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122nd International Workshop on HRR and O2k-Fluorometry and TRACT training course

2017 June 26 - July 01
Schröcken, Vorarlberg, Austria

The **122nd Workshop on High-Resolution Respirometry (HRR)** is the **37th** International Oxygraph Course held in Schroecken since 1988. We provide an overview of the **O2k-Fluorometer**, with real-time analysis by **DatLab 7 (*new*)** and applications of the **TIP2k**. O2k-Demo experiments 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 can be stored and shipped on dry-ice – introducing the MitoFit Proficiency Test. **Instrumental setup** and service of the polarographic oxygen sensor (**OroboPOS**) are demonstrated, followed by hands-on practice in 10 teams. A wide range of mitochondrial topics is 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 seven O2k (14 chambers). The **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 Walks & Talks, enjoying the refreshing scenery of the secluded alpine environment or using spare time for individual practice. Join for a visit to the *Alpmuseum*.

Lecturers and tutors

Doerrier-Velasco Carolina	CSO, OROBOROS INSTRUMENTS
Garcia e Souza Luiz	PhD student, OROBOROS INSTRUMENTS
Gnaiger Erich	CEO, OROBOROS INSTRUMENTS
Laner Verena	COO, OROBOROS INSTRUMENTS
Velika Beata	Pavol Jozef Šafárik University in Kosice, Republic Slovakia
Wohlfarter Yvonne	Internship, OROBOROS INSTRUMENTS

Programme

1 Monday, Jun 26

*printed in workshop materials

	Arrival	Weblink
15:00	**Arrival in Bregenz:** Meeting point Bregenz train station at 3:00 pm; approx. 1 h bus drive to Schröcken and Hochtannberg (Salober); walk to Hotel Körbersee (approx. 40 min)	IOC-travel
18:30-19:30	*Welcome reception at Hotel Körbersee* & ***get-together:*** Introduction of participants and their research interests - a welcome by OROBOROS INSTRUMENTS	Schroecken
19:30	*Dinner*	

2 Tuesday, Jun 27

	Workshop 1		Weblink
07:30-08:30	*Breakfast*		
08:30-09:30	**Challenges of innovation and continuation: transition to O2k-Series H and DatLab 7** O2k instrumental setup – overview with video clips		O2k-Videosupport
09:30-11:30	**Hands-on (10 groups)**		
	O2k instrumental setup	**OroboPOS service**	O2k-Start
09:30-10:15	Groups 1-5	Groups 6-10	POS Service
10:15	*Coffee / Tea*		
	O2k instrumental setup	**OroboPOS service**	POS Service
10:45-11:30	Groups 6-10	Groups 1-5	O2k-Start
11:30-12:30	**Oxygen calibration (instrumental quality control 1)** DL-Protocol O2-calibration air		Gnaiger 2008 POS SOP: O2-calibration
12:30	*Lunch packages/ Walk & Talk* *Alternative:* individual O2k-tasks		
14:30-15:30	**Cell respiration and simultaneous measurement of H_2O_2 production** (Demo-Experiment)		O_2-Flux Analysis
15:30	*Coffee / Tea*		

16:00-18:00	**Hands-on (7 groups): Oxygen calibration and cell respiration** Cell respiration and simultaneous measurement of H_2O_2 production.	MiPNet15.09 Yeast reference assay
18:30	*Dinner*	
20:00-21:00	**DatLab analysis:** Reproducibility of technical repeats	POS-Calibration-SOP O2 background

3 Wednesday, Jun 28

	Workshop 2	Weblink
07:30-08:30	*Breakfast*	
08:30-10:00	**Experimental design:** Pathway and coupling control of mitochondrial respiration	MitoPedia: Respiratory states
10:00	*Coffee / Tea*	
10:30-11:30	**O2k-Demo experiment**: Respiration of permeabilized cells: Measurement of oxygen consumption (O2k-Core) with RP1 and RP2.	SUIT reference protocol
11:30-12:00	**Hands-on (7 groups) - getting started with an O2k experiment:** washing, stirrer test, air calibration	O2k-calibration
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 (RP1 and RP2) with 7 Power-O2k	SUIT Reference Protocols
16:00	*Coffee / Tea*	
16:30-17:45	**DatLab analysis and SUIT protocols** Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	MitoPedia: Respiratory control ratios MitoPedia: SUIT
17:45-18:45	**DatLab analysis: hands-on in teams** Analysis of the hands-on experiment with permeabilized cells.	DatLab Flux Analysis MitoPedia: DatLab
19:00	*Dinner + registration for the walk to the Alpmuseum*	
20:30-21:30	**O2k perspectives:** 10+5 min presentations of abstracts 1-4	

4 Thursday, Jun 29

	Workshop 3	Weblink
07:30-08:30	*Breakfast*	
08:30-09:00	**From isolated mitochondria to tissue fibres and tissue homogenate preparation:** The PBI-Shredder (Demonstration)	MiPNet17.03 Shredder vs Fibres
09:00-10:00	**Introduction to instrumental O2 background** (Demo-Experiment), using the TIP2k DL-Protocol: Instrumental O2 background_TIP2k.	SOP: O2 background TIP2k manual
10:00	*Coffee / Tea*	
10:30-12:00	**Instrumental quality control 2:** O2 background test with the TIP2k; analysis of oxygen flux; O2 background from air saturation to zero oxygen concentration; or for permeabilized muscle fibres in the high-oxygen range of 500 – 200 µM.	
12:00	*Lunch packages / walk & talk* *alternative:* individual O2k-tasks	
14:30-15:00	**Tutorial on the Bioblast wiki** www.bioblast.at	O2k-Network www.bioblast.at
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	**SUIT protocols**	MitoPedia: SUIT
18:30	*Dinner*	
20:00-21:15	**O2k perspectives:** 10+5 min presentations of abstracts 5-9	

5 Friday, Jun 30

	Workshop 4	Weblink
07:30-08:30	*Breakfast*	
08:30-10:00	**Hands-on (7 groups): Coupling control protocol for intact cells in 7 O2ks** Advanced groups: CCP for intact cells with measurement of H_2O_2.	Coupling control protocol
10:00	*Coffee / Tea*	MiPNet18.10 O2kvsMultiwell*
10:30-12:00	**Data analysis**	The Blue Book* pp 43-57
12:00	*Lunch packages*	
12:30-15:30	*Walk to the Alpmuseum - guided tour and reception: € 15.-*	Alpmuseum*
15:30	*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 support**	O2k-technical support
17:50-18:45	**OXPHOS analysis: diagnosis of respiratory defects**	
19:00	*Dinner*	
20:00	*Feedback discussion: Next steps in the individual projects*	

6 Saturday, Jul 01

	Departure
06:30-7:30	*Breakfast*
	Early morning: departure from Hotel Körbersee at 08:15 am, bus departure 9.00 am at Salober.

Participants

Participant	Institution
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*Asteriks indicate the number of O2k instruments in the participant's lab.

OROBOROS: O2k in numbers

- **25 years** - since 1992
- **>900** instruments world-wide
- **>570** O2k-Network Labs in 49 countries
- **>2,200** O2k-Publications: www.oroboros.at
- **OROBOROS-Team: 20**
- **122** O2k-Workshops

OROBOROS INSTRUMENTS O2k Mitochondria and cell research

MiPNet22.01 Abstracts IOC122: 10+5 min

O2k perspectives

1. Dolezelova E, Kunzova M, Panicucci B, Zikova A (2017) Mitochondrion remodeling during *T.b.brucei* developmental differentiation. Mitochondr Physiol Network 22.01.

Trypanosoma brucei undergoes a complex life cycle as it alternates between a mammalian host and the blood-feeding insect vector, a tsetse fly. Due to the different environments, the distinct life stages differ in their energy metabolism, i.e. insect stage (procyclic cells, PS) depends on mitochondrial oxidative phosphorylation (OXPHOS) for ATP production while the bloodstream stage (BS) gains energy by aerobic glycolysis. The dramatic switch from the OXPHOS to glycolysis happens during the complex development of the PS in the tsetse fly. This development differentiation is characterized by extensive remodeling of mitochondrion structure and changes in mitochondrial bioenergetics. Importantly, the molecular mechanism behind this process is completely unknown. We have established the *in vitro* differentiation system, in which the transition from PS to epimastigotes followed by differentiation to transmission-ready metacylic trypanosomes is triggered by RNA binding protein 6 (RBP6) expression. This *in vitro* induced differentiation of PF cells takes 8 days. The appearance of epimastigotes and metacyclic trypanosomes in the culture was mapped using light and fluorescent microscopy. The whole cell proteome of cell culture harvested every day after the RBP6 induction was identified by label-free quantitative mass spectrometry. This proteomic data serves as a resource for further detailed characterization of changes happening in the parasite mitochondrion as well as identification of possible candidates involved in the PS differentiation.

2. Ghanim M, Mok K, Kelly V (2017) HAMLET derivatives as a pre-operative therapy in oesophageal cancer. Mitochondr Physiol Network 22.01.

Oral and oesophageal cancers are aggressive tumours associated with high morbidity and mortality. Lack of early detection strategies is one of the reasons for late diagnosis, since these cancers often do not exhibit any symptoms until entering advanced stages. Their pathogenesis is still unclear, thus there are few satisfactory therapies. Difficulties in operating on oral cavity or oesophagus, as well as applying radiotherapy and often occurring chemotherapy resistance in these cancers is a major set back in increasing the survival rate of the patients. Due to the unfortunate placement of the tumours, patients experience difficulties with swallowing, chewing, breathing and talking, during the disease as well as during the therapy. Surgeries often greatly lower the comfort of living of patients, leave scars and the overall survival rate still remains low. Development of an effective adjuvant therapeutic agent that would gently ease the symptoms and quickly reduce the size of the tumour before applying more invasive therapies could greatly increase the comfort of patients and ideally increase the survival rate.

HAMLET - **H**uman **A**lpha-lactalbumin **M**ade **LE**thal to **T**umour cells – is a complex formed from partially unfolded α-lactalbumin and oleic acid. Discovered by Catharina Svanborg and her group while studying the anti-bacterial properties of human milk on human lung cancer cells, HAMLET has since been proven to selectively target tumour cells and cause their death while having no such effect on healthy differentiated cells. It is the first identified protein that has a defined function in its native site and acquires a new beneficial function after partial unfolding. Other similar protein-fatty acid complexes that exhibit similar cytotoxic properties have been since identified.

The project aims to decipher the cellular mechanism that makes HAMLET selectively toxic to cancer cells. In particular, the research will focus on the process of metabolic transformation including aerobic glycolysis, extracellular acidification and membrane hyperpolarisation. It is hoped that by identifying the critical characteristics that selectively kill cancer cells, that it will be possible to further enhance HAMLET's efficacy as an adjuvant therapy.

3. Goudie L, Mancini NL, Wang A, McKay DM, Shearer J (2017) Inhibition of mitochondrial fission as a novel treatment for IBD. Mitochondr Physiol Network 22.01.

Inflammatory bowel disease (IBD) encompasses a group of disorders that involve an exaggerated immune response to intestinal microbes. Recently we, and others, have assessed the possibility that excessive mitochondrial fission affects epithelial-microbial interactions, decreases epithelial barrier function and contribute to enteric inflammation. Excessive fission, mediated by DRP1 and Fis1, promotes a remodeling of mitochondrial networks into more punctate mitochondria that generate more reactive oxygen species and can affect energy and cell death pathways. Hypothesising that elevated mitochondrial fission would occur in enteric inflammation, male Balb/c mice were given dextran sulfate sodium (DSS) (5% (w./v.) 5 days, 3 days water) or dinitrobenzene sulfonic acid (DNBS) (3 mg, intrarectally.) ± an inhibitor of DRP1 and Fis1, P110 (3 mg/Kg, intraperitoneally.) daily. On necropsy DSS and DNBS displayed the characteristic signs of colitis associated with these models. Disease was substantially less in P110-treated mice as gauged by (i) macroscopic disease scores, (ii) shortening of the colon and (iii) colon motility (i.e. bead extrusion) (n=8-12). Analysis of histopathology on H&E stained sections of mid-colon revealed some improvement in P110 treated mice, but this was not a statistically significant result. Thus, systemic administration of a selective inhibitor of mitochondrial fission reduced the severity of disease in two different, commonly used murine models of colitis. Studies are required to define the mechanism of this effect in terms of the target cell (e.g. epithelium vs. macrophage) and systemic vs. local effects of the P110. We conclude that inhibition of DRP1 and Fis1 interaction provides a novel approach to mitigating IBD.

4. Han WH, Kuny S, Sauve Y, Lemieux H (2017) Retinal mitochondrial respiration defects precede hyperglycemia onset in type 2 diabetes. Mitochondr Physiol Network 22.01.

There is increasing evidence linking retinal mitochondria defects with development and progression of diabetic retinopathy. We tested the hypothesis that defects in retinal mitochondrial oxidative phosphorylation (OXPHOS) might precede the development of hyperglycemia and associated vascular changes in type 2 diabetes.

We used male Nile grass rats (*Arvicanthis niloticus*), which when fed standard rodent chow, undergo hyperinsulinemia at 2 month, followed by hyperglycemia by 6 month and retinal pericyte drop at 18 month. Controls were fed a high fiber low-calorie diet, which prevented hyperglycemia up to 18 month. High-resolution respirometery (Oxygraph 2k; OROBOROS) allowed measuring mitochondrial function in retina homogenates isolated from individual Nile grass rats (n=6-11 animals per group). Specific aspects of mitochondrial respiration were isolated using a multiple substrates-inhibitor protocol: 1) NADH- and succinate-dependent respiration pathway (N- and S-pathway); 2) maximal cytochrome c oxidase (COX) capacity; 3) integrity of the outer mitochondrial membrane under addition of exogenous cytochrome c, cytochrome c control factor (CcCF). Respirometery parameters data were expressed as flux control ratios (FCR), the respiration rate was normalized against maximal OXPHOS capacity. Citrate synthase activity was measured to estimate mitochondrial content. Data are expressed as mean ± SEM. Significance between groups was set at $p < 0.05$ using the non-parametric Mann-Whitney U-test.

Retinal mitochondrial defects were detected in 2 mo animals that maintained normoglycemia but displayed hyperinsulinemia. An increase in CcCF, indicating compromised membrane integrity, was observed in these animals when compared to controls (5.2 ± 1.5% vs. 1.0 ± 0.5%; $p = 0.007$; of note: larger value imply less integrity in mitochondrial membranes). Unexpectedly, at 6 mo, hyperglycemic animals had higher membrane integrity relative to control animals ($p=0.009$). The FCR showed an increase contribution of the N-pathway to overall mitochondrial respiration (0.64 ± 0.01 vs. 0.60 ± 0.01, respectively, $p = 0.011$).

Prior to hyperglycemia development, hyperinsulinemia is associated with reduced outer membrane integrity and increased N-pathway driven respiration in retinal mitochondria. These findings support that targeting of mitochondria, prior to hyperglycemia, might prevent diabetic retinopathy.

5. Karavyraki M, Porter RK (2017) Mitochondrial function and morphology linked to metabolic differences in normal, dysplastic and cancerous oral cells. Mitochondr Physiol Network 22.01.

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer in the world and accounts for more than 90% of oral malignancies. OSCC is usually preceded by the oral premalignant lesions, mainly oral leukoplakia (OLK) after repeated insults of carcinogens, tobacco. Dysplastic oral keratinocyte (DOK) cells were firstly isolated from a 57-year-old man who was a heavy smoker prior to the appearance of a white patch on his tongue. Eleven years later a squamous-cell carcinoma developed at the site and was excised. Subsequently the remaining dysplasia was removed, and it was from a piece of this that the primary cell cultures which eventually gave rise to DOK were initiated. The DOK line has been single-cell cloned and is apparently immortal (SCC) [1]. Mitochondria and mitochondrial proteins are undoubtedly potential anti-cancer targets. Mitochondria are the site of oxidative phosphorylation. Mitochondrial morphology is sensitive to stress and respond dynamically to the changes in their cellular microenvironment. Mitochondrial dysfunction is also a hallmark of many diseases. For instance Complex I subunit mutations and citric acid cycle enzyme mutations are associated with several cancers [2,3].

The aim of this project is to characterize differential mitochondrial function/morphology in comparisons of normal, dysplastic and cancerous oral cells.

Our initial focus will be on a mitochondrial functional/morphological comparison of normal tongue cells, the dysplastic tongue cell line (DOK) and tongue carcinoma cell line (SCC-4). Cells will be characterised for invasiveness, migration, anoikis resistance and hypoxia while their bioenergetic profiling will be examined by OROBOROS high-resolution respirometry and Seahorse Extracellular Flux Analysis. Further to their mitochondrial morphology and dynamics, Confocal Microscopy will be applied, while Quantitative RT-PCR & immunoblotting will be used to analyze their mitochondrial protein expression levels. Through chemotherapy sensitivity will be analyzed their differential drug profiling of defined stages of OSCC correlated to mitochondrial function, while NMR metabolite analysis will be used to investigate their metabolic profiling.

The predicted outcome of this project will be the discovery of differential mitochondrial abundance, morphology, functional proteins involved in mitochondrial dynamics and metabolic differences in normal, dysplastic and oral cancer cells. These discoveries will lead to the identification of novel therapeutic targets.

6. O'Brien KA, Horscroft JAH, Lindsay RT, Philp A, Harridge SDR, Murray AJ (2017) PPARα independent effects of nitrate supplementation on skeletal muscle mitochondrial function in hypoxia. Mitochondr Physiol Network 22.01.

Oxygen insufficiency (hypoxia), either in response to environmental exposure or pathological states, induces metabolic stress and remodelling the details of which remain ill-defined. A controversial aspect of acclimation is skeletal muscle metabolic remodelling, a process that may be aided by nitrate supplementation. Mechanisms of nitrate action have been demonstrated previously in skeletal muscle to involve interaction with a master regulator of fat metabolism, peroxisome proliferator-activated receptor alpha (PPARα)[1]. In the present study, the potential for dietary nitrate supplementation to aid hypoxic acclimatisation through protection of skeletal muscle mitochondrial function and the requirement for PPARα in this response were investigated. Hypoxia induced a 26% decrease ($p \leq 0.001$) in mass specific long chain fatty acid LEAK state respiration and a 23% decrease ($p \leq 0.01$) in carbohydrate oxidative phosphorylation capacity in control (chloride treated) mice of both $PPAR\alpha^{+/+}$ and $PPAR\alpha^{-/-}$ genotypes. These significant decreases were not apparent in nitrate supplemented mice, indicating a nitrate dependent recovery of mitochondrial function. A nitrate effect was observed in both $PPAR\alpha^{+/+}$ and $PPAR\alpha^{-/-}$ mice, suggesting a mechanism acting independently of PPARα. Our results confirm previous reports of hypoxia suppressing skeletal muscle mitochondrial function and show this effect can be partially alleviated through dietary nitrate supplementation. Whilst the signalling mechanisms remain uncertain, this process appears to occur independently of PPARα.

7. Skolik RA, Menze MA (2017) What can we learn from different sugars as substrates for cancer cells? Mitochondr Physiol Network 22.01.

Otto Warburg described 61 years ago how energy production in highly proliferating cancer cells shifts from oxidative phosphorylation (OXPHOS) to glycolysis even in presence of oxygen concentrations high enough to support mitochondrial OXPHOS. When glucose is replaced by the monosaccharaide galactose, alternative energy substrates such a glutamine are utilized. This simple change dramatically shifts energy metabolism towards the mitochondrion by engaging higher rates of OXPHOS. These increased rates of OXPHOS are dependent upon cofactors such as the iron-sulfur clusters found in several of the respiratory Complexes. Recent findings have revealed that the family of mitochondrial-associated NEET (CISD1 and CISD2) proteins contains labile 2Fe-2S clusters capable of transfer to apo-acceptor enzymes. Galactose treatments are currently used both as models for aging and to increase sensitivity of cancer cells to mitochondrial toxins for drug development (4). Surprisingly, a comprehensive understanding of the impact of galactose on cancer cells remains unknown. Furthermore, the impact of galactose on NEET protein expression and function has not been explored.

We hypothesize that the observed increase in OXPHOS after replacing glucose with galactose as a carbon source relies on shifts in the expression patterns of mitochondrial dehydrogenases and redox active proteins.

Here we show that HepG2 cells cultured in presence of dialyzed FBS (dFBS) and galactose show dramatic shifts in metabolism, gene expression, and mitochondrial activity. The response to galactose exposure was time dependent, with longer exposure to galactose resulting in more pronounced increases in OXPHOS capacity. Additionally, we reveal that utilization of dFBS can have broad implications on cellular physiology by changing expression of CISD1, thought to be involved in a variety of processes ranging from protection from oxidative stress to the regulation of cellular bioenergetics.

8. Truu L, Chekulayev V, Klepinin A, Ounpuu L, Tepp K, Puurand M, Koit A, Shevchuk I, Kaambre T (2017) Bioenergetics of colorectal cancer. Mitochondr Physiol Network 22.01.

Bioenergetics is a fast growing field in cancer research, where many promising outcomes could provide targeted cancer treatment. Energy metabolism specific literature is characterized by many contradictions, concluding that cancer cells metabolize their increased glucose uptake via glycolysis rather than more energy efficient oxidative phosphorylation (OXPHOS). Furthermore, the majority of these conclusions are the outcome of only *in vitro* studies on cell culture models, without taking into consideration the factors arising from the tumor microenvironment giving significant effects *in vivo*. We have conducted quantitative cellular respiration analysis on normal colon tissue, colorectal cancer (HCC) clinical tissue samples and CaCo-2 cell cultures. Our results show that HCC is not a fully glycolytic tumor and OXPHOS system might be the main source of ATP. Comparing healthy colon, HCC tissue and CaCo-2 cells, we found elevated rates of maximal ADP-activated respiration and greater activity of respiratory Complex CII over CI in both HCC and CaCo-2 cells, whereas the opposite result in healthy tissue was present. These results indicate that the bioenergetic profile of Caco-2 cells corresponds generally to HCC tissue. Further research is in progress to generate a full cancer development model consisting of cell cultures, clinical polyps and malignant versus healthy tissue samples.

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

>2,200 O2k-Publications – http://wiki.oroboros.at/index.php/O2k-Publications: Topics

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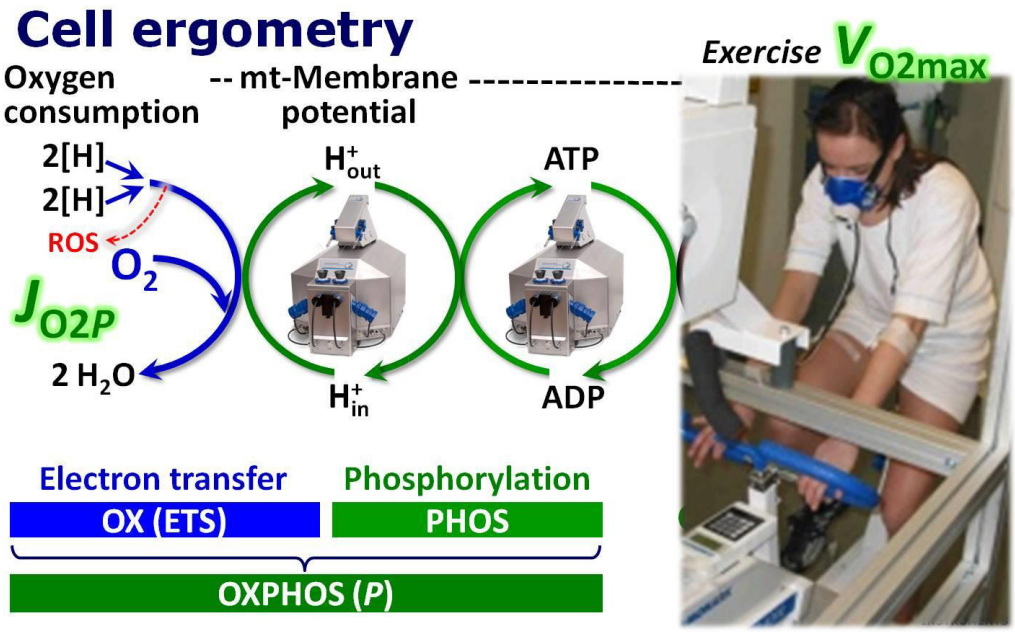
Mitochondria and cell research

O2k-Workshops are listed as MitoGlobal Events

MitoFit - diagnostic standard



From spiroergometry (V_{O2max}) to cell ergometry for MitoFit scoring.

The O2k-Core and O2k-Fluorometer represent the gold standard for generating reliable quantitative respirometric data to develop the MitoFit Knowledge Management Platform (KMP) and MitoFit database.

- **Reference sample of cryopreserved mitochondria:** The availability of a reference sample for respirometry will provide enormous benefits for scientific research and open up new perspectives on clinical applications. Its use enables a new level of quality control in respiratory studies to be attained.
- **MitoFit proficiency test:** A ring test allows evaluation of the proficiency of a laboratory by measuring respiration of reference samples at pre-defined times and following standard experimental protocols. Reporting the reproducibility of measurements is a quality control for the evaluation of compliance with defined standard requirements.
- **MitoFit test on human blood cells:** Tissue biopsy for the study of mitochondrial function is a practical but invasive approach. Measurement of mitochondrial performance in human blood cells allows a noninvasive sampling procedure, enabling collection and cryopreservation of samples for later measurement and analysis. This will widen the applicability of respirometry for the study of human physiology immensly, permitting routine screening and repeated monitoring of the MitoFit score.

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high-resolution respirometry
Schöpfstr 18, A-6020 Innsbruck, Austria
www.mitofit.org

COST Action CA15203
MITOEAGLE

Evolution **A**ge **G**ender **L**ifestyle **E**nvironment
Mitochondrial fitness mapping – Quality management network

The MITOEAGLE Network aims at:

- Improving our knowledge on mitochondrial function in health and disease with regard to **E**volution, **A**ge, **G**ender, **L**ifestyle and **E**nvironment
- Interrelating results of studies performed world-wide with the help of a MITOEAGLE data management system
- Providing standardized measures to link mitochondrial and physiological performance to understand the myriad of factors that play a role in mitochondrial physiology

Join the COST Action MITOEAGLE any time and contribute to the quality management network

More information:
www.mitoeagle.org