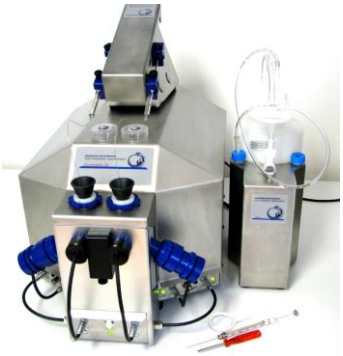
IOC96. *Mitochondrial Physiology Network* 19.11: 1-8 (2014)
http://www.bioblast.at/index.php/MiPNet19.11_IOC96

Version 2: 2014-10-07

96th International Workshop on HRR and O2k-Fluorometry

2014 October 07-12

Schröcken, Vorarlberg, Austria



The **96th Workshop on High-Resolution Respirometry (HRR)** is the **32nd** International Oxygraph Course held in Schroecken since 1988. A practical overview is provided of the **Oxygraph-2k and O2k-Fluorescence LED2-Module**, with real-time analysis by **DatLab** and applications of the **TIP2k**. A demo experiment illustrates the principle and shows the unique advantage of simultaneous monitoring of oxygen concentration, respiration and **hydrogen peroxide production**. Yeast cells are used as a biological reference material obtained as freeze dried samples. 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 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 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.

Lecturers and tutors

[Gnaiger Erich]
[Fontana-Ayoub Mona]
[Krumschnabel Gerhard]

The Universe of Mitochondrial Physiology in 2 ml

Programme IOC96

1 Tuesday, Oct 07

*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 - a welcome by OROBOROS INSTRUMENTS	IOC96

2 Wednesday, Oct 08

	Workshop 1		Weblink
07:30-08:30	*Breakfast*		
	Principles of high-resolution respirometry and O2k-Fluorometry - from switching on the Oxygraph-2k to the experimental result		Gnaiger 2008 POS*
08:30-09:15	**Get O2k-Connected with OROBOROS:** a guided tour to the Oxygraph-2k		get O2k-Connected
09:15-10:00	**Introduction to a DemoExperiment with the O2k**		Pesta 2012 Methods Mol Biol*
10:00	*Coffee / Tea*		
10:30-12:00	**O2k-Demo experiment:** Introduction to DatLab 6, respiration of intact cells: Simultaneous measurement of oxygen consumption (O2k-Core) and H_2O_2 production (O2k-Fluorescence LED2-Module)		MiPNet18.06_Amplex-Yeast*
12:00	*Lunch - Walk & Talk*		
15:00-16:00	**O2k instrumental setup and sensor service** - overview		O2k-Manual
16:00	*Coffee / Tea*		
	O2k instrumental setup	**OroboPOS service**	
16:30-17:15	Groups 1-5	Groups 6-10	O2k-Start
17:15-18:00	Groups 6-10	Groups 1-5	POS Service
18:30	*Dinner*		
20:00-21:00	**Hot MiP-Topics 1:** 10+5 min presentations of abstracts 1-3		IOC96 Abstracts MiPNet19.11

IOC 96

3 Thursday, Oct 09

	Workshop 2	Weblink
07:30-08:30	*Breakfast*	
08:30-09:15	**Experimental design 1:** Coupling control protocol with intact cells: ROUTINE, LEAK, ETS, ROX	Cells: PCP
09:15-10:00	**Experimental design 2:** Substrate and coupling control of mitochondrial respiration - MitoPathways through CI&II	The Blue Book* pp 43-57
10:00	*Coffee / Tea*	
10:30-12:00	**SUIT protocol with DatLab Analysis and guide through Excel templates**	DatLab Flux Analysis
12:00	*Lunch - Fishing*	The Blue Book p 56*
14:00-15:00	**Tissue homogenate preparation:** The PBI-Shredder	MiPNet17.03 Shredder vs Fibres*
15:00	*Coffee / Tea*	
15:30-18:00	**O2k-experiment:** Respiration with tissue homogenate: Two SUIT protocols with 6 Power-O2k	Krumschnabel 2013 Abstract MiP2013: 26-27*
18:30	*Dinner*	
20:00-21:00	**Hot MiP-Topics 2:** 10+5 min presentations of abstracts 4-7	IOC96 Abstracts MiPNet19.11

4 Friday, Oct 10

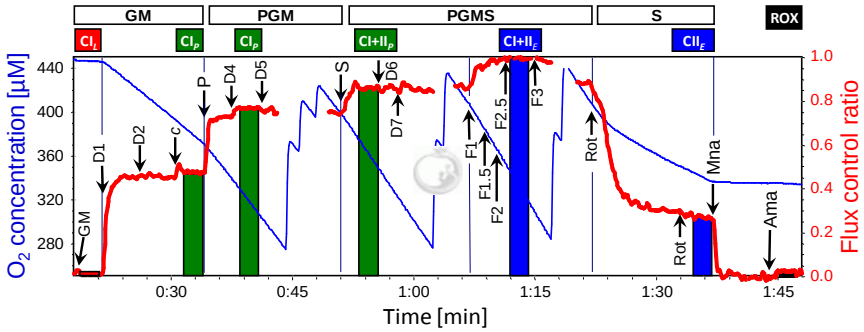
	Workshop 3	Weblink
07:30-08:30	*Breakfast*	
08:30-09:15	**DatLab O_2 flux analysis:** Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	Glossary: Respiratory states
09:15-10:00	**DatLab guide through the menus:** DL-Demo files and DL-Excel templates	DatLab Guide
10:00	*Coffee / Tea*	
10:30-12:00	**DatLab Analysis: hands-on in 10 teams**	DatLab Flux Analysis
12:00	*Lunch - Walk & Talk*	
15:00-15:45	**O2k-MultiSensor overview and O2k-Fluorometry applications:** Amplex™ red, safranin and TMRM	MiPNet17.17_Amplex-Mouse-brain*
15:45	*Coffee / Tea*	
16:15-17:00	**Prepare O2ks for O2k-Fluorometry experiments: TMRM, safranin, Amplex Ultrared.**	Krumschnabel 2014 Methods Enzymol
17:00-18:30	**6 O2k-Fluorometry experiments: respiration, mt-membrane potential and H_2O_2 production.**	
18:30	*Dinner*	
20:00-21:00	**DatLab analysis: diagnosis of respiratory defects.**	Krumschnabel 2014 Methods Enzymol

5 Saturday, Oct 11

	Workshop 4	*Weblink*
07:30-08:30	*Breakfast*	
08:30-09:30	**Instrumental quality control 1: The oxygen sensor OroboPOS - calibration, stability testing, and evaluation of sensitivity to measure oxygen flux.**	O2k-Calibration
09:30-10:15	**Instrumental quality control 2: O2k-Background test and on-line analysis of oxygen flux.**	O2k-Background*
10:15	*Coffee / Tea*	MiPNet18.10 O2kvsMultiwell*
10:45-11:45	**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.**	TIP2k User Manual
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*
16:45-17:15	**IOC-Questions - discussion of '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**	O2k-Feedback
	Farewell party	

6 Sunday, Oct 12

	Departure / Fish project	
	Breakfast	
	Early morning: Departure	Gnaiger 1993 Verh Dtsch Zool Ges*



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

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MiPNet19.11 Abstracts IOC96: 10+5 min

Hot topics in Mitochondrial Physiology

1.1. Bednarczyk P (2014) Coupling of the mitochondrial BKCa channel to the respiratory chain.

Potassium channels as present in the plasma membrane of various cells have also been found in the inner mitochondrial membrane. Potassium channels have been proposed to regulate the mitochondrial membrane potential, respiration, matrix volume and Ca2+ ion homeostasis. It has been suggested that mitochondrial potassium channels participate in ischemic preconditioning and neurodegenerative disorders. In our study single channel activity of a large conductance Ca2+-regulated potassium channel was measured by patch-clamp of mitoplasts isolated from an astrocytoma cell line. A potassium selective current was recorded with a mean conductance of 290 pS in symmetrical 150 mM KCl solution. The channel was activated by Ca2+ at micromolar

concentrations and inhibited irreversibly by iberiotoxin, an selective inhibitor of the BKCa channel. Additionally, we showed that substrates of the respiratory chain like NADH, succinate, and glutamate/malate, decrease the activity of the channel at positive voltages. The effect was abolished by rotenone, antimycin and cyanide, being inhibitors of respiratory chain. Our findings indicate that mitochondrial large conductance Ca2+-regulated potassium channels with properties similar to the surface membrane BKCa channel are present in human astrocytoma mitochondria and can be stimulated by redox status of the respiratory chain.

1.2. Dennerlein S (2014) Insights into COX-assembly: An important but not the only OXPHOS complex.

Within the last decades the development of a broad range of diagnostic methodologies led to the identification of an increasing number of human mitochondrial disease genes. Many patients present defects in the mitochondrial oxidative phosphorylation system (OXPHOS), the main energy source of the cell. The OXPHOS system is composed of the ATP producing ATP synthase (complex V), and of four multisubunit enzyme complexes, the mitochondrial respiratory chain (MRC) or complexes I-IV. In addition to the 82 structural components, an increasing number of associated factors, required for complex assembly, have been described. In contrast to the nuclear-encoded structural components, mutations in several COX assembly factors have been reported and were shown to be associated with various disorders. However, beside the growing number of identified MRC assembly factors, the understanding of the molecular mechanisms of complex IV assembly and maturation is far from complete.

We have recently identified a novel player, MITRAC12, which was shown to be in association with SURF1, a COX assembly factor, mutated in Leigh syndrome. This finding led to the identification of the MITRAC complex, a COX assembly intermediate. We have shown that MITRAC components feed back to COX1 translation and further facilitate the integration of newly imported nuclear encoded COX-subunits in the maturing enzyme via TIM21. However, the data support also an involvement of TIM21 in the maturation of all other OXPHOS complexes. Hence it is essential to get quantitative data of the activity and functionality of these complexes.

1.3. Xu R (2014) Exploring the role of IKKε in cancer metabolism.

Inflammation is a hallmark of cancer considered to be responsible for tumour survival and proliferation. Emerging evidence indicates that IκB kinase ε (IKKε) plays an important role both in inflammation and cancer. As a kinase, IKKε was previously reported to activate the transcription factor NF-κB downstream of Toll-like receptors [1], and also to be involved in the regulation of interferon (IFN) signalling by phosphorylating IRF-3 and IRF-7 [2]. Moreover, IKKε was recognised as an oncogene and is reported to be overexpressed in 30% of breast cancers and breast cancer cell lines [3]. More recently, IKKε has been also implicated in the regulation of energy balance in obese mice [4].

To investigate the role of IKKε in inflammation and understand more about its role as oncogene, we generated a 293-FLIP-IN cell line, in which expression of IKKε is induced by addition of doxycycline. In this cell line IKKε induction leads to phosphorylation of IRF-3 and production of IFN-β, confirming that the cell line is a perfectly suitable model to study IKKε. Our data show that the tumour promoting mechanism of IKKe is not limited to the activation of NFkB, but can also involve alterations in cellular metabolism. Preliminary data indicate that, when IKKe is constitutively active - being the driving oncogene – it diverts glucose from being fully oxidised in the mitochondria to other metabolic pathways, thus contributing to the Warburg effect, supporting cell proliferation.

Beside its role as an oncogene, IKKe could also contribute to malignant transformation upon activation of the innate immune response by rewiring cellular metabolism, highlighting a new aspect of how inflammation can contribute to tumorigenesis.

2.4. Calabria E (2014) High-intensity interval training (HIT) in aging: changes in cardiovascular fitness and cardiometabolic risk factors.

High Intensity Interval Training (HIT) has been shown to improve cardiovascular fitness and seems to induce beneficial modifications of cardiometabolic risk factors in healthy subjects and patients. Less is known about the efficacy of HIT applied to healthy older adults, and the adaptations induced at the central and periprheral level.

This study tested the hypothesis that 8 weeks of HIT can induce significant improvements of cardiovascular fitness, exercise capacity and of selected cardiometabolic risk factors in healthy older adults. In 12 healthy elderly male volunteers, we measured V'O2max, gas exchange threshold (GET), respiratory compensation point (RCP), resting mean, systolic and diastolic blood pressures (MBP, SDP, DBP), fasting blood glucose concentration (GLU), total cholesterol/HDL ratio (CHOLtot/HDL), % body fat (BF) and waist circumference (WC) before (PRE) and after (POST) an

8-week of HIT. The training program consisted of 7 bouts of 2-min near-maximal cycling (i.e. 85-90%V'O2max) interspersed with 2 minutes of recovery performed 3 times a week.

Absolute and relative V'O significantly increased by 5.4% and 11.7% respectively. V'O at GET and RCP increased by 7.2% respectively. MBP and SDP significantly decreased by 7% and 9% respectively. GLU was diminished by 7% and TC/HDL decreased by 5%. BF and WC decreased by 4% and 1.4% respectively. Surprisingly, analysis of the quadriceps muscle showed that both CSA and muscle volume significantly increased (5.0 and 5.4% respectively). Thus we can conclude that 8 weeks of HIT promote significant changes of maximal aerobic power and exercise resistance in healthy, male, elderly subjects. In addition, they induce significant improvements of some selected cardiometabolic risk factors.

Further studies are now needed to investigate how peripheral modifications in the skeletal muscle tissue may contribute to the described adaptations. Changes in the mass and/or in the function of the mitochondrial network will be evaluated on skeletal muscle biopsies in association to the assessment of muscle fiber type expression and capillary density.

2.5. Dhillon RS (2014) Acetylation and mitochondrial respiratory function in aging and calorie-restricted mice.

Recent studies have demonstrated widespread reprogramming of the mitochondrial proteome via post-translational modifications (PTMs) in response to a number of treatments that can affect metabolism. However, little is known of how these PTMs modify electron transport chain function. The objectives of our research are to examine the global acetylome in aging and caloric restriction in wild-type and sirtuin 3 knockout mice. We hypothesize that hyperacetylation seen in sirtuin 3 knockout or control diet mice will adversely affect mitochondrial respiration, and that caloric restriction may offset mitochondrial defects due to aging. Previous studies in our lab have demonstrated an increase in sirtuin 3 protein expression in the livers of calorie-restricted mice. Furthermore, all tissues do not respond to calorie restriction in a similar manner, yet how this affects mitochondrial metabolism has not been determined. We aim to explore these questions and incorporate a comparative approach using aging models (naked mole rats) and caloric-restriction models (hibernating mammals) to further elucidate the evolutionary mechanisms involved in post-translational modifications and metabolic phenotypes.

2.6. Ojuka E (2014) Excess fructose consumption: effects on mitochondrial function and insulin resistance.

Fructose is the master of all foods in its ability to cause fat accumulation and insulin resistance. The lecture discusses how these properties of fructose are exploited by some mammals and birds to survive periods of food scarcity and also why these same properties have contributed to the alarming increase in the incidence of type II diabetes in man today. Mechanisms to explain why small amounts of fructose enhance glucose metabolism but excess consumption leads to de novo lipogenesis, ectopic lipid deposition, and insulin resistance are explored in detail. The acute effects of fructose overload on the mitochondrial function along with the adaptive responses of the organelle to prolonged exposure are also examined. Lastly the thrifty gene hypothesis is presented as a possible mechanism to explain why humans are so prone to fructose-induced obesity and diabetes.

2.7. Karabatsiakis A (2014) Consequences of depression on respiratory activity in peripheral blood mononuclear cells.

Stress and trauma are contributing factors to the etiology and pathophysiology of depression. Although the precise underlying biological mechanisms are not completely known, the role of mitochondrial physiology reaches increasing focus of attention in this context. Clinical symptoms of depression include a lack of energy and motivation, sleep disturbances, cognitive deficits and reduced immunity, all linking to changes in energy metabolism. In this study we investigated the respiratory activity of peripheral blood mononuclear cells (PBMCs), an established model to investigate the psychoimmunological consequences of depression.

In total 44 participants (n=22 acute depressed patients, n=22 non-depressed healthy controls) participated in the study. Peripheral blood was collected by venous punction and PBMCs were isolated using Ficoll-based dense gradient centrifugation following storage at -80° C until analysis. After thawing cells were transferred into an Oroboros high-resolution oxygraph for the characterization of respiratory activity.

PBMCs of acute depressed subjects showed significantly lower respiratory activity: routine and uncoupled respiration as well as spare respiratory capacity, coupling efficiency and ATP turnover-related respiration were significantly decreased. Further, impaired respiration of PBMCs showed a significantly negative correlation with clinical symptom severity of depression.

Here we not only show that mitochondrial activity in immune cells is impaired in acute depressed patients but also that symptom severity is significantly correlated with mitochondrial respiration. Our results strengthen the perspective of a contributing role of mitochondria to the pathophysiology of depression. Impaired immunity, a health consequence in depression, might be linked to decreased energy metabolism thus providing a new therapeutical target in the treatment of the disorder an immune-related comorbidities.

Accommodation and Location

Hotel Körbersee www.koerbersee.at
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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 – www.oroboros.at/?O2k-Manual
O2k-Protocols – www.oroboros.at/?O2k-Protocols
>1,300 O2k-Publications – www.bioblast.at/index.php/O2k-Publications

Acknowledgements

Programme prepared for printing by S Fleischmann, V Erhart and E Gnaiger, OROBOROS INSTRUMENTS Corp.

Contribution to K-Regio project *MitoCom Tyrol*, funded in part by the Tyrolian Government and the European Regional Development Fund (ERDF).
www.oroboros.at/?MitoCom-Tyrol

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