



## MitoPathways: Mitochondrial Respiratory Control by Substrates, Functional Diagnosis of Mitochondrial Defects and Tissue Characterization

MitoPathways Workshop, April 2007 (Schroecken, Austria)

### Programme

**Wednesday, 18. April 2007**

#### 1. Introduction

- 09:04 Erich Gnaiger (Innsbruck, AT) MitoPathways – Pathways to the Workshop.  
Chairman: Zdenek Drahotka (Prague, CZ)
- 09:10 Chuck Hoppel (Cleveland, US) Protocol: Oxidative phosphorylation method: Human skeletal muscle or liver mitochondria.
- 10:30 Coffee/Tea
- 10:54 Erich Gnaiger (Innsbruck, AT) MitoPathways – an introduction to the concept of the 'Q-junction': Rational and development of multiple substrate/inhibitor protocols.

#### 2. Muscle: Permeabilized fibers and Isolated Mitochondria

- 12:25 Hélène Lemieux (Innsbruck, AT; Rimouski, CA) Tissue-specific variations in mitochondrial respiratory control by multiple substrates in permeabilized muscle fibers.
- 13:00 Lunch – Walk & Talk  
Chairman: Chuck Hoppel (Cleveland, US)
- 14:40 Robert Boushel (Copenhagen, DK; Montreal, CA) MitoPathways-Complex I+II: the Q-Junction.
- 15:00 Patrick Schrauwen (Maastricht, NL) In vivo and ex vivo mitochondrial function in human type 2 diabetes mellitus.
- 16:25 Wouter van Marken Lichtenbelt (Maastricht, NL) Mitochondrial function in permeabilized human skeletal muscle fibers: Role of fatty acids and calcium cycling.
- 17:07 Coffee/Tea
- 17:20 Matthijs Hesselink (Maastricht, NL) Fat accumulation and mitochondrial function during the development of diabetes in ZDF rats.

- 18:15 Richard Haas (San Diego, US) Methodological issues in the use of high-resolution respirometry for the diagnosis of mitochondrial disease.
- 19:30 Dinner  
After dinner:  
**Considerations on the workshop structure of day 2**, that may include: Further presentations, discussion topics, summary, further steps.

## Thursday, 19. April 2007

### 3. Liver: Tissue homogenate and isolated mitochondria

Chairman: Eskil Elmér (Lund, SE)

- 08:40 Zdenek Drahotá (Prague, CZ) Specific sensitivity of various mitochondrial enzymes to peroxidative damage.
- 09:20 Andrey Kozlov (Vienna, AT) Application of homogenates versus isolated mitochondria for functional diagnosis of rat liver respiratory capacity and P:O ratios.
- 10:00 Coffee/Tea

### 4. Isolated Mitochondria and Intact cells

- 10:30 Antonio Galina (Rio de Janeiro, BR) Mitochondrial and bioenergetic dysfunction in human hepatic cells infected with dengue 2 virus.
- 11:00 Rodrigue Rossignol (Bordeaux, FR) Mitochondrial bioenergetics and structural network organization.
- 11:55 Chuck Hoppel (Cleveland, US) Age-related changes in human skeletal muscle mitochondrial energy metabolism.
- 12:30 Lunch

### 5. Mitochondrial Function and Dysfunction

Chairman: Rodrigue Rossignol (Bordeaux, FR)

- 14:40 Mariana Rosca (Cleveland, US) Heart failure causes specific alterations in the phosphorylation apparatus of skeletal muscle subsarcolemmal and intermyofibrillar mitochondria.
- 15:10 Flemming Dela (Copenhagen, DK) Insulin resistance, mitochondrial function and mitochondrial morphology.
- 16:00 Robert Boushel (Copenhagen, DK; Montreal, CA) Reduced skeletal muscle mitochondrial respiratory capacity in AMPK  $\alpha 2$  deficient mice.
- 16:15 Regitze Kraunsoe (Copenhagen, DK) High-resolution respirometry in rat and human abdominal adipose tissue.
- 16:40 Coffee/Tea

Quote of the day: 'In terms of trends it is conclusive'!  
E. Gnaiger, Schroecken 2007

### 6. General Session: Questions Related to O2k-Applications

Chairman: Flemming Dela (Copenhagen, DK)

- 17:00 Eskil Emér & Magnus Hansson (Lund, SE) Respiratory studies of blood cells from sepsis patients (pilot studies)  
Calcium handling and respiration in rat brain mitochondria.
- *Topics on high-resolution respirometry*
  - calcium insults in intact and permeabilised cells;
  - the suitability of using BSA when detecting mitochondrial dysfunction following sepsis/ischemia/Huntingtons disease/neurotoxicology;
  - the buffer and pH buffering to use with human platelets and white blood cells and the pH buffer to use when cultured cells are transferred from a 5% CO<sub>2</sub> atmosphere to the O<sub>2</sub>k;
  - cover slip holder/stopper for the O<sub>2</sub>k (to be able to mount multiple coverslips with attached cells in the O<sub>2</sub>k);
  - MultiSensor developments.

## 7. General Summary

17:45 Topics/presentations that emerged during the workshop.

18:00 General Summary Discussion – Outlook

- Special issue (possibly): *Mitochondrion*
- Title: **Integrated Mitochondrial Function: OXPHOS and Mitochondrial Respiration**
- Introductory chapters: MitoPathways
- Respirometric protocols – standard format
  - (a) Introduction: Area of interest of project (general)
  - (b) Methods: Sample preparation (detailed), sample storage (detailed), chemicals and media
  - (c) Protocol(s): Representative Oxygraph trace, conceptual explanation, [possibly: quantitative result for control group]. *2 pages. Template*
  - (d) Discussion of limitations, potential for further improvement of protocol, possible comparison with supplementary approaches (spectrophotometry, membrane potential, ...)

20:15 Dinner / After dinner: Conclusions

## The Group

- Austria, Innsbruck: [Erich Gnaiger](#), [Hélène Lemieux](#), [Francesca Scandurra](#)
- Austria, Vienna: [Andrey Kozlov](#)
- Brazil, Rio de Janeiro: [Antonio Galina](#)
- Czech Republic, Prague: [Zdenek Drahotka](#)
- Denmark, Copenhagen: [Flemming Dela](#), [Robert Boushel](#), [Regitze Kraunsoe](#), [Steen Larsen](#)
- France, Bordeaux: [Rodrigue Rossignol](#), [Katarina Smolkova](#)
- Netherlands, Maastricht: [Patrick Schrauwen](#), [Matthijs Hesselink](#), [Wouter van Marken Lichtenbelt](#), [Joris Hoeks](#)
- Sweden, Lund: [Eskil Elmer](#), [Magnus Hansson](#)
- USA, Cleveland : [Charles Hoppel](#), Mariana Rosca, Vazquez Edwin
- USA, San Diego: [Richard Haas](#)

## Suggestions for presentations

1. Send the title of your contribution(s). An abstract is not mandatory, although a summary will be helpful for the workshop (for internal circulation, only).
2. Send in advance the fundamental details of the protocol of your study: experimental temperature, respiration medium, details on biopsies, preparation of sample, cell culture, permeabilization, range of sample size,...
3. Present plenty of oxygraph traces that show the 'representative experiment' and explain the final protocol(s). In addition, show key experiments leading to the final protocol, and illustrate the rational how experimental problems have been solved.
4. Discuss the protocol(s) and interpretations in terms of mitochondrial pathways and mitochondrial defects.
5. Provide a summary of results (if available) related to the diagnostic problem.
6. Relate the quantitative results on respiratory capacity in various states (of the control group) to data in the literature (as far as possible and meaningful).

The long-term goal might be - if we can agree - the preparation of a review for an appropriate high-level journal. This long-term goal will require prior publication of primary papers by several (all?) groups involved in MitoPathways.

Input of material that may be circulated internally to all participants is very welcome.