IOC100. Mitochondrial Physiology Network 20.01(01): 1-8 (2015)
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100th International Workshop on HRR, O2k-Fluorometry and TPP

2015 April 09-14

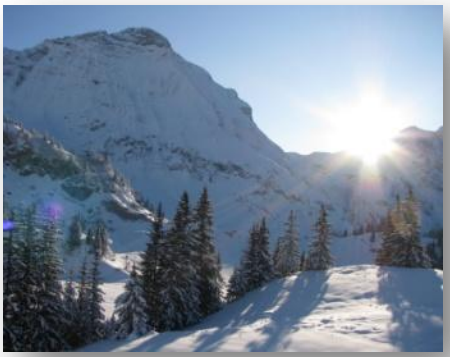
Schröcken, Vorarlberg, Austria

The **100th Workshop on High-Resolution Respirometry (HRR)** is the **33rd** International Oxygraph Course held in Schroecken since 1988. A practical overview is provided of the **Oxygraph-2k and O2k-Fluorometer**, with real-time analysis by **DatLab** and applications of the **TIP2k**. Demo experiments illustrate the principle and show the unique advantages and limitations of simultaneous monitoring of oxygen concentration, respiration, hydrogen peroxide production or mt-membrane potential. HEK 293T cells are used as a biological reference sample, which are used world-wide and can be stored on dry-ice.

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 invariably asked for a detailed discussion of protocol design. The **Blue Book** provides a basic introduction to mitochondrial physiology and is 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 six O2k (12 chambers). **O2k-MultiSensor** and particularly O2k-Fluorometry has become an integral part of the O2k-Workshop. Optimization of protocol design for various O2k-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. A special-interest group will focus on TPP^+.

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



Programme

1 Thursday, Apr 09

*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*	
20:30-21:15	**Get-together:** introduction of participants and their research interests - a welcome by OROBOROS INSTRUMENTS	IOC100

2 Friday, Apr 10

	Workshop 1			Weblink
07:30-08:30	*Breakfast*			
08:00	*Organize loan of skiing equipment*			
	Principles of high-resolution respirometry and O2k-Fluorometry - from switching on the Oxygraph-2k to the experimental result			Gnaiger 2008 POS
08:30-09:30	**Get O2k-connected with OROBOROS:** a guided tour to the Oxygraph-2k			O2k-Manual
09:30-11:00	**Hands-on (6 groups) introduction to DatLab:** DL Installation, DL-Demo files and DL-Excel templates, with video support for the O2k-Manual			DatLab Guide DatLab Flux Analysis
11:00	*Lunch packages/ Practice: skiing / walk & talk / alternative:* individual O2k-tasks			
15:00	*Coffee / Tea*			
	Standard programme		**Special interest group**	
	O2k instrumental setup service	**OroboPOS**	**TPP**	
15:30-16:00	Groups 1-5	Groups 6-10 Groups 1-5	Introduction, Assembly of the ISE	O2k-Start POS Service TPP-Electrode
16:00-16:30	Groups 6-10			
16:30	*Coffee / Tea*			
17:00-18:45	**Hands-on (6 groups) Getting started with an O2k experiment 1:** washing procedures, stirrer test, air calibration		Calibration of the TPP^+ electrodes	DatLab Guide TPP-Electrode
19:00	*Dinner*			

20:30-21:15	**Hot Topics 1:** 10+5 min presentations of abstracts. Campbell Matthew, Smenes Benedikte Therese, Ost Mario Chairs: Pablo Garcia-Roves, Zuzana Sumbalova	IOC100 Abstracts

3 Saturday, Apr 11

	Workshop 2		Weblink
07:30-08:30	*Breakfast*		
08:30-09:15	**Tissue homogenate preparation:** the PBI-Shredder and general discussion of sample preparation		MiPNet17.03 Shredder vs Fibres O2k-Start
09:15-10:00	**Hands-on (6 groups) getting started with an O2k experiment 2:** washing, stirrer test, air calibration		
10:00	*Lunch packages/ Practice: skiing / walk & talk / alternative:* individual O2k-tasks		
14:30	*Coffee / Tea*		
	Standard programme	**Special interest group**	
15:00-16.30	**Hands-on (6 groups) O2k-experiment with cell lines:** SUIT protocol with HEK 293T cells and real-time DatLab analysis	**Special interest group: TPP** Calibration of the TPP$^+$ electrodes, experiment with cells	Pesta 2012 Methods Mol Biol TPP-Electrode
16:30	*Coffee / Tea*		MiPNet18.10 O2kvsMultiwell*
17:00-18:15	**Hands-on: SUIT experiment continued with DatLab Analysis and Excel templates**	**Special interest group: TPP** Evaluation of the membrane potential	DatLab Flux Analysis TPP-Electrode
18:15-19:00	**Experimental design 1:** Substrate control of mitochondrial respiration - MitoPathways through CI and CII		The Blue Book*
19:00	*Dinner*		
20:30-21:00	**Hot Topics 2:** 10+5 min presentations of abstracts. Oliveira Marcos Tulio, Purhonen Janne Chairs: Anthony Hickey, Carolina Doerrier		IOC100 Abstracts

4 Sunday, Apr 12

	Workshop 3	Weblink
07:30-08:30	*Breakfast*	
08:30-09:15	**Experimental design 2:** Coupling control protocol with intact cells vs. mt-preparations: OXPHOS, ROUTINE, ETS, LEAK	Cells: PCP
09:15-10:00	**DatLab Analysis:** Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	Glossary: Respiratory states
10:00	*Lunch packages/ Practice: skiing / walk & talk / alternative:* individual O2k-tasks	
14:30	*Coffee / Tea*	
15:00-16:30	**Hands-on (6 groups) Multisensor O2k-experiment 1:** combined respirometry and fluorometric detection of mitochondrial membrane potential with TMRM and safranin using permeabilized HEK 293T cells	Krumschnabel 2014 Methods Enzymol MiPNet19.19* Safranin Data Acquisition and Analysis*
16:30	*Coffee / Tea*	

17:00-19:00	**Hands-on (6 groups) Multisensor O2k-experiment 2:** combined respirometry and fluorometric detection of H_2O_2 production with Amplex Red using permeabilized HEK 293T cells	MiPNet19.20 Amplex Red Data Acquisition and Analysis*
19:00	*Dinner*	
20:30-21:15	**Round table with our O2k-Nework guest scientists: Pablo Garcia-Roves, Anthony Hickey, Zuzana Sumbalova**	

5 Monday, Apr 13

	Workshop 4	***Weblink***
07:30-08:30	*Breakfast*	
08:30-09:15	**Instrumental quality control 1:** The oxygen sensor OroboPOS - calibration, stability testing, and evaluation of sensitivity to measure oxygen flux	O2k-Calibration
09:15-10:00	**Instrumental quality control 2:** O2k-Background test with TIP2k; analysis of oxygen flux	O2k-Background
10:00	*Coffee / Tea*	
10:30-12:00	**Hands-on (6 groups): O2k-Background from 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
12:00	*Lunch packages*	
12:30-15:30	*Walk to the Alpmuseum: Guided tour and reception*	http://www.alpmuseum.at
16:00	*Coffee / Tea*	
16:00-16:45	**Working groups: Elaborate answers to the 'Questions for the O2k-Workshop'** - *come prepared* **Special interest group TPP: questions and answers**	IOC-Questions*
16:45-17:15	**IOC-Questions - discussion of 'Answers' Special interest group TPP: questions and answers**	
17:15-18:00	**Introduction to trouble shooting**	O2k-Troubleshooting
18:00-18:45	**The O2k-Workshop continues with the Bioblast wiki - in the spirit of Gentle Science**	www.bioblast.at
19:00	*Dinner*	
20:30-21:00	**Panel Discussion - Feedback IOC100**	O2k-Feedback*
	Farewell party	

6 Tuesday, Apr 14

	Departure
	Breakfast
	Early morning: Departure

Lecturers and tutors

Doerrier Carolina	Post-doctoral scientist, OROBOROS INSTRUMENTS
Garcia-Roves Pablo M	O2k-Network Lab: ES Barcelona Garcia-Roves PM
Gnaiger Erich	CEO, OROBOROS INSTRUMENTS
Hickey Anthony J	O2k-Network Lab: NZ Auckland Hickey AJ
Laner Verena	Chief Operating Officer (COO), OROBOROS INSTRUMENTS
Sumbalova Zuzana	O2k-Network Lab: SK Bratislava Sumbalova Z

Participants

Participant	Institution	Special interest
Al Shahrani Mesfer	National Hospital for Neurology & Neurosurgery, Neurometabolic Unit (**UK London Land JM**)	
Campbell Matthew	University of Washington, Department of Radiology (**US WA Seattle Marcinek DJ**)	TPP
Campesan Susanna	Department of Genetics, University of Leicester (**UK Leicester Moisoi N**)	TPP
Cedikova Miroslava	Charles University in Prague, Faculty of Medicine in Plzen, Dept. Physiology (**CZ Plzen Matejovic M**)	TPP
Chavanelle Vivien	Lab Adaptations Métaboliques à l'Exercice en conditions Physiologiques et Pathologiques, AME2P, Univ Blaise Pascal (**FR Aubiere Sirvent P**)	
Dal-Pizzol Felipe	Universidade do extremo sul Catarinense (**BR Florianopolis Dal-Pizzol F**)	TPP
Donner Verena	German Institute of Human Nutrition, DIfE (**DE Nuthetal Klaus S**)	
Gopala Srinivas	Sree Chitra Tirunal Inst Med Sciences and Technology (**IN Kerala Gopala S**)	(TPP)
Guida Marianna	European Academy of Bozen/Bolzano, EURAC (**IT Bolzano Pichler I**)	
Hargreaves Iain	National Hospital for Neurology & Neurosurgery, Neurometabolic Unit (**UK London Land JM**)	
Kuncova Jitka	Charles University in Prague, Faculty of Medicine in Plzen, Dept. Physiology (**CZ Plzen Matejovic M**)	TPP
Lie Ingunn	Norwegian University of Science and Technology, Institute for circulation and medical imaging (**NO Trondheim Rognmo O**)	
Martin Daniel	Centre for Altitude, Space and Extreme Environment Medicine, UCL (**UK London Martin D**)	
McKenna Helen	Centre for Altitude, Space and Extreme Environment Medicine, UCL (**UK London Martin D**)	
Mercan Sercan	Ankara Diskapi Training and Research Hospital (**TR Ankara Alpaslan Pinarli F**)	
Michalak Slawomir	Department of Neurochemistry and Neuropathology, Poznan University (**PL Poznan Michalak S**)	
Oliveira Marcos Tulio	Departamento de Tecnologia - FCAV, Universidade Estadual Paulista (**BR Jaboticabal Oliveira MT**)	
Ost Mario	German Institute of Human Nutrition, DIfE (**DE Nuthetal Klaus S**)	
Pearce Linda	Univ. Pittsburgh - Department of Environmental and Occupational Health (**US PA Pittsburgh Peterson J**)	TPP
Polyak Erzsebet	**US PA Philadelphia Falk MJ:** Children's Hospital of Philadelphia (PA, US)	TPP
Purhonen Janne	University of Helsinki, Biomedicum Helsinki (**FI Helsinki Purhonen J**)	
Raat Harold	Lab of Exp. Anesthesiology, Dept. Anesthesiology, Erasmus Medical Center (**NL Rotterdam Sluiter W**)	TPP
Repici Mariaelena	Department of Genetics, University of Leicester (**UK Leicester Moisoi N**)	

Smenes Benedikte Therese	Institute for circulation and medical imaging, Norwegian University of Science and Technology (NTNU) (**NO Trondheim Rognmo O**)	
Sobotka Ondrej	Charles University in Prague, Faculty of Medicine, Dept. Physiology (**CZ Hradec Kralove Cervinkova Z**)	TPP
Spinazzi Marco	KU Leuven Center for Human Genetics (**BE Leuven Spinazzi M**)	(TPP)
Tharyan Rebecca George	Max-Planck-Institute for Biology of Ageing (**DE Cologne Antebi A**)	

MiPNet20.01 Abstracts IOC100: 10+5 min

Hot topics in Mitochondrial Physiology

Hot Topics 1

Campbell MD (2015) Improved redox state increases aged skeletal muscle performance. Mitochondr Physiol Network 20.01.

The decline in skeletal muscle performance with age (sarcopenia) is a significant public health concern due to the effect on quality of life and loss of independence. Mitochondrial oxidative stress has been thought to be a key mediator of age-related degeneration in skeletal muscle, although recent reports have challenged this view. Previously our lab has demonstrated that directly targeting mitochondrial oxidative stress and energetics using a single acute treatment with the mitochondrial-targeted peptide SS-31 reduces oxidative stress and improves muscle function in aged mice. However, whether long-term treatment with SS-31 peptide prevents sarcopenia and improves exercise tolerance is still unknown. We used osmotically controlled pumps to deliver a dose of SS-31 peptide equivalent to 3 mg/kg of body mass per day for 4 or 8 weeks to 7 and 26-month old mice. After 4 and 8 weeks SS-31 treatment led to an increase in both running distance and time in 26-month old mice using a ramped treadmill protocol compared to saline treated controls. The increase in exercise tolerance also correlated with an improvement of in-situ fatigue resistance among aged mice treated with SS-31 peptide for 8 weeks. To test whether changes in redox signaling could underlie mitochondrial and muscle deficits with age we used a thiol redox proteomics approach. Aging results in a significant increase in protein S-glutathionylation (PSSG) that was partially reversed with SS-31 treatment. Cysteine residues sensitive to PSSG with age and reversal by SS-31 treatment exist in many different cellular systems including but not limited to cellular contractility, glycolysis, oxidative phosphorylation, membrane repair, and control of redox signaling. Our preliminary data supports the conclusion that SS-31 represents a novel intervention with excellent translational potential to improve skeletal muscle function in the elderly.

Smenes BT, Bakkerud F, Hassel E, Wohlwend M, Slagsvold KH, Rognmo O, Wahba A (2015) Mitochondrial function in patients exercising prior to coronary surgery – A single blinded randomized controlled trial. Mitochondr Physiol Network 20.01.

During open heart surgery, the myocardium suffers from ischemia-reperfusion injury (IR) despite perioperative cardioprotection. This type of injury impairs mitochondrial function and leads to cell death, which impairs cardiac function and negatively affects patient outcome [1]. We previously demonstrated that remote ischemic preconditioning preserves mitochondrial function during cardiac surgery [2,3], and animal studies have shown that as little as a few bouts of endurance exercise can protect the heart from IR [4]. Up until now, exercise as preconditioning has not been studied in humans in a clinical setting.

20 patients undergoing elective isolated primary coronary artery bypass surgery at St. Olav's University Hospital, Norway will be included. The patients are randomized to either one bout of incremental treadmill running 24 hours preoperatively (N=10), or to prepare for surgery according to standard procedures (N=10). Atrial and ventricular biopsies will be collected at two time points: Before and after aortic cross-clamping during extracorporeal circulation. The Oxygraph-2k (OROBOROS Instruments, Innsbruck, Austria) will be used to assess mitochondrial respiration and the fluorescence module allows us to measure H_2O_2 production. A substrate-uncoupler-inhibitor-titration protocol will be used to assess different aspects of integrated TCA cycle function and electron transport system.

Due to blinding, we are not able to present any preliminary data before data collection is completed. We hypothesize that one bout of exercise one day prior to open heart surgery is sufficient to induce cardioprotection and preserve mitochondrial function.

Ost M (2015) "Mitokines" and the organismal role of mitochondrial function in energy homeostasis & metabolism. Mitochondr Physiol Network 20.01.

Recent studies have expanded our view of mitochondria beyond their cell autonomous roles, showing that an impaired mitochondrial function in one tissue (e.g. skeletal muscle) has strong metabolic consequences for the whole organism.

To identify key molecular mechanisms in response to chronic mitochondrial distress, we studied transgenic mice with ectopic expression of uncoupling protein 1 in skeletal muscle (UCP1-TG), as a model of muscle-specific mitochondrial perturbation. *Ex vivo* analysis of functional respiratory capacity performed on permeabilized *Soleus* muscle fibers revealed an elevated LEAK control ratio and reduced OXPHOS coupling efficiency in transgenic muscles. However, this compromised mitochondrial function promotes an increased total energy expenditure, delayed diet-induced obesity development, improved glucose homeostasis, and even longevity. The exact physiological mechanisms underlying this metabolic improvement have not yet been resolved.

Strikingly, we were able to show that impaired mitochondrial respiratory capacity affects not only muscle itself, but also white adipose tissues (WATs), which show an increased metabolic activity (including mitochondrial COX activity and browning). This suggests a cross-talk between muscle and WAT, possibly mediated by myokines. Very recently, we were able to proof an increased expression and secretion of muscle fibroblast growth factor 21 (FGF21) in UCP1-TG mice. FGF21 has emerged as an important regulator of whole body metabolic processes and its secretion from muscle seems to be related to mitochondrial function, thereby the term "mitokine" has been proposed. Therefore, we conclude that the metabolic improvements of UCP1-TG mice are linked to endocrine effects of FGF21 as a "mitokine" that signals mitochondrial distress to the whole organism in a cell non-autonomous manner, which will be addressed in future studies.

Hot Topics 2

Oliveira MT, Saari S, Andjelkovic A, Jacobs HT (2015) Investigating the metabolic alterations caused by the transgenic expression of the mitochondrial alternative oxidase of *Ciona intestinalis* in *Drosophila melanogaster*. Mitochondr Physiol Network 20.01.

The alternative respiratory enzymes constitute additional pathways to the mitochondrial oxidative phosphorylation (OXPHOS) system, in which oxygen consumption is uncoupled from ATP production. The expression of alternative oxidases and alternative NADH dehydrogenases from tunicates and fungi in the fruitfly *Drosophila melanogaster*, in the mouse and in cultured human cells has proven to be benign and to counteract deleterious effects of defective OXPHOS systems, such as the high production of reactive oxygen species. The observed benefits are in paradox with the fact that genes for alternative enzymes were lost independently, early in the evolution of Vertebrata and Arthropoda (Figure 1). We thus propose to investigate the functions of an alternative enzyme from *Ciona intestinalis* (Tunicata: Ascidiacea), the alternative oxidase (AOX), when expressed in transgenic fruitflies. In preliminary thermal and nutritional stress experiments that challenge mitochondrial metabolism, AOX-expressing flies showed developmental problems (low rate of pupa eclosion) whose intensity appears to be related to the amount of carbohydrates (especially glucose) in the diet. Interestingly, we also found that strong expression of AOX in the fly testes causes low accumulation of mature spermatozoids, which in turn causes reproductive disadvantages. We are using a variety of molecular biology techniques (including RNA-Seq and high-resolution mass spectrometry) to investigate the changes in cell transcriptomics and mitochondrial proteomics and respiration, providing data about the physiological alterations caused by AOX and insights into the evolution of the mitochondrial alternative pathways in animals.

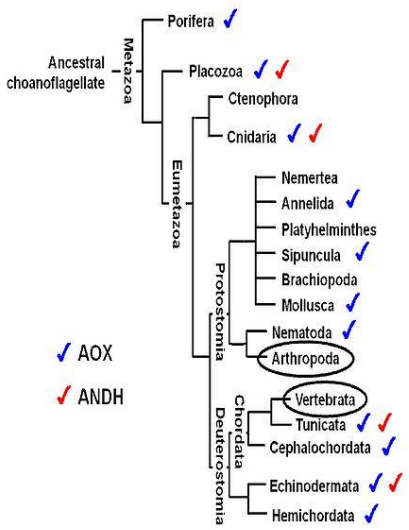


Figure 1. Phylogenetic relationships among animal taxa (according to [1]), indicating the lineages for which alternative enzyme genes have been found. Although vertebrates and arthropods are groups with the highest number of complete genomes sequenced (black circles), there is no evidence for the presence of the alternative oxidase (AOX) and the alternative NADH dehydrogenase (ANDH) in these animals. For taxa with no indications, such as Ctenophora, Nemertea, Platyhelminthes and Brachiopoda, there are not enough biochemical or genomic sequence data available to determine presence or absence of the alternative enzymes. Figure adapted from [2].

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. »Open Access

O2k-Manual – http://wiki.oroboros.at/index.php/O2k-Manual

O2k-Protocols – http://wiki.oroboros.at/index.php/O2k-Protocols

>1,400 O2k-Publications – http://wiki.oroboros.at/index.php/O2k-Publications: Topics

Acknowledgements

MEDIZINISCHE UNIVERSITÄT INNSBRUCK

Mitochondrial Physiology D Swarovski Research Laboratory

WGT ELEKTRONIK

OROBOROS INSTRUMENTS

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
Homepage: www.oroboros.at

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