



## Course on High-Resolution Respirometry

IOC44. Mitochondrial Physiology Network 12.24: 1-8 (2007)

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# 44<sup>th</sup> International Course on High-Resolution Respirometry



12-16 Dec 2007

Schröcken, Vorarlberg, Austria

The 44<sup>th</sup> O2k-Course on High-Resolution Respirometry includes experiments with permeabilized muscle fibers (horse skeletal muscle), providing a practical overview of the **Oxygraph-2k**, with integrated on-line analysis by **DatLab 4.2 (new update)**, and demonstration of the **TIP-2k**. Emphasis will be placed on hands-on applications by all participants and a general introduction into O2k-high-resolution respirometry.



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.

Right: Dr. Dominique Votion (Belgium), veterinary specialist on horse muscle pathophysiology, at an O2k-experiment (IOC36).



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, or use the spare time for specific tutorials.

Snowfall may contribute to or interfere with outdoor activities, but performance of the OROBOROS Oxygraph-2k is weather-independent. 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 Research Lab., Innsbruck, Austria; and OROBOROS INSTRUMENTS, Austria

- **Fasching Mario**, PhD, [mario.fasching@oroboros.at](mailto:mario.fasching@oroboros.at) (*tutor*)
- **Gnaiger Erich**, PhD, [erich.gnaiger@i-med.ac.at](mailto:erich.gnaiger@i-med.ac.at) (*lecturer & tutor*)
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- **Subarsky Patrick**, PhD, [patrick.subarsky@oroboros.at](mailto:patrick.subarsky@oroboros.at) (*tutor*)

## Guest lecturer

- **Anderson Ethan J.**, PhD, Department of Exercise and Sport Science & Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC, USA.

## Programme

### Wednesday, 12. December

**Participants arriving in Bregenz:** Meeting point at Bregenz train station, 1.1 hour drive to Schröcken.

**Afternoon/Evening** Check in at Hotel Mohnenfluh.

19:30 Dinner

After dinner: Welcome; introduction of participants.



### Day 2: Thursday, 13. December

**08:45 - 11:45**

#### **Principles of high-resolution respirometry - from switching on the Oxygraph-2k to the experimental result.**

- Oxygraph-2k demo experiment with DatLab 4.2 (new update).
- Oxygen calibration of the polarographic oxygen sensors (POS).

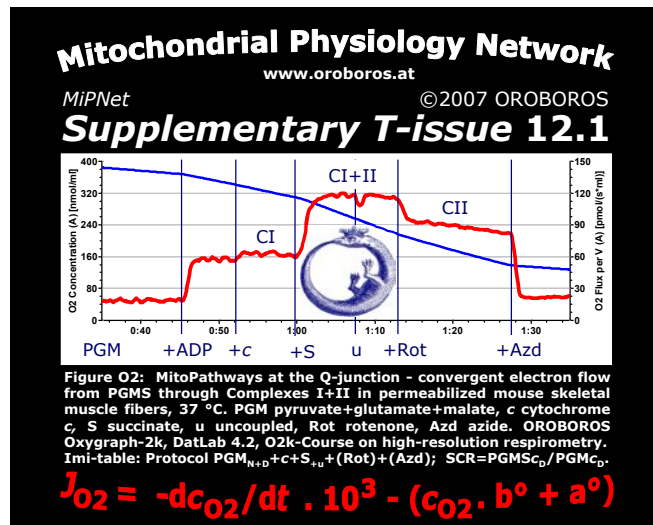


- Preparation of permeabilized muscle fibers.
- Determination of fiber wet weight with **METTLER TOLEDO** microbalance **XS205DU** (1 to 3 mg per chamber).
- Addition of permeabilized skeletal muscle fibers, closing the chamber.
- Multiple substrate OXPHOS titration protocol: ADP control ratio, succinate control ratio, phosphorylation control ratio.



12:00 - 16:00

Lunch break; skiing (bus leaves at 12:22 from Hotel Mohnenfluh).



**16:15 - 19:00 Working group session 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.

19:30 Dinner

**21:00 MiPNet Session 1.**

**Erich Gnaiger** (Innsbruck, AT): High-resolution respirometry and OXPHOS titration protocols: Flux Control Ratios.

**Discussion of results.**

**Day 3: Friday, 14. December**

**Calibrate O2**

Active plot in graph 1 O2 Concentration (A)

Calibration source: Active file PDS # 6001

Oxygen concentration cO2 [µM]	Select Mark	POS signal Recorded [V]	Slope uncorrected [pmol/(s.ml)]	Temperature [°C]	Barometric pressure pb [kPa]
Air calibration: c1 180.97	R1	R1 9.7958	0.17	37.0002	95.20
Zero calibration: c0 0.000	R0	R0 0.0278		37.0007	95.10

O2 solubility factor of medium, FM 0.920 Medium MIR05

O2 Calibration Info

Concentration

Calibration factor for concentration [µM/V] Fc 18.53 Fc = (c1-c0) / (R1-R0)

Calibration offset [V] ac 0.0278 ac = (c1-R0-c0-R1) / (c1-c0)

Pressure

Oxygen pressure pO2 [kPa]	POS signal Current I [µA]	Oxygen consumption by POS J'O2(POS) [pmol/(s.ml)]
Air calibration: p1 18.626	I1 2.4489	I1=R1/G 3.16J*1 = 2.591-(I1-ap) / V
Zero calibration: p0 0.0000	I0 0.0069	I0=R0/G

Calibration factor for pressure [kPa/µA] Fp 7.627 Fp = (p1-p0) / (I1-I0)

Calibration offset [µA] ap 0.0069 ap = (p1-I0-p0-I1) / (p1-p0)

O2 solubility, SO2 9.72 cO2 = pO2·SO2 Gain, G [V/µA] 4

H2O vapor pressure pH2O\* [kPa] 6.27 pO2\* = (pb-pH2O\*)·0.20946 O2k Chamber volume, V [ml] 2.00

Volume fraction of O2 in dry air 0.20946

Open / Close calibration info Cancel Calibrate and Copy to Clipboard

**08:45 - 11:45 Working group session 2:**

Hands-on experiments with the Oxygraph-2k - instrumental performance: O2k-calibration and background test, on-line DatLab analysis.

12:00 - 16:00 Lunch break, skiing

**16:15 - 19:00 Working group session 3:**

High-resolution respirometry and DatLab 4; Instrumental setup and service. Parallel special interest group: DatLab 4 Analysis and respiratory protocols.

19:30 Dinner

21:00

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

**Ethan J. Anderson** (Greenville, US): Excess dietary fat depletes glutathione, increases mitochondrial oxidant emitting potential and shifts redox balance to a more oxidized state in human skeletal muscle.

21:30

**Hélène Lemieux** (Innsbruck, AT) Respirometry with permeabilized cultured cells: Method and application.

**Day 4 (Saturday, 15. December)**

08:45 - 12:30



**Working group session 4:** High-resolution respirometry and DatLab 4; Instrumental setup, POS service.

Parallel special interest group: TIP-2k titration and injection, feedback control and steady-state.



**Special Topic: Francesca Scandurra** (Innsbruck, AT) Oxygen limitation of cellular respiration: Aerobic-anoxic transitions and hypoxic steady-states.



Alpmuseum uf m Tannberg, Batzen [www.alpmuseum.at](http://www.alpmuseum.at)

13:00 - 17:00

Snowshoe walk to a welcome at the Alpmuseum uf m Tannberg (we keep the details of timing flexible according to weather conditions).



**17:30 -19:30 Working group session 5**

Open topics: Problems and solutions.  
Special interest groups: Inhibitor titration and flux control / Experimental regimes / Oxygen kinetics.

20:00 Dinner

22:00

*Discussion - Summary – Conclusions*



**Sunday, 16. December**

Departure to Bregenz and Innsbruck

**Tuesday, 18. December - OROBOROS MiPart - Dec. 18, 18:18, Schöpfstr. 18**

**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**

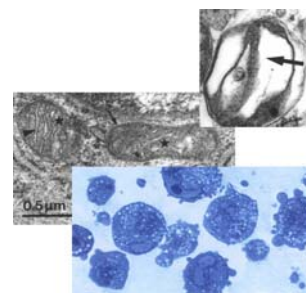
- Instrumental background: measurement and correction as a function of  $pO_2$ .
- High resolution of respiratory flux at various steady-states.
- The Titration-Injection microPump TIP-2k: automatic titrations.
- Conceptual and methodological advantages of measurement at physiological low levels of oxygen.
- 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 1<sup>st</sup> ed ISBN 978-3-9502399-0-4 – [www.orooboros.at](http://www.orooboros.at)



## Accommodation and Location

**Hotel Mohnenfluh** [www.mohnenfluh.at](http://www.mohnenfluh.at); Tel.: +43 5519 203; [hotel@mohnenfluh.at](mailto:hotel@mohnenfluh.at). The course takes place at Hotel Mohnenfluh (Sylvia Schramm-Strolz, right), including accommodation for all participants breakfast, meals and coffee breaks.

### Skiing



Warth-Schröcken - <http://www.snowworld.at/>.

The skiing area Salober is reached by a free bus service, leaving at 12:20/12:22 at Hotel Tannberg / Hotel Mohnenfluh. The following information relates to prices in 2006/07, which may have changed: For the afternoon after 12:30, the skiing pass is € 22.50 for the skiing lifts of Salober and Warth. 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. Top ski (+boots) is € 16.- (+7.-; 1 day), 30.- (+12.-; 2 days), 42.- (+17.-; 3 days) or 52.- (+22.-; 4 days). You can return to Schröcken on skis (depending on snow conditions) or by the free bus (leaving 15:30 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.orooboros.at](http://www.orooboros.at).

## Contact

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OROBOROS INSTRUMENTS  
high-resolution respirometry

Oxygraph-2k



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Cooperation and Feedback in Science

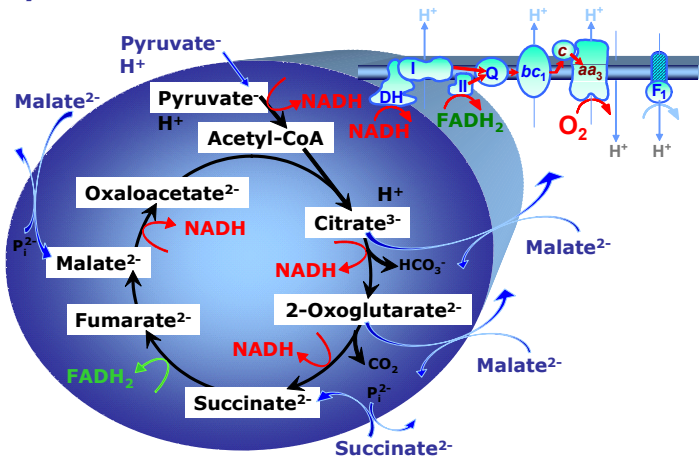
## Participants

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# Hot topics in Mitochondrial Physiology – MiPNet Abstracts

## MiPNet 1. High-resolution respirometry and OXPHOS titration protocols: Flux Control Ratios.

### Mitochondrial Pathways Pyruvate+Malate+Succinate

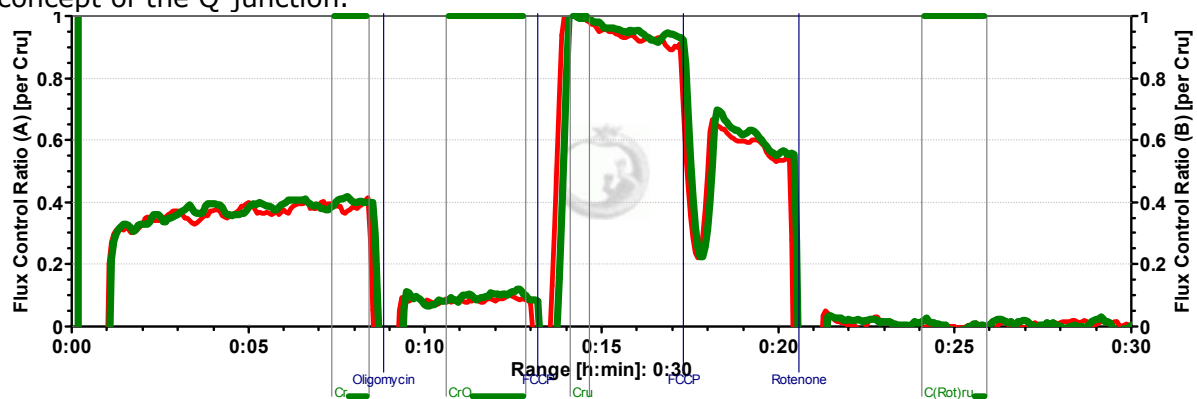


Erich Gnaiger<sup>1,2</sup>

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Electron flow in the mitochondrial respiratory chain drives proton translocation through the inner mitochondrial membrane, building a membrane potential and proton motive force which in turn fosters the power for oxidative phosphorylation. Metabolic maps in bioenergetics carefully point out that, in contrast to a

linear arrangement of respiratory complexes, input into the electron transport chain converges from Complexes I and II (CI+II, and other flavoproteins) into the Q-cycle. The implications of this Q-junction on mitochondrial respiratory control are not sufficiently recognized in bioenergetics and metabolic flux control analysis. The Q-junction emerges now as a novel paradigm of respiratory control in mitochondrial physiology, based on high-resolution respirometry (OROBOROS Oxygraph-2k [1]) in permeabilized cells and tissue preparations. ADP-activated respiration with malate+glutamate or pyruvate (classical State 3) increases up to 2-fold after addition of succinate [1]. Parallel electron input converging at the Q-junction shares flux control with the phosphorylation system, and corresponds to mitochondrial substrate supply *in vivo*. By establishing the reference state of maximum coupled respiration, convergent electron input into the Q-junction provides the proper basis for (i) quantifying excess capacities, metabolic thresholds, and interpreting flux control by various enzymes (e.g. COX) and functional units (phosphorylation system), and (ii) evaluation of specific enzymatic defects in mitochondrial respiratory physiology and pathology. The design is discussed of a reference protocol for multisubstrate/inhibitor titrations, which takes into account the concept of the Q-junction.



The new version of DatLab 4.2 includes an option for plotting Flux Control Ratios (FCR). Interpretation of such flux control ratios is discussed in comparison to the classical respiratory control (RCR) or uncoupling control ratios (UCR).

1. Gnaiger E, ed (2007) *Mitochondrial Pathways and Respiratory Control*. OROBOROS MiPNet Publications, Innsbruck: 96 pp.

**MiPNet 2.**

**Excess dietary fat depletes glutathione, increases mitochondrial oxidant emitting potential and shifts redox balance to a more oxidized state in human skeletal muscle system**

Ethan J. Anderson, Kristen E. Boyle, Joseph A. Houmard, P. Darrell Neuffer  
 Exercise Science & Physiology, East Carolina University, Greenville, NC.-  
 andersonet@ecu.edu

The worldwide epidemic of Type 2 Diabetes is linked to modern lifestyle, of which high dietary fat intake is a substantial component. To examine the molecular impact of a high fat diet on human skeletal muscle mitochondria, male (18-34 yrs) subjects were recruited and muscle biopsies were obtained following a 12-hour fast (A), 4 hours after a single high-fat meal (65-70 % fat content, B), and after 5 days of high-fat diet/12-hour fast (C). Using a method to measure in situ mitochondrial respiration and H<sub>2</sub>O<sub>2</sub> emission (mH<sub>2</sub>O<sub>2</sub>, an index of reactive oxygen species) in permeabilized muscle fibers supported by glutamate+malate (GM), succinate (S), and palmitoyl-carnitine (Pc), we observed from A to both B and C a striking 2.5- and 3-fold (*P*<0.05) increase in V<sub>max</sub> of G+M/S and Pc-supported mH<sub>2</sub>O<sub>2</sub> emission, respectively. Surprisingly, from A to C the total glutathione content (GSH<sub>t</sub>) was decreased by 40 % (*P*<0.05), and the GSH/GSSG ratio decreased by 2.5-fold (*P*<0.05). Respiration with all substrates remained unchanged from A to B, but basal and maximal G+M/S-supported respiration was decreased by 30 % (*P*<0.05) at C.

These data demonstrate that an excess dietary fat intake (1) increases skeletal muscle mH<sub>2</sub>O<sub>2</sub> emission and shifts redox balance to a more oxidized state, (2) decreases capacity for scavenging mH<sub>2</sub>O<sub>2</sub> by depleting GSH<sub>t</sub>, (3) decreases mitochondrial respiration, all potentially contributing to the etiology of Type 2 diabetes.

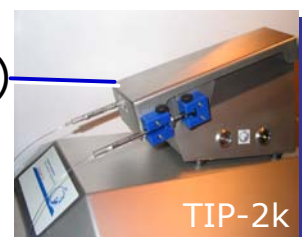
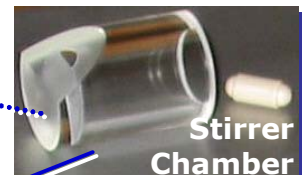
OROBOROS INSTRUMENTS  
 high-resolution respirometry



**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



Gnaiger (2001) *Respir. Physiol.* 128: 277-297.