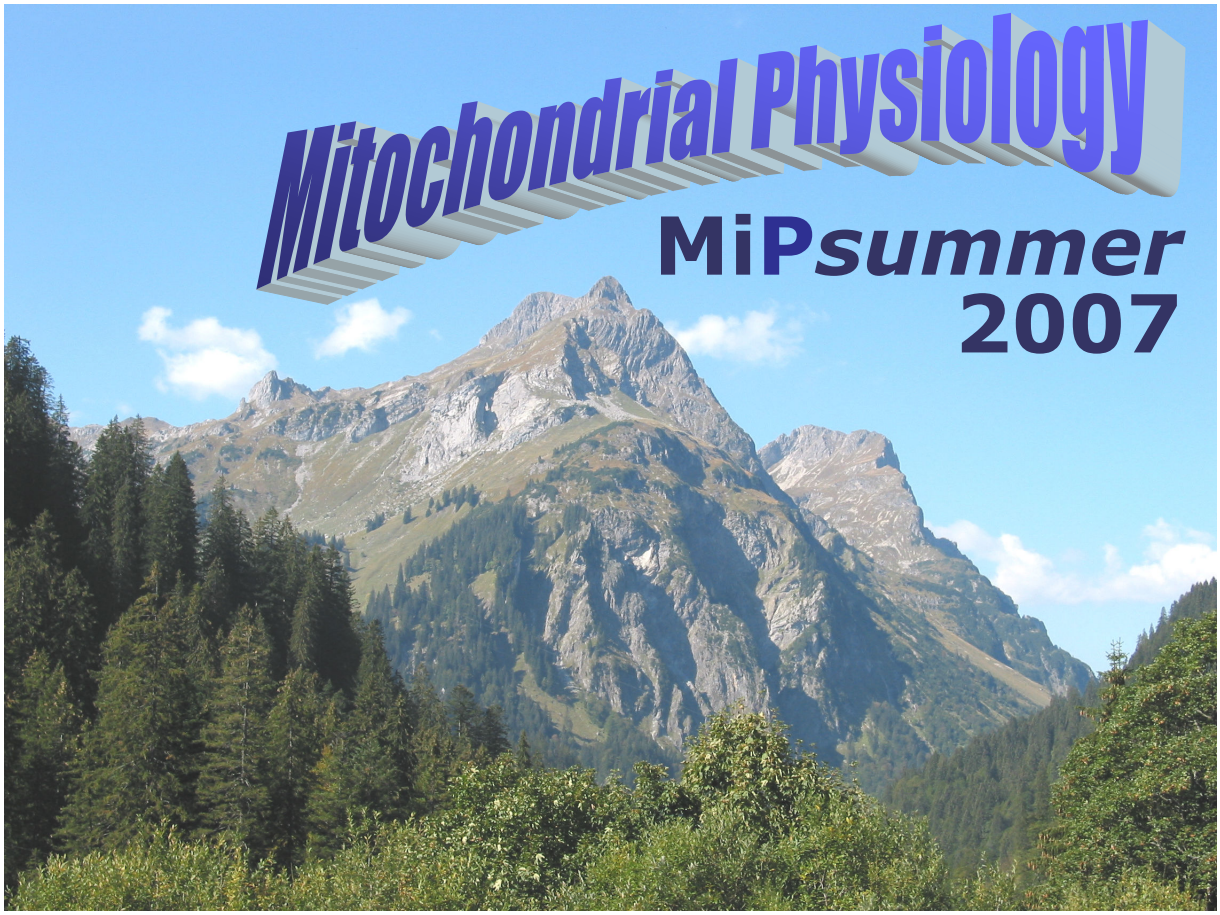

Mitochondrial Physiology Network **12.18**: 1-28 (2007) Last update: 2007-07-12

MiP*summer* 2007

1st MiP*summer* Schol on
Mitochondrial Respiratory Physiology
12-18 July 2007 Schröcken, Vorarlberg, Austria

Programme and Abstracts

Local Organizers

Erich Gnaiger (Innsbruck, Austria)
Hélène Lemieux (Innsbruck, Austria)
Francesca Scandurra (Innsbruck, Austria)

MiP*summer* Partners

MiP*summer* Financial Support:

Novartis Pharma AG, CH-4002 Basel, Switzerland. - Novartis Institutes for BioMedical Research (NIBR)

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Program overview **Lectures: 45+15 min; Poster flashes: 5+5 min**

Thursday, 12. July

Afternoon	Registration at Hotel Mohnenfluh, check-in
19:00	Welcome reception and dinner at Hotel Mohnehnfluh

Friday, 13. July – Tuesday, 17. July

07:30-08:45	Breakfast
	Morning lectures: Gemeindesaal Schröcken (Lecture Hall)
09:00-10:00	Lecture 1
10:00-11:00	Lecture 2
11:00-11:45	Coffee break
11:45-12:45	Lecture 3
13:00-15:00	Break: Lunch; alpine walks and talks.
15:00-19:00	Workshops; Poster flash presentations and poster sessions; Special interest sessions; Discussions, with coffee break
20:00	Dinner
Monday, 16. July	*(in case of favourable weather conditions):*
12:00	**MiP*summer* Walk** to the cheese and wine reception at the *Alpmuseum uf m Tannberg*; refreshment in the lake Körebersee or at Hotel Körbersee; **MiP*summer* Party.**
Wednesd., 18. July	Departure in the early morning

MiP*summer* 2007

The programme of the 1st MiP*summer* School on "Mitochondrial Respiratory Phyisology" provides a balance between introductions into the basic concepts, advanced methodological and scientific approaches, and specific applications, with a focus on mitochondrial respiratory function linked to the general theme of MiP with basic scientific and clinical perspectives.

Sessions Page

MiP*summer* Abstracts Page

Special MiP*summer* Events

Thursday, 12. July

19:00 Welcome reception and dinner at Hotel Mohnehnfluh

Sunday, 15. July

20:15 **Open air folk music** – garden of Hotel Mohnenfluh.

Monday, 16. July

12:00 **MiP*summer* Walk** to the cheese and wine reception at the *Alpmuseum uf m Tannberg*; refreshment in the lake Körebersee or at Hotel Körbersee.

18:30 **MiP*summer* Grill Party.**

21:00 **Meeting of the MiP*board*** – MiP2008 (round table conference), MiP2009 (joint conference with UMDF), future perspectives (open for all participants).

Tuesday, 17. July

22:00 After dinner MiP*summer* Lecture by **Charles Hoppel** (Cleveland, US) Inner mitochondrial membrane and outer space.

Wednesday 18. – Sunday 22. July

Satellite: 41st International Course on High-Resolution Respirometry. Hotel Mohnenfluh, Schröcken, Austria.
OROBOROS INSTRUMENTS – www.oroboros.at

1 - Friday, 13. July

MiP*summer* Morning Lectures. **Mitochondrial respiratory capacity, coupling and respiratory control.**

Chair: Steven Hand (US), Susana Cadenas (ES)

1L-01. 09:00-10:00 **Erich Gnaiger** (Innsbruck, AT) MitoPathways and mitochondrial respiration in top gear – an introduction to the concept of the 'Q-junction' and respiratory control with multiple substrates.
Reading: Gnaiger E (2007) in: *Mitochondrial Pathways and Respiratory Control.* OROBOROS MiPNet Publications, Innsbruck: pp. 21-37.

1L-02. 10:00-11:00 **Erich Gnaiger** (Innsbruck, AT) P/O, respiratory and uncoupling control ratios – established concepts re-visited.
Reading: Gnaiger E (2001) *Respir. Physiol.* 128: 277-297.

11:00 Coffee

1L-03. 11:45-12:45 **Kathrin Renner** (Regensburg, DE) Development of a respiratory protocol to detect mitochondrial defects under drug treatment.
Reading: Renner *et al* (2003) *Biochim. Biophys. Acta* 1642: 115-123.

13:00 Lunch

MiP*summer* Afternoon Workshop. **Permeabilized muscle fibers and high-resolution respirometry.**

1W. 15:00-19:00 **Hélène Lemieux**, Erich Gnaiger (Innsbruck, AT)

1. Permeabilized fiber preparation: Mouse heart
2. Multiple substrate/inhibitor protocol: A lecture hall experiment on-line
3. Cytochrome *c* oxidase inhibitor titration – biochemical threshold and excess capacity

17:00-18:15 Coffee and Posters

Posters:

1P-01. **Marco Alves**, Paulo J Oliveira, Rui A Carvalho (PT) Preservation of cardiac mitochondrial function during ischemia and ischemia-reperfusion: role of the cardioplegic protection by Celsior and Histidine buffer solution.

1P-02. **Dominique-Marie Votion** (BE), Hélène Lemieux, Ange Mouithys-Mickalad, Didier Serteyn, Erich Gnaiger. Study of the mitochondrial respiration in the equine muscle with high-resolution respirometry: feasibility, preliminary results and potential applications.

1L-04. 18:15-19:00 **Hélène Lemieux** (Innsbruck, AT) High excess capacity of cytochrome *c* oxidase in permeabilized fibers of the mouse heart.
Reading: Lemeux H, Gnaiger E (2007) in: *Mitochondrial Pathways and Respiratory Control.* OROBOROS MiPNet Publications, Innsbruck: pp. 38-42.

20:00 Dinner at Hotel Mohnenfluh

2 - Saturday, 14. July

MiP*summer* Morning Lectures. **Basic science and clinical reality**

Chair: Kathrin Renner (DE), Erich Gnaiger (AT)

2L-01. 09:00-10:00 **Hong Kyu Lee** (Seoul, KR) The metabolic syndrome and mitochondrial function - towards an international mtDNA cybrid study group.

2L-02. 10:00-11:00 **Jaap Keijer** (Wageningen, NL) Bioactive food components and energy metabolism.

11:00 Coffee

2L-03. 11:45-12:45 **Vilma Borutaite** (Kaunas, LT) Mitochondrial regulation of cell death: NO and cytochrome *c* redox state.
Reading: Mander P *et al.*(2005) *J. Neurosci. Res.* 79: 208-215.

13:00 Lunch

MiP*summer* Special Topic: **Basic science and clinical reality**

2L-04. 15:00-15:45 **Hong Kyu Lee** (Seoul, KR) Genetic susceptibility of type 2 diabetes: genetic factors related to mitochondrial function and risk of diabetes mellitus.

Flash presentations of posters:

2P-01. 15:50-16:00 **Sandra Amaral**, Luís Martins, Paula C. Mota, Paulo J. Oliveira, João Ramalho-Santos (PT) Mitochondrial testicular function: Effects of age and diabetes.

2P-02. 16:00-16:10 **Timothy R Koves**, Noland RC, Slentz DS, Ilkayeva O, Newgard CB, Muoio DM (US) Mitochondrial dysfunction and metabolic inflexibility in models of aging and diet-induced obesity.

2P-03. 16:10-16:20 **Joris Hoeks**, JJ Briedé, J de Vogel, G Schaart, M Nabben, E Moonen-Kornips, MKC Hesselink, P Schrauwen (NL) Mitochondrial function, -content and -ROS production in rat skeletal muscle: effect of high-fat feeding.

2P-04. 16:20-16:30 **Maxi Meissner**, G Henk Visser, Frans Stellaard, F Kuipers (NL) Bile acid signaling, FXR and energy expenditure – novel perspectives for the treatment of metabolic diseases.

16:30–19:00 Coffee and Posters

2P-05. **Ruwen Böhm**, L Schild, Th Herdegen (DE) Different approaches to assessing mitochondrial function and their utility and accuracy for pharmaceutical testing.

2P-06. Peter Lindinger, **Martine Calame**, Matthias Hoch, Alex N Eberle, Ralph Peterli, Thomas Peters, Sjouke Hoving, Hans Voshol, Ian Fearnley, John E Walker (CH) Proteome analysis of mitochondria in adipose tissues of Bariatric patients: Identification of risk factors and/or predictors for the treatment outcome.

2P-07. **Alena Čížková**, Stránecký V, Ivánek R, Hartmannová H, Nosková L, Piherová L, Tesařová M, Hansíková H, Honzík T, Zeman J, Divina P, Paul J, Houštěk J, Kmoch S (CZ) h-MitoArray and gene expression analysis in 13 patients with mitochondrial ATP synthase deficiency.

2P-08. **Daniela Fornuskova**, Stiburek L, Pejznochova M, Zeman J (CZ) Depletion of COX5A subunit in HEK293 cells.

2P-09. **Virginie Rhein**, G Baysang, F Meier, L Ozmen, H Bluethmann, E Savaskan, F Müller-Spahn, C Czech, J Götz, A Eckert (CH) Changes in mitochondrial respiration in brain cells of transgenic mice models of Alzheimer's disease

2P-10. **Olga Brantova**, J Sladkova, H Hansikova, J Zeman (CZ) Fibroblasts cell culture as model for apoptosis detection.

2P-11. **Karin Eberhart**, Ireen Ritter, Birgit Timischl, Reinhard Kofler, Peter Oefner, Kathrin Renner (DE) Synergistic effect of glucocorticoids and 2-deoxyglucose - energy metabolism as a new target in the treatment of leukemia.

2P-12. **Zdeněk Paris**, Julius Lukeš (CZ) Functional analysis of genes encoding mitochondrial proteins in *Trypanosoma brucei.*

20:00 Dinner at Hotel Mohnenfluh

3 - Sunday, 15. July

MiP*summer* Morning Lectures: **Oxygen kinetics of mitochondrial respiration and inhibition by nitric oxide**

Chair: Vilma Borutaite (LT), Jaap Kejer (NL)

3L-01. 09:00-10:00 **Erich Gnaiger** (Innsbruck, AT) Respiratory control by oxygen pressure under normoxia and hypoxia.
Reading: Gnaiger E (2003) *Adv. Exp. Med. Biol.* 543: 39-55.

3L-02. 10:00-11:00 **Alessandro Giuffre** (Rome, IT) Inhibition of respiratory oxidases by NO: Mechanistic and physiological aspects.
Reading: Brunori M *et al* (2006) *Biochim. Biophys. Acta.* 1757:1144-1154.

11:00 Coffee

3L-03. 11:45-12:45 **Susana Cadenas** (Madrid, ES) Inhibition of cellular respiration by edogenous nitric oxide and relative sensitivity of the main two cellular targets of nitric oxide.
Reading: Rodríguez-Juárez *et al* (2007) *Biochem. J.* 405: 223–231.

13:00 Lunch

MiP*summer* Afternoon Workshop: **Spectrophotometry and oxygen kinetics. – NO and kinetic models.**

3W. 15:00-17:00 **Natascha Sommer** (DE), Francesca Scandurra, Thomas Derfuss, Erich Gnaiger. Simultaneous remission spectrophotometry and high-resolution respirometry. Respiratory control by oxygen and redox states of mitochondrial cytochromes in cells: Instrument demonstration.

17:00-18:00 Coffee and Posters

3P-01. **Enara Aguirre**, F Rodríguez-Juárez, E Gnaiger, S Cadenas (ES) Inhibition of cellular respiration by nitric oxide during normoxia and hypoxia.

3P-02. **Felix Rodríguez-Juárez**, E Aguirre, S Cadenas (ES) Relative sensitivity of soluble guanylate cyclase and mitochondrial respiration to endogenous nitric oxide at physiological oxygen concentration.

3D. 18:00-18:30 **Discussion: Oxygen kinetics, NO and models of competitive and non-competitive inhibition.**

19:00 Dinner at Hotel Mohnenfluh.

20:15 **Open air folk music** – garden of Hotel Mohnenfluh.

4 - Monday, 16. July

MiP*summer* Morning Lectures: **Mitochondrial membrane potential and respiratory control**

Chair: Rodrigue Rossignol (FR), Alessandro Giuffre (IT)

4L-01 09:00-10:00 **Vilma Borutaite** (Kaunas, LT) Mitochondrial membrane potential and respiratory control.

4L-02 10:00-11:00 **Steven C Hand** (Baton Rouge, US) Mitochondrial membrane potential, pH gradients, and redox state.
Reading: Menze MA *et al* (2005) *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289: R68–R76.

11:00 Coffee and Posters

Posters:

4P-01. **Magdalena Labieniec**, Cezary Watala, Dominika Malinska (PL) Resorcylidene aminoguanidine (RAG) affects calcium mobilization and transmembrane potential in isolated rat liver mitochondria without impairments of respiratory chain.

Flash presentations and posters:

4P-02. 11:30-11:40 **Dominika Malińska**, Adam Szewczyk (PL) Mitochondrial potassium channel opener BMS-191095l – cytoprotective properties and the influence on mitochondrial physiology.

4P-03. 11:40-11:50 **David Julian**, Allman Rollins (US) Mitochondrial movement is by Brownian motion in coelomocytes from an annelid worm.

In case of favourable weather conditions:

12:00 **MiP*summer* Walk** to the cheese and wine reception at the *Alpmuseum uf m Tannberg*; refreshment in the lake Körebersee or at Hotel Körbersee.

18:30 **MiP*summer* Grill Party.**

21:00 **Meeting of the MiP*board*** – MiP2008 (round table conference), MiP2009 (joint conference with UMDF), future perspectives (open for all participants).

5 - Tuesday, 17. July

MiP*summer* Morning Lectures: **Basic science and clinical reality**

Chair: Hong Kyu Lee (KR), Dominique-Marie Votion (BE)

5L-01. 09:00-10:00 **Rodrigue Rossignol** (Bordeaux, FR) Mitochondrial bioenergetics and structural network organization.

5L-02. 10:00-11:00 **Enrico Calzia** (Ulm, DE) Small and large animal models of sepsis and I/R-injury - how to combine basic science and clinical reality.

11:00 Coffee

5L-03. 11:45-12:45 **Charles Hoppel** (Cleveland, US) Integrated mitochondrial function and the clinical utility of polarographic analysis.

13:00 Lunch

15:00 **MiP*summer*** Afternoon Workshop: Lectures on open topics; Round table discussion; Summary - evaluation

20:00 MiP*summer* Dinner at Hotel Mohnenfluh

5A. 22:00 **Charles Hoppel** (Cleveland, US) Inner mitochondrial membrane and outer space.

MiP*summer* Abstracts

Day 1: Cardiac and Skeletal Muscle Mitochondria

1L-01. MitoPathways and mitochondrial respiration in top gear – an introduction to the concept of the 'Q-junction' and respiratory control with multiple substrates.

Erich Gnaiger (Innsbruck, AT)
Medical University of Innsbruck, Dept. General and Transplant Surgery, D. Swarovski Research Laboratory, A-6020 Innsbruck, Austria. – erich.gnaiger@i-med.ac.at

Oxidative phosphorylation (OXPHOS) is a key element of bioenergetics, extensively studied to resolve the mechanisms of energy transduction in the mitochondrial electron transport system and analyze various modes of mitochondrial respiratory control. OXPHOS flux control is exerted by (i) coupling of electron transport to proton translocation and ATP synthesis mediated by the proton motive force, (ii) catalytic capacities of respiratory complexes, carriers and transporters, (iii) kinetic regulation by ADP, oxygen and various substrates feeding electrons into the respiratory chain, and (iv) specific inhibitors such as NO. Electrons flow to oxygen along linear thermodynamic cascades (electron transport chains) from either Complex I with three coupling sites, or from Complex II with two coupling sites. These pathways of electron transport are conventionally separated by using either NADH-linked substrates, such as pyruvate+malate, or the classical succinate+rotenone combination, to analyze site-specific H^+/e and P/O ratios or defects of specific respiratory complexes in functional diagnosis. The experimental separation of various electron transport pathways is common to the extent of establishing a bioenergetic paradigm in studies of OXPHOS. This bioenergetic paradigm is extended by a perspective of mitochondrial physiology emerging from a series of studies based on high-resolution respirometry (OROBOROS Oxygraph-2k).

Proper understanding and evaluation of the functional design of the OXPHOS system requires a transition from analyses of the electron transport *chain* (ETC) to a perspective of the convergent structure of electron flow to the Q-junction in the mitochondrial electron transport *system* (ETS). Electron transport capacity of cells *in vivo* is generally underestimated on the basis of the 'State 3 paradigm' and conventional respiratory protocols applied with isolated mitochondria, permeabilized cells or tissues, with profound implications on studies of biochemical thresholds, excess capacities and flux control coefficients of various mitochondrial enzymes.

The present overview on 'Mitochondrial Pathways and Respiratory Control' combines concepts of bioenergetics and cell metabolism leading to a new understanding of mitochondrial respiratory physiology and application of substrate combinations in novel Oxygraph assays. (i) Convergent electron flow through respiratory Complexes I and II (CI+II e-input) and through glycerophosphate dehydrogenase and electron-transferring flavoprotein exert additive effects on respiratory flux, increasing coupled respiration 1.3- to 2-fold relative to State 3 with Complex I substrates. (ii) Uncoupled respiration in living cells represents OXPHOS capacity only in cases when the phosphorylation system (adenylate nucleotide translocase, phosphate carrier, ATP synthase) does not exert control over coupled respiration. Mitochondrial oxidation *in vivo* is coupled to phosphorylation. A shift towards control by the phosphorylation system is observed when electron transport capacity is increased by convergent electron input into the Q-junction. (iii) Respiratory control ratios and uncoupling control ratios need to be combined for proper evaluation of coupling in OXPHOS.

Convergent CI+II e-input corresponds to the operation of the citric acid cycle and mitochondrial substrate supply *in vivo*. By establishing the reference state of maximum coupled respiration, convergent CI+II e-input provides the proper basis for (i) quantifying excess capacities of Complexes III and IV, (ii) interpreting flux control by various components such as the phosphorylation system or COX, and (iii) for evaluation of specific enzymatic defects in the context of mitochondrial respiratory physiology and pathology. Applicaton of substrate combinations in multiple substrate/inhibitor titration

protocols extends conventional bioenergetic studies to the level of mitochondrial physiology applied for the diagnosis of respiratory control in health and disease.

Gnaiger E (2007) Mitochondrial pathways through Complexes I+II: Convergent electron transport at the Q-junction and additive effect of substrate combinations. In: *Mitochondrial Pathways and Respiratory Control.* OROBOROS MiPNet Publications, Innsbruck: pp. 21-37.

1L-02. P/O, respiratory and uncoupling control ratios – established concepts re-visited.

Erich Gnaiger (Innsbruck, AT)

Medical University of Innsbruck, Dept. General and Transplant Surgery, D. Swarovski Research Laboratory, A-6020 Innsbruck, Austria. – erich.gnaiger@i-med.ac.at

Respiratory steady states have been defined by Chance and Williams (1956) according to a protocol for oxygraphic experiments with isolated mitochondria, for studies of mitochondrial respiratory control. As simple as they may appear, measurements of respiratory States (2-3-4) and respiratory control ratios (RCR) need to be well designed and concepts to be clarified. With proper expression of normalized respiratory fluxes (the inverse of the conventional RCR), a linear relationship exists between P/O ratios and 'RCR'. Changes of the 'RCR' may not merely be caused by uncoupling, but frequently are caused by alterations of catalytic OXPHOS capacities, including the phosphorylation system. Interpretation of 'RCR', therefore, is complicated in cases of multiple mitochondrial defects. Experimental examples will be presented on measurements of P/O ratios and respiratory and uncoupling control ratios, with discussion and critical evaluation of results.

Gnaiger E (2007) Respiratory states and flux control ratios. In: *Mitochondrial Pathways and Respiratory Control.* OROBOROS MiPNet Publications, Innsbruck: pp. 43-50.

Hütter E, Renner K, Pfister G, Stöckl P, Jansen-Dürr P, Gnaiger E (2004) Senescence-associated changes in respiration and oxidative phosphorylation in primary human fibroblasts. *Biochem. J.* 380: 919-928.

Kuznetsov AV, Schneeberger S, Seiler R, Brandacher G, Mark W, Steurer W, Saks V, Usson Y, Margreiter R, Gnaiger E (2004) Mitochondrial defects and heterogeneous cytochrome *c* release after cardiac cold ischemia and reperfusion. *Am. J. Physiol. Heart Circ. Physiol.* 286: H1633–H1641.

Stadlmann S, Rieger G, Amberger A, Kuznetsov AV, Margreiter R, Gnaiger E (2002) H_2O_2-mediated oxidative stress versus cold ischemia-reperfusion: mitochondrial respiratory defects in cultured human endothelial cells. *Transplantation* 74: 1800-1803.

1L-04. High excess capacity of cytochrome *c* oxidase in permeabilized fibers of the mouse heart.

Hélène Lemieux, Erich Gnaiger

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Metabolic flux control analysis and the concept of excess capacity of enzymes over pathway flux are related by the functional threshold, at which damage or inhibition of an enzyme reduces excess capacity to a minimum and start to affect overall flux of the pathway. Excess capacity of cytochrome *c* oxidase (COX) varies between tissues, but little is known about differences between species. In particular, information is lacking on mitochondrial respiratory function in the mouse heart, despite the fact that mutant mice provide increasingly important animal models. Permeabilized muscle fibers were prepared from the left ventricle of a single mouse heart, and measured in OROBOROS Oxygraph-2k instruments in parallel at 4, 25, 30, 37 and 40 °C ($N \geq 4$)[1]. Threshold plots were constructed from azide titrations of flux through the electron transport chain (parallel e-input into complexes I+II with malate+pyruvate+glutamate+succinate and uncoupling by FCCP), versus COX (0.5 mM TMPD+2 mM ascorbate after uncoupling and inhibition by rotenone+malonate+antimycin)[2] A). Azide was used, since inhibition of COX by cyanide is reversed by pyruvate particularly at low oxygen levels. The inhibition constant, K_i, of COX for azide was 0.1 mM at 37 °C, increasing from 4 to 40 °C over two orders of magnitude. COX velocity measured with TMPD+ascorbate was 1.3-fold of maximum electron transport capacity of the respiratory chain at 25 to 40 °C, and 3.3-

fold at 4 °C. In contrast, linear extrapolations of the threshold plots revealed a COX excess capacity of 1.6-fold over pathway flux in the range of 30 to 40 °C, increasing to 1.8- and 7.6-fold at 25 °C and 4 °C, respectively. Application of complex I substrates only, would yield an apparent COX excess capacity of >3-fold over pathway flux (at 30 and 37 °C), since parallel e-input through complex I+II doubled flux compared to complex I substrates. Taken together, COX excess capacity in myocardial fibers of the mouse was significantly higher than in fibers of rat heart or human skeletal muscle. Results obtained under hypothermic incubation conditions of permeabilzed fibers may be extrapolated to physiological temperature of 37 °C with caution only. The very high COX excess capacity under hypothermia (4 °C) may compensate for hypothermic hypoxia by decreasing the p_{50} of mitochondrial respiration in parallel to the decreased p_{50} of hemoglobin and myoglobin. The present study yields an important baseline for further investigations of mitochondrial function in the mouse heart, including genetic models of acquired and inherited mitochondrial defects.

1. Lemieux H, Garedew A, Blier PU, Tardif J-C, Gnaiger E (2006) Temperature effects on the control and capacity of mitochondrial respiration in permeabilized fibers of the mouse heart. *Biochim. Biophys. Acta* 1757 (5-6, Suppl. 1): 201-202.
2. Garedew A, Lemieux H, Schachner T, Blier PU, Tardif J-C, Gnaiger E (2006) High excess capacity of cytochrome *c* oxidase in permeabilized fibers of the mouse heart. *Biochim. Biophys. Acta* 1757 (5-6, Suppl. 1): 167-168

1P-01. Preservation of cardiac mitochondrial function during ischemia and ischemia-reperfusion: Role of the cardioplegic protection by Celsior and Histidine buffer solution.

Marco Alves[2], Paulo J. Oliveira[2], Rui A. Carvalho[1]

[1] *Department of Biochemistry, NMR Center, University of Coimbra, Portugal*

[2]*Center for Neurosciences and Cell Biology, University of Coimbra, Portugal* - *alvesmarc@gmail.com*

Cardiac ischemia occurs when a reduced coronary blood flow occurs, which results in a decrease of oxygen and nutrients supply contributing to several pathophysiologies (e.g. myocardial infarction, peripheral vascular insufficiency, stroke and hypovolemic shock). The restoration of blood flow to an ischemic organ is essential to prevent irreversible injury. Paradoxically, reperfusion induces more damage to the tissue than ischemia alone. During heart transplantation, various stressful conditions occur which include long periods of ischemic organ storage in cold cardioplegic solutions followed by transplant and reperfusion. Since ATP production in mitochondria is essential to the heart in order to maintain contractile activity, mitochondrial function may be the main mediator of ischemia and ischemia-reperfusion injury.

We focused on two selected cardioplegic solutions: Histidine Buffer Solution (HBS) and Celsior Solution (CS) and their ability to protect cardiac mitochondrial function. A standard Krebs-Henseleit (K-H) Solution was used as negative control. Male and Female Wistar rats (6-8 weeks) were each divided in 2 sets of groups. Isolated hearts from both sets were subjected to different ischemia periods (0, 4 and 6 h) and immersed in the different preservation solutions at 4 ºC. Subsequently, heart mitochondria (HM) of one experimental group were immediately isolated using standard procedures, while the hearts from another group were subjected to 30 minutes reperfusion in a Langendorf column prior to HM isolation. Two different control groups were used. In one of them HM were immediately isolated after animal sacrifice. In the second group (reperfusion control), hearts were excised and reperfused during 30 minutes. A Clark-type oxygen electrode was used to measure mitochondrial respiration parameters. HM transmembrane electric potential was measured with a TPP^+- selective electrode.

Ischemia, particularly for 6 h, induced damage in some mitochondrial parameters (e.g. ADP-induced depolarization and lag phase), although the majority of indexes evaluated were unchanged for both 4 and 6 h. Reperfusion particularly induced damage to some mitochondrial parameters (e.g. lag phase, state 3 and FCCP respiration or maximum potential attained). Some parameters such as the Respiratory Control Rate, FCCP-stimulated respiration or Potential Recuperation recovery after ADP phosphorylation

with complex I substrates were especially affected by reperfusion, which suggests that this complex is particularly affected by reperfusion.

The data confirms reperfusion as the main damage generator to HM. Most importantly, the results suggest that the CS is more effective in preventing HM damage.

Supported by FCT: SFRH/BD/31655/2006; POCI/SAU-OBS/55802/2004

1P-02. Study of the mitochondrial respiration in the equine muscle with high-resolution respirometry: Feasibility, preliminary results and potential applications.

Dominique-Marie Votion[1a,b], Hélène Lemieux[2], Ange Mouithys-Mickalad[3], Didier Serteyn[1a], Erich Gnaiger[2]

[1a] *Equine Teaching Hospital, Department of Clinical Sciences, Faculty of Veterinary Medicine and* [1b]*Equine European Centre of Mont-le-Soie, University of Liège, Sart Tilman, B-4000 Liège, Belgium*

[2] *Medical University of Innsbruck, Dept. General and Transplant Surgery, D. Swarovski Research Laboratory, A-6020 Innsbruck, Austria.*

[3] *Centre of Oxygen: Research and Development (C.O.R.D.), University of Liège, Sart Tilman, B-4000 Liège, Belgium* - dominique.votion@ulg.ac.be

Equines have been bred since time in memoriam to a large variety of uses of high specificity. Regardless of their intended use, horses are able to perform physical activities at a level that surpasses other animals of similar body size. The athletic potential of an individual relates to oxygen (O_2) transport and utilisation[1]. As regard to the maximal oxygen consumption (VO_{2max}) which is considered as an index of exercise's capacity[2], the horse is an amazing athletic animal. For example, when expressed on a mass specific basis, race horses have values for VO_{2max} *twice* those of elite human athletes[3,4]. During high-intensity exercise, the large VO_{2max} is achieved as a result of remarkable cardiopulmonary adaptations. It might be expected that the respiratory capacity of equine muscle would be also proportionately higher than the one of human. Measurements of the maximal respiratory capacity of the equine skeletal muscle are scarce[5]. However, it has always been the ambition of physiologists to determine athletic suitability for specific disciplines and to predict athletic performance.

Up to now, cellular energetics of muscles has been based upon the histochemical and biochemical analyses of large biopsy samples[6] (200 to 300 mg of muscle). Given the little practicality of performing muscle biopsy in performance horses, the technique has been limited to scientific protocols or to the evaluation of horses with suspected myopathy[7].

High-resolution respirometry offers the opportunity to perform bioenergetics' studies with a minimal amount of sample using permeabilised tissue[8]. This technique precludes the use of time-consuming preparation of isolated mitochondria and enables the study of mitochondrial function within a preserved intramitochondrial environment. We aimed at studying the feasibility of high-resolution respirometry for investigating the mitochondrial respiratory function of the equine muscles with permeabilised fibres obtained by microsampling.

Microbiopsies were performed in the *triceps brachii* of 3 horses with a 14 G biopsy needle that enables the sampling of 20 to 40 mg of muscle. The specimens were prepared according to the Oroboros Oxygraph-2k manual. From 1 to 4 mg wet weight of sample were put in Oxygraph-2k chambers filled with media warmed at 37°C in presence of malate plus glutamate. Oxygen was injected into the chambers in order to reach a 500 nmol/ml O_2 concentration. Then, the experiment starts with the measurement of *State 2* respiration after steady-state respiratory flux. *State 3* of respiration was initiated by adding ADP in excess. Integrity of mitochondria was estimated by assaying the mixture with Cyt *c*. A further increase of respiration was stimulated by adding succinate. After that, uncoupling of oxidative phosphorylation by stepwise titration of FCCP was used to obtain the maximal stimulation of flux. Finally, the respiration was inhibited by the addition of rotenone (inhibition of complex I) and antimycin A (inhibition of complex III). All measurements were performed at steady-state.

A single biopsy enabled to perform at least 4 titration protocols that was completed within 1.5 hour. The O_2 concentration remained above 240 nmol/ml for all the procedure

which may of importance to avoid O_2 dependence of results. Integrity of mitochondrial function in samples preserved for 4 days (at 4°C in BIOPS) did not demonstrate any alteration thus enabling to delay analysis (and open many perspectives such as sampling during field trials). These preliminary results demonstrated the feasibility of studying mitochondrial respiration in the equine muscle based on microsamples which may, with no doubt, be performed on performance horses without consequence (no scar, no pain, no sedation required). It is now necessary to evaluate the variability between runs, to determine reference ranges taking into account all the parameters that may influence results (age, gender, breed, level of training etc.) and to compare mitochondrial physiology with the one of other athletic and non athletic species.
Such a database will offer the possibility to study the relationship between mitochondrial function and parameters of athletic capacity as well as to the pathophysiological mechanism underlying equine myopathic disorders.

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1. Poole DC. Current concepts of oxygen transport during exercise. *Equine and Comparative Exercise Physiol.* 2004; 1:5-22
2. Bassett DR Jr, Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc.* 2000; 32:70-84.
3. Hörnicke H, Meixner R, Pollmann U. Respiration in exercising horses. In: Snow DH, Persson SGB, Rose RJ, eds. Equine Exercise Physiology. (Proceedings of the first International Conference on Equine Exercise Physiology, Oxford: Great Britain), Cambridge: Granta Editions. 1982; 7-16.
4. Saltin B, Åstrand PO. Maximal oxygen uptake in athletes. *J Appl Physiol.* 1967; 23:353-358.
5. Gollnick PD, Bertocci LA, Kelso TB, Witt EH, Hodgson DR. The effect of high-intensity exercise on the respiratory capacity of skeletal muscle. *Pflugers Arch.* 1990; 415:407-13.
6. Votion DM, Navet R, Lacombe VA, Sluse F, Essén-Gustavsson B, Hinchcliff KW, Rivero JLL, Serteyn D, Valberg SJ. Muscle energetics in exercising horses. *Equine and Comparative Exercise Physiology.* 2007; *In Press.*
7. Ledwith A, McGowan CM. Muscle biopsy: a routine diagnostic procedure. *Equine Vet Ed*. 2004; 16:62-67.
8. Kuznetsov AV, Strobl D, Ruttmann E, Königsrainer A, Margreiter R., Gnaiger E. Evaluation of mitochondrial respiratory function in small biopsies of liver. *Analyt Biochem*. 2002; 305:186-194.
9. Valberg SJ, Carlson GP, Cardinet GH, Birks EK, Jones JH, Chomyn A, DiMauro S. Skeletal muscle mitochondrial myopathy as a cause of exercise intolerance in a horse. *Muscle Nerve*. 1994; 17:305-312.

Day 2: Basic Science and Clinical Reality

2L-01. The metabolic syndrome and mitochondrial function - towards an international mtDNA cybrid study group.

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Reading:

Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. Diabetes. 2006 Dec;55 Suppl 2:S9-S15
Cho YM, Park KS, Lee HK. Genetic factors related to mitochondrial function and risk of diabetes mellitus. Diabetes Res Clin Pract. 2007 Apr 20;
Fuku N, Park KS, Yamada Y, Nishigaki Y, Cho YM, Matsuo H, Segawa T, Watanabe S, Kato K, Yokoi K, Nozawa Y, Lee HK, Tanaka M. Mitochondrial haplogroup N9a confers resistance against type 2 diabetes in Asians. Am J Hum Genet. 2007 Mar;80(3):407-15
Lee HK, Park KS, Cho YM, Lee YY, Pak YK. Mitochondria-based model for fetal origin of adult disease and insulin resistance. Ann N Y Acad Sci. 2005 May;1042:1-18.
West GB, Woodruff WH, Brown JH. Allometric scaling of metabolic rate from molecules and mitochondria to cells and mammals. Proc Natl Acad Sci U S A. 2002 Feb 19;99 Suppl 1:2473-8.
West GB, Brown JH, Enquist BJ. A general model for the origin of allometric scaling laws in biology. Science. 1997 Apr 4;276(5309):122-6.

Lee DH, Lee IK, Jin SH, Steffes M, Jacobs DR Jr. Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. Diabetes Care. 2007 Mar;30(3):622-8.

Shertzer HG, Genter MB, Shen D, Nebert DW, Chen Y, Dalton TP. TCDD decreases ATP levels and increases reactive oxygen production through changes in mitochondrial F(0)F(1)-ATP synthase and ubiquinone. Toxicol Appl Pharmacol. 2006 Dec 15;217(3):363-74.

2P-01. Mitochondrial testicular function: Effects of age and diabetes.

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Mitochondrial oxidative phosphorylation is the major ATP synthesis pathway in eukaryotes. In this process, oxygen consumption is coupled to ADP phosphorylation with the intermediate generation of a pH and electric gradient across the inner mitochondrial membrane (delta pH and delta psi), respectively. Testicular dysfunction, decreased fertility potential, variations in semen quality and abnormalities in spermatogenesis in male animals have been described in both aging and in chronic diseases linked to oxidative stress, such as Diabetes Mellitus. Also, and most importantly, mitochondrial function is altered during diabetes. Taking into account that sperm motility depends on proper mitochondrial function, we analysed the bioenergetics of isolated rat testicular mitochondria by measuring oxygen consumption and the generation of the mitochondrial delta psi. We examined respiratory parameters and membrane potential with substrates for both complex I (NADH oxidation), and complex II (succinate oxidation) of the membrane respiratory chain. Additionally, we also assessed mitochondrial calcium loading capacity. Changes in mitochondrial function caused by aging were monitored in control rats (2 and 5 months old), while diabetes-induced changes were evaluated in a type I (streptozotocin-induced) diabetic rat model. The respiratory control ratio, an index of mitochondrial coupling between respiration and phosphorylation, was lower in older animals ($P \leq 0.01$) regardless of the substrate used, suggesting that mitochondrial functionality is affected in the testis of older males. In accordance with this finding, the maximal oxygen consumption sustained by the respiratory chain (uncoupled respiration) and delta-psi generated was also lower in older animals ($P \leq 0.01$), although their calcium loading capacity was increased, suggesting possible adaptations to an age-related increase in oxidative stress. Interestingly, respiratory function was similar in diabetic and control rats, again suggesting adaptations to the deleterious effects of the disease. This idea is reinforced by both the higher uncoupled respiratory rate, and the lower resting oxygen consumption (state 4) in diabetic animals ($P \leq 0.05$), the latter of which suggests a decrease in membrane proton leak. Taken together our results suggest that mitochondrial testicular function declines with age in rats. However, in previous work we found that older rats have higher sperm count and motility. Thus, it is reasonable to speculate that when mitochondrial function is affected by age, rodent sperm may switch to alternative metabolic pathways. On the other hand, diabetic animals appear to have some testicular mitochondrial adaptations to overcome the disease process.

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2P-02. Mitochondrial dysfunction and metabolic inflexibility in models of aging and diet-induced obesity.

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Obesity, diabetes, and aging are associated with declining skeletal muscle function and are often characterized by accumulation of intramuscular lipids and insulin resistance. Emerging data suggests that mitochondrial dysfunction may provide a common link among these pathophysiologic states. In ongoing studies using mass spectrometry-based metabolic profiling, we show that rodent models of aging and diet-

induced obesity exhibit muscle accumulation of most short, medium, and long-chain fatty acylcarnitine species, implying a heavy mitochondrial lipid burden. In addition, muscle levels of long and medium chain acylcarnitines correlated negatively with insulin sensitivity. Studies in isolated skeletal muscle mitochondria from aged animals fed on a year-long high fat diet displayed low rates of complete fat oxidation but high rates of incomplete oxidation along with "metabolic inflexibility" (impaired substrate switching from fatty acid to a carbohydrate-derived fuel). We propose that excessive β-oxidation and impaired mitochondrial substrate switching might contribute to skeletal muscle insulin resistance through mechanisms of oxidative stress. These possibilities, as well as the molecular basis of metabolic inflexibility, are under investigation

2P-03. Mitochondrial function, -content and -ROS production in rat skeletal muscle: effect of high-fat feeding.

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Aims/hypothesis: The accumulation of fat in skeletal muscle is involved in the development of type 2 diabetes mellitus and a diminished mitochondrial function has been suggested to be a primary cause of this muscular fat accumulation. Although the cause of this mitochondrial dysfunction remains unclear, there are indications that a high intake of dietary fat negatively affects indirect parameters of mitochondrial function. Whether a high intake of dietary fat diminishes intrinsic mitochondrial functioning is however unknown and subject of the present study.

Methods: Male Wistar rats were subjected to an 8-week low- vs. high-fat dietary intervention after which isolated skeletal muscle mitochondria were analyzed for functional capacity by respirometry and for ROS production by electron spin resonance spectroscopy. In addition, intramyocellular lipids (IMCL) were assessed by Oil Red O staining, peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PPARGC1A) protein and markers for mitochondrial content by western blotting.

Results: The high-fat dietary intervention did not affect intrinsic functioning of skeletal muscle mitochondria, neither on a carbohydrate- nor on a lipid substrate. Interestingly, in muscle homogenates PPARGC1A protein increased by ~2-fold upon high-fat feeding and the protein content of structural subunits of 4 different complexes in the respiratory chain tended to increase as well. Protein content of all these subunits correlated tightly with PPARGC1A protein content. IMCL levels increased significantly while mitochondrial ROS production remained unaffected.

Conclusions/interpretation: Despite normal (functional) or even improved (content) mitochondrial function, IMCL levels increased upon high fat feeding. This illustrates that a reduced mitochondrial function is not a prerequisite for muscular fat accumulation.

2P-04. Bile acid signaling, FXR and energy expenditure – novel perspectives for the treatment of metabolic diseases.

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The aim of this project is to elucidate the role of bile acid signaling via FXR in the modulation of energy expenditure to potentially allow for novel treatments of metabolic diseases, such as insulin resistance and type 2 diabetes.

The farnesoid X receptor (FXR) is a nuclear receptor that is predominantly expressed in enterohepatic tissues, as these are exposed to its ligands: bile acids. FXR has

applicability in metabolic diseases, for example: Hepatic expression of FXR is reduced in animal models of diabetes. FXR deficient mice display elevated circulating FFAs, which has been associated with elevated serum glucose levels, impaired glucose and insulin tolerance. FXR acts to maintain bile acid homeostasis, control glucose and lipo-protein metabolism, and regulate fuel availability upon fasting / refeeding. Thus FXR is an important metabolic regulator. Furthermore, FXR has recently been shown to be involved in the regulation of energy expenditure. Bile acid signaling appears to have a crucial role herein. However, the precise mechanisms and whether FXR plays a direct or indirect part in the regulation of energy expenditure are not yet understood and need careful investigation.

Using the doubly labeled water (DLW) technique to measure daily energy expenditure (DEE), 5 $Fxr^{-/-}$ and WT male mice were injected with DLW in a pilot study. Blood samples were taken via the tail at 25 hours to determine basal energy expenditure and upon a following 24 hour fast to determine fasted energy expenditure. Moreover using the DLW technique, the effect of bile acid sequestration on energy expenditure was studied in ob/ob and db/db mice after a 10 day diet supplemented with a bile acid binding compound. First results will be presented.

To further elucidate the role of bile acid signaling and FXR in energy expenditure, research perspective include to investigate mitochondrial function in metabolically active tissues and its relation to energy expenditure in WT and Fxr deficient mice that are on a normal diet, a high fat diet, supplemented with an FXR agonist or a bile acid sequestrant and in a model of cholestasis.

2P-05. Different approaches to assessing mitochondrial function and their utility and accuracy for pharmaceutical testing

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Mitochondria play a pivotal role for energy homoeostasis and apoptosis. Evidence has accumulated that these two primary functions are interacting with each other, e.g. disrupted oxidative phosphorylation might contribute to neurodegeneration in Parkinson's or Alzheimer's disease. Traditionally, oxidative phosphorylation is evaluated best using the Clark type electrode. The disadvantage for pharmaceutical testing comes from that substances have to be processed separately in a time consuming manner. Thus optical methods using resazurine or JC-1 were established. We have used these methods to quantify the impact of two c-Jun N-terminal kinases (JNK) inhibitors on mitochondrial function because JNKs are involved in apoptotic processes and cytochrome C release from mitochondria. However, the apparently interesting results turned out to be artifacts caused by the optical activity of the substances or biochemical reactions between resazurine and adjuvants / stabilizing agents like ascorbic acid. Furthermore, results obtained by these methods do not only reflect the oxidative phosphorylation function but also the function of other pathways like the tricarboxylic acid cycle or NADH-depleting reactions. Thus optical methods are inferior to the Clark type electrode concerning quantitative pharmaceutical testing.

2P-06. Analysis of mitochondria in adipose tissues of bariatric patients: Identification of risk factors and/or predictors for the treatment outcome.

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At the present bariatric surgery is recognized as the only reliable treatment modality to achieve weight loss in a majority of morbidly obese patients. However, complications

and/or absence of weight loss are observed in a number of cases, and this may be related to deficits in metabolic regulation. Recently, impaired mitochondrial activity was shown to be linked with the development of obesity and cardiovascular disease in an animal model (Wisløff *et al*, 2005). So far there are no predictors for the outcome of bariatric surgery. As a consequence we propose to analyse mitochondria at the sites where energy is stored and produced, i.e. in the human adipose tissue, with a particular focus on protein expression

This research project focuses on two major aims: (I) Identification of potential predictors for the outcome of bariatric intervention (proteome analysis of patients with vs. without weight loss), and (II) identification of novel mitochondria-based risk factors related to energy metabolism and obesity (comparison of morbidly obese patients before and after weight loss vs. lean control patients).

Isolated mitochondria populations are subjected to a series of enzymatic assays to characterize functionality of mitochondrial respiratory chain (Citrate synthase, individual complexes I-V of the respiratory chain, ATP production). In addition the mitochondrial count per adipocyte is under investigation using Taqman quantification. The differential proteomic analysis is based on two complementary techniques: (I) 2D Gel-Electrophoresis with isoelectric focusing and (II) pre-fractionation of the proteome by anion-exchange. MS identification will be performed for protein spots of interest.

In summary this project covers the combination of a carefully characterized patient population and a detailed molecular analysis of human fat tissue. Therefore, this integrated approach considering various important parameters may lead to better criteria for bariatric surgery and eventually pave the basis for a pharmacotherapy of morbid obesity.

2P-07. h-MitoArray and gene expression analysis in 13 patients with mitochondrial ATP synthase deficiency.

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Mitochondria generate much of the energy of the cell, produce most of the endogenous reactive oxygen species, regulate cellular redox state, cytosolic concentration of calcium and integrate many of the signals for initiating of apoptosis.

Defects of mitochondrial functions lead in humans to vast array of usually multisystemic pathologies. Several hundreds of diseases resulting from various defects of mitochondrial biogenesis and maintenance, defects of respiratory chain complexes (OXPHOS) or defects of individual mitochondrial proteins are known. To strengthen diagnostic work-up for various mitopathies we designed focused oligonucleotide microarray which allows expression profiling of 1632 human mitochondria-related genes and tested its performance in analysis of genetically heterogeneous group of 13 patients with biochemically proven ATP synthase deficiency. Gene expression data analysis allowed classification of patients into several distinct groups, provided information on subgroup and patient specific gene expression profiles, defined candidate disease causing genes and gave basic information on pathogenic mechanisms associated with ATP synthase deficiency.

2P-08. Depletion of COX5A subunit in HEK293 cells.

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COX5A is one of 13 structural subunits of cytochrome c oxidase (COX), the terminal enzyme of respiratory chain. We used RNAi to down-regulate steady-state level of COX5A

subunit and analyzed impact of its knock-down on COX assembly. We chose plasmid with short hairpins embedded within a miRNA transcript from the naturally occurring miR30 to mimic a natural microRNA primary transcript. The final transcript contains also GFP and Neomycin Phosphotransferase coding sequences situated tandemly, upstream of the miR30-like hairpins. For success in experiments based on RNAi an appropriate, high-throughput screening method estimating silencing potential of candidate shRNAs is important. We constructed seven derivatives of the plasmid coding for hairpins aimed at different positions of *COX5A* mRNA.

A long halflife of COX5A (the level of the protein is at least 48 hours post nucleofection unchanged on Western Blot) contradict the screening on protein level by imunodetection of endogenous COX5A. To circumvent the problem, we introduced COX5A coding sequence into the maxFP-Red – N plasmid to encode fusion protein. The marker plasmid was cotransfected with RNAi-mediating plasmid derivatives (the higher the level of fusion protein the higher the leakage of individual hairpin-mediated RISC systems). A fluorescence of fusion protein was found rapidly lower compared to maxFPRed marker and complicated a setting of FACS measurements, but Western Blot of fusion protein with COX5A antibody gave an acceptable result. To optimize the detection of RNAi-potential through fluorescence by FACS, we re-cloned COX5A coding sequence into 3´UTR of maxFPRed marker. The final transcript contains target sequence for RNAi but leads to translation of merely maxFPRed marker. Fluorescence intensities were comparable with that obtained at empty plasmid.

Based on the above-mentioned methods, we chose three candidate shRNAs and prepared stable cell lines, where depletion of COX5A level was confirmed to some extent using SDS immunoblotting. Also specific activity of COX was revealed decreased. BN-PAGE showed diminished level of COX holoenzyme and its assembly intermediates.

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2P-09. Changes in mitochondrial respiration in brain cells of transgenic mice models of Alzheimer's disease.

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Alzheimer's disease (AD) is the most frequent form of dementia among the elderly and is characterized by neuropathological hallmarks of extracellular amyloid plaques and intracellular neurofibrillary tangles in the brain of AD patients. Amyloid plaques are composed of the amyloid-beta (Aβ) protein, derived from its precursor protein APP. Neurofibrillary lesions are formed from paired helical filaments composed of hyperphosphorylated tau protein, a microtubule-associated-protein.

In addition, mitochondrial dysfunction and energy metabolism deficiencies are recognized as earliest events and correlated with impairments of cognitive abilities in AD. Nevertheless, the specific mechanisms leading to mitochondrial failure in AD are not well understood.

Interestingly, accruing data indicate that Aβ as well as tau pathology lead to mitochondrial dysfunction and increased levels of reactive oxygen species (ROS). Reduced ATP levels, impairments of mitochondrial membrane potential including reduced cytochrome c oxidase activity in APP transgenic mice and reduced complex I activity in P301L tau transgenic mice have been observed. However, how tau pathology mediates these changes and its role within the amyloid cascade remains unclear.

To better understand the direct impact of Aβ/tau interplay on mitochondria, we are currently investigating the brains of double (APP (KM670/671NL) / PS2 (N141I)) and triple (APP (KM670/671NL) / PS2 (N141I) / Tau (P301L)) transgenic mice at the age of 7-8, 12 and 16 months. Mitochondrial respiration is studied by the measurement of the oxygen consumption at 37° using an Oxygraph-2k system equipped with two chambers and Datlab software. State 2 respiration is measured after addition of malate/glutamate. Then, ADP is added to measure state 3 respiration. After determining coupled respiration,

FCCP is added and respiration is measured in the absence of a proton gradient. To check the integrity of the mitochondrial membrane cytochrome c is added. In order to inhibit complex I and III activities rotenone and antimycine A, respectively are added. Then, ascorbate and TMPD are added and respiration is measured. Finally, sodium azide is added to inhibit complex IV activity. Moreover, enzyme activities of these complexes, mitochondrial membrane potential, ATP levels and ROS production are determined.

First evidence indicates that the respiration of mitochondria of brain cells from the triple transgenic mice is reduced compared to the double transgenic mice without tau mutation. Based on our preliminary findings, we hypothesize that at the level of mitochondria, the two defining neuropathological AD proteins, tau and Aβ, seem to act in a synergistic or additive way finally leading to/accelerating neurodegenerative mechanisms.

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2P-10. Fibroblasts cell culture as model for apoptosis detection.

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Introduction: Abnormal apoptosis is one of the primary cause of various diseases such cancer, degenerative diseases, and autoimunne diseases.
Increased susceptibility to apoptosis has been shown in many models of mitochondrial defects but its relevance to human diseases is still discussed. As well, the potential role of apoptotic cell death in the development of the cellular and tissue lessions seen in lysosomal storage diseases, and particularly in neurological diseases is often discussed. The aim of our work was to introduce and to improve the methods for induction and detection of apoptosis in fibroblasts cell culture.

Material and methods: The study was performed on fibroblasts cell lines. Staurosporine, potent inhibitor of phospholipid/calcium-dependent protein kinase and Actinomycin D, an antineoplastic antibiotic inducing apoptosis were used as chemical apoptogen. Hydrogen peroxid (30uM), UV radiation (15 min.), serum starvation and heat shock (43°C for define time) were used for simulation of environmental stress.

Results: Hydrogen peroxid induced apoptosis during 2 hours of cultivation in contrast to staurosporine, which induced first apoptotic changes after 5 hours of cultivation. Serum starvation presents large delay between apotosis induction and appearance of first apoptotic events. Changes in morphology, such a cell shrinkage or membrane blebing under oxidative stress, advert to first apoptotic events and is visible in phase contrast. Mitochondrial membrane potential was measured by MitoTracker ROS or a dual-emission potential-sensitive probe JC-1. Western blot or cytochemistry exploitaging pattern of specific antibodies was used for detection of activated caspase 9 and caspase 3, which cleave cellular substrates in answer to realizing of cytochrome c in cytoplasma. Chromatin condensation and changes in nuclear morphology as apoptotic event was visualized by fluorescent dye Hoechst 33258. Ultrastructural changes in cell during apoptotic events reveals transmission electron microscopy.

Conclusion: We have verified most of mentioned methods on fibroblast cell culture. In our first approach, we came across some nonspecific difficulties during apoptosis induction such a heterogeneity in apoptotic start in one cell culture under same conditions, divergent sensitivity to one apoptogen in on culture, most of all dependent on localization of cells in cultivation flask. Furthermore fibroblasts present slow growth rate and lower susceptibility to apoptogen in comparison to transformed cell lines and demand very accurate confluence in all tested culture. Despite mentioned disadvantages, we conclude, that under precise defined conditions, fibroblast cell culture represent useful model for apoptosis induction, pursuing of apoptotic kinetics and detection of large scale of apoptotic event on structural, biochemical and ultrastructural level. Consequently, we are going to test suitability of another methods, relating to apoptose, for fibroblast cell culture.

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2P-11. Synergistic effect of glucocorticoids and 2-deoxyglucose - energy metabolism as a new target in the treatment of leukemia.

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Glucocorticoids (GC) are used in the therapy of acute lymphoblastic leukemia (ALL) in combination with other chemotherapeutics because of their ability to induce cell cycle arrest and apoptosis. Despite a relatively high survival rate, the therapy has severe side effects and resistance develops frequently. The underlying mechanism of GC induced apoptosis is still unclear. Recent gene expression profiles of patients undergoing GC-monotherapy have revealed the enzyme PFKFBP-2 (Schmidt et al. 2006) as promising candidate gene indicating the contribution of metabolic disturbances to GC-induced apoptosis. The glucose analog 2-deoxyglucose (2-DG) that accumulates in the cell as 2 deoxyglucose phosphate (2-DGP) but cannot be further metabolized is often used experimentally to mimic glucose and energy deprivation. Important metabolic pathways, glycolysis and subsequently the citrate acid cycle and oxidative phosphorylation as well as the pentose phosphate pathway can be disturbed by the administration of the glucose analog. It is also reported that through the accumulation of 2-DGP hexokinase II is released from the outer mitochondrial membrane (Krieglstein et al. 1982). Resulting membrane alterations could lead to the release of proapoptotic factors (cytochrome *c*, Smac/Diablo, AIF), which is the on-set of the intrinsic pathway, leading to apoptosis. Expecting a synergism, we explored the effect of co-administration of 2-DG and the GC dexamethasone in T- and B-ALL cells.

Co-administration of 2-DG and GC significantly enhanced the kinetic of apoptosis and the sensitivity to dexamethasone in acute lymphoblastic T- and pre B-cells. ATP levels were significant earlier reduced by the co-administration of 2-DG and dexamethasone compared to sole dexamethasone or 2-DG treatment. The two ATP producing pathways glycolysis and oxidative phosphorylation contributed to a different extent to the ATP decline in the two systems. The ATP levels in B-cells were more dependent on changes in the glycolytic flux, whereas in T-ALL cells oxidative phosphorylation was more affected. Although we cannot distinguish between a higher ATP turn-over or less production, it is described that low ATP levels induce apoptosis (Garland et al. 1997).

Reduced mitochondrial capacity was measured only after digitonin treatment and furthermore single respiratory enzyme activities and a slight cytochrome *c* release were detected suggesting mitochondrial membrane alterations. These effects were even more pronounced in T-ALL cells, in B-ALL cells mitochondria partially recovered over time. Whether these changes lead to the release of pro-apoptotic factors or are the consequences of the detachment of the hexokinase needs to be clarified. The significantly higher intracellular 2-DGP concentration after co-administration of 2-DG and dexamethasone compared to 2-DG alone supports the latter hypothesis. Disturbances in the energy metabolism seem to play a major role in the earlier on-set of 2-DG and dexamethasone induced apoptosis, since we can abolish the enhanced sensitivity by the administration of pyruvate.

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2P-12. Functional analysis of genes encoding mitochondrial proteins in Trypanosoma brucei.

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Trypanosoma brucei is a parasitic protist that causes African sleeping sickness in humans and livestock. The unicellular parasite is transmitted by the tse-tse fly. During their life cycle, trypanosomes alternate between the final vertebrate and the intermediate invertebrate hosts. The transmission occurs via the inoculation of flagellates with the tse-tse saliva into the final host. In order to survive in substantially different environments of both hosts, the parasite's mitochondrion undergoes dramatic changes. The organelle is

strongly downregulated in the vertebrate (bloodstream stage), whereas it is fully active in the insect host (procyclic stage).

In our lab, we are using RNA interference to analyze the function of genes encoding mitochondrial proteins in procyclic *T. brucei*. We mainly focus on proteins involved in: i/ mitochondrial mRNA stability and editing ii/ subunits of respiratory complexes, and iii/ iron-sulfur cluster assembly proteins. The ensuing phenotypes are studied by a wide range of methods, by which different mitochondrial functions are followed. To identify novel components of these complex pathways, several approaches are being used, such as homology searches and TAP-tagging of proteins followed by mass spectrometry. Novel approaches for the identification of candidate genes, by forward genetics using RNA interference library of *T. brucei*, will be discussed.

Day 3: Oxygen Kinetics of Mitochondrial Respiration and Inhibition by Nitric Oxide

3L-01. Respiratory control by oxygen under normoxia and hypoxia.

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Oxygen limitation is generally considered as impairment of mitochondrial respiration under hypoxia and ischemia.[1] Low intracellular oxygen levels under normoxia, however, imply mild oxygen limitation, provide protection from oxidative stress, and result from economical strategies for oxygen transport through the respiratory cascade to cytochrome *c* oxidase. Both perspectives relate to the critical oxygen pressure which inhibits mitochondrial respiration. Mitochondrial oxygen affinities ($1/p_{50}$) are related to the kinetics of cytochrome *c* oxidase which is a function of turnover under the control of ADP.[2] The p_{50}, therefore, increases in active states. ADP kinetics is related to the respiratory control ratio.[3] ADP/O_2 flux ratios are high even under severe oxygen limitation, as demonstrated by calorespirometry.[4] Oxygen limitation reduces the uncoupled respiration observed under control by ADP, as shown by relationships derived between ADP/O_2 flux ratios, respiratory control ratios, and ADP kinetics. Bioenergetics at low oxygen versus oxidative stress must be considered in the context of limitation of maximum aerobic activity, ischemia-reperfusion injury, mitochondrial signalling to apoptosis, and mitochondrial theories of ageing.

1. Gnaiger E (2003) Oxygen conformance of cellular respiration. A perspective of mitochondrial physiology. *Adv. Exp. Med. Biol.* 543: 39-55.
2. Gnaiger E, Lassnig B, Kuznetsov AV, Margreiter R (1998) Mitochondrial respiration in the low oxygen environment of the cell: Effect of ADP on oxygen kinetics. *Biochim. Biophys. Acta* 1365: 249-254.
3. Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. *Respir. Physiol* 128: 277-297.
4. Gnaiger E, Méndez G, Hand SC (2000) High phosphorylation efficiency and depression of uncoupled respiration in mitochondria under hypoxia. *Proc. Natl. Acad. Sci. USA* 97: 11080-11085.

3W. Respiratory control by oxygen and redox states of mitochondrial cytochromes in living cells.

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Simultaneous determination of respiratory redox states and rates has been essential in contributing to our understanding of respiratory control in isolated mitochondria. To extend these concepts to intact cells, increased sensitivity is required in a combined detection system. In a closed chamber containing 5-10 Mio cells/ml (such as fibroblasts or promyeloid cells 32D) it takes about 60 s for the transition from 10 µM O_2 to zero

oxygen. This raises the controversial issue of differences between results obtained at steady state conditions versus rapid transitions. Conventional gas/aqueous phase systems, on the other hand, have the problem of oxygen gradients at severe oxygen limitation. To address these methodological challenges, we compared mitochondrial oxygen kinetics derived from transitions with steady states obtained by continuous injection of air saturated aqueous medium (TIP-2k) into the chamber of an OROBOROS Oxygraph-2k (Austria). Redox states of mitochondrial cytochromes (cytochrome *b*, *c*, aa_3) were determined by measurement of difference spectra at wavelengths of 405-630 nm through a flexible glass-fibre light guide of the O2C spectrophotometer (LEA Medizintechnik, Germany). The apparent K_m for oxygen was 0.3-0.4 µM O_2 obtained from aerobic-anoxic transitions, and fully agreed with the steady state kinetics observed over 120-600 s of continuous oxygen injection. Oxygen concentrations were maintained at constant levels of 400 down to 50 nM O_2. A progressive reduction was monitored of mitochondrial cytochromes, corresponding to steady state respiration of 10-50 % of maximal rates. This combination of methods provides a valid reference for interpreting cytochrome spectra obtained in the intact organ during hypoxia and pathological states.

3P-01. Inhibition of cellular respiration by nitric oxide during normoxia and hypoxia.

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Physiological concentrations of nitric oxide (NO) inhibit the mitochondrial respiratory chain enzyme cytochrome *c* oxidase (COX) in a reversible manner that is competitive with oxygen (1,2). Thus, mitochondrial respiration is controlled by the NO-COX signalling pathway. Using a system in which NO is produced inside the cells in a finely controlled manner (3), we performed a detailed respirometric study of inhibition of respiration by NO at physiological oxygen levels (30 µM) and oxygen kinetics in the low oxygen range at concentrations of NO up to 1.8 µM, corresponding to pathological conditions. An electrochemical NO electrode (ISO-NOP, World Precision Instruments, Stevenage, Herts., U.K.) was introduced into the chamber of a high-resolution respirometer (Oroboros Oxygraph-2k, Innsbruck, Austria). The higher sensitivity and reproducibility of oxygen concentration and respiration measurements at low oxygen concentrations (hypoxia) and during transitions to anoxia provided by high-resolution respirometry is in part achieved by on-line correction for instrumental background oxygen dynamics and for the time response of the oxygen sensor (4). The combined technology allows simultaneous recording of respiration as a function of oxygen concentration and NO production evaluated by extracellular measurement. Oxygen flux at 30 µM oxygen in control cells was 16 ± 0.9 pmol.s^{-1}.10^{-6} cells (n = 55), decreasing to 4.6 ± 0.9 pmol.s^{-1}.10^{-6} cells (n = 7) in the presence of 1.8 µM NO. Hyperbolic oxygen kinetics of respiration was characterized by the p_{50} of 0.071 ± 0.004 kPa and J_{max} of 15.5 ± 1.6 pmol.s^{-1}.10^{-6} cells (n = 5) in the absence of NO. Induction of the cells to produce 1.8 µM NO, decreases the affinity of COX for oxygen and significantly alters energy metabolism which may have important pathophysiological consequences.

1. Brown G.C. and Cooper C.E. (1994) FEBS Lett. 356, 295-298
2. Cleeter M.W., Cooper J.M., Darley-Usmar V.M., Moncada S. and Shapira A.H. (1994) FEBS Lett. 345, 50-54
3. Rodriguez-Juarez F., Aguirre E. and Cadenas S. (2007) Biochem. J. Immediate Publication, doi: 10.142/BJ20070033
4. Gnaiger E. (2001) Respir. Physiol. 128, 277-297

3P-02. **Relative sensitivity of soluble guanylate cyclase and mitochondrial respiration to endogenous nitric oxide at physiological oxygen concentration**

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The physiological actions of nitric oxide (NO) are mediated by activation of the enzyme soluble guanylate cyclase and the subsequent increase in the levels of cyclic GMP. NO also binds to the binuclear centre of cytochrome *c* oxidase (COX) and inhibits mitochondrial respiration in competition with oxygen and in a reversible manner [1,2]. Although sGC is more sensitive to endogenous NO than COX at atmospheric oxygen tension [3], the more relevant question is which enzyme is more sensitive at physiological oxygen concentration (30 μM O_2). We used HEK 293 cells transfected with the inducible isoform of the NO synthase (iNOS) gene under the control of a tetracycline-inducible promoter. In this system, NO is generated inside the cells at different levels for extended periods of time. NO-stimulated cGMP production was measured by immunoassay inside hypoxic chambers at 30 μM O_2, while mitochondrial oxygen consumption was determined by high-resolution respirometry (Oroboros Oxygraph-2k). Our results show that the concentration of NO that causes EC_{50} of sGC was approx. 2.9 nM, whereas that required to achieve IC_{50} of respiration was 141 nM [the basal oxygen consumption in the absence of NO was 14 ± 0.8 $O_2.s^{-1}.(10^6 \text{ cells})^{-1}$]. In agreement with this, the NO-cGMP signalling transduction pathway was activated at lower NO concentrations than the AMPK (AMP-activated protein kinase) pathway. We conclude that guanylate cyclase is approx. 50-fold more sensitive than cellular respiration to endogenous NO under our experimental conditions.

1. Brown GC, Cooper CE (1994) Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal cytochrome oxidase respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett.* 356: 295-298.
2. Cleeter MWJ, Cooper JM, Darley-Usmar VM, Moncada S, Shapira AHV (1994) Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. *FEBS Lett*. 345: 50-54.
3. Bellamy TC, Griffiths C, Garthwaite J (2002) Differential sensitivity of guanylyl cyclase and mitochondrial respiration to nitric oxide measured using clamped concentrations. *J. Biol. Chem.* 277:31801-31807.

Day 4: Mitochondrial Membrane Potential and Respiratory Control

4P-01. **Resorcylidene aminoguanidine (RAG) affects calcium mobilization and transmembrane potential in isolated rat liver mitochondria without impairments of respiratory chain.**

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Ca^{2+} uptake and energy production are crucial for maintaining cell functionality. They are both critically dependent on mitochondrial membrane potential. The purpose of our study was to evaluate the effect of resorcylidene aminoguanidine (RAG), a novel agent with coronary vasodilator activity, on rat liver mitochondria.

The effects of RAG on permeability of mitochondrial membrane(s) were studied using multichannel flow cytometry. Mitochondrial membrane potential was monitored with the fluorescence cationic dye, rhodamine 123 (Rh123). The increased Rh123 fluorescence

indicates that RAG, used at the concentration of 50, 100 or 200 ðM (10 min), hyperpolarizes mitochondrial membrane.

The influence of RAG on Ca^{2+} uptake was monitored with Fluo-3AM. RAG used at all studied concentrations caused an increased influx of calcium ions into energized mitochondria. The obtained data suggest that RAG may interact with plasma and subcellular membranes.

Oxygen uptake by mitochondria in the presence of RAG, measured with a Clarck oxygen electrode using the OROBOROS Oxygraph-2k, revealed that RAG did not significantly affect the respiratory chain at all used concentrations.

Our preliminary observations lead us to a speculation that RAG may contribute the mitochondrial membrane rearrangement and thus it is able to facilitate the penetration of other compounds but has no detectable effect on the energy production by mitochondria.

4P-02. Mitochondrial potassium channel opener BMS-191095l – cytoprotective properties and the influence on mitochondrial physiology

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ATP-regulated potassium channels (K_{ATP}), first identified in plasma membrane, are also present in the inner mitochondrial membrane. Opening of the mitochondrial potassium channels affects mitochondrial respiration, membrane potential and matrix volume. An evidence exists that mitochondrial K_{ATP} channel ($mitoK_{ATP}$) is involved in cytoprotection, as the openers of the channel can mimic the effects of ischemic preconditioning. The protective effect of $mitoK_{ATP}$ openers was demonstrated in brain, heart and skeletal muscle, yet the exact mechanism of the protection is still unknown.

We have shown that BMS-191095, an opener selective towards $mitoK_{ATP}$, and not plasma membrane K_{ATP}, protects C2C12 myoblasts against calcium ionophore A23187-induced cell death. Similarly to what was previously shown on isolated rat skeletal muscle mitochondria, BMS-191095 dose-dependently increased the respiration rate in C2C12 myoblasts, which confirms that mitochondria are the site of action of this chemical.

Disruption of calcium homeostasis is one of the events responsible for ischemia-reperfusion induced cell injury. Thus, explaining the link between observed protection and modulation of mitochondrial functioning caused by BMS-191095 can clarify the mechanism of $mitoK_{ATP}$–mediated cytoprotection.

4P-03. Mitochondrial movement is by Brownian motion in coelomocytes from an annelid worm

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Mitochondria in neurons of both vertebrates and invertebrates and in hepatocytes from rats show non-random movement as a result of being attached to cytoskeletal components. This provides them the capacity to move to specific locations within cells using ATP-dependent motors. We investigated mitochondrial motion in coelomocytes (nucleated "blood cells") from the marine, mudflat annelid *Glycera dibranchiata* to determine whether mitochondria in these cells are also "tethered" to the cytoskeleton and whether exposure to varying salinities, which causes short-term changes of cell dimensions, alters mitochondrial movement. We analyzed mitochondrial motion using high-resolution fluorescence video microscopy of live coelomocytes labeled with MitoTracker Green FM (Invitrogen). Cells were exposed to normal seawater (1000 mOsm/L) or mixtures of seawater and distilled water to 750, 500 and 250 mOsm/L (all containing 10 mM HEPES and 0.1% glucose). Motion tracks were analyzed in the X and Y axes in a split plot, fully nested design, with 5 mitochondria from each of 5 cells from each of 4 worms at each of the 4 dilutions (400 total mitochondria). Surprisingly, at 1000 mOsm/L the characteristic mitochondrial movement was continuous and random,

characteristic of Brownian motion. Diluting the incubation medium increased the average length of the motion "track" for each mitochondrion during the recoding period ($P<0.0001$), and this increase was approximately inversely proportional to osmolarity, with mitochondria at 250 mOsm/L moving 2.8 times farther, on average, than mitochondria at 1000 mOsm/L. Furthermore, the average total displacement of the mitochondria during the recording period also increased significantly and proportionally with dilution ($P<0.001$). Therefore, the mitochondria demonstrated Brownian motion and presented no evidence of being tethered to the cytoskeleton, but the mechanism by which dilution increased motion is still unknown. It is unclear whether mitochondria being untethered represents a specific adaptation to the gas transfer functions of annelid coelomocytes, or is instead a general feature of non-neuronal invertebrate cells.

Day 5: Basic Science and Clinical Reality

5L-01. **Mitochondrial bioenergetics and structural network organization.**
Rodrigue Rossignol (Bordeaux, FR)

5L-02. **Small and large animal models of sepsis and I/R-injury – how to combine basic science and clinical reality.**
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Animal models should be the obvious link between basic science and clinical reality. However, as will be discussed in more details in the scheduled lecture, depending on the technique used to induce a specific pathologic condition, the medical treatment of the animal during the experiment, and of course the animal species itself, the degree to which the experiment may simulate a true clinical situation widely varies. For example, in pigs, i.e. the most "popular" large laboratory animal species, common abdominal disorders and abdominal sepsis can adequately be simulated, as both the anatomy and the physiology of the abdominal organs of pigs are closely similar to the human ones. In contrast, when studying lung pathologies in pigs, one should be aware of the marked differences between this species and humans, in particular of the high susceptibility to hypoxia and of the relatively large functional units of the pig lung.

In our institution we recently developed a clinically relevant model of faecal peritonitis-induced abdominal sepsis thus mimicking one of the most frequent conditions encountered in critically ill patients. This model is closer to clinical reality than the previous endotoxin shock model [1,2] in as much as the pulmonary circulation is less abruptly compromised. This fecal peritonitis model was used to study the effects of different inspired oxygen concentrations on the organ's function and inflammatory response; surprisingly, we demonstrated that hyperoxia better maintains organ's function than normoxia without raising the inflammatory response and even reducing the number of apoptotic cells in lungs and liver.

An analogous peritonitis model was established in mice; in that case fecal peritonitis is induced by a so-called cecal ligation and puncture (CLP) technique [3,4], which works reliably in the mouse but not in the pig. Despite of the very small dimensions, even in mice a large spectrum of miniaturised organ's monitoring and support techniques can be employed to simulate the conditions of an intensive care unit (Mice ICU). Obviously, the most important advantage of the mouse-model is that it allows studying genetically modified animals. For example, this experimental setup was used to investigate the inflammatory response and the role of anti-oxidant enzymes in faecal peritonitis, surprisingly demonstrating that even in SOD-overexpressing mice the SOD activity decreases over time, thus failing to enhance the anti-oxidant defense.

In order to simulate I/R injury, over the last years a large animal model of thoracic aortic cross-clamping has been developed [5,6]. In that case, the most serious problem imposed by the experimental setup is to prevent left ventricular failure by tight control of

blood pressure, which is achieved by continuous infusions of several anti-hypertonic drugs during the clamping manoeuvre.

By means of this model we observed a beneficial influence of an intrarenal Parecoxib infusion on kidney function, which was unrelated to macrocirculation. In a further experiment pre-treatment with a superoxide-dismutase (SOD) containing oral diet reduced (1) DNA-damage related to surgery and I/R-injury, (2) apoptosis in the spinal cord white matter, and (3) regional venous acidosis, without, however, ameliorating organ function [7].

In conclusion, animal models of sepsis and I/R-injury are standardized tool for testing therapeutic strategies developed by basic science in a realistic context, but many details of the specific experimental setup crucially influence the validity of the obtained results.

1 Asfar P et al (2002) Crit Care Med 30: 1826 - 1833
2 Asfar P et al (2004) Intensive Care Med 30: 957 – 964
3. Albuszies G et al (2005) Crit Care Med 33: 2332 – 2338
4. Barth E et al (2006) Crit Care Med 34: 307 - 313
5. Hauser B et al (2005) Shock 24: 476 – 481
6. Hauser B et al (2006) Shock 25: 633 – 640
7. Maier C et al (2007) Intensive Care Med 33: 845 – 850

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