OROBOROS O2k-Workshops

Mitochondrial Physiology Network 21.01(01):1-6 (2016)
Version 01: 2016-04-01
Updates: http://wiki.oroboros.at/index.php/MiPNet21.01_IOC109_Schroecken_AT

109th International Workshop on HRR and O2k-Fluorometry

2016 April 03-08

Schröcken, Vorarlberg, Austria

The **109th Workshop on High-Resolution Respirometry (HRR)** is the **35th** 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.

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.

Lecturers and tutors

Gnaiger Erich	CEO, OROBOROS INSTRUMENTS
Laner Verena	Chief Operating Officer (COO), OROBOROS INSTRUMENTS
Lamberti Giorgia	Post-doctoral Scientist, OROBOROS INSTRUMENTS
Sobotka Ondrej	CZ Hradec Kralove Cervinkova Z: Faculty of Medicine, Charles University Prague (CZ)
Sumbalova Zuzana	Principal Investigator (PI), OROBOROS INSTRUMENTS
Volska Kristine	LV Riga Makrecka-Kuka M: Latvian Institute of Organic Synthesis, Laboratory of Pharmaceutical Pharmacology

Programme

1 Sunday, Apr 03

*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); 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	IOC109
	Organization of loan skiing equipment @ Verena	

2 Monday, Apr 04

	Workshop 1		Weblink
07:30-08:30	*Breakfast*		
8:30-09:30	**O2k instrumental setup** – overview with video clips		O2k-Manual
09:30-11:00	**Hands-on (10 groups)**		
	O2k instrumental setup	**OroboPOS service**	
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	*Lunch packages/ Practice: skiing / walk & talk / alternative:* individual O2k-tasks (depending on the weather)		
15:00	*Coffee / Tea*		
15:30-16:15	**Instrumental quality control 1:** O_2 calibration and the O2k quality control system		Gnaiger 2008 POS O2k-Calibration

16:15-17:00	**Instrumental quality control 2:** O2k-background test with the TIP2k; analysis of oxygen flux	O_2-Flux Analysis
17:00-18:30	**Hands-on (6 groups) - O2k calibration and background test:** air saturation to zero oxygen concentration; O2k-background with automatic TIP2k	O2k-Background TIP2k User Manual
18:30	*Dinner*	
20:00-20:45	**DatLab analysis:** O2k-calibration and instrumental background flux	POS-Calibration-SOP
20:45-21:00	**Group reports**	O2 Background

3 Tuesday, Apr 05

	Workshop 2	*Weblink*
07:30-08:30	*Breakfast*	
08:30-10:00	**DatLab guide through the menus and DatLab O_2 flux analysis:** DL-Demo files and DL-Excel templates; Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	DatLab Guide Glossary: Respiratory states
10:00	*Coffee / Tea*	
10:30-11:00	**Hands-on (6 groups) - getting started with an O2k experiment:** washing, stirrer test, air calibration	O2k-Calibration
11:00-12:00	**O2k-Demo experiment**: Respiration of intact cells: Simultaneous measurement of oxygen consumption (O2k-Core) and H_2O_2 production (O2k-Fluo LED2-Module) in HEK cells.	Makrecka-Kuka 2015 Biomolecules
12:00	*Lunch packages / Practice: walk & talk / alternative:* individual O2k-tasks Individual interviews: Bioblast questionnaire	*The Blue Book p 56** Bioblast questionnaire
14:00-16:00	**Hands-on (7 groups) - O2k-experiment:** Respiration with permeabilized cells: SUIT protocols with 7 Power-O2k Advanced group (4 O2ks): incl. measurement of H_2O_2 production	
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 1-3	IOC109 Abstracts

4 Wednesday, Apr 06

	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) - SOPs with the O2k:** 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 / Practice: skiing / walk & talk / alternative:* individual O2k-tasks Individual interviews: Bioblast questionnaire	Bioblast questionnaire
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**
17:15-18:00	**OXPHOS analysis: diagnosis of respiratory defects**
18:30	*Dinner*
20:00-21:00	**O2k-Perspectives:** 10+5 min presentations of abstracts 4-6
21:00	*Registration for the Walk to the Alpmuseum @ Verena*

5 Thursday, Apr 07

	Workshop 4	*Weblink*
07:30-08:30	*Breakfast*	
08:30-10:00	**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 TIP2k User Manual
10:00	*Coffee / Tea*	MiPNet18.10 O2kvsMultiwell*
10:30-12:00	**Experimental design:** Substrate and coupling control of mitochondrial respiration; intact cells vs. mt-preparations: OXPHOS, ROUTINE, ETS, LEAK – MitoPathways	The Blue Book* pp 43-57 Cells: CCP Coupling control state
12:00	*Lunch packages*	
12:30-15:30	*Walk to the Alpmuseum - guided tour and reception: € 15.-*	Alpmuseum*
16:00	*Coffee / Tea*	
16:00-17:00	**Working groups: elaborate answers to the 'Questions for the O2k-Workshop'** - *come prepared*	IOC-Questions*
17:00-17:45	**IOC-questions - discussion of 'Answers', Introduction to O2k-technical service and the MitoFit proficiency test**	O2k-Technical support
17:50-18:45	**The O2k-Workshop continues with the Bioblast wiki - in the spirit of Gentle Science:** beyond the O2k-Network to MITOEAGLE	O2k-Network www.bioblast.at
19:00	*Dinner*	
20:30-21:00	**Panel discussion - Feedback IOC109** *Participants to be nominated*	O2k-Feedback*
	Farewell party	

6 Friday, Apr 08

	Departure
06:30-7:30	*Breakfast*
	Early morning: departure from Hotel Körbersee at 7.45 am

Participants

Participant	Institution
Bianchi Lucas*	**FR Paris Munnich A:** Institut Imagine, DR1 INSERM Génétique des Maladies Mitochondriales (FR)
Bhaskar Monica***	**CH Bern Djafarzadeh S:** Inselspital Bern, Department of Intensive Care Medicine (CH)
Grellier Tiia	**EE Tartu Paju K:** University of Tartu, Department of Pathophysiology (EE)
Hoefler Saskia*	**DE Hannover Hildebrandt T:** University of Hannover, Institute of Plant Genetics (DE)
Hoffmann Christoph*	**DE Tuebingen Weigert C:** University Hospital Tuebingen, Internal Medicine IV (DE)
Houzelle Alex***	**NL Maastricht Schrauwen P:** Maastricht University (NL)
Jain Aakriti*	**UK London Anastasiou D:** The Francis Crick Institute (UK)
Jha Rajan Kumar*	**IN Hyderabad Thangaraj K:** Evolutionary and Medical Genetics Laboratory, Centre for Cellular and Molecular Biology, CCMB (IN)
Jocken Johan***	**NL Maastricht Schrauwen P:** Maastricht University (NL)
Jourde Benjamin*	**CH Basel Das S:** Novartis Pharma AG (CH)
Kappler Lisa*	**DE Tuebingen Weigert C:** University Hospital Tuebingen, Internal Medicine IV (DE)
Kjaer Laura Kofoed*	**DK Copenhagen Poulsen HE:** Rigshospitalet Copenhagen (DK)
Klaus Katherine***	**US MN Rochester Nair KS:** Mayo Clinic School of Medicine (MN, US)
Kurz Sandra*	**DE Munich Knolle PA:** Klinikum rechts der Isar, Institute of Molecular Immunology (DE)
LaBarge Simon**	**US CA San Diego Schenk S:** University of California, UC San Diego (CA, US)
Lohr Kerstin*	**DE Munich Knolle PA:** Klinikum rechts der Isar, Institute of Molecular Immunology (DE)
Markova Michaela****	**CZ Pilsen Kuncova J:** Charles University in Prague, Dept. of Physiology (CZ)
Martin Eugenia*	**UK Glasgow Metcalfe NB:** University of Glasgow, Institute of Biodiversity, Animal Health & Comparative Medicine, College of Medical, Veterinary and Life Sciences (UK)
Melser Sue*	**FR Bordeaux Marsicano G:** Bordeau University, Laboratoire MRGM, Rare Diseases: Genetics and Metabolism, Université Bordeaux (FR)
Nielsen Birgitte*	**DK Copenhagen Poulsen HE:** Rigshospitalet Copenhagen, Lab of clinical Pharamacology (DK)
Segerer Gabriela*	
Siewiera Karolina**	**PL Lodz Watala C:** Medical University of Lodz, Department of Haemostasis and Haemostatic Disorders (PL)
Soendergaard Stine*********	**DK Copenhagen Dela F:** University of Copenhagen, Faculty Of Health Sciences (DK)
Sparagna Genevieve**	**US CO Boulder Sparagna G:** University of Colorado, Anschutz Medical Center (CO, US)
Walter Bjoern*	**DE Essen Rauen U:** Universitätsklinikum Essen, Institut für Physiologische Chemie (DE)
Wetterwald Celine*****	**FR Angers Gueguen N:** Ingénieur hospitalier, Biochimie Métabolique et Hormonologie, Département de Biochimie et Génétique (FR)

MiPNet21.01 Abstracts IOC109: 10+5 min

O2k perspectives

1. Kurz S, Wohlleber D, Knolle PA (2016) Mitochondria as immune sensors of viral infection in hepatocytes. Mitochondr Physiol Network 21.01.

Hepatotropic viruses, such as Hepatitis B virus (HBV) and Hepatitis C virus (HCV), are a global health problem and can induce severe liver disease. However, HBV and HCV are non-cytopathic viruses rather the antiviral immune response induces the liver inflammation and disease. Recognition and elimination of virus-infected hepatocytes occurs by antigen presentation of viral antigens on MHC class I molecules to virus-specific CD8 T effector cells (CTLs). Therefore, some viruses gained strategies to evade the immune response through interfering with MHC class I mediated antigen presentation.

Wohlleber et al. (2012) demonstrated a novel cytotoxic CTL effector mechanisms, the non-canonical CTL effector function. Thereby cross-presentation of viral antigens by non-infected liver sinusoidal endothelial cells (LSEC) stimulates CTLs to secrete TNF. CTL-derived TNF selectively induces apoptosis in infected hepatocytes but not in non-infected hepatocytes. As non-infected LSEC cross-present hepatocyte derived viral antigens this circumvents viral immune evasion in infected hepatocytes.

Based on the specificity of TNF-induced apoptosis for virus-infected hepatocytes, viral infection sensitizes hepatocytes towards TNF-induced apoptosis. Several observations indicate a central role of mitochondria in this pro-apoptotic signaling. Mitochondria are the central organelles for energy metabolism and Ca2+ homeostasis but also in the apoptotic signaling cascade in hepatocytes with which the viral infection interferes. In addition, upon infection the isolated mitochondria show an alteration in their calcium buffering capacity and sensitivity against calcium. Furthermore, viral infection also interferes with the oxidative phosphorylation in primary hepatocytes. However, the detailed molecular mechanism and role of mitochondria in sensitizing hepatocytes is still under investigation.

2. Siewiera K, Kassassir H, Watala C (2016) Potential role of mitochondria as modulators of blood platelet activation and reactivity in diabetes. Mitochondr Physiol Network 21.01.

Numerous studies have shown that cardiovascular complications are one of the major consequences of *diabetes mellitus*. They are responsible for two of three death events among this group of patients. Blood platelet dysfunctions are strongly involved in the development of the micro- and macrovascular complications in types 1 and 2 diabetes mellitus. However, mechanisms of abnormal platelet activation and their hypersensitivity in diabetes are still far from complete understanding. We believe that the changes in the functioning of the platelet mitochondria may largely underlie such phenomena.

WHAT WE KNOW: Platelet mitochondria provide energy that may be used for platelet activation and platelet-mediated blood clotting. Both platelet activation and intraplatelet granule secretion are energy-dependent processes. Moreover, in the course of platelet activation the rates of both glycolysis and oxidative phosphorylation increase, thus reflecting the increased energy requirements for the functioning of blood platelets [1]. The role of mitochondria as a supplier of ATP is so important in the course of platelet response to stimulating agents, that the inhibition of mitochondrial respiration has been found to reduce dense granule secretion and aggregation of platelets [2]. Therefore, the impaired functioning of platelet mitochondria may lead to altered platelet reactivity profile and relevantly altered responses of stimulated platelets.

OUR RESULTS AND CONCLUSIONS: Greater activation of circulating (resting) blood platelets was observed in streptozotocin-diabetic rats compared to their non-diabetic littermates. Diabetic platelets were also characterized by significantly elevated mitochondria mass, increased mitochondrial membrane potential and enhanced respiration, although the respiration control ratios appear to remain unchanged. Moreover, higher mitochondrial membrane potential and elevated mitochondrial respiration were closely related to the excessive activation of circulating platelets in diabetic animals. Interestingly, diabetic and healthy blood platelets differed also in response to insulin treatment. The incubation of diabetic platelets with insulin resulted in the elevated mitochondrial respiration, whereas no changes were observed in healthy platelets [3]. Our results indicate that long-term untreated diabetes may change blood platelet bioenergetics. These phenomena may be the result of the adaptation of blood platelets to much higher availability of energy substrates in diabetes. Observed alterations may be a potential underlying cause of abnormal platelet functioning in *diabetes mellitus*, however further research to validate this conclusion seems mandatory.

3. Soendergaard SD (2016) Acute exposure of human muscle fibers to EPO or Actovegin has a marked effect on the mitochondrial respiratory capacity. Mitochondr Physiol Network 21.01.

Actovegin, a drug made from the deproteinized hemodialysate of calf blood increases the mitochondrial respiratory capacity of untrained and overweight subjects, indicating that Actovegin may have the potential to improve performance. These findings are interesting because the drug is not on the World Anti-Doping Agency's prohibited list, but used by athletes. Therefore, we wanted to investigate whether Actovegin had the same effect in trained subjects. Also, we wanted to

compare the effect of Actovegin with the effect of erythropoietin (EPO; a banned substance) on the mitochondrial respiratory capacity.

We obtained basal muscle biopsies (m. vastus lateralis) from 8 trained subjects (VO2max: 54±2ml/min/kg). The skeletal muscle fibers were acutely exposed to either Actovegin (50µl/ml) or EPO (50µl/ml, 2000IU) during permeabilization, washing of the fibers and the respiratory analysis, resulting in a ~2h exposure time. Mitochondrial respiratory capacity was measured with high-resolution respirometry (Oxygraph-2k; OROBOROS, Innsbruck, Austria) and by sequential addition of malate, glutamate, ADP, succinate and FCCP.

EPO and Actovegin increased maximal complex I activity ($P<0.05$) compared to control (22±4, 43±3, 61±5pmol/mg/s) with a significant difference between EPO and Actovegin (43±3, 61±5pmol/mg/s, respectively). Only Actovegin increased the maximal oxidative phosphorylation capacity significantly (72±5, 82±8, 95±4 pmol/mg/s), but both EPO and Actovegin increased the maximal electron transport system capacity (77±5, 101±9, 112±10pmol/mg/s) ($P<0.05$). In regards to ADP kinetics, Vmax was significantly increased by EPO and Actovegin (18±2, 33±3, 50±4pmol/mg/s) ($P<0.05$), whereas Km was unaltered by EPO, but significantly increased by Actovegin (0.18±0.04, 0.21±0.04, 0.72±0.31mM).

The study demonstrates that acute exposure of human muscle fibers to EPO or Actovegin increases the mitochondrial respiratory capacity of trained subjects. The mechanism(s) are not clear, but EPO has been found to increase the NAD+ levels and the NAD+/NADH ratio in myoblasts (1), which could explain the observed increased complex I respiration with EPO (2). Actovegin contains succinate which in part can explain the effect of Actovegin on the mitochondrial respiration. It is not known whether Actovegin also contains NAD+, but it is intriguing to think that Actovegin and EPO might modulate mitochondrial function through the same mechanism, but this is only speculations.

4. Sparagna G (2016) Measuring cardiac mitochondrial function in pediatric patients before and after implantation of a left ventricular assist device. Mitochondr Physiol Network 21.01.

Heart failure is a multifaceted disease involving both energetic and mechanical deficits. In order to help the heart mechanically, a left ventricular assist device (LVAD) is often employed in heart failure patients. This device unloads the heart and patients usually see improvements in several functional parameters. In this study, the cardiac mitochondrial function of two pediatric patients with LVADs was assessed before insertion of the LVAD and then in the explanted heart after the patients received a heart transplant.

A small piece of LV tissue was recovered at the time of LVAD implantation for both patients. Later, when these patients received a transplant, a sample from the explanted heart was used for this study. In both instances, heart tissue was rapidly harvested in the operating room, placed in BIOPS solution, kept at 4°C, and mitochondrial respirometry by OROBOROS Oxygraph-2k was performed.

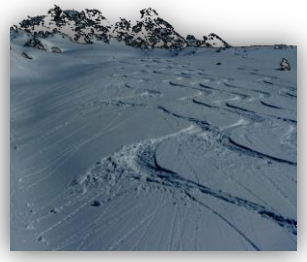
In the first patient, a 14.3 year old female with an LVAD for 7 days, O2k respirometry in BIOPS preserved tissue fibers showed a trend for increased respiratory control ratio ($p=0.07$), but no improvements in the other measured parameters using pyruvate/malate, glutamate or succinate as substrates. In contrast, the second patient, a 16 year old female, had her LVAD for 45 days and did show significant improvements in respiration rate with pyruvate/malate as well as glutamate. Respiration with succinate and the respiratory control ratio in the second patient also showed a trend for increase (both $p=0.06$) with the LVAD.

In summary, mitochondrial function can be measured in small pieces of heart tissue extracted during LVAD implantation. Although the LVAD is a mechanical device, its implantation results in an energetic improvement with a positive effect on mitochondrial function. This mitochondrial improvement, however, seems to take longer than a week to occur in a measurable manner.

5. Lohr K, Knolle PA (2016) Mitochondrial function and t-cell differentiation

6. Labarge S (2016) *Title to be announced*

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. » Full text in Bioblast

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

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

>1,700 O2k-Publications – http://wiki.oroboros.at/index.php/O2k-Publications: Topics

Acknowledgements

Programme prepared for printing by S Fleischmann, E Gnaiger, G Lamberti and V Laner, OROBOROS INSTRUMENTS.

Contribution to K-Regio project MitoFit.
The project MitoFit is funded by the Land Tirol within the program K-Regio of Standortagentur Tirol.
www.mitofit.org

Contact

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

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

NextGen-O2k
a project supported by the "Technologieförderungsprogramm Tiroler Innovationsförderung" of the Tyrolean Government.

COST Action CA15203 Mitochondrial fitness mapping
MITOEAGLE: Evolution - **A**ge - **G**ender - **L**ifestyle - **E**nvironment

O2k-Workshops are listed as MitoGlobal Events