IOC50. *Mitochondrial Physiology Network* 14.3: 1-16 (2009) ©2009 OROBOROS®
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50th International Course on High-Resolution Respirometry

18-22 April 2009


Schröcken, Vorarlberg, Austria

The **50th O2k-Course** is the 18th presentation of high-resolution respirometry (HRR) in Schröcken since 1988. This O2k-Course includes experiments with permeabilized muscle fibers and cells, providing a practical overview of the **Oxygraph-2k**, with integrated on-line analysis by **DatLab 4.3** (new upgrade) and application of the **TIP-2k** for high-resolution respirometry. The O2k-system is introduced with specific perspectives of mitochondrial physiology. Emphasis is placed on hands-on applications by all participants.

Experienced tutors guide small working groups step-by-step through the approach of HRR. Five fully upgraded Oxygraph-2k are available for a do-it-yourself application of both hardware and software. Combined with an introduction and demo experiment, it is best to put the O2k into action yourself.

During lunch breaks, sufficient time is available for skiing (first day) or relaxing walks and talks, to enjoy the refreshing scenery of the alpine environment, or use the spare time for specific tutorials. With DatLab 4.3 we accomplish data analysis on-line during the experiment, providing final results and their graphical presentation by the end of an experimental run. Thus we gain sufficient time to see the Titration-Injection microPump TIP-2k with new feedback-control in action and practice its simple and automatic operation.

Support

MITOFOOD COST Action Number FA0602 (Coordinator: Dr. Jaap Keijer, RIKILT-Institute of Food Safety, Wageningen University, The Netherlands.

Tutors

- **Mario Fasching,** PhD, mario.fasching@oroboros.at
- **Erich Gnaiger**, PhD, erich.gnaiger@i-med.ac.at
- **Kathrin Renner**, PhD, kathrin.renner@oroboros.at
- **Simone Köfler**, Mag, simone.koefler@oroboros.at (*admin.*)

Coupled Guest Tutors IOC44-IOC50

- **Kane Dan A**, MSc, East Carolina University, Brody School of Medicine, Department of Exercise and Sport Science & Department of Physiology, East Carolina, Greenville, USA. - dak1021@ecu.edu
- **Tweedie Constance**, MSc, Faculty of Medicine, University of Calgary, Calgary, AB, Canada. – cltweedi@ucalgary.ca

Constance and Dan participated at IOC44 in Schröcken, Dec. 2007, where they met for the first time. Recently, an additional meaning of 'coupling' was introduced to our courses on HRR, when they became engaged. We are celebrating a high level of coupling when Conny and Dan return to the IOC50 as experienced tutors.

Programme IOC50

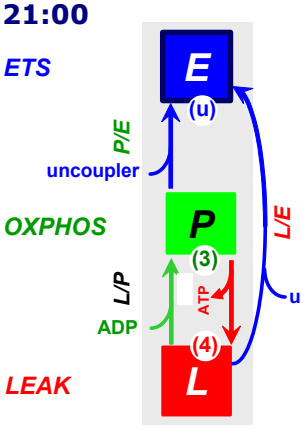
Day 1: Saturday, 18. April

16:00 Participants arriving in Bregenz: Meeting point at 4:00 pm in Bregenz train station; 1.1 hour drive to Schröcken. Check in at Hotel Mohnenfluh.

18:30 **Welcome reception**

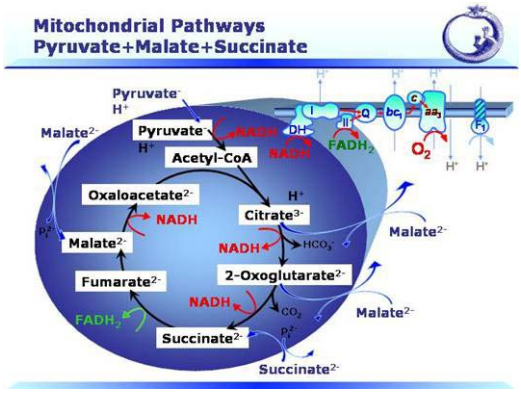
19:00 Dinner

21:00



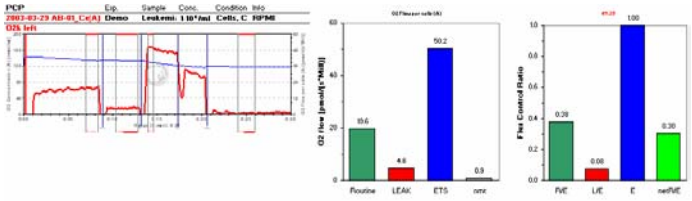
Erich Gnaiger (*Innsbruck, AT*) Electron transport system (*ETS*), *OXPHOS* capacity and *LEAK* respiration: Experimental advances with high-resolution respirometry.

L/E versus *L/P* coupling control ratio



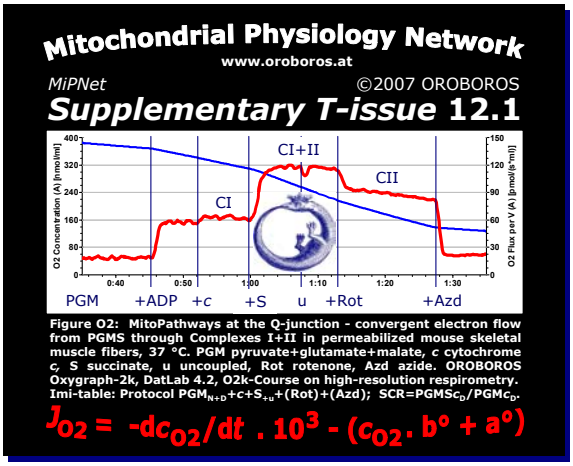
Day 2: Sunday, 19. April

08:30 **Principles of high-resolution respirometry - from switching on the Oxygraph-2k to the experimental result (demo experiment).**



- Oxygraph-2k demo experiment with DatLab 4.3.
- Oxygen calibration of the polarographic oxygen sensors (POS).
- Demo experiment: Phosphorylation control titration with intact cells: *ROUTINE, LEAK, ETS, ROX*

12:00 - 16:00 Lunch break; skiing (bus leaves at 12:14 from Hotel Mohnenfluh).



16:15 -18:45 **Parallel group sessions:** Hands-on with the Oxygraph-2k (5 O2k - 10 chambers):

A. Oxygen calibration, Phosphorylation Control Titration with intact cells; on-line DatLab analysis.

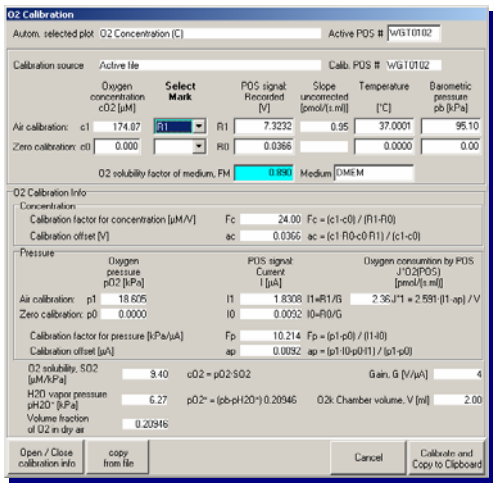
B. Preparation of permeabilized skeletal muscle fibers; oxygen calibration, *OXPHOS* titration protocol; on-line DatLab analysis.

19:00 Dinner

IOC44

21:00 Discussion of results of the demo experiment.

Day 3: Monday, 20. April



08:30 – 09:00 **Introduction:**
Instrumental Background

09:00 – 12:00 **Parallel group sessions:**
Hands-on experiments with the Oxygraph-2k - instrumental performance: O2k-calibration; instrumental background *competition*, DatLab analysis.
Washing and filling the O2k chambers with experimental media; air calibration
Demo: Preparing the TIP-2k for dithionite titration.
DatLab Oxygen calibration of the POS.

A. Instrumental background test for expeirments with cells and isolated mitochondria, from air saturation to zero oxygen concentration, wth automatic TIP-2k titration protocol.
B. Instrumental background test for experiments with permeabilized muscle fibers, in the high-oxygen range. Manual titration of hydrogen peroxide into MiR06 (MiR05 with catalase).

12:00 – 15:00 Lunch break – walk and talk (skiing lifts are closed)

15:15 – 16:00 **Parallel group sessions: Instrumental Background Analysis**
Parallel group sessions:

	Setup	POS Service	DatLab Analysis; general topics A	B	C
16:00 – 16:45	**Gr. 1**	**Gr. 2**	**Gr. 3**	**Gr. 4**	**Gr. 5**
16:45 – 17:30	**Gr. 2**	**Gr. 3**	**Gr. 4**	**Gr. 5**	**Gr. 1**

17:30 Coffee

	Setup	POS Service	DatLab Analysis; general topics A	B	C
18:00 - 18:45	**Gr. 3**	**Gr. 4**	**Gr. 5**	**Gr. 1**	**Gr. 2**

19:00 Dinner

Hot topics: MiPNet Session (10+10 min)
Chair: Constance Tweedie and Dan A Kane

21:00-21:20 **MiPNet 1: Dan A Kane** (*Greenville, USA*) Effect of blebbistatin on ADP-stimulated respiratory kinetics in permeabilized myofibers.

21:20-21:40 **MiPNet 2: Malou Friederich** (*Uppsala, SE*) Mitochondria function in the type 2 diabetic kidney; influence of oxidative stress on Uncoupling Protein-2 activation.

21:40-22:00 **MiPNet 3: Walid Fazeli** (*Hamburg, DE*) Impairment of respiratory capacity and control of isolated mitochondria during the development of hypertrophy in rat heart.

Day 4 Tuesday, 21. April

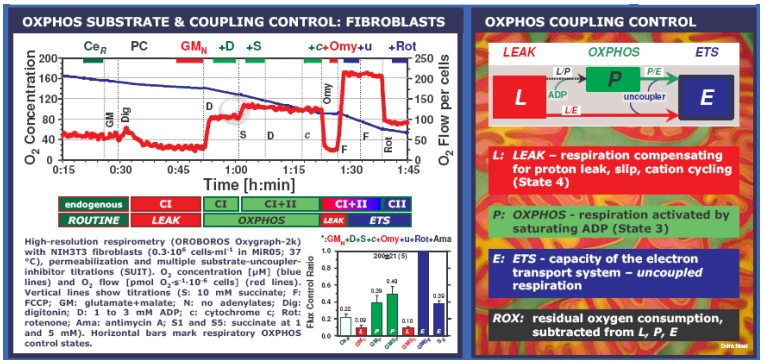
	Setup	POS Service	DatLab Analysis; general topics A	B	C
08:30 – 09:15	**Gr. 4**	**Gr. 5**	**Gr. 1**	**Gr. 2**	**Gr. 3**
09:15 – 10:00	**Gr. 5**	**Gr. 1**	**Gr. 2**	**Gr. 3**	**Gr. 4**

10:00 Coffee

10:30-11:30 Mario Fasching (*Innsbruck, AT*): Trouble Shooting

11:30 - 16:00 Snowshoe walk to a welcome at the Alpmuseum, lunch at Hotel Körbersee (*we keep the details of timing flexible according to weather conditions (rental of snowshoes and guided tour: 15.- Euro).*

Alpmuseum uf m Tannberg, Batzen www.alpmuseum.at



17:00-18:30 Erich Gnaiger (*Innsbruck, Austria*) Experimental protocols with intact cells and mitochondrial preparations.

19:00 Dinner

21:00 ***Discussion - Summary – Conclusions***
Farewell party of IOC50

Day 5: Wednesday, 22. April

Departure or continuation at MultiSensor Workshop IOC51

MiPNet Abstracts–
Hot topics in Mitochondrial Physiology

MiPNet 1. Effect of blebbistatin on ADP-stimulated respiratory kinetics in permeabilized myofibers.

Kane DA, Perry CGR, Lin CT, Anderson EA, Neufer PD.
The Metabolic Institute & Departments of Exercise and Sport Science & Physiology, East Carolina University, Greenville, NC, USA.

Permeabilized myofibers allow *in situ* assessments of mitochondrial respiratory function with minimal perturbation to the mitochondrial reticular structure. However, myofibril contraction may limit respiratory kinetics by attenuating the diffusion of oxygen and/or exogenously added substrates into the mitochondrial reticulum. ADP-stimulated respiration was therefore assessed in the absence (C) or presence of vehicle (V, DMSO) or the myosin II inhibitor blebbistatin (BLEB) dissolved in V in saponin-permeabilized red gastrocnemius myofibers from Sprague Dawley rats. State 3 respiration with pyruvate+malate (5mM+2mM) was significantly greater in BLEB vs V under normoxia and hyperoxia at 37 °C. The K'_m for ADP was ~10-fold higher with BLEB vs C in normoxia at 37 °C. Thus, inhibiting contraction alters *in situ* assessments of ADP-stimulated respiration kinetics in permeabilized myofibers. This may be due to a

preserved mitochondrial reticular structure concomitant with improved intracellular diffusion and mitochondrial uptake of oxygen and/or substrates.
Supported by NIH DK073488.

MiPNet 2. Mitochondria function in the type 2 diabetic kidney; influence of oxidative stress on Uncoupling Protein-2 activation.

Friederich M[1], Sergiu-Bogdan C[2], Fasching A[1], Brismar K[2], Palm F[1,3]
[1]Institution of Medical Cell Biology, Uppsala University, Sweden; [2]Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden; [3]Department of Medicine, Division of Hypertension and Nephrology, Georgetown Medical Center, Washington DC, USA

Background: Diabetes is closely associated with increased oxidative stress and the prevalence of diabetes type 2 together with complications, such as kidney damage, is rapidly increasing throughout the world. Recently, uncoupling protein-2 (UCP-2) has gained increasing interest since it has been shown to regulate mitochondria function and oxygen utilization the type 1 diabetic kidney. Studies have shown that UCP-2 is activated by superoxide, making UCP-2 a possible link between increased oxidative stress and altered mitochondria function.

Aim: To investigate the function of kidney mitochondria from type 2 diabetic db/db mice, and the potential role of UCP-2 and oxidative stress.

Methods: Four groups of mice were used; C57/bl and db/db, with and without antioxidant treatment by Q10 for two weeks prior to mitochondria isolation. Oxygen consumption was measured using the Oxygraph-2k (OROBOROS) during baseline and after sequential addition of the Krebs cycle substrate glutamate (10 mM), the adenosine triphosphate (ATP) synthase substrate adenosine diphosphate (ADP; 300 µM), the ATP synthase inhibitor oligomycin (12µg/mg protein) and the specific UCP-2 inhibitor guanosine diphosphate (GDP; 50 µM). Protein expression of UCP-2 was measured from kidney tissue homogenate by standard western blot using a primary antibody against UCP-2 and a secondary antibody conjugated to HRP. UCP-2 protein expressions were corrected for β-actin.

Results: Weight and blood glucose was significantly increased in both db/db groups compared to both control groups (45.6±2.7 g and 29.7±1.5 mM vs. 27.6±1.2 g and 7.3±0.5 mM, respectively). Q10 did not alter any of these parameters. Control mice did not respond to glutamate stimulation, whereas diabetic mice increased oxygen consumption by 24.2±6.9% in response to glutamate. Q10 treatment to diabetic mice prevented this stimulation. Addition of GDP reduced oxygen consumption in the diabetic group, whereas it had no effect to either Q10-treated diabetics or any of the control groups.

Baseline UCP-2 protein levels were similar in both untreated controls and untreated diabetics, and Q10 reduced the protein expression by approximately 50% in both groups.

Discussion: Mitochondria from type 2 diabetic db/db mice kidneys display glutamate-stimulated oxygen consumption. This effect is inhibited by GDP, which indicates a pivotal involvement of UCP-2 for the glutamate-stimulated oxygen consumption in the diabetic kidneys. Since this effect is prevented by Q10, it is likely that UCP-2 is activated by the increased oxidative stress commonly associated with sustained hyperglycemia.

Conclusion: Kidney mitochondria from diabetic db/db-mice display glutamate-stimulated oxygen consumption due to increased oxidative stress, which activates the uncoupling function of UCP-2.

MiPNet 3. Impairment of respiratory capacity and control of isolated mitochondria during the development of hypertrophy in rat heart.

Fazeli Walid
Sektion Neonatologie und Pädiatrische Intensivmedizin, Universitätsklinikum Eppendorf (UKE), Hamburg, Germany.

Objective: Increasing myocardial afterload results in hypertrophy associated with increased need for ATP production. We speculated that during the development of

hypertrophy respiratory capacity of isolated mitochondria would increase to match energy demand.

Methods: Hypertrophy was induced by aortic banding in 50 g rats. Mitochondria were isolated 2, 6 and 10 weeks after banding. The degree of hypertrophy was assessed by echocardiography. Subsarcolemmal (SSM) and interfibrillar (IFM) mitochondria were isolated by differential centrifugation. Citrate synthase activity was used as mitochondrial marker enzyme. Maximal ADP-stimulated oxygen consumption (State 3) as well as the respiratory control index and ADP/O ratio were measured with a Clark-type electrode and glutamate and succinate as substrates.

Results: Aortic banding resulted in a continuously increasing afterload as rats grew which resulted in significant hypertrophy which was verified by echo and by an increase in the heart to body weight ratio from 2.28±0.08 to 5.63±0.36. Citrate synthase activity was unaltered by hypertrophy. State 3 respiration of both IFM and SSM decreased continuously after aortic banding. State 3 respiration of IFM was significantly reduced 10 weeks after aortic banding. The respiratory control index was significantly reduced 10 weeks after increasing afterload. The ADP/O ratio was reduced 2 weeks after aortic banding, and unchanged after 6 and 10 weeks.

Conclusion: The development of hypertrophy is not associated with an increase in maximal respiratory capacity, but instead with an impairment in maximal ATP producing capacity, which may contribute to the onset of heart failure. This concerns especially the IFM. The datas shown could be explained by the well described substrate switch during the development of hypertrophy. We suggest that the impact of reduced fatty acid oxidation of hypertrophied hearts is stronger on IFM than on SSM.

Questions for the O2k-Course

The O2k-Manual (# refers to Chapter numbers in the O2k-Manual) provides the answers to many of these questions – and you find more information on www.oroboros.at …

Oxygraph-2k assembly (O2k-Manual #1.O2k.A)

- What is the most important consideration for positioning the glass chamber during assembly of the O2k?
- How do I detect a leak in the chamber?

Polarographic oxygen sensor (POS)

- Why is it important to check the non-calibrated raw signal (voltage, after current-to-voltage conversion) of the polarographic oxygen sensor?
- Why is it important to maintain an extremely constant temperature in and around the O2k-chamber?
- Does the POS respond to oxygen concentration, c_{O2} [µmol·dm^{-3} = µM], or partial oxygen pressure p_{O2} [kPa]? (#1.4.A)

POS calibration (O2k-Manual #1.O2k.D)

- How many calibration points are required for proper calibration of the polarographic oxygen sensor (POS)?
- During POS calibration, should the chamber be open or closed?
- What is an acceptable voltage (raw signal) of the POS at (a) air calibration, and (b) zero oxygen calibration, and how are these raw signals affected by the gain setting?
- Why should you check the raw voltage during calibration?
- The sensor voltage is above 9.9 V. What should I do?
- What does the stirrer test tell me?
- How do I perform a zero oxygen calibration?
- The oxygen solubility, S_{O2} [µM·kPa^{-1}], relates oxygen concentration to partial pressure. Which variables need to be considered for estimation of the oxygen solubility of an aqeous solution, for example of a respiration medium? (#1.4.A)
- When is the oxygen calibration of a POS preferentially performed?

- How long does it take approximately (5, 15, 30 or 45 min) to perform an oxygen calibration at air saturation, after the O2k is switched on (at experimental temperature in the range of 20 to 37 °C)?
- Do you need to consider the instrumental background when performing an oxygen calibration of the POS at zero oxygen concentration?
- Do you need to consider the instrumental background when performing an oxygen calibration of the POS at air saturation?
- Does the oxygen signal have to be stable for an oxygen calibration of the POS?
- How do you define POS signal stability? (#1.1.D)
- Do you have to perform a zero oxygen calibration of the POS before air calibration?
- Can you perform an oxygen calibration of the POS with biological sample and respiratory activity in the aqueous solution, when equilibration is performed with a gas phase in the chamber and stability of the signal is observed?
- What is the difference between static calibration (#1.O2k.D) and dynamic sensor calibration (#1.O2k.G; time constant – for advanced users)? How can I use a dynamic calibration (stirrer test) as a quick sensor test? (#1.O2k.G)

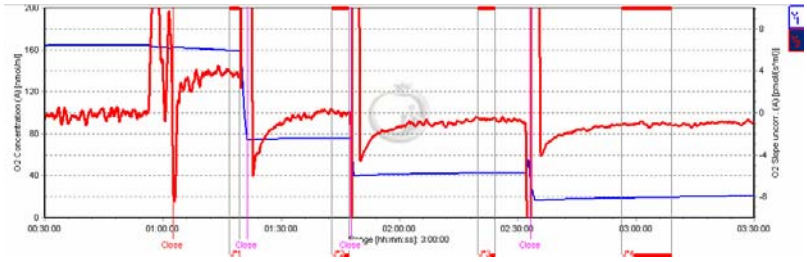
POS Service (O2k-Manual #1.O2k.B)

- What should I do if the sensor connector threads appear dark and dirty?
- The POS membrane box appears to have two types of membranes, which one should be applied to the sensor?
- How can I avoid creating bubbles when filling the electrolyte reservoir of the POS?
- Can I repeat the ammonia treatment?
- How can I check sensor performance?
- What precautions should be taken when handling the sensor connector?

Cleaning of the Chamber (O2k-Manual #1.5.C)

- Which solution should be placed in the chamber when the O2k is not in use (i.e. overnight, for a few days)?
- Can detergents be used to clean the chamber and the PVDF stoppers?
- What is the recommended cleaning procedure between experimental runs?
- The glass chambers appear to have surface residue. Can this be removed, what is the procedure?
- The stirring bar gets stuck. What can I do?

Instrumental background test (O2k-Manual #1.O2k.E)



- Does the oxygen signal have to be stable for setting a mark in an instrumental background test?
- Does the oxygen flux have to be constant for for setting a mark an instrumental background test?
- How do we define flux stability? Is a flat red line always an indication of a stable flux?
- Do I need to calibrate instrumental background flux at air saturation and zero oxygen concentration?
- Do I need to calibrate the POS before performing an instrumental background calibration?
- We use the symbol *a*° for the intercept at zero oxygen concentration, and the symbol *b*° for the slope of background oxygen flux as a function of oxygen concentration. In

the analysis of instrumental background, we have obtained 0.022 and -1.7. Which value is *a*° and *b*°?
- Does the background-corrected flux have to be zero when the oxygen signal is stable?
- How often do I have to check the instrumental background?

Literature

Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial function. In: Mitochondrial Dysfunction in Drug-Induced Toxicity (Dykens JA, Will Y, eds) John Wiley: 327-352. – *A methodological introduction into high-resolution respirometr, with focus on*
- Polarographic oxygen sensor and traditional oxygraphy
- High-resolution respirometry: The Oxygraph-2k
- Calibration of Polarographic Oxygen Sensors and Oxygen Concentration in Respiration Media at Air Saturation
- From Oxygraph Slopes to Respiratory Flux Corrected for Background Effects
- Phosphorylation control protocol with intact cells
- Titration Steps of the PC Protocol
- Experimental Example for the PC Protocol
- Flux Control Ratios from the PC Protocol
- Intact cells, permeabilized cells and tissue, or isolated mitochondria?

Gnaiger E (2009) Capacity of oxidative phosphorylation in human skeletal muscle. New perspectives of mitochondrial physiology. *Int. J. Biochem. Cell Biol.* doi:10.1016/j.biocel.2009.03.013
- Respirometry with permeabilized fibres and isolated mitochondria
- Convergent CI+II electron input and *OXPHOS* capacity
- Tissue-*OXPHOS* capacity in human permeabilized muscle fibres and isolated mitochondria
- Tissue-OXPHOS capacity and functional diversity

Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. *Respir. Physiol.* 128: 277-297. – *A detailed introduction into high-resolution respirrometry with particular emphasis on kinetics and measurements at low oxygen:*
- Mitochondrial kinetics measured by high-resolution respirometry
- Calibrations and corrections for response time and instrumental background
- Steady-state injection respirometry
- Mitochondrial respiratory control at low oxygen
- Apparent oxygen affinity and catalytic efficiency of mitochondrial respiration
- Effect of ADP and oxygen limitation on ADP/O2 flux ratios
- The low-oxygen environment of the cell: Mitochondria between hypoxic and oxidative stress

Gnaiger E, Kuznetsov AV, Schneeberger S, Seiler R, Brandacher G, Steurer W, Margreiter R (2000) Mitochondria in the cold. In: *Life in the Cold* (Heldmaier G, Klingenspor M, eds) Springer, Heidelberg, Berlin, New York: 431-442. – *Isolated mitochondria and permeabilized muscle fibers, MiR05*
- Optimization of mitochondrial cold storage
- Mitochondrial respiration medium, MiR05
- Mitochondrial cold ischemia-reperfusion injury

Renner K, Amberger A, Konwalinka G, Kofler R, Gnaiger E (2003) Changes of mitochondrial respiration, mitochondrial content and cell size after induction of apoptosis in leukemia cells. *Biochim. Biophys. Acta* 1642: 115-123. – *Intact cells, cytochrome c oxidase, cytochrome c test, respiration per million cells, per citrate synthase, per mg protein, or per cytochrome c oxidase activity*

Further information: Introductory course material is available on our homepage www.oroboros.at, with the following sections:
1. Oxygraph-2k and Manual
2. MiPNet Protocols - www.oroboros.at/index.php?o2k-protocols
3. O2k-Publications
4. WorldWide Mitochondrial Physiology Network

Accomodation and Location

Hotel Mohnenfluh www.mohnenfluh.at; Tel.: +43 5519 2031; hotel@mohnenfluh.at. The course takes place at Hotel Mohnenfluh, including accomodation for all participants with breakfast, meals and coffee breaks.

Participants and Areas of Interest

Achten Jelle, Department of Clinical Genetics, Maastricht University Hospital, Maastricht, The Netherlands. – jelle.achten@gen.unimaas.nl *(Inborn errors of metabolism; diagnosis of defects in oxidative phosphorylation)*

Bierau Jorgen Ph.D, Laboratory of Biochemical Genetics, Department of Clinical Genetics, Maastricht University Hospital, Maastricht, The Netherlands. – jorgen.bierau@gen.unimaas.nl *(Inborn errors of metabolism; diagnosis of defects in oxidative phosphorylation)*

Castelein Natascha, PhD Student, Department of Biology, Ghent University, Ghent, Belgium. - natascha.castelein@ugent.be *(Research on mitochondrial function in aging C. elegans; metabolism, mitochondrial function)*

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Doblander-Gruber Christine, Mag.Dr., D. Swarovski Research Laboratory, Dept. General Transplant Surgery, Medical University Innsbruck, Austria. - christine.doblander@i-med.ac.at *(respiration, Crabtree effect, hypoxia, oxygen sensing and ROS production in cancer cells)*

Fasching Angelica, PhD, Department of Medical Cell Biology, Division of Integrative Physiology, Uppsala University, Uppsala, Sweden. – angelica.fasching@mcb.uu.se *(Diabetes, oxygen handling, kidney, mitochondrial function in the diabetic kidney)*

Fasching Mario, PhD, OROBOROS INSTRUMENTS, Innsbruck, Austria. - mario.fasching@oroboros.at *(Lecturer, tutor)*

Fazeli Walid, Dr., Sektion Neonatologie und Pädiatrische Intensivmedizin, Universitätsklinikum Eppendorf (UKE), Hamburg, Germany. – walid.fazeli@gmx.de *(Onset and suppression of metabolic size allometry in neonatal mammals. Respiratory capacity of (isolated) mitochondria in the context of fatty acid oxidation deficiency)*

Fernández Iglesias Anabel, Departament de Bioquímica i Biotecnologia, Universitat Rovira i Virgili, Campus Sescelades, Tarragona, Spain. - anabel.fernandez@urv.cat *(Oxydative stress)*

Friederich Malou, PhD student, M.Sc., Department of Medical Cell Biology, Division of Integrative Physiology, Uppsala University, Uppsala, Sweden. – malou.friederich@mcb.uu.se *(Diabetes, oxygen handling, kidney, mitochondrial function in the diabetic kidney)*

Gnaiger Erich, PhD, Prof., D. Swarovski Research Laboratory, Dept. General Transplant Surgery, Medical University Innsbruck; and OROBOROS INSTRUMENTS; Austria. – erich.gnaiger@oroboros.at *(Lecturer, tutor)*

Haberberger Birgit, PhD Student, Institut für Humangenetik, Helmholtz Zentrum München, Neuherberg, Germany. - birgit.haberberger@helmholtz-muenchen.de *(Respiratory chain activity measurements and complex deficiencies in patients with mitochondriopathy; cell line: fibroblasts, mutations, biochemical activity]*

Hagve Martin, Department of Medical Physiology, Institute of Medical Biology, University, of Tromsø, Tromsø, Norway. – martin.hagve@gmail.com *(heart metabolism, free fatty acids, oxygen consumption, uncoupling; measurement of oxygen consumption from T2D animals (mainly heart mitochondria but also skeletal muscle mitochondria); mitochondria exposed to free fatty acids.*

Hempenstall Sarah, PhD Student, Zoology, School of Biological Sciences, Aberdeen, Scotland, UK. – sarah.hempenstall@abdn.ac.uk *(Caloric*

restriction, autophagic response, ROS production, mitochondrial respiration, JNK, FOXO; investigating the underlying mechanisms of lifespan extension in male C57BL/6 mice exposed to early- and midlife caloric restriction)

Kaiser Sabine, Sanofi-Aventis Deutschland GmbH, Hottersheim, Germany. – sabine.kaiser@sanofi-aventis.com

Kane Dan, MSc, East Carolina University, Brody School of Medicine, Department of Exercise and Sport Science & Department of Physiology, East Carolina, Greenville, USA. - DAK1021@ecu.edu (*Coupled* guest tutor*)

Krohne Christian, PhD Student, Institut für Anästhesiologie & Operative Intensivmedizin, Klinikum Mannheim, Mannheim, Germany. - christian.krohne@medma.uni-heidelberg.de *(MURF1-dependent regulation of carbohydrate metabolism as revealed from transgenic mouse studies; Diabetes, muscle glycolysis, pyruvate dehydrogenase)*

Larsen Filip, GIH, Stockholm University, College of P.E. and Sports, Stockholm, Sweden. - filip.larsen@ki.se *(Regulation of mitochondrial respiration by nitrite/nitric oxide]*

Lazarescu Adrian, University of Medicine and Pharmacy, Timisoara, Romania. - fiziopatologie@umft.ro *(Involvement of mitochondria in ischemia/reperfusion injury of the heart and cardioprotective strategies; ischemic and pharmacological pre/postconditioning, physical activity; skeletal muscle adaption to exercise)*

Leloup Corinne, PhD, Université Paul Sabatier, Toulouse , France. - leloup@cict.fr *(Mitochondrial superoxide anion production depending on nutritional status and metabolic fluxes in the hypothalamus; rat; brain metabolism, hypothalamus, mitochondria, ROS, mitochondrial dynamics, fission, fusion)*

Pesta Dominik, Mag., D. Swarovski Research Laboratory, Dept. General Transplant Surgery, Medical University Innsbruck; and OROBOROS INSTRUMENTS; Austria. – dominik.pesta@student.uibk.ac.at *(exercise and mitochondrial respiratory function in permeabilized muscle fibers)*

Pontoglio Alessandro, PhD student, Istituto di Biochimica e Biochimica Clinica, Policlinico A. Gemelli - Università Cattolica, Roma, Italy. - espeedy@libero.it *(re-evaluation of Warburg effect on cancer cells]*

Perkhofer Susanne, Dr., Inst. Hygiene, Mikrobiologie und Sozialmedizin, Medical University Innsbruck, Austria. - susanne.perkhofer@i-med.ac.at *(Influence of human platelets on mitochondrial activity of Aspergillus gumigates)*

Renner Kathrin, Mag.Dr., Regensburg, Germany. – kathrin.renner@oroboros.at (*Tutor*)

Schmidtke Peter, Dr., Sektion Neonatologie und Pädiatrische Intensivmedizin, Universitätsklinikum Eppendorf (UKE), Hamburg, Germany. – peter.schmidtke@gmail.com *(Onset and suppression of metabolic size allometry in neonatal mammals)*

Sartori Bettina, Inst. Hygiene, Mikrobiologie und Sozialmedizin, Medical University Innsbruck, Austria. - bettina.sartori@i-med.ac.at *(Influence of human platelets on mitochondrial activity of Aspergillus gumigates)*

Thouas George, PHD, Faculty of Engineering, Monash University, Clayton Victoria, Australia. - george.thouas@eng.monash.edu.au

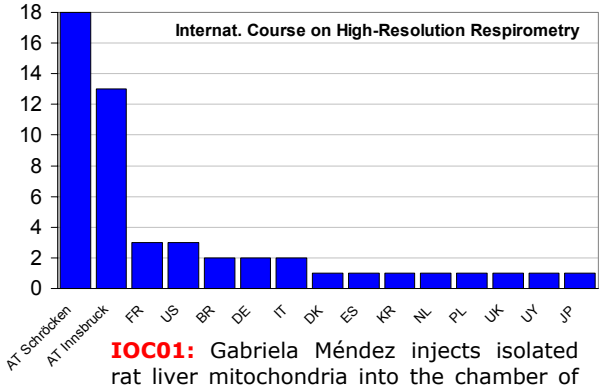
Tweedie Constance, MSc, Faculty of Medicine, University of Calgary, Calgary, Canada. - cltweedi@ucalgary.ca (*Coupled* guest tutor*)

Vienne Jean-Claude, Dr., Technicien de laboratoire, Centre de Biologie-Pathologie, Pôle de Biochimie-Biologie Moléculaire, CHRU de Lille, France. - jc-vienne@chru-lille.fr *(1. Diagnostic of mitochondrial cytopathies on permeabilized muscle fibers and cells; pediatric samples. 2. Collaborative research programmes on the mitochondria: Mitochondrial cytophathies, OXPHOS, metabolism, respirometry)*

Wang Xiaoyang, M.D. Ph.D., Department of Physiology, Gothenburg University, Gothenburg, Sweden. – xiaoyang.wang@fysiologi.gu.se

Wessels Bart, PhD Student, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands. - b.wessels@tue.nl *(Mitochondrial function in type 2 diabetes, onset of insulin restistance, Use of ^{31}P-NMR spectroscopy combined with in vitro mitochondrial respiration measurements)*

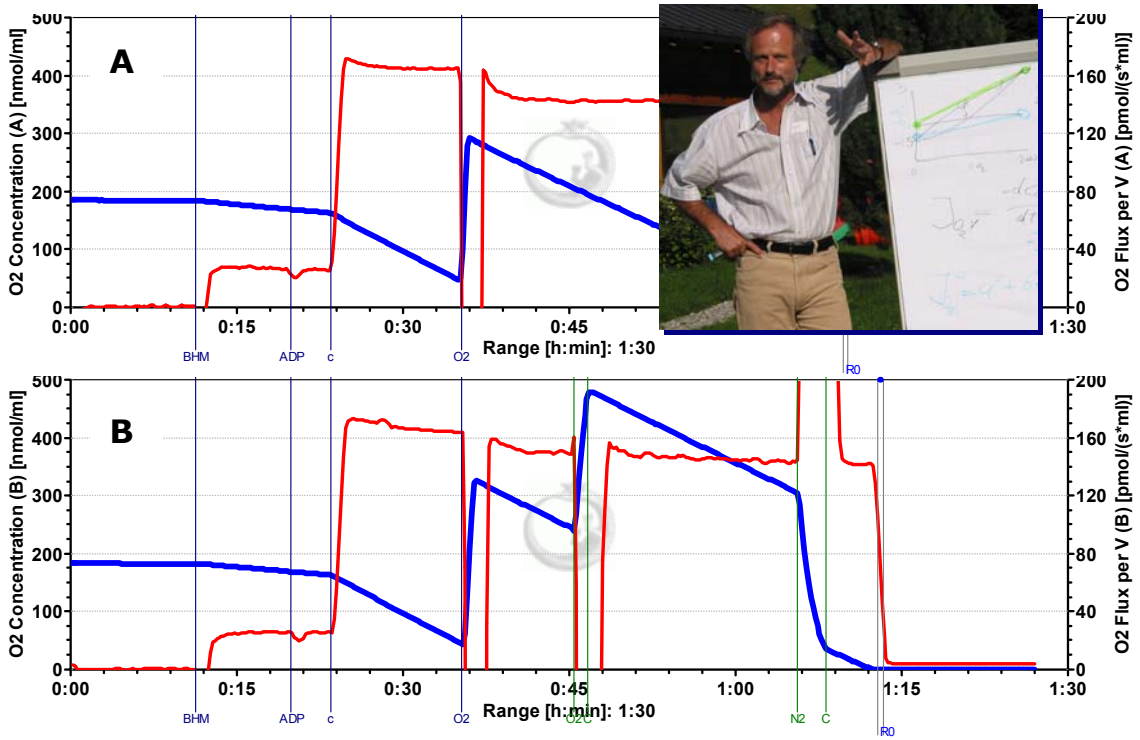
50 Courses on High-Resolution Respirometry 1991-2009



IOC01: Gabriela Méndez injects isolated rat liver mitochondria into the chamber of the Oroboros Oxygraph (O2P, Paar, Graz) at the International Summer Course, Innsbruck 1991.

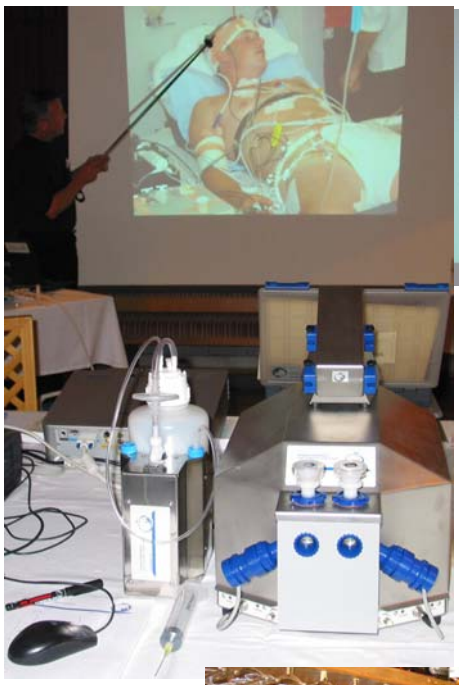

Salober, April 2007


IOC41, July 2007



IOC40 – San Diego, USA. OROBOROS Oxygraph-2k (2007-06-12). Left chamber (A) and right chamber (B), with simultaneous display of **oxygen concentration** (thick blue lines) and **oxygen flux** (respiratory rate, thin red lines; negative time derivative of oxygen concentration). Isolated beaf heart mitochondria (BHM).

IOC36-participants and high-jump on the way from Salober/lake Kalbelesee to lake Körbersee, the peak of Widderstein in the background.





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Cooperation and Feedback in Science