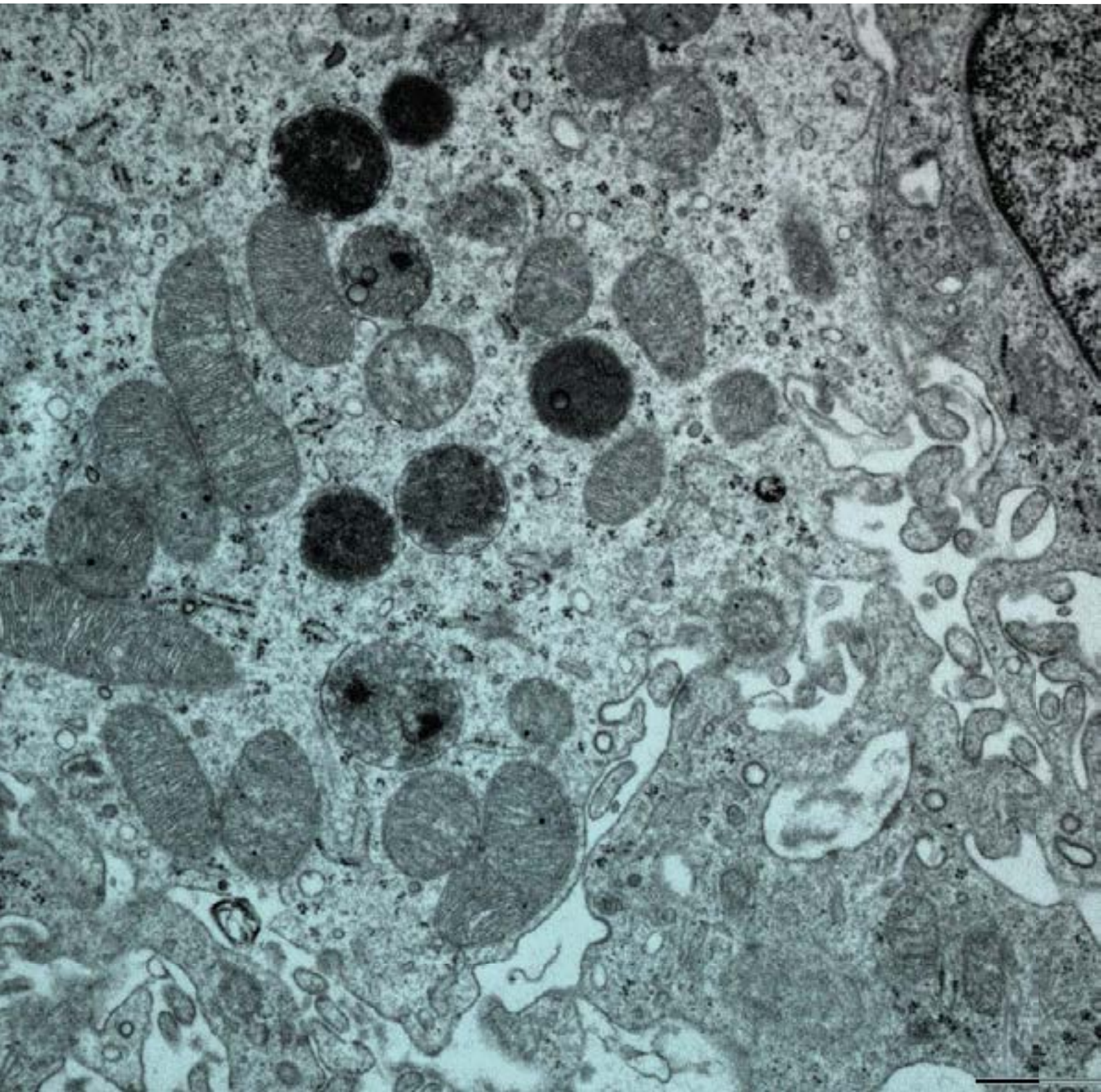
COPENHAGEN

MiP SUMMER 2013

MITOCHONDRIAL PHYSIOLOGY: THEORY & PRAXIS

ACTA PHYSIOLOGICA SYMPOSIUM



Index

MiPs - The Mitochondrial Physiology Society – MiPsociety

Welcome

MiP*summer* 2013 - the Mitochondrial Physiology Summer School taking place in Copenhagen in August 2013 – is the 6th MiP*summer* School organized by the Mitochondrial Physiology Society. Starting at the birth place of the MiP*society* (2003) in Schröcken in 2007, MiP*summer* developed towards a firmly established MiP*event* in our scientific community. Following the successful MiP*summer* Schools 2007 and 2008 (Schröcken, Austria; organized by Erich Gnaiger), 2009 (Louisiana State University, Baton Rouge, USA; organized by Steven Hand), 2010 (Druskininkai, Lithuania; organized by Vilma Borutaite), and 2011 (University of Cambridge, UK; organized as a FEBS Workshop by Guy Brown), Flemming Dela kindly agreed to continue our tradition by organizing MiP*summer* 2013 at The Panum Institute, University of Copenhagen.

The MiP*summer* School is open to graduate students, postdocs, scientists from academia and biotechnology/pharmaceutical companies and related entities. Our aim is to provide information on current concepts and trends in mitochondrial physiology ranging from basic to biomedical and clinical perspectives. Lecturers and students may have different views on '*What is mitochondrial physiology*'. The physiological focus emphasizes the integration of mitochondrial function in cellular metabolism, and principles of bioenergetics provide a fundamental basis for mitochondrial physiology. In addition to introductory lectures and practical demonstrations of cutting-edge techniques applied in mitochondrial physiology, ample time is given to student presentations making MiP*summer* a truly interactive event. Potential controversies among proponents of various lines of thought should lead to stimulating discussions, which provide a unique opportunity for critical evaluation of methods and concepts and may be considered as the signature of an actively growing field of research.

MiP*summer* is international. Despite its strong base in Europe this MiP*event* should spread further to more continents, with a global perspective in cooperation with national and international organizations. To support these aims, we invite all participants of MiP*summer* 2013 – including students and faculty - to join and become members of the MiP*society*, providing a continuing feedback, stimulation, motivation and active participation in integrating mitochondrial physiological research into the big picture of science and medicine.

Erich Gnaiger
Chair, Mitochondrial Physiology Society
www.mitophysiology.org

Medical University of Innsbruck
Department of Visceral, Transplant and Thoracic Surgery
D. Swarovski Research Laboratory
A-6020 Innsbruck, Austria

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Welcome by the local organizer

August 2013

Dear participants in the MiP*summer* 2013

It is my pleasure to welcome you all to this Mitochondrial Physiology Summer School in Copenhagen.

The focus of this year's MiP*summer* is to combine state-of-art lectures with hands-on analyses of aspects of mitochondrial function and morphology using different technologies. We will present you with several choices of mitochondrial analyses that you can consider in your own research. We do not pretend to provide you with a full training course in these techniques, but hopefully you will by the end of the week have gained a better understanding of the limitations, pit-falls and advantages of many of the currently available techniques to study mitochondria.

An important part of the summer school is to present your own research interest to the other participants, which will hopefully facilitate scientific discussions and enlarge your "mitochondrial network". The poster presentations will take place on Tuesday 27th and Thursday 29th from 13:30-14:30. Remember to mount your poster already in the morning on the day you should present, which you can see elsewhere in the program. The Poster walk will be chaired, and all presenters are given 3 min to present their poster, with additionally 2 min for questions/answers.

Tuesday and Thursday evening, after the scientific program, we will organize small groups which in an informal way will be guided to areas in the neighbourhood with café's, restaurants and bars (not covered by the registration fee). Join at the registration table after the scientific program!

Bikes are at your disposal all week. After you have registered you will be guided to the bike area. The bikes can be used for your transportation during the week, and shall be returned on Friday morning.

Wednesday afternoon we are delighted to invite you all on a guided bike ride through Copenhagen. Pending the weather conditions, there will be a possibility for you to take a swim in the (very clean) water in the harbour! We will end this bike tour with a dinner at a restaurant (included in the registration fee).

Thursday evening the speakers dinner will take place at 19:00. Please look in your conference bag for further details.

If you have questions, please do not hesitate to contact one of the staff members, which are here only to help you!

MiP*summer* 2013 course credit: 4.0 ECTS

Welcome to MiP*summer* 2013 in Copenhagen.

Flemming Dela

Finding your way at the Panum Institute

The Panum Institute has its main entrance at Nørre Allé 20.

When you enter the Panum Institute you will come to a main hall with numbers.
The Panum Institute is divided into several buildings, the numbers indicate the buildings.

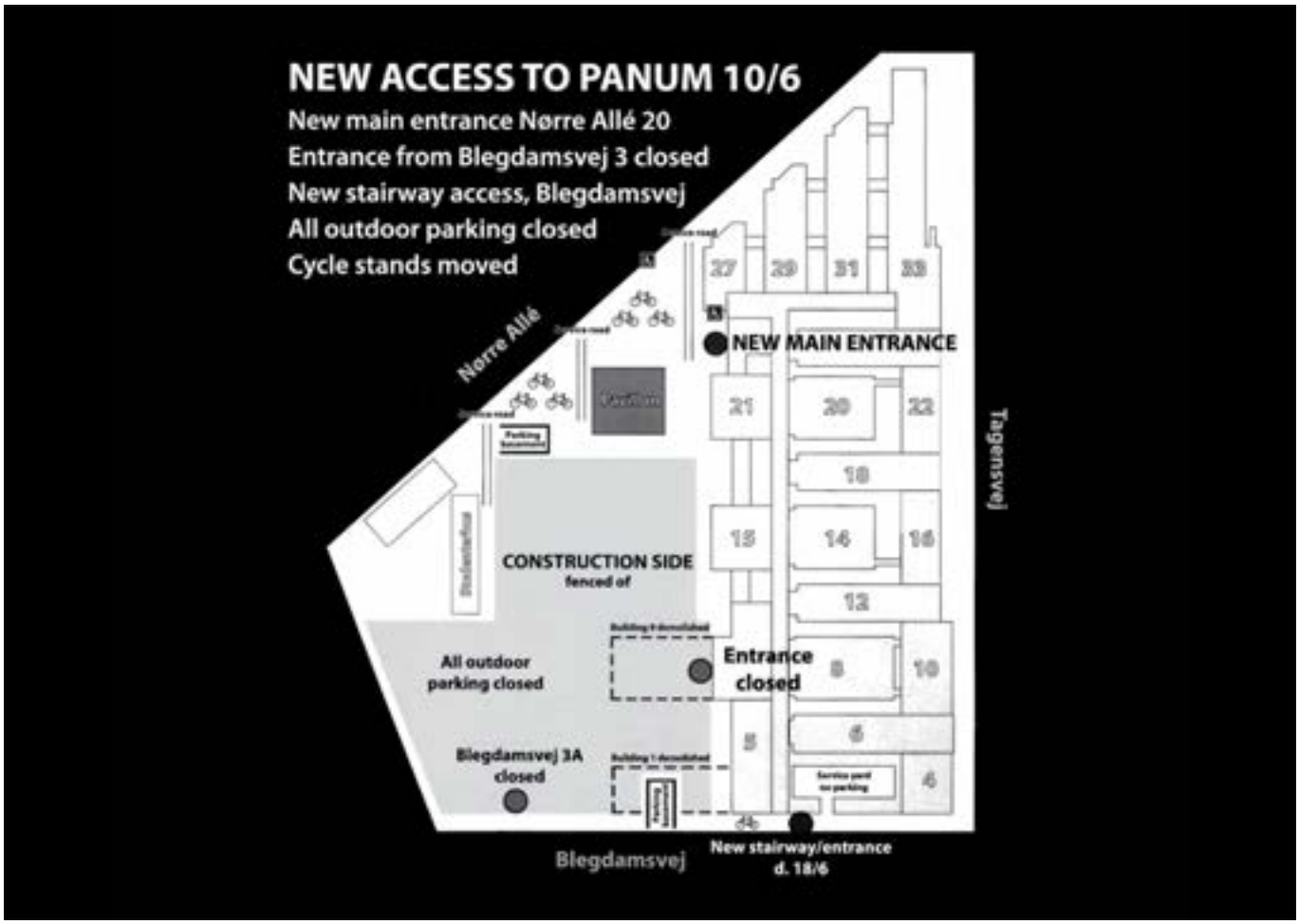
Haderup auditorium
(Building 20 – see map of Panum below)

The Haderup auditorium is situated in the main hall between building 18 and 24 (please follow the Haderup auditorium and the MiP*summer* 2013 signs).
- Registration takes place outside the Haderup auditorium.
- All lectures take place in the Haderup auditorium.

All workshops except the "Mitochondrial Imaging" workshop and the "NMR" workshop take place on the first floor of building 12 (indicated as 12.1.) The last number indicates the room number, i.e. 12.1.42 is the first floor of building 12, room number 42.

The "Mitochondrial Imaging" workshop takes place at the Core facility for Microscopy at the ground floor. Please follow the Core facility and MiP*summer* 2013 signs.

The "NMR" workshop takes place at the NMR center. The NMR center is situated on the ground floor near the entrance of the Panum Institute and will be indicated with MiP*summer* 2013 signs.



General information

Congress secretary
Jacqueline van Hall
Building 12.4.10
Tel. (+45) 3532 7423
Mobile (+45) 2875 7457

Late arrival
Please contact the congress secretary in case of late arrival.

Hotels
Delegates:
Hotel Østerport
Oslo Plads 5
2100 Copenhagen Ø
Tel.: (+45) 3311 2266

Hotel Østerport is located near the Østerport station and is 10 minutes away from the Copenhagen city centre. It is situated 25 minutes from the airport with direct train and bus connections. The hotel is only a few minutes walk from shopping streets.

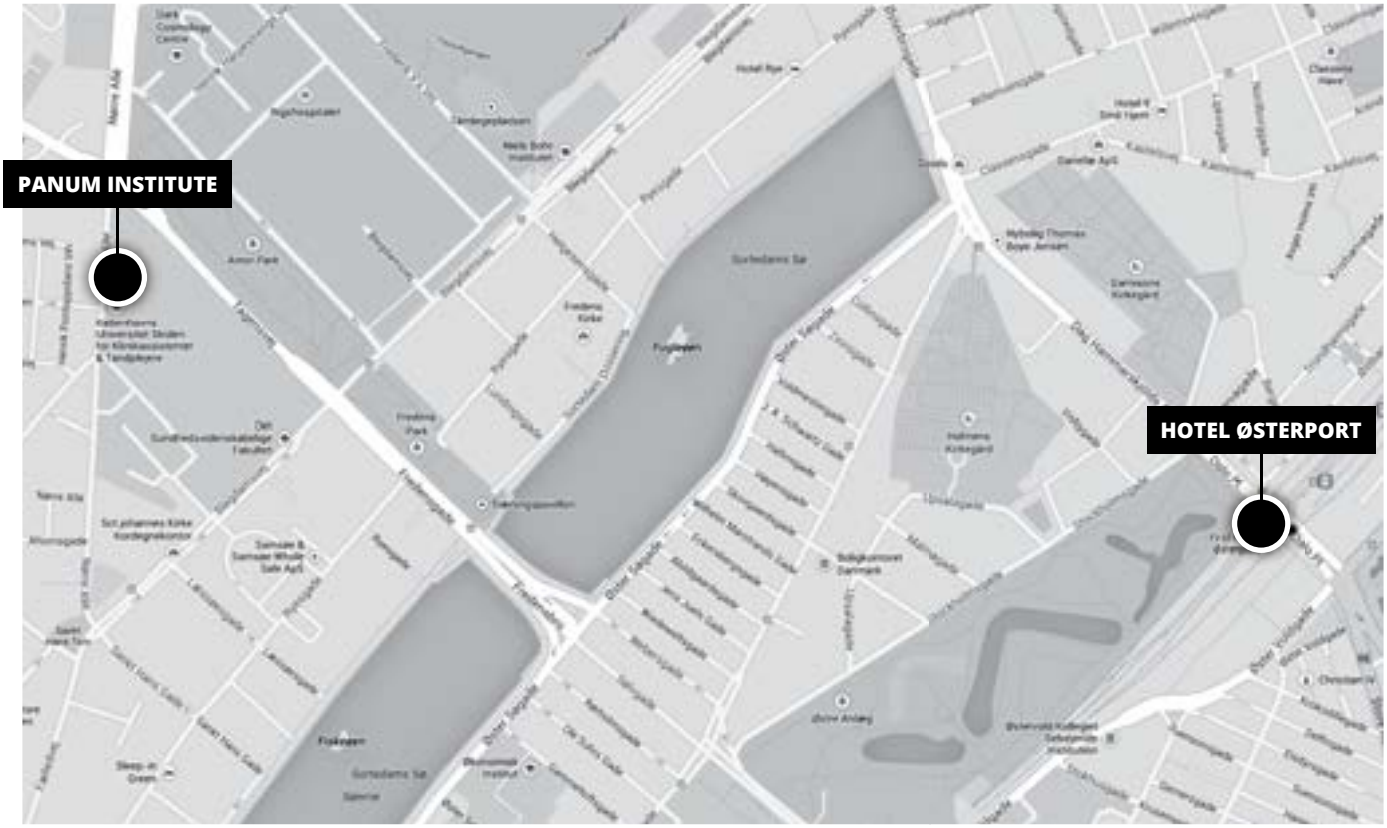
Invited speakers:
Hotel Nora
Nørrebrogade 18B
2200 København N
Tel.: (+45) 3537 2021
www.hotelnora.dk

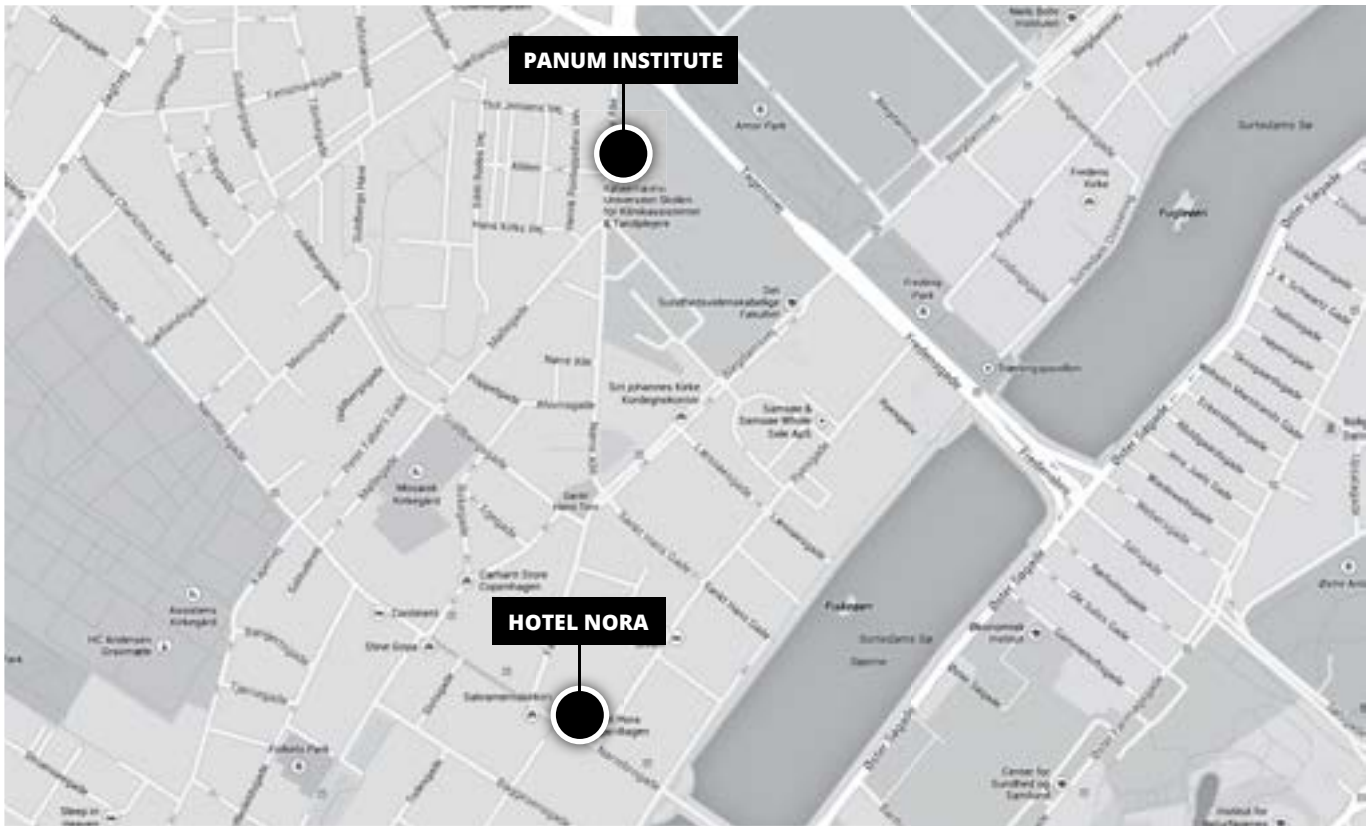
Journey planner
You can find information about public transportation at www.rejseplanen.dk

Taxi services
There is a taxi stand close to the Panum Institute, in front of the University Hospital, Rigshospitalet, Blegdamsvej 9. In addition, the information desk at Panum and the guards at the main entrance will be happy to assist you with calling a taxi.

If you want to call a taxi, here is contact info to some taxi companies:
• Taxa 4x35: (+45) 35353535
• Dantaxa: (+45) 70252525
• Amager-Øbro Taxa: (+45) 32515151

ATM machines around Panum institute
If you need cash, the closest ATM is at the intersection between Nørregade and Fælledvej – Danske Bank.


PANUM INSTITUTE
HOTEL ØSTERPORT


PANUM INSTITUTE
HOTEL NORA

Morning programme

<table>
<tr><th>Monday 26th
Morning</th><th>Tuesday 27th
Morning 09:00 – 12:00</th><th>Wednesday 28th
Morning 09:00 – 12:00</th><th>Thursday 29th
Morning 09:00 – 12:00</th><th>Friday 30th</th></tr>
<tr><td rowspan="5">Arrival
Registration from 11:00

Bicycles for participants.</td><td>Human blood sampling/ muscle biopsies
Room: 12.1.26</td><td>Human blood sampling/ muscle biopsies
Room: 12.1.26</td><td>Human blood sampling/ muscle biopsies
Room: 12.1.26</td><td rowspan="2">09:00 – 09:45
A role of mitochondria in heart failure?
Renée Ventura-Clapier (France)</td></tr>
<tr><td colspan="3">Mitochondrial imaging [Core facility for microscopy] (Clara Prats) Room: 21.01</td></tr>
<tr><td colspan="3">Mitochondrial respiration in circulating blood cells using the Oroboros 2k, 2 machines (Eskil Elmér + crew)
Room: 12.1.42</td><td rowspan="2">09:45 – 10:30
Intracellular Energetic Units in cardiac cells
Rita Guzun (France)</td></tr>
<tr><td colspan="3">Mitochondrial respiration in permeabilized human muscle fibers. [Oroboros 2k, 2 machines] (Regitze Kraunsøe; Michael Lund)
Room: 12.1.30</td></tr>
<tr><td colspan="3">A. Mitochondrial / Cellular ROS production in human muscle fibers : 1 SAFAS machine + 1 O2k-Fluorescence LED2-Module (Martin Gram, Steen Larsen)
Room: 12.1.31

B. Cells 1 O2k-Fluorescence LED2-Module (Erich Gnaiger + Verena Laner)
Room: 12.1.39</td><td>10:30 – 11:00
Break</td></tr>
<tr><td>12:00 – 12:30
Welcome.
Course Information.
Flemming Dela</td><td colspan="3">NMR determination of skeletal muscle mitochondrial function [NMR centre] / Isolation of liver mitochondria (Bjørn Quistorff)
NMR Center</td><td rowspan="2">11:00 – 11:30
Ischemic – reperfusion injury in cardiac muscle.
Takashi Yokota (Japan)</td></tr>
<tr><td>Opening speech by the Chair of the Executive committee, The Mitochondrial Physiology Society. Erich Gnaiger (Austria)</td><td colspan="3">Mitochondrial respiration and Glycolysis in microtiter plate format using the XFe analyzer. (Claus Desler/ Sofia Vikström)
Room: 12.1.29</td></tr>
<tr><td>12:30 – 13.15
The Bioenergetic basics.
David G. Nicholls (USA)</td><td colspan="3">Mitochondrial function in intact and permeabilized circulating blood cells using the XFe analyzer (Claus Desler/ Sofia Vikström)
Room: 12.1.21</td><td>11:30 – 12:00
Mitochondria in regulation of cell death in cardiovascular pathologies
Vilma Borutaite, (Lithuania)</td></tr>
<tr><td>13:15 – 13.45
Break / Lunch</td><td colspan="3">Lunch & Poster viewing 12:00 – 13:30</td><td>12:00 – 12:15
Closing
Flemming Dela</td></tr>
</table>

Afternoon programme

<table>
<tr><th>Monday 26th
Afternoon</th><th colspan="4">Tuesday 27th
Afternoon</th><th>Wednesday 28th
Afternoon</th><th colspan="3">Thursday 29th
Afternoon</th><th>Friday 30th
Afternoon</th></tr>
<tr><td rowspan="2">13:45 – 14:15
Studying mitochondrial bioenergetics in intact cells
David G. Nicholls (USA)</td><td colspan="4">13:30 – 14:30
Guided poster presentations (3 + 2 min)</td><td rowspan="2">13:30 – 14:15
“Ex vivo respiratory capacity of human blood cells - a SUITable application of the O2k Oxygraph”.
Eskil Elmér (Sweden)</td><td colspan="3">13:30 – 14:30
Guided poster presentations (3 + 2 min)</td><td rowspan="11">Departures</td></tr>
<tr><td>#1-8 Skeletal muscle & Exercise</td><td>#9-15 Aging, Development, Cancer & Cell death</td><td>#16-23 Neuro</td><td>#24-29 Heart</td><td>#30-36 Models, Structure and Function</td><td>#37-42 Inflammation, Oxidative stress and ROS</td><td>#43-51 Metabolism</td></tr>
<tr><td>14:15 – 15:00
Determination of skeletal muscle mitochondrial function by NMR techniques
Graham J Kemp (UK)</td><td colspan="4">14:30 – 15:00
Pro´s and Con´s on mitochondrial preps: Isolated mitochondria – permeabilized cells and tissue – tissue homogenate – intact cells.
Erich Gnaiger (Austria)</td><td>14:15 – 15:00
Brain energy metabolism during aging
Albert Gjedde (Denmark)</td><td colspan="3">14:30 – 15:00
Mitochondrial superoxide production in isolated mitochondria – sites of production and relative site contribution
Martin Hey-Mogensen (Denmark)</td></tr>
<tr><td>15:00 – 15:30
Break (coffee/tea)</td><td colspan="4" rowspan="2">15:00 – 15:30
ATP turnover in human hepatic tissue.
Julia Szendrödi (Germany)</td><td>15:00 – 15:20
Brain Mitochondrial Dysfunction in a mouse model of premature aging.
Takashi Yokota (Japan)</td><td colspan="3" rowspan="2">15:00 – 15:30
Immunohistochemistry as a tool to measure mitochondrial content and investigate structural organization.
Clara Prats (Denmark)</td></tr>
<tr><td>15:30 – 16:00
OXPHOS Analysis: High-resolution-respirometry and mitochondrial physiology beyond the intact cell
Erich Gnaiger (Oroboros Instruments)</td><td rowspan="5">SOCIAL PROGRAM
→ → → →</td></tr>
<tr><td>16:00 - 16:30
Measuring Mitochondrial Function Using the Seahorse XF Flux Analyzer
Sofia Vikström (Seahorse Bioscience)</td><td colspan="4">15:30 – 16:00
The role of mitochondrial respiration in Insulin resistance
Esther Phielix (The Netherlands)</td><td colspan="3">15:30 – 16:00
Break (coffee/tea)</td></tr>
<tr><td rowspan="2">16:30 – 17:15
Consideration of experimental parameters and other indices of mitochondrial function
Darrell Neufer (USA)</td><td colspan="4">16:00 – 16:30
Break (coffee/tea)</td><td colspan="3">16:00 – 16:45
Fission and Fusion of Mitochondria
Antonio Zorzano (Spain)</td></tr>
<tr><td colspan="4">16:30 – 17:00
The role of mitochondria in insulin secretion.
David G. Nicholls (USA)</td><td colspan="3">16:45 – 17:15
Mitochondrial respiration as biomarker for Fatigue and Cognition.
Scott Maynard (Denmark)</td></tr>
<tr><td>17:15 – 17:30
Instructions for next day practical experiments
Flemming Dela</td><td colspan="4">17:00 – 17:30
Exercise training and mitochondrial biogenesis and function.
Kent Sahlin (Sweden)</td><td colspan="3">19:00 –
Speakers dinner</td></tr>
</table>

Social program

Tuesday evening:
- Informal guide tours to the neighbourhood café's, restaurants and bars. We meet at the registration desk after the last lecture and form smaller groups.

Wednesday:
- After the last lecture (~ 15:30) we meet at the registration desk. Smaller groups of 8-10 persons will be formed, and then we use the bikes for a tour in/outside Copenhagen. We will end at a restaurant around 18:00 for food, beer and wine! Casual ware. Instructions/guidance for your own return to the hotels will be given.

Thursday evening:
- Informal guide tours to the neighbourhood café's, restaurants and bars. We meet at the registration desk after the last lecture and form smaller groups.
- Speakers dinner: please look in your conference bag.

Poster walk/talk

The poster sessions take place outside the Auditorium. Please make sure to mount your poster as early as possible.

On Tuesday and Thursday from 13:30 to 14:30 there will be a guided poster walk. Present your poster in max 3 mins and prepare for a 2 min discussion of your poster. The abstracts have been allocated to the various categories on the two days:

Tuesday 27th Abstract/poster:
1-8: Skeletal muscle & Exercise
#9-15: Aging, Development, Cancer & Cell death
#16-23: Neuro
#24-29: Heart

Thursday 29th Abstract/poster:
30-36: Models, Structure and Function
#37-42: Inflammation, Oxidative stress and ROS
#43-51: Metabolism

See your abstract/poster number on pages 26-83.

Overview of workshops

Workshop 1 (Imaging)
Mitochondrial imaging [Core facility for microscopy] (Clara Prats) **Room: 21.01**

Workshop 2 (Oroboros - blood)
Mitochondrial respiration in circulating blood cells using the Oroboros 2k, 2 machines (E. Elmér + crew) **Room: 12.1.42**

Workshop 3 (Oroboros - muscle)
Mitochondrial respiration in permeabilized human muscle fibers. [Oroboros 2k, 2 machines] (Regitze Kraunsoe; Michael Lund) **Room: 12.1.30**

Workshop 4 (ROS)
A.
Mitochondrial / Cellular ROS production in human muscle fibers: 1 SAFAS machine + 1 O2k-Fluorescence LED2-Module (Martin Gram, Steen Larsen) **Room: 12.1.31**

B.
Cells 1 O2k-Fluorescence LED2-Module (Erich Gnaiger + Verena Laner) **Room: 12.1.39**

Workshop 5 (NMR)
NMR determination of skeletal muscle mitochondrial function [NMR centre] / Isolation of liver mitochondria (Bjørn Quistorff) **NMR Center**

Workshop 6 (Seahorse)
Mitochondrial respiration and Glycolysis in microtiter plate format using the XFe analyzer. (Claus Desler / Sofia Vikström) **Room: 12.1.29**

Workshop 7 (Seahorse - blood)
Mitochondrial function in intact and permeabilized circulating blood cells using the XFe analyzer (Claus Desler / Sofia Vikström) **Room: 12.1.21**

Overview of workshops

Tuesday, August 27

Workshop 1 (Imaging)
1. Andreas Bergdahl
2. Jaap Keijer
3. Alba Gonzalez-Franquesa
4. Niklas Psilander
5. Per Frank
6. Martin Wohlwend
7. Thomas Kalvik
8. Anne Lykkegaard Widlund
9. Andreas Kalsen Petersen
10. Christiane Gam
11. Morten Hostrup

Workshop 2 (Oroboros - blood)
1. Blair Aldridge
2. Terri Pietka
3. Christian Silva Platas
4. Li Meng
5. Loes Duivenvoorde
6. Adelheid Weidinger
7. Jérôme Piquereau
8. Giada Zurlo
9. Merethe Hansen
10. Maria Angleys

Workshop 3 (Oroboros - muscle)
1. Brenna Osborne
2. Sam Shepherd
3. Andrea Kodde
4. Miranda Nabben
5. Sandra Eggimann
6. Jukka Kallijärvi
7. Ina Katrine Nitschke Pettersen
8. Fredrik Hoel
9. Bodil Bjørndal
10. Brian Lindegaard Pedersen
11. Ole Hyldegaard

Workshop 4 A. + B. (ROS)
1. Hana Kratochvílová
2. Fabian Peters
3. Michelle Desforges
4. Saima Ajaz
5. Neta Amior
6. Oleg Pak
7. Jim Lund
8. Michel van Schaardenburgh
9. Fredrik Bækkerud
10. Marie Sannes Ramsvik
11. Henriette Kihl

Workshop 5 (NMR)
1. Daniil Popov
2. Natalia Komelina
3. Thomas Agnew
4. Marco Spinazzi
5. Giulia Girolimetti
6. Vineta Fellman
7. Magnus J. Hansson
8. Johannes Ehinger
9. Michael Karlsson
10. Sarah Piel
11. Liselotte Christiansen

Workshop 6 (Seahorse)
1. Christopher Perry
2. Chin San Liu
3. Katerina Hejzlarová
4. Nikola Kovárová
5. Jaroslaw Walczak
6. Jennifer Wettmarshausen
7. Mina Davoudi
8. Sofie Lange
9. Andreas Vigelsø Hansen
10. Christa Funch Jensen
11. Marianne Andersen

Workshop 7 (Seahorse - blood)
1. David Busija
2. Andreas Koenig
3. Gerardo de Jésus Garcia-Rivas
4. Aleksandr Klepinin
5. Chris Gaffney
6. Susana Pereira
7. Kristian Karstoft
8. Anelise Tonin

Overview of workshops

Wednesday, August 28

Workshop 1 (Imaging)
1. Hanna Kratochvilová
2. Fabian Peters
3. Michelle Desforges
4. Oleg Pak
5. Magnus Hansson
6. Johannes Ehinger
7. Michael Karlsson
8. Sarah Piel
9. Ina Katrine Nitschke
10. Fredrik Hoel
11. Sofie Lange

Workshop 2 (Oroboros - blood)
1. Brenna Osborne
2. Gerardo de Jésus Garcia-Rivas
3. Katerina Hejzlarová
4. Nikola Kovárová
5. Susana Pereira
6. Bodil Bjørndal
7. Anders Kalsen
8. Christiane Gam
9. Andreas Vigelsø Hansen
10. Liselotte Christiansen

Workshop 3 (Oroboros - muscle)
1. Andreas Bergdahl
2. Daniil Popov
3. Neta Amior
4. Li Meng
5. Loes Duivenvoorde
6. Jaap Keijer
7. Jim Lund
8. Fredrik Bækkerud
9. Marie Sannes Ramsvik
10. Kristian Karstoft
11. Marianne Andersen

Workshop 4 A. + B. (ROS)
1. Adelheid Weidinger
2. Alba Gonzalez-Franquesa
3. Jérôme Piquereau
4. Giada Zurlo
5. Niklas Psilander
6. Per Frank
7. Martin Wohlwend
8. Thomas Kalvik
9. Morten Hostrup
10. Merethe Hansen
11. Christa Funch Jensen

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3. Jaroslaw Walczak
4. Chris Gaffney
5. Sam Shepherd
6. Andrea Kodde
7. Miranda Nabben
8. Jennifer Wettmarshausen
9. Michel van Schaardenburgh
10. Anne Lykkegaard Widlund
11. Brian Lindegaard Pedersen

Workshop 6 (Seahorse)
1. David Busija
2. Andreas Koenig
3. Aleksandr Klepinin
4. Thomas Agnew
5. Marco Spinazzi
6. Giulia Girolimetti
7. Sandra Eggimann
8. Vineta Fellman
9. Jukka Kallijärvi
10. Ole Hyldegaard
11. Anelise Tonin

Workshop 7 (Seahorse - blood)
1. Blair Aldridge
2. Terri Pietka
3. Natalia Komelina
4. Chin San Liu
5. Saima Ajaz
6. Mina Davoudi
7. Henriette Kihl
8. Maria Angleys

Overview of workshops

Thursday, August 29

Workshop 1 (Imaging)
1. Andreas Koenig
2. Christopher Perry
3. Gerardo de Jésus Garcia-Rivas
4. Nikola Kovárová
5. Sam Shepherd
6. Saima Ajaz
7. Miranda Nabben
8. Jim Lund
9. Michel van Schaardenburgh
10. Fredrik Bækkerud
11. Brian Lindegaard Pedersen

Workshop 2 (Oroboros - blood)
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3. Jennifer Wettmarshausen
4. Magnus Hansson
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8. Bodil Bjørndal
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Workshop 7 (Seahorse - blood)
1. Thomas Agnew
2. Li Meng
3. Adelheid Weidinger
4. Sandra Eggimann
5. Jukka Kallijärvi
6. Thomas Kalvik
7. Christiane Gam
8. Liselotte Christiansen
9. Ole Hyldegaard

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Tuesday 27th

Skeletal Muscle & Exercise

Abstracts # 1 - 8

Abstract # 1

Nicotinamide phosphoribosyltransferase knockdown impairs mitochondrial function in mouse myoblasts

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In skeletal muscle proper mitochondrial biogenesis and function is critical for maintaining insulin sensitivity as mitochondrial dysfunction is associated with development of metabolic disorders such as insulin resistance and type 2 diabetes. A family of energy sensing enzymes called sirtuins deacetylates and thus activates transcription factors involved in mitochondrial biogenesis. Nicotinamide adenine dinucleotide (NAD) is a required substrate for sirtuin activation which results in conversion of NAD to nicotinamide (NAM). NAM directly inhibits sirtuin activity, and re-synthesis of NAD is likely essential for maintaining sirtuin activity. Nicotinamide phosphoribosyltransferase (Nampt) is the rate-limiting enzyme in the recycling pathway that converts NAM to NAD, but evidence for a role of Nampt in maintaining sirtuin activity and mitochondrial function is lacking. The aim of the current study was to determine the role of Nampt in maintaining mitochondrial function in response to metabolic stress such as the transition between substrate utilization in skeletal muscle cells. We hypothesized that mitochondrial function would be impaired in absence of Nampt.

We have generated a stable Nampt knockdown (KD) mouse myoblast (C2C12) cell line using a shRNA lentiviral approach. Nampt mRNA expression and protein abundance was reduced by ≈80% in the KD cells. Mitochondrial function was determined using a Seahorse X-96 Extracellular Flux Analyzer. Basal mitochondrial respiration was unaffected by Nampt KD compared to cells expressing non-sense shRNA (control). However, maximal respiratory capacity induced by the oxidative phosphorylation uncoupler Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) was significantly ($p < 0.01$) decreased by ≈60% in the Nampt KD cells. This reduction was not due to altered protein abundance of proteins involved in the electron transport chain. Moreover, palmitate-induced fatty acid oxidation was significantly ($p < 0.01$) decreased by ≈25% in Nampt KD cells compared to control cells. Protein levels of Carnitine Palmitoyltransferase 1, the rate-limiting enzyme transporting long chain fatty acid across mitochondrial membrane, were similar in all cell lines. These findings suggest that Nampt is required for optimal oxidation of fatty acids and Nampt may play a role for maintaining mitochondrial function in skeletal muscle cells.

Abstract # 2

Acute exercise reverses starvation-mediated insulin resistance in humans

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Background Within 2-3 days of starvation pronounced insulin resistance develops, possibly mediated by increased lipid load. Here we show that one exercise bout increases mitochondrial fatty acid (FA) oxidation and reverses starvation induced insulin resistance.

Methods Nine healthy subjects underwent 75 h starvation on two occasions: with no exercise (NE) or with one exercise session at the end of the starvation period (EX). Glucose tolerance and insulin sensitivity were measured with an intravenous glucose tolerance test (IVGTT). Muscle biopsies were analyzed for mitochondrial function, contents of glycogen and phosphorylation of regulatory proteins.

Results Glucose tolerance and insulin sensitivity were impaired after starvation but in EX the response was attenuated or abolished. Glycogen stores were reduced and plasma FA increased in both conditions with a more pronounced effect in EX. After starvation mitochondrial respiration decreased with complex I substrate (NE and EX) but in EX there was an increased respiration with complex I+II substrate. EX altered regulatory proteins associated with increases in glucose disposal (decreased phosphorylation of glycogen synthase, GS), glucose transport (increased phosphorylation of Akt substrate of 160 kDa, AS160) and FA oxidation (increased phosphorylation of acetyl-CoA carboxylase, ACC).

Conclusion Exercise reversed starvation-induced insulin resistance and was accompanied by reduced glycogen stores, increased lipid oxidation capacity, and activation of signaling proteins involved in glucose transport and FA metabolism.

Abstract # 3

Aerobic interval training reverses differences in mitochondrial function between rats inbred for low- and high aerobic capacity

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Background Statistical association between low aerobic capacity and all-cause morbidity and mortality has been shown[1]. Moreover, inbreeding rats for low- and high intrinsic aerobic capacity was linked to manifestation of cardiovascular risk factors[2]. These findings point towards an impaired oxygen metabolism segregating healthy function from dysfunction. However, mechanistically association between low oxygen metabolism and low health remains unresolved.
Objectives Mitochondrial oxidative phosphorylation (OXPHOS) capacity was measured in 22 female rats artificially inbred for low- and high aerobic capacity (LCR; HCR, respectively) for 30 generations. The rats were randomized to either sedate or aerobic interval training (AIT) sessions 5 times a week for 1 month and then 2 times a week for 8 months.
Methods Mitochondrial OXPHOS capacity was assessed using high-resolution respirometry in permeabilized muscle fibers of gastrocnemius (GASTRO) and left ventricle (LV) of the heart. The measurements were blinded and corrected for wet-weight, but not yet adjusted for mitochondrial content.
Results *Baseline results for GASTRO* in sedate LCR compared to sedate HCR showed reduced respiration rates for fatty acid ($p \leq 0.01$), complex I ($p<0.05$) and complex II ($p<0.05$) linked respiration alone, combined complex I+II linked respiration ($p<0.05$) and electron transport chain (ETS) capacity ($p<0.05$). Three months of AIT improved respiration in LCR, such that there was no statistical difference between trained LCR and sedentary HCR (all $p>0.05$) or trained HCR (all $p>0.05$) in any measured variable. *Baseline results for LV* showed lower combined I+II linked respiration in sedentary LCR compared to sedentary HCR ($p<0.05$). Three months of AIT improved combined I+II linked respiration in LCR and vanished the statistical difference compared to both sedentary ($p>0.05$) and trained HCR ($p>0.05$). We also found an increase in fatty acid oxidation after AIT such that trained LCR rats had higher fatty acid oxidation compared to sedate HCR rats ($p<0.05$).
Conclusion Three months sedentary rats that contrast in intrinsic low- and high aerobic capacity differ significantly in OXHPOS in the Gastrocnemius as well as in the heart. The Gastrocnemius revealed broad initial impairments in LCR whereas the heart seemed to be able to preserve more of the respiratory chain complexes. Three months of AIT was able to reverse all these initial impairments of mitochondrial function both in the heart and in the periphery. These findings might explain some of the low health features of low capacity rats.

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Abstract # 4

Expression of PGC-1α transcript variants after acute aerobic exercise in trained human skeletal muscle

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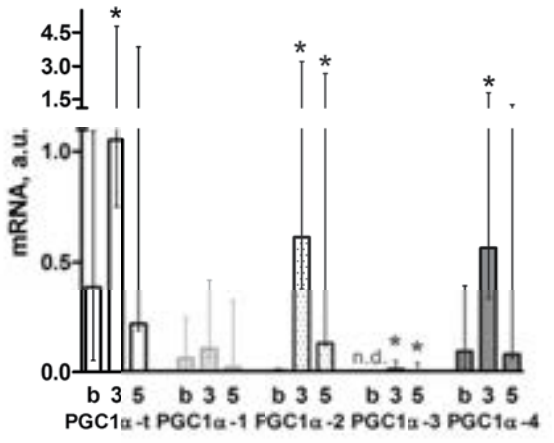
Background There are at least four transcript variants of PGC-1α mRNA in a human skeletal muscle. PGC-1α-1 isoform is transcribed from the canonical proximal promoter and PGC-1α-2, -3 and -4 are transcribed from the alternative upstream promoter, which is placed ~ 14 kb proximal of canonical one. The rodent studies have shown that an acute aerobic exercise induces an increase in the expression of PGC-1α-1, -2 and -3 mRNA variants [1]. Recently the increased expression of PGC-1α-4 mRNA associated with skeletal muscle hypertrophy has been recorded in mouse skeletal muscle. Moreover 8 weeks strength training has been shown to increase expression of PGC-1α-4 mRNA in a resting human skeletal muscle whereas aerobic training had not such an effect [2].

Objectives PGC-1α mRNA isoforms expression was measured in *m. vastus lateralis* of seven endurance athletes ($V'o_{2max}$ 66(60-70) ml/kg/min) before, 3 and 5 h after 90 min cycling (60% $V'o_{2max}$).

Methods The expression of PGC-1α mRNA isoforms was evaluated by qPCR. Phosphorylation of PGC-1α upstream kinases p38 (Thr180+Tyr182) and AMPK (Thr172) was measured by Western blot.



Results The abundance of PGC-1α-1 and -4 mRNA in human skeletal muscle was shown to be higher and PGC-1α-3 mRNA lower than PGC-1α-2 mRNA isoform at rest. The acute aerobic exercise led to a tremendous (>200-fold) increase of PGC-1α-2 and 3 mRNA levels at the 3d-5th h of recovery. At that period PGC-1α-2 mRNA abundance constituted ~50% of the total PGC-1α mRNA. The exercise session led to a 6-fold increase of PGC-1α-4 mRNA during recovery. No significant correlations were found between the changes of upstream kinases (p-p38 and p-AMPK) level and PGC-1α mRNA isoforms abundance.

Conclusion The substantial increase of PGC-1α-2 mRNA abundance during recovery allows us to speculate that PGC-1α-2 has more important functional role for the increase of mitochondrial biogenesis in human skeletal muscle than other isoforms.

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Abstract # 5

The progressive effects of exercise training on the acute regulation of creatine-independent respiratory sensitivity to ADP in human skeletal muscle

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Background: Creatine-independent regulation of apparent mitochondrial respiratory sensitivity (K_{mapp}) to ADP is thought to occur through diffusion of adenine nucleotides between the cytoplasm and mitochondrial matrix via specific membrane transporters. While creatine-dependent phosphate shuttling was demonstrated to be regulated by *in situ* contraction of permeabilized muscle fibres[1] and exercise in humans[2], creatine-independent K_{mapp} to ADP became impaired following moderate intensity exercise[2]. This surprise deficiency would appear to be a limitation to maintaining energy homeostasis during exercise and may therefore be subject to improvement with continual exercise challenges.

Objectives: To determine the progressive effects of exercise training on the response of creatine-independent K_{mapp} to acute exercise challenges in human skeletal muscle.

Methods: High-resolution respirometry (Oroboros, Oxygraph-2k) was used to determine K_{mapp} to ADP (Complex I-supported ADP titrations) in relaxed (25uM Blebbistatin, a myosin II ATPase inhibitor[1,2]) permeabilized muscle fibres at 37°C prepared from human vastus lateralis biopsies sampled Pre, immediately Post and 3hr-Post exercise (1hr, 10 x 4 min intervals of cycling at 91% maximum heart rate separated by 2 min rest) in ten healthy but un-trained men (age, body weight and VO_2peak were 24+/- 1 yr, 74.6 +/- 3.5 kg, 52.1 +/- 1.9 ml/kg/min respectively). Participants then completed eight additional exercise sessions with the biopsy trial repeated at the 5th and 9th session.

Results: K_{mapp} to ADP was quite insensitive pre-training (Pre, 687 μM) but improved following the 1st exercise challenge (Post, 478 μM, $p<0.05$ vs Pre; 3hr-Post, 506 μM, n.s. vs Pre). By the 5th session, K_{mapp} to ADP was unchanged before exercise vs. the 1st session (Pre, 579) and did not change following exercise (Post, 586 μM; 3hr-Post 604 μM). However, by the 9th session K_{mapp} to ADP was improved by 41% in the basal state (Pre, 403 μM, $p<0.05$ vs Pre1st) such that no further improvements occurred after exercise (Post, 319 μM; 3hr-Post 383 μM).

Conclusion: Unlike the impairments in creatine-independent K_{mapp} to ADP following long moderate intensity exercise[2], a single high intensity interval-based challenge to muscle energy homeostasis may improve mitochondrial creatine-independent adenine nucleotide transport. This improved respiratory control progressively manifests in even the basal, non-exercised condition in trained muscle suggesting a new constant state of preparedness for subsequent challenges to energy homeostasis.

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Abstract # 6

The effect of two weeks of immobilization and 6 weeks of subsequent aerobic training on mitochondrial respiration in young healthy men

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Introduction
Mitochondrial dysfunction has been related to lifestyle conditions such as obesity and type-2 diabetes. It is well known that these conditions are strongly affected by physical inactivity. Even so there is limited information on the effect of physical inactivity on mitochondrial function in human subjects. Thus, the aim of the present study is to investigate whether 2 weeks of unilateral immobilization and 6 weeks of aerobic retraining affect mitochondrial respiration in young healthy men. Also, we wanted to investigate if the inactive or trained state affects the mitochondrial ability to response to acute exercise.

Methods
We recruited 12 young healthy recreationally active males. Subjects had one leg randomly immobilized with a DonJoy knee brace for 2 weeks. After the immobilization period subjects performed 6 weeks of supervised cycle ergometer training (20 sessions of 55 min). After immobilization and after the retraining period, subjects performed acute exercise for 45 min on a kicking ergometer. Muscle biopsies were taken before and after immobilization and after the retraining period, and before and after acute exercise in the immobilized and retrained state. Mitochondrial function was determined as respiration in saponin-permeabilized muscle fibers. Furthermore maximal voluntary contraction; VO2max and leg lean mass were measured.

Results
Maximal voluntary contraction and leg lean mass decreased from baseline to immobilization and increased to baseline again after retraining in the immobilized leg with no change in the control leg. VO2max was reduced after immobilization and was significantly higher than basal level after retraining. Immobilization resulted in a significant decrease in the ratio between succinate and glutamate supported respiration. This ratio returned to basal values after retraining. Furthermore, retraining caused an increase in succinate supported respiration and State 4o. Acute exercise resulted in a decreased state 2 and antimycin A inhibited respiration in the non-immobilized leg with no change in the immobilized leg.

Discussion
The major finding of the present study was that immobilization caused intrinsic changes in mitochondrial function, shown as a significant decrease in the substrate control ratio (succinate vs. glutamate supported respiration). Acute exercise altered the mitochondrial function in the control leg but this was not present in the immobilized leg. Based on these results we hypothesize that the acute exercise induced increase in oxidative stress, caused a mitochondrial adaptation resulting in a lower mitochondrial free radical production in the control leg; an adaptation which was blunted in the immobilized leg.

Abstract # 7

Exercise with low glycogen increases mitochondrial biogenesis signaling in human skeletal muscle

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Recent studies suggest that carbohydrate (CHO) restriction can improve the training-induced adaptation of muscle oxidative capacity. However, the importance of low muscle glycogen on the molecular signaling of mitochondrial biogenesis remains unclear. Here, we compare the effects of exercise with low (LG) and normal (NG) glycogen on different molecular factors involved in the regulation of mitochondrial biogenesis. Ten highly trained cyclists (VO_{2max} 65 ± 1 mL kg^{-1} min^{-1}, W_{max} 387 ± 8 W) exercised for 60 min at approximately 64% VO_{2max} with either low (166 ± 21 mmol kg^{-1} dry weight) or normal (478 ± 33 mmol kg^{-1} dry weight) muscle glycogen levels achieved by prior exercise/diet intervention. Muscle biopsies were taken before, and 3 h after, exercise. The mRNA of peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1α) was enhanced to a greater extent when exercise was performed with low compared with normal glycogen levels (8.1-fold vs. 2.5-fold increase). Cytochrome c oxidase subunit I (COX-I) and pyruvate dehydrogenease kinase isozyme 4 (PDK4) mRNA were increased after LG (1.3- and 114-fold increase, respectively), but not after NG. Phosphorylation of AMP-activated protein kinase (AMPK), p38 mitogen-activated protein kinases (MAPK) and acetyl-CoA carboxylase (ACC) was not changed 3 h post exercise. Mitochondrial reactive oxygen species (ROS) production and glutathione oxidative status tended to be reduced 3 h post exercise. We conclude that exercise with low glycogen levels amplifies the expression of the major genetic marker for mitochondrial biogenesis in highly-trained cyclists. The results suggest that low glycogen exercise may be beneficial for improving muscle oxidative capacity.

This study was supported by The Swedish National Centre for Research in Sports.

Abstract # 8

Changes in citrate synthase activity is related to change in whole body oxidative capacity independent of HIT or endurance training intervention

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Background

Citrate synthase (CS) activity is routinely used as a marker of mitochondrial quantity after exercise training interventions. This has been done in studies using short term high-intensity interval training (HIT) and endurance training (ET) of long duration and lower intensity. In the literature a relationship between changes in whole body aerobic capacity and changes in CS activity is often assumed despite different training interventions. However, this needs to be elucidated.

Methods

A systematic search of literature, published from 1983 to 2012, was done on PubMed. We used a search profile including: *citrate, synthase, human, skeletal, muscle, training, not electrical stimulation, not in-vitro, not rats*. We included studies that reported changes in citrate synthase activity and aerobic capacity in humans. We limited inclusion to studies where whole body oxygen uptake (VO_{2max} absolute or relative) was reported.

Results

The search returned 180 publications. Of these 105 did not fulfil our inclusion criteria. From the 85 remaining publications we identified 114 intervention groups that were included in this review. There was a positive (R = 0.48) correlation (P < 0.001) between the relative change in aerobic capacity and the relative change in CS activity in all 114 groups, figure 1.

Studies that used ET as intervention (n=77) correlated positively (P < 0.001, R = 0.414). Studies that used HIT (n=37) training also correlated positively (P < 0.001, R = 0.53).

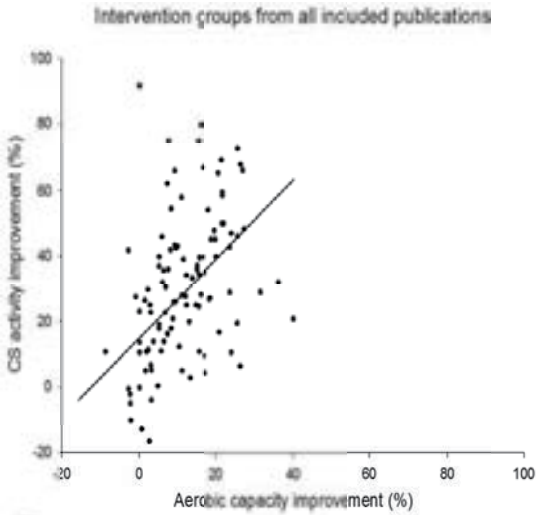

Figure 1

Conclusion

This literature review suggests that changes in whole body oxidative capacity are matched by changes in muscle CS activity. This is independent of the type of training.

Tuesday 27th

Aging, Development, Cancer & Cell death

Abstracts # 9 – 15

Abstract # 9

Effect of resveratrol on mitochondrial function in whole cells

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Background Resveratrol (Resv) has been shown to impact the function of isolated mitochondria, but its modulation of mitochondria in whole cells remains poorly defined. In experiments with isolated mitochondria, Resv decreases several complexes in the electrons transport chain (ETC)[1,2]. Other experiments show an increase in mitochondrial activity by Resv[3,4]. Changes in cellular metabolism are early indicators of processes such as cell proliferation, differentiation and apoptosis. Seahorse Bioscience has developed an in vitro real time bioenergetics assay for determination and quantification of ETC activity. Previous experiments in isolated mitochondria using this technology have demonstrated an increased basal respiration rate following exposure to Resv[5].

Objectives Investigate the effect of Resv on mitochondrial activity in HeLa (human cervical cancer) and C2C12 (mouse myoblast) cell lines.

Methods The Beckman Coulter, Z2 Coulter particle count and size analyzer was used to analyze cell proliferation and cell size. The relative cell number was determined after analysis of oxygen consumption by using a Neutral Red method (NR). The Seahorse XF-24 analyzer was used to test effect of Resv on mitochondrial activity.

Conclusion Resv increases the cell size of HeLa WT but not HeLa Rho0, showing that functional mitochondria are a prerequisite for the regulation of cell size of Resv. Resv decreases the cell number in HeLa WT and HeLa Rho0, and the presence or absence of functional mitochondria did not interfere with effect of Resv on cell proliferation. In HeLa WT Resv decrease the mitochondrial activity about 20% when exposed to 10-200 μM in an acute state. C2C12 myotubes exposed to high Resv concentrations (100 – 300 μM) both decrease cell size and cell number indicating cytotoxic effects. Resv at high concentrations after 24 hr decrease mitochondrial activity in C2C12 myotubes, whereas low Resv concentrations (10 - 30 μM) for 24 and120 hr had no effects. Resv (100 μM for 24 hr) change the inhibitory effect of different ETC inhibitors indicating significant effects on complex V, I and III.

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Abstract # 10

Overexpression of the mitochondrial deacetylase SIRT3 in liver has no effect on metabolic parameters in mice fed a high fat diet, despite increased oxygen consumption in isolated hepatocytes

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Background SIRT3, is a member of the sirtuin family of NAD^+-dependent deacylases and the main mitochondrial deacetylase. SIRT3 has been shown to directly regulate a range of mitochondrial proteins, suggesting a key role for this enzyme in energy metabolism. Studies in SIRT3 knockout mice and high fat diet (HFD) fed mice have suggested a link between deleted or low SIRT3 protein and detrimental metabolic outcomes, including accelerated development of metabolic syndrome[1] and fatty-acid oxidation disorders[2].

Objectives Given the detrimental effects of SIRT3 deletion, the aim of this study was to determine if hepatic overexpression of SIRT3 could protect against metabolic defects induced by excess lipid in mice.

Methods C57BL6 mice were subjected to hydrodynamic tail vein injection of a plasmid containing SIRT3-FLAG to produce liver-specific overexpression. Primary hepatocytes were isolated and assessed for oxygen consumption (Seahorse XF Analyzer), triglyceride accumulation, and insulin-stimulated glycogen synthesis, with or without overnight fatty acid treatment. Mice with liver overexpression of SIRT3 were also maintained on either a chow or high fat diet (HFD) and glucose tolerance, adiposity and liver triglyceride accumulation were assessed.

Results SIRT3 was effectively overexpressed at both the mRNA and protein level in liver. Isolated hepatocytes overexpressing SIRT3 showed significantly increased basal oxygen consumption over controls (>50 percent increase, $p<0.02$). SIRT3 overexpressing hepatocytes also showed 20% less triglyceride accumulation in the non-fatty acid treated group ($p= 0.02$), and improvements in insulin action in fatty acid treated hepatocytes, as indicated by insulin-stimulated glycogen synthesis (4.58 in CON to 7.144 nmol/hr/mg in SIRT3). Despite the effects seen in isolated hepatocytes, *in vivo* liver SIRT3 overexpressing mice displayed similar glucose intolerance, adiposity levels and triglyceride accumulation as control animals in response to HFD.

Conclusion These results show that despite beneficial effects in hepatocytes *in vitro*, acute overexpression of SIRT3 in mouse liver was not protective against metabolic defects induced by a HFD. This suggests that other components of the sirtuin pathway, eg. NAD^+ availability may be critical for sirtuin regulation of metabolism *in vivo*.

References

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Abstract # 11

Identification of a novel mutation in *Wars2* that causes reduced fat mass in ageing mice.

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Overall life expectancy in humans is on the increase. As a result, diseases associated with ageing pose an ever-increasing social and financial burden on society. The Harwell Ageing Screen is the first large-scale project to employ ENU-mutagenesis and phenotyping programmes to specifically generate new recessive mouse models of late onset or age-related disease. Mutant Pedigree Cohort 151 (MPC-151) was identified from the Harwell Ageing Screen as a potential model of resistance to age related obesity.

Affected MPC-151 mice plateau in weight at 9 months of age and are significantly lighter than littermates at all subsequent time points. At 15 months of age affected mice had a mean weight of 31.8g compared with 53.0g in control mice. Body composition analysis using EchoMRI indicated that the reduction in weight is representative of significantly reduced fat mass with affected mice having a mean total fat mass of 5.7g compared with 21.2g in control mice.

Single nucleotide polymorphism (SNP) analysis mapped the MPC-151 lean phenotype to a 73Mb region of chromosome 3 that contains 1023 genes. DNA from one affected MPC-151 mouse was sent for whole genome sequencing and identified a novel non-synonymous mutation in *Wars2* that causes a Valine117Leucine substitution. The mutation also affects splicing as it is located at the boundary between intron 2 and exon 3. *Wars2* encodes mitochondrial tryptophanyl tRNA synthetase ($_{mt}$TrpRS). Interestingly, *Wars2* has recently been identified in human genome wide association studies as one of 13 loci associated with waist hip ratio (WHR) independent of body mass index in humans. WHR is a measure of body fat distribution and is used as a predictor of the complications associated with obesity such as type 2 diabetes, coronary heart disease and chronic inflammation [1].

Preliminary RT-PCR results have indicated that two *Wars2* mRNA splice variants are present in MPC-151 mice that are homozygous or heterozygous for the *Wars2* mutation. The larger product corresponds to full length Wars2 whereas the second product corresponds to a smaller *Wars2* transcript where exon 3 has been skipped. This smaller transcript is not observed in WT mice and we have not yet confirmed whether the product of the shorter splice variant is stable or whether it retains any functional ability.

We are investigating the effect of the *Wars2* mutation by characterizing the respiratory function of mitochondria in mouse embryonic fibroblast cell cultures generated from mice that are homozygous for the *Wars2* mutation. We hypothesize that altered functional ability of $_{mt}$TrpRS expressed in $Wars2^{V117L/V117L}$ mice will result in reduced respiratory function of mitochondria due to reduced expression of the mitochondrial genome.

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Abstract # 12

Physical Properties of Dietary Lipid Droplets in Early Life Program Adipose Tissue Development and Gene Expression in Adult Mice

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Background Feeding mice in early life a Concept infant milk formula (IMF) with large, phospholipid coated lipid droplets (Nuturis®) reduces fat accumulation in adult life upon an obesogenic diet.[1] It has remained unclear to what extent (pre)adipocyte proliferation and/or differentiation are involved in this unique type of nutritional programming of adult body composition. We hypothesized that early feeding with Concept IMF reduces hyperplastic and hyperthrophic growth of adipocytes in adulthood, compared with Control IMF.

Methods Male mice received a diet containing either Nuturis® (Concept IMF) or standard formula (Control IMF) from postnatal day (PN) 16-42. Subsequently, mice were challenged with a moderate Western style diet (WSD; 20% w/w fat, 0.1% w/w cholesterol) until dissection at PN98. Body composition was monitored by dual-energy x-ray absorptiometry analysis during WSD challenge. We assessed weight of four adipose tissue depots, adipocyte number and size distribution, and gene expression of epididymal adipose tissue.

Results Concept IMF reduced fat accumulation during the WSD challenge by 34%, compared with Control IMF group. In Concept IMF exposed rats, adipocyte size was reduced compared to control IMF, whereas adipocyte numbers were similar. Gene expression of adipocyte size markers (leptin, Mest/Peg1) was lower in Concept IMF compared to Control IMF mice. Expression of the pre-adipocyte marker Pref1 was unaffected, indicating similar pre-adipocyte numbers in both groups.

Conclussion Nuturis® Concept IMF diet in early life alters adipocyte functionality, resulting in reduced growth of white adipose tissue in adulthood upon a moderate WSD challenge. Nuturis® Concept IMF exposure limited hypertrophic growth but did not affect hyperplastic growth of adipocytes during a moderate WSD challenge. These data indicate that physical structure of dietary lipids in early life has a programming potential towards adipocyte functionality and risk on obesity later in life.

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Abstract # 13

Maternal Nutrient Restriction and the Kidney: Implications in Mitochondrial Gene Expression.

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Background Epidemiological studies have established that early-life malnutrition results in structural alterations in the kidneys, predisposing the offspring to later life renal dysfunction. In agreement, examination of renal autopsies of adults born with a low birth weight revealed substantial variation in renal composition.

Objectives Due to the role of mitochondrial bioenergetics in energy metabolism and kidney function, the objective of this study was to analyze the impact of maternal nutrition restriction (MNR) on fetal renal mitochondrial transcript expression.

Methods Female baboons were fed normal chow (C) or 70% of C diet (MNR) and fetuses were harvested via c-section at 165 days (0.9 gestation). Kidney samples were analyzed in terms of mRNA for mitochondrial-relevant genes and for immunohistochemistry.

Results MNR did not affect fetal kidney:body weight ratio, although the plasma amino acid profile was altered in MNR fetuses with a decrease on essential and non-essential amino acids, accompanied by increased plasma cortisol. Differential expression of mRNA encoding proteins related with mitochondrial metabolite transport and dynamics was found in female (F) *vs* male (M) fetuses (Down: TIMM17A, SLC25A17, OPA1; Up: COX6A1, IMMP1L and SFN). MNR-related alterations in gene expression were more evident in F, with 16 transcripts significantly altered, 14 down-regulated (including NDUFV3, NDUFS5, COX6A2, COX7B, ATP5J and ATP12A) and 2 up-regulated (CDKN2A and SLC25A15). A total of 9 transcripts were different in M fetuses, with 7 down-regulated (specifically coding for complex I and IV and TIM/TOM complex subunits) and 2 up-regulated (SFN and TIMM9) in the MNR group.

Conclusion Renal transcripts encoding for key mitochondrial energy metabolism proteins are susceptible to MNR in a gender-dependent manner. We speculate that these differences can lead to decreased mitochondrial fitness that, in turn, impacts normal kidney development and susceptibility to mitochondrial-mediated renal pathologies in the adult life.

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Abstract # 14

MITOCHONDRIAL RHOMBOID PARL AS A CRITICAL REGULATOR OF CELL DEATH

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Background: PARL is a mitochondrial serine protease belonging to the large family of rhomboid intramembrane proteases with an unknown function in the mitochondrion.

Objectives: to define the clinical, pathological, and biochemical phenotype of the *Parl*$^{-/-}$ mice, and of the conditional knock out *Parl*$^{nestincre-/-}$.

Methods: *Parl*$^{-/-}$ mice were obtained using a PGK-driven Cre-recombinase. Animals carrying a null allele in the brain (Parl $^{nestincre-/-}$) were obtained by breeding with transgenic females expressing a Nestin-driven Cre-recombinase. Apoptosis was evaluated through TUNEL staining in cells and tissues. Mitochondrial respiration was evaluated in isolated mitochondria from MEF using a Clarke-type electrode. Mitochondrial morphology was evaluated by confocal microscopy in MEF transfected with mtYFP.

Results: *Parl*$^{-/-}$ mice show a striking multisystem disorder with generalized cachexia, atrophy of thymus and spleen and early death before the age of 3 months. Increased apoptosis is detected in the immune system, and cultured cells are more vulnerable to release cytochrome c after challenge with intrinsic apoptotic inducers, implicating PARL as an important regulator of cell death. Surprisingly, oxygen consumption measurements on isolated mitochondria from MEF are normal, and *Parl*$^{-/-}$ cells did not show alterations in mitochondrial morphology or fusion rates.

Conclusions: PARL is indispensable for life. Deletion of PARL leads to increased susceptibility to apoptotic stimuli through mechanism which warrant further evaluations.

Abstract # 15

Mitochondrial DNA genotyping and methodological approaches to quantify heteroplasmy of mitochondrial mutations reveal synchronous nature of simultaneously detected endometrial and ovarian cancers

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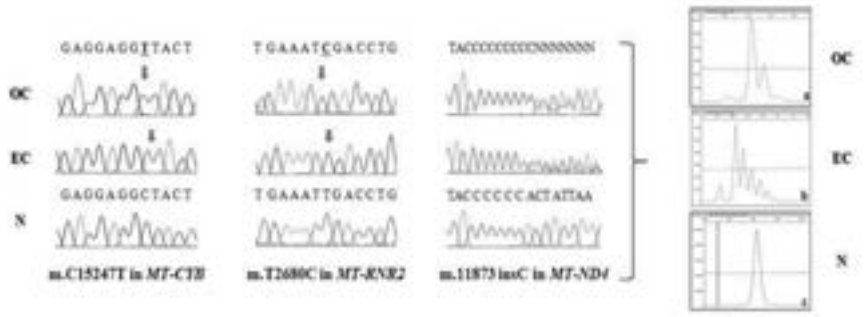
Background Simultaneous independent primary tumors of the female genital tract occur in 1–2% of gynecological cancer patients. Guidelines for determining the nature of simultaneously detected tumors are often ambiguous and may require further molecular analyses[1]. We demonstrated in a previous case of synchronous endometrial (EC) and ovarian carcinoma (OC) the important role of mitochondrial DNA (mtDNA) sequencing in diagnosis[2]. In fact, it is plausible to think that the detection of a random somatic mtDNA mutation in both EC and OC of the same patient may be considered as an unequivocal marker of clonality of the two lesions, since it is virtually impossible that the same mutation may arise in two independent tumors. When investigating mtDNA mutations, the peculiar aspects of mitochondrial genetics, such as heteroplasmy, require suitable approaches which must be sensitive enough to detect low mutation loads[3].

Objectives We aim to demonstrate how the use of mtDNA mutations screening for diagnosis of synchrony in simultaneously diagnosed EC and OC may be informative when the usual guideline approaches are unsuccessful.

Methods Mitochondrial DNA sequencing was performed in 11 samples of women with suspected synchronous cancer. In order to establish the tumor specificity and the heteroplasmy level of the mutations, different methods were used: Fluorescent PCR (F-PCR), denaturing High Performance Liquid Chromatography (dHPLC), quantitative Real-Time PCR (qRTPCR).



Results Mitochondrial tumor specific mutations at different heteroplasmy levels were found in 5/11 cases therefore this analysis was informative for 45% of patients. In these 5 women mtDNA analysis indicated metastatic cancer diagnosis (Figure).

Conclusion Mitochondrial DNA sequencing supported by more sensitive approaches to detect low-level heteroplasmy may provide a cheap and useful contribution to indisputably recognize the metastatic nature of simultaneously detected carcinomas.

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Tuesday 27th

Neuro

Abstracts # 16 – 23

Abstract # 16

Mitochondrial physiology and dynamics in primary fibroblasts derived from patients with amyotrophic lateral sclerosis (ALS)

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Background Mitochondrial dysfunctions play a crucial role in the pathogenesis of various neurodegenerative disorders. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease which affects upper and lower motor neurons. The majority of ALS cases are sporadic (sALS) while only around 10% are familial (fALS). Among the familial forms the most studied and very common disease-causing mutations are those found in *SOD1* gene.

Objectives In order to verify presence of mitochondrial stress and compare sporadic and familiar form of ALS we investigated numerous parameters of mitochondrial physiology in primary fibroblast cultures derived from patients with both forms of ALS and age matched control subjects.

Methods The mitochondrial membrane potential, generation of ROS and cytosolic calcium levels were measured in living cells with laser scanning cytometer. The mitochondrial structure and cytoskeleton organization were visualized by confocal microscopy. Complex I and IV titration and measurement of respiration rates were performed on Oroboros oxygraph. Levels of proteins involved in dynamics of mitochondria (Opa1, Mfn1, Mfn2, Drp1, Fis1) were determined by western blot method.

Results The mitochondrial membrane potential was slightly decreased and cytosolic calcium level was raised in fibroblasts derived from patients with fALS in comparison to controls. Preliminary results showed no significant changes in intracellular ROS levels. Data from oxygraph indicated that activity of complex IV in fibroblasts of fALS seems to be reduced when compared to controls. The profile of proteins responsible for the dynamics of mitochondria were different in investigated cell lines in comparison to controls.

Conclusion Our preliminary results indicate that mitochondrial stress might be present in fibroblasts derived from patients suffering from both familial and sporadic form of ALS.

Abstract # 17

Mitochondrial complex IV dysfunction in blood cells from ALS patients

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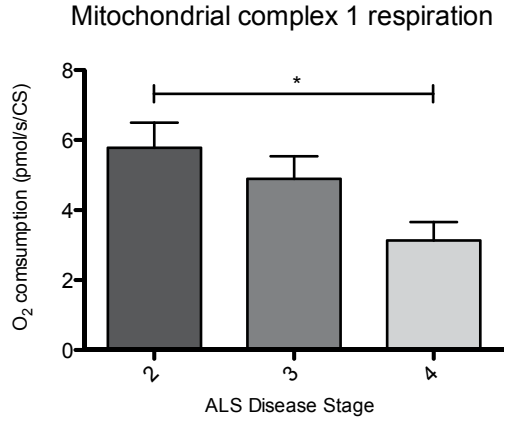
Background Mitochondrial dysfunction is implicated in Amyotrophic Lateral Sclerosis (ALS) but the exact role of the mitochondria in the pathogenesis is not known. ALS is suspected to be a systemic disease with its primary symptoms from the nervous system and abnormalities in both CNS- and muscle mitochondria have been shown in ALS patients.

Objectives We hypothesized that mitochondrial dysfunction could be detected in peripheral blood mononuclear cells (PBMC) and platelets from ALS patients.

Methods Blood samples were acquired from 24 patients diagnosed with ALS and age-matched controls. Thrombocytes and PBMC were isolated and mitochondrial oxygen consumption was measured in intact and permeabilized cells with addition of mitochondrial substrates, inhibitors and uncouplers. Respiratory values were normalized for cell count, citrate synthase activity (CS) and mitochondrial DNA content respectively.

Results The activity of mitochondrial Complex I (CI) per cell was increased in thrombocytes from ALS patients. When normalized for CS, Complex IV (CIV) activity was decreased in ALS patients in both cell types and no difference was detected in CI. However, a significant decrease in CI activity in stage 4 ALS patients compared to stage 2 was seen in PBMCs (see figure) CS per cell was significantly higher in ALS patient than control platelets.

Conclusions CIV-activity per mitochondria is reduced in ALS patients compared to control and there appears to be a compensatory increase in mitochondrial content in cells from ALS patient. The results indicate that mitochondrial dysfunction is more pronounced in late stage ALS disease.



Abstract # 18

Mitochondrial respiratory chain enzymatic activities and UCP3 expression in muscles of patients with hereditary and sporadic amyotrophic lateral sclerosis

Marco Spinazzi, Alberto Casarin, Elisabetta Tasca, Leonardo Salviati, Valentina Cima, Bruno Gavassini, Elena Pegoraro, Gianni Sorarù, Corrado Angelini.

Background: Amyotrophic lateral sclerosis is a complex neuromuscular disease characterized by progressive neurodegeneration of motor neurons with an unclear pathogenesis. A primary dysfunction of the mitochondrial respiratory chain (RC), more prominent at the level of CIV, has been previously reported in muscles from ALS patients,[1] but the results are controversial.[2] Moreover, a dramatic increase of the mitochondrial uncoupling protein 3 (UCP3) has been reported in muscles of ALS patients by a single study.[3]

Objective: We wished to verify if mitochondrial respiratory chain enzymatic dysfunction and elevated UCP3 expression occur in muscles of patients with sporadic and hereditary ALS.

Methods: Activities of the mitochondrial RC enzymes (complex I, II, III, IV, I+III, II+III) as well as of the matrix enzyme citrate synthase were measured using the optimized spectrophotometric assays recently described by our group[4] in muscles from controls (n=15) and different types of sporadic (n=11, of which 5 with a predominant upper motor neuron phenotype, 6 with significant denervation) and hereditary ALS patients (2 with a SOD1 gene mutation, and 3 with a c9ORF72 mutation).
Muscle sections of patients and controls were processed with a combined SDH/COX staining for quantification of COX negative fibers and ragged-blue fibers. The examiner evaluated over 350 fibers for each individual, blinded to the diagnosis.
Protein expression of UCP3 was measured by western blotting.

Results: The activities of the mitochondrial RC enzymes did not differ between ALS patients and controls except in the coupled assay for complex II+III ($p<0.05$).
The frequence of COX negative and ragged red fibers was also not different between patients and controls. Expression of the muscle mitochondrial uncoupling protein 3, was very variably expressed in both patients and controls, without any significant difference.

Conclusion: Our data argue against a significant mitochondrial dysfunction as a unifying pathogenetic mechanisms in muscles of sporadic and hereditary ALS patients. UCP3 is not a useful biomarker for ALS. We speculate that the isolated mild defect of the coupled assay for complex II+III could arise from a secondary reduction in coenzyme Q10 pools in muscles of ALS patients, but this would require further investigations.

References

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Abstract # 19

Over Expression of OPA1 and PINK1 Genes in Patients with Spinocerebellar Ataxia Type III

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Background Mitochondrial fusion, fission and mitophagy may be involved in the neuropathology of spinocerebellar ataxia type 3 (SCA3).

Methods Ten healthy subjects and twenty SCA3 patients were recruited. The scale of the Assessment and Rating of Ataxia was applied as a clinical evaluation. The CAG repeat number of ataxin-3 and expressions of mitochondrial fusion genes (*OPA1* and *MFN2*), fission genes (*DNM1L* and *FIS1*) and mitophagy genes (*PINK1*, *PARK2* and *RHOT1*) were measured by RT-PCR.

Results Significantly increased gene expressions of *OPA1* ($p < 0.05$) and *PINK1* ($p < 0.05$) were noted in leukocytes of SCA3 patients compared with healthy subjects and corresponded to an increase in the CAG repeat number in the ataxin-3 gene and the disease severity of SCA3.

Conclusion Mitochondrial fusion and mitophagy are related to the pathogenesis of SCA3, which may capitalize the mechanism of mitochondrial recycling and quality control. *OPA1* and *PINK1* can serve as two effective biomarkers to predict the progress of SCA3 and also provide a basis for new drug development in the treatment of SCA3.

Abstract # 20

Cyclic AMP Signaling in Brain Mitochondria Affects ATP Production

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Background Mitochondria are the primary source of ATP production. When adapting to the bioenergetic demand of the cell, mitochondria adjust the ATP production according to cellular needs. A cAMP signaling chain within mitochondria has been proposed in the liver[(1)], however the mechanism for metabolic adaptation of ATP demand and production in the brain remains elusive.

Objectives Our objective was to characterize the role of mitochondrial cAMP signaling in adapting ATP production to cellular energy demands.

Methods The presence of signaling proteins in mitochondria was confirmed by Western Blotting. ATP production in isolated mitochondria was measured by luminescence using the luciferin-luciferase method in a plate-reader format. Mitochondrial membrane potential changes in cultured neurons were assayed using the cationic dye TMRM.

Results Presence of the cAMP-generating enzyme soluble andenylyl cyclase (sAC) and its downstream effector PKA was shown in isolated mitochondria. We increased intramitochondrial cAMP by the membrane-permeable analogue 8Br-cAMP and measured ATP production in isolated mitochondria, as well as inhibiting PKA with the inhibitor H89. Inhibition of oxidative phosphorylation by the ATP synthase inhibitor oligomycin abolished ATP production.

Conclusion A signaling pathway contained within mitochondria producing cAMP holds the ability to regulate oxidative phosphorylation, ultimately affecting mitochondrial ATP production.

References

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Cyclic AMP Produced inside Mitochondria Regulates Oxidative Phosphorylation

Cell Metabolism, Volume 9, Issue 3, 4 March 2009, Pages 265–276

Abstract # 21

Flux control analysis of the mitochondrial oxidative phosphorylation in neuroblastoma cells

Aleksandr Klepinin, Vladimir Chekulayev, Natalja Timohhina, Kersti Tepp, Igor Shevchuk, Andrus Kaldma, Andre Koit, and Tuuli Kaambre

Laboratory of Bioenergetics, the National Institute of Chemical Physics and Biophysics, Tallinn, Estonia

Background Neuroblastoma (NB) is a severe pediatric tumor arising from any neural crest element. According to the established idea, NB is considered as a glycolytic tumor of the Warburg phenotype, but little is known about the bioenergetic capacity of mitochondria in NB cells.

Objectives The aim of this study was to clarify the regulation of mitochondrial respiration and main energy fluxes in NB cells.

Methods Studies were performed on murine NB cells of the line Neuro-2a (N2a), and same cells differentiated with all-trans-retinoic acid (RA) (dN2a) served as an *in vitro* model of normal neurons. Oxygraphy and Metabolic Control Analysis were applied to characterize the function of oxidative phosphorylation (OXPHOS) in NB cells. Additionally, the activities of hexokinase (HK), creatine kinase (CK) and adenylate kinase (AK) were assayed. Flux control coefficients (FCC) for all components of the OXPHOS system were measured by their titration with specific inhibitors in the presence of exogenously-added ADP as described in (*Kaambre et al., JBB, 2012, 44, 539-558*).

Results Respiration rates of undifferentiated N2a cells (uN2a) and the activity of Complex-II in the cells were found to be appreciably lower comparing to N2a cells. Nevertheless, our results indicate that NB is not a fully glycolytic tumor and could produce a significant part of ATP *via* OXPHOS. Studies suggested that in NB cells the HK2 and AK2 could play an important role in the generation of ATP. MCA showed that in uN2A cells the key sites in the metabolic control of the energy production are Complex-I, Complex-II and IV, whereas in dN2a cells are Complex-II and IV. Results obtained for the phosphate and adenine nucleotide carriers showed that in dN2a cells these carriers exert lower control over the OXPHOS than in undifferentiated cells. The sum of FCC calculated for uN2a cells (5.06) was found to exceed considerably that for normal cells (close to 1). This suggested that in the mitochondria of NB cells some respiratory chain complexes could be assembled into large supercomplexes (respirasomes). The HK, AK and CK activities in dN2A cells were found to exceed clearly those measured for undifferentiated cells showing that RA-treated NB cells have higher metabolic rates.

Conclusion Our studies support the view that in high-risk NB the role of OXPHOS in generation of ATP oxidative phosphorylation (OXPHOS) is transiently suppressed compared to normal neural tissue. Therefore, mitochondria are now considered as potential targets for anti-cancer therapy and tentative strategies.

Further work is needed to uncover the structure of respiratory supercomplexes in NB cells. The formation of respirasomes could be one of the ways for NB cells to avoid apoptosis, as the cytochrome-c, which should be released during the process is fixed in the supercomplex.

Abstract # 22

Augmented cerebrovascular responses to diazoxide, an opener of mitoK$_{ATP}$ channels, following experimental strokes in rats

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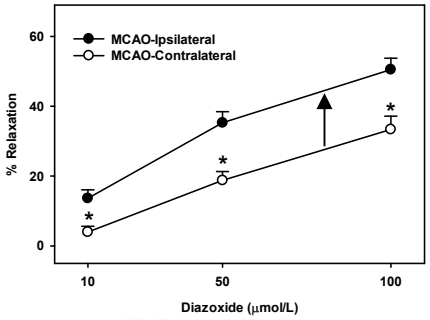
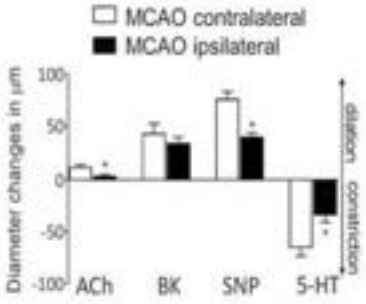
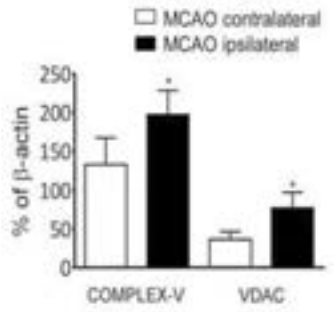
Background Ischemic stress has well know detrimental effects on responsiveness of cerebral arteries. Previous research has not examined the effects of ischemia on cerebral vascular responses to mitochondrial activation by opening mitoK$_{ATP}$ channels. We have previously shown that mitochondrial activation by diazoxide induces pre-conditioning of neurons, astroglia, and cerebral endothelial cells via signaling events that also alter tone of cerebral arteries.[1,2]

Objectives We investigated the role and mechanisms of mitochondrial-derived vasoreactivity in the middle cerebral arteries (MCAs) of male SD rats 48 h following 90 min of *transient* MCA occlusion (MCAO), a widely accepted experimental stroke model.

Methods MCAO caused ipsilateral ischemia and the contralateral side was non-ischemic. Diameters of pressurized, isolated MCAs were measured. Mitochondrial membrane potential was characterized using TMRE and mitochondrial proteins were determined using western blot.







Results Dilation to diazoxide was greatly enhanced on the MCAO side compared to the control side (Left) while responses were reduce on the MCAO side to several other stimuli (Middle). Associated with increased diazoxide-induced dilation was an increase in mitochondrial proteins (Right). Futhermore, TMRE fluorescence was greater under baseline conditions on the MCAO side and in the presence of diazoxide the decrease on the MCAO side was greater than on the contralateral side (165±7 to 70±4 versus 100%--normalized standard to 80±3, respectively).

Conclusion Our results demonstrate a surprising resilience of mitochondrial mechanisms controlling vascular tone in cerebral arteries following experimental strokes at a time when other vascular responses are reduced. The underlying basis for enhanced dilation appears to involve increased mitochondrial mass since mitochondrial proteins and TMRE fluorescence are greater on the MCAO side. We speculate that targeting mitochondria may be useful therapy for improving outcome in stroke patients.

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Abstract # 23

Developing models for the study of Periodic Paralysis

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Periodic Paralysis is caused by mutations of skeletal voltage gated sodium and calcium channels. The direct effects of these mutations are well characterised, leading to an understanding of the initial disease phenotype (attacks of paralysis). However, many patients develop a progressive untreatable myopathy that may render them wheelchair-bound. The pathophysiological mechanisms that link muscle membrane ion channel dysfunction to myopathy remain obscure. We propose that dysregulation of cellular calcium signaling and downstream effects on mitochondrial biology play a central role. Study of these pathways requires the direct analysis of functional muscle cells carrying the mutations. Patient muscle biopsies are not available for the study of such diseases and to this end three alternative models are being developed.

1: Myotubes generated from patient and control fibroblasts. The fibroblasts are transduced to myoblasts by lentiviral delivery of MyoD to the cells. Culture of these cells in low serum medium in a matrigel matrix with addition of Agrin promotes differentiation into myotubes.

2: An immortalised myoblast cell line. A similar differentiation protocol gives rise to myotubes in which control or mutant sodium channels will be over-expressed.

3: Muscle is dissected from mutant and control mice. Single fibres are released from the Flexor Digitorum Brevis for confocal imaging and fibre bundles from the Soleus for analysis of oxygen consumption.

In each case, dynamic live cell imaging is used to characterise changes in intracellular calcium signaling and mitochondrial function while respirometry is used to assess the activity of mitochondrial complexes. This will help to determine the impact of mutations on intracellular calcium handling and mitochondrial structure and function.

Tuesday 27th

Heart

Abstracts # 24 – 29

Abstract # 24

Reperfusion-induced mitochondrial dysfunction in the porcine heart is reduced by TRO40303 in the area at risk predominantly through preservation of outer mitochondrial membrane intactness

M.J. Hansson[1, 2], S. Morota[1], S. Koul[3], R. Jablonowski[2], J. van der Pals[3], M. Kanski[2], P. Gilje[3], H. Engblom[2] and D. Erlinge[3].

1. Mitochondrial Pathophysiology Unit, 2. Department of Clinical Physiology and 3. Department of Cardiology, Lund University, Lund, Sweden.

Background Mitochondria are considered to play critical roles in cell death pathways following ischemia-reperfusion injury.

Objectives The objective of the present study was to perform a functional assessment of mitochondria following ischemia-reperfusion injury of the porcine heart as well as to evaluate mitochondrial effects of hypothermia and the outer membrane translocator protein (TSPO) ligand TRO40303.

Methods Pigs were subjected to 40 min occlusion of the left anterior descending artery followed by 4 hours of reperfusion [1]. Three groups of pigs were treated either by administration of 15 mg/kg TRO40303 or the same volume of saline (1 ml/kg) at normothermia 5 min before reperfusion, or by hypothermia (32°C) initiated prior to occlusion, n=8 for all groups. Transmural needle biopsies were taken from the non-ischemic area in the left lateral wall, from the area at risk in the midventricular anterior wall and from the ischemic core area in the apical anterior wall. Mitochondrial respiratory function was evaluated polarographically in skinned heart fibers using specific substrates and inhibitors [2,3]. In the control group, heart fibers from both ischemic areas demonstrated a general reduction of respiratory states. However, respiration linked to respiratory complex I was more affected than that to complex II indicating loss of soluble matrix components such as NAD(H). Addition of exogenous cytochrome *c* (CytC) increased the level of respiration several fold in both ischemic areas indicating increased permeability of the outer membrane and that CytC loss contributed to the reduced levels of respiration. These changes were diminished by hypothermia in both ischemic areas. TRO40303 attenuated inhibition of respiration involving complex II and reduced the stimulatory effects of CytC in the area at risk, but did not significantly reduce the altered ratio of complex I- and II-mediated respiration, and was without effect in the ischemic core area.

Conclusion It is concluded that mitochondria in both the ischemic core area and the area at risk undergo significant alterations in respiratory function following ischemia-reperfusion injury consistent with both inner and outer mitochondrial membrane permeabilization which can be inhibited by hypothermia initiated prior to occlusion. Administration of TRO40303 prior to reperfusion appears to reduce reperfusion-induced mitochondrial dysfunction in the area at risk mainly by preserving outer membrane intactness and limiting CytC release.

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Abstract # 25

Mitochondrial Dysfunction and Oxidative Stress in Naturally-Occurring Feline Hypertrophic Cardiomyopathy

L. Christiansen[1], Flemming Dela[2], Jørgen Koch[1], and Takashi Yokota[2]

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Background: Hypertrophic cardiomyopathy (HCM) is a primary myocardial disease, characterized by unexplained hypertrophy of the left ventricle. HCM features similar clinical and pathological characteristics in human beings and cats. Mitochondrial dysfunction and oxidative stress are well known to play a role in heart failure and in various cardiovascular diseases. However, in the pathogenesis of naturally occurring HCM, the role of mitochondrial function and oxidative stress is not known.

Objectives: The study aimed at evaluating the involvement of mitochondrial dysfunction in naturally-occurring feline HCM before severe heart failure develops.

Methods: Cardiac muscle was obtained from nine cats diagnosed with HCM (6 males; 2-10 years old, 6.6 ± 0.8 (mean ± SEM)) and from fifteen age-matched control cats (CON) (4 males; 2-11 years, 4.9 ± 0.7). High-resolution respirometry was used to measure mitochondrial function in permeabilized, cardiac muscle fibres. Oxidative stress was assessed by measurements of mitochondrial H_2O_2 generation and thiobarbituric acid reactive substances (TBARS).

Results: In heart muscle of HCM cats, complex-I-linked state 3-respiration was significantly decreased (30 ± 6 pmol s^{-1} mg^{-1}) compared to CON (65 ± 7 pmol s^{-1} mg^{-1}) (P=0.002). Fatty acid oxidation with palmitoyl-carnitine and octanoyl-carnitine was significantly decreased in HCM hearts (12 ± 2 pmol s^{-1} mg^{-1}) and (15 ± 1 pmol s^{-1} mg^{-1}) compared to CON (30 ± 2 pmol s^{-1} mg^{-1}) and (45 ± 5 pmol s^{-1} mg^{-1}), respectively (P=0,002 and P=0.0001). Mitochondrial H_2O_2 generation during state 3, with complex I-linked substrates, was significantly higher in HCM hearts compared to CON ($P<0.05$). TBARS were increased in HCM cats compared to CON.

Conclusion: Findings of the study indicate that mitochondrial dysfunction and enhanced oxidative stress may play an important role in the pathogenesis of feline HCM in the occult stage of disease.

Abstract # 26

Myocardial Fatty Acid Oxidation is Regulated by CD36-Mediated FA Signaling

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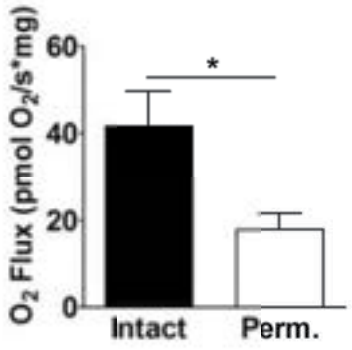
Background: The multifunctional scavenger receptor CD36 facilitates cellular long chain fatty acid (FA) uptake and mediates FA-related intracellular signaling. The CD36-deficient (CD36KO) heart, fed or fasted utilizes less palmitate and more glucose indicating impaired fuel flexibility (1, 2) and we recently showed that CD36KO mice subjected to an overnight fast develop electrical abnormalities and altered lipid metabolism (3).
Objectives: We hypothesized that FA-signaling via CD36 at the plasma membrane plays a key role in regulating fatty acid oxidation and investigated the impact of altered acylcarnitine production in the CD36 deficient heart.
Methods: Acylcarnitine content was determined in fasted wild-type (WT) and CD36KO hearts using multidimensional shotgun lipidomics. OXPHOS capacity and protein content of respiratory complex subunits were measured in isolated mitochondria from WT and CD36KO hearts. Palmitate induced activation of AMP kinase (AMPK, p-T172) protein was measured in control and CD36 knockdown C_2C_{12} myotubes and fasted WT and CD36KO mouse hearts by western blotting. Finally, palmitate induced oxidation was measured in intact and saponin-permeabilized soleus muscle fibers from wild-type mice.
Results: Consistent with the known impairment of FA oxidation in the fasted CD36KO heart (1, 2), we found that the fasted CD36KO heart is deficient in the ability to increase acylcarnitine production.



Additionally, there were no defects in mitochondrial oxidative capacity or respiratory chain protein subunits in myocardial mitochondria from the CD36KO mouse compared to wild-type. Interestingly, despite the lack of increased FA oxidation or acylcarnitine production, CD36 deficiency increased basal levels and activation of AMPK in the heart. Similar results were found with CD36 knockdown C_2C_{12} cells, which had a higher basal activation of AMPK that was unresponsive to the palmitate induced activation of AMPK observed in control C_2C_{12} cells. Thus, the lack of FA oxidation in the fasted CD36 deficient heart likely reflects a limiting role of plasma membrane-mediated FA supply and deficient FA-induced signaling and is not due to insufficient AMPK activation. To test this theory, O_2 consumption induced by palmitate oxidation was measured in intact and saponin-permeabilized wild-type mouse soleus muscle fibers. We found that permeabilization resulted in a ~50% decrease in respiration (Figure), indicating that FA induced uptake/signaling at the plasma membrane drives FA oxidation.
Conclusion: Fatty acid binding to CD36 triggers intracellular signaling events that ultimately lead to activation of AMPK and results in enhanced fatty acid oxidation.

References:

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Abstract # 27

Exercise prevent obesity-induced myocardial oxygen waste and development of left ventricular dysfunction - also when the heart is challenged with a high fat load

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Background Mechanical inefficiency and increased myocardial oxygen consumption are hallmarks of diabetes-induced cardiomyopathy. We have recently shown that exercise training counteract obesity-induced impairment of left ventricular (LV) function and mechanoenergetics, by decreasing work-independent myocardial oxygen consumption (MVO_2).

Objectives As acute elevations in the supply of fatty acids (FA) are also known to induce myocardial oxygen waste, we have investigated whether exercise-induced reduction in MVO_2 is maintained in the presence of elevated FA.

Methods and Results Diet-induced insulin resistant obese C57BL/6J (DIO) mice, subjected to 8-10 weeks of high intensity training (interval running, 10x4 min at 85-95% of VO_{2max}), showed increased aerobic capacity, improved glucose tolerance and reduced obesity. LV function and work-independent MVO_2 were measured in isolated perfused hearts in the presence of high fatty acid levels (2.4 mM) and mitochondrial respiration was measured using high resolution respirometry in isolated cardiac mitochondria using malate and glutamate as substrates. Sedentary DIO mice exhibit LV diastolic and systolic dysfunction, accompanied with a 20% ($p<0.02$) increase in work-independent MVO_2, as well as reduced respiratory capacity and oxygen cost for ATP production (P/O ratio). HIT prevented the diet-induced impairment of work-independent MVO_2, and normalized both respiratory capacity and P/O ratio.

Conclusion Exercise prevents obesity-related development of LV dysfunction and normalizes work-independent MVO_2 - also when the heart is challenged with a high fat load.

Abstract # 28

Time dependence of diet-induced conduction disturbances in the heart

Christa Funch Jensen[1], Lasse Skibsbye[1], Emil Daniel Bartels[2], Lars Bo Nielsen[2], Niels-Henrik Holstein-Rathlou[1], Thomas Jespersen[1], Morten Schak Nielsen[1], and Lene Nygaard Axelsen[1]

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Background: Diabetes and metabolic syndrome causes cardiac complications, including electrical abnormalities that increase the risk for cardiac arrhythmias and sudden death. The pathogenesis, however, remains unknown. We have previously established fructose and fat fed rats (FFFRs) as a pre-diabetic model, which within 6 weeks of feeding exhibit intramyocardial lipid accumulation, decreased cardiac conduction velocity (CV), and increased risk of ventricular fibrillation following ischemia-reperfusion. The CV slowing is not explained by Na^+-channel, K^+-channel or gap junction conductance levels or kinetics, however, CV may be altered by a depolarized resting membrane potential resulting in decreased Na^+ channel availability. Furthermore, acute cardiac lipid loading does not reduce CV per se, but changes in cardiac lipid profile may contribute to CV slowing over time. Therefore, the aim of this study is to investigate the time dependence of 1) diet-induced cardiac CV disturbances and 2) changes in resting membrane potential and action potential (AP) morphology.

Methods: Age-matched male Sprague-Dawley rats are divided into 2 groups. One group is fed unlimited normal chow and the other a special diet consisting of 60 % fat in the chow and 10 % fructose in the drinking water. Following 1, 3 and 6 weeks of feeding, cardiac CV, AP morphology as well as lipid content is determined.

CV is measured by placing a ventricular tissue strip in a fluid chamber. A stimulation electrode is placed on the apex end, propagating an impulse from apex to base. Two measuring electrodes are placed in series on a muscle fiber bundle, and the conduction delay is determined. CV is thus calculated as the interelectrode distance divided by the impulse delay.

Another ventricular tissue strip is placed in a separate fluid chamber, for resting membrane potential and AP morphology characterization. A bipolar silver electrode is used for tissue stimulation and APs are recorded with standard intracellular microelectrodes.

Ventricular triglyceride levels are quantified by thin layer chromatography following lipid extraction. Lipid droplet volume percentage and intracellular localization are determined by confocal microscopy subsequent to BODIPY staining.

Results: Preliminary data indicate that CV slowing occurs in FFFRs somewhere between 3 and 6 weeks of feeding, which indicate that diet-induced CV disturbances takes relatively long time to develop. The results on resting membrane potential and AP morphology are pending.

Abstract # 29

The Implications of Apolipoprotein E Deficiency on Cardiac Mitochondrial Oxygen Consumption.

C. Rocha[1], C. Scheede-Bergdahl[2] and A. Bergdahl[1]

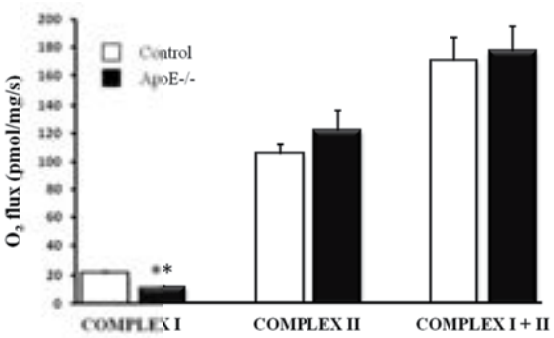
[1]Department of Exercise Science, Concordia University, Montreal, Canada, [2]Department of Kinesiology and Physical Education, McGill University, Montreal, Canada,

Background: Apolipoprotein E (ApoE) is a class of macromolecules found in chylomicrons and low- and intermediate-density lipoproteins. They are essential for normal catabolism of triglyceride-rich substances and are, as such, determinants of lipid metabolism. Absence or structural mutations of ApoE will result in impaired plasma clearance of cholesterol and triglycerides. As a consequence, lipids will be shunted into non-oxidative pathways that ultimately disrupt normal cellular function.

Objectives: The objective for this project was to determine the effects of disturbed ApoE mediated metabolism on mitochondrial oxidative capacity.

Methods: 2 month old female, age-matched C57Bl/6 (Control) or ApoE-/- (Experimental) mice were used. Mitochondrial OXPHOS capacity was measured in permeabilized cardiomyocytes by high-resolution respirometry in a cross-sectional design. Mitochondrial content was assessed by citrate synthase activity and immunoblottings of the voltage-dependent anion channel. Further immunoblottings were done to determine the relative expressions of the 5 OXPHOS complexes.

Results: The in-situ study of mitochondrial function revealed a highly significant, 50% decrease (p=0.002) state 2 respiration (basal, ADP-restricted) in the ApoE-/- mice whereas the state 3 (maximal, ADP stimulated) respiration was similar in both groups. Furthermore the respiratory control ratio was 15% higher (p=0.05) in the heart tissue from the ApoE-/- mice while the mitochondrial leak, estimated as antimycin A flux subtracted by oligomycin flux was reduced by 11% (p=0.05).



Conclusion: The results indicate that lack of ApoE, even in young animals, will affect the coupling between oxidation and phosphorylation within the cardiac mitochondria. Additional work is necessary to clarify the potential damaging effects of this reprogramming of bioenergetics. Modified substrate metabolism following a lack of ApoE and subsequent augmentation of cholesterol and triglyceride levels, seems to initially alter the energetic demands of the heart. Additional studies need to be performed to define the functional underpinnings and whether this condition ultimately contributes to heart failure.

Thursday 29th

Models, Structure and Function

Abstracts # 30 – 36

Abstract # 30

Characterization of complex III deficiency caused by myxothiazol administration in wild type mice

Mina Davoudi[1], Jukka Kallijärvi[2], Sanna Marjavaara[2], Eva Hanson, Per Levéen[1,2], Heike Kotarsky[1], Vineta Fellman[1,2,3]

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A common cause of complex III (CIII) deficiency with liver disorder is mutations in the assembly factor BCS1L leading to depletion of Rieske iron sulfur protein (RISP) in CIII. We wanted to study the impact of RISP inhibition on the formation of respiratory chain complexes and supercomplexes. By administering myxothiazol to inhibit RISP in 5-6 wks old wild type mice (0.56 mg/kg body mass i.p 1- 5 times) we induced CIII deficiency for liver studies. Control mice were given saline with DMSO. After 24, 48, 72, and 74 h animals (n=3/group) were sacrificed and liver mitochondria isolated. CIII-activity was decreased to 50% of control. In the main supercomplexes, myxothiazol treated mice demonstrated increased amount of both complex III (containing RISP) and complex I. Free complex I was also increased. We conclude that myxothiazol induced CIII inhibition causes a compensatory increase of complex I and III content in the supercomplexes.

Abstract # 31

Generation of a *Drosophila* model for GRACILE, a mitochondrial complex III disorder

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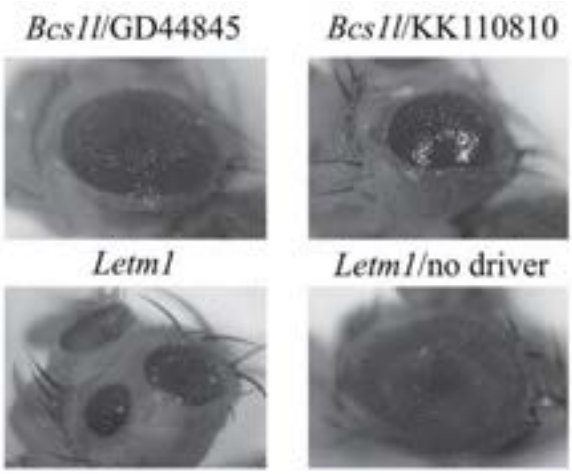
Background The nuclear *BCS1L* gene encodes a mitochondrial AAA-family protein that it acts as a chaperone and incorporates the Rieske iron-sulfur protein into complex III of the respiratory chain[1]. Different *BCS1L* mutations cause a wide spectrum of phenotypes in humans, the most severe of which is a fatal hepatopathy of newborns called GRACILE syndrome[2]. The GRACILE phenotype provides an excellent model for CIII deficiency studies in humans and mice[3].



Objectives The fruit fly (*Drosophila*) is an increasingly important model organism in mitochondrial and metabolic research. Here, we have used *in vivo* RNAi approach to develop a *Drosophila* model for GRACILE and for the study tissue-specific functions of the Bcs1l protein.

Methods RNAi lines were obtained from Vienna *Drosophila* RNAi Center and Gal4 driver and balancer lines either from Bloomington *Drosophila* Stock Center or as gifts from other researchers. Fly maintenance and genetic crosses were performed according to standard protocols.

Results We found that silencing of *DmBcs1l* in the developing eye caused a mild to severe rough eye phenotype. Severe eye malfomation was caused by silencing of *DmLetm1*, which encodes an essential mitochondrial protein, the mammalian homolog of which is known to interact with BCS1L.

Conclusions Simple RNAi silencing of *Bcs1l* in the *Drosophila* eye yields a non-lethal and easily scorable phenotype that may prove useful in testing genetic interactions of *Bcs1l*, and as readout in dietary and pharmacological experiments.

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Abstract # 32

The mechanism by which mitochondrial DNA 9205delTA mutation alters the structure and function of ATP synthase and cytochrome *c* oxidase

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Background Missense mutations in mtDNA *MT-ATP6* gene frequently cause severe mitochondrial disorders. A different type of *MT-ATP6* mutation is represented by 9205delTA microdeletion which cancels the STOP codon of *MT-ATP6* gene and affects the cleavage site in *MT-ATP8/MT-ATP6/MT-CO3* polycistronic transcript, thereby interfering with the processing of mRNAs for ATP6 subunit (F_o-a) of the ATP synthase and Cox3 subunit of the cytochrome *c* oxidase (COX).

Objectives To gain more insight into the pathogenic mechanism, we investigated changes in the structure (subunit levels and composition of the native enzymes) and function (respiration, membrane potential and enzymatic activities) of ATP synthase and COX in control and 9205delTA homoplasmic cybrids.

Methods All experiments were performed in control and 9205delTA homoplasmic cybrid cell lines and some in ρ^+ and ρ^0 cells. Structural changes of ATP synthase and COX were analyzed using SDS-PAGE, BNE- or hrCNE1-PAGE and WB or activity staining in gel. ADP-stimulated respiration was measured by high-resolution respirometry (Oroboros, Oxygraph-2k), $\Delta\Psi_m$ was measured with TPP^+-selective electrode, ATP synthesis was determined in a luciferin-luciferase reaction and ATP hydrolysis in ATP-regenerating system, COX activity was measured spectrophotometrically.

Results We found that 9205delTA mutation diminishes the synthesis of F_o-a protein and reduces the content of Cox1, Cox2 and Cox3 proteins, alters the structure but not the content of ATP synthase, decreases the content of COX, and prevents most of mitochondrial ATP production. The ATP synthase complex was assembled without F_o-a subunit but it was rather labile and unable to synthesize ATP. The complex retained ATP hydrolytic activity that was unexpectedly oligomycin-sensitive, thus questioning the involvement of F_o-a subunit in the inhibitor effect.

Conclusion The pathogenic mechanism of 9205delTA mutation is caused by combine defect of both ATP synthase and COX enzymes, perhaps the COX deficiency is the primary and more critical for the overall mitochondrial energy provision.

Acknowledgement This work was supported by the Grant Agency of the Czech Republic (303/11/0970, 303/12/1363) and Ministry of Education, Youth and Sports of the Czech Republic (AV0Z 50110509, RVO:67985823).

Abstract # 33

The human N-terminal acetyltransferase hNaa30: *in vivo* substrates and its role in maintaining the integrity and structure of the Golgi Apparatus and Mitochondria.

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Background N-terminal acetylation (Nt-acetylation) is among the most common protein modifications in eukaryotes. Despite its emerging roles in regulating protein degradation, targeting and complex formation, the biological roles of Nt-acetylation remain largely unexplored. The NatC complex is one of the three major N-terminal acetyltransferases (NATs)[1].

Objectives Characterize the *in vivo* substrate profile of hNatC by positional proteomics. Findings from the substrate profile are to be studied with immunofluorescence microscopy to uncover the biological function of hNatC.

Methods The hNatC complex was depleted from SILAC labeled A431 cells by RNAi, fractionated into cytosolic and organellar fractions and analyzed with combined fractional diagonal chromatography. Follow-up studies was performed in hNatC depleted CAL62 and HeLa cells by RNAi and analyzed with immunofluorescence microscopy.

Results Here we define the *in vivo* human NatC Nt- acetylome by knockdown of the catalytic subunit hNaa30 in A431 cells and positional proteomics. This revealed 50 human NatC substrates and expanded our current knowledge on the substrate repertoire of NatC. Interestingly, Nt-acetylation of Met-Lys N-termini which is mostly occurring in higher eukaryotes is now also attributed by NatC. NatC may thus contribute to the increased levels of Nt-acetylation observed in higher versus lower eukaryotes, together with NatF1. The substrates of NatC included cytosolic as well as a large fraction of organellar proteins, in particular mitochondrial proteins. Several organellar proteins were found to be significantly regulated. Among the regulated proteins 33% was mitochondrial proteins, suggesting a role for hNatC in mitochondria. Further investigation revealed that knockdown of hNaa30 induces fragmentation of the mitochondrial network and the *cis*-Golgi stack. The observed *cis*-Golgi fragmentation in hNaa30p-depleted cells is not caused by a general disruption of microtubules, or by disruption of endoplasmic reticulum-to- Golgi transport. Further, the evaluated cells were non-apoptotic stressing that the observed phenotype was not due to apoptosis.

Conclusions A large variety of human cytosolic and organellar proteins, in particular mitochondrial proteins, are Nt-acetylated by NatC and this NAT is essential for *cis*-Golgi integrity in a microtubule- and ER-independent manner. Future efforts are focused on elucidating the effect of Nt-acetylation on mitochondrial hNatC substrates and the mitochondrial network itself.

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Abstract # 34

High molecular weight forms of mammalian respiratory chain complex II

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Background Well estabilished supercomplexes of the mitochondrial respiratory chain are formed by complexes I, III, IV. An involvement of complex II (CII), linking respiratory chain with tricarboxylic acid (TCA) cycle, in these supercomplexes is questionable so far. We focused at higher molecular weight forms of CII, which can be detectable after mild detergent solubilisation and clear native electrophoresis, and on to specific interactions of CII with complexes of oxidative phosphorylation pathway (OXPHOS) or TCA cycle.

Objectives Detection of higher molecular weight forms of complex II (CII_{hmw}) in cell lines and tissues (as the representative cell line/tissue we used human fibroblasts and rat heart), and their characterization.

Methods Digitonin-solubilised mitochondrial proteins were separated by native electrophoresis[1] or two-dimensional electrophoretic systems and immunodetected after western blot. In-gel Activities of OXPHOS complexes were measured. CII and complex V (CV) were immunoprecipitated from rat heart and human fibroblasts mitochondria using an antibody against SDHA subunit of CII and a rabbit polyclonal antibody to CV.

Results Native electrophoresis in combination with immunoblot revealed existence of CII_{hmw} structures, which are enzymatically active and differ in electrophoretic mobility between tissues (500 – over 1000 kDa) and cultured cells (400 – 670 kDa). These CII_{hmw} are unstable and readily dissociate on to CII monomer and individual subunits. Isolated defects of OXPHOS complexes have no effect on CII_{hmw} formation, but they are destabilised in mtDNA-depleted, rho0 cells. Immunoprecipitation and electrophoretic experiments indicate, that one of the binding partner of CII could be CV, interactions with other OXPHOS or TCA cycle complexes were not detected.

Conclusion We demonstrated that under sufficiently mild conditions, CII associates into higher molecular structures. Formation of CII_{hmw} depends on the presence of the functional respiratory chain and their lability point out to weak interactions responsible for their formation. Although our experiments clearly indicate the association of CII with CV, there is possibility that CII or it´s subunits may have a role beyond direct involvement in the mitochondrial bioenergetics.

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Abstract # 35

Mitochondrial membrane assembly and multiple forms of TMEM70 protein

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TMEM70 is a 21 kDa protein functioning as specific, ancillary factor of mammalian ATP synthase. It is localized in the inner mitochondrial membrane and its dysfunction inhibits biosynthesis of ATP synthase and represents the frequent cause of fatal mitochondrial disease, autosomal recessive encephalo-cardiomyopathy.

We have investigated membrane assembly of TMEM70 protein using GFP- and FLAG-tagged forms of TMEM70 expressed in HEK293 cells. Based on accessibility to trypsin or membrane-impermeable dye Trypan blue we demonstrated that TMEM70 protein has a hairpin structure with N- and C- termini oriented towards mitochondrial matrix. When solubilized with mild detergents and resolved by BN-PAGE, TMEM70 is detected in multiple homooligomeric forms, dimers and higher oligomers. Variable portion of tagged TMEM70 is found in high molecular weight region, partly overlapping with assembled ATP synthase. Extensive crosslinking and immunoprecipitation studies as well as immunogold electron microscopy confirmed interactions between TMEM70 molecules but no direct interactions with ATP synthase subunits indicating that the biological function of TMEM70 in ATP synthase biogenesis may be mediated through interaction with some other protein.

This work was supported by the Grant Agency of the Czech Republic P303/11/0970, Charles University in Prague UNCE 204011; Ministry of Education, Youth and Sports of the Czech Republic (AV0Z50110509, RVO-VFN64165); IGA MZ ČR NT 11186-5 and IGA MZ ČR NT 13114-4.

Abstract # 36

Hyperactivation of UNC-105 causes loss of mitochondrial membrane potential and impaired mitochondrial ATP production in *C. elegans*

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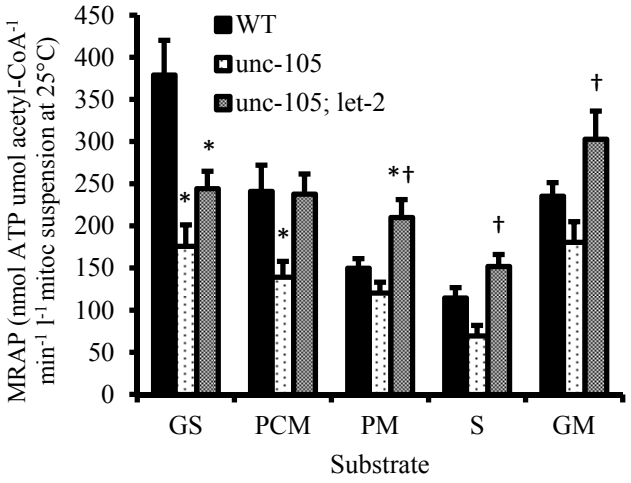
Background *C. elegans* is a free-living nematode known for its utility in genomics research. A dominant gain-of-function mutation in *unc-105,* a putative mechano-sensitive ion channel of the ENaC/Degenerin family, causes mitochondrial fragmentation in body-wall muscles (Szewczyk *et al*. unpublished data) and a movement defect[1]. These defects are ameliorated in *unc-105; let-2* suppressed mutants (*let-2* encodes a collagen).

Objectives Mitochondrial membrane potential and maximal rates of ATP production (MRAP) were determined in wild-type (WT) and *unc-105* mutants to quantify disruption, if any, of mitochondrial function. The *unc-105; let-2* mutants were also assessed to quantify any potential rescue of mitochondrial function.

Methods Any disruption of mitochondrial membrane potential was quantified using JC-10 and Mitotracker® CMXRos *in vivo* staining, which exhibit potential-dependent accumulation in mitochondria. JC-10 fluorescence was quantified using ImageJ. MRAP and maximal citrate synthase (CS) activity were determined in mitochondria isolated from mixed-age worms (n≈300 per assay). MRAP was determined through incubation with a bioluminescent luciferase-based monitoring reagent, and a combination of respiratory substrates and ADP.

Results Mitochondrial accumulation of JC-10 was reduced in *unc-105* versus WT (P <0.001), suggesting the loss of membrane potential in *unc-105* mutants. Similarly, Mitotracker® CMXRos accumulated in mitochondria from WT (P <0.01) and *unc-105; let-2* suppressed mutants (P <0.01), but not *unc-105* mutants. MRAP were reduced in *unc-105* mutants versus WT and *unc-105; let-2* suppressed mutants (Figure). Substrate combinations were glutamate and succinate (GS), palmitoyl-L-carnitine and malate (PCM), pyruvate and malate (PM), succinate (S) and glutamate and malate (GM). Data were normalised for maximal CS activity to account for mitochondrial content and were analysed using one-way ANOVA. * P <0.05 vs. WT and † P <0.05 vs. *unc-105*.



Conclusion The *unc-105* mutants showed evidence of disrupted mitochondrial membrane potential compared to WT, which was accompanied by impaired MRAP with GS and PCM. In the *unc-105; let-2* suppressed mutants, mitochondrial membrane potential and MRAP were better conserved. Thus, as in mammalian cells, constitutive cationic influx leads to pathological changes in mitochondrial function in *C. elegans* muscle. These results establish that it is possible to use *C. elegans* to understand genomic control of mitochondrial ATP production.

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Thursday 29th

Inflammation, Oxidative stress and ROS

Abstracts # 37 – 42

Abstract # 37

Interplay between Mitochondrial Reactive Oxygene Species and Gene Expression

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Background and aim

Excessive systemic inflammatory response (SIR) causes multiple organ dysfunction and often death. SIR induces elevated mitochondrial reactive oxygen species (mtROS) production. mtROS have been shown to play an important role in intracellular signal-transduction. These reactive species can be selectively trapped by mitochondria-targeted antioxidants (mtAOX). The aim of the study was to compare biological effects of Mito-Tempo, a water-soluble mtAOX, and SkQ1*, a fat-soluble mtAOX on inflammation.

Methods Inflammation in Sprague-Dawley rats was induced by lipopolysaccharide (8 mg/kg). After 8 hrs, mtROS, lipid peroxidation products (TBA-RS), mitochondrial function and gene expression were determined by electron paramagnetic resonance, calorimetry, high resolution respirometry and RT-PCR, respectively.

Results Preliminary experiments revealed optimal concentrations of mtAOX which do not impair mitochondrial function. These concentrations were 5 nmol/kg for SkQ1 and 50 nmol/kg for Mito-Tempo. Inflammatory response resulted in increased mtROS generation and in elevated TBA-RS levels. mtROS generation was inhibited only by Mito-Tempo but not by SkQ1, while both substances led to a decrease of TBA-RS levels. Both, SkQ1 and Mito-Tempo regulated expression of MnSOD, GRP78 and other enzymes related to SIR.

Conclusion Our data suggests that mtAOX may attenuate gene expression particularly those relevant to SIR.

*SkQ1 was generously donated by V.P. Skulachev.

Abstract # 38

Evidence that fibroblasts from patients affected by Medium-Chain Acyl-Coa Dehydrogenase Deficiency (MCADD) are under chronic oxidative stress

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Background Mutations in the *ACADM* gene causes MCADD. The clinical features are variable and the physiopathology has been related to energy deficiency, accumulation of toxic metabolites and presence or lack of misfolded MCAD protein, resulting in oxidative stress.

Objectives To evaluate the extent of mitochondrial oxidative stress under different metabolic conditions in cultured skin fibroblasts of controls and MCADD patients carrying distinct *ACADM* mutations.

Results Fibroblasts were grown in standard glucose concentration (11mmol/L) and after 24hrs the media were replaced by galactose (11mmol/L) or galactose and palmitate (100µmol/L). At standard glucose concentration, patient fibroblasts presented higher levels of mitochondrial superoxide compared to controls fibroblasts. In galactose media, the percentage of stressed cells in all groups increased, but the level of superoxide was still higher in the patient fibroblasts. When galactose plus palmitate media was used, patient fibroblasts with the c.[199T>C] + [985A>G] genotype, associated with mild disease, seem to have higher superoxide levels than patients carrying null mutations.

Conclusion These results indicate that fibroblasts of MCADD patients are under chronic oxidative stress. It could be speculated that such mild stress exposure could induce an adaptive response, protecting cells against oxidative damage during pathological situations of metabolic stress like feverish infections.

Abstract # 39

Diverse and tissue specific mitochondrial response in a mouse model of sepsis-induced multiple organ failure

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Background The pathogenesis of sepsis-induced multiple organ failure is complex and our understanding is incomplete. It has been shown that sepsis can induce brain mitochondrial dysfunction by increased uncoupling of the oxidative phosphorylation system [1]. However we have previously demonstrated increasing respiration in human platelets during the first week of sepsis following admission to the intensive care unit [2].

Objectives The objective of the present study was to evaluate the early temporal dynamics of brain and liver mitochondrial function in a mouse model of sepsis-induced multiple organ failure.

Methods The ethical committee at Tokyo Medical University (H-24013) approved the study. Sepsis was induced by cecal ligation and puncture (CLP) and controls were sham operated. Using high-resolution respirometry (Oroboros Oxygraph-2k) brain and liver homogenates from 31 C57BL/6 mice were analyzed at either 6 h or 24 h. ROS-production was measured in brain samples using fluorometry (Oroboros LED2 module). A substrate-uncoupler-inhibitor-titration protocol examined the role of the individual complexes as well as the uncoupled maximal respiratory capacity not restricted by phosphorylation. Citrate synthase was measured in the samples as a mitochondrial marker.

Results The brain exhibited an early decreased respiratory efficiency, the 6-hour group showed an increase in the LEAK state in septic mice ($P = 0.037$) and a trend to lower complex I function in the sepsis group ($P = 0.124$) resulting in a decreased respiratory control ratio ($P = 0.043$). At the 24-hour time point there was also a trend to lower complex I function in the sepsis group ($P = 0.077$) and a non-significant increase in the LEAK state ($P = 0.262$) resulting in a decreased respiratory control ratio
($P = 0.029$). There was no difference in brain ROS-production between the groups. In the liver at 24-hours, there was an increased LEAK state in septic mice ($P = 0{,}0007$) as seen in brain homogenates, however complex I and complex I+II function in liver homogenates from septic mice was also increased ($P = 0{,}0007$ and $P < 0.0001$) compared to controls. There was no significant increase in citrate synthase activity.

Conclusion The present study demonstrates a diverse and tissue specific mitochondrial response to sepsis. The brain displays an early decreased respiratory control ratio; conversely the liver showed increased OXPHOS capacity compared to controls at 24 hours. It should be noted that the liver displayed a significant difference in the control groups between time-points, perhaps due to the insult of a sham operation. This needs to be further evaluated before any final conclusions can be made.

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Abstract # 40

Linking placental mitochondrial dysfunction and taurine deficiency to development of pregnancy complications in maternal obesity.

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Background: The last decade has seen an alarming rise in the number of obese women of reproductive age. Maternal obesity increases the risk of developing pre-eclampsia (PE), fetal growth restriction (FGR) and stillbirth but reasons for this are unclear. Each of these serious pregnancy complications is associated with increased oxidative stress and placental pathology, particularly in the outer syncytiotrophoblast (STB) layer and underlying cytotrophoblast (CTB). STB is renewed to preserve its function as a solute transporting epithelium and endocrine/paracrine organ, maintaining nutrient delivery to the fetus and producing hormones that sustain pregnancy. STB renewal occurs by CTB that proliferate, differentiate, and fuse with the multinucleated STB. In PE and FGR there is reduced CTB proliferation and fusion, and increased CTB apoptosis which leads to placental insufficiency. Reduced STB taurine transporter (TauT) activity is also a feature of PE and FGR as well as maternal obesity during the first trimester and at term[1]. In non-placental cells, taurine protects against damage caused by factors which are elevated in obesity such as inflammatory cytokines and reactive oxygen species. Emerging evidence suggests taurine's cytoprotective role is related to its ability to promote mitochondrial function[2]. Interestingly, mitochondrial dysfunction has been demonstrated in placentas from PE and FGR[3]. In addition to supplying cellular energy, mitochondria are involved in a range of processes important for maintenance and function of STB, such as intracellular signalling, cellular differentiation, and cell death. This project is beginning to test the hypothesis that reduced placental taurine transport causes mitochondrial dysfunction which leads to compromised STB renewal and increased susceptibility to damage, providing a link between maternal obesity and development of pregnancy complications.

Objective: To investigate CTB differentiation and susceptibility of STB/CTB to death and damage following inhibition of TauT in term human placenta.

Methods: Two *in vitro* approaches were used: i) STB model: CTB isolated from normal pregnancy (NP) were maintained in primary culture for 66 hrs during which time they differentiate into multinucleated STB. TauT was inhibited using siRNA technology. Immunofluorescent staining of desmosomes allowed visualisation of multinucleation (used to assess differentiation). In parallel experiments to investigate susceptibility to cell death, prior to fixation at 66 hrs, cells were cultured overnight +/- the inflammatory cytokine TNFα. Apoptosis was measured by presence of cleaved cytokeratin 18, detected by immunohistochemistry (IHC). ii) Explant model: Placental villous explants from NP were maintained in culture for 7 days. TauT was inhibited by incubating explants with the taurine antagonist β-alanine. To investigate susceptibility to damage, explants were treated +/- H_2O_2 from day 5 and oxidative damage to DNA was assessed by 8-hydroxyguanosine IHC.

Results: siRNA-mediated TauT knockdown in CTBs inhibits their differentiation into multinucleated syncytia ($n=7$, $p<0.01$) and increases TNFα-induced apoptosis ($n=4$, $p<0.05$)[4]. Oxidative DNA damage in STB nuclei and cytoplasm (i.e. mitochondrial DNA) is significantly higher in explants treated with H_2O_2 following TauT inhibition by β-alanine ($n=4$, $p<0.05$).

Conclusion: These data suggest taurine is important for maintaining STB and protecting against damage caused by inflammatory stimuli and oxidative stress. Future work will explore whether taurine mediates these effects by promoting placental mitochondrial function.

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Abstract # 41

Superoxide dismutase 2 and thioredoxin reductase 2 in the adult rat ovary during follicular development

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Background Reactive oxygen species (ROS) and their scavenging systems play important physiological roles in the ovary[1]. How these processes are exactly regulated during follicular development and atresia is still unclear. Superoxide dismutase 2 (SOD2), located in the mitochondria, metabolizes superoxide radicals to hydrogen peroxide, which is further detoxified to water and oxygen by catalase or glutathione peroxidase. Although SOD2 has been localized in the human and bovine ovary, its localization in the rat and mouse ovary during follicular development and atresia is less clear. Thioredoxin reductase 2 (TXNRD2), a reductase of thioredoxin2 (TXN2), provides a major line of defence against mitochondrial ROS, nevertheless, not much is known about the localization of TXNRD2 in the mammalian ovary[2].

Objectives To identify the localization of SOD2 and TXNRD2 in ovaries of adult rats in order to understand their role in normal follicle development and atresia.

Methods SOD2 and TXNRD2 protein levels were measured in ovaries from 100-day-old adult rats, using western-blotting on homogenates of whole ovaries. The cellular localization of SOD2 and TXNRD2 was determined by immunohistochemistry using 5 μm thick paraffin sections. The sections were stained using a rabbit polyclonal antibody against SOD2 (diluted 1:1000, Abcam) and TXNRD2 (diluted 1:200, Novus). Staining was performed in ovaries of at least 6 animals. For western blotting the same antibodies were used at dilutions of 1:5000 and 1:500 for SOD2 and TXNRD2, respectively.

Results In the western blots, specific bands for SOD2 and TXNRD2 were detected at the expected molecular weight of 25 kD and 56 kD, respectively.
Immunohistochemical analysis showed the prominent presence of SOD2 in healthy preantral and preovulatory follicles (staining in theca and granulosa cells, and oocyte) and in the luteal cells of the corpora lutea (CL). Staining was faint to absent in granulosa cells of atretic preantral and antral follicles. TXNRD2 immunostaining was observed in the granulosa and theca cells of healthy preantral and antral, and conspicuously present in the steroidogenic cells of the CL. TXNRD2 staining was faint to absent in granulosa and theca cells of atretic preantral and antral follicles.

Conclusion SOD2 and TXNRD2 are present in granulosa and theca cells of developing healthy follicles in the adult rat ovary, but staining is faint to absent in atretic follicles. It is possible that the levels of these proteins in granulosa cells of atretic follicles are not high enough to scavenge superoxide radicals, resulting in increased superoxide radical levels which may cause cell death leading to follicular atresia.

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Abstract # 42

CVB3-induced oxidative stress regulates cell metabolism and innate immune response to infection via mitochondrial antiviral signaling protein (MAVS)

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The RNA virus Coxsackie B3 (CVB3) is an important human pathogen, which is one of the leading causes of severe alimentary tract infection and viral myocarditis. In addition to activating "classical" innate and adaptive immune responses, CVB 3 infection stimulates other cellular activities such as production of reactive oxygen species (ROS). There is little knowledge on the cross-talk between ROS and the innate antiviral response. Our data suggest that CVB3 infection leads to the production of ROS in T cells, and that ROS plays a critical role in activation of mitochondria associated innate immune RIG-I pathway. In this pathway the cytosolic RIG-I like helicases recognize the viral RNA and upon interaction with the mitochondrial antiviral signaling protein (MAVS), recruit signaling molecules that activate the transcription factors NF-κB and IRF-3/7 to promote secretion of type I interferon (IFN). The exact mechanism and regulation downstream of MAVS-sequestered complex remains unclear but our preliminary and published data suggest that pro-apoptotic caspase-3 and -9 may regulate signaling of the RIG-I pathway in MAVS dependent fashion via ROS stimulated glutathionylation, which promotes redox-sensitive signaling. Furthermore, our data suggest that CVB3 affects cellular metabolism by depleting MAVS from the mitochondrial membrane, which in turn influences T cell proliferation and expansion. Our data supports the idea that the innate immune system not only detects specific viral molecules but also senses the cellular oxidative stress level, and integrates this into the innate immune response to infections.

Thursday 29th

Metabolism

Abstracts # 43 – 51

Abstract # 43

Metabolic flexibility before and after lifestyle intervention in a diet-induced type 2 diabetes animal model

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Background
Sedentary habits along with an excess of macronutrients intake are spreading obesity-related type 2 diabetes hence, becoming an epidemic global problem. Metabolic flexibility(1) is defined by Kelley *et al*. as the "clear capacity to utilize lipid and carbohydrate fuels and to transition between them". The disruption of this ability, the so-called "metabolic inflexibility", could play an important role during the early onset of type 2 diabetes when there is already evidence of fasting hyperinsulinemia, hyperglycemia and hyperleptinemia.

Objective
The aim of this project is to assess the metabolic adaptations on an animal model of diet-induced type II diabetes and the effect of a lifestyle intervention programme in reverting them.

Methods
C57BL/6J male mice were used for the study and divided in three groups: a control (*Ctrl*) group (fed for 16 weeks with chow standard diet), a high fat diet (*HFD-pathological*) group (fed for 16 weeks with 45% HFD) and an intervention (*Int*) group (in which a lifestyle intervention was performed after feeding the animals with 45% HFD for 16 weeks). This lifestyle intervention consisted of calorie restriction, modification of the 45% HFD with mono- and poly-unsaturated fatty acids, and exercise training for 5 weeks.

Results
The mice in the *HFD-pathological group* were glucose intolerant and had disrupted insulin sensitivity when compared with their littermates, *Ctrl group*. Those *HFD-pathological* mice were overweight and hyperinsulinemic and hyperleptinemic after overnight fasting. Morphological analyses of the pancreas showed that the *HFD-pathological* mice had more and bigger pancreatic islets than the *Ctrl* littermate mice. Isolated islets from *HFD-pathological* mice had an increased *in vitro* glucose-stimulated insulin secretion. When the lifestyle intervention was performed in the *HFD-pathological* mice, the *Int group* mice reversed most of the phenotype previously defined for the *HFD-pathological group* mice and showed a general improvement in glucose homeostasis; thus, reversing the deleterious effects that led them to a type II diabetic-like state. This communication will be presenting how mitochondrial function will be responding to the different experimental conditions. Mitochondrial function is being evaluated in liver, hypothalamus, glycolytic and oxidative skeletal muscle, and white and brown adipose tissue.

Conclusion
At this point of the study, we have defined the phenotype of the three experimental groups; demonstrated that our lifestyle intervention is able to re-establish glucose homeostasis and body composition; and finally, performed mitochondrial function studies in key metabolic tissues.

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Abstract # 44

Assessment of metabolic flexibility of aged and adult mice using three non-invasive, indirect calorimetry-based methods.

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Background Indirect calorimetry (InCa) is a non-invasive method that can potentially be used to assess metabolic and age-related flexibility as a measure for metabolic health.

Objective To test three InCa-based methods to assess their use for health assessment of mice.

Methods Three InCa methods; i) diurnal pattern of respiratory exchange ratio (RER), ii) RER response to a glucose bolus and iii) response to mild oxygen restriction (OxR, 14.5 % O_2), were tested in aged (72 weeks at start) and adult (10 weeks at start) wild-type male C57Bl/6JOlaHsd mice. Analysis was repeated to assess response stability.

Results Aged mice showed higher adiposity and lower white adipose tissue mitochondrial density, indicative of age-impaired metabolic health. First, natural diurnal patterns of respiratory exchange ratio were followed for 24 hours under standard conditions. These were not stable with time. Second, fasted mice received a glucose bolus to test switch-effectiveness from fat to glucose oxidation. No differences between adult and aged mice were seen. Third, mice were challenged with OxR and adaptation was assessed. In contrast to adult mice, aged mice did not maintain reduced oxygen consumption under OxR at both time periods. OxR thus appeared most sensitive to stably detect differences between both groups. Gene expression and biochemical analyses showed that OxR affected glucose and lactate homeostasis in liver and adipose tissue, supporting the observed differences in oxygen consumption.

Conclusion InCa analysis of the response to OxR is a sensitive and reproducible method to non-invasively measure age-impaired metabolic health in aged mice.

Abstract # 45

Dietary restriction of mice on a high-fat diet induces substrate efficiency and improves metabolic health.

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Background High energy intake and, specifically, high dietary fat intake challenge the mammalian metabolism and correlate with many metabolic disorders such as obesity and diabetes. Dietary restriction (DR) is known to prevent the development of metabolic disorders. The current western diets are highly enriched in fat, and it is as yet unclear whether DR on a certain high-fat diet elicits similar beneficial effects on health.

Objective: To assess health beneficial responses of DR of a HFD

Methods: C57BL/6J mice were given either an *libitum* moderate high fat diet (30 en %; HF) or 30 % restriction of the same diet (HF-DR). Metabolic parameters were analysed and whole genome microarray analysis of white adipose tissue (WAT) was performed.

Results. Already after five weeks of restriction, the serum levels of cholesterol and leptin were significantly decreased in HF-DR mice, whereas their glucose sensitivity and serum adiponectin levels were increased. The body weight and measured serum parameters remained stable in the following 7 weeks of restriction, implying metabolic adaptation. Gene expression in WAT was strongly influenced by HF-DR; in total, 8643 genes were differentially expressed between both groups of mice, with a major role for genes involved in lipid metabolism, mitochondrial functioning and metabolic flexibility. This was confirmed by quantitative real-time reverse transcription-PCR and substantiated by increase in mitochondrial density in WAT of HF-DR mice.

Conclusions. DR of HF improves health parameters and gene expression profiles related to substrate efficiency and metabolic flexibility. This is associated with increased mitochondrial density, which may be a marker for adipose tissue health.

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Abstract # 46

INSULIN SENSITIVITY BEFORE AND AFTER GASTRIC BYPASS IN TYPE 2 DIABETES PATIENTS AND NORMAL GLUCOSE TOLERANCE SUBJECTS.

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Introduction

Gastric bypass surgery (GBP) is known to bring 75-80% of type 2 diabetes patients into remission shortly after their operation.

We studied insulin sensitivity and body composition before and after GBP in morbidly obese patients with type 2 diabetes (DM2) and patients with normal glucose tolerance (NGT).

The aim was to determine how insulin sensitivity is affected by uncontrolled weight loss before operation and resulting from GBP.

Methods

11 patients (7Female/4Male, 6NGT/5DM2) were recruited. Patients were studied 3 times: At baseline (A); after 10-12% weight loss by diet (before surgery) (B); and 4 months post surgery (C). Body composition was measured by Dual X-ray Energy Absorption (DXA) and peripheral insulin sensitivity by hyperinsulinemic (80mU/m²/min), euglycemic clamp.

Results

In the NGT there was no difference in GIR/FFM from A-B and B-C ($p>0,05$) and in spite of a weight loss (-27.5±2kg) from A-C, GIR/FFM did not increase (11.0±0.9 vs. 13.5±1.3 mg/min/kgFFM ($p=0.07$)).

In DM2 GIR/FFM did not change from A-B (4.5±0.5 vs. 6.4±1.0 mg/min/kgFFM ($p>0.05$)) but from B-C (6.4±1.0 vs. 9.2±0.9 mg/min/kgFFM ($p=0.02$)) and A-C (4.4±0.5 vs. 9.2±0.9 mg/min/kgFFM ($p=0.002$)) GIR/FFM increased significantly.

The size of weight loss in NGT vs. DM2 from A-B (5.7±1.0 vs. 5.2±1.0 kg) and B-C (21.8±1.0 vs. 22.8±3.0 kg) was similar ($p>0.05$).

Conclusion

GBP does not improve GIR/FFM in obese patients without DM2; most likely due to initially preserved insulin sensitivity.

A minor (diet induced) weight loss does not improve GIR/FFM in DM2, while a more massive GBP induced weight loss improves peripheral insulin sensitivity.

Abstract # 47

Insulin sensitivity does not correlate with mitochondrial respiration: Studies in gastric bypass patients

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Introduction and aim

Obesity and type 2 diabetes are accompanied by intramyocellular lipid accumulation. It has been hypothesized that this leads to mitochondrial dysfunction and insulin resistance. Our aim was to compare changes in peripheral insulin sensitivity and mitochondrial respiration after a diet induced weight loss and subsequently by a Roux-En-Y gastric bypass induced weight loss in obese patients with or without type 2 diabetes (T2D).

	Obese			T2DM		
	A	B	C	A	B	C
N	10	-	-	6	-	-
Age (years)	38±10	-	-	40±7	-	-
Weight (kg)	130±6	-	-	120±6	-	-
BMI (kg/m^2)	43±1	-	-	41±2	-	-
Weight loss (kg)	-	6±1	27±1	-	5±1	28±4
Body fat (%)	50±2	48±2	40±2*	45±2	44±2*	35±3*$^{\phi}$
VO_{2max} (l/min)	2.7±0.2	2.9±0.3	2.7±0.3	2.7±0.3	2.5±0.2	2.5±0.3

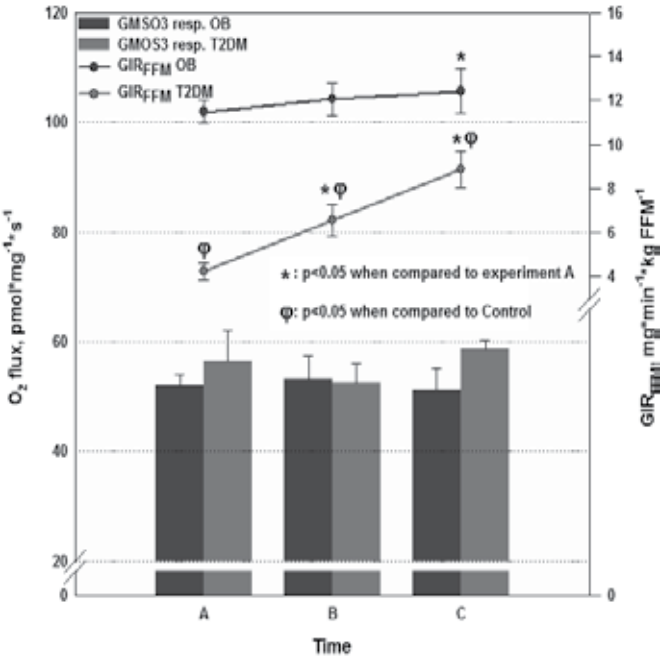
*: p<0.05 when compared to A, ϕ: p<0.05 when compared to B.
Changes were compared by two way repeated measures analysis of variance.

Materials and methods:

16 subjects (4M/12F; 6 with (T2DM) and 10 (OB) without T2D) reported thrice to the lab after an overnight fast: Prior to weight loss (A), 2 mo later just prior to operation (B) and 4 mo after operation (C). At each visit tree tests were performed: Day 1: DEXA scan for body composition and a graded bicycle test for VO_2max. Day 2: Hyperinsulinaemic euglycemic clamp to measure peripheral insulin sensitivity. A biopsy was taken from m. vastus lateralis for respirometry prior to the clamp. Muscle fibers were split, permeabilised and transferred to the respirometer.

Results:

Anthropometric data is shown in the table. Insulin sensitivity measured per fat free mass (GIR/FFM) and maximally coupled respiration data are shown in the figure.



Conclusion:

In spite of the marked difference in insulin sensitivity between OB and T2DM, maximally coupled mito-chondrial respiration was similar in the two groups. Moreover, with marked improvements in T2DM insulin sensitivity, due to the massive weight loss, mitochondrial respiration remained unchanged. These results speak against an association of mitochondrial respiratory capacity and insulin resistance in skeletal muscle in OB and T2DM.

Abstract # 48

The effects and physiological mechanisms of free-living interval-walking training on glycaemic control in type 2 diabetes patients: a randomized, controlled trial

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Background In type 2 diabetes patients, free-living walking training is feasible but shows limited effect upon glycaemic control variables. On the other hand, interval training methods have shown huge improvements in glycaemic control but suffer from lower adherence rates.

Objectives In this study, we first evaluated the feasibility of free-living walking training in type 2 diabetes patients; secondly, we investigated the effects of interval-walking versus continuous-walking training upon glycaemic control; and thirdly, we assessed the underlying physiological mechanisms of changes in glycaemic control.

Methods Subjects with type 2 diabetes (58.7 ± 1.4 years, 29.5 ± 0.9 kg/m^2) were randomized to a control group (n=8), a continuous-walking training group (n=12), or an interval-walking training group (n=12). Training groups were instructed to train 5 sessions per week, 60 minutes per session and were controlled with an accelerometer and a heart rate monitor. Before and after the 4 month intervention, maximal oxygen consumption (VO_2max) was assessed, glycaemic control was measured using continuous glucose monitoring (CGM), and insulin secretion/sensitivity was measured using a hyperglycaemic clamp (5.4 mmol/l above fasting glucose concentration).

Results Training groups demonstrated high and equal training adherence (89 ± 4%), and training energy-expenditure and mean training intensity were comparable. VO_2max was unchanged in the control group and continuous-walking group, but increased in the interval-walking group (16 ± 4 %, $P<0.05$). Glycaemic control (mean CGM glucose levels) worsened in the control group (delta mean CGM glucose = 1.2 ± 0.4 mmol/l, $P<0.05$), whereas mean and maximum CGM glucose levels decreased in the interval-walking training group (delta mean CGM glucose = -0.8 ± 0.3 mmol/l, $P=0.05$, delta maximum CGM glucose = -2.8 ± 0.8 mmol/l, $P<0.05$). The continuous-walking training group showed no changes in glycaemic control.
In the interval walking training group, the insulin sensitivity (57 ± 17 %, $P<0.05$) increased, whereas the insulin secretion did not change (3 ± 6 %, $P>0.05$). The disposition index increased comparable to the insulin sensitivity (60 ± 16 %, $P<0.05$). In the continuous-walking and control group, no changes were seen in any of these parameters.

Conclusion Free-living walking training is feasible in type 2 diabetes patients and interval-walking training is superior to energy-expenditure matched continuous-walking training upon improving glycaemic control. Furthermore, interval-walking induced improvements in glycaemic control seem to be dependent on improvements in insulin sensitivity and increased disposition.

Abstract # 49

Mitochondrial toxicity of Metformin and Phenformin assessed by respirometry of human peripheral blood cells

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Purpose: Metformin and Phenformin are anti-diabetic drugs. Phenformin has been banned from the market due to the high incidence of lactic acidosis (LA).[1] Metformin is generally considered as safe but the development of LA has been reported in rare cases.[2] In both cases, LA is thought to be linked to drug-induced mitochondrial dysfunction in the liver and other tissues.[1] Using high-resolution respirometry it was investigated whether Metformin- and Phenformin-induced mitochondrial toxicity can be detected in human peripheral blood cells.

Methods: Thrombocytes were isolated according to Sjövall *et al*[3] and white blood cells were isolated by Ficoll gradient centrifugation[4]. The integrated function of mitochondria in both intact and permeabilized blood cells was studied using high-resolution respirometry. The cells were treated with a wide concentration range of Metformin, Phenformin or vehicle to assess direct effects by the compounds on respiration. Thereto the time-dependent effect of Metformin on endogenous respiration in intact thrombocytes was followed for 60 minutes. In permeabilized cells, a multiple substrate-uncoupler-inhibitor-titration (SUIT) protocol was used in order to determine maximal respiratory capacities and the site of toxicity of Metformin and Phenformin.

Results: Intact thrombocytes showed a decreasing routine respiration and maximal electron transfer system (ETS) capacity with increasing doses of Metformin compared to controls. In permeabilized thrombocytes, Metformin and Phenformin induced a dose-dependent reduction of maximal oxidative phosphorylation and uncoupled ETS capacity predominantly through inhibition of complex I. Lower concentrations of the drugs were required to induce respiratory inhibition in permeabilized cells compared to intact, but the toxic effect in intact cells developed over time. Although a qualitatively similar toxic effect was seen for both drug treatments, the cells were more sensitive to treatment with Phenformin than Metformin when using the same concentrations. The toxic effect of Metformin was also similar in permeabilized white blood cells.

Conclusion: Metformin and Phenformin have a direct toxic effect on mitochondrial function in human peripheral blood cells. The reduction of respiration is predominantly mediated through a dose-dependent inhibition of complex I. Respirometry of peripheral blood cells may be a suitable assay to predict mitochondrial toxicity of drugs in humans.

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Abstract # 50

MITOCHONDRIAL CARRIER PROTEINS IN FATTY ACID ACTIVATED UNCOUPLING IN DIFFERENT TISSUES OF HIBERNATING GROUND SQUIRRELS

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Background.

Fatty acid - induced uncoupling of mitochondria is known to be mediated by some anion carriers, as uncoupling proteins (UCPs), adenine nucleotide translocase (ANT) and aspartate/glutamate antiporter. Well-studied UCP1 in brown fat produce heat using this mechanism. UCP1 activity is tightly regulated, being inhibited by purine nucleotides and activated by free fatty acids. It is supposed that similar properties are inherent in UCP2 and UCP3 homologues in different tissues. ANT is also involved in the fatty acid-induced uncoupling and responsible for the major part of the thermoregulatory uncoupling in heart- and skeletal- muscle mitochondria of cold-exposure rats and hibernating ground squirrels.

Objectives.

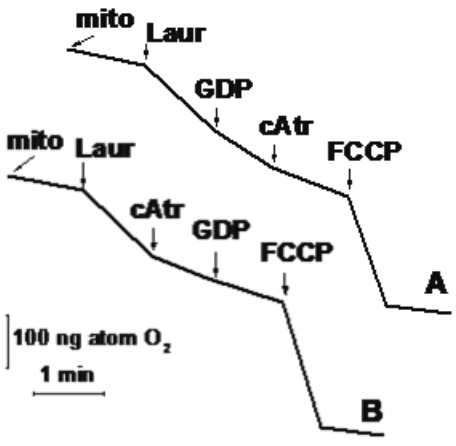
The aim of our investigation was to reveal functional activity of UCP2 and UCP3 using recoupling effect of purine nucleotides and to differentiate them from the contribution of ANT in the uncoupling using its high-specific inhibitor - carboxyatractylate.

Methods.

We measured respiration rate of mitochondria in the presence of oligomycin and rotenone under addition of 100μM laurate. We tried different order of additions of inhibitors to demonstrate their independent effects. We used recoupling effect of GDP to indicate activity of UCP3 in skeletal muscle and UCP2 in lung and spleen of long-tailed hibernating ground squirrels. Mitochondria of liver were taken as negative control, since there are no any UCPs at significant level. Hibernating ground squirrels as the object ensured increased level of all UCPs during winter season.

Results.

We found that GDP in millimolar concentrations had a slight recoupling effect on respiration rate and membrane potential in all studied mitochondria of hibernating ground squirrels, including skeletal muscle, lung, spleen and liver mitochondria. However, GDP had no effect, if carboxyatractylate in micromolar concentration was added previously. No crucial differences were found in compare to liver mitochondria. At the same time, evident and independent recoupling effect of glutamate before as well as after carboxyatractylate, demonstrate activity of aspartate/glutamate antiporter in fatty acid – induced uncoupling.



Conclusion.

We concluded that recoupling effect of purine nucleotides in skeletal muscle, lung, spleen and liver mitochondria of hibernating ground squirrels can be explained by their interaction with ANT rather then by functioning of UCP2 and UCP3.

Acknowledgments.

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Abstract # 51

Altered mitochondrial DNA content in patients with diabetic nephropathy

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Background: It is estimated that 360 million people are living with diabetes in the world and one third of these patients develop diabetic nephropathy (DN), a serious complication of diabetes which can lead to end-stage renal disease. In recent years it has become apparent that mitochondrial dysfunction contributes to the development of DN. Changes in mitochondrial DNA (MtDNA) content and integrity have been proposed to be related to mitochondrial dysfunction and oxidative stress (1, 2).

Objectives: The aim of this project is to investigate the hypothesis that diabetes induced changes in MtDNA content and integrity play a role in oxidative stress and diabetes complications.

Methods: Blood and urine samples from diabetes patients with and without nephropathy were used to extract DNA and RNA. MtDNA content was determined as the mitochondrial to nuclear genome ratio (Mt/N) using real time quantitative PCR (3). Mitochondrial biogenesis was measured by quantifying mRNA expression of mitochondrial transcription factors TFAM and PGC1- α relative to GAPDH in total RNA isolated from whole blood by using absolute real time quantitative PCR.

Results : Circulating MtDNA content was increased ($P<0.05$) in patients with diabetic nephropathy compared to controls. MtDNA content was significantly higher ($P<0.05$) in urine pellets (1.9± 1.87) compared to whole blood (0.8 ± 0.49) in diabetes patients without nephropathy. In contrast, in DN patients MtDNA content was significantly lower ($P<0.05$) in the urine pellets (1.47±0.85) when compared with whole blood (2.75 ± 1.11). Both TFAM and PGC1alpha mRNAs were detected in whole blood in all patients but values were the highest in diabetic nephropathy group compared to the non nephropathy or control groups however this difference was not significant ($P>0.05$).

Conclusion: We have observed an alteration in MtDNA content in blood and urine of DN patients and also a slight but statistically non-significant increase in mitochondrial biogenesis. In order to further elucidate the relationship between mitochondria and DN, we will measure oxidative stress markers and undertake mitochondrial functional studies in the future. These preliminary data support the hypothesis that mitochondrial dysfunction is associated with DN and show that alterations in MtDNA can be detected in blood and urine samples from patients.

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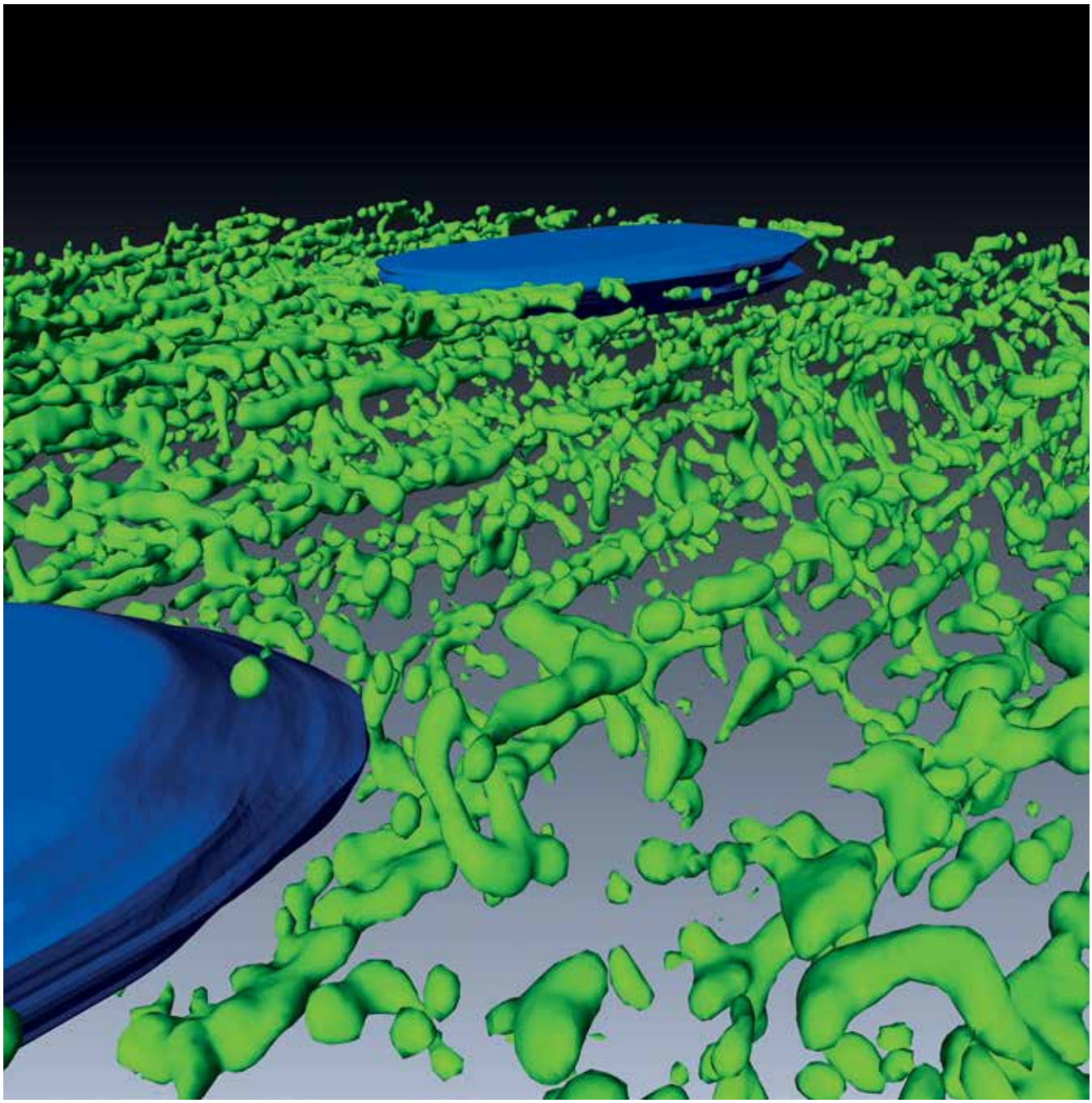

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