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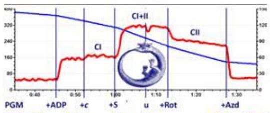
106th International Workshop on O2k high-resolution respirometry and O2k-Fluorometry

2015 October 06-11

Schröcken, Vorarlberg, Austria



O2k-Workshops HRR

The **106th Workshop on O2k high-resolution respirometry (HRR)** is the **34th** International Oxygraph Course held in Schroecken since 1988. An overview is provided of the **Oxygraph-2k, TIP2k, and O2k-Fluo LED2-Module**, with real-time analysis by **DatLab 6.2 (*new*)**. A demo experiment illustrates the principle and shows the unique advantage of simultaneous monitoring of oxygen concentration, respiration and **hydrogen peroxide production**. Cryopreserved cells are used as a biological reference sample (MitoFit proficiency test). Respiration and **mt-membrane potential** will be determined in hands-on experiments with cardiac tissue homogenate.

Instrumental setup and service of the polarographic oxygen sensor (**OroboPOS**) are demonstrated, followed by hands-on practice in 10 teams. In the evenings, general mitochondrial topics are covered; abstracts and experimental experiences are presented by participants.

IOC participants asked invariably for detailed discussions of protocol design. The **Blue Book** provides a basic introduction to mitochondrial physiology, complemented by overview presentations with examples, including **DatLab Analysis** of demo files. **Instrumental quality control** is a fundamental component of HRR and will be put to the practical test in teams using seven O2k (14 chambers). **O2k-MultiSensor** and particularly O2k-Fluorometry has become an integral part of the O2k-Workshop. Optimization of protocol design for various MultiSensor applications helps to critically evaluate basic principles of mitochondrial physiology. You will also see the **Titration-Injection microPump TIP2k** with feedback-control in action and practice its simple and automatic operation.

Lunch breaks provide an opportunity for relaxing walks and talks, enjoying the refreshing scenery of the secluded alpine environment, offer a visit to the Alpmuseum, or give sufficient spare time for individual practice.

instruments@oroboros.at
www.oroboros.at

Lecturers and tutors

Doerrier Carolina	Mitochondrial Application Specialist, NextGen-O2k, OROBOROS
Gnaiger Erich	CEO, OROBOROS
Hoppel Florian	PhD Student, MitoFit, OROBOROS
Laner Verena	Chief Operating Officer (COO), OROBOROS
McManus Meagan	O2k-Network Lab: US PA Philadelphia Wallace DC
Sumbalova Zuzana	O2k-Network Lab: SK Bratislava Sumbalova Z; OROBOROS

Programme IOC106

1 Tuesday, Oct 06

* *printed in workshop materials*

	Arrival	Weblink
15:00	**Arrival in Bregenz:** Meeting point Bregenz train station at 3:00 pm; approx. 1 hour bus drive to Schröcken and Hochtannberg (Salober). Transfer/walk to Hotel Körbersee.	IOC-travel
18:30	*Welcome reception at Hotel Körbersee*	Schroecken
19:00	*Dinner*	
20:30-21:00	**Get-together:** introduction of participants and their research interests – define working groups	IOC106

2 Wednesday, Oct 07

	Workshop 1		Weblink
07:30-08:30	*Breakfast*		
08:30-09:30	**O2k instrumental setup** – overview with video clips		O2k-Manual
	Hands-on (10 groups) **O2k instrumental setup**	**OroboPOS service**	Special task group: **SUIT protocols**
09:30-10:15	Groups 1-5	Groups 6-10	O2k-Start
10:15-11:00	Groups 6-10	Groups 1-5	POS Service
11:00	*Coffee / Tea*		
11:30-12:00	**Get O2k-Connected with OROBOROS:** a guided tour to the O2k		Oxygraph-2k
12:00	*Lunch packages/ walk & talk / alternative: individual O2k-tasks*		
14:30-15:30	**Instrumental quality control 1:** O_2 calibration and the O2k quality control system		Gnaiger 2008 POS* O2k-Calibration
15:30	*Coffee / Tea*		
16:00-16:30	**Instrumental quality control 2:** Analysis of oxygen flux; O2k-background test with the TIP2k		O2-Flux Analysis
16:30-18:00	**Hands-on (7 groups): O2k calibration and background test:** air saturation to zero oxygen concentration; or for permeabilized muscle fibres in the high-oxygen range of 500 - 200 µM. O2k-background with automatic TIP2k or manual titrations. Special interest group: O2k-background with TPP^+ electrodes		O2k-Background TIP2k User Manual
18:30	*Dinner*		
20:00-21:00	**DatLab analysis and group reports:** O2k-calibration and instrumental background		POS-Calibration-SOP O2 Background

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3 Thursday, Oct 08

	Workshop 2	Weblink
07:30-08:30	*Breakfast*	
08:30-09:15	**DatLab O_2 flux analysis:** Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	Glossary: Respiratory states
09:15-10:00	**DatLab guide through the menus:** DL-Demo files and DL-Excel templates	DatLab Guide
10:00	*Coffee / Tea*	
10:00-10:45	**Hands-on (7 groups) getting started with an O2k-experiment:** washing, stirrer test, air calibration	O2k-Calibration
10:45-12:00	**O2k-Demo experiment**: Respiration of intact cells: Simultaneous measurement of oxygen consumption (O2k-Core) and H_2O_2 production (O2k-Fluo LED2-Module)	Makrecka-Kuka 2015 Biomolecules
12:00	*Lunch packages/ walk & talk / alternative: individual O2k-tasks*	The Blue Book p 56*
14:00-16:00	**Hands-on (7 groups). O2k-experiment:** Respiration with permeabilized cells: SUIT protocols with 7 Power-O2k	
16:00	*Coffee / Tea*	
16:30-18:00	**SUIT protocol and DatLab analysis with Excel templates**	DatLab Flux Analysis
18:30	*Dinner*	
20:00-21:00	**O2k perspectives:** 10+5 min presentations of abstracts 1-6	IOC106 Abstracts MiPNet20.10

4 Friday, Oct 09

	Workshop 3	Weblink
07:30-08:30	*Breakfast*	
08:30-09:30	**From isolated mitochondria to tissue fibres and tissue homogenate preparation:** The PBI-Shredder	MiPNet17.03 Shredder vs Fibres*
09:30-10:00	**Hands-on (7 groups) getting started with an O2k experiment 2:** washing, stirrer test, air calibration	O2k-cleaning and ISS
10:00	*Coffee / Tea*	
10:30-12:00	**O2k-experiment:** Respiration with tissue homogenate: Two SUIT protocols with 7 Power-O2k	Krumschnabel 2013 Abstract MiP2013: 26-27*
12:00	*Lunch packages/ walk & talk / alternative: individual O2k-tasks*	
15:00-16:00	**DatLab analysis: hands-on in teams**	DatLab Flux Analysis
16:00	*Coffee / Tea*	
16:30-17:15	**DatLab analysis: summary discussion**	DatLab Flux Analysis
17:15-18:00	**O2k-Fluorometry demo: Calibration for H_2O_2 production.**	MiPNet20.14_AmplexRed_H2O2-production
18:30	*Dinner*	
20:00-21:00	**DatLab analysis: diagnosis of respiratory defects.**	

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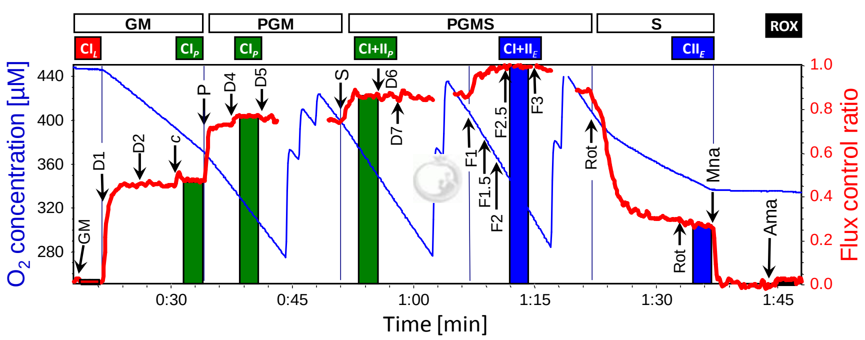
5 Saturday, Oct 10

	Workshop 4	***Weblink***
07:30-08:30	*Breakfast*	
08:30-09:15	**Experimental design 1:** Substrate and coupling control of mitochondrial respiration - MitoPathways through CI&II	The Blue Book* pp 43-57
09:15-10:00	**Experimental design 2:** Coupling control protocols: ROUTINE, LEAK, ETS, ROX	Cells: CCP Coupling control state
10:00	*Coffee / Tea*	MiPNet18.10 O2kvsMultiwell*
10:30-12:00	**O2k-MultiSensor overview and O2k-Fluorometry applications:** Amplex™ red, safranin and TMRM	MiPNet17.17_Amplex-Mouse-brain*
12:00	*Lunch packages*	
12:30-15:30	*Walk to the Alpmuseum: Guided tour and reception: 15 €*	Alpmuseum*
16:00	*Coffee / Tea*	
16:00-16:45	**Working groups: Elaborate answers to the 'Questions for the O2k-Workshop'**	IOC-Questions*
16:45-17:30	**IOC-Questions - discussion of 'Answers' - technical support**	O2k-Technical support
17:30-18:15	O2k-Network, MitoGlobal EAGLE and future O2k-plans (5+5 min) Anthony Molina, Meagan McManus, *and participants to be nominated*	O2k-Network
18:15-18:45	**The O2k-Workshop continues with the Bioblast wiki - in the spirit of Gentle Science**	www.bioblast.at
19:00	*Dinner*	
20:30-21:30	**Panel Discussion - Feedback**	O2k-Feedback
	Farewell party	

6 Sunday, Oct 12

	Departure / Fish project
	Breakfast
	Early morning: Departure



SUIT protocol with trout heart homogenate in a high oxygen concentration regime (MiR06Cr, 15 °C, reox with H_2O_2; Krumschnabel 2013 Abstract MiP2013)*.

Participants

Participant	Institution
Ball Darran**	**UK Southampton Grocott MP:** University of Southampton, Academic Unit of Cancer Sciences (UK)
Bezuidenhout Nicole**	**ZA Cape Town Ojuka EO:** University of Cape Town, Institute of South Africa Newlands, ESSM UCT Dept of Human Biology Sports Science (ZA)
Coker Robert*	**US AK Fairbanks Coker R:** University of Alaska, Institute for Arctic Biology (US)
Droescher Stephanie	**AT Innsbruck OROBOROS:** OROBOROS INSTRUMENTS (AT)
Einer Claudia*	**DE Munich Zischka H:** Helmholtz Zentrum München, Institute of Molecular Toxicology and Pharmacology (DE)
Garnham Jack**	**UK Leeds Peers C:** LIGHT, University of Leeds, Cardiovascular & Neuronal Remodelling (UK)
Gudiksen Anders **	**DK Copenhagen Pilegaard H:** University of Copenhagen (DK)
Haas Clarissa ***	**BR Porto Alegre Souza DOG:** Universidade Federal do Rio Grande do Sul, Biochemistry Department (BR)
Halling Jens F**	**DK Copenhagen Pilegaard H:** University of Copenhagen (DK)
Hansen Allan**	**DK Copenhagen Poulsen HE:** Rigshospitalet Copenhagen, Clinical Pharmacology (DK)
Jackson Christopher	**FI Helsinki Wartiovaara A:** Biomedicum Helsinki, Research Program for Molecular Neurology (FI)
Lamberti Giorgia	**AT Innsbruck Huber L:** Medical University of Innsbruck, Biocenter (AT)
Lindquist Carine*	**NO Bergen Berge RK:** University of Bergen, Department of Clinical Science (NO)
Matsumoto Junichi**	**JP Sapporo Yokota T :** Hokkaido University, Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine (JP)
Molina Anthony JA****	**US NC Winston-Salem Molina AJA:** Department of Internal Medicine, Section on Gerontology and Geriatric Medicine, Wake Forest School of Medicine (US, NC)
Nakajima Takayuki**	**JP Sapporo Yokota T :** Hokkaido University, Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine (JP)
Overby Peter**	**DK Copenhagen Pilegaard H:** University of Copenhagen (DK)
Pirinen Eija	**FI Helsinki Pirinen E:** University of Helsinki, Research Program for Molecular Neurology (FI)
Scaini Giselli*	**US TX Houston Reus GZ:** Departmentof Psychiatry and Behavioral Sciences, Center for Experimental Models in Psychiatry, UT Health Science Center at Houston (TX, US)
Schmitt Sabine*	**DE Munich Zischka H:** Helmholtz Zentrum München, Institute of Molecular Toxicology and Pharmacology (DE)
Sharaf Mahmoud*	**CA Charlottetown Kamunde C:** University of Prince Edward Island, Department of Biomedical Sciences (CA)
Smith James**	**ZA Cape Town Ojuka EO:** University of Cape Town, Institute of South Africa Newlands, ESSM UCT Dept of Human Biology Sports Science (ZA)
Takada Shingo**	**JP Sapporo Yokota T :** Hokkaido University, Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine (JP)
Tyrrell Daniel ****	**US NC Winston-Salem Molina AJA:** Department of Internal Medicine, Section on Gerontology and Geriatric Medicine, Wake Forest School of Medicine (US, NC)
Volani Chiara	**AT Innsbruck Weiss G:** Medical University of Innsbruck, Department of Inner Medicine (AT)
Ward Marie Louise*	**NZ Auckland Ward ML:** The University of Auckland, Department of Physiology, Faculty of Medical and Health Sciences (NZ)
Weiss Alexander*	**AT Innsbruck Jansen-Duerr P:** Medical University of Innsbruck, Research Institute for Biomedical Aging Research (AT)
West Malcolm**	**UK Southampton Grocott MP:** University of Southampton, Academic Unit of Cancer Sciences (UK)

MiPNet20.10 Abstracts IOC106: 10+5 min

O2k perspectives

1. Alteraas EK, Lindquist C, Berge RK, Bjorndal B (2015) OXPHOS capacity in mouse liver altered by modified fatty acids that inhibit or stimulate beta-oxidation. Mitochondr Physiol Network 20.10.

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in developed countries, characterized with high accumulation of triglycerides in the liver. NAFLD is associated with obesity, insulin resistance and dyslipidemia, and has been linked to reduced mitochondrial function. Tetradecylthiopropionic acid (TTP) and Tetradecylthioacetic acid (TTA) are artificial fatty acids, shown to inhibit and increase mitochondrial β-oxidation respectively. They were used to investigate the effect of β-oxidation on mitochondrial respiration as well as important mitochondrial factors. In the present study, C57BL/6JBomTac male mice (n = 8-10) were fed a low-fat diet for three weeks supplemented with TTP, TTA, or combined TTP and TTA. As expected, β-oxidation was significantly higher in liver homogenate from TTA-treated mice. TTP treatment decreased hepatic β-oxidation, although not significantly. In accordance with the tendency of inhibited β-oxidation, plasma carnitine and acetylcarnitine levels were lower in TTP-treated mice than in controls, while palmitoylcarnitine levels increased. Concomitantly, liver TAG and cholesterol levels were increased. In contrast, TTA increased plasma carnitine levels, but did not affect liver lipid levels. Interestingly, the combined intervention of TTP + TTA increased β-oxidation and prevented liver TAG and cholesterol accumulation, but despite this an accumulation of plasma palmitoylcarnitine and a decrease in acetylcarnitine was observed. Our results show significantly higher oxidative phosphorylation and maximal capacity of the electron transport system in liver samples from TTA-treated mice. This increase was mainly due to increased complex I activity. TTP did not significantly alter mitochondrial respiration. Strikingly, in mice treated with a combination of TTP and TTA, oxidative phosphorylation was significantly decreased. Our findings suggest that TTA impact the mitochondrial respiration and fatty acid catabolism positively, while TTP did not have any effect on the mitochondrial respiration. Combined, TTA was able to prevent the TTP-induced liver lipid accumulation, but this was linked to a reduced OXPHOS capacity, which could indicate mitochondrial malfunction. More analyses and studies should be done to elaborate this further.

2. Power A, Crossman D, Hickey AJ, Ward M (2015) Investigating mitochondrial function in a rat model of right ventricular hypertrophy. Mitochondr Physiol Network 20.10.

Background: The heart is a highly aerobic organ with more than 90% of ATP regenerated by oxidative phosphorylation (OXPHOS) in the mitochondria. Mitochondrial dysfunction has been identified as a hallmark in the transition of compensatory hypertrophy to heart failure. However, the contribution of mitochondrial dysfunction to the contractile deficit is debated.

Objectives: To determine if mitochondrial energy supply compromises contractile function in right ventricular (RV) hypertrophy.

Methods: Rats were injected with monocrotaline (MCT) to induce pulmonary artery hypertension and RV hypertrophy, or saline (CON) for age-matched controls. Four weeks post-injection, multicellular cardiac trabeculae (length 2-3 mm, diameter 150-250 µm) were micro-dissected from the RV and mounted on an inverted microscope between a force transducer and a length changer. Steady-state force and intracellular Ca^{2+} transients were measured prior to saponin "skinning" of the sarcolemma to allow buffer access to the cytosol without damaging organelle membranes. Contraction and relaxation of the trabeculae was then assessed using buffered Ca^{2+} solutions, with and without exogenous ATP added to the superfusate.

Results: MCT trabeculae produced similar force to CON despite having lower Ca^{2+} transients. Following skinning, CON trabeculae showed no change in the maximum Ca^{2+}-activated force when exogenous ATP was removed from superfusate, while MCT trabeculae showed smaller contractures without exogenous ATP when stimulated with saturating Ca^{2+}.

Discussion: In this MCT model of compensated right ventricular hypertrophy there appears to be only a small contribution of mitochondrial dysfunction to contraction/relaxation when intracellular Ca^{2+} is controlled. This protocol can be used to further examine energy specific deficits in the failing heart, and to investigate the effects of drugs that modulate mitochondrial energy supply on contractile function.

3. Scaini G, Gomes LM, Carvalho-Silva M, Arent CO, Mariot E, Quevedo J, Streck EL (2015) Co-administration of omega-3 fatty acids and mood stabilizers reverses the impairment of bioenergetic parameters induced by fenproporex administration in hippocampus of rats. Mitochondr Physiol Network 20.10.

Bipolar disorder (BD) presents a complex alternating clinical course with recurrent mood changes including manic and depressive episodes. Moreover, studies show that changes in energy metabolism are involved in the pathophysiology of BD and that omega-3 (ω3) fatty acids have beneficial properties in the central nervous system, by modulate energy metabolism. Thus, in the present stud we evaluate the effect of ω3 fatty acids alone or in combination with lithium or valproate on bioenergetic parameters, namely respiratory complexes (CI, CII, CII–III, CIV), malate dehydrogenase, succinate dehydrogenase, citrate synthase and creatine kinase activities. We observed a significant decrease of succinate dehydrogenase, CII and CIV and creatine kinase activities in hippocampus of animals submitted to fenproporex administration, as compared to the control group. Additionally, the ω3 fatty acids in combination with VPA or Li were able to reverse the decrease in succinate dehydrogenase, CII and CIV activities. However, the decrease in CK activity was reversed only with ω3 fatty acids in association with VPA. The present findings support the idea that ω3 fatty acids plays an important role in the modulation of energy metabolism, and exercise essential antioxidant capacity in the central nervous system, suggesting that the ω3 fatty acids may be a possible contributing in BD therapy.

4. Sharaf MS, Van den Heuvel MR, Stevens D, Kamunde C (2015) Mechanisms of mitochondrial import and interactions of zinc and calcium. Mitochondr Physiol Network 20.10.

Zinc and calcium have highly interwoven functions that are essential for cellular homeostasis. Here, we studied the mechanisms of their import into mitochondria and interactions on oxidative phosphorylation (OXPHOS) and membrane potential (ΔΨm). We showed that while the two cations permeated the mitochondrial inner membrane via different mechanisms they synergistically inhibited OXPHOS but antagonistically dissipated ΔΨm. Ruthenium red completely prevented calcium-induced OXPHOS inhibition suggesting that exclusive entry of calcium into the matrix via mitochondrial calcium uniporter is a fundamental step for the functional impairment caused by this cation. Overall, these data indicate that interactions of zinc and calcium on mitochondrial function result from mechanisms other than competition for their uptake pathways.

5. Volani C, Haschka D, Demetz E, Doerrier C, Gnaiger E, Weiss G (2015) Determination of mitochondrial respiration in peripheral blood mononuclear cells. Mitochondr Physiol Network 20.10.

Mitochondria are dynamic organelles, involved in fundamental cell processes, including oxidative phosphorylation [1, 2]. Iron plays a decisive role in these processes because it is central part of mitochondrial enzyme complexes but also regulates citric acid cycle activity by modulating mitochondrial aconitase expression. Hence, imbalances of iron homeostasis impact on mitochondrial activity and, thus, on cell and organ functions [3]. So far, little information is available on how to best measure mitochondrial activity and its interaction with iron homeostasis in vivo; therefore we questioned whether determination of mitochondrial respiration in peripheral blood mononuclear cells (PBMCs) could be a good surrogate marker for that.

Human PBMCs were collected from buffy coats, purified cells ($2x10^6$ cells/ml) were resuspended in mitochondrial respiration medium (MiR05), and mitochondrial activity was assessed by high resolution respirometry (OROBOROS INSTRUMENTS, Austria). Moreover, to access the impact of iron on mitochondrial respiration we studied mitochondrial respiration in hearts and livers of mice, receiving either iron deficient- or standard iron-diet one week before being sacrificed. Organs were collected and stored in Custadiol prior to homogenization in MiR05. Mitochondrial routine respiration, complex I and II maximal oxidative phosphorylation together with non-coupled respiration of the homogenates were assessed at a final concentration between 1 and 2 mg.

Our ongoing experiments indicate that mitochondrial function testing can be successfully performed in human PBMCs as well as in mouse tissues. Analyses of organ samples from mice indicate that dietary iron supplementation leads to enhanced oxidative phosphorylation. Furthermore, it is plausible to hypothesize that PBMCs mitochondrial activity can reflect this organ increase. In conclusion, the use of high-resolution respirometry (OROBOROS INSTRUMENTS, Austria) represents a powerful and reliable tool to investigate mitochondrial respiration in PBMCs and tissues, and to systemically study the effects of iron homeostasis on mitochondrial function. Moreover, determination of mitochondrial function in PBMCs might provide useful information on mitochondrial activity in tissues.

Accommodation and Location

Hotel Körbersee www.koerbersee.at
T +43 5519 265; hotel@koerbersee.at

More detail

Gnaiger E (2014) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 4th ed. Mitochondr Physiol Network 19.12. OROBOROS MiPNet Publications, Innsbruck:80 pp. »Bioblast link«

O2k-Manual – http://wiki.oroboros.at/index.php/O2k-Manual
O2k-Protocols – http://wiki.oroboros.at/index.php/O2k-Protocols
>1,500 O2k-Publications – www.bioblast.at/index.php/O2k-Publications

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www.mitofit.org

Contact

Erich Gnaiger, PhD
Medical University of Innsbruck
D. Swarovski Research Laboratory
A-6020 Innsbruck, Austria
www.bioblast.at

OROBOROS INSTRUMENTS
Schöpfstrasse 18
A-6020 INNSBRUCK, Austria
T +43 512 566796 F +43 512 566796 20
Email instruments@oroboros.at
www.oroboros.at
Cooperation and Feedback in Science

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O2k-Workshops are listed as MitoGlobal Events