J[1], Rodenburg RJ[4,5], Smeitink JAM[4], Riksen NP[3], Willems PGHM[1], Adjobo-Hermans MJW[1], Zischka H[2,6], Koopman Werner JH[4,7]

1. Dept Biochem (286), Radboud Inst Molec Life Sci, Radboud Ctr Mitochondr Med, Radboud Univ Med Ctr, Nijmegen, NL
2. Inst Molec Toxicol Pharmacol, Helmholtz Ctr Munich, German Res Ctr Environ Health, Neuherberg, DE
3. Dept Intern Med (463), Radboud Inst Molec Life Sci, Radboud Univ Med Ctr, Nijmegen, NL
4. Dept Pediatr, Amalia Children's Hospital, Radboud Inst Molec Life Sci, Radboud Ctr Mitochondr Med, Radboud Univ Med Ctr, Nijmegen, NL
5. Translational Metabol Lab, Radboud Ctr Mitochondr Med, Radboud Univ Med Ctr, Nijmegen, NL
6. Inst Toxicol Environ Hygiene, Tech Univ Munich, School Med, Munich, DE.
7. Dept Human Animal Physiol, Wageningen Univ & Res, Wageningen, NL - werner.koopman@radboudumc.nl

Attachment of cargo molecules to lipophilic triphenylphosphonium (TPP^+) cations is a widely applied key technology for mitochondrial targeting. We previously demonstrated that the vitamin E-derived antioxidant (Trolox; 500 nM; 96 h) increases the levels of active mitochondrial Complex I (CI), the first complex of the electron transfer system (ETS), in primary human skin fibroblasts (PHSFs) of Leigh Syndrome (LS) patients with isolated CI deficiency.

Primed by this finding, we here studied the cellular effects of mitochondria-targeted Trolox (MitoE10), mitochondria-targeted ubiquinone (MitoQ10) and their mitochondria-targeting moiety decylTPP (C_{10}-TPP^+). Relative to vehicle (DMSO), chronic treatment (100 nM, 96 h) with these molecules of PHSFs from a healthy subject and an LS patient with isolated CI deficiency (*NDUFS7-V122M* mutation) did not greatly affect cell viability.

Unexpectedly, this treatment significantly reduced CI levels/activity, lowered the amount of ETS supercomplexes, inhibited mitochondrial oxygen consumption, increased extracellular acidification, altered mitochondrial morphology and stimulated the levels of hydroethidine-oxidizing ROS.

We conclude that the mitochondria-targeting decylTPP moiety is responsible for the observed effects and advocate that every study employing alkylTPP-mediated mitochondrial targeting should routinely include control experiments with the corresponding alkylTPP moiety.

Keywords: Complex I; Trolox; decylTPP; mitochondrial targeting; supercomplexes; glycolysis

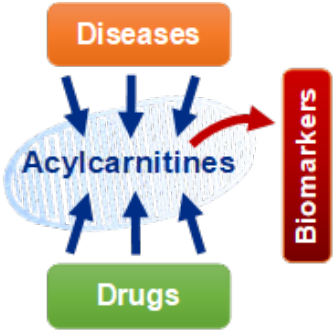
Cite: Bulthuis EP, Einer C, Distelmaier F, Groh L, van Emst-de Vries SE, van de Westerlo E, van de Wal M, Wagenaars J, Rodenburg RJ, Smeitink JAM, Riksen NP, Willems PGHM, Adjobo-Hermans MJW, Zischka H, Koopman WJH (2022) The decylTPP mitochondria-targeting moiety lowers electron transport system supercomplex levels in primary human skin fibroblasts. In: https://doi.org/10.26124/bec:2022-0001

F-03

Bioblast link

Mitochondrial metabolites acylcarnitines: therapeutic potential and drug targets.

Dambrova Maija[1,2], Vilskersts R[1,2], Liepinsh E[1]
1. Latvian Institute of Organic Synthesis, Riga, LV
2. Riga Stradins University, Riga, LV



Acylcarnitines are esters of L-carnitine that emerge from the energy metabolism pathways of fatty acids in mitochondria and peroxisomes [1]. Depending on the length of the acyl chain, acylcarnitines can be grouped as short-, medium-, long- and very long-chain acylcarnitines. Metabolomic profiling assays that investigate disease and nutrition states often include measurements of different acylcarnitines. This has resulted in increased interest regarding the consequences of elevated/decreased levels of plasma acylcarnitine concentrations and the mechanisms associated with these changes.

Altered acylcarnitine metabolome is characteristic for certain inborn errors of fatty acid metabolism, as well as cardiovascular, metabolic and neurological diseases, and some forms of cancer. Acylcarnitines are considered as biomarkers for such diseases and pathological conditions as insulin resistance, heart failure and fatty acid oxidation metabolism-related inherited diseases. Long-chain acylcarnitines accumulate under conditions of insufficient mitochondrial functionality and can reach tissue levels that can affect enzyme and ion channel activities and impact energy metabolism pathways and cellular homeostasis. These detrimental processes directly impact mitochondrial physiology and can exaggerate arrhythmia, insulin insufficiency, neurodegenerative and neuropsychiatric conditions.

Dietary and pharmacological means can be used to regulate synthesis and transport pathways of acylcarnitines and thus counteract the detrimental effects of their accumulation or reverse deficits. The most abundant acylcarnitines, acetylcarnitine and propionylcarnitine, are used as food supplements to tackle neurological and cardiovascular conditions.

Better understanding of biochemical and molecular mechanisms behind increased/decreased acylcarnitine levels and their physiological and pathological roles forms basis for therapeutic target selection and preclinical drug discovery in future and also explains off-target effects of some clinically used drugs [2].

1. Dambrova M et al (2022) Acylcarnitines: nomenclature, biomarkers, therapeutic potential, drug targets and clinical trials. Pharmacol Rev 74:1-50.
2. Dambrova M, Cecatto C, Vilskersts R, Liepinsh E (2022) Mitochondrial metabolites acylcarnitines: therapeutic potential and drug targets. https://doi.org/10.26124/mitofit:2022-0020

Keywords: acylcarnitine; mitochondrial energy metabolism; biomarker; cardiometabolic diseases

Cite: Dambrova M, Vilskersts R, Liepinsh E (2022) Mitochondrial metabolites acylcarnitines: therapeutic potential and drug targets. In: https://doi.org/10.26124/bec:2022-0001

F-04

Bioblast link

Nano-encapsulated dichloroacetophenone (DAP), a regulator of essential mitochondrial enzyme, is a potential inhibitor of prostate cancer cell growth.

Subramaniam S[1,2,5], Jeet V[1,2,5], Gunter JH[1,2,5], Clements JA[1,2,5], Popat A[3,4,5], Batra Jyotsna[1,2,3]

1. School of Biomedical Sciences; Faculty of Health, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia
2. Australian Prostate Cancer Research Centre-Queensland (APCRC-Q), Translational Research Institute, Queensland University of Technology, Woolloongabba, Australia
3. School of Pharmacy, The University of Queensland, Brisbane, QLD, Australia
4. Mater Research Institute, Woolloongabba, QLD, Australia
5. Translational Research Institute, Woolloongabba, QLD, Australia - Jyotsna.batra@qut.edu.au

Our previous genetic studies identified Pyruvate Dehydrogenase Kinase 1 (PDK1) as a key gene regulated by microRNA in an allele dependent manner. Metabolic reprogramming is beneficial for tumour cell. We showed that PDK1 is an oncogene and plays a major role in glycolytic pathway in prostate cancer. Recently, targeting metabolic pathways with drugs has emerged as potential therapy in prostate cancer. In this study we found that DAP is more potent than dichloroacetate (DCA) in inhibiting prostate cancer cell proliferation, migration, colony formation and induced apoptosis. Further, DAP reduced extra cellular acidification rate in prostate cancer cells. In addition, lactoferrin conjugated DAP particle inhibited proliferation of prostate cancer cells at a low dose compared to DAP alone. DAP and lactoferrin conjugated DAP nanoparticles selectively caused a reduction in prostate cancer cell proliferation compared to normal derived cell line. Furthermore, lactoferrin conjugated DAP particles suppressed both glycolytic and oxidative phosphorylation pathway in prostate cancer cells. DAP and lactoferrin conjugated DAP particles suppressed the cell viability of docetaxel resistant cell line, PC3 RX-DT2R in a dose dependent manner. Overall, our results demonstrate that targeting glycolytic pathway via PDK1 by DAP could be therapeutic strategy in prostate cancer. Nanoparticle based DAP delivery may improve the efficiency in targeting prostate tumour metabolism.

Keywords: metabolism; pyruvate dehydrogenase kinase 1; Dichloroacetophenone; prostate cancer; lactoferrin nanoparticle

Cite: Subramaniam S, Jeet V, Gunter JH, Clements JA, Popat A, Batra J (2022) Nano-encapsulated dichloroacetophenone (DAP), a regulator of essential mitochondrial enzyme, is a potential inhibitor of prostate cancer cell growth. In: https://doi.org/10.26124/bec:2022-0001

F-05 *not presented*

Bioblast link

Cytotoxicity of mitochondrial Complex I inhibitor rotenone: a complex interplay of cell death pathways.

Ganguly U[1], Bir A[2], Chakrabarti Sasanka[1]

1. Dept of Biochemistry and Central Research Laboratory, Maharishi Markandeshwar Institute of Medical Sciences and Research, Maharishi Markandeshwar University (Deemed to be), Mullana, Ambala, India - profschakrabarti95@gmail.com
2. Dept of Biochemistry, Dr. B.C. Roy Multispeciality Medical Research Centre, IIT Kharagpur, India

Ferroptosis has been identified as a type of regulated cell death triggered by a diverse set of agents with implications in various diseases like cancer and neurodegenerative diseases. Ferroptosis is iron-dependent and accompanied by an accumulation of reactive oxygen species (ROS) and lipid oxidation products, a depletion of reduced glutathione, mitochondrial morphological alterations and the rupture of cell membrane; the process is inhibited by specific antioxidants like ferrostatin-1 and liproxstatin-1 and by other general antioxidants like the iron-chelator deferoxamine, vitamin E and N-acetylcysteine. However, the mechanism of cell death in ferroptosis subsequent to the accumulation of ROS and lipid oxidation products is not clearly established. We show here that the classical mitochondrial Complex I inhibitor rotenone (0.5 μM) causes death of SH-SY5Y cells (a human neuroblastoma cell line) over a period of 48 h accompanied by mitochondrial membrane depolarization and intracellular ATP depletion. This is associated with an intracellular accumulation of ROS and the lipid oxidation product malondialdehyde or MDA and a decrease in reduced glutathione content. All these processes are inhibited very conspicuously by specific inhibitors of ferroptosis such as ferrostatin-1 and liproxstatin-1. However, the decrease in Complex I activity upon rotenone-treatment of SH-SY5Y cells is not significantly recovered by ferrostatin-1 and liproxstatin-1. When the rotenone-treated cells are analyzed morphologically by Hoechst 33258 and propidium iodide (PI) staining, a mixed picture is noticed with densely fluorescent and condensed nuclei indicating apoptotic death of cells (Hoechst 33258) and also significant numbers of necrotic cells with bright red nuclei (PI staining) [1].

1. Ganguly U, Bir A, Chakrabarti S (2022) Cytotoxicity of mitochondrial Complex I inhibitor rotenone: a complex interplay of cell death pathways. https://doi.org/10.26124/mitofit:2022-0013

Keywords: rotenone; mitochondria; ferroptosis; reactive oxygen species; neurodegeneration

Cite: Ganguly U, Bir A, Chakrabarti S (2022) Cytotoxicity of mitochondrial Complex I inhibitor rotenone: a complex interplay of cell death pathways. In: https://doi.org/10.26124/bec:2022-0001

F-06

Bioblast link

Sterile activation of innate immunity specifically target Complex I activity and flight dispersal in the major arbovirus vector *Aedes aegypti*.

Gaviraghi A[1,2], Barletta ABF[2,3,4], Alves e Silva TL[2,3,5], Oliveira MP[1,2,6], Sorgine MHF[2,3], Oliveira Marcus F[1,3]

1. Laboratório de Bioquímica de Resposta ao Estresse, Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-590, Brazil.
2. Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (INCT-EM), Rio de Janeiro, RJ 21941-590, Brazil.
3. Laboratório de Bioquímica de Artrópodes Hematófagos, Instituto de Bioquímica Médica Leopoldo de Meis, Programa de Biologia Molecular e Biotecnologia, Universidade Federal do Rio de Janeiro, RJ 21941-590, Brazil. - maroli@bioqmed.ufrj.br

Aedes aegypti females are natural vectors of important arboviruses including Dengue, Zika and yellow fever. Mosquitoes activate innate immune response signaling pathways upon infection, which target the pathogens and limit their propagation. Despite the beneficial effects of immune activation for insect vectors, there are phenotypic costs that ultimately affect their fitness. However, the underlying mechanisms that mediate these fitness costs remain poorly understood. Given the high energy required to mount a proper immune response, we hypothesized that systemic activation of innate immunity would impair flight muscle mitochondrial function, compromising tissue energy demand and flight activity. Here, we investigated the dynamic effects of activation of innate immunity by intra-thoracic zymosan injection on *A. aegypti* flight muscle mitochondrial metabolism. Zymosan injection significantly increased defensin expression in fat bodies in a time-dependent manner and ultimately affecting induced flight activity. Although oxidant levels in flight muscle were hardly altered, ATP-linked and maximal mitochondrial oxygen consumption rates were significantly reduced at 24h upon zymosan injection. These effects were parallel to significant and specific reductions in Complex I activity upon zymosan treatment. Finally, the magnitude of defensin up-regulation negatively correlated with maximal, ATP-linked, and NADH-linked respiratory rates in flight muscles. Despite strong reductions were observed in proline and reserve capacity respiratory rates 24h upon zymosan injection, this effect was not correlated to the magnitude of innate immune response activation. Collectively, we demonstrate that activation of innate immunity in fat body strongly associates to reduced flight muscle Complex I activity with direct consequences to mitochondrial physiology and dispersal. Remarkably, our results indicate that a trade-off between dispersal and immunity exists in an insect vector, underscoring the potential consequences of disrupted flight muscle mitochondrial energy metabolism to arbovirus transmission.

Keywords: metabolism; mitochondria; Zika; Dengue; bioenergetics; respiration; muscle; immunity; fitness; dispersal; flight; transmission

Cite: Gaviraghi A, Barletta ABF, Alves e Silva TL, Oliveira MP, Sorgine MHF, Oliveira MF (2022) Sterile activation of innate immunity specifically target Complex I activity and flight dispersal in the major arbovirus vector *Aedes aegypti*. In: https://doi.org/10.26124/bec:2022-0001

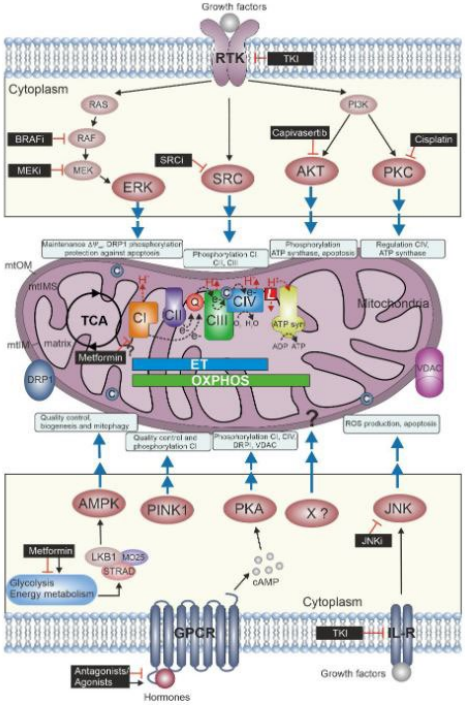
F-07

Bioblast link

Kinase perturbations redirect mitochondrial function.

Torres-Quesada Omar[1,2], Strich S[1], Stefan E[1,2]

1. Tyrolean Cancer Research Institute, Innrain 66, 6020 Innsbruck, Austria - omar.torres@tkfi.at
2. Inst of Biochemistry and Center for Molecular Biosciences, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria



Protein kinases take the center stage in numerous signaling pathways by phosphorylating compartmentalized protein substrates for controlling cell proliferation, cell cycle and metabolism. Kinase dysfunctions have been linked to numerous human diseases such as cancer. This has led to the development of kinase inhibitors which aim to target oncogenic kinase activities. The specificity of the cancer blockers depends on the range of targeted kinases. Therefore, the question arises of how cell-type-specific off-target effects impair the specificities of cancer drugs. Blockade of kinase activities has been shown to converge on the energetic organelle, the mitochondria. In this review, we highlight examples of selected major kinases which impact mitochondrial signaling. Further, we discuss pharmacological strategies to target kinase activities which are linked to cancer progression and redirecting mitochondrial function. Finally, we propose that cell-based recordings of mitochondrial bioenergetic states might predict off-target or identify specific on-target effects of kinase inhibitors [1].

1. Torres-Quesada O, Strich S, Stefan E (2022) Kinase perturbations redirect mitochondrial function. https://doi.org/10.26124/mitofit:2022-0011

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

Cite: Torres-Quesada O, Strich S, Stefan E (2022) Kinase perturbations redirect mitochondrial function. In: https://doi.org/10.26124/bec:2022-0001

F-08

Bioblast link

Tracking patient-mutation and lead-molecule driven alterations of kinase activity conformations.

Stefan Eduard[1,2]

1. Institute of Biochemistry and Center for Molecular Biosciences, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria
2. Tyrolean Cancer Research Institute & KinCon biolabs, Innrain 66, 6020 Innsbruck, Austria

Kinases function as molecular switches for coordinating spatiotemporal signal transmission. Genomic alterations affect kinase abundance and/or their activities which contribute to the etiology and progression of diseases such as distinct cancers and Parkinson's disease (PD). Thus, major drug discovery efforts aim to identify lead molecules targeting the clinically relevant kinase entity. The concept of personalized medicine aims to apply the therapeutic agent with the highest efficacy towards a patient-specific target protein mutation. We have recently implemented a cell-based reporter system which fosters the decision-making process for identifying and selecting efficient lead molecules. We have engineered a modular kinase conformation (KinCon) biosensor platform for live-cell analyses of kinase activity states. This biosensor facilitates the recording of kinase activity conformations of the wild-type and the respective mutated kinase upon lead-molecule or approved-drug exposure. First, in proof-of-principle studies we have demonstrated that this technology is suitable for the systematic determination of melanoma drug efficacies using the full-length KinCon reporters for BRAF and MEK which harbor distinct cancer patient mutations (Röck et al., Science Advances 2019, Mayrhofer et al., PNAS 2020, Fleischmann et al., Biomolecules 2021). Second, we have extended KinCon reporters to quantify the activity-relevant formation of multimeric kinase complexes, involving members of the RIPK [inflammation] or CDK [cancer] kinase families. Third, recently we have engineered mitochondria associated biosensors to analyze and categorize PD kinase mutations. Thus, with new KinCon reporters we are setting out to characterize PD causing kinase gain-of-function mutations and to reactivate a PD kinase displaying a collection of loss-of-function mutations, directly in an intact cell setting. Finally, we would like to underline that this precision-medicine-oriented KinCon biosensor concept is not restricted to recordings of kinase drug efficacies/specificities. We have first evidence that such conformation reporter can extended to other (pseudo)enzyme categories.

Keywords: molecular switch; kinase biosensor; undruggable; off-target effects; drug efficacies

Cite: Stefan E (2022) Tracking patient-mutation and lead-molecule driven alterations of kinase activity conformations. In: https://doi.org/10.26124/bec:2022-0001

F-09

Bioblast link

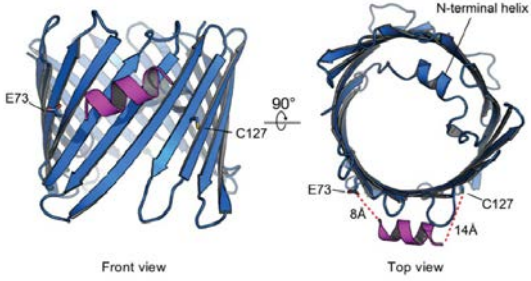
Modulation of mitochondrial respiration in ALS cells by hexokinase-based peptides: a novel therapeutic approach to fight neurodegeneration.

Magrì Andrea[1,2], Cubisino SAM[3], De Pinto V[2,3], Messina A[1,2]

1. Dept Biological, Geological, Environmental Sciences, Univ of Catania, Italy
2. we.MirtoBiotech s.r.l., Catania, Italy
3. Dept Biomedical and Biotechnological Sciences, Univ of Catania, Italy - andrea.magri@unict.it

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease which affects motor neurons (MNs). Many familial and sporadic forms correlate with mutations in the gene encoding the antioxidant enzyme Cu/Zn Superoxide Dismutase (SOD1). Among mutants, the dismutase-active SOD1 G93A forms toxic aggregates on the cytosolic surface of outer mitochondrial membrane (OMM), using the Voltage-Dependent Anion Channel 1 (VDAC1) as binding site [1]. VDAC1 is the most abundant OMM pore-forming protein and allows the trafficking of metabolites (pyruvate, malate), ions, NAD^+/NADH and ATP/ADP across the membrane; furthermore, it serves as an anchor for many cytosolic proteins, mostly for Hexokinases (HKs) [2]. However, in ALS MNs, the mitochondrial accumulation of SOD1 G93A impairs molecules exchange through VDAC1 and displaces HKs from mitochondria, promoting the organelle dysfunction and cell death [1-2].

By a means of *in vitro* and *in cellulo* approaches, we previously demonstrated that HK1 and SOD1 G93A compete for the same mitochondrial binding site, VDAC1 [3]. Based on these observations, we developed a small synthetic peptide corresponding to the first 11 amino acid residues of the HK1 N-terminal domain (NHK1) [3]. NHK1 is able to modulates VDAC1 activity when it is reconstituted in artificial membranes; when added to ALS MNs, the peptide promotes a complete recovery of the cell viability in a dose-response manner [3-4]. By using High-Resolution Respirometry (HRR), we then analyzed the mitochondrial respiration profile of MN-like cells NSC34 stably expressing SOD1 G93A. Our results indicate that NHK1 promotes a partial increase of oxygen consumption corresponding to ROUTINE and OXPHOS state. As demonstrated by FCRs analysis, the peptide stimulates a significative decrease of the LEAK respiration while increases net respiration and coupling efficiency linked to OXPHOS state [4]. This effect is probably due to the reduction of ~70 % of VDAC1-SOD1 G93A aggregates observed in the mitochondrial fraction of cells treated with NHK1 [4].



In conclusion, our results suggest that NHK1 drives the recovery of compromised mitochondrial respiration typical of ALS and provide new insights into the development

of therapeutic molecules to fight the disease. Overall, our work helps to better understand the relationship between altered mitochondrial metabolism and MNs death.

1. Israelson A, Arbel N, da Cruz S, Ilieva H, Yamanaka K, Shoshan-Barmatz V, Cleveland DW (2010) Misfolded mutant SOD1 directly inhibits VDAC1 conductance in a mouse model of inherited ALS. https://doi.org/10.1016/j.neuron.2010.07.019
2. Magrì A, Reina S, De Pinto V (2018) VDAC1 as pharmacological target in cancer and neurodegeneration: focus on its role in apoptosis. https://doi.org/10.3389/fchem.2018.00108
3. Magrì A, Belfiore R, Reina S, Tomasello MF, Di Rosa MC, Guarino F, Leggio L, De Pinto V, Messina A (2016) Hexokinase I N-terminal based peptide prevents the VDAC1-SOD1 G93A interaction and re-establishes ALS cell viability. https://doi.org/10.1038/srep34802
4. Magrì A, Risiglione P, Caccamo A, Formicola B, Tomasello MF, Arrigoni C, Zimbone S, Guarino F, Re F, Messina A (2021) Small Hexokinase 1 peptide against toxic SOD1 G93A mitochondrial accumulation in ALS rescues the ATP-related respiration. https://doi.org/10.3390/biomedicines9080948

Keywords: substrate preference; metabolic conductance; kidney; Complex I; respirometry

Cite: Magrì A, Cubisino SAM, De Pinto V, Messina A (2022) Modulation of mitochondrial respiration in ALS cells by hexokinase-based peptides: a novel therapeutic approach to fight neurodegeneration. In: https://doi.org/10.26124/bec:2022-0001

F-10

Bioblast link

Ubiquinol supplementation accelerates the recovery of mitochondrial health of patients with post COVID-19 syndrome on mountain spa rehabilitation.

Sumbalová Zuzana[1], Kucharská J[1], Rausová Z[1], Palacka P[2], Kovalčíková E[3], Takácsová T[3], Mojto V[4], Navas P[5], López-Lluch G[5], Gvozdjáková A[1]

1. Comenius Univ in Bratislava, Fac of Medicine, Pharmacobiochemical Lab of 3rd Dept of Internal Medicine, Bratislava, Slovakia
2. Comenius Univ in Bratislava, Fac of Medicine, 2nd Dept of Oncology, Bratislava, Slovakia
3. Sanatorium of Dr. Guhr, High Tatras, Tatranská Polianka, Slovakia
4. Comenius Univ in Bratislava, Fac of Medicine, 3rd Dept of Internal Medicine, Bratislava, Slovakia
5. Centro Andaluz de Biología del Desarrollo, Univ Pablo de Olavide-CSIC-JA, and CIBERER, Inst de Salud Carlos III, Sevilla, Spain

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After overcoming COVID-19, some people develop a variety of mid- and long-term effects like fatigue, breathlessness, cognitive dysfunction as part of post COVID-19 condition. These symptoms might persist from the initial illness or develop after the recovery. Spa rehabilitation is recommended for patients with post COVID-19 syndrome. In our previous study deficit of CI-linked mitochondrial function and reduced endogenous

coenzyme Q_{10} (CoQ_{10}) concentration was found in platelets of non-hospitalized, non-vaccinated patients 3 – 6 weeks after acute COVID-19 [1].

After overcoming COVID-19, some people develop a variety of mid- and long-term effects like fatigue, breathlessness, cognitive dysfunction as part of post COVID-19 condition. These symptoms might persist from the initial illness or develop after the recovery. Spa rehabilitation is recommended for patients with post COVID-19 syndrome. In our previous study deficit of CI-linked mitochondrial function and reduced endogenous coenzyme Q_{10} (CoQ_{10}) concentration was found in platelets of non-hospitalized, non-vaccinated patients 3 – 6 weeks after acute COVID-19 [1].

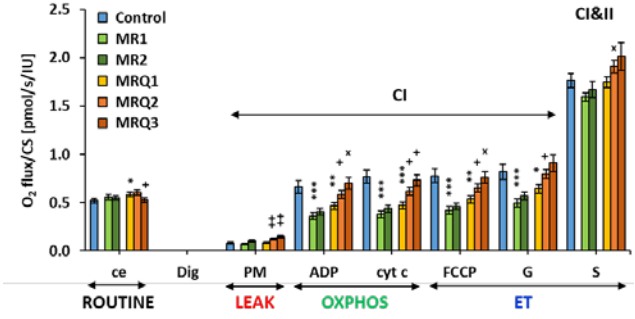

Figure 1: The effect of mountain spa rehabilitation and CoQ10 supplementation on platelet mitochondrial respiration of patients with post COVID-19 syndrome. Freshly isolated platelets (250 x 106) were used in a 2 mL chamber of an O2k-Respirometer (Oroboros Instruments, Austria) filled with mitochondrial respiration medium MiR05 with 20 mM creatine at 37 °C. Substrate-uncoupler-inhibitor titration protocol 1 [2] was applied. The columns show mean ± sem of the *Rox*-corrected respiratory capacities after titration steps indicated on the x-axis. ce: intact cells; Dig: digitonin; PM: pyruvate plus malate; ADP: adenosine diphosphate; cyt c: cytochrome c; FCCP: uncoupler; G: glutamate; S: succinate. All substrates were titrated in kinetically saturating concentrations, the uncoupler FCCP was titrated in optimum concentration to reach the maximum flux. *Rox* – O_2 flux after digitonin. *p<0.05, **p<0.01, ***p<0.001 vs Control; xp<0.1, +p<0.05, ++p<0.01, +++p<0.001 vs. before MR (MR1 or MRQ1).

In this project we studied effects of mountain spa rehabilitation (MR) and MR combined with ubiquinol (reduced form of CoQ10) supplementation (MRQ) on pulmonary function, clinical and psychological symptoms, endogenous CoQ_{10} levels, and platelet mitochondrial bioenergetics of patients with post COVID-19 syndrome.

In total, 36 patients with post COVID-19 syndrome and 15 healthy volunteers (control group) were included in the study. The patients acomplished mountain spa rehabilitation in Sanatorium of Dr. Guhr in Tatranská Polianka, High Tatras, Slovakia with individual therapeutic program including special respiratory physiotherapy procedures, mental well-being, nutrition counseling and adequate exercise therapy. Fourteen patients were on mountain spa rehabilitation (MR) lasting 16 – 18 days and 22 patients were on MR with simultaneous supplementation with ubiquinol (2x100 mg/day) lasting 16 – 18 days and on ubiquinol supplementation for next 12 – 14 days after leaving the spa. Pulmonary function by 6-minute walking test (6MWT), exercise dyspnea by Borg scale (BS), oxygen saturation (SpO_2) and clinical symptoms by questionnaire were evaluated before and after 16 – 18 days of MR. Platelet bioenergetics by high-resolution respirometry, plasma TBARS concentration, and CoQ_{10} concentration in blood, plasma and platelets were evaluated before (MR1 and MRQ1 groups) and after MR (MR2 and MRQ2 groups), and additionally in 8 patients with CoQ_{10} supplementation 12 – 14 days after MR (MRQ3 group).

Platelet mitochondrial Complex I (CI)-linked oxidative phosphorylation (OXPHOS) and electron transfer (ET) capacity was markedly reduced in patients with post COVID-19 syndrome vs the control group (Fig. 1). After 16 – 18 days of MR these parameters improved in both groups vs before MR. The improvement in the group of patients supplemented with ubiquinol was higher than in the non-supplemented group. CI-linked OXPHOS and ET capacity increased further after additional 12 – 14 days of CoQ_{10} supplementation at home (MRQ3 group).

The CoQ_{10} concentration markedly raised after 16 – 18 days of supplementation with ubiquinol in platelets (+60%, p <0.0001), blood (+185%, p<0.0001), and plasma (+232%, p<0.0001) reflecting high bioavailability of supplemented CoQ_{10}. The increase of platelet mitochondrial CI-linked OXPHOS and ET capacity correlated with the increase of CoQ_{10} in platelets and there was a trend to positive correlation between the improvement of pulmonary function and the increase of CoQ_{10} in platelets.

These data show significant role of supplemented ubiquinol in acceleration of mitochondrial health regeneration in patients with post COVID-19 syndrome. Mountain spa rehabilitation with coenzyme Q_{10} supplementation could be recommended to the patients with post COVID-19 syndrome.

1. Sumbalová Z, Kucharská J, Palacka P, Rausová Z, Langsjoen P, Langsjoen AM, Gvozdjáková A (2022) Platelet mitochondrial function and endogenous coenzyme Q_{10} levels are reduced in patients after COVID-19. https://doi.org/10.4149/BLL_2022_002
2. SUIT-001 O2 ce-pce D004

Keywords: post COVID-19 syndrome; lungs function; platelets mitochondrial bioenergetics; coenzyme Q10; mountain spa rehabilitation

Cite: Sumbalová Z, Kucharská J, Rausová Z, Palacka P, Kovalčíková E, Takácsová T, Mojto V, Navas P, López-Lluch G, Gvozdjáková A (2022) Ubiquinol supplementation accelerates the recovery of mitochondrial health of patients with post COVID-19 syndrome on mountain spa rehabilitation. In: https://doi.org/10.26124/bec:2022-0001

F-11 *poster*

Bioblast link

Effects of metformin on mitochondrial function in skeletal muscle cells: differences between therapeutic and suprapharmacological concentrations.

Pavlovic Kasja[1], Krako Jakovljevic N[1], Isakovic AM[2], Ivanovic T[1,3], Markovic I[2], Lalic NM[1,3]

1. Clinic for Endocrinology, Diabetes and Metabolic Diseases, University Clinical Center of Serbia – kasja.pavlovic@med.bg.ac.rs
2. Inst of Medical and Clinical Biochemistry, Faculty of Medicine, Univ of Belgrade
3. Faculty of Medicine, Univ of Belgrade

Metformin is an oral antidiabetic drug that has been widely used in clinical practice for over 60 years. Despite of this, the molecular mechanisms of metformin action are still not completely understood. Although metformin-induced inhibition of mitochondrial respiratory Complex I has been observed in many studies, published data is inconsistent. Furthermore, metformin concentrations used for in vitro studies and their pharmacological relevance are a continuing topic of debate, as concentrations required to

cause mitochondrial Complex I inhibition are significantly higher than the plasma concentrations detected in patients on oral therapy [1]. The aim of this study was to explore the effects of therapeutic metformin concentrations on mitochondrial function in muscle cells in vitro, and compare the effects with those of higher concentrations, that have already been established to affect mitochondrial function. We conducted all experiments in conditions of high and low glucose, in order to evaluate the role of glucose availability on metformin action.

C2C12 mouse skeletal muscle cells were cultured in either high (25 mM) or low glucose DMEM (5.5 mM). Mitochondrial respiration was measured by high-resolution respirometry (Oroboros O2k) while total ROS, superoxide production and mitochondrial membrane potential were measured by flow cytometry (FACS Calibur).

Mitochondrial respiration was measured in permeabilized cells treated with growing concentrations of metformin for 24 h. ROUTINE respiration decreased only in cells treated with the highest concentration (5 mM), while OXPHOS capacity (N-pathway) decreased both in cells treated with 1 mM and 5 mM metformin. Cells cultured in low glucose medium were more sensitive to metformin treatment – untreated cells had significantly higher OXPHOS capacity than cells grown in high glucose medium, and when treated with 5 mM metformin the decrease in respiration was more pronounced (68 % for high glucose and 85 % for low glucose). No differences were observed in LEAK respiration, OXPHOS capacity (S-pathway) or residual oxygen consumption (*Rox*). Measuring respiration of living cells, we observed a decrease in ROUTINE and LEAK respiration and ET capacity in cells treated with 5 mM (but not 50 μM) metformin. We observed no changes in mitochondrial respiration of differentiated and undifferentiated cells treated with 50 μM metformin for 5 days, in any of the respiratory states or either cell culture medium. There was no difference between citrate synthase activity of untreated and cells treated with metformin for 5 days. 5 mM metformin increased total ROS (DHR) and superoxide (DHE) production, which was more pronounced for high glucose compared to low glucose-cultured cells. 5 mM metformin caused depolarization of the mitochondrial inner membrane in both media, the effect being more pronounced in low glucose medium-grown cells. According to our results, micromolar, therapeutic metformin treatment did not cause changes in mitochondrial respiration, ROS production or mitochondrial membrane potential. OXPHOS capacity was higher in untreated low glucose, compared to high glucose medium-cultured cells, which can be explained by the Crabtree effect [2], and was previously shown for C2C12 cells [3]. Higher sensitivity of low glucose-cultured cells to metformin treatment could be a consequence of circumventing the Crabtree effect, by lowering the glucose concentration in cell medium [4]. In conclusion, while suprapharmacological metformin concentrations cause Complex I inhibition in skeletal muscle cells in vitro, therapeutic concentrations cause no such effect in these cells. This suggests the need to further clarify the mechanisms that are relevant for therapeutic effects of metformin in skeletal muscle.

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2. de Kok M, Schaapherder AF, Wüst R, Zuiderwijk M, Bakker JA, Lindeman J, Le Dévédec SE (2021) Circumventing the Crabtree effect in cell culture: A systematic review. https://doi.org/10.1016/j.mito.2021.03.014
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Keywords: metformin; concentration; glucose; Complex I; muscle

Cite: Pavlovic K, Krako Jakovljevic N, Isakovic AM, Ivanovic T, Markovic I, Lalic NM (2022) Effects of metformin on mitochondrial function in skeletal muscle cells: differences between therapeutic and suprapharmacological concentrations. In: https://doi.org/10.26124/bec:2022-0001

F-12 *poster*

Bioblast link

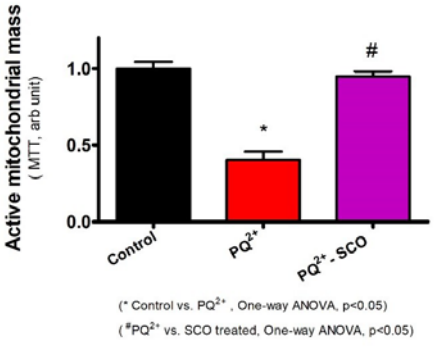
Effects of *Scenedesmus rubescens* extract on Paraquat induced parkinsonism model in SH-SY5Y cells and mitochondrial dysfunction.

Kolonics Attila[1], Léner V[2], Kutasi B[3], Kutasi J[3]

1. Univ of Physical Education, Budapest, Hungary - kolonics.attila@tf.hu
2. Dept of Experimental Pharmacology, National Institute of Oncology, Budapest, Hungary
3. Albitech Biotechnological Ltd., Budapest, Hungary

Parkinson's disease (PD), a common neurodegenerative disease is characterized by the progressive loss of dopaminergic neurons in the substantia nigra. PD is an age related neurodegenerative disorder believed to originate in part via reactive oxygen species overproduction, leading to oxidative stress and mitochondrial dysfunction [1]. Paraquat (PQ^{2+}), a widely used herbicide, is an oxidative stress inducer that has been implicated as a potential risk factor for the development of PD [2]. *Scenedesmus sp.* have been suggested for human nutraceutical application due to their content of the nutritious fatty acids and antioxidant pigments like astaxanthin, β-carotene, lutein and other vitamins. These pigments are naturally occurring polyphenolic compounds that display a variety of therapeutic properties in oxidative stress [3].

The effect of *Scenedesmus rubens* (single cell, green algae) DMSO-extract (SCO) on PQ^{2+}-induced cellular toxicity was evaluated by measuring cell viability by MTT assay. Cellular ATP production was measured by bioluminescent luciferase assay (FLAAM) in SH-SY5Y cells after 3 days of PQ^{2+} treatment. Direct mitochondrial effects of PQ^{2+} vs SCO was evaluated in intact murine brain mitochondria by monitoring basal and succinate induced ROS production, monitoring H_2O_2-induced fluorescence of 1 μM Amplex Red (ex. 560 nm / em. 584 nm) in the presence of horseradish peroxidase (10 IU); $\Delta\Psi_{mt}$ (TMRE); NADH-level via its endogenous fluorescence and ubiquinol cytochrome *c* oxidoreductase activity.



SCO treatment increased the viability of PQ^{2+} exposed SH-SY5Y cells and elevated cellular ATP levels in the OXPHOS state. PQ^{2+}-induced elevation of basal and succinate-mediated ROS production and decrease of complex III activity was ameliorated by SCO. Succinate induced mitochondrial NADH increase was not challenged by PQ^{2+} but it was decreased by SCO suggesting inhibition of RET.

In conclusion, hydrophobic molecular species of *Scenedesmus rubescens* may exhibit neuroprotection against PQ^{2+}-induced neurotoxicity and one of the potential explanantions is a bypass of mitochondrial electron flow that can be therapeutically beneficial in Parkinson's disease but also in vascular diseases affected by ischemia / reperfusion.

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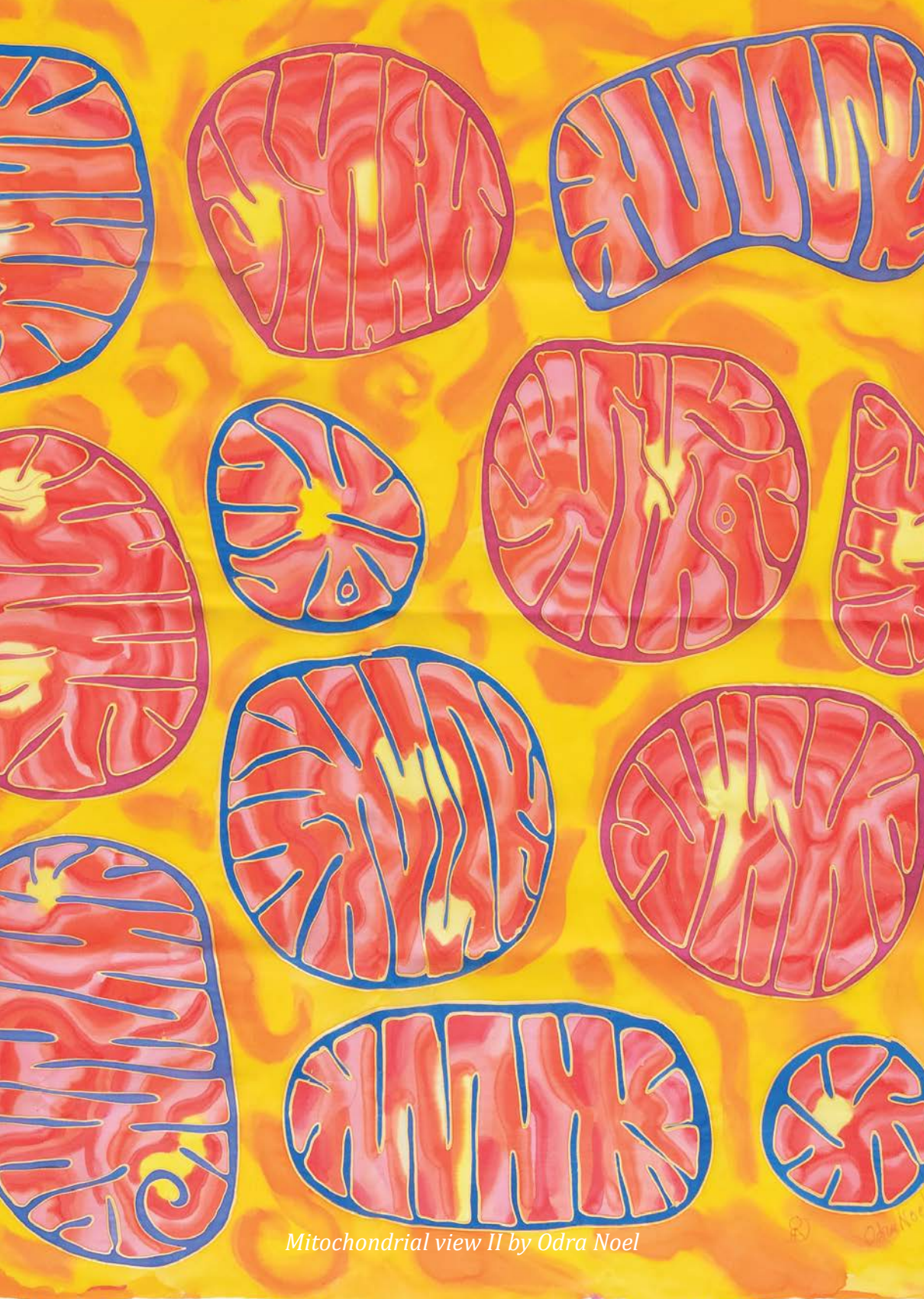
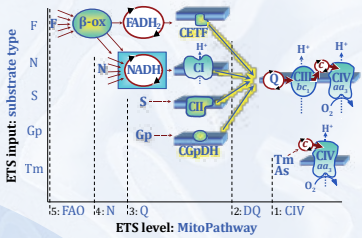
Cite: Kolonics A, Léner V, Kutasi B, Kutasi J (2022) Effects of *Scenedesmus rubescens* extract on Paraquat induced parkinsonism model in SH-SY5Y cells and mitochondrial dysfunction. In: https://doi.org/10.26124/bec:2022-0001



Mitochondrial view II by Odra Noel

MitoPathways: OXPHOS analysis

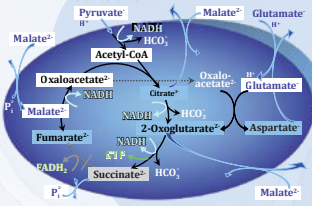
OROBOROS INSTRUMENTS



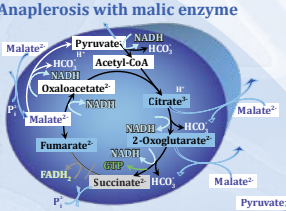
N-pathway

PGM

Anaplerosis with malic enzyme

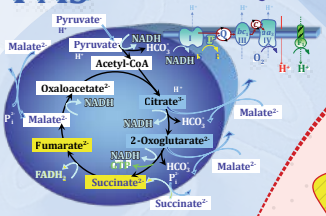


M

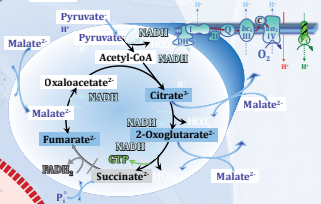


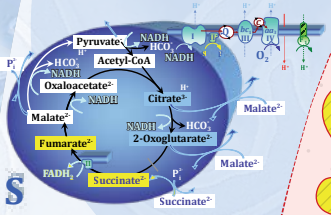
PMS

NS-pathway



PM



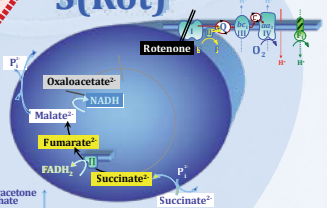


S

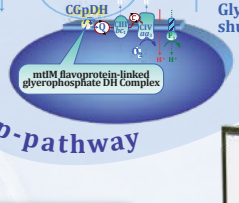
Succinate with malic enzyme

S-pathway

S(Rot)



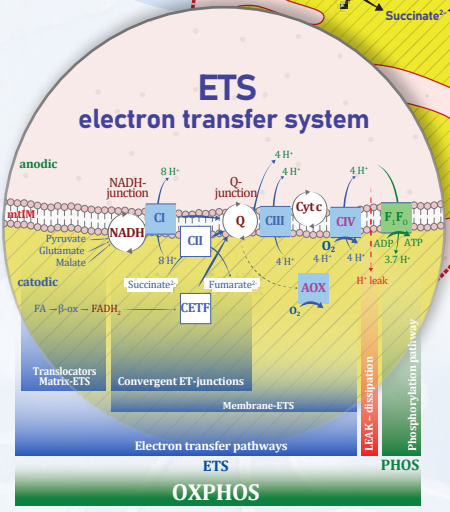
Gp-pathway

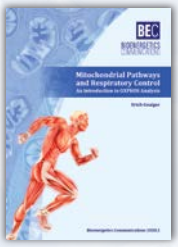


Glycerophosphate shuttle

ETS

electron transfer system





MitoPathways: https://doi.org/10.26124/bec:2020-0002
MitoEAGLE: https://doi.org/10.26124/bec:2020-0001.v1

NextGen O2k

MitoEAGLE

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Fields of Energy I by Odra Noel

Bioblast link

A collection of stories on project reality: How does a project change organizational identity?

Gnaiger Carolina
Oroboros Instruments, Innsbruck, Austria

The project NextGen-O2k has received funding from the European Union's Horizon 2020 research and innovation programme, SME instrument phase 2, under grant agreement No. 859770.

The journal *Bioenergetics Communications* (BEC) was launched in the context of the work package 'Communication and Dissemination' of the H2020 project NextGen-O2k.

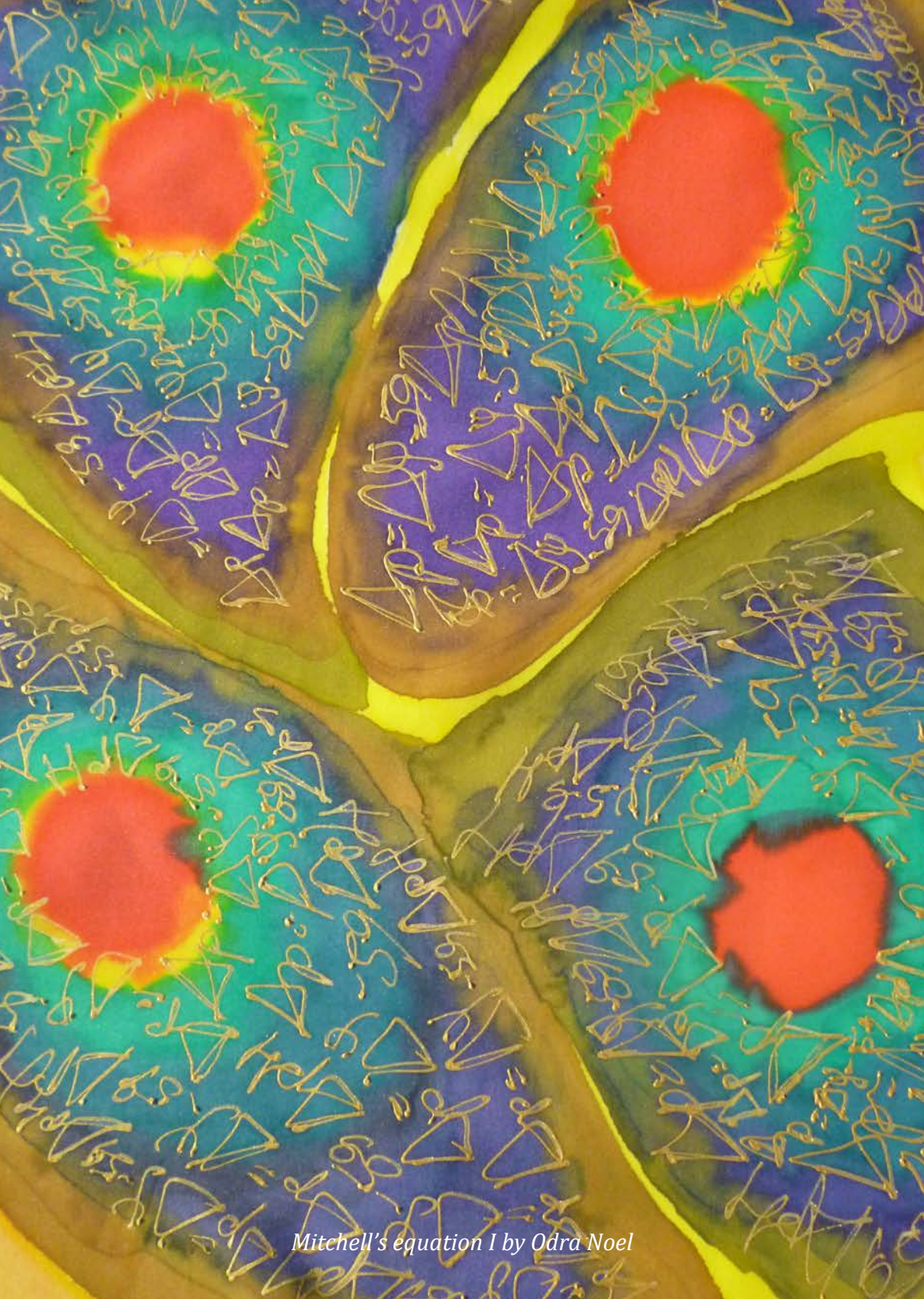
With projects as attractive organizing tools, organizations increasingly work by projects and adopt project management methods for their work. As more and more project-based organizations exist across business areas, knowing more about the impact projects exert on organizations becomes important. How does a project change organizational identity throughout the project life cycle [1]? In the empirical context of the company Oroboros Instruments GmbH and the successfully completed project "NextGen-O2k", the thematically analyzed project stories of members of the organizational team reveal four group narratives on project reality: the project (*1*) as *identity reinforcer* can underscore the continuity of an organization's identity, (*2*) as *analytical lens* gives impetus to reflect about identity, (*3*) as *hero* may help to see difficulties as opportunities in overcoming crises, and (*4*) as *authoritative figure* affects how organizations carry out their work. The interpretation of the narrative constructions confirms that organizational and individual identity change in an interplay. The type of project and the level of prioritization it receives within the organization is decisive in what happens to organizational and individual identity. For the organizational team of Oroboros Instruments, "*this project has taken over everything*", in the sense of Bioblast - the Oroboros Ecosystem.

While the main body of this investigation focused on organizational identity of a company [1], an international scientific journal — such as BEC — requires similar emphasis on identification with its mission. Can *Bioenergetics Communications* take over?

1. Gnaiger C (2022) A collection of stories on project reality: How does a project change organizational SME identity? Master thesis, University of Innsbruck.

Keywords: Projects; Management; Organizations; Organizational identity

Cite: Gnaiger C (2022) A collection of stories on project reality: How does a project change organizational identity? In: https://doi.org/10.26124/bec:2022-0001



Mitchell's equation I by Odra Noel

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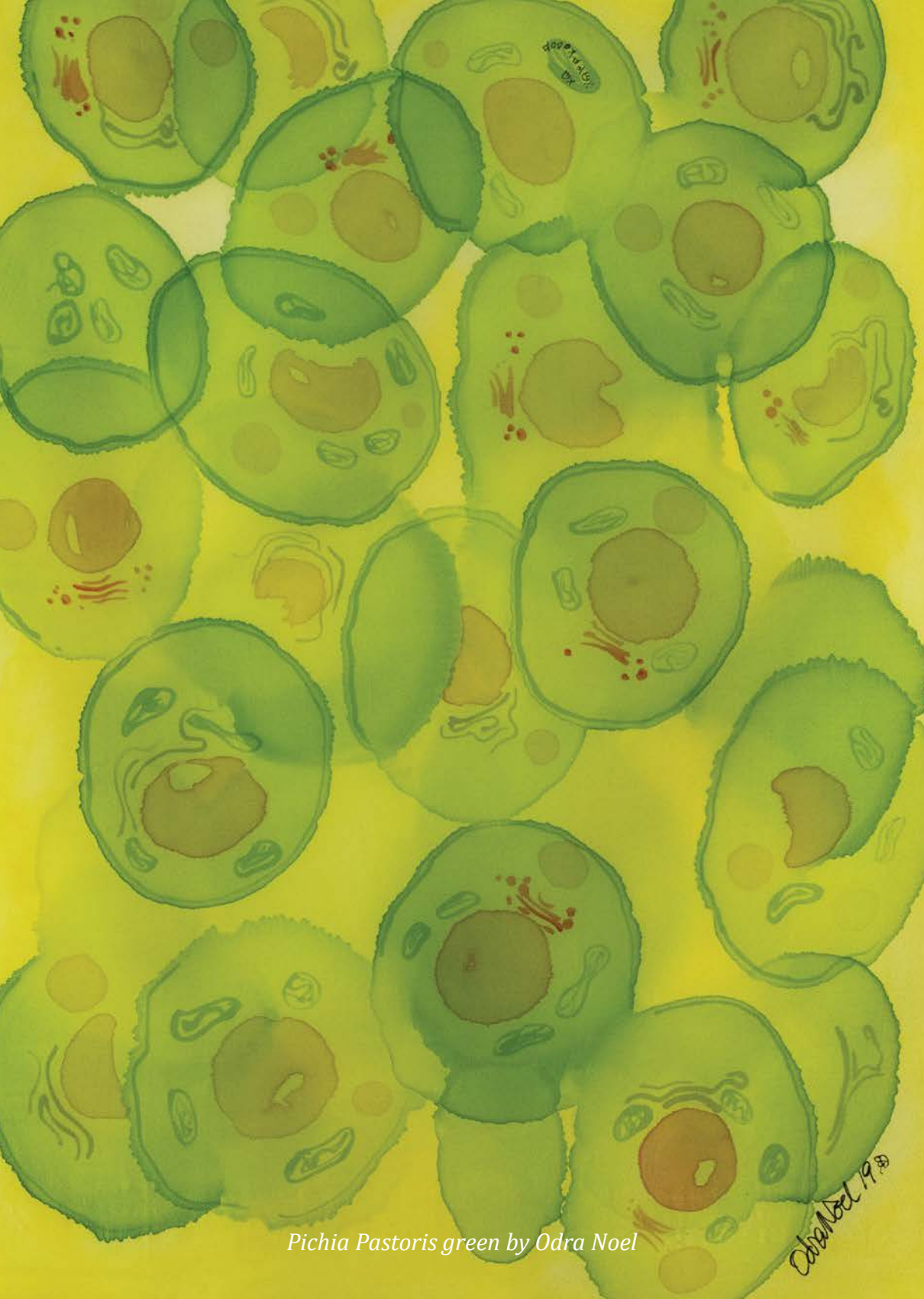

Pichia Pastoris green by Odra Noel

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