Oroboros O2k-Workshop

Mitochondrial Physiology Network 28.01(01):1-12 (2023)
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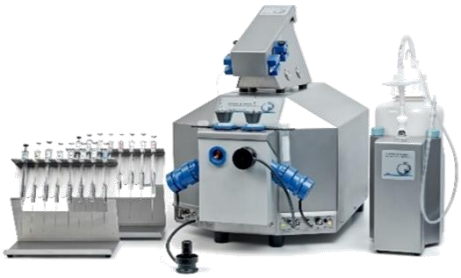
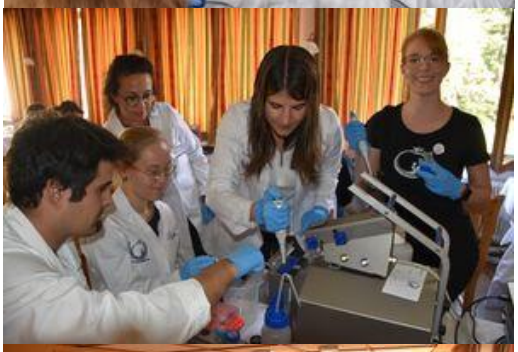
160th O2k-Workshop on High-Resolution Respirometry



2023 June 19–24
Schroecken, Vorarlberg, Austria



The **160th O2k-Workshop on high-resolution respirometry (HRR)** is the **44th** International Oxygraph Course (IOC) held in Schroecken since 1988. We provide an overview of the **O2k-FluoRespirometer**, with real-time analysis by **DatLab 7.4 or DatLab 8 (*new*)** and applications of the **Titration-Injection microPump TIP2k**.
Instrumental setup and service of the polarographic oxygen sensor (**OroboPOS**) are demonstrated, followed by hands-on practice in teams. **Instrumental quality control** is a fundamental component of HRR and will be put to practical test in teams using multiple O2k.
A wide range of mitochondrial topics is covered; abstracts and experimental experiences can be presented by participants. IOC participants invariably asked for a detailed discussion of protocol design. The **Blue Book** (5th edition) and the **Mitochondrial physiology** provide a basic introduction to mitochondrial bioenergetics, complementing the training course, and therefore we recommend reading them beforehand.
The O2k-Workshop will give an introduction of the **O2k-Applications** using **fluorescence**. The hands-on will include Amplex UltraRed experiments with yeast cells, demonstrating the unique advantages and limitations of simultaneous monitoring of oxygen concentration, respiration, and hydrogen peroxide production.
Finally, the new applications of the **NextGen-O2k** will be presented: the Q-Module to assess coenzyme Q-redox state and NADH-Module to assess NAD-redox state. It is possible to join for a visit to the *Alpmuseum*, and lunch breaks provide an opportunity for relaxing *Walks&Talks*, enjoying the refreshing scenery of the secluded alpine environment or using spare time for individual practice.

Lecturers and tutors

Cardoso Luiza	Mitochondrial Wizard, Oroboros Instruments
Donnelly Chris	Guest tutor, Institute of Sport Sciences, University of Lausanne
Gnaiger Erich	CEO, Innovation Alchemist, Oroboros Instruments
Grings Mateus	Mitochondrial Jedi, Oroboros Instruments
Leo Elettra	Mitochondrial Mermaid, Oroboros Instruments
Schmitt Sabine	Mitochondrial Detective, Oroboros Instruments

List of participants

Participant	Institution	Team
Abella Gioscia Pilar	UY Montevideo Radi R - Hospital de Clínicas, UY ***	6
Baelde Rianne	NL_Amsterdam_Nollet EE - Amsterdam UMC, NL **	2
Bercion Sylvie	FR Fort de France Neviere R - University Hospital of Martinique, FR **	5
Boner Winifred	UK_Glasgow_Metcalfe NB - University of Glasgow, UK ***	1
Brendel Heike	DE_Dresden_Birkenfeld AL - Universitätsklinikum Carl Gustav Carus, DE *	4
Dubois Marie-Daniela	FR Fort de France Neviere R - University Hospital of Martinique, FR **	6
Edman Sebastian	SE Stockholm Larsen FJ - GIH The Swedish School of Sport and Health Sciences / Karolinska Institute, SE **	1
Goropashnaya Anya	US AK Fairbanks O'Brien K - University of Alaska Fairbanks, US *	2
Hardorp Rebecka	AT Innsbruck VASCage - VASCage GmbH, AT	8
Kindl Franziska	AT Innsbruck Oroboros – Oroboros Instruments	8
Lauritzen Knut	NO Oslo Eide L - Oslo University Hospital, NO **	4
Liu Tianshi	SE_Lund_Elmer E - Lund University, SE ******	5
Magierecka Agnieszka	UK_Glasgow_Metcalfe NB - University of Glasgow, UK ***	2
McLennan Darryl	UK_Glasgow_Metcalfe NB - University of Glasgow, UK ***	3
Müller Michael	DE_Goettingen_Mueller M - Universitätsmedizin Goettingen, DE *	7
Rice Sarah	US AK Fairbanks O'Brien K - University of Alaska Fairbanks, US *	3
Robertson Danielle	US_TX Dallas_Robertson D - UT Southwestern Medical Cente, US	7
Romero Martinez Alejandra	AT Innsbruck VASCage - VASCage GmbH, AT	5
Sekine Shusuke	SE_Lund_Elmer E - Lund University, SE ******	6
van Agen Laura	DE_Goettingen_Mueller M - Universitätsmedizin Goettingen, DE *	4
Warnaar Vincent	NL_Amsterdam_Nollet EE - Amsterdam UMC, NL **	1
	DE Heidelberg *	7

Asterisks indicate the number of O2k instruments in the participant's lab.

Program

1 Monday, Jun 19

* printed in workshop materials

	Arrival	Weblink
15:00	**Arrival in Bregenz:** Meeting point Bregenz train station at 15:00; approx. 1 h bus drive to Schröcken and Hochtannberg (Salober); walk to Hotel Körbersee (approx. 40 min)	IOC-travel
18:00-19:00	*Welcome reception at Hotel Körbersee & **get-together:*** Introduction of participants and their research interests - a welcome by Oroboros Instruments	Schroecken
19:00	*Dinner*	

2 Tuesday, Jun 20

	Workshop 1		Weblink
07:30-08:30	*Breakfast*		
08:30-09:30	**O2k-Series I and XA and DatLab 7.4 and 8.0** **O2k instrumental setup – overview with video clips** *Mateus Grings*		O2k-FluoRespirometer NextGen-O2k MitoPedia: DatLab DL-Protocols O2k-Videosupport
09:30-12:30	**Hands-on: chamber assembly, volume calibration and OroboPOS service**		
09:30-10:45	Teams **1–4** Chamber assembly	Teams **5–8** OroboPOS service	POS Service O2k FluoRespirometer Manual NextGen-O2k Manual
10:45-11:15	*Coffee / Tea*		
11:15-12:30	Teams **5–8** Chamber assembly	Teams **1–4** OroboPOS service	
12:30-14:30	*Lunch packages / Walk & Talk*		
14:30-15:00	**Oxygen calibration** *Mateus Grings*		SOP: POS-calibration Gnaiger 2008 POS Baglivo BEC 2022.8 *
15:00-15:30	**Hands-on (8 teams): Oxygen calibration (instrumental quality control 1)** DL-Protocol (Instrumental): O2k-cleaning BeforeUse DL-Protocol (Instrumental): O2 calibration air		SOP: O2k-cleaning_and_ISS O2k-Start
15:30-16:00	*Coffee / Tea*		
16:00-19:00	**Hands-on (8 teams): Digitonin test –** Determination of the optimum digitonin concentration for permeabilization of plasma membrane DL-Protocol (O2): SUIT-010 O2 ce-pce D008 DL-Protocol (Instrumental): O2k-cleaning AfterUse		SOP Hamilton microsyringes MiPNet09.12 O2k-Titrations SUIT-010_O2_ce-pce_D008 Video: How to perform an experiment with a SUIT DL-Protocol (DLP)
19:00-20:30	*Dinner*		

20:30-21:30	**O2k perspectives:** 10+5 min presentations of abstracts: Baelde, Warnaar, Mueller, Magierecka	

3 Wednesday, Jun 21

	Workshop 2	Weblink
07:30-08:30	*Breakfast*	
08:30-09:45	**Experimental design:** Pathway and coupling control of mitochondrial respiration *Erich Gnaiger*	MitoPedia: Respiratory states Mitochondrial Physiology * Blue Book *
09:45-10:15	**Substrate-uncoupler-inhibitor titration (SUIT) protocols –** fundamental principles *Luiza Cardoso*	MitoPedia: SUIT
10:15-10:45	*Coffee / Tea* *Registration for the walk to the Alpmuseum*	
10:45-11:00	**Oroboros SUITbrowser:** How to find a DL-Protocol (DLP) *Luiza Cardoso*	Oroboros SUITbrowser Video: How to find a DL-Protocol (DLP)
11:00-11:30	**Experiment traces overview**: Respiration of permeabilized cells - Measurement of oxygen consumption with Reference Protocols RP1 (SUIT-001) and RP2 (SUIT-002) *Luiza Cardoso*	SUIT reference protocol SUIT-001_O2_ce-pce_D003 SUIT-002_O2_ce-pce_D007
11:30-12:30	**Hands-on (8 teams) - getting started with an O2k experiment:** washing, stirrer test, air calibration DL-Protocol (Instrumental): O2k-cleaning BeforeUse DL-Protocol (Instrumental): O2 calibration air	SOP: O2k-cleaning_and_ISS SOP: POS-calibration
12:30-14:00	*Lunch packages / Walk & Talk*	
14:00-16:00	**Hands-on (8 teams) - O2k-experiment:** Respiration with permeabilized HEK 293T cells - SUIT protocols 001 and 002 (RP1 and RP2) DL-Protocol (O2): SUIT-001 O2 ce-pce D003 DL-Protocol (O2): SUIT-002 O2 ce-pce D007 DL-Protocol (Instrumental): O2k-cleaning AfterUse _inhibitors	SUIT-001_O2_ce-pce_D003 SUIT-002_O2_ce-pce_D007
16:00-16:30	*Coffee / Tea – Take turns in your team to continue the experiment*	
16:30-17:45	**DatLab analysis and SUIT protocols** Flux per volume, flux per mass, flow per cell, flux control ratios, flux control efficiencies *Luiza Cardoso*	MitoPedia: Respiratory control ratios MitoPedia: SUIT
17:45-19:00	**Hands-on: DatLab analysis – O_2 flux** Analysis of the hands-on experiment with permeabilized cells.	O_2-Flux Analysis MitoPedia: DatLab
19:00-20:30	*Dinner*	
20:30-21:30	**O2k perspectives:** 10+5 min presentations of abstracts: Edman, McLennan, Abella, Rice	

4 Thursday, Jun 22

	Workshop 3	Weblink
07:30-08:30	*Breakfast*	
08:30-09:00	**Hands-on (8 teams): - getting started with an O2k experiment:** washing, stirrer test, air calibration DL-Protocol (Instrumental): O2k-cleaning BeforeUse DL-Protocol (Instrumental): O2 calibration air	SOP: O2k-cleaning and ISS SOP: POS-calibration
09:00-09:30	**The ABC of hypoxia – what is the norm** *Chris Donnelly*	Donnelly BEC 2022.12
09:30-10:00	**Introduction to H_2O_2 measurements** *Sabine Schmitt*	Amplex UltraRed H_2O_2 Komlodi BEC 2021.4 *
10:00-10:30	**Hands-on (8 teams): H_2O_2 protocol for living cells** Oxygen dependence of H_2O_2 production with baker´s yeast DL-Protocol (O2&AmR): AmR calibration DL-Protocol (O2&AmR): SUIT-013 AmR ce D023	SUIT-013 AmR ce D023
10:30-11:00	*Coffee / Tea – Take turns in your team to continue the experiment*	
11:00-12:30	**Hands-on (8 teams): continuation** DL-Protocol (O2&AmR): SUIT-013 AmR ce D023 DL-Protocol (Instrumental): O2k-cleaning AfterUse	SUIT-013 AmR ce D023
12:30-14:30	*Lunch packages / Walk & Talk*	
14:30-15:30	**H_2O_2 data analysis** *Sabine Schmitt*	H_2O_2-Flux Analysis
15:30-16:30	**Hands-on: H_2O_2 data analysis**	H_2O_2-Flux Analysis
16:30-17:00	*Coffee / Tea*	
17:00-18:00	**Data interpretation using SUIT protocols OXPHOS analysis: diagnosis of respiratory defects** *Erich Gnaiger*	MitoPedia: SUIT
18:00-18:30	**Results obtained with SUIT-001 and 002 presentation and discussion** *Luiza Cardoso*	
19:00-20:30	*Dinner*	
20:30-21:30	**O2k perspectives:** 10+5 min presentation of abstract: Robertson **SUIT Quiz**	

5 Friday, Jun 23

	Workshop 4	Weblink
07:30-08:30	*Breakfast*	

08:30-09:00	**Introduction to instrumental O2 background** (traces overview), using the TIP2k *Elettra Leo*	MiPNet14.06 Instrumental O2 background
09:00-11:00	**Hands-on (8 teams): Instrumental O2 background (instrumental quality control 2)** O2 background test with the TIP2k; analysis of oxygen flux; O2 background from air saturation to zero oxygen concentration DL-Protocol (Instrumental): O2k-cleaning BeforeUse DL-Protocol (Instrumental): Instrumental O2 background TIP2k	TIP2k manual
10:30-11:00	*Coffee / Tea – Take turns in your team to continue the experiment*	
11:00-12:00	**Hands-on (8 teams): Data analysis - instrumental O_2 background flux**	
12:00-12:30	*Lunch packages*	
12:30-15:30	*Walk to the Alpmuseum – guided tour and reception: € 15*	Alpmuseum
15:30-16:00	*Coffee / Tea*	
17:00-17:30	**Introduction to permeabilized fibers** *Erich Gnaiger*	MiPNet14.14 Permeabilized Fiber Preparation
17:30-18:30	**Oroboros Ecosystem - Tutorial on the Bioblast wiki** *Erich Gnaiger*	Bioblast O2k-Network O2k-Publications
18:30-18:15	**NextGen-O2k: Q-Module** *Luiza Cardoso*	Q-Module
18:15-19:00	**NextGen-O2k: NADH-Module** *Mateus Grings*	NADH-Module
19:00-20:30	*Dinner*	
20:30-21:30	*Feedback discussion: Next steps in the individual projects*	

6 Saturday, Jun 24

	Departure	
06:30-7:30	*Breakfast*	
08:15	**Departure from Hotel Körbersee, bus departure 9:00 at Salober**	

O2k perspectives abstracts: 10+5 min

Abella P[1], Valez V[2], Vaamonde L[1], Blasina F[1], Rodriguez-Rey M[1,2] (2023) Severe acute neonatal hypoxia: mitochondrial dysfunction at an animal model and the role of nitric oxide. Mitochondr Physiol Network 28.01.

1. Hospital de Clínicas University Hospital, School of Medicine, University of the Uruguayan Republic, UDELAR, Uruguay
2. Centro de Investigaciones Biomédicas CEINBIO. School of Medicine, University of the Uruguayan Republic, UDELAR, Uruguay

Hypoxic-ischemic events due to intrapartum complications are the second leading cause of neonatal mortality and initiate an acute brain disorder known as hypoxic-ischemic encephalopathy (HIE). In HIE, the brain undergoes primary and secondary mitochondrial energy failure phases, between there is a latent phase where partial neuronal recovery is observed. At neuronal level, the entry of calcium due to hypoxia-ischemia, activates neuronal nitric oxide synthase (nNOS) resulting in the production of nitric oxide (•NO). This leads to accumulation of reactive oxygen and nitrogen species, causing mitochondrial damage. Mitochondrial dysfunction exacerbates the injury caused by hypoxia. Pharmacological treatments targeting mitochondria or inhibiting •NO production plays a key role in improving mitochondrial function, consequently, neuroprotection. 2-iminobiotin (2IB) inhibits nNOS and is currently in study as a neuroprotective agent.
The aim of this study is to investigate the effect of hypoxia on the developing brain in a neonatal piglet model and the pharmacological neuroprotection provided by 2IB as a modulator of neuronal •NO production.
For this purpose, a 24-48-hour-old newborn piglet (*Sus scrofa domestica*) model is used. The animals are anesthetized and placed on mechanical ventilatory support with FiO2 of 0.21 (normoxia). Throughout the experiment, they are continuously monitored using pulse oximetry and regional cerebral near-infrared spectroscopy (NIRS), invasive blood pressure measurement, integrated amplitude electroencephalogram (aEEG), central temperature, and serial blood gasses analysis. Hypoxia is induced by obstructing the endotracheal tube for 4 minutes, repeating this procedure 3 times every 30 minutes. Between each hypoxia, re ventilation with FiO2. 0.21 The administration of 2IB is done immediately after hypoxia (intravenous 0.2 mg/kg of 2IB). After 4 hours, the animal is sacrificed. Brain biopsies are taken to measure mitochondrial function. Mitochondrial respiration is measured in brain biopsies using an Oroboros Oxygraph at 37°C.
At present, this project is under development. Some experimental procedures have been already done. During hypoxia it was observed hemodynamic affectation shown by bradycardia, increased blood pressure, and decreased oxygen saturation and regional cerebral oxygen saturation, recovering between each hypoxia. On the aEEG, a voltage decrease is observed during hypoxia with subsequent recovery. In blood gasses analysis it is observed a sustained increase in lactate without recovery between hypoxia. Regarding mitochondrial function, a decrease in all respiratory indices was observed in the hypoxia group compared to the control group. We observe significant differences on maximum respiration, reserve capacity and non-mitochondrial consumption. Until now we do not have 2IB results.

This project is being developed on the perinatal unit of the University Hospital, at the neonatal pathology area working with a neonatal piglet model with high translational value, with partnership of CEINBIO.

Baelde R[1], Fortes Monteiro A[1], Nollet E[1], Galli R[1], Strom J[2], van der Velden J[1], Ottenheijm C[1], de Winter J[1] (2023) Kbtbd13^{R408C}-knockin mouse model elucidates mitochondrial pathomechanism in NEM6. Mitochondr Physiol Network 28.01.

1. Dept of Physiology, Amsterdam UMC, location VUmc, The Netherlands
2. Dept of Cellular and Molecular Medicine, University of Arizona, Tucson, USA

Nemaline Myopathy (NEM) is among the most common non-dystrophic congenital myopathies. Nemaline Myopathy type 6 (NEM6) is characterized by muscle weakness and muscle slowness

and caused by variants in Kelch-repeat-and-BTB-(POZ)-Domain-Containing-13 (*KBTBD13*). The majority of the NEM6 patients harbors the Dutch founder mutation *KBTBD13R408C* (c.1222C>T, p.Arg408Cys). Histological characterization of NEM6 patient biopsies by NADH staining showed the presence of cores, indicating the absence of complex I (NADH) activity, suggesting mitochondrial dysfunction. Here, we aimed to investigate whether mitochondrial dysfunction contributes to NEM6 disease pathology. Therefore, we used the *Kbtbd13R408C*-knockin mouse model that phenocopies NEM6 hallmarks, e.g muscle weakness, impaired muscle relaxation kinetics and the presence of nemaline bodies. First, we used metabolic treadmill experiments to investigate mitochondrial function *in vivo*. 9 months old homozygous *Kbtbd13R408C*-knockin mice showed a significant impaired running performance, decreased VO2max and increased respiratory exchange ratio (RER) compared to wildtype (WT) mice. Second, mitochondrial respiration was investigated at the muscle tissue level by *in vitro* respirometry experiments in oxidative muscle (soleus). Soleus muscle of *Kbtbd13R408C*-knockin mice showed significant decreased complex I (NADH) linked respiration and total oxidative phosphorylation compared to WT mice, indicating that mitochondrial dysfunction contributes to the lower VO2max and running performance assessed *in vivo.* Third, we performed enzymatic NADH and SDH stainings on cryosections of soleus muscle of 9 months old WT and *Kbtbd13R408C*-knockin mice to assess enzymatic activity in both slow-twitch (type I) and intermediate/fast-twitch (type IIa) myofibers. Soleus muscle of *Kbtbd13R408C*-knockin mice showed cores in both type I and type IIa myofibers. To conclude, the presence of cores in myofibers of *Kbtbd13R408C*-knockin mice phenocopies NEM6 patients. The *Kbtbd13R408C*-knockin mouse model revealed that mitochondrial dysfunction contributes to NEM6 disease pathology. Next, we will use the *Kbtbd13R408C*-knockin mouse model to study the onset and progression of mitochondrial dysfunction in NEM6 and test interventions that target mitochondrial function.

Edman S[1,2], Flockhart M[1], Larsen FJ[1,4*], Apro W[1,3,4*] (2023) Need for Speed: Human fast-twitch mitochondria favor power over efficiency. Mitochondr Physiol Network 28.01.

1. The Åstrand Laboratory, Department of Physiology, Nutrition and Biomechanics, The Swedish School of Sport and Health Sciences, Stockholm, Sweden
2. Department of Women's and Children's Health, Karolinska Institute, Stockholm, Sweden
3. Department of Clinical Sciences, Intervention and Technology, Karolinska Institute, Stockholm, Sweden
* These authors contributed equally

At the request of the author, this abstract is not made available online.

Magierecka A, McLennan D, Dawson N, Millet C, Metcalfe NB (2023) Sustained swimming performance is independent of organism-level and mitochondrial-level metabolism in European minnows. Mitochondr Physiol Network 28.01.

School of Biodiversity, One Health and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK

Metabolic rate is a fundamental trait that influences and constraints the behaviour and performance of animals. However, conventional measures of metabolic rate, based on whole-animal oxygen consumption, often fail to show the predicted relationships with measures of animal performance since it is unclear what proportion of consumed oxygen is associated with ATP production. Thus the efficiency with which the mitochondria convert oxygen into ATP can be a better determinant of an animal performance capacity. In this study we examined whether mitochondrial efficiency predicts sustained swimming performance in the European minnow (*Phoxinus phoxinus*), a riverine fish. We measured individual critical swimming speed (Ucrit) followed by a measurement of maximum metabolic rate (MMR), standard metabolic rate (SMR) and mitochondrial function, predicting that measures of mitochondrial performance such as the ATP produced per molecule O_2 consumed will be positively related to U_{crit} and that the variation in U_{crit} explained by mitochondrial function will be greater than any explained by MMR and SMR.

Maximal rates of oxidative phosphorylation at the mitochondrial level were positively correlated with maximal oxidative metabolism of the whole fish. However, contrary to our predictions, swimming performance was unrelated to both organism-level and our measures of mitochondrial level metabolism, with U_{crit}, MMR and SMR being influenced by individual body mass only. This suggests that, while mitochondrial function predicts whole-animal metabolism, it does not necessarily constrain animal locomotor performance, and the maximum sustained swimming speed is not determined by energy or oxygen supply. Further research is needed to determine the limits to physical performance.

McLennan D (2023) Maximal rates of mitochondrial oxidative phosphorylation predict territorial performance in brown trout. Mitochondr Physiol Network 28.01.

School of Biodiversity, One Health and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK

In social hierarchies, establishing dominance over competing conspecifics confers better access to food, shelter, and reproductive opportunity. Dominance status has previously been linked to body size and prior residency (i.e., who was there first); however, experimental studies have shown that even when opponents are size- and residency-matched, clear dominance hierarchies often still become established. One explanation for this may be among-individual variation in metabolic rate, since a higher metabolic rate could allow a greater allocation of energy to dominance-related traits, such as aggressive behaviours. In this study, size-matched brown trout (Salmo trutta) were tested for their ability to fight for territories in a landscaped artificial stream tank, based on two days of behavioural observations on colouration, aggressive behaviour and food acquisition. Each individual faced 3 opponents, so that each fish could be ranked from most competitive (winning all three territorial battles) to least (losing all three). We then measured their mitochondrial function. Mitochondrial efficiency (i.e., the number of ATP produced per molecule O2 consumed) was unrelated to any of our measured dominance-related traits. However, we found that maximal rates of oxidative phosphorylation at the mitochondrial level were positively correlated with an individual's territorial performance – individuals with higher phosphorylation were better at acquiring food, were more aggressive and were therefore more likely to establish overall dominance. This study exemplifies how there can be clear links between mitochondrial bioenergetics and an individual's overall performance.

Mueller M (2023) Deciphering the role of cerebral redox imbalance and mitochondrial dysfunction in Rett Syndrome. Mitochondr Physiol Network 28.01.

Inst Neuro- und Sensory Physiology, University Medical Center Goettingen, Germany

At the request of the author, this abstract is not made available online.

Rice S (2023) Metabolism at Extremes: *in vivo* kinetics of metabolites in hibernation. Mitochondr Physiol Network 28.01.

Institute of Arctic Biology, University of Alaska Fairbanks, US

At the request of the author, this abstract is not made available online.

Robertson DM (2023) Dysregulation of mitochondrial homeostasis in ocular disease. Mitochondr Physiol Network 28.01.

Department of Ophthalmology, UT Southwestern Medical Center, Dallas, Texas, USA

In the eye, it is now widely recognized that dysregulation of mitochondrial homeostasis is a key pathophysiological event in many ocular diseases. The ocular surface is comprised of two contiguous epithelia that cover the anterior most surface of the eye. These include the corneal and conjunctival epithelium. Together, they form the stratified epithelial surface that functions to prevent pathogen invasion and, with respect to the corneal epithelia, is critical for the refraction of light. Different types of stress negatively impact the ocular surface. These include

hyperglycemic stress (as seen in diabetes), hyperosmolar stress (as seen in dry eye disease), inflammatory stress, drug and pollutant toxicity, contact lens wear, and stress-induced by host-pathogen interactions. To investigate these effects on the corneal and conjunctival epithelium, we are focusing on changes in mitochondrial and metabolic homeostasis, including mitochondrial dynamics and mitophagy. To investigate changes in cell metabolism, we have coupled metabolomics with real time Seahorse metabolic flux analysis. In this presentation, we will present our current state of knowledge on metabolic changes that occur during hyperosmolar stress, inflammatory stress, and infection. For the latter, we will discuss changes in metabolism that occur during infection by the opportunistic gram-negative pathogen, *Pseudomonas aeruginosa*. Our overall goal is to identify how specific metabolic pathways are impacted in response to pathological stress and ultimately develop novel therapeutics designed to enhance mitochondrial metabolism and promote disease resolution.

Warnaar V[1], Nassar A[1], Nollet E[1], Michels M[2], Kuster D[1], Ochala J[3], Buikema JW[1,4], van der Velden J[1] (2023) Effect of Mavacamten on the myofilament-mitochondrial axis in hypertrophic cardiomyopathy with and without sarcomere mutation. Mitochondr Physiol Network 28.01.

1. Amsterdam Cardiovascular Sciences, Department of Physiology, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
2. Department of Cardiology, Erasmus MC, Rotterdam, The Netherlands
3. Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark
4. Heart Center, Department of Cardiology, Amsterdam UMC, Amsterdam, The Netherlands

Hypertrophic cardiomyopathy (HCM) is the most common autosomal dominant inherited form of cardiomyopathies. A lack of knowledge about the key regulators that are involved in the disease onset and progression results in limited therapeutic strategies to prevent or cure HCM. This study focuses on the role of mitochondrial (dys)function in the pathophysiology of HCM, and test the effect of the myosin inhibitor Mavacamten (MAVA). Previous research has shown that MAVA corrects the so-called Super Relaxed (SRX) / Disorded-relaxed (DRX) ratio of myosin. MAVA shifts myosin heads from the high energy consuming DRX to an energy-saving SRX state. To define both the acute and chronic effect of MAVA treatment studies in cardiac tissue slices from HCM patients, stem-cell derived engineered heart tissue (EHT) and an established HCM mouse model will be combined.

To study MAVA-mediated PREVENTIVE effects we will set up a human in vitro model for HCM. Therefore, we will generate EHTs from human induced pluripotent stem cell lines with Crispr/Cas9-induced MYBPC3 (c.2373insG) founder mutation, the isogenic control line and two patient-derived cell lines. Next, ex vivo tissue slices, of cardiac samples from HCM patients, will be kept in culture to study the REVERSIBILITY of the altered myofilament-mitochondrial axis by chronic MAVA treatment. To proof the effects of MAVA treatment on the heart an in vivo mouse model with the same Dutch founder mutation is used to link the in vitro data on the myofilament-mitochondrial axis with in-depth in vivo characterization of the heart. Functional readouts such as contraction and relaxation parameters are obtained and subsequent analyses include mitochondrial function, SRX/DRX ratio analyses. It is expected that MAVA reduces ATP demand and improves mitochondrial function by normalizing the SRX/DRX ratio. Subsequent improved mitochondrial function, and the coincident reduced oxidative stress, exerts positive effects on cardiac remodeling.

Funding was provided by BMS and NWO-VICI.

Accommodation and location

Hotel Körbersee https://www.koerbersee.at/en
T +43 5519 265 hotel@koerbersee.at

More detail?

Gnaiger E (2020) **Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis**. 5th ed. Bioenerg Commun 2020.2. https://doi.org/10.26124/bec:2020-0002

Gnaiger E et al — MitoEAGLE Task Group (2020) **Mitochondrial physiology**. Bioenerg Commun 2020.1. https://doi.org/10.26124/bec:2020-0001.v1

O2k-Manual – http://wiki.oroboros.at/index.php/O2k-Manual
O2k-Procedures – http://wiki.oroboros.at/index.php/O2k-Procedures
>4,500 O2k-Publications – http://wiki.oroboros.at/index.php/O2k-Publications:_Topics

MitoFit Preprints

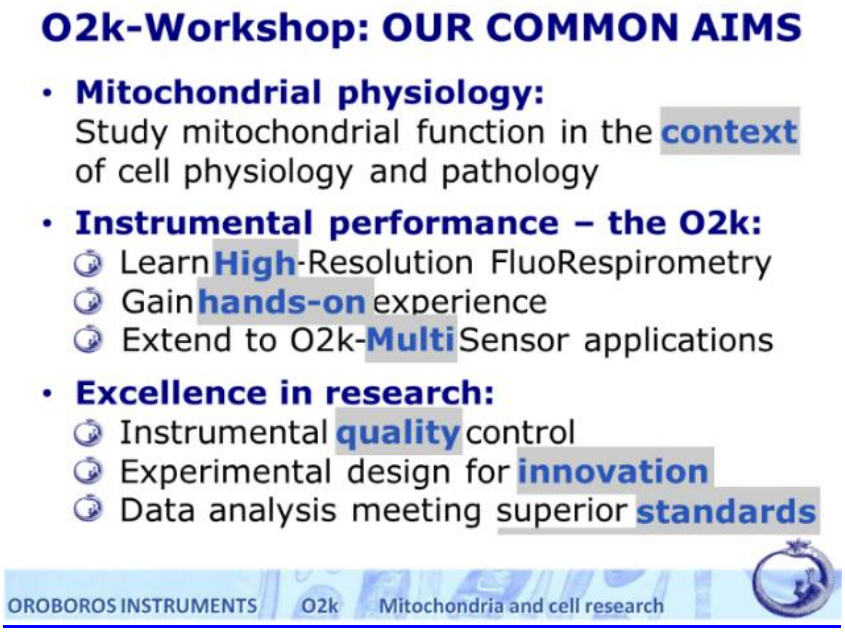
The Open Access preprint server for mitochondrial physiology and bioenergetics

» https://www.mitofit.org/index.php/MitoFit_Preprints

Bioenergetics Communications

The Open Access journal for publishing scientific and technical advances in bioenergetics and mitochondrial physiology as Living Communications

» https://www.bioenergetics-communications.org



Acknowledgements

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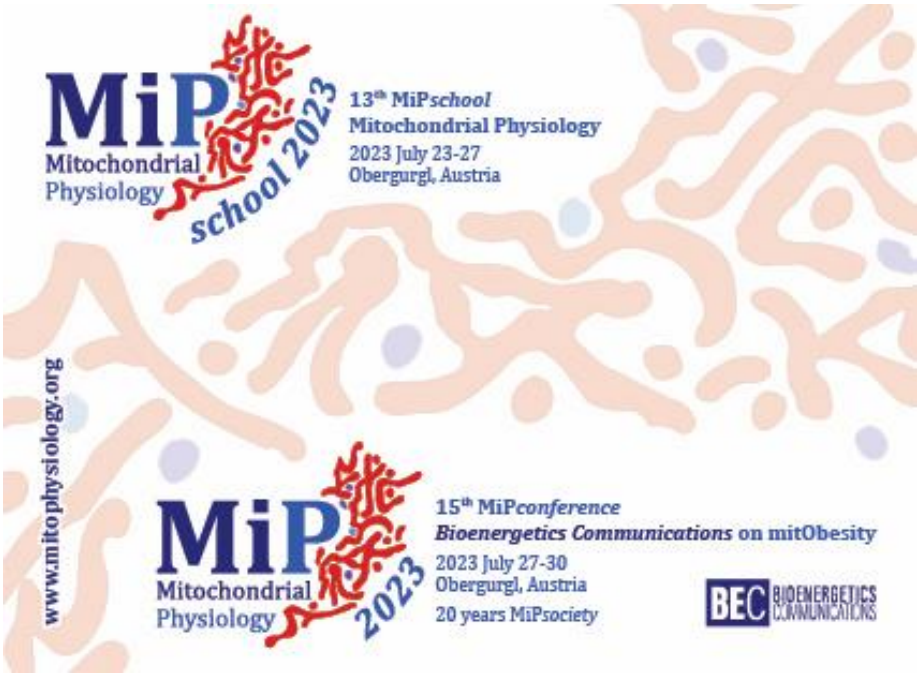

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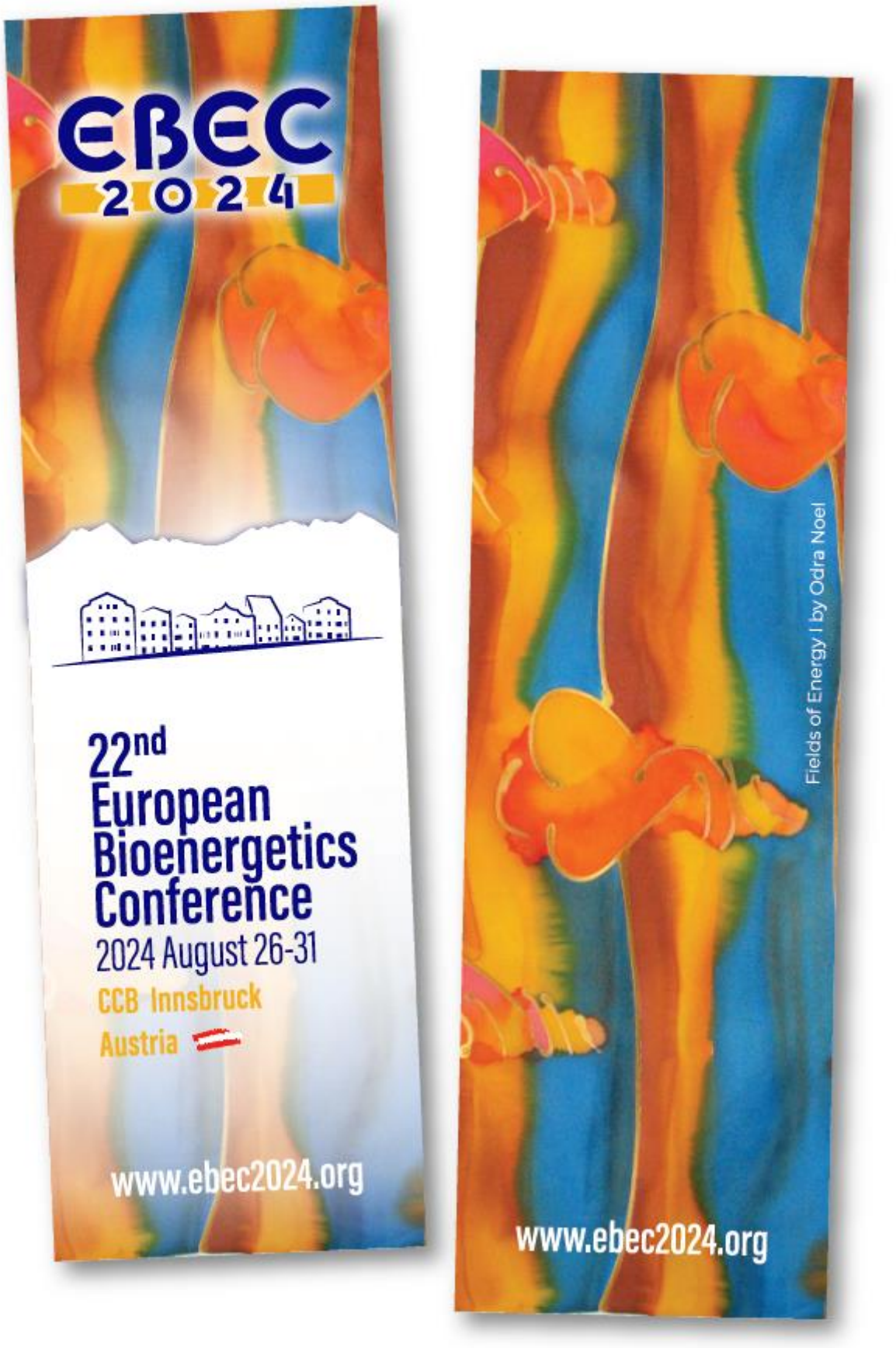
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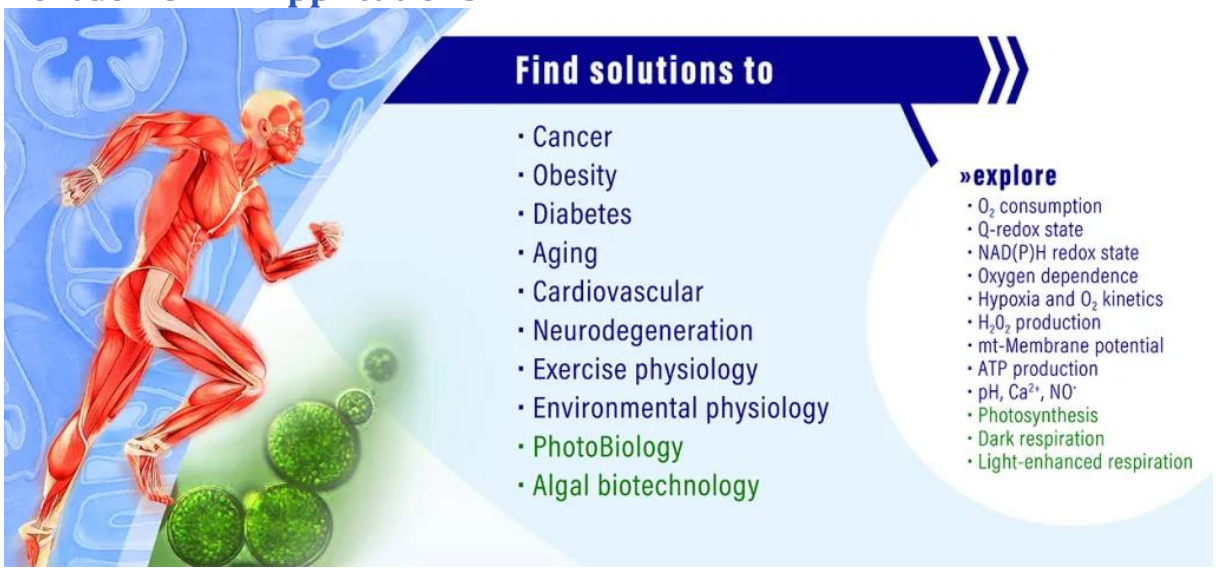

Oroboros - as a driving force in mitochondrial physiology - extends the analytical and diagnostic power of high-resolution respirometry by integration of NADH- and Q-redox monitoring in the **NextGen-O2k**. We aim at establishing the Oroboros quality control management for dissemination to our worldwide O2k-Network laboratories. This will become an effective contribution to address the acute *reproducibility crisis* of scientific investigation. In the spirit of Open Science and global networking, we will enable data sharing across projects and institutions in an Open Access database on mitochondrial physiology and pathology, to resolve the *inflation crisis* and ultimately the *value-impact crisis* of present academic publication. This will support key developments in mitochondrial medicine. In addition, we expand our business to algal biotechnology and ecology with the NextGen-O2k PhotoBiology-Module, widening our focus from medicine to environment and climate.

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Mitochondria and cell research

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