IOC-27. *Mitochondrial Physiology Network* 9.2: 1-13

Last update: 2004-06-09

International Course and Workshop on High-Resolution Respirometry

14-18 April, 2004

Schröcken, Vorarlberg, Austria

Including the International Workshop: Mitochondrial Physiology Events

Programme

Wednesday, 14. April Registration and informal evening at Hotel Mohnenfluh.

Thursday, 15. April

08:30 – 11:45 **Erich Gnaiger** (Innsbruck, AT): www.oroboros.at - the Oxygraph-2k and hot topics in MiP. Introduction and practical approach.

12:00 Ski break (bus leaves at 12:11 from Hotel Tannberg)

16:15 -19:00 **Erich Gnaiger and Brigitte Haffner** (Innsbruck, AT): Oxygraph-2k: Instrument demonstration.

19:00 Dinner

20:30 **Hot topics in Mitochondrial Physiology - MiPNet Session 1.**

Assegid Garedew (Würzburg, DE): Respirometric investigations on malignant and foetal human cells with special emphasis on the apparent oxygen affinity. MiP-Net 1.

Karl J. Tronstad (Bergen, NO): Mitochondrial-targeted fatty acid analog induces apoptosis with selective loss of mitochondrial glutathione in leukemia cells. MiP-Net 2.

Anne Devin (Bordeaux, FR): Yeast mitochondrial biogenesis is regulated via the cAMP protein kinase TPK3. MiP-Net 3.

Friday, 16. April

08:30 - 11:45 **Hands-on experiments with the Oxygraph-2k.**

12:00 Ski break (bus leaves at 12:11 from Hotel Tannberg)

16:15 -16:45 ***Special guest lecture*:**
Veronica Hollis (London, UK): Studies of mitochondrial activity in whole cells using respirometry and light absorption spectroscopy. MiP-Net 4.

17:15 – 18:30 Parallel Sessions
a) Working Groups: DatLab Analysis and high-resolution respirometry.
b) MiP Events: Project Application Marie Curie Conferences & Training Courses

19:00 Dinner

20:30 **Hot topics in Mitochondrial Physiology - MiPNet Session 2.**

Martin J. Kushmerick (Seattle, US): High-resolution respirometry of single mouse muscles: regulation of mitochondrial respiration in a complex intact and integrated system. MiP-Net 5.

David J. Marcinek (Seattle, US): Mitochondrial coupling and function in vivo: new, non-invasive approaches. MiP-Net 6.

Erich Gnaiger (Innsbruck, AT): CMRC Greenland 2004 expedition. High-resolution respirometry in muscle biopsies from arm versus leg of Inuit hunters and a Danish control group. MiP-Net 7.

Saturday, 17. April

08:30 - 12:00 **Working Groups: High-resolution respirometry / DatLab Analysis.**

12:00 Lunch

13:30 -16:45 Parallel Sessions
a) Working Groups: DatLab Analysis and high-resolution respirometry.
b) MiP Events: Project Application Marie Curie Conferences & Training Courses

17:00 - 23:00 Snowshoe walk and evening in the *Alpmuseum uf m Tannberg*

From Schröcken via Alp Felle to Alp Batzen (2 h walk).

19:00 Welcome at the *Alpmuseum.*

20:30 Dinner at Hotel Körbersee - www.koerbersee.at.
Hiking boots are recommended.

Batzen www.alpmuseum.at

Josef Staggl (Schröcken) takes the lead: Snowshoe walk of the Oxygraph-2k course in April 2004

Sunday, 18. April

09:30 - 11:45 **Course Session 7 and Conclusions**
12:00 **Departure**

CONTENTS: OVERVIEW ON HIGH-RESOLUTION RESPIROMETRY

Erich Gnaiger, Innsbruck (for further information on courses see: www.oroboros.at and www.mitophysiology.org)

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

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

- Low oxygen and measurement of cellular oxygen consumption – pushing the limits of detection.
- Optimum system design - the OROBOROS Oxygraph-2k.
- On-line recording of oxygen concentration and flux; linear slope versus oxygen flux as a function of time.
- The concept of high-resolution calibrations – overview on instrument demonstration.

OROBOROS Oxygraph-2k*:* On-line instrumental performance

- Instrumental background: measurement and correction as a function of p_{O_2}.
- High resolution of respiratory flux at various steady-states.
- 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 and Oxygraph service

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

DatLab – the specialized software for High-Resolution Respirometry: Data acquisition and analysis.

Travel

Arrivals: Wednesday, 14. April 2004

Munich-Schröcken: Transfer Innsbruck-Schröcken (c. 2.5 h by car; meeting points and departure times according to specified arrival times in Innsbruck).

Zurich-Schröcken: Train from Zurich to Bregenz; transfer Bregenz-Schröcken (departure time from Bregenz train station according to specified arrival times; c. 1 h and 15 min by car; in case of late changes call

Hotel Mohnenfluh: Tel. +43 5519-203; in Austria: Tel. 05519-203).

Departure: Sunday, 18. April 2004
Transfer Schröcken-Innsbruck (Schröcken-Bregenz), departures according to departure times in Innsbruck (Bregenz).

Registration

Registration fee: Euro 350.-
Presentations: A beamer, overhead projector and flip chart will be available for presentations. Coffee breaks are included during the programme.

Accomodation and Location

Schröcken: www.snowworld.net/

Hotel Registration: Organized through registration. The number of rooms in Hotel Mohnenfluh is limited. If necessary, further accomodation may be arranged in the nearby Hotel Tannberg or in appartment houses. All meals are arranged jointly in Hotel Mohnenfluh.

Hotel Mohnenfluh www.mohnenfluh.at; Tel.: +43 5519 203; hotel@mohnenfluh.at
1. Two-room appartment, one participant per room; EURO 65.- per night per participant.
2. Single room: EURO 75.- per night.
3. Two-room appartment shared by four participants: EURO 60,- per night per participant.
In all cases included: Breakfast, lunch, dinner, coffee and cakes.

Skiing www.intermaps.com/skimaps/snowworld.
Bus trips are free from Schröcken to the skiing area of Salober, leaving at 12:11 at Hotel Tannberg (near Hotel Mohnenfluh; or at 11:06 from Hotel Mohnenfluh). For the afternoon after 12:30, the skiing pass is EURO 22.50 for the skiing lifts of Salober and Warth. There is also excellent crosscountry skiing around lake Kalbelesee and Körbersee, as well as easy walking in magnificent winter scenery. Ski rental is available in Schröcken and at Salober. Top ski (+boots) is Euro 16.- (+7.-; 1 day), 30.- (+12.-; 2 days), 42.- (+17.-; 3 days) or 52.- (+22.-; 4 days). You can return to Schröcken by skiing (depending on snow conditions) or by the free bus (leaving 15:30 at Salober).

Weather

Sub-freezing temperatures are normal in April, but sunny days in spring may be warm. Sunshine is very strong – bring sunglasses and sunscreen, even if you do not plan to go skiing. Protect yourself against wind and potential snowfalls (gloves, jacket, etc.).

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OROBOROS INSTRUMENTS
high-resolution respirometry
Oxygraph-2k

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Mitochondrial Physiology: www.mitophyiology.org

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Hot topics in Mitochondrial Physiology – MiP-Net Abstracts

MiP-Net 1. Respirometric investigations on malignant and foetal human cells with special emphasis on the apparent oxygen affinity

Assegid Garedew, Ulrike Kämmerer, Dominique Singer
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Malignant and foetal cells are confronted with hypoxic *in situ* situations due to the limited supply of oxygen. The aim of our study was to investigate as to whether these cells have metabolic depression leading to hypoxic hypometabolism and/or higher oxygen affinities.

Respirometric investigations were made using the Oroboros Oxygraph-2k on malignant breast cancer (HBL-100), ovarian cancer (PA1, SKOV3, SCHPL), skin cancer (BML) and benign cells (HUVEC, peripheral blood monocytes-PBMC from healthy donors as well as leucocytes from umbilical cord blood and maternal placental blood) at various cell densities with special emphasis on p_{50} and I_{O2} (oxygen flux per million cells).

The malignant cell lines (HBL-100, PA1, BML, SKOV3) showed oxygen consumption rates, I_{O2}, that ranged from 10 to 61 pmol s^{-1} (10^6 cells)$^{-1}$, generally higher than that of the benign cell line HUVEC with 10 to 21 pmol s^{-1} (10^6 cells)$^{-1}$. Regardless of the variation in I_{O2} among the different cell lines (both malignant and benign), the p_{50} values remained fairly constant from 0.5 to 0.9 µM. The oxygen consumption rates of the malignant patient material (SCHPL, an ovarian carcinoma that metastasises at the pleural cavity) were lower than that of the malignant cell lines. They ranged from 8 to 16 pmol s^{-1}(10^6 cells)$^{-1}$ at cell densities of 1 or 2 million cells ml^{-1} but very high (19.8 to 40) at 0.5 million cells ml^{-1}. This same pattern was also observed for PA1 (an ovarian carcinoma that metastasises at the ascite) with 10 to 16 and 29 to 49 pmol s^{-1}(10^6 cells)$^{-1}$ at cell densities of 1 or 2 and 0.5 million cells ml^{-1}, respectively.

PBMC displayed lower I O_2 values of 3 to 6 pmol s^{-1}(10^6 cells)$^{-1}$ and reduced p_{50} values (0.1 to 0.27 µM). The oxygen consumption rates of freshly isolated umbilical cord blood leucocytes ranged from 3.5 to 5 pmol s^{-1} (10^6 cells)$^{-1}$ with p_{50} values of 0.16 to 0.27 µM. Whilst the I O_2 values of maternal placental blood leucocytes were 0.6 to 1.3, far lower than their foetal counter parts, with p_{50} values from 0.09 to 0.12 µM.

Our study demonstrated that malignant cells have higher oxygen consumption rates and elevated p_{50} values than benign cells, confirming that they do not have higher oxygen affinity as an adaptation to hypoxia. The oxygen consumption rates showed a reverse dependence on cell density (0.5, 1, and 2 x 10^6 cells ml^{-1}) for all but HBL and BML, displaying a crowding effect and competition for limited resources. It is interesting to observe that the foetal and maternal placental derived leucocytes display different oxygen consumption rates. This might be explained by the differences in the pO_2 in the foetal (ca 3.3 kPa) and maternal (ca 13.3 kPa) side of the placenta. The low p_{O2} could lead to intrauterine metabolic suppression by the foetal cells and can be activated at birth.

MiP-Net 2. Mitochondrial-targeted fatty acid analog induces apoptosis with selective loss of mitochondrial glutathione in leukemia cells.

Karl J Tronstad, Bjørn T Gjertsen, Camilla Krakstad, Kjetil Berge, Odd T Brustugun, Stein O Døskeland, Øystein Bruserud, Rolf K Berge
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Some fatty acids and derivatives are known to induce cell death in cancer cells. Mitochondria may have important roles in the death process. Therefore, we investigated the mitochondrial contribution in cell death induced by a modified fatty acid, tetradecylthioacetic acid (TTA), which cannot be β-oxidized. TTA treatment induced apoptosis in IPC-81 leukemia cells via depolarization of the mitochondrial membrane potential ($\Delta\psi$) and early release of cytochrome *c*, accompanied by depletion of mitochondrial glutathione. Caspase-3 activation and cleavage of poly (ADP-ribose) polymerase (PARP) occurred at a late stage, but the broad-spectra caspase inhibitor zVAD-fmk did not block TTA-induced apoptosis. Overexpression of Bcl-2 partially prevented TTA-induced apoptosis, whereas cAMP-induced cell death was completely blocked. In conclusion, TTA seems to trigger apoptosis through mitochondrial-mediated mechanisms and selective modulation of the mitochondrial redox equilibrium.

MiP-Net 3. Yeast mitochondrial biogenesis is regulated via the cAMP protein kinase TPK3.

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In most organisms, the cellular mitochondrial content is modulated by environmental stimuli and/or in response to energy demand changes. Several cAMP targets and transcription factors seem to be involved in the up-modulation of mitochondrial biogenesis when energy demand increases. In the yeast *Saccharomyces cerevisiae*, the Ras/cAMP/protein kinase pathway is involved in many physiological

adaptations of cells upon environmental changes. Among the adaptation processes occurring during the transition between exponential growth to stationary phase, the down-modulation of the cytochrome content and of the respiratory activity of yeast cells plays a role in the maintenance of high growth yield. The question is therefore raised as to the role of the Ras/cAMP-protein kinase A pathway in this adaptative process. We have previously shown that mutant strains exhibiting an increased or decreased activity of this pathway have a coordinated change in mitochondrial enzyme equipment. The question thus arose as to whether a specific kinase might be involved in this process. We show that out of the three cAMP protein kinases in yeast, TPK3 is the one involved in mitochondrial biogenesis. Indeed, when grown on non-fermentescible carbon source TPK3- cells have a significantly decreased amount of mitochondria. Respiratory rates, enzymatic equipment as well as mitochondrial DNA amount are decreased in this strain. Moreover, signaling to mitochondrial biogenesis through TPK3 is dependent on ROS production.

MiP-Net 4. Studies of mitochondrial activity in whole cells using respirometry and light absorption spectroscopy

Veronica S Hollis, Miriam Palacios-Callender, Marisol Quintero, Roger J Springett,[†] Salvador Moncada
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We have developed a system to study mitochondrial function in whole cells that couples respirometric and spectroscopic measurements. The system detects changes in optical attenuation in the visible region during cellular respiration and, simultaneously, monitors oxygen (O_2) and nitric oxide (NO) concentrations by polarographic methods. Using mitochondrial inhibitors we have previously demonstrated the ability of the system to distinguish, from the spectroscopic measurements, changes in redox state of the cytochromes comprising various electron transport chain (ETC) enzymes [1].

Recently we have investigated the effects of endogenously generated NO on the cytochrome redox states and the consequences for cell signalling [2]. We have observed a decrease in cytochromes aa_3 of cytochrome *c* oxidase (CcO, complex IV) at O_2 concentrations at which O_2 consumption (VO_2) is still maximal. Moreover, the O_2 concentration at which the cytochrome reduction occurred was significantly lowered by treating the cells with an inhibitor of NO synthase (NOS). We also observed an increase in superoxide (O_2^-) production in cells incubated at 3% O_2 compared to 21%, which was accompanied by a translocation to the nucleus of the transcription factor NF-kB. Here we demonstrate how basal levels of NO, by enhancing the reduction of the ETC cytochromes at low O_2 concentrations, play a role in the observed increase in O_2^- and the subsequent activation of NF-kB, which has been linked to hypoxic signalling responses [3].

In order to investigate further the effects of NO on mitochondrial function, we are currently involved in development of the system, some ideas for which are presented here. Specifically, we aim to modify the system to enable measurements to be made at constant O_2 concentrations by providing a continuous supply of O_2 to match VO_2 at any O_2 concentration. This will enable us to study cell bioenergetics under more physiological conditions than the current system allows. Our goal is also to integrate into the system other optical measurements, such as that of NADH redox state, reactive oxygen species production and mitochondrial membrane potential. These additional information will broaden our understanding of mitochondrial physiology as a whole, and, in particular, the interaction between NO and the ETC and the subsequent cellular responses.

1. Hollis VS, Palacios-Callender M, Springett RJ, Delpy DT, Moncada S (2003) Monitoring cytochrome redox changes in the mitochondria of intact cells using multi-wavelength visible light spectroscopy. *Biochim*. *Biophys*. *Acta* 1607: 191-202.
2. Palacios-Callender M, Quintero M, Hollis VS, Springett RJ, Moncada S (2004) Basal levels of NO regulate superoxide production at low oxygen concentrations by modifying the redox state of cytochrome c oxidase. *Proc*. *Natl*. *Acad*. *Sci*., in press.
3. Pearlstein DP, Ali MH, Mungai PT, Hynes KL, Gewertz BL, Schumacker PT (2002) Role of mitochondrial oxidant generation in endothelial cell responses to hypoxia. *Arterioscler*. *Thromb*. *Vasc*. *Biol*. 22: 525-527.

MiP-Net 5. Mitochondrial coupling and function in vivo: new, non-invasive approaches.

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Mitochondria play a critical role in integrating cellular metabolism and controlling cell survival. Studies of isolated mitochondria and cells have provided many insights into mitochondrial function, however extrapolating these studies to the intact physiological system is challenging. The sensitivity of mitochondria to the intracellular environment, which is affected by both local and systemic factors, makes an in vivo analysis critical to understanding mitochondrial function in both normal and pathological states. We have developed a non-invasive approach, combining optical and magnetic resonance spectroscopies, to quantify mitochondrial coupling (P/O) and oxygen sensitivity of mitochondrial respiration in skeletal muscle. The novel development that allows this analysis is the ability to separate Hb and Mb saturations from in vivo optical spectra, thereby providing a quantitative measure of local oxygen consumption. This non-invasive approach yields a P/O of 2.16 ± 0.24 for resting mouse skeletal muscle, which approaches the theoretical maximum for mitochondrial oxidative phosphorylation (2.33 – not including substrate level phosphorylation). Systemic treatment with 2,4-dinitrophenol reduces this value to 1.37 ± 0.22. Application of

this non-invasive strategy to aging mice indicates that P/O is reduced to 1.30 ± 0.50 in 30-month old animals. Our value for resting P/O agrees is consistent with most analyses of mitochondrial coupling in isolated tissues, but is higher than typically reported from isolated cells and mitochondria. In vivo analysis also indicates that mitochondrial respiration is insensitive to intracellular PO_2s down to 2-3 mm Hg. Above this level there is no significant effect of intracellular PO_2 on O_2 flux, [PCr], or intracellular pH. This new approach to mitochondrial function provides the necessary tools to understand mitochondrial function in the intact organism and has the potential to serve as a powerful tool in the diagnosis and treatment of mitochondrial pathologies. (supported by NIH grants AG-00057, AR-45184, AR-36281).

MiP-Net 6. High resolution respirometry of single mouse muscles: regulation of mitochondrial respiration in a complex intact and integrated system.

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Single mouse muscles were studied while at rest and stimulated to give isometric twitch contractions at 20° C by high resolution respirometry (Oroboros Oxygraph). The apparatus was modified to enable a single soleus (slow twitch type) or extensor digitorum longus (fast twitch type) muscle of young mice to be mounted at resting length. Isometric twitches induced by electrical stimulation without electrolysis of water. Twitch force was recorded during steady state stimulation lasting up to 30 min. Oxygen consumption flux (J_{O2}) was obtained from the time course of changes in partial pressure of oxygen versus time and knowledge of the apparatus baseline. These measurements were possible because of a long diffusion path to the external connections effectively closed the chamber to the oxygen diffusion from the ambient even when chamber p_{O2} ~ 700 torr (93 kPa). Isometric twitches were studied at frequencies up to 5 Hz in muscles that weighed typically 10 – 15 mg (wet) each. Steady state J_{O2} linearly increased with tension time integral in the steady state as stimulation frequency increased. Total energy cost was proportional to the time integral of tension. In these series of twitches lasting ~ 15 minutes, the respiration rate increased above baseline at the onset of stimulation monoexponentially to a new rate; monoexponential time constant 180 sec. When the stimulation stopped the respiration rate returned to baseline more rapidly; time constant 90 sec. The lack of dependence of J_{O2} at fixed frequency with muscle thickness as well as diffusion modeling showed no part of the muscle was oxygen limited. These results exclude simple equilibrium mechanisms for respiratory control which requires symmetrical on and off rates e.g. (2, 3), The linear dependence of respiration rate with isometric force produced shows that J_{O2} did not reach its maximal rate. This means that the energy balance system is limited not by oxygen or substrate but by achievable cellular ATPase flux. The hypothesis that exogenous pyruvate would increase mitochondrial supply of NADH and influence the kinetics of cellular respiration was tested. Adding 10 mM pyruvate to the medium did not change the steady state J_{O2} (relative to tension time integral) which means that the overall coupling of tension-dependent ATPase to respiration does not depend on manipulations of mitochondrial redox potential (and presumably protonmotive force). On the other hand 10 mM pyruvate dramatically increased the kinetics of increase in J_{O2} and return of J_{O2} to baseline: pyruvate increased the kinetics of the "on" and "off" rate 2-fold. The rate of phosphorylation was directly measured by ^{31}P NMR in the absence of substrate; kinetics of phosphorylation agreed quantitatively with the cell respiration. All these results are consistent with a simple model of energy balance in muscle cells (human and rodent) in which ADP is the primary controller of respiration and creatine kinase system operates as a simple temporal and spatial buffer at near equilibrium with cellular substrate and product concentrations (1, 4). Supported by ideas and work by my many past and present colleagues who are coauthors of our papers and financially by NIH (AR36281 and AR36281).

1. Kushmerick MJ. Energy balance in muscle activity: Simulations of ATPase coupled to oxidative phosphorylation and to creatine kinase. *Comp Biochem Physiol* 120B: 109 - 123, 1998.
2. Meyer RA. A linear model of muscle respiration explains monoexponential phosphocreatine changes. *American Journal of Physiology* 254: C548-C553, 1988.
3. Meyer RA and Foley JM. Cellular Processes Integrating the Metabolic Response to Exercise. In: *Handbook of Physiology.*, edited by Rowell LB and Shepherd JT. New York: Oxford University Press, 1996, p. 841-869.
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MiP-Net 7. CMRC Greenland 2004 expedition. High-resolution respirometry in muscle biopsies from arm versus leg of Inuit hunters and a Danish control group.

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The adaptation pattern after long-term whole body exercise differes markedly between arm and leg muscle [1], and differences persist in aerobic and anaerobic capacities of deltoid and vastus lateralis even in elite cross-country skiers who have well-trained arm and leg muscles [2]. To further study such differences in the larger frame of the CMRC Greenland 2004 expedition, a protocol for high-resolution respirometry (30 °C; three Oroboros Oxygraph-2k operated in parallel) was

designed for quantification of mitochondrial respiratory capacities in permeabilized muscle fibers obtained from needle biopsies.

Since respiration of permeabilized muscle fibers is oxygen limited even close to air saturation [3], measurements were performed in the range of 20 to 50 kPa oxygen pressure (c. 210 to 530 µM). ADP stimulated respiration was determined in a sequence of substrate titrations (Fig. 1), of malate and octanoyl-carnitine, glutamate, and succinate. At this stage, cytochrome *c* was added and did not exert any stimulatory effect on respiration, indicating the intactness of the outer mitochondrial membrane in the permeabilized muscle fibers. (Fig. 1). Subsequently complex I was inhibited by rotenone, to compare respiratory capacity with substrates for complex I+II, and complex II alone. Mitochondrial coupling was evaluated after further inhibition of ATP synthase by oligomycin, and uncoupling by a multiple-step FCCP titration (Fig. 1). Antmycin A, ascorbate and TMPD were titrated for final determination of the capacity of cytochrome *c* oxidase. In the range up to 50 kPa, autoxidation of 2 mM ascorbate and 0.5 mM TMPD was biphasic hyperbolic, as determined in the absence of a biological sample in mitochondrial medium MiR05 and after addition of the other substrates and inhibitors, including 10 µM cytochrome *c*. For the high- and low-affinity components, maximum fluxes, $J°'_{max}$, were 6.8 and 60.8 pmol $O_2 \cdot s^{-1} \cdot ml^{-1}$, and the corresponding values of $K°'_m$ were 2.7 and 169 µM. This may reflect the different effects of the two main components (ascorbate and TMPD) contributing to the chemical background, and seved as a basis for the oxygen-dependent correction of chemical background oxygen flux.

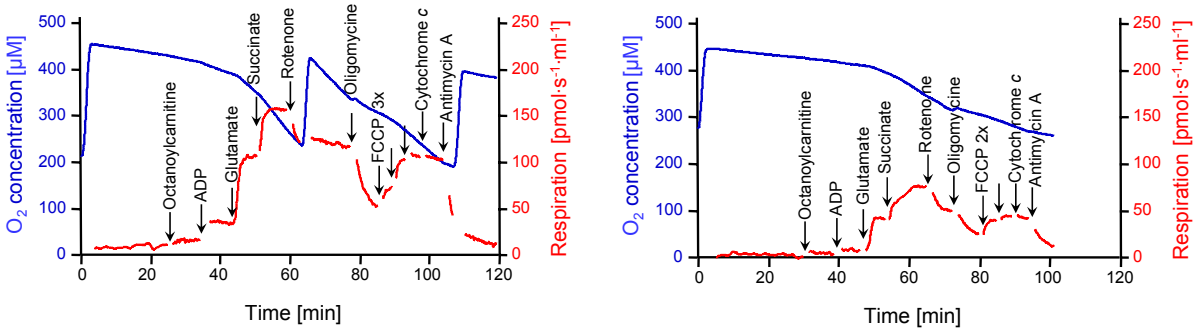

Fig. 1. Oxygen flux per volume (respiration) and oxygen concentration of permeabilized fibers, illustrating the titration regime after addition of the sample to mitochondrial respiration medium (MiR05) containing malate. After inhibition of ocmplex III by antimycin A, complex IV activity was measured with ascorbate and TMPD (not shown). Left and right show an experiment with vastus lateralis (5.0 mg wet weight) and deltoid (5.2 mg wet weight), respectively (Danish Subject 03, baseline). On the trace of oxygen flux, sections are eliminated which were distorted by either titrations (arrows9 or reoxygenations. Oxygenations were performed initially with pure oxygen purged into the gas phase of the intermittently opened chamber. To avoid a drop of oxygen below air-saturation levels, further reoxygenations were necessary in the vastus lateralis experiment owing to the higher respiratory activity of leg muscle.

In the titration regime shown in Figure 1, the respiratory control ratio after addition of ADP was low, and does not quantify coupling but reflects substrate limitation of respiration with octanoyl-carnitine. This was clearly indicated by the large stimulatory effect of glutamate. The parallel electron input from complex I and II (after addition of succinate before rotenone) resulted a higher respiratory flux compared to substrates for either complex alone. This provides evidence of the excess capacity of the respiratory chain downstream of complexes I and II, particularly of complex IV without application of the artificial substrates ascorbate and TMPD [4]. Adenylate control and coupling were quantified by inhibition of respiration by oligomycine (state 4o) and subsequent uncoupling by FCCP (state 3u; Fig. 1). The respiratory control ratio (state 3 divided by state 4o respiration) was significantly higher in the leg muscle. Whereas the general pattern of respiration was similar in vastus lateralis and deltoid, the absolute level of mitochondrial respiratory capacity was higher in the leg than arm muscle in the Danisch control group, analyzed shortly before the Greenland expedition (Fig. 1). This agrees with differences in citrase activity between arm and leg, which remains idential after muscle adaptation during skiing in the cold [1]. In contrast, first analyses of traditional Inuit hunters indicate an even mitochondrial respiratory capacity in leg and arm muscle, which may be genetically fixed or determined by dominant factors of life style.

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Ouroboros: Kirkegaard graveyard, Copenhagen

Photos
International Oxygraph-2k Workshop, Schröcken, Austria, April 2004

***Top**:* Kari Williams, Suhel Parvz, Lukas Gradl, Assegid Garedew and Erich Gnaiger taking a look at the screen. ***Right**:* Four Oxygraph-2k ready for the workshop in Hotel Mohnenfluh. ***Below**:* Karl Johan Tronstad and Kari Williams in action.

***Right**:* Erich Gnaiger explains to Johan Tronstad, Kari Williams and Carsten Werner, Marty Kushmerick in the background.

***Right**:* Carolien Vink and Pavla Krivakova practice the membrane application on the oxygen sensor under the guidance of Brigitte Haffner.

***Below - a toast**:* Veronica Hollis, Luca Martino, Eugen Faist, Irmi Frajo-Apor, Pavla Krivakova, Suhel Parvez, ..

***Right – ready for the snowshoe walk**:* Carolien Vink, Brigitte Haffner, Assegid Garedew, Suhel Parvez, Eugen Faist, Pavla Krivakova, Kari Williams, David Marcinek, Veronica Hollis, Jerzy Duszynski, Pat Kushmerick, Luca Martino, Marty Kushmerick, Carsten Werner, Karl Johann Tronstad; *in front:* Josef Staggl, Erich Gnaiger.

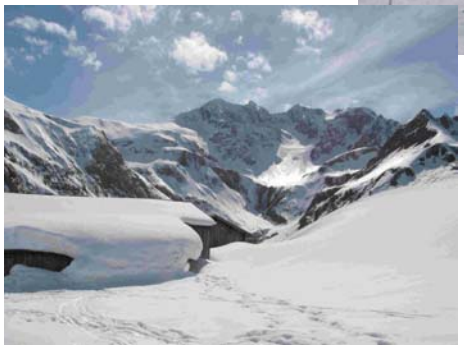
International Oxygraph-2k Workshop, Skibreak at Salober

***Top**:* Alexandra and Eugen in action.
***Below**:* Suhel, Luca, Erich, Assegid, Caroline and Pavla.

Left and below: David, Karl Johan, Anne and Alexandra take off to an adventure on the steep slopes.

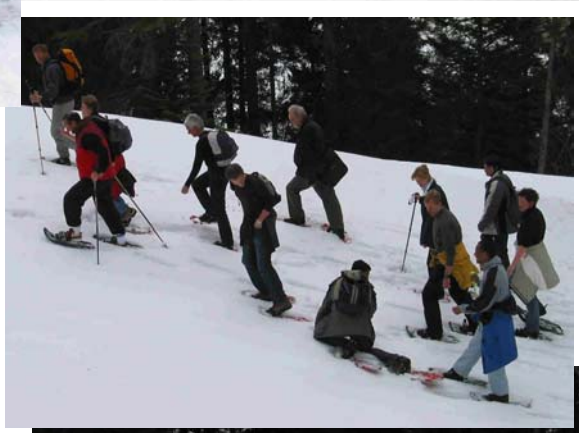
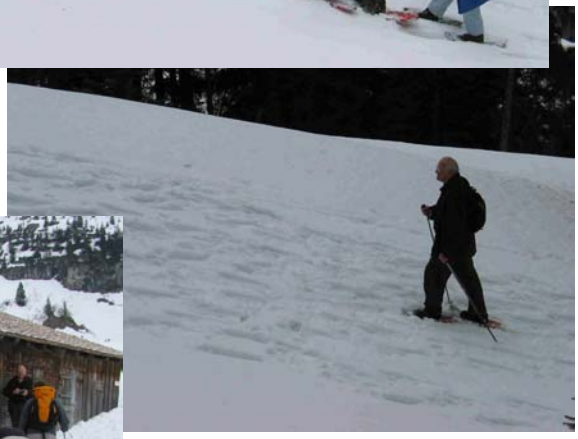
***Below**:* Eugen in style.

***Top**:* Brigitte and Karl Johann.
Right: Elegant Anne.
Below: A view on Alp Büel and the peak of Braunarl, from Hotel Körbersee to Schröcken.



***Top right**:* Pavla, Luca, Jerzy, Brigitte, Assegid, Suhel, Kari and Anne - a break from the skiing break.

***Right**:* Veronica and Jerzy – with snowshoes at Alp Batzen.


Snowshoe walk to the "Alpmuseum uf m Tannberg", Alp Batzen. *Above right:* Edwin Schwarzmann guides us through the Alpmuseum, while (*left*) Schnapps, alp cheese and spicy sossages are being served in an international atmosphere.

Correspondence

"Thank you for a wonderful workshop in Schröcken. Everything was very well organized, and both science and skiing were very enjoyable. There was a good repartition between explanations and hands-on and I learned a lot." *Anne Devin*

"I just wanted to say thank you again for inviting me to the MiP workshop, I really enjoyed myself. Please also thank Irmi, Brigitte and Lukas for their help." *Veronica Hollis*

"I must acknowledge that I gained a lot from the workshop and at present doing experiments on the oxygraph without any problem, It is all beacuse of the excellent training from the workshop." *Suhel Parvez*

"Thanks a lot for the highly educational and entertaining wokshop." *Assegid Garedew*

"… the course was excellent." *Karl Johan Tronstad*

"The Oxygraph Workshop was an excellent way for me to become familiar with the Oxygraph as Marty and I gear up for the next stage of experiments dealing with mitochondrial function in muscle. Thanks for the brief introduction to such a beautiful part of the world. I hope to be back soon." *David Marcinek*