IOC46. *Mitochondrial Physiology Network* 13.2: 1-12 (2008)

46th International Course on High-Resolution Respirometry

4-8 April 2008

Schröcken, Vorarlberg, Austria

The 46th O2k-Course is the 15th presentation of high-resolution respirometry 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 4.2** (new update), application of the **TIP-2k** and discussion of developments of **MultiSensor modules** 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 high-resolution respirometry. Five Oxygraph-2k, three TIP-2k and several PCs are available for a do-it-yourself application of both hardware and software.

During lunch breaks, sufficient time is available for relaxing walks and talks, skiing and snow shoe walking, to enjoy the refreshing scenery of the alpine environment with excellent show-conditions in April, or use the spare time for specific tutorials.

With DatLab 4.2 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

Med. Univ. Innsbruck, Dept. General Transplant Surgery, D. Swarovski Res: Lab., Innsbruck; and OROBOROS INST., Austria

- **Mario Fasching,** PhD, mario.fasching@oroboros.at
- **Erich Gnaiger**, PhD, erich.gnaiger@i-med.ac.at
- **Simone Köfler**, Mag, simone.koefler@oroboros.at (*admin.*)
- **Hélène Lemieux**, PhD, helene.lemieux@oroboros.at
- **Francesca Scandurra**, PhD, francesca.scandurra@oroboros.at
- **Patrick Subarsky**, PhD, patrick.subarsky@oroboros.at

Guest lecturers

- **Pierre Blier** (Rimouski, CA)
- **John Boyle** (Leeds, UK)
- **Charles Darveaux** (Toledo, CA)
- **Christophe Rocher** (Bordeaux, FR)
- **Colin Selman** (Aberdeen, UK)
- **Russell Swerdlow** (Kansas, US)
- **Nathalia Timokhina, Kersti Tepp** (Tallinn, EE)
- **Demidmaa Tuvdendorj** (Galveston, US)

Programme IOC46

Friday, 4. April

16:15 Participants arriving in Bregenz: Meeting point at Bregenz train station, 1.1 hour drive to Schröcken. Check in at Hotel Mohnenfluh.

18:30 **Welcome reception** – Introduction of participants

19:00 Dinner

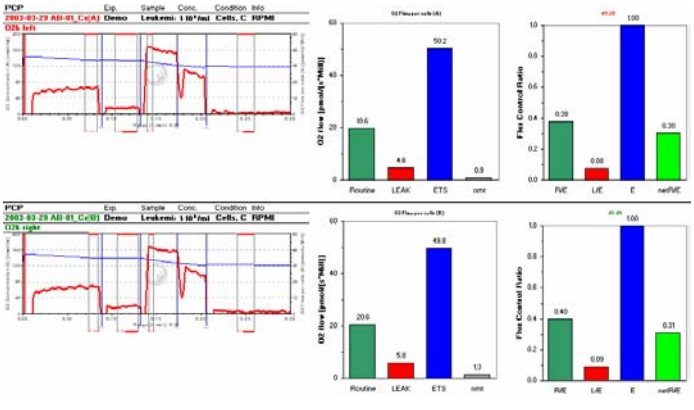
21:00 **MiPNet Session 1**

Erich Gnaiger (Innsbruck, AT) O2k-Courses in the spirit of OROBOROS.

21:15 (10+5) **Pierre Blier** (Rimouski, CA) Mussels and sex: a mitochondrial tale.

21:30 (7+3) **Colin Selman** (Aberdeen, UK) Lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice.

21:40 (7+3) **Nathalia Timokhina, Kersti Tepp** (Tallinn, EE) Steady state kinetics of regulation of respiration in permeabilized cardiac cells in situ: Evidence for local restrictions of ATP and ADP diffusion across outer mitochondrial membrane and for central role of mtCK.



Day 2: Saturday, 5. April

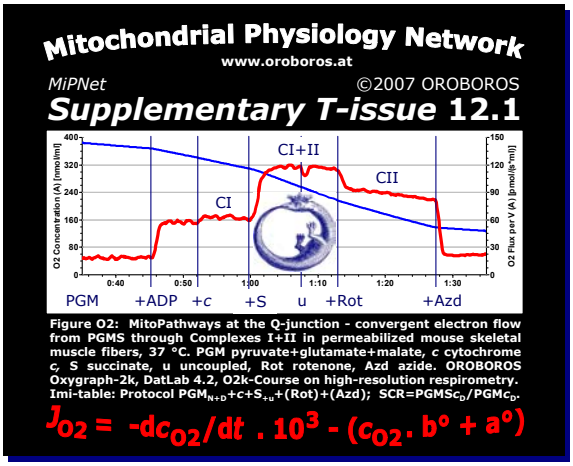
08:30 Erich Gnaiger (Innsbruck, AT)

Electron transport system (*ETS*), *OXPHOS* capacity and *LEAK* respiration: An experimental protocol for high-resolution respirometry.

09:15 - 11:45 **Principles of high-resolution respirometry - from switching on the Oxygraph-2k to the experimental result (demo experiment).**

- Oxygraph-2k demo experiment with DatLab 4.2.
- Oxygen calibration of the polarographic oxygen sensors (POS).
- Preparation of permeabilized muscle fibers.
- Determination of fiber wet weight with METTLER TOLEDO microbalance XS205DU (1 to 3 mg per chamber).
- Addition of muscle fibers, closing the chamber.
- Demo experiment: Multiple substrate-uncoupler-inhibitor titration and on-line DatLab analysis.

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



16:15 -18:45 **Parallel group sessions 1:**
Hands-on with the Oxygraph-2k (four instruments - eight parallel chambers): Preparation of permeabilized skeletal muscle fibers; OXPHOS titration protocols. On-line DatLab analysis.

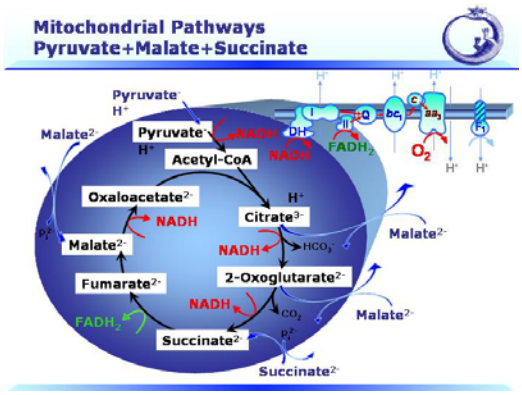
19:00 Dinner

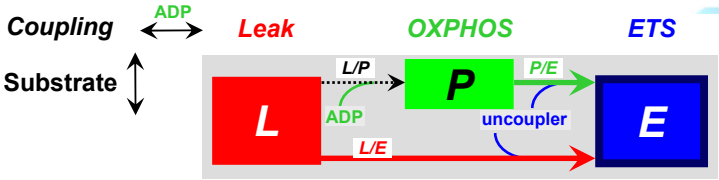
21:00 **Hot topics in Mitochondrial Physiology: MiPNet Session 2.**

21:00 (7+3) **Demidmaa Tuvdendorj** (Galveston, US) Skeletal muscle mitochondrial fat oxidation in burn injury. Effect of peroxisome proliferator-activated receptor alpha agonist fenofibrate on mitochondrial capacity to oxidize fatty acids.

21:10 **Hélène Lemieux, Patrick Subarsky, Erich Gnaiger** (Innsbruck, AT):

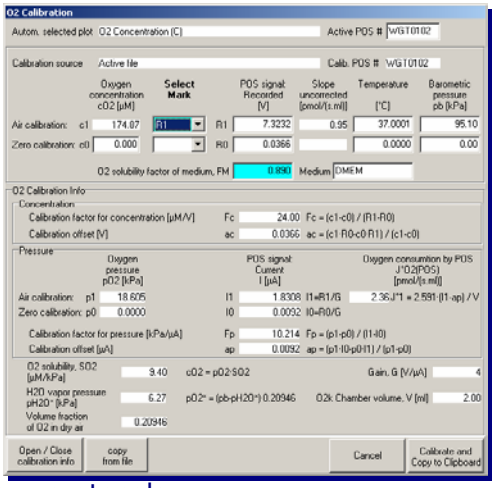
High-resolution respirometry and OXPHOS titration protocols: Flux Control Ratios in permeabilized fibers and cultured cells.





Discussion of results of the demo experiment.

Day 3: Sunday, 6. April



08:30 - 11:45 **Parallel group sessions 2:** Hands-on experiments with the Oxygraph-2k - instrumental performance: O2k-calibration and background, DatLab analysis.

12:00 – 16:00 Lunch break, skiing: bus leaves at 12:14

16:15 - 18:45 **Parallel group sessions 3:** High-resolution respirometry and DatLab 4; Instrumental setup and service.
DatLab 4 Analysis and respiratory protocols.

19:00 Dinner

Hot topics in Mitochondrial Physiology: MiPNet Session 3.

21:00 (7+3) **Russell Swerdlow** (Kansas, US) Cybrid modeling of human mitochondriopathies.

21:10 (7+3) **Christophe Rocher** (Bordeaux, FR) Influence of mitochondrial DNA level on cellular energy metabolism: implications for mitochondrial diseases.

21:20 (20+10) **John Boyle** (Leeds, UK) Hypoxia-induced reactive oxygen species generation as a cell signalling event.

Day 4 (Monday, 7. April)

08:30 - 11:45 **Parellel group sessions 4:** High-resolution respirometry and DatLab 4; Instrumental setup, POS service.
TIP-2k titration and injection, feedback control and steady-state.

12:00 - 17:00 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

17:30 -18:30 **Parallel group sessions 5**
Open topics: Problems and solutions. Special interest groups: Inhibitor titration and flux control / Experimental regimes / Oxygen kinetics.

19:00 Dinner

21:00 (30+10) **Charles Darveau** (Ottawa, CA): Diversity and evolution of animal energetics.

21:40 ***Discussion - Summary – Conclusions***

Tuesday, 8. April

Departure to Bregenz and Innsbruck

CONTENTS: OVERVIEW ON HIGH-RESOLUTION RESPIROMETRY

Introduction: Mitochondrial and cellular respiratory physiology – new challenges for high instrumental performance.

High-resolution respirometry – what makes the difference? Presentation of the OROBOROS Oxygraph-2k

- Low oxygen and measurement of cellular oxygen consumption – pushing the limits of detection.
- Optimum system design - the OROBOROS Oxygraph-2k.
- DatLab 4.2: on-line recording of oxygen concentration and flux; linear slope versus oxygen flux as a function of time.
- DatLab 4.2: the specialized software for high-resolution respirometry; high-resolution calibrations.

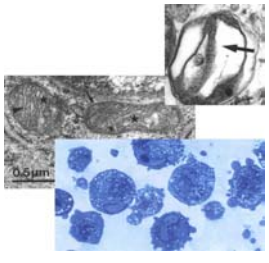
OROBOROS Oxygraph-2k and TIP-2k: On-line instrumental performance

- Instrumental background: measurement and correction as a function of pO_2.
- High resolution of respiratory flux at various steady-states.
- The Titration-Injection microPump TIP-2k: automatic titrations.
- Conceptual and methodological advantages of measurement at physiological low levels of oxygen.
- High time resolution for kinetic analyses: Determination of the time constant, dynamic corrections.

Polarographic oxygen sensor (POS) and O2k service

- Cleaning of anode and cathode.
- Electrolyte and membrane application.
- Oxygraph-2k and TIP-2k: instrumental maintenance.

Protocols for the O2k Demo Experiment



Gnaiger E, ed (2007) *Mitochondrial Pathways and Respiratory Control*. OROBOROS MiPNet Publications, Innsbruck: 96 pp. Electronic 1st ed ISBN 978-3-9502399-0-4 – www.oroboros.at

IOC 46

Accomodation and Location

Hotel Mohnenfluh www.mohnenfluh.at; Tel.: +43 5519 203; hotel@mohnenfluh.at. The course takes place at Hotel Mohnenfluh, including accomodation for all participants with breakfast, meals and coffee breaks.

Skiing

Warth-Schröcken - http://www.snowworld.at/.
The skiing area Salober (Schröcken and Warth) is reached by a free bus service, leaving at 11:44, 12:14 and 12:44 at Hotel Mohnenfluh. There is also excellent crosscountry skiing around lakes Kalbelesee and Körbersee, as well as easy walking in magnificent winter scenery. Ski rental is available in Schröcken and at the skiing lift Salober. You can return to Schröcken on skies or by the free bus (leaving 14:59, 15:29 and 15:59 at Salober).

Weather Snowfall and sub-freezing temperatures are expected in December. Sunshine may be strong – bring sunglasses and sunscreen, even if you do not plan to go skiing. Protect yourself against wind and potential snowfall or rain (gloves, jacket, etc.).

Further information Introductory course material is available on our homepage www.oroboros.at.

Contact

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OROBOROS INSTRUMENTS
high-resolution respirometry
Oxygraph-2k

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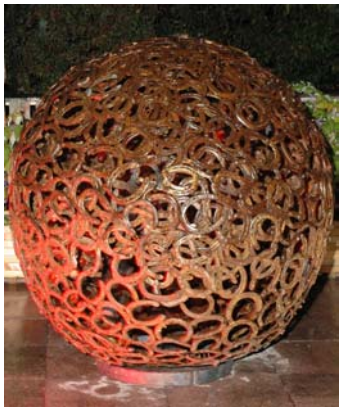
Cooperation and Feedback in Science

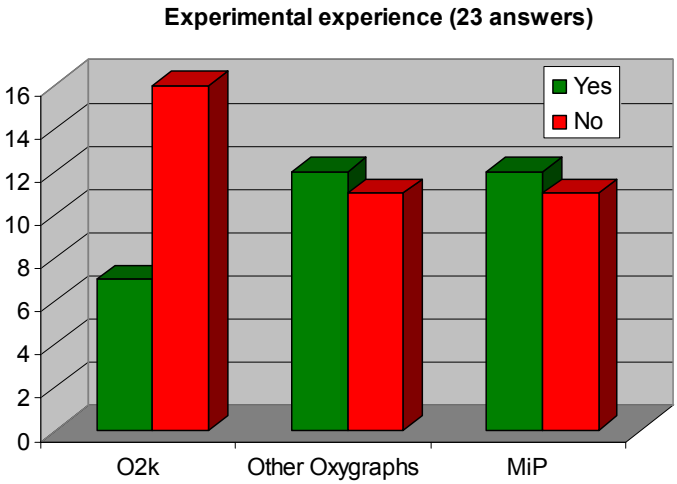


Friday, 18. April

OROBOROS *MiPArt*

'an open door'

Apr. 18 - 18:18 - Schöpfstr. 18



Participants and Areas of Interest

Blier Pierre, PhD Prof., Département de Biologie Chimie et des Sciences de la Santé, Université du Québec à Rimouski, Rimouski, Qc, Canada. - pierre_blier@uqar.qc.ca
Thermal sensitivity of mitochondrial metabolism and the impact of the evolution of mtDNA on the functional properties of mitochondria (evolution, ectotherms, cytochrome oxidase, excess, PDH, fish, mussels, ROS, adaptive selection).

Boyle John, Dr., Division of Cardiovascular and Neuronal Remodelling, Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, UK. - j.p.boyle@leeds.ac.uk
Hypoxia-induced reactive oxygen species generation as a cell signalling event and its possible role in neurodegenerative disease (astrocytes, neurones, acute hypoxia, chronic hypoxia, ROS, Alzheimer's disease, Parkinson's disease).

Clarke Kieren J, Department of Biochemistry, Trinity College of Dublin, Dublin, Ireland. – clarkekj@tcd.ie
The role of UCP's in mitochondrial function (mitochondria, UCP's).

Darveau Charles-A., Dr., Assistant Professor, Dept of Biology, University of Ottawa, Ottawa, Ontario, Canada. - cdarveau@uottawa.ca
Evolution of animal energetics. I study diversity and evolution of metabolic rate during flight in insects, and the underlying mechanisms at various levels of organisation, including flight muscle metabolism and biochemistry (metabolism, evolution, flight, insects, biomechanics, wing morphology, enzymes, mitochondria, phylogeny, comparative and evolutionary physiology).

Tuvdendorj Demidmaa, PhD, Department of Surgery, Shriners Burn Hospital, Galveston, Texas, USA. - detuvden@utmb.edu
Muscle mitochondrial beta-oxidation pathway in burn related injury (burn, beta-oxidation, mitochondria).

De Palma Clara, Dr., LITA-Università degli studi di Milano, Lab Farmacologia Cellulare, Milan, Italy. – clara.depalma@guest.unimi.it
Evaluation of the difference in mitochondria metabolism between wt and dystrophic mice and role of NO-donor (nitric oxide, mitochondria, muscle fibers).

Ehinger Johannes, PhD Student, Lund University, Laboratory for Experimental Brain Research, Wallenberg Neuroscience Center, Lund, Sweden. - johannes@ehinger.net
Will be working in Dr. Elmérs group in Lund, on mitochondrial function in sepsis (platelets), status epilepticus (brain homogenates and isolated mito), cell culture (fibroblasts from children metabolic disease and glial cell lines) - also muscle biopsies from children (brain, calcium, permeability transition, sepsis, human, multiple titration, TIP, calcium electrode, pH electrode, muscle biopsy, fibroblast, status epilepticus).

Fisar Zdenek, Dr., Charles University, Department of Psychiatry 1st Faculty of Medicine, Prague. - zfisar@lf1.cuni.cz

Effects of mental disorders and psychotropic drugs on mitochondrial functions (synaptosomes; mitochondria; platelets; lymphocytes; depression; antidepressants; antipsychotics, drug abuse; respiratory chain; membrane potential; nitric oxide; reactive oxygen substances; apoptosis).

Gómez Durán Aurora, Dpto Bioquímica, Biología Molecular y Celular, University of Zaragoza, Zaragoza, Spain. – auroragd@unizar.es
Characterization of mtDNA's poblational variants (mtDNA, haplogroups, cybrids, OXPHOS).

Kim Miseon, Sookmyung Women's University, Seoul, Republic of Korea. - erate@sookmyung.ac.kr
Mitochondrial RCR and uncoupling phenomenon in iron overloaded mitochondria of heart (iron, heart, mitochondria).

Koziel Rafal, Dr., Austrian Academy of Sciences, Institute for Biomedical Aging Research, Innsbruck, Austria. - rafko@necki.gov.pl

Latini Alexandra, Prof. Dr., Dept. Biochemistry, Center of Biological Sciences, UFSC, Universidade Federal de Santa Catarina, Campus Universitario Trindede, Florionopolis - SC, Brazil. - alatini@ccb.ufsc.br
Investigation of the mitochondrial function in experimental models of toxicity, (1) to get a fundamental introduction into the technical details of high-resolution respirometry for mitochondrial, cellular and tissues studies; (2) to perform experiments to optimize a multiple-substrate respiratory protocol for application to brain homogenate or a brain permeabilized tissue preparation; (3) to apply the 'Q-junction protocol' for studing brain mitochondrial respiration.

Leadsham Jane, Dr., Dept. of Biosciences, University of Kent, Canterbury, Kent, UK. - j.e.leadsham@kent.ac.uk
Investigating the generation of ROS and mitochondrial dysfunction in relation to the onset of apoptosis in *Saccharomyces cerevisae*. Inappropriate activation of the RAS/cAMP/PKA pathway in *S. cerevisiae* leads to actin aggregation and mitochondrial generated ROS, resulting in apoptotic cell death (apoptosis, ROS, actin aggregation).

Morota Saori, Ph.D., Lund University, Laboratory for Experimenta. Brain Research, Wallenberg Neuroscience Center, Lund, Sweden. - saori.morota@med.lu.se
Will be working in Dr. Elmérs group in Lund, on mitochondrial function in sepsis (platelets), status epilepticus (brain homogenates and isolated mito), cell culture (fibroblasts from children metabolic disease and glial cell lines) - also muscle biopsies from children (brain, calcium, permeability transition, sepsis, human, multiple titration, TIP, calcium electrode, pH electrode, muscle biopsy, fibroblast, status epilepticus).

Onyango Isaac, University of Kansas School of Medicine, Landon Center on Aging, Kansas City, KS, USA. - ionyango@kumc.edu
Mitochondria in aging and neurodegeneration (cybrids, electron transport system, neurodegenerative diseases, aging).

Paju Kalju, Dr. Assoc. Prof., Faculty of Medicine, Pathophysiology, University of Tartu, Estonia. - kalju.paju(at)ut.ee
Expression and function of creatine kinase, adenylate kinase and hexokinase in relation to their role in regulation of oxidative phosphorylation and intracellular energy transfer in myocardium and cultured cardiac HL-1 cells (OXPHOS, energy transfer, heart, muscle cells, HL-1 cells, ATPases).

Pertuiset Claire, INSERM U688, Université Victor Segalen-Bordeaux, Bordeaux, France. – claire.pertuiset@u-bordeaux2.fr

Mitochondrial physiopathology (bioenergetics, mitochindria, ADNmt).

Rocher Christophe, Dr., INSERM U688, Université Victor Segalen-Bordeaux, Bordeaux, France. - christophe.rocher@u-bordeaux2.fr

Schmidtke Peter, Sektion Neonatologie und Pädiatrische Intensivmedizin, Zentrum Frauen-, Kinder- und Jugendmedizin, Universitätsklinikum Eppendorf (UKE), Hamburg, Germany. - peter.schmidtke@gmail.com
Onset and suppression of metabolic size allometry (after birth and in response to hypoxia) in neonatal mammals – a regulatory role of varying oxygen tensions on tissue respiration?

Segales Jessica, PhD Student, Institute for Research in Biomedicine, University of Barcelona, Barcelona, Spain. - jessica.segales@irbbarcelona.org
Role of mitofusin-2 in mitochondrial metabolism.

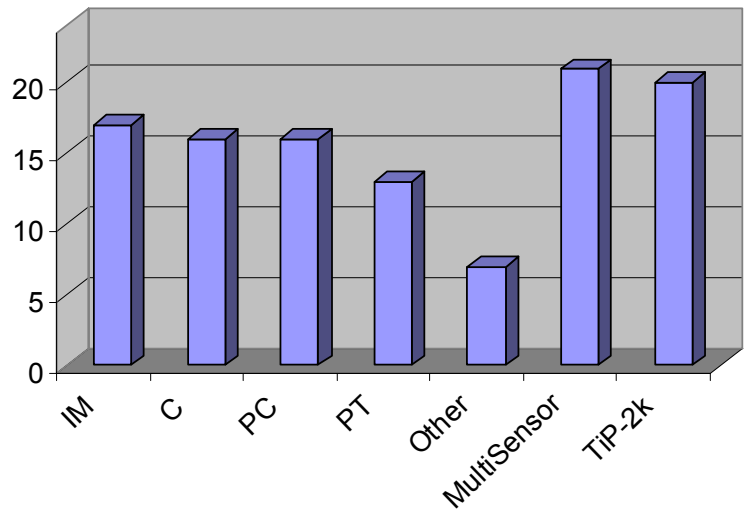
Selman Colin, Dr., Integrative Physiology, School of Biological Sciences, University of Aberdeen, Aberdeen, UK. - c.selman@abdn.ac.uk
The effects of caloric restriction and intermittent feeding on mitochondrial function and ROS production (ageing, oxidative stress, reactive oxygen species, autophagy).

Swerdlow H. Russell, MD, Professor of Neurology, Molecular and Integrative Physiology, University of Kansas School of Medicine, Landon Center on Aging, Kansas City, KS, USA. - rswerdlow@kumc.edu
Mitochondria in aging and neurodegeneration (cybrids, electron transport system, neurodegenerative diseases, aging).

Tepp Kersti, Phd Student, Laboratory of Bioenergetics, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia. - kersti.tepp@kbfi.ee
Possible role of cytoskeletonin in intracellular arrangement and regulation of mitochondrial respiration (ICEU, mitochondria, creatine kinase, ATPases).

Timokhina Natalja, Phd Student, Laboratory of Bioenergetics, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia. - ovetshko@kbfi.ee
Molecular system bioenergetics in heart cells (regulation of respiration, cardiomyocytes, creatine kinase).

Application interests (22 answers)



Model systems:

Isolated Mitochondria	IM
Intact cell	C
Permeabilized cells	PC
Permeabilized tissue	PT

Hot topics in Mitochondrial Physiology – MiPNet Abstracts

MiPNet 1. Cybrid modeling of human mitochondriopathies

Russell H. Swerdlow, Isaac G. Onyango
University of Kansas, Kansas City, USA

For non-Mendelian disorders in which systemic mitochondrial dysfunction occurs, mitochondrial DNA (mtDNA) may contribute to the underlying mitochondriopathy. The cytoplasmic hybrid (cybrid) technique allows investigators to screen the functional integrity of mtDNA in candidate diseases. In cases where mtDNA functional integrity is compromised, cybrid cell lines facilitate studies of downstream functional consequences. The cybrid approach was previously used to model mitochondrial dysfunction in a number of maternally inherited encephalomyopathy disorders and several sporadic neurodegenerative diseases, and has provided insight into mitochondrial genetics, physiology, and disease relevance.

MiPNet 2. Influence of mitochondrial DNA level on cellular energy metabolism: Implications for mitochondrial diseases

Christophe Rocher[1], Jan-Willem Taanman[2], Denis Pierron[1], Benjamin Faustin[1], Giovani Benard[1], Rodrigue Rossignol[1], Monique Malgat[1], Laurence Pedespan[3], Thierry Letellier[1*]
[1]U688 – Univ Victor Segalen-Bordeaux 2, Bordeaux-cedex, France, [2]Univ Dept Clinical Neurosciences, Royal Free and Univ Coll Med School, Univ College London, London, United Kingdom; [3]Hôpital Pellegrin enfants, Bordeaux-Cedex, France

The total amount of cellular mitochondrial DNA (mtDNA) varies widely and seems to be related to the nature and metabolic state of tissues and cells in culture. It is not known, however, whether this variation has any significance in vivo, and to which extent it regulates energy production. To better understand the importance of the cellular mtDNA level, we studied the influence of a gradual reduction of mtDNA copy number on oxidative phosphorylation in two models: i) a control human cell line treated with different concentrations of 2', 3'-dideoxycytidine, a nucleoside analogue that inhibits mtDNA replication by interfering with mitochondrial DNA polymerase γ, and ii) a cell line derived from a patient presenting mtDNA depletion. The two models were used to construct biochemical and phenotypic threshold curves. Our results show that oxidative phosphorylation activities are under a tight control by the amount of mtDNA in the cell, and that the full complement of mtDNA molecules are necessary to maintain a normal energy production level.

MiPNet 3. Steady state kinetics of regulation of respiration in permeabilized cardiac cells in situ: Evidence for local restrictions of ATP and ADP diffusion across outer mitochondrial membrane and for central role of MTCK

Kersti Tepp, Nathalia Timokhina
Laboratory of Bioenergetics, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia

Mitochondrial ATP production and fine regulation of energy fluxes are of central importance for normal cell functioning, especially for cells of tissues with high energy demand, such as the heart, brain, skeletal and smooth muscle. The purpose of our work was to perform steady state kinetic analysis of regulation of mitochondrial respiration in permeabilized cardiomyocytes to quantitatively evaluate the role of mitochondrial outer membrane (MOM) in the restriction of diffusion of ADP in the cells, and the complete kinetic description of the role mitochondrial creatine kinase (MtCK) in regulation of respiration in the cells in situ, necessary for mathematical modelling of energy

metabolism in the cells. Complete kinetic analysis of the MtCK reaction in permeabilized cardiac cells in situ was performed in the presence of powerful ADP trapping system consisting of pyruvate kinase (PK) and phosphoenol pyruvate (PEP) to exclude influence of free extramitochondrial ADP produced by MgATPases. Complete kinetic analysis of the MtCK - activated respiration showed striking differences between mitochondria in situ and in vitro. Remarkably, the apparent dissociation constants of MgATP from its binary and ternary complexes with MtCK, Kia and Ka, respectively, were increased by several orders of magnitude in comparison with the values of these constants in vitro, showing very much decreased apparent affinities for this substrate in the medium in situ due to diffusion restrictions at the MOM level. This strongly increases the effective compartmentation of ATP and ADP in the intermembrane space of mitochondria in situ. Interestingly, the apparent kinetic constants for creatine were significantly decreased in situ, showing that this substrate is a very effective regulator of respiration in the cells in vivo. (Key words: respiration, cardiomyocytes, mitochondria, creatine kinase, creatine).

MiPNet 4. **Lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice.**

Colin Selman
Integrative Physiology, School of Biological Sciences, University of Aberdeen, Scotland, UK

Ageing research has been revolutionized during the last few years with the use of model organisms where genetic alterations can extend lifespan. For example, in several mouse mutants, impairment of the growth hormone (GH)/IGF1 axis increases life span and insulin sensitivity. In addition, single gene mutations in the insulin and insulin-like growth-factor signalling pathways increase lifespan in worms, flies and mice and have recently been correlated with long-life in humans. This implies an evolutionary conservation of mechanisms. We recently demonstrated that female Irs1-/- mice are long-lived and display resistance to a range of age-sensitive markers of aging including skin, bone, immune, and motor dysfunction. These improvements in health were seen despite mild, lifelong insulin resistance. Irs1-/- female mice also displayed normal anterior pituitary function, distinguishing them from long-lived somatotrophic axis mutants. In addition, whole-mouse genome microarrays identified many mitochondrial gene ontology categories significantly up-regulated relative to control mice. In contrast, Irs2-/- mice were short-lived, whereas Irs1+/- and Irs2+/- mice of both sexes showed normal life spans. Our results therefore suggest that IRS1 signalling is an evolutionarily conserved pathway regulating mammalian life span and may be a point of intervention for therapies with the potential to delay age-related processes.

MiPNet 5. **Skeletal muscle mitochondrial fat oxidation in burn injury. Effect of peroxisome proliferator-activated receptor alpha agonist fenofibrate on mitochondrial capacity to oxidize fatty acids.**

Tuvdendorj Demidmaa[1], Aarsland A[1,2]
[1]Metabolism Unit, Department of Surgery, Shriners Children Hospital, [2]Department of Anesthesiology, The University of Texas Medical Branch, Galveston, TX, USA.

Severe burn injury results in hyperglycemia and insulin resistance (1). Retrospective analyses have indicated that hyperglycemia correlates with higher mortality rate and increased graft loss and sepsis. The insulin resistance in burn patients affects multiple tissues, but primarily liver and muscle. Our group is mainly interested in insulin resistance in relation to muscle tissue. It is evident that in muscle, the inability of insulin to stimulate glucose uptake through the insulin sensitive GLUT-4 transporter is the cause of insulin resistance. Fat metabolism is also affected in burn patients; free fatty acid (FFA) flux is increased. It's been reported that in burns fatty acid oxidation is increased but if the delivery exceeds the oxidation rate, this could lead to increased intracellular TG accumulation. Peroxisome proliferator-activated receptor α (PPAR-α) is one of the nuclear

receptors that, when stimulated by endogenous lipids, activate specific genes involved in fat metabolism. PPAR-α agonists were reported to lower plasma triglycerides, increase peripheral glucose metabolism and mitochondrial FFA β-oxidation. Our group has conducted a study on evaluating the effect of short-term (14 days) PPAR-α agonist – fenofibrate (FEN) – treatment in burn children (2,3). Results: Whole body palmitate oxidation measured by stable-isotope method was significantly increased in FEN group compared to placebo (PLA). Mitochondrial oxidation of pyruvate and ATP production after pyruvate and palmitoyl-CoA significantly increased in FEN group. The activity of citrate synthase and cytochrome C did not change in FEN group but significantly decreased in the propranolol treated patient group, and the differences between the two groups were significant. However there was no change in muscle intracellular TG accumulation. There was no change in basal glucose and insulin levels after the treatment. However, glucose clearance during insulin clamp significantly increased in post-treatment FEN group. Conclusion: Fenofibrate treatment significantly improves fatty acid oxidation and mitochondrial function. Lack in detection changes in basal glucose and insulin levels may be due to short period of intervention. Due to persistence of insulin resistance in burn patients up to 1-2 years post-burn, long-term fenofibrate intervention studies are of interest.

1. Wolfe RR, MJ Durkot, JR Allsop, JF Burke (1979) Glucose metabolism in severely burned patients. Metabolism 28: 1031-1039.
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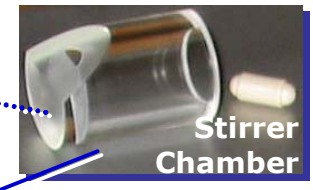
OROBOROS INSTRUMENTS

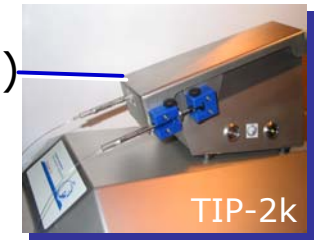
high-resolution respirometry

Oxygraph-2k: Integrative System's Approach

High-resolution respirometry rests on a system of tightly connected features which are integral to a **scientific concept** of methodological development:

1. Polarographic oxygen sensor
2. Mechanics
3. Electronics, MultiSensor Upgradig
4. The Software DatLab
5. Calibrations: Sensor and chamber
6. Titration-Injection microPump (TIP-2k)
7. Scientific support





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