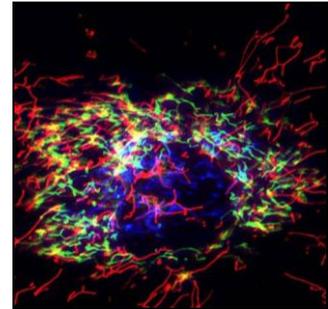


Cover

Front *Human Skin Fibroblast* by Werner J Koopman (MiPart at MiP2010).

Three confocal sections (bottom to top: red, green and blue) of a living cell stained with rhodamine 123. Dept Biochemistry (286) NCMLS, Radboud University Medical Centre Nijmegen, The Netherlands.

MiP2010: Mitochondrial physiology. The many functions of the organism in our cells. *Mitochondr Physiol Network* 15.6: 128 pp.



Back *World Health Worries* by Odra Noel, London. MiPart Gallery, OROBOROS, Innsbruck, Austria (Bioblast 2012).

The world is worried about health. This map provides a high level view of the human tissues where major health concerns are, at the beginning of the XXI Century.

Most developed and developing countries have cardiovascular diseases as their main cause of death. North America struggles with obesity (adipose tissue), with the harmful effects of alcohol (hepatic tissue) represented in Alaska. Europe, with its ageing population, has a heavy load of neurodegenerative and psychiatric conditions (neurones). The Middle East and a good portion of the Far East have to deal with cardiovascular conditions (represented by cardiac muscle), while a wave of diabetes expands over the rest of the Far East and the Pacific (pancreatic acinus tissue). Australia struggles with high levels of digestive track cancers (intestinal villi). Africa is the place where communicable diseases (infectious and parasitic diseases) are serious serial killers (represented by blood: erythrocytes and white blood cells). South America has its fair share of cardiac problems and diabetes, but also infectious diseases, particularly respiratory infections (lung tissue).

As curiosities, hidden among the tissues are several mitochondria, a key to the future understanding and research into health and ageing. Greenland, with its very small population highlights a male infertility concern (sperm), and the only visible artery of the composition is right in the heart of Amazonia.

Based on data on causes of death and burden of disease (World Health Organisation, public website. Data from 2008, accessed October 2012).

Bioblast 2012: Mitochondrial Competence. *Mitochondr Physiol Network* 17.12: 96 pp.



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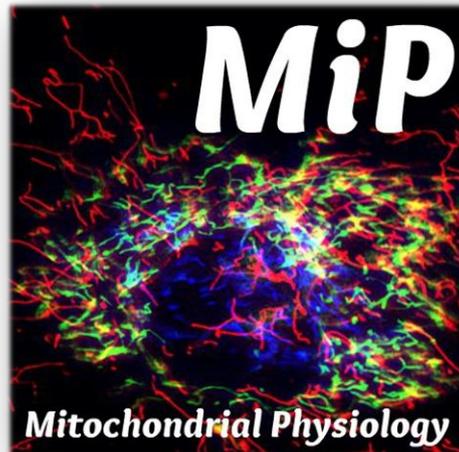
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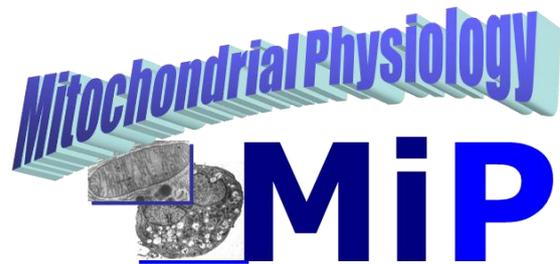
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MiP2013
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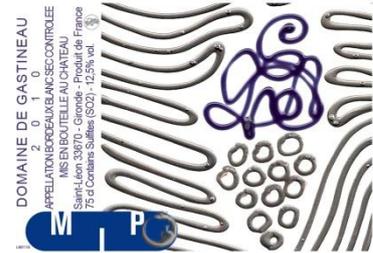
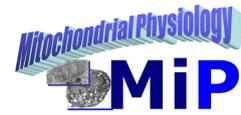
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**9th Conference on
Mitochondrial Physiology
The Mitochondrial Physiology Society**

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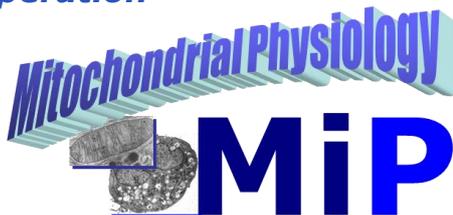
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MitoPedia Glossary: Terms and abbreviations

CI	Complex I, NADH:ubiquinone oxidoreductase; CI-linked substrate state.
CII	Complex II, succinate:ubiquinone oxidoreductase; CII-linked substrate state.
CI+II	CI plus CII linked substrate state with convergent electron transfer at the Q-junction.
CIII	Complex III, ubiquinol:cytochrome c reductase.
CIV	Complex IV, cytochrome c oxidase; CIV-linked substrate state.
CV	Complex V, ATP synthase.
ETS, E	Electron transfer system; ETS state; ETS capacity.
J_{O_2}	Oxygen flux, respiration expressed as moles per second per unit system size, e.g. per volume, per mg protein, or per mg wet weight (size-specific quantity).
I_{O_2}	Oxygen flow, respiration expressed per system, e.g. per cell (10^6 cells), per instrumental chamber (extensive quantity).
LEAK, L	Resting respiration compensating mainly for proton leak; LEAK state.
mt	Mitochondrial, mitochondria.
OXPHOS, P	Oxidative phosphorylation; OXPHOS state; OXPHOS capacity.
p_{O_2}	Partial pressure of oxygen [kPa]; 1 mmHg = 0.133322 kPa.
Q	Coenzyme Q, redox system (ubiquinol/ubiquinone) of the ETS.
RCR, P/L	Respiratory acceptor control ratio.
ROS	Reactive oxygen species.
TCA cycle	Tricarboxylic acid cycle.
$V_{O_{2max}}$	Maximal oxygen consumption in an ergometric test.
$\Delta\Psi_{mt}$, mtMP	Mitochondrial membrane potential (intensive quantity).

>> For further information, see **MitoPedia** in the **Bioblast Wiki** www.bioblast.at



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The *information synthase*
for mitochondrial physiology

MiP2013 in the MiPMap:

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Comparative MiP2013 - Opening

What is mitochondrial physiology – why comparative?

Erich Gnaiger

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How mitochondria work

10 years after setting the foundations of the Mitochondrial Physiology Society (MiP2003, Schröcken, Austria) our search continues as to what mitochondrial physiology is. Mitochondrial physiology is the study of “how mitochondria work”.

Animal physiology is the study of “how animals work” - says the title of a textbook by Knut Schmidt-Nielsen. Comparative physiology derives its fascination from the diversity of form and function. Organismic variation is studied in diverse environments and in extremes of physiological performance, with explosive activities and high power output in short bursts or endurance over prolonged periods of time with high efficiency. Diversity is nature’s treasure and the subject of comparative physiology. The famous August Krogh principle – Krogh received the Nobel Prize in 1920 - is frequently cited [1,2]: “*For a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied.*” This principle was first formulated in 1975 by another Nobel laureate who received the Prize in 1953 for the metabolic cycle that carries his name, Sir Hans Krebs [3,4]. This direct link between one of the most famous mitochondrial biochemists and the August Krogh principle that “*epitomized the very essence of comparative physiology*” [2] immediately raises the question: Why was comparative mitochondrial physiology not established some 30 to 40 years ago?

The world as a laboratory – the Kjell Johansen principle

In comparative mitochondrial physiology two disciplines converge which were not in immediate conceptual and methodological contact: bioenergetics and comparative physiology. Ladd Prosser divided physiology into cellular physiology, physiology of special groups and comparative physiology, noting for the first category that “*at the cellular level all organisms have more in common than in difference*” [5]. Accordingly, the physiology and biochemistry of the cell and the bioenergetics of the mitochondrion are focused primarily on unifying principles: the genetic code, the protonmotive force. If there is little variation in the way how mitochondria work, if muscle from horse to mouse simply “*builds more mitochondria of the same kind*” upon increased energy demand [6], then diversity is missing as an essential substrate for a comparative science to develop. If the same bricks are used for building libraries and garages, then there is little interest in comparison of bricks. Taking the *world as a laboratory* [2] mitochondrial diversity is discovered along with conserved traits: Sir John Walker (Nobel Prize 1997) unravels unity and diversity in *Generating the fuel of life* (page 8). A paradigm change in mitochondrial physiology comes to light (*Kjell Johansen Memorial Session, A1; page 9*).

Peter Hochachka and his students, colleagues and competitors brought biochemistry into comparative physiology. The influential book by Peter Hochachka and George Somero (1st ed 1973; [7]) reviews mitochondrial respiration in the global size range from the molecule cytochrome c oxidase to *Gaia*, and explores exotic animals living in exotic places and adapted to extreme conditions of temperature, hydrogen sulfide concentrations or metabolic shutdown (*Session A2*). These are excellent examples of the Kjell Johansen principle: “*If those species are from Cleveland, choose another problem*” (Roy Weber, personal communication; *A1-01*) or “*if you can study an organism in Cleveland, study something else*” [8]. The toxic hydrogen sulfide in the extreme habitat of hydrothermal vents was discovered as a substrate for mitochondrial respiration [7]. The pathways were elucidated further in less ‘exotic’ animals, rat liver mitochondria and the intertidal lugworm [9]. Today we discuss mt-respiratory control by H₂S in mammalian cells from the extreme environment of the alimentary tract or in the setting of the intensive care unit (*Session B1*).

Comparative and environmental MiP

Temperature and hypoxia stand out as prominent chapters in the work of Kjell Johansen and Peter Hochachka. These important topics are largely ignored in mitochondrial bioenergetics, when respiration or ROS production of mitochondria from rat, mouse or human tissues is studied at room temperature or 30 °C (without discussing hypothermia in homeotherms) or at atmospheric oxygen levels (without concern of hyperoxia relative to intracellular p_{O_2} [10]). Current discussions on the mitochondrial free radical theory of aging (*Session C3*) rely strongly on comparative studies of nematodes, insects, birds and mammals [11]. Naked mole rats with a 25-30 years maximum life span are compared with mice (3-4 years). The naked mole rat lives in its burrows in Kenya in a natural thermostat with a core body temperature of about 30 °C [2]. Keep this in mind when selecting the temperature in your next experiment on mammalian mitochondria. Experimental temperature comes to mind as a hot MiPchallenge for ectothermic species experiencing a global experiment of climate change (*Session A3*).

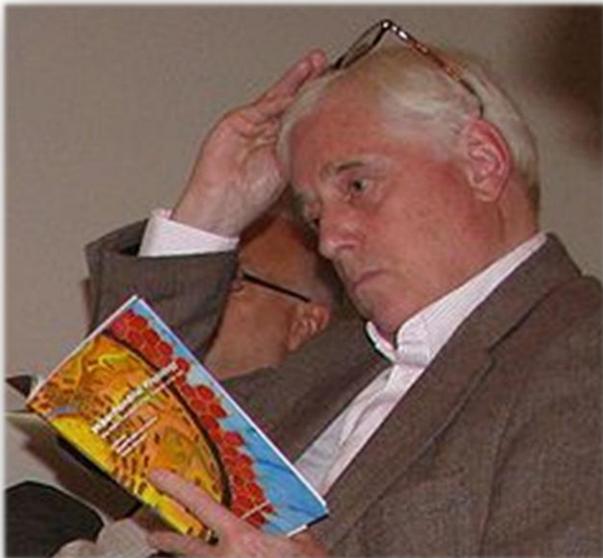
Mitochondrial respiratory control - from unity to diversity

The diversity of mt-respiratory control currently discovered in different species, tissues and cell types is both exciting and disturbing. Disturbing because we have not developed a unifying hypothesis to understand the differential adaptive advantages of the diverse tissue- and species-specific patterns of mitochondrial respiratory control, of variations in ROS production, supercomplex formation and very generally differences in mitochondrial form and function. This presents a challenge to mitochondrial physiology to operate in the intellectual framework of comparative physiology [12].

One lesson of comparative mitochondrial physiology is straightforward, appears to be simple and important, hence cannot be emphasized enough: If you are interested in humans, study humans and compare. If you want to know about mitochondrial function in heart, study heart and compare.

"Mitochondrial functional diversity will represent a gold mine for mitochondrial physiology, to learn more about mitochondrial function, mitochondrial health and disease" [13]. Three years later this promise is put to the test: Will MiP2013 emerge as a milestone linking comparative concepts with mitochondrial physiology and pathology (*Section C*), even if important contributions [14] are missing at a small conference? An energized international mitochondrial network is at work with fission and fusion, excitement, efficiency and high potential to bring comparative mitochondrial physiology [15] to the forefront as a driving force in mitochondrial research and medicine.

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15. The Mitochondrial Physiology Map – MiPMap: <http://www.bioblast.at/index.php/MiPMap>



MiP2013 Keynote Lecture

**Professor
Sir John E Walker**

Generating the fuel of life.

Walker John E

*Medical Research Council Mitochondrial
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- john.walker@mrc-mbu.cam.ac.uk

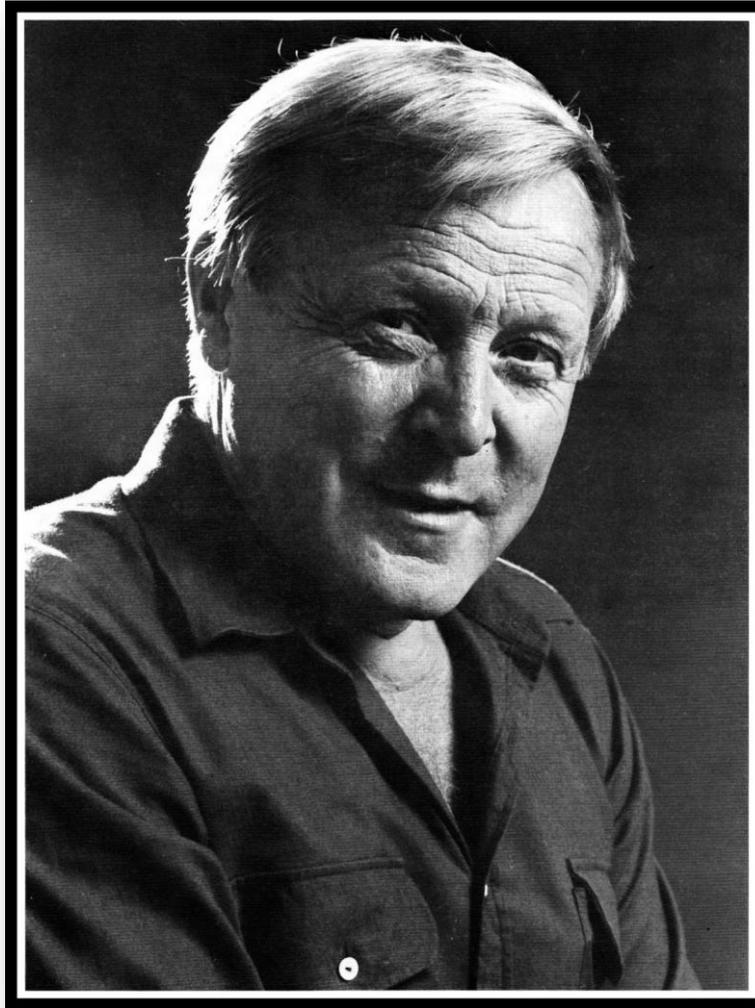
The lecture will be devoted to the topic of how the biological world supplies itself with energy to make biology work, and what medical consequences ensue when

the energy supply chain in our bodies is damaged or defective. We derive our energy from sunlight, which, via photosynthesis in green plants, provides high energy components in the foods that we ingest. We harvest that energy, effectively by “burning” (oxidising) the high energy components, releasing cellular energy in a controlled way to generate the fuel of life, in the form of the molecule known as adenosine triphosphate (or ATP for short). The key steps in this process take place in the mitochondria inside the cells that make up our tissues. They serve as biological “power stations” that contain millions of tiny molecular turbines, the ATP synthase, that rotate rather like man-made turbines churning out the cellular fuel in massive quantities, which is then delivered to all parts of our bodies to provide the energy to make them function. Each of us makes and expends about 60 kg of this fuel every day of our lives. Defects in the fuel supply process are increasingly being recognised as important components of complex human diseases such as cancer, neurodegeneration and neuromuscular diseases, and they may also be part of the process of ageing.

The ATP synthases found in mitochondria eubacteria and chloroplasts have many common features. Their overall architectures are similar, and they all consist of two rotary motors linked by a stator and a flexible rotor. When rotation of the membrane bound rotor is driven by proton motive force, the direction of rotation ensures that ATP is made from ADP and phosphate in the globular catalytic domain. When ATP serves as the source of energy and is hydrolysed in the catalytic domain, the rotor turns in the opposite sense and protons are pumped outwards through the membrane domain, and away from the catalytic domain. The lecture will describe the common features of their catalytic mechanisms. However, the ATP synthase from mitochondria, eubacteria and chloroplasts differ most fundamentally in the energy cost that is paid to make each ATP molecule. The most efficient ATP synthase is found in the mitochondria from multicellular animals. The ATP synthases in unicellular organisms, and chloroplasts, pay various higher costs that seem to reflect the supply of available energy in the biological niches that they inhabit. The ATP synthases also differ significantly in the way they are regulated. Eubacteria have evolved a range of mechanisms of regulation, and the chloroplast enzyme is rendered inactive by a redox mechanism in the hours darkness. Mitochondria contain an inhibitor protein, IF_1 , that inhibits ATP hydrolysis but not ATP synthesis. Its *in vitro* mechanism has been studied in great detail, but its *in vivo* role is mysterious, and suppression of expression of the protein appears not to influence respiration.



In mitochondria the ATP synthase is organised in rows of dimers along the edges of the cristae, and as will be discussed, it has been suggested that the permeability transition pore involved in apoptosis resides in the dimeric enzyme.

**Kjell Johansen 20 Sep 1932 – 4 Mar 1987**

Scan by Roy Weber from the portrait in the seminar room, Zoophysiology, Department of Bioscience, Aarhus University. The photograph was published in the Aarhus University 1987 Yearbook.

In this context I cannot help recalling a discussion I overheard years ago in the Arctic between Larry Irving and some fellow physiologists. The discussion got heated when Larry Irving refused to recognize the white laboratory rat as an animal. He argued that the white rat with food and water ad libitum and a thermostatted cage placed in a regulated light-dark cycle for literally thousands of generations could and should not qualify as an animal. I think Irving won the discussion [1] – and Kjell was right [2].

*Beija flor is
very much alive –*

the Power of Life



*As always
Kjell*

**Kjell Johansen
Viking and Physiologist**

1. Johansen K (1987) The August Krogh Lecture: The world as a laboratory. Physiological insights from nature's experiments. In: Advances in physiological research (McLennan H, Ledsoe JR, McIntosh CHS, Jones DR, eds). Plenum Publishing Corporation: 377-396.
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A1

MiPcomparative: Oxygen delivery and muscle OXPHOS

Kjell Johansen Memorial Session

Kjell Johansen lecture



A1-01 Hemoglobins: molecular adaptations safeguarding mitochondrial O₂ supply.

Weber Roy E

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Vertebrate hemoglobins (Hb) are exquisitely designed to transport O₂ from the respiratory organs to the tissues, thereby safeguarding mitochondrial O₂ supply and aerobic metabolism in the face of wide and independent variations in O₂ tensions and temperature at the sites for loading and unloading of O₂ [1-3].

In transporting O₂, vertebrate Hbs (composed of 2 α and 2 β globin chains) switch between the T (tense, low O₂-affinity, deoxygenated) structure that predominates in the tissues, and the R (relaxed, high-affinity, oxygenated) structure that predominates in the lungs and gills. The T-R shift is basic to cooperativity between the O₂-binding heme groups that increases O₂ (un)loading for a given change in O₂ tension - and is reflected in the sigmoid shape of O₂ binding curves. Hb's *in vivo* O₂ binding properties are a product of its intrinsic O₂ affinity and its interaction with red cell allosteric effectors that decreases Hb-O₂ affinity by stabilizing the T-structure. Apart from protons and CO₂ (that facilitate O₂ unloading in the acid tissues via the "Bohr-effect") these effectors include chloride ions and organic phosphates [ATP in lower vertebrates, IPP (inositol pentaphosphate) in birds and DPG (diphosphoglycerate) in mammals]. The interaction with effectors varies between and within individual species and plays a key role in adjusting O₂ transport in response to changes in environmental conditions, metabolic requirements, and mode of life. The decrease in Hb-O₂ affinity with rising temperature mandated by the exothermic nature of heme oxygenation, enhances O₂ unloading in warm tissues that require more O₂, but may become maladaptive - and thus commonly is reduced - in regional heterothermic species where it may hamper O₂ unloading (in cold extremities of arctic mammals) or cause excessive O₂ release (in warm muscles, brains or eyes of fast-swimming fish).

Based on case studies (Hbs from estivating fish, fast-swimming gamefish, high-altitude Andean frogs, geese that scale the Himalayas, Rocky Mountain Deer mice and Hb recreated from extinct mammoths [4-6]) the treatise analyses the molecular mechanisms for Hb's role in securing mitochondrial O₂ supply under stressful conditions - illustrating the key significance of molecular interactions to understanding physiological ecology.

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A1-02 Mitochondrial adaptations to hypoxia in high-altitude birds and mammals.

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The hypoxic and cold environment at high altitudes requires endothermic animals to sustain high rates of O₂ consumption for both locomotion and thermogenesis while facing a diminished O₂ supply. We are examining the mitochondrial mechanisms of genotypic adaptation and phenotypic plasticity that help maintain ATP supply during hypoxia in high-altitude birds and mammals. Respiratory capacity, cytochrome oxidase activity, phosphorylation efficiency, oxygen kinetics, and several other variables were measured in mitochondria isolated from the flight muscle of bar-headed geese and the hindlimb muscle of highland deer mice, and each were compared to closely-related lowland taxa. Our results suggest that several mitochondrial adaptations, coupled with enhanced mitochondrial O₂ supply, contribute to performance in hypoxia.

Supported by NSERC of Canada.



A1-03 Comparative muscle mitochondrial physiology of the northern elephant seal.

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²Cell and Molecular Biology Program; ³Dept Biology, Colorado State
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The Northern Elephant seal (*Mirounga angustirostris*; E-seal) is known for its remarkable capacity for diving in cold water to hunt for up to 2 hours without resurfacing for air. E-seal muscle contains very large quantities of myoglobin, which helps to maintain muscle p_{O_2} prior to and during long dives, but the muscle mitochondrial respiratory phenotype of this species has not been characterized. Ongoing studies in our lab have sought to understand the effects of species, sex and environment on muscle mitochondrial function in E-seals by performing high resolution respirometry (HRR) on samples obtained from individuals at various stages of their life cycle, compared to data obtained from human vastus lateralis muscle biopsies in our laboratory. Seal biopsies were taken from the primary swimming muscle (*M. longissimus dorsi*) with a 6 mm biopsy cannula and stored on ice-cold BIOPS (0-4 days) prior to being saponin permeabilized for HRR using a variety of SUIT protocols on an Oroboros Oxygraph-2k.

Compared to fibers from young adult humans, young adult E-seal fibers exhibit ~50% lower mass-specific values of maximal ETF+CI and CII-linked OXPHOS and ETS capacity, with trends for higher values in male vs. female E-seals. Despite this, OXPHOS rates with palmitoylcarnitine+malate (PalM) were similar in E-Seals and humans, with a trend for higher rates in female vs. male seals. Respiratory adenylate control ratios (P/L_N) with PalM and pyruvate+malate are similar between seals and humans, with trends for higher values in male vs. female seals. ETF+CI OXPHOS flux normalized to ETS capacity (P/E) is also similar in seals and humans, but tends to be higher in female vs. male seals. Interestingly, despite similar values of LEAK respiration in the absence of adenylates (L_N), E-seal fibers consistently exhibit 50-80% higher indices of CII-linked oligomycin-induced LEAK respiration normalized to OXPHOS or ETS capacities (L_{Omy}/E and L_{Omy}/P) compared to humans, indicating a much greater capacity for LEAK respiration in the presence of adenylates. Respiratory LEAK indices are greater in female vs. male seals, despite similar P/L_N and higher P/E values. Notably, data from newborn E-seal pups show markedly higher mass-specific OXPHOS and ETS capacities and lower indices of LEAK compared to young adults, with values similar to those seen in adult humans, suggesting a strong environmental and/or developmental component to the differences seen in adults.

Taken together, our studies indicate that E-Seals maintain a high capacity for fatty acid oxidation and phosphorylation control of respiration despite a lower overall OXPHOS

and ETS capacity and a much greater capacity for LEAK respiration compared to humans. This remarkable mitochondrial phenotype may serve to meet both bioenergetic and thermal demands of extended cold water dives routine to this mammalian species. Observed differences between sexes and life-cycle stages parallel known aspects of E-seal biology, suggesting that muscle mitochondrial adaptations likely result from a combination of genetic, hormonal and/or environmental factors.

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A1-04 Biochemical coupling efficiency in permeabilized fibres from arm and leg muscle in Inuit versus Caucasians: a functional test of the uncoupling hypothesis in Greenland.

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10 years ago the uncoupling hypothesis was presented for mitochondrial haplogroups of arctic populations suggesting that lower coupling of mitochondrial respiration to ATP production was selected for in favor of higher heat dissipation as an adaptation to cold climates [1,2]. Up to date no actual tests have been published to compare mitochondrial coupling in tissues obtained from human populations with regional mtDNA variations. Analysis of oxidative phosphorylation (OXPHOS) is a major component of mitochondrial phenotyping [3]. We studied mitochondrial coupling in small biopsies of arm and leg muscle of Inuit of the Thule and Dorset haplogroups in northern Greenland compared to Danes from western Europe haplogroups. Inuit had a higher capacity to oxidize fat substrate in leg and arm muscle, yet mitochondrial respiration compensating for proton leak was proportionate with OXPHOS capacity. Biochemical coupling efficiency was preserved across variations in muscle fibre type and uncoupling protein-3 content. After 42 days of skiing on the sea ice in northern Greenland, Danes demonstrated adaptive substrate control through an increase in fatty acid oxidation approaching the level of the Inuit, yet coupling control of oxidative phosphorylation was conserved. Our findings reveal that coupled ATP production is of primary evolutionary significance for muscle tissue independent of adaptations to the cold.

Our functional test of the uncoupling hypothesis was based on a substrate-uncoupler-inhibitor titration (SUIT) protocol using high-resolution respirometry designed to interrogate coupling control in different substrate states (ETF-linked octanoylcarnitine+malate and CII-linked succinate) and compare OXPHOS capacities for ETF-, CI-, CI+II and CII-linked substrate states [3,4]. Various coupling control ratios can be derived from such experiments (respiratory acceptor control ratio, $RCR=P/L$; RCR is the inverse of the OXPHOS control ratio, L/P) which are related to but do not directly express biochemical coupling efficiency [3]. A general concept of normalization of mt-respiration is presented here, which provides a consistent expression of biochemical coupling efficiency, Δj_{E-L} , ranging from 0.0 at zero coupling to 1.0 at the limit of a completely coupled system.

Flux control ratios, j , are oxygen fluxes normalized relative to a common respiratory reference state [2]. For a given protocol or set of respiratory protocols, flux control ratios provide a fingerprint of coupling and substrate control independent of (i) mt-content of cells or tissues, (ii) purification in preparations of isolated mitochondria, and (iii) assay conditions for determination of tissue mass or mt-markers external to a respiratory protocol (CS, protein, stereology, etc.). Complementary to the concept of flux control ratios and analogous to elasticities of metabolic control analysis [3], **flux control factors** express the control of respiration by a specific metabolic variable, X , as a dimensionless (normalized) fractional change of flux, Δj . Z is the reference state with high (stimulated or un-inhibited) flux; Y is the background state at low flux, upon which X acts ($j_Y = Y/Z$); X is either added (stimulation, activation) or removed (reversal of inhibition) to yield a flux Z from background Y . Note that X , Y and Z denote both, the metabolic control variable (X) or respiratory state (Y , Z) and the corresponding respiratory flux, $X = Z - Y$. Experimentally, inhibitors are added rather than removed; then Z is the reference state and Y the background state in the presence of the inhibitor. The flux control factor of X upon background Y is expressed as the change of flux from Y to Z , normalized for the reference state Z :

$$\Delta j_{Z-Y} = (Z - Y)/Z = 1 - j_Y$$

Substrate control factors express the relative change of oxygen flux in response to a transition of substrate availability in a defined coupling state. Coupling and phosphorylation control are determined in an ETS-competent substrate state.

With ETF-linked or CII-linked substrates, ETS capacity (E) was not in excess of phosphorylation system capacity, such that $P = E$ or $P/E = 1.0$. The corresponding flux control factor is the **biochemical coupling efficiency**. This coupling efficiency is simply related to the respiratory acceptor control ratio, $\Delta j_{E-L} = (RCR - 1)/RCR = (P - L)/P$. In the present example, LEAK respiration is the background state ($Y = L$) and $P = E$ is the reference state (Z). Biochemical coupling efficiencies measured with ETF-linked and CII-linked substrates were identical in Inuit and Europeans, arm and leg, and before and after the 42 days adaptation to life in the cold.

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A1-05 Convective O₂ delivery, diffusion and mitochondrial OXPHOS components of V_{O₂max} during exercise in health and disease.

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Maximal oxygen consumption ($V_{O_{2max}}$) in the organism is defined by the integrated functional capacities of multiple organ systems and characterized by proportionate design of the structural components of the O₂ cascade from lung to mitochondria across a wide range of species. Yet, humans exhibit an excess capacity of muscle mitochondrial OXPHOS capacity relative to convective O₂ delivery [1, 2]. This pattern holds with aging and in chronic diseases such as chronic obstructive lung disease and type 2 diabetes despite a lower expression and/or dysfunction of mitochondria. Muscle diffusional O₂ conductance (D_{O_2}) is largely dependent on the capillary volume:muscle interface which influences the mean transit time of erythrocytes and off-loading of O₂ from haemoglobin

at a given p_{50} and blood flow. Convective O_2 delivery remains a dominant factor defining maximal V_{O_2} as revealed by comparison of muscle D_{O_2} , mean capillary p_{O_2} , and V_{O_2} during exercise engaging small to large muscle groups. The increase in muscle V_{O_2} with endurance training in young, healthy humans is characterized by proportional increases in O_2 convection and diffusion, with variable enhancement of mitochondrial OXPHOS capacity, which remains in excess to maximize the p_{O_2} gradient from the red blood cell to cytochrome c oxidase.

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A1-06 The effects of hypoxic training on aerobic performance in normoxia and moderate hypoxia: a randomized, double blind, placebo controlled study.

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The effects of hypoxic training on exercise performance remain controversial. To test the hypotheses that (i) hypoxic training possesses ergogenic effects at sea-level and altitude, and (ii) the benefits are primarily mediated by improved mitochondrial function of skeletal muscle, we determined aerobic performance (incremental test to exhaustion and time trial) in moderately-trained subjects undergoing six weeks of endurance training (3-4 times/week, 60 min/session) in normoxia (placebo, $N=8$) or normobaric hypoxia ($F_{IO_2}=0.15$; $N=9$) using a double blinded and randomized design. Exercise tests were performed in normoxia and acute hypoxia ($F_{IO_2}=0.15$). Mitochondrial oxidative capacity and efficiency were quantified from skeletal muscle biopsies. Total hemoglobin mass (Hb_{mass}) was measured by carbon-monoxide rebreathing. Neither maximal capacity of oxidative phosphorylation nor mitochondrial efficiency was altered by training or hypoxia. Hypoxic training increased Hb_{mass} more than placebo (8.4 vs 3.3%, $P=0.03$). In normoxia, hypoxic training had no additive effect on maximal oxygen uptake ($V_{O_{2max}}$), time trial performance or cycling efficiency. In acute hypoxia, hypoxic training conferred no advantage on $V_{O_{2max}}$, but improved cycling efficiency, and tended to enhance time trial performance (52 vs 32%, $P=0.09$). Our data suggest that training in hypoxia possesses no ergogenic effect at sea level, but may confer some advantage for endurance performance at moderate altitude, possibly through Hb_{mass} expansion.



A1-07 Improvements in exercise performance with high-intensity interval training are facilitated by an increase in skeletal muscle mitochondria content.

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Six sessions of high-intensity interval training (HIT) completed over a two-week span is sufficient to improve exercise capacity [1,2]. The mechanisms explaining such improvements are not fully understood. Accordingly, the aim of the present study was to

perform a comprehensive evaluation of physiologically relevant adaptations occurring after 6 sessions of HIT in sedentary young adults and determine the mechanisms explaining improvements in exercise performance. Sixteen sedentary subjects completed 6 sessions of repeated [8-12] 60 s intervals of high-intensity cycling (100% peak power output elicited during incremental maximal exercise test) intermixed with 90 s of recovery cycling at a low intensity (30 W) over a 2-week period. Potential training-induced alterations in skeletal muscle respiratory capacity, mitochondrial content, skeletal muscle oxygenation, cardiac capacity, blood volumes, and peripheral fatigue resistance were assessed. Training improved maximal oxygen consumption ($V_{O_{2max}}$; $\sim 8\%$; $p=0.026$) and cycling time to complete a set amount of work ($\sim 5\%$; $p=0.008$). Skeletal muscle respiratory capacity improved, most likely as a result of an increased content of skeletal muscle mitochondria ($\sim 20\%$, $p=0.026$). Maximal tissue oxygenation improved by 10% while maximal cardiac output, blood volumes, oxygen carrying capacity, and relative measures of peripheral fatigue resistance were all unaltered with training. These results suggest that acute increases in mitochondrial content following two-weeks of HIT may facilitate improvements in respiratory capacity, tissue oxygenation, and ultimately are responsible for the improvements in both maximal whole-body exercise capacity as well as endurance performance in previously sedentary individuals.

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A1-08 High intensity training decreases mitochondrial ADP sensitivity in human adipose and skeletal muscle tissue.

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It has previously been reported that mitochondrial ADP sensitivity decreases (higher K_m') with endurance training in human skeletal muscle [1]. This decrease was accompanied by an increased maximal ADP stimulated mt-respiration (J_{max}) and an increased maximal oxygen uptake ($V_{O_{2max}}$). It is not known whether high intensity training (HIT) has the same effect on mitochondria and if these changes also occur in mitochondria from human adipose tissue. The aim of this project was to investigate mitochondrial ADP sensitivity and maximal ADP stimulated respiration in human adipose and skeletal muscle tissue after six weeks of HIT. Twelve healthy overweight subjects (7 F/5 M) (age: 40 ± 2 yrs, BMI: 32 ± 2 , $V_{O_{2max}}$: 2612 ± 166 ml/min) were included in the study. The subjects underwent six weeks (3 times a week) of HIT. $V_{O_{2max}}$ was measured pre and post training. Mitochondrial ADP sensitivity (K_m') and J_{max} was measured pre and post HIT in adipose tissue and permeabilized muscle fibers by high-resolution respirometry (Oxygraph-2k, Oroboros, Innsbruck, Austria). The adipose tissue was permeabilized with digitonin and the skeletal muscle fibers with saponin. The protocol used for respirometry was as follows: Malate (2 mM), Glutamate (10 mM) and ADP titrated in the following steps (0.05 – 0.10 – 0.25 – 0.50 – 1.00 – 2.50 – 5.00 mM). SigmaPlot was used to determine K_m' for ADP and J_{max} .

$V_{O_{2max}}$ was significantly improved after HIT. Mitochondrial ADP sensitivity was significantly ($P < 0.05$) decreased after HIT in permeabilized skeletal muscle (0.14 ± 0.02 mM vs. 0.29 ± 0.03 mM), and the same trend was seen in adipose tissue although not significant ($P = 0.056$; 0.11 ± 0.02 mM vs. 0.16 ± 0.04 mM). J_{max} was similar after HIT in permeabilized skeletal muscle (21 ± 1 pmol \cdot s $^{-1}\cdot$ mg $^{-1}$ vs. 22 ± 1 pmol \cdot s $^{-1}\cdot$ mg $^{-1}$) as well as in

adipose tissue ($0.36 \pm 0.03 \text{ pmol} \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ vs. $0.36 \pm 0.03 \text{ pmol} \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$), before vs. after, respectively.

ADP sensitivity decreased in permeabilized human skeletal muscle and the same trend was seen in adipose tissue. This was accompanied by a similar maximal ADP stimulated respiration pre and post training in both skeletal muscle and adipose tissue. This is the first time that ADP sensitivity has been investigated in adipose tissue. Interestingly the HIT training adaptation in mitochondria from adipose tissue is similar to that observed in skeletal muscle although not with the same magnitude.

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A1-09 Dietary inorganic nitrate reduces basal metabolic rate in man.

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According to the rate-of-living and oxidative damage theory of aging, extended lifespan is predicted by low energy metabolism and low reactive oxygen species production rates. Recently, several studies show that dietary inorganic nitrate mainly present in vegetables can reduce oxygen consumption during physical exercise in humans [1] and contribute to attenuated oxidative stress in animal models of disease [2]. Nitrate accumulates in saliva and is bioactivated through reduction to nitrite by oral bacteria.

We examined the effects of dietary nitrate on basal metabolic rate (BMR) and markers of oxidative stress in man using a double-blind, randomized cross over design. 15 young healthy males volunteered and indirect calorimetry was used to determine basal metabolic rate after three days of dietary intervention with sodium nitrate (NaNO_3 , $0.1 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) or placebo (NaCl). The administered amount of nitrate resembles what is found in 100-300 g of nitrate rich vegetables such as beetroot or spinach.

The intervention reduced BMR by 4.3% after nitrate administration compared with placebo ($p < 0.02$). A strong negative correlation was found between the change in salivary nitrate and the change in BMR ($r^2 = 0.72$; $p < 0.002$). In addition, nitrate supplementation reduced plasma levels of malondialdehyde, indicating lower oxidative stress as a result of the intervention. Thyroid hormone status was unaffected.

The cuisines of cultures known for their longevity are usually rich in vegetables and future studies will reveal whether this life span extension is linked to the high nitrate content in this food group.

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A1-10 Human mitochondria have a unique response to ischemia-reperfusion injury compared to mitochondria from rat, mouse and pig.

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Mitochondrial function and respiratory dysfunction after short periods of ischemia has been implicated as major determinants of ischemic cell injury [1]. *In vitro* models of isolated mitochondria subjected to anoxia-reoxygenation are routinely used to study mitochondrial abnormalities and pharmacological approaches to attenuate ischemic injury [2]. Mice and rat mitochondria are by far the most common animal model in this area of research.

We show here, in agreement with a plethora of other studies, that respiration of mice and rat mitochondria decreases after a brief period of *in vitro* anoxia-reoxygenation, an effect most often related to ROS injury of Complex I. Conversely, human mitochondria demonstrate a unique feature with increased respiration after anoxia-reoxygenation. This effect is increasing with longer anoxic periods, independent of uncoupling of the mitochondrial membrane potential or potassium channel activation and cannot be inhibited by *in vitro* application of antioxidants. The increase in respiration is also present regardless of the route of electron-entry into the ETS and cannot be inhibited by any known inhibitors of the mitochondrial complexes. Instead, the differing responses seem to be associated with interspecific differences in the function of cytochrome c oxidase.

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A1-11 Functional plasticity of interfibrillary mitochondria (IFM) as cardiac response mechanism to stress.

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A morphological hallmark of the failing human heart is a devastating autophagic degradation of cellular structures starting from the perinuclear region, proposed to actively shift the heart into a decompensated state [1]. We studied heart samples from different species, i.e. a mouse model of cardiac specific expression of MCP1 that autonomously develops heart failure [2], hibernating Syrian hamsters [3] and a pig model of mitochondrial dysfunction exposed to hyperbaric oxygen.

Our data reveal an age-dependent increase of perinuclear degradation in mouse hearts that occurred prior to the onset of cardiac dysfunction. These center core-like lesions in the myofibrillar compartment are most likely the end-stage result of a vicious cycle that starts with a physiological response to lowered levels of cardiac workload. Accordingly we found that in hibernating Syrian hamsters under conditions of depressed metabolism interfibrillary mitochondria are reversibly silenced whilst subsarcolemmal mitochondria remain more active. Central remodeling of cardiomyocyte compartments is a phenomenon primarily known in the hibernating myocardium [4]. Here we show in pig hearts that the isolated impairment of the interfibrillary compartment can be fully re-activated upon treatment with hyperbaric oxygen.

We conclude that differential compartment regulation by switching the activity status of mitochondrial sub-populations from on to off and vice versa might provide a hitherto unnoticed flexible on-demand plasticity in cardiomyocytes. Such alterations make proper myofibril contraction in the silenced compartment unlikely. Silenced mitochondria can be re-activated on demand. Only long-lasting mitochondrial silencing, e.g. upon chronic cardiac overload, might increase the risk of adverse cardiomyocyte remodeling.

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A1-12 Remodeling of skeletal muscle mitochondria in response to exercise training in *taz* shRNA mouse model of human Barth syndrome.

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Barth syndrome is a mitochondrial disease associated with exercise intolerance and cardioskeletal myopathy resulting from mutations in the tafazzin (*taz*) gene. *Taz* encodes a phospholipid transacylase believed to be important for the remodeling of cardiolipin and maintaining optimal mitochondrial membrane function. The present study characterized skeletal muscle mitochondrial function and exercise capacity of a new *taz* shRNA mouse model of Barth syndrome (90% *taz*-deficient), and examined the effect of exercise training on these parameters.

Mitochondrial respiratory function was assessed in mitochondria freshly isolated from hindlimb muscles using an Oroboros Oxygraph-2k with pyruvate+malate as substrates. A pre-training treadmill graded exercise test (GXT) revealed profound exercise intolerance in *taz* mice, which corresponded to reduced respiratory capacity, citrate synthase (CS) and ETS Complex I protein content of muscle mitochondria in the *taz* vs. age-matched wild-type (WT) mice. Based on the pre-training GXT, exercise training was conducted at 12-17 m/min, 0% grade for 60 min/d, 5 d/wk. Exercise training elicited a 99% increase in GXT run time in the *taz* mice ($P < 0.01$ vs. pre-training), but failed to increase levels to that of sedentary WT mice.

Unexpectedly, training significantly decreased OXPHOS capacity of isolated muscle mitochondria from exercised mice (WTS: 4993 ± 371 , WTX: 3780 ± 561 , TazS: 2979 ± 384 , TazS: 1828 ± 525 (pmol/(s*mg), $P = 0.02$ Sed. vs. Ex.), and significantly decreased mitochondrial CS activity in *taz* mice (WTS: 4.48 ± 0.51 , WTX: 3.87 ± 0.69 , TazS: 3.21 ± 0.54 , TazX: 1.63 ± 0.69 (RU/g), $P = 0.01$). Training tended to reduce mitochondrial lactate dehydrogenase (LDH) and monocarboxylate transporter 1 (MCT1) activities, MnSOD content, and 4-hydroxynonenal-protein adducts (index of oxidative stress), but tended to increase mitochondrial UCP3 in exercised WT and *taz* mice. Interestingly, training significantly increased CS activity in total muscle homogenates (WTS: 1.491 ± 0.112 , WTX: 1.792 ± 0.143 , TazS: 1.325 ± 0.108 , TazX: 1.550 ± 0.143 (RU/g), $P = 0.05$ Sed. v. Ex.), suggesting a training-induced increase in whole-muscle oxidative capacity despite a lower OXPHOS capacity per mg protein of isolated mitochondria. This study indicates that exercise training improves functional capacity of *taz* deficient mice despite persistent mitochondrial respiratory dysfunction, and induces selective remodeling of mitochondria in skeletal muscle perhaps to mitigate oxidant production from a dysfunctional respiratory system while adapting to increased metabolic demand.

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A1-13 Skeletal muscle mitochondrial function in ovariectomized rats: A time course study and the role of estrogen replacement.

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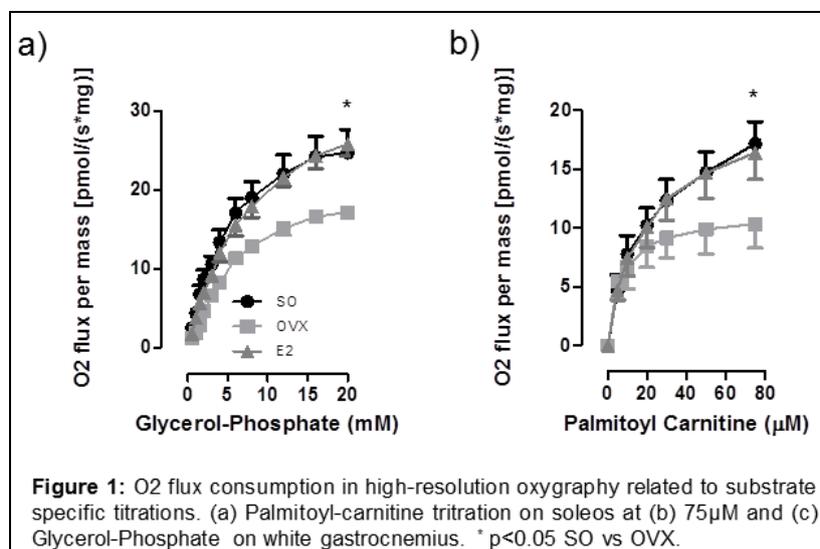
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In rodents, bilateral ovariectomy (OVX) is a good model for understanding the consequences of female steroid gonadal hormones loss. Many studies have shown that OVX decreases energy expenditure (EE), and this decrease could be the cause of obesity in the model. Forty percent of total body EE is assigned to skeletal muscle (SM), and alterations of SM mitochondrial function are associated with decreased EE, but the effect of OVX on SM mitochondrial function it is still unknown. The aim of this study was to evaluate the effect of OVX and estrogen replacement on SM mitochondrial function in rats.

Female Wistar rats (2-3 months old) were divided in three groups: sham-operated (SH); ovariectomized (OVX); OVX treated with estradiol (0.7 µg/100 g per day) (E2). The animals were weighed each week until the eighth week. *In vivo* oxygen consumption was determined during 16 hours (8 h dark/ 8 h light). After the third and eighth weeks (3 W and 8 W respectively), the soleus (SOL) and gastrocnemius muscles (WG) were gently excised, and either frozen in liquid nitrogen or used immediately to test mitochondrial respiration function on high-resolution respirometry (Oroboros Instruments, Oxygraph-2k, Innsbruck, Austria). Muscle tissues were homogenized, and RNA was isolated for analysis of PGC1-α gene expression by qRT-PCR. One-way ANOVA with Dunnett’s post-test was used to compare the groups.

Body mass was already greater in OVX rats after the second week of surgery, as compared to the E2 and SH operated groups. Visceral fat compartment was greater in the OVX than in the SH and E2 groups at 3 W and 8 W. We found no differences in whole body oxygen consumption at any time. Coupled respiration of SOL mitochondria was lower in OVX than in SH or E2 animals at 8 W ($P<0.05$), but no differences were detected at the 3 W after castration either in SOL or in the WG muscles. Proton leak did not change in any group. Respiration related to ATP synthesis was lower after 8 W of castration in SOL, in relation to SH and E2 ($P<0.05$) and WG muscles ($P<0.05$), but no differences were detected at 3 W. However, already at 3 W, there was a decrease of coupled respiration when lipids-derived substrate, palmitoyl-carnitine and glycerol-phosphate, were used in OVX muscles ($P<0.05$; Figure 1). PGC1-α was already 65% lower in OVX than SH and E2 at 3 W after castration.

Short-term OVX leads to skeletal muscle mitochondrial dysfunction that is related to lipid substrates utilization. On the other hand, long-term OVX promotes mitochondrial dysfunction on OXPHOS level. The estrogen replacement restores all these disorders. Early mitochondrial events related to estrogen deficiency seem to be responsible for the decrease of later mitochondrial biogenesis. **Figure 1**





Peter Hochachka 9 Mar 1937 – 16 Sep 2002

Peru, September 1996, on the Inca trail after a 4.5 h walk & talk.

At the *International Summer Course Innsbruck. Calorimetry, respirometry, and biological energetics: from the organelle to the organism* (1991) Peter Hochachka presented an "Overview of metabolic suppression in defence against environmental stress: the hypoxia example" - a 4.5 h talk. On the next days, full morning lectures were continued by Steve Hand (*Anabolic and catabolic arrest during invertebrate dormancy*) and Erich Gnaiger (*Muscle and mitochondria at rest and work: Adenylate control and the concept of chemical reaction pressure*). During the evening party of the last day, Peter's enthusiasm caught everybody in an unforgettable slide show: *A global perspective of environmental physiology* - in the spirit of Kjell Johansen's "The world as a laboratory". The marathon lectures received an overwhelmingly positive response by the 55 students. With this in mind, Peter invited me to the World Congress of High Altitude Medicine and Physiology in Cusco (telephone call at 3 am), with the condition to join a pre-conference workshop in Machu Picchu with five participants: In a 4.5 h walk&talk up the Inca trail I talked thermodynamic efficiency [1] which he enjoyed (photo by EG), whereas Peter expanded on biochemical efficiency in Quechuas on the way down [2] which was definitely more 'efficient'. Much later I received a surprising and disturbing letter, where Peter suggested a personal contract to be made: he or me - whoever may survive the other - should organize an event to commemorate the other. For more than a decade I kept this in mind. Outstanding events were organized in Peter's name. I did not feel prepared to add something living up to his expectations. The present emergence of a new 'Comparative Mitochondrial Physiology' is just right to fulfill my contract with Peter.

Innsbruck, Sep 2013 - Erich Gnaiger

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A2

MiPcomparative: Hypometabolism and mt-density

Peter Hochachka Memorial Session 1

Peter Hochachka lecture



A2-01 The role of mitochondria in low oxygen signaling in turtle brain.

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Earth's changing environment has been a major evolutionary force shaping the diversity of species both in the past and present. In particular, seasonal ice cover in northern latitudes has selected for hypoxia and anoxia tolerance in some species, such as freshwater turtles. At the northern reaches of their range North American western painted turtles spend 4 months or more buried in the mud bottom of ice covered lakes and ponds [1]. This offers a unique opportunity to understand how a vertebrate brain, an organ extremely sensitive to reduced oxygen availability in mammals, can function without oxygen [2]. Through oxidative phosphorylation mitochondria fuel the inherently high energetic demands of brain and in mammals mitochondria also play a key role in injury from hypoxic stress – including loss of calcium homeostasis and production of reactive oxygen species (ROS) leading to apoptosis and necrosis. Hypoxic or anoxic stress does not signal stress in turtle brain but rather protective mechanisms with the onset of anoxia. Indeed our data show that mitochondria play a key role in low oxygen signaling in turtle brain by a reduction in mitochondrial membrane potential and release of a relatively small but significant amount of calcium. The increase in cytosolic calcium signals a phosphatase based mechanism to decrease whole-cell glutamatergic (NMDA and AMPA) excitatory currents in pyramidal neurons. While in stellate neurons anoxia results in a large reduction in mitochondrial ROS production that increases the magnitude of GABAergic inhibitory neurotransmission. The increased GABA activity produces a chloride based shunting current that “arrests” action potentials in pyramidal cells resulting in metabolic depression and neuroprotection.

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A2-02 Defense against ATP depletion during the energy-limited state of diapause.

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Gastrula-stage embryos of *Artemia franciscana* (brine shrimp) undergo dramatic respiratory depression and developmental arrest upon release from the adult female as they enter a state of hypometabolism termed diapause [1]. Metabolism as measured by respiration rate declines by over 99% during entry into diapause across a 26-day time course [2]. The primary basis for the inhibition is a restriction of oxidative substrate to the mitochondrion that involves an orchestrated interplay at multiple enzymatic sites including trehalase, hexokinase, pyruvate kinase and pyruvate dehydrogenase [2].

While a substantial decrease in embryo ATP occurs during diapause, a significant amount of ATP remains [e.g., ATP:ADP ratio = 1.306 ± 0.036 (mean \pm SE, $N = 10$)]. This observation is noteworthy when one considers that proton conductances of mitochondria isolated from diapause and post-diapause embryos are identical when compared as a function of the driving force ($\Delta\psi_{mt}$) [2]. Thus proton leak apparently is not downregulated during diapause, and as a consequence, $\Delta\psi_{mt}$ is likely severely compromised because respiration of intact embryos is depressed far below that required to compensate for leak. Under such conditions, one would predict that the F_1F_0 -ATP synthase could reverse and fully deplete cellular ATP. Because ATP is not depleted, we predict that the F_1F_0 -ATP synthase is blocked during diapause by the F_1 -ATPase inhibitor protein IF1. This 9.6 kDa protein binds to the ATP synthase at the F_1 catalytic domain and inhibits the hydrolytic activity of the enzyme under conditions where $\Delta\psi_{mt}$ is low [3]. Further, acidic pH is known to promote the formation of the active dimeric state of IF1 and a stable complex with the enzyme [3]. It is likely that intracellular pH of *A. franciscana* embryos may decline during diapause as the metabolic depression phase progresses.

IF1 could potentially explain the conservation of adenylates in diapause. Affinity purification [4] and characterization of the ATP synthase from *A. franciscana* and its interaction with IF1 is underway.

Supported by NSF grant IOS-0920254 and NIH grant 2 RO1 DK046270-14A1.



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A2-03 Mechanisms of mitochondrial metabolic suppression in hibernation.

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In winter mammalian hibernators, such as the 13-lined ground squirrel (*Ictidomys tridecemlineatus*), enter a state of torpor where metabolic rate is suppressed by ca. 90%, and body temperature (T_b) subsequently declines towards freezing.

These changes are fully reversed during arousals, which occur spontaneously every 5-14 days throughout the winter. Arousals return animals to full euthermia for ca. 1 day before they enter another bout of torpor. This cycle is apparently driven by endogenous signals, and offers an excellent natural model to study plasticity of many physiological functions, including mechanisms of metabolic regulation.

The reversible suppression of whole-animal metabolism in hibernation is mirrored by some aspects of mitochondrial oxidative phosphorylation. In liver mitochondria isolated from torpid animals, OXPHOS capacity fueled by succinate oxidation is ca. 70% lower than in euthermic animals, even when measured at the same *in vitro* temperature (37 °C). Torpid suppression of mitochondrial respiration may conserve endogenous fuels and minimize damage by ROS, but it is not evident in all tissues, including cardiac muscle and forebrain. Moreover this suppression depends on several factors, including oxidative substrate and assay temperature. For example, in skeletal muscle mitochondria respiration is suppressed in torpor by ca. 35%, but only with succinate, and only when measured at 37 °C [1].

In liver mitochondria suppression of OXPHOS capacity occurs early during entrance into torpor, declining by 70% before T_b falls to 30 °C [2]. Conversely, reversal of suppression during arousal occurs only gradually; even after T_b rises from 5 °C to 30 °C, respiration is only 50% of values of fully aroused animals [3]. This "fast in, slow out" pattern suggests that temperature-sensitive mechanisms are responsible for the reversible suppression of mitochondrial metabolism.

In torpor, both intact mitochondria and succinate dehydrogenase (SDH) have higher apparent affinity for succinate, but SDH is inhibited by ca. 25%, probably by oxaloacetate (OAA) [1]. Reversal of OAA inhibition restores SDH activity, but does not fully "rescue" OXPHOS capacity to euthermic levels [3]. Recent phosphoproteomic analyses revealed seasonal (i.e. summer vs. winter) differences in the phosphorylation state of several mitochondrial proteins, but no differences between torpor and arousal. Future experiments will examine potential torpid inhibition of oxidative phosphorylation complexes by measuring their redox state in intact mitochondria using rapid-scanning optical spectrometry. We will also examine changes in mitochondrial protein acetylation state using immunoblot analysis.

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A2-04 Diversity and evolution of mitochondrial metabolism: proline as a metabolic reward for pollinators.

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Insect flight is the most energy demanding activity performed by animals. Flight muscle tissue is extremely diverse, ranging in contraction frequency depending on the species and lifestyle, to metabolic fuels used as a function of metabolic flux rate and ecological opportunities. Pollinators such as bees have developed an intimate relationship with flowering plants and coevolved. Bee flight requires high metabolic rate to perform maneuvering flight, and are thought to have become specialized in using carbohydrate as sole metabolic fuel. Plant physiologists have recently documented that a considerable amount of energy is invested in producing the amino acid proline in plant nectar. Yet, the most common bee model species, the honeybee, does not appear to be capable of oxidizing proline as muscle metabolic fuel. Using permeabilized insect flight muscle fibers, we investigated the diversity and evolution of proline as a metabolic fuel in bees and wasps. I will show how the honeybee is not representative of hymenopterans as a group, as it cannot oxidize proline while its sister species, a bumblebee, and a representative of their ancestral group, a wasp, can greatly increase muscle respiration using proline. We further described muscle metabolic phenotype of the bumblebee and show how proline greatly enhance oxidation of pyruvate. This work illustrates how proline can be used as a metabolic reward for pollinators, and the diversity and evolution of mitochondrial metabolism will be discussed in this context. I will integrate this new aspect of flight muscle metabolism evolution with the work I had started as a graduate student with Peter Hochachka.



A2-05 Mitochondrial function, a proximate mechanism underlying the pace-of-life?

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Life histories describe how organisms grow, mature, reproduce and senesce, and evidence is accumulating that most of the variation in the pace of life of animals can be arranged along a slow-fast continuum. Natural variation in life history traits has largely been studied from an evolutionary viewpoint, highlighting their links to ecological factors and their fitness consequences [1]. It is only recently that researchers have focused their interest on the physiological mechanisms underlying variation in the pace of life [2]. In this context, we proposed that mitochondrial function, the main generator of both energy (adenosine triphosphate, ATP) and reactive oxygen species (ROS), may act as the keystone between the optimization of energy availability for life-history traits and the resulting oxidative cost.

Using both descriptive and experimental approaches, we investigated the relationship between energy flow, oxidative stress and growth in an amphibian anuran model, the common frog *Rana temporaria*. We hypothesized that mitochondrial uncoupling (resulting in less ATP but also reduced ROS production) induces a slow pace of life (slow growth and slow ageing).

Chronic exposure to an uncoupler (2,4-dinitrophenol) during tadpole development induced a lower growth rate associated with a decreased mitochondrial efficiency of energy transduction and an improved oxidative status (lower ROS production and low oxidative damage despite low antioxidant defenses). In addition, a comparison of

physiological parameters of wild frogs exhibiting dissimilar body size showed a higher energy efficiency transduction in frogs exhibiting high growth rate.

Our results suggest that (i) the mitochondrial efficiency of energy transduction could drive the amount of energy available for animal growth, and (ii) studying both facets of mitochondrial function (ATP and ROS production) allows a better understanding of the proximate mechanisms underlying life history trade-offs. Mitochondrial function under the regulation of numerous metabolites and endocrine factors, might represent the physiological link between environmental condition (temperature, resource, stressor events...) and energy allocated to animal performance. This approach is opening new avenues within an evolutionary ecological framework that aims at explaining the diversification of paces-of-life in response to environmental conditions.

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A2-06 Respiration of tissue and mitochondria from Atlantic salmon (*Salmo salar*, L) liver, muscle, heart, brain and blood.

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Salmon aquaculture production in Norway amounted to 1.2 million tons in 2012. For growth salmon utilize today a diversity of food containing different supplies of fat and protein. When aquaculture started around 1970, approx. 3 kg dry feed would give 1 kg fish. Today approx. 1 kg dry feed gives 1 kg salmon. The feed are utilized for both growth and energy production. Cellular and mitochondrial respiration has now gained interest since better formulation of feed and additives to be used are of importance.

Salmon in the wild store large amounts of fat when food supplies are abundant, but the animal can easily starve for several weeks, losing weight without any visible harm. In our opinion salmon might therefore be used as a model for studying the metabolic changes of animals that gain or lose weight. We assume that changes in respiratory properties of different tissues show large changes when these conditions change. We also propose that studying salmon physiology might even be important for understanding obesity in humans [1]. A problem in aquaculture is cardiac arrest in large salmon (4-7 kg). At present this is an unexplained event, but we think it might be coupled to the mitochondrial capacity of the heart muscle.

In the present study we have compared respiration in perfused tissue from liver, muscle, heart, brain and blood, and a mitochondrial fraction isolated by differential centrifugation from these tissues [2]. The following protocol was used for characterization of respiration. Glutamate+malate were followed by ADP and phosphorylation was inhibited by oligomycin followed by uncoupling with FCCP. Inhibition of Complex I was studied using rotenone followed by addition of antimycin A for determination of residual oxygen consumption (ROX).

The respiratory capacity of perfused tissue and the respective mitochondrial fraction decreased in the order heart, brain, liver, muscle and blood. Endogenous respiration in brain tissue and mitochondria was substantially higher than in the other tissues. In the presence of glutamate+malate respiratory control varied and was highest in mitochondria from heart where the mitochondria were highly coupled. Mitochondrial respiration in the presence of ADP was higher than the respiration achieved after addition of oligomycin and FCCP. However, respiration after inhibition with rotenone and antimycin A was high, indicating that ROX might explain the cardiac arrest in grown up salmon. In brain and the other tissues ROX was lower. High ROX in salmon would be deleterious for quality due to rancid taste development from all the polyunsaturated fatty acids present. Blood cells of fish contain mitochondria, but their respiratory capacity in salmon appear to be limited

[3]. The present study was performed at 25 °C. This is a temperature that salmon avoid in nature, and our further studies will look into the effect of temperature on respiration.

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A2-07 Tissue homogenates for respiratory OXPHOS analysis in comparative mitochondrial physiology: mouse and trout – heart and liver.

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OXPHOS analysis is based on measurement of mitochondrial (mt) respiration in various steady-states of substrate supply and coupling of electron transfer to phosphorylation of ADP. To secure full accessibility of flux control variables (substrates, ADP, etc.) to the mt organelle, mitochondria are either isolated from their cellular containment or plasma membranes are mechanically or chemically permeabilized. However, while permeabilized muscle fibres represent excellent mt-preparations, they require incubation at artificially high oxygen levels to avoid oxygen diffusion limitation [1]. On the other hand, mt-H₂O₂ production is oxygen dependent over a wide range of oxygen pressure [2]. Uncontrolled oxygen gradients in permeabilized muscle fibres, therefore, argue against application of this model for the combined study of ROS production and respiration. A high-quality preparation of tissue homogenate could eliminate diffusion restrictions and thus the need for elevated oxygen levels. This may provide an optimum compromise for a variety of respirometric and fluorometric studies. Therefore, we evaluated the PBI-Shredder (Fig. 1) as an auxiliary HRR-tool for a standardized preparation of homogenates from various tissues and species.

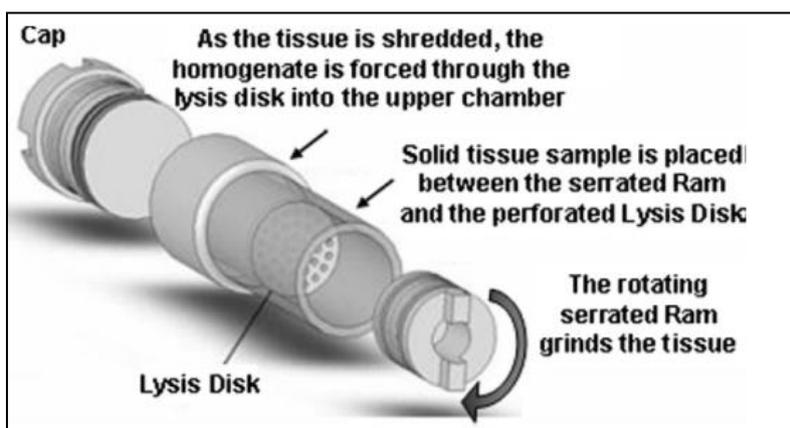


Figure 1. FT500-PS Shredder Pulse Tube for use with the PBI Shredder, reproduced from ref [3].

In the present study we applied high-resolution respirometry (HRR) to characterize and compare homogenate preparations from heart and liver of trout and mouse at the respective physiologically relevant temperature of 15 °C and 37

°C [4]. In trout heart (Fig. 2) and liver biochemical coupling efficiency with Complex I (CI)-linked substrates was identical in the two tissues. CI-linked substrate control (OXPHOS) was higher whereas CII-linked succinate control was lower in heart than liver. Pyruvate enhanced glutamate+malate stimulated OXPHOS capacity to a larger extent in heart than liver. The ADP-ATP phosphorylation system exerted a higher control over OXPHOS (CI+II) in heart (Fig. 2) than liver, making trout heart in this respect a better mt-model for human mt-cardiac function [5] than mouse heart. Mouse heart and liver homogenates were measured at 37 °C using an identical substrate-uncoupler-inhibitor titration (SUIT) protocol. The cytochrome c test (<5% stimulation in healthy controls)

indicated outer mt-membrane integrity in all cases, following an optimization of the PBI-Shredder application with high reproducibility of complete mt-yield and preservation of mitochondrial respiratory control compared to permeabilized fibres.

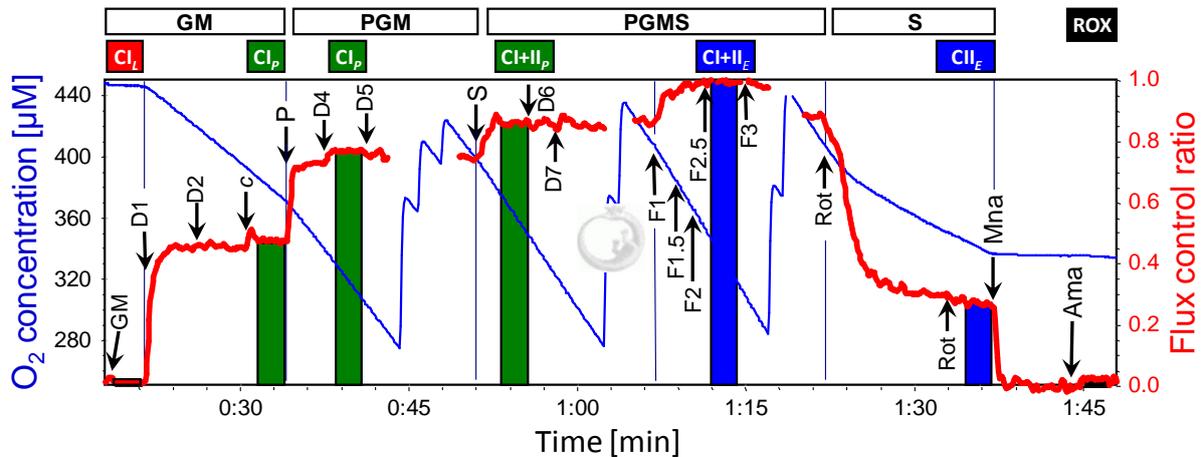


Figure 2. SUIIT protocol with trout heart homogenate (4 mg W_w/ml , MiR06Cr, 15 °C): LEAK with glutamate+malate, CI_L ; 1 mM ADP (D1) was saturating for OXPHOS capacity, CI_p (compare D2), very small cytochrome c effect (c); strong stimulation by pyruvate, P, after which a higher ADP concentration (D4) was required for maximum flux; small stimulation by succinate, $CI+II_p$, followed by a significant uncoupling effect (low P/E ratio), indicating limitation of OXPHOS by the phosphorylation system relative to ETS capacity, $CI+II_E$. Optimum FCCP concentration was higher than in liver homogenate (2.5 versus 1.0 μM), but 3 μM exerted a pronounced time-dependent inhibitory effect (not due to limitation by oxygen, as shown by the lower flux after reoxygenation). After rotenone (Rot) low CII linked ETS capacity, CII_E , and very low residual oxygen consumption, ROX, in the homogenate (malonate and antimycin A, Mna and Ama). Exp 2011-10-05 A-02.

In mouse heart homogenate, oxygen consumption and hydrogen peroxide production were monitored simultaneously by the modular extension of the OROBOROS Oxygraph-2k with the O2k-Fluorescence LED2-Module and application of Amplex™ Ultrared using minimum amounts of tissue (2 mg wet weight per chamber) and MiR05Cr (no catalase). The oxygen-independent range was significantly extended in homogenate compared to permeabilized fibres. The strong H_2O_2 scavenger pyruvate was excluded from the protocol. H_2O_2 production showed a reversible dependence on oxygen concentration that exceeded by far the effects of various substrate and coupling control states on the rate of hydrogen peroxide formation, in striking contrast to mouse brain mitochondria [6].

The remarkable species- and tissue-specific diversity of OXPHOS (substrate and coupling) control patterns will be discussed in relation to selecting appropriate models for comparative mitochondrial physiology and pathology, and for a variety of O2k-MultiSensor protocols applied for functional diagnosis of mitochondrial performance. Tissue homogenate as a fast and reproducible mt-preparation (Fig. 2) opens up new perspectives for comparative mitochondrial physiology.

Supported by K-Regio project *MitoCom Tyrol*.

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Peter Hochachka 9 March 1937 – 16 September 2002

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Somero G, Suarez RK (2005) Peter Hochachka: Adventures in biochemical adaptation.
Annu Rev Physiol 67: 25-37.

A3

MiPcomparative: Temperature – a hot MiPchallenge

Peter Hochachka Memorial Session 2



A3-01 Mitochondrial function and the development of endothermy in the precocial Pekin duck (*Anas pekin*).

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Development of endothermy is associated with maturation of aerobic capacity in developing avian species. In the embryonic state, precocial species, such as the Pekin ducks (*Anas pekin*), display an ectothermic phenotype. Aerobic capacity increases and ducks become endothermic rapidly after hatching. A major source of heat generation is shivering thermogenesis of leg muscles. Development of endothermy involves maturation of multiple organs and systems involved in O₂ delivery to the tissues and maturation of mitochondrial function. Here we examined the role of mitochondrial function during attainment of endothermy in precocial Pekin ducks by first measuring whole animal O₂ consumption (V_{O₂}) as ambient temperature decreased from 35 down to 15 °C. Mitochondrial function was assessed at the site of shivering thermogenesis, the thigh flexor muscles, and a muscle group not associated with shivering thermogenesis, breast muscle. To characterize development of mitochondrial function associated with obtaining endothermy, we measured citrate synthase activity as a proxy for mitochondria content and mitochondrial O₂ flux of permeabilized skeletal muscle. Animals were examined at the prehatch, internally pipped (IP) and externally pipped (EP) stages through 19 h post hatching.

Animals obtained an endothermic phenotype rapidly after hatching. The V_{O₂} of IP and EP embryos decreased during gradual cooling from 35 to 15 °C. In hatchlings as early as 0 h post hatching, V_{O₂} increased significantly during gradual cooling from 35 °C down to 15 °C. While body mass did not differ between EP embryos and hatchlings, whole ventricle mass increased significantly upon hatching. Citrate synthase activity of thigh flexor muscle increased significantly with age. In contrast, citrate synthase activity of breast muscle did not change upon hatching and was significantly lower than that of thigh muscle. Mitochondrial O₂ flux in permeabilized thigh flexor muscle increased significantly upon hatching. There were no changes in LEAK respiration between IP, EP, and hatchling muscle fibers. There was a significant increase in mitochondrial oxidative phosphorylation (OXPHOS) capacity through Complex I in muscle fibers from EP embryos and hatchlings when compared with IP embryo fibers. OXPHOS capacity through Complex I and II and maximal ETS capacity were significantly greater in hatchling muscle fibers when compared with both IP and EP fibers. Increased metabolic capacity necessary to attain endothermy was associated with increasing metabolic capacity of the tissue and increasing O₂ delivery capacity. This change in aerobic capacity was obtained rapidly upon hatching as evident by the increase in whole animal V_{O₂} and OXPHOS capacity of thigh muscle fibers in hatchlings.

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A3-02 Holding our breath in our modern world: are mitochondria keeping the pace with global changes?

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Changes in environmental temperature can pose significant challenges to animals. Shifts in thermal habitat have been shown to be a major force driving species adaptation. These adaptations have been the focus of major research efforts to delineate the physiological or metabolic constraints related to temperature and to reveal the phenotypic characters that can or should adjust. Considering the current consensus on climate change, the focus of research will likely move on questioning if they will survive to future modifications of their thermal niches. Adjustments to temperature can either be through physiological plasticity (e.g. acclimation) or via genetic adaptation. Therefore we will have to specify what are the genetic and phenotypic attributes (at the level of individual, population and species) that could grant survival success. These questions are particularly important for ectotherms, which are in thermal equilibrium with the surrounding environment. To start answering these queries, we should wonder if any physiological or metabolic function set the temperature impact on organisms. Some recent developments point to mitochondria as a key metabolic structure that partly delineates the thermal range that organism can tolerate [1]. The catalytic capacity of mitochondria is highly sensitive to thermal variation and therefore should partly dictate the temperature dependence of biological functions. Mitochondria are a complex network of pathways of different enzymatic reactions that synergistically interact. The fine regulation of both ATP and ROS production depends on this integration of different enzymes and pathways. Here, we will scrutinize the temperature dependence of different parts of the mitochondrial pathways and evaluate the evolutionary challenges that should be overcome to insure mitochondrial adaptations to new thermal environments.

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A3-03 The effect of low-temperature acclimation on mitochondrial function in the common killifish (*Fundulus heteroclitus*), a top-down elasticity analysis.

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Based on its presumed role in altering whole-animal metabolic rate, the mitochondrion has become the focus of hypotheses that address the process of thermal adaptation. It has been proposed that temperature-induced limitations on mitochondrial function (due to passive thermal effects on biochemical activity or an imbalance between O₂ supply and demand at low temperatures) affect whole organism performance and, as a result, cold-adapted or -acclimated species compensate with increased mitochondrial density and/or activity [1]. To address this hypothesis we use *Fundulus heteroclitus*, a teleost species with genetically distinct, locally adapted subpopulations (Northern, Southern, and Hybrid) which reside over a large thermal gradient. During acute high temperature shifts (37 °C), liver mitochondria isolated from 5 °C acclimated Northern *Fundulus heteroclitus* lose the capacity to perform oxidative phosphorylation. This phenomenon is not observed with fish acclimated to 15 and 25 °C, which is indicative of a cost of acclimation to low temperatures [2].

To investigate the functional differences in mitochondrial properties as a result of low temperature acclimation we have acclimated Northern and Southern *Fundulus heteroclitus* to 5, 15 and 33 °C. We compare the kinetics of liver mitochondrial ADP-phosphorylation, proton conductance, and substrate oxidation during acute shifts to 5, 15, and 33 °C. In addition, we compare the rates of basal and maximum reactive oxygen species (ROS) production to assess its contribution as a result of proton conductance.

Our current results indicate that during acute shifts to high temperature, cold-acclimated Northern killifish exhibit equivalent levels of LEAK respiration (i.e., proton leak) as room- and warm-temperature acclimated killifish while maintaining a lower membrane potential. This equivalent level of proton leak is reflected in no difference in ROS production when compared to the 15 °C acclimation. In addition, warm-acclimation appears to result in increased basal ROS production, while lowering maximal ROS. These results indicate that there are changes in mitochondrial function associated with low-temperature acclimation.

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A3-04 Mitochondria in a changing climate? The role of mitochondrial in hyperthermic heart failure in different fish species.

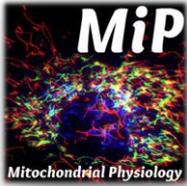
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Predictions of climate change mediated rises in ocean temperatures suggest that ectothermic hearts may place tight constraints on species. For many aquatic species, the upper temperature limit (T_{max}) and the heart failure (HF) temperature (T_{HF}) is only a few degrees away from their current environmental temperatures [1,2]. For tropical fishes this window between T_{max} and T_{HF} appears to be narrower than for temperate fishes, where only slight temperature increases induce heart failure (HF) [3]. Why heat stress induces HF remains unresolved, and for fishes elevated temperatures may result from energy and/or oxygen supply disruptions to and from cardiac mitochondria [2]. Damaged/stressed mitochondria may release ROS, trigger apoptosis, and/or simply may fail to produce enough ATP to sustain a heartbeat. Recent work within a common New Zealand wrasse (*Notolabrus celidotus*) found that ATP synthesis capacity collapses prior to T_{HF} alongside loss of cytochrome c [4]. However, is this effect limited to one species from one thermal habitat? We therefore compared heart mitochondria from three wrasse species that occupy cold temperate (*N. cinctus*), temperate (*N. celidotus*) and tropical (*Thalassoma lunare*) habitats. In all three species a drop in phosphorylation efficiency (inferred from RCR) occurred below the T_{HF} indicating that heart mitochondrial ATP supply is compromised at elevated temperatures. While acclimation of *N. celidotus* at their winter low of 15 °C and summer high of 21 °C revealed that the RCR values of heart mitochondria from warm acclimated animals adjusts, this appears to come at a cost of an increased dependence on anaerobic metabolism and an increased sensitivity of flux with Complex I and II substrates. These data indicate that mitochondrial integrity may well play a role in thermal stress tolerance and limit species distributions in our warming world.

Supported by a Royal Society Marsden Grant to AJ H.

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A3-05 Thermal sensitivity of mitochondria from arctic charr heart: Mitochondrial respiration and ROS production.

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In the context of climate change, it is of paramount importance to investigate the thermal sensitivity of aquatic ectotherms [1]. Oxidative phosphorylation in mitochondria is one of the key processes of energy production and is known to be influenced by temperature [2,3]. The aim of our study was to shed light on the specific steps of the electron transfer system (ETS) that contribute to the adaptation of fish to temperature changes. For this purpose, we measured oxygen consumption and hydrogen peroxide production at two different temperatures (10 and 15 °C) in mitochondria isolated from arctic charr heart (*Salvelinus alpinus*) raised at 10 °C. Activities of citrate synthase and cytochrome c oxidase (COX) were also measured at the same temperatures. Specifically, respiration rates of Complex I and Complex II in both coupled and uncoupled states were determined separately by adding either pyruvate, malate, ADP and FCCP for Complex I or succinate, ADP and FCCP for Complex II. Moreover, respiration rates were also measured in the presence of pyruvate+malate+succinate+ADP allowing the evaluation of Complexes I+II together.

Our preliminary results showed that the Complex I and Complex II respiration rates (taken together) were higher when measured separately than when both complexes worked simultaneously. However, this difference was only significant at 15 °C. This may concomitantly occur with a higher reactive oxygen species production at elevated temperatures, and potentially a disruption of mitochondrial integrity. Subsequent analyses of hydrogen peroxide production, citrate synthase and COX activity and another series of experiments at 20 °C will give us further insights into the thermal sensitivity of arctic charr heart mitochondria.

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A3-06 Mitochondrially-encoded protein Var1 promotes loss of respiratory function in *Saccharomyces cerevisiae* under stressful conditions.

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The yeast *Saccharomyces cerevisiae* cells easily lose their mtDNA, especially when under stress. After the loss, the cells acquire resistances to a variety of harsh conditions. This suggests that the loss of mtDNA is driven by an active process. To test this we measured the frequency of petite transformation under stresses (ethidium bromide treatment and heat-shock) and found that an inhibitor of mitochondrial translation erythromycin reduces the frequency of the petite transformation. Apparently, the simplest explanation is that under the stresses some mitochondrially-encoded protein(s) induces further damage of mtDNA. Most of the proteins encoded by mtDNA are membrane-associated, strongly hydrophobic and thus unlikely to interact with DNA. On the contrary, Var1, a component of mitochondrial ribosomes, is hydrophilic, positively charged and prone to aggregation. It appeared that in cells expressing Var 1 construct in the nucleus erythromycin had no effect on the frequency of the stress-induced petite transformation. We also found that the addition of DNase altered Var1 content in the preparation of mitochondrial nucleoids, which is indicative of binding. Importantly, Var1 is conserved only in yeast. Together, it suggests that Var1 physically interacts with mtDNA and under stress negatively regulates its maintenance [1].

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B1

MiPregulatory: Sulfide and structure



B1-01 Adaptation of colonocyte mitochondria to intense hydrogen sulfide exposure.

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Sulfide (H_2S , HS^- , S^{2-}) is highly toxic because it is an inhibitor of mitochondrial Complex IV similar to cyanide, NO or CO [1] and high nanomolar concentration of sulfide are sufficient to inhibit 50% of cytochrome oxidase activity [1,2]. However, when respiration of intact cells is measured, concentrations above 10 μM are needed to reach a significant inhibition. In mammals the metabolism of sulfur containing amino acids releases sulfide that if unchecked would be rising to micromolar values within minutes. Moreover, the anaerobic metabolism of the bacteria in the colonic lumen raises concentrations of free sulfide to 60 μM or even to mM concentrations if one considers the bound sulfide. Protection of cells and particularly of colonocytes against sulfide toxicity is thus required [3,4].

Sulfide at least in the H_2S form (20-30% of free sulfide at physiological pH) is a hydrogen donor and is used by the mitochondrial respiratory system as a substrate [4,5]. The enzyme involved is the sulfide quinone reductase (SQR), associated with two other enzymes (a dioxygenase and a sulfur transferase) to release thiosulfate ($\text{H}_2\text{S}_2\text{O}_3$). Initiation of SQR activity is detectable with nM concentration of sulfide. Two sulfide molecules are engaged, one molecule of dioxygen is used by the dioxygenase and one atom of oxygen by the cytochrome oxidase and thus for the same electron transfer through Complexes III and IV three times more oxygen are needed. The SQR activity was first demonstrated in colonocytes [4] but later on it was recognized that it is present in a large variety of cells/organs [5]. It is thought to reflect the need of a continuous sulfide disposal to eliminate endogenous release. Moreover, sulfide like NO or CO is thought to be a gasotransmitter and SQR activity appears relevant to sulfide signaling/pharmacology [6].

As external concentration requires to be lower than 10 μM the establishment of a steady state for sulfide oxidation requires a continuous infusion of sulfide [4]. Because of the stoichiometry of sulfide oxidation this infusion increases the oxygen consumption of the cells/mitochondria even if a "normal" carbon based oxidation was taking place [5]. If the flux of sulfide ($J_{\text{H}_2\text{S}}$) is too high inhibition of respiration and of oxygen consumption develops quickly [5]. Therefore according to the intensity of sulfide exposure (concentration and flux) sulfide could either stimulate or inhibit cellular oxygen consumption [5]. A convenient way to express the sulfide challenge is the ratio between the flux of sulfide delivered and the respiratory rate before sulfide infusion ($J_{\text{H}_2\text{S}}/J_{\text{O}_2}$) indicating a relative sulfide exposure [5]. A few cell types including colonocytes tolerate values above 2 [5].

Colonocytes are adapted to maximize their sulfide oxidation capacity:

- 1) As in other cells sulfide oxidation takes priority over the use of other substrates [5].
- 2) SQR expression and activity makes it able to saturate the mitochondrial respiratory system with electrons from sulfide [5].
- 3) Differentiation of colonocytes increases the content in respiratory enzymes but paradoxically not in SQR [7].
- 4) In contrast to the other cells studied so far, colonocytes use reverse electron transfer in Complex I to increase further their sulfide disposal rate [5]. However, when the sulfide exposure is too high the colonocytes switch towards a glycolytic metabolism [2].

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B1-02 Studying mitochondrial effects of sulfide. Does the species matter?

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Several investigations demonstrated that a hibernation-like metabolic state characterized by reduced energy expenditure and hypothermia can be induced in mice that have inhaled 80-100 ppm of H₂S [1,2,3]. These observations suggest the exciting prospect of pharmacologically controlling energy expenditure in severe trauma and disease states by protecting against transitory hypoxia. However, equivocal data available from studies on large animals still raise serious doubts on the genuine effects of H₂S on cell metabolism and mitochondrial function. In fact, data from sheep or swine failed to show any metabolic effect [4,5], and the effects observed injecting the intravenous sulfide donor Na₂S in pigs are still controversial [6,7]. Obviously, body temperature is a major confounder in most animal studies on sulfide, as it can be easily modified, deliberately or accidentally, in murine experiments, but is hard to control in larger species. Therefore, it is still not clear which impact non-toxic doses of sulfide may have *in-vivo* on metabolic or mitochondrial functions beyond eventual temperature related effects.

In the last few years we performed experiments on the effects of sulfide in anesthetized and mechanically ventilated mice using a setting closely resembling an intensive care unit [8]. A closed loop control of body temperature allowed to efficiently separate genuine effects of sulfide and body temperature. Our data obtained *ex-vivo* by measuring mitochondrial respiration in small liver biopsies by means of high-resolution respirometry performed with the Oxygraph-2k (Oroboros Instruments, Innsbruck, AT) suggest that low (non-toxic) sulfide concentrations *per se* do not suppress mitochondrial respiratory activity (Figure 1, A and B). Nevertheless, sulfide normalised the amount of LEAK-respiration, which was increased in septic animals under normothermia, an effect which was not related to any change in body temperature (Figure 1, C and D).

In conclusion, the main premise for studying mitochondrial effects of sulfide in animal models should be an efficient control of body temperature in order to clearly separate the effects of both factors. Technically, this can be done most easily in small animals like mice. Of course, the question remains to which extent data obtained in mice may be translated to larger mammalian species. Further preliminary data obtained on tissue biopsies from mice and pigs, however, indicate that sulfide is processed in a very similar way at the cellular level in different mammalian species, thus suggesting that the effects of sulfide at the mitochondrial level may be comparable between the species.

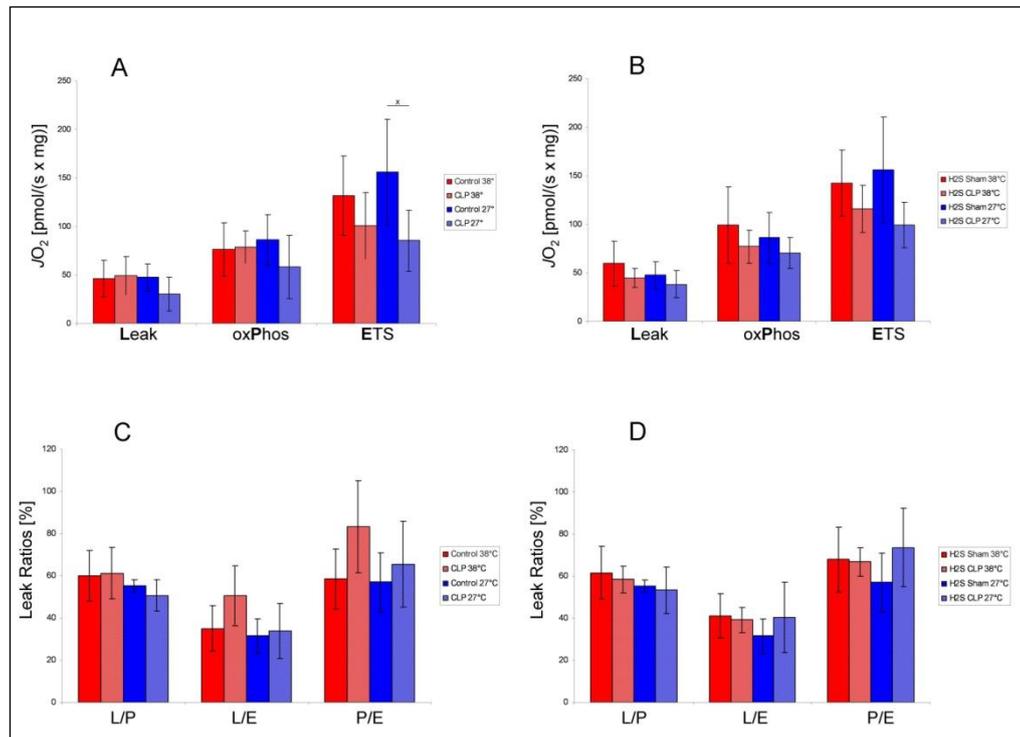


Figure 1: Mitochondrial respiration in mice ex-vivo after 5 h of mechanical ventilation. Treatments were control (closed bars) vs. sepsis (CLP-induced, light bars), intravenous Na_2S (panels B and D) vs. placebo (diagrams A and C), and 38 °C (red bars) vs. 27 °C (blue bars). All data are means and SD. LEAK, OXPHOS, and ETS respiration were almost equal regardless of the sulfide treatment (A vs. B), but LEAK ratios (L/E) as well as the P/E ratio were significantly increased at 38 °C in the septic animals (light red bars in panel C) but normalised under sulfide treatment (panel D). Mitochondrial respiration was measured at 37 °C in all groups regardless of the temperature of 38 °C or 27 °C of the animals under anesthesia and mechanical ventilation.

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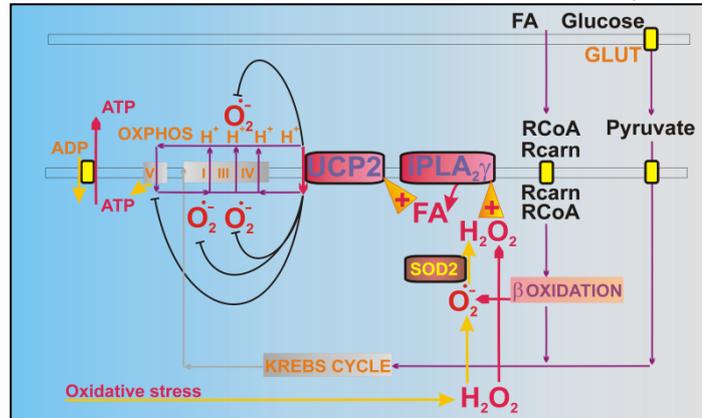
B1-03 Antioxidant synergy of mitochondrial uncoupling protein UCP2 and phospholipase iPLA2 γ .

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Recently, a modified mechanism [1] of originally suggested fatty acid cycling for mitochondrial uncoupling protein-1 (UCP1) confirmed all our original findings [2], including the necessity of fatty acid to approach UCP after its cleavage from membrane phospholipids [3]. We now tested a hypothesis that ubiquitous UCP2 provides feedback downregulation of oxidative stress *in vivo* via synergy with an H₂O₂-activated mitochondrial calcium-independent phospholipase A2 (mt-iPLA2), which cleaves off free fatty acids required for UCP2 as cycling substrates. We demonstrated UCP2 activation by free fatty acids (detected by gas chromatography/mass spectrometry) while observing that bovine serum albumin or GTP inhibit the tert-butylhydroperoxide- or H₂O₂-induced increase in respiration and decrease in membrane potential in lung and spleen mitochondria from control but not UCP2-knockout mice [4]. Upon induction of uncoupling, mitochondrial superoxide formation decreased instantly, but not when mt-iPLA2 γ was blocked by R-bromo-enol lactone (R-BEL). Mt- iPLA2 γ was alternatively activated by H₂O₂ produced in conjunction with the electron-transferring flavoprotein:ubiquinone oxidoreductase (ETFQOR), acting in fatty acid β -oxidation. We have also obtained similar findings in insulinoma INS-1E cells silenced for either UCP2 or iPLA2 γ . Preliminary data showed that mt-iPLA2 γ is directly activated by H₂O₂. The evidence that this cytoprotective mechanism also exists *in vivo* stems from the observed increases in protein carbonylation in lung and spleen tissues and mitochondria, potentiated by R-BEL in wild-type but not in UCP2-knockout mice. We thus demonstrate for the first time a synergic antioxidant role between H₂O₂-activated mt- iPLA2 γ and UCP2, because ablation of either mt- iPLA2 γ or UCP2 prevented such a role. Our *in vitro* simulations also show for the first time that UCP2, functional due to fatty acids released by redox-activated mt- iPLA2 γ , can suppress mitochondrial superoxide production by its uncoupling action.

H₂O₂-activated mt- iPLA2 γ and UCP2 act in concert to protect against oxidative stress *in vivo* (cf. Figure).



Supported by GACR grants P302/10/0346 (PJ), P304/10/P204 (AD) and P303/11/P320 (JJ)

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B1-04 Mitochondrial network and cristae remodeling upon hypoxia.

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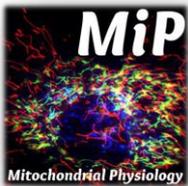
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We have observed thinning of mitochondrial matrix space (mtMS) at negligible changes of mitochondrial outer membrane (mtOM) tubules in HepG2 cells during hypoxic adaptation at 5% oxygen by means of various methods such as 3D high-resolution 4Pi microscopy, 3D super-resolution fluorescent photo-activated localization (BiplaneFPALM) microscopy [1] and cryo-electron microscopy (EM). This phenotype is hypoxia-induced-factor- (HIF-) dependent (maximal HIF1 α stabilization is around 5th hour in hypoxia [2,3]) and is independent of the type of energy metabolism, i.e. sole aerobic glycolysis (glycemic cells) or oxidative phosphorylation (OXPHOS cells). More extensive mtMS shrinkage inside the mtOM reflected the expansion of intermembrane space (IMS), documented by EM as shrinkage of matrix space of cristae in parallel to IMS visualization using Eos-conjugated truncated lactamase- β . This remodeling proceeded along the predominant length of mitochondrial network tubules. The hypoxic IMS expansion resembles Hackenbrock's classic observation of condensed cristae conformation for isolated mitochondria at phosphorylating state OXPHOS, however it is not encountered *in situ* [4]. Confirmation of paradoxical observations of orthodox cristae, in cells undoubtedly phosphorylating in atmospheric conditions, were observed by electron microscopy. The mitochondria had more shrunken IMS and expanded mtMS. In turn, upon hypoxia, the IMS expansion reflected the established Hackenbrock's condensed cristae conformation [5]. We suggest that easier oxygen diffusion throughout the expanded IMS benefits the cells upon hypoxic adaptation.

Supported by GACR grant P302/10/0346 (to JP) and Czech Ministry of education LH11055 (to PHL).

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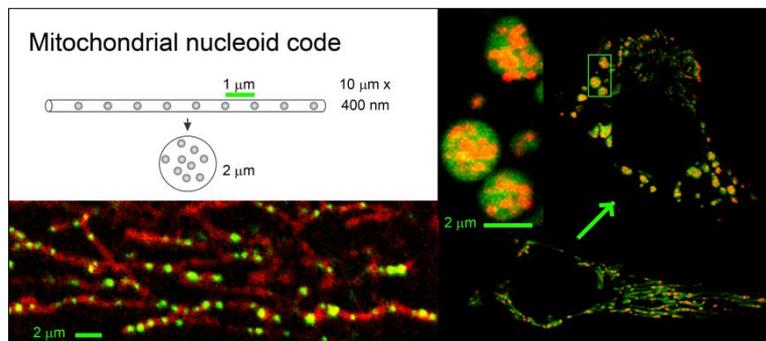


B1-05 Distribution of nucleoids of mitochondrial DNA.

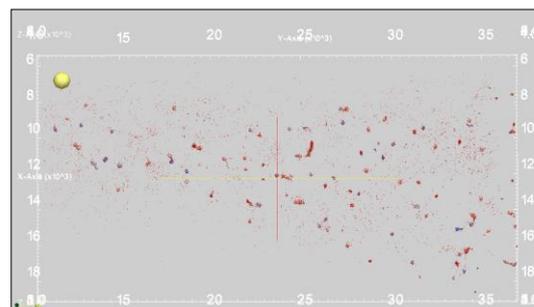
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Robustness of the mitochondrial (mt) genome is given by thousand to hundred thousand (e.g. in mammalian eggs) copies of double stranded mtDNA (~16.5 kD in humans) in a single cell, while mtDNA is organized in nucleoids containing accessory proteins and recruited proteins of the mt replication/transcription machinery. It has been disputed whether a single nucleoid contains just a single mtDNA molecule [1] or up to 6 mtDNA molecules on average. Also a uniform size of nucleoids was disputed. Since the mt network undergoes locally frequent fission (fragmentation) and fusion events, and when integrated over time, a highly interconnected mitochondrial reticulum is seen, and since upon certain insults and/or pathological states a fragmented network may appear all the time, mt nucleoid (re-)distribution is very important also related to mitophagy. Using conventional confocal microscopy [2], we found only two major morphological states: *i*) a tubular state of the mitochondrial network with equidistant nucleoid spacing, 1.10 ± 0.2 nucleoids per Δm , and *ii*) a fragmented state of solitary spheroid objects in which several nucleoids were clustered. We rarely observed singular mitochondrial fragments with a single nucleoid inside and very seldom observed empty fragments. Reintegration of fragments into the mitochondrial network re-established the tubular state with equidistant nucleoid spacing. The two major morphological states coexisted at intermediate stages. These observations suggest that both mitochondrial network fission and reconnection of the fragmented network are nucleoid-centric, *i.e.*, fission and new mitochondrial tubule formation are initiated around nucleoids.



We have also used 3D super-resolution fluorescence photoactivated localization microscopy Biplane FPALM to screen a distribution of nucleoid diameters hepatoma HEPG2 cells and found that rather a range of sizes exists up to 300 nm dimensions. We thus have confirmed previous results obtained by 3D interference PALM [3] and excluded simplified data obtained by 2D STED (stimulated depletion emission) [1].



Supported by GACR grant 13-02033S.

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B2

MiPregulatory: Calcium, potassium and topics of regulation



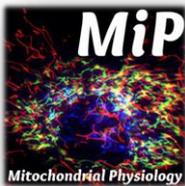
B2-01 Mitochondrial respiration, Ca²⁺ transport and oxidative stress: Fungi, trypanosomes, plants and mammals.

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I will present an overview on our contributions to the field of mitochondrial bioenergetics in fungi, trypanosomes, plants and mammals. I will start by describing the experimental conditions that allowed for the stoichiometric measurements between oxygen consumption, electron flow through the respiratory system, Ca²⁺ uptake by mitochondria and the outward pumping of protons by rat heart mitochondria respiring on NAD-linked substrates. We showed that the transfer of two electrons from NADH to O₂ was accompanied by the outward pumping of 10-12 H⁺. These experiments also originated the first observation indicating that Ca²⁺ release from mitochondria could be stimulated by the oxidized state of mitochondrial pyridine nucleotides. Further studies on these mechanisms provided evidence that Ca²⁺ plus prooxidants, including NAD(P)H oxidants, open the mitochondrial permeability transition pore (PTP) suggesting the redox nature of the PTP. These studies have significantly contributed to the current understanding on the participation of mitochondria in the process of cell death that occurs under conditions of tissue oxidative stress. The studies with mammalian mitochondria provided the background for research carried out in plants, fungi and protozoa. In plants and fungi we discovered the plant mitochondrial uncoupling protein (PUMP) and that plants have a family of uncoupling mitochondrial proteins, consisting of up to six members that are fundamental for plant defence against biotic and abiotic stresses. In protozoa, the use of digitonin to permeabilize the cell membrane to small molecules, nucleotides and probes permitted the characterization of energy-linked mitochondrial functions in situ including the description of the Ca²⁺ uniporter in these parasites. In addition, I will present more recent studies characterizing molecular mechanisms of mitochondrial participation in the processes of degenerative diseases such as hyperlipidemias.

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B2-02 The mitochondrial gas pedal, a unique property of neurons exists also in heart and skeletal muscle but not in astrocytes. New evidences by *in silico* investigations and (patho-) physiological consequences.

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Cytosolic calcium (Ca²⁺_{cyt}) has a strategic task in co-ordinating cellular work load and ATP regeneration but the mechanisms are not completely understood. Up to now the paradigmatic view was that Ca²⁺ after its uptake by the Ca²⁺ uniporter activates PDH,

ICDH and α -KGDH within the matrix. However we have shown by computer simulation that these effects are insufficient to explain the *in vivo* results [1]. Moreover, we have recently shown that $\text{Ca}^{2+}_{\text{cyt}}$ in low nM concentration range ($S_{0.05} = 225 \pm 22$ nM) exclusively regulates the glutamate-dependent OXPHOS capacity (glutamate+malate) of isolated brain mitochondria (BM) via aralar [2-7], the mitochondrial glutamate/aspartate carrier. Whereas OXPHOS capacity with pyruvate+malate is only slightly influenced by $\text{Ca}^{2+}_{\text{cyt}}$ we detected that pyruvate *formation* by the malate aspartate shuttle (MAS) is tightly controlled by $\text{Ca}^{2+}_{\text{cyt}}$ [6,7]. Through its common substrate couple NADH/NAD⁺, the formation of pyruvate by LDH or glycolysis is linked to MAS with aralar as a central component. A rise of $\text{Ca}^{2+}_{\text{cyt}}$ in a reconstituted MAS causes an up to five-fold enhancement of OXPHOS due to an increased substrate supply, acting as a metabolic pyruvate supply unit which we called "gas pedal". In contrast, intramitochondrial Ca^{2+} modulates the oxidation rates only of those substrate molecules which are already present within the mitochondrial matrix. Moreover a substantial Ca^{2+} -uptake by BM requires $[\text{Ca}^{2+}_{\text{cyt}}] > 500$ nM indicating that changes of $\text{Ca}^{2+}_{\text{cyt}}$ in the low nM concentration range should not have an effect on intramitochondrial Ca^{2+} .

In a recent *in silico* work we developed a simple computer model comprising MAS, pyruvate production by glycolysis or LDH, tricarboxylic acid cycle and OXPHOS. It is demonstrated, that MAS is absolutely necessary for pyruvate supply and OXPHOS to take place. It is shown that a direct MAS activation can itself significantly elevate the OXPHOS flux and thus oxygen consumption. Finally, it is demonstrated that an activation of MAS in parallel with a direct activation of the remaining components helps to increase effectively the oxygen flux and to maintain intermediate metabolism homeostasis. The model helps to understand directly and intuitively the properties of the system.

We further found that the gas pedal is also a property of heart and skeletal muscle. However, the largest extent of gas pedal we detected in neurons, therefore and since neuronal mitochondria are not able to oxidize fatty acids the gas pedal is a unique property of neurons. In contrast astrocytic mitochondria do not contain aralar therefore they have no gas pedal. These cell specific differences allow to specify the contribution of neurons and astrocytes to the function of isolated brain mitochondria.

The gas pedal concept includes a hypothesis on the occurrence of de-energized neuronal mitochondria at low $\text{Ca}^{2+}_{\text{cyt}}$ (120 nM) at night and of energized mitochondria at elevated $\text{Ca}^{2+}_{\text{cyt}}$ (440 nM) at day as it was measured recently during circadian $\text{Ca}^{2+}_{\text{cyt}}$ oscillations in single neurons of nucleus suprachiasmaticus [8]. Our concept also explains mitochondrial dysfunction caused by permanently diminished $\text{Ca}^{2+}_{\text{cyt}}$ causing de-energized mitochondria with subsequent cellular energetic depression or by largely and permanently increased $\text{Ca}^{2+}_{\text{cyt}}$ causing over-energization also at night with the danger of enlarged ROS formation.

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B2-03 Bioenergetic aspects of postnatal development of cardiac cells: Formation of a structure-function relationship.

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In adult cardiac muscle cells mitochondria are positioned in a crystal-like pattern and are integrated into functional complexes, intracellular energetic units (ICEU), which regulate energy metabolism in striated muscles [1]. In postnatal cardiomyocytes the cellular cytoarchitecture is significantly less organized, ICEUs have not been established and these cells are relatively more dependent on anaerobic glycolysis [2]. In this work we demonstrate that during heart postnatal development exhaustive changes are taking place both at the functional and structural level of energy metabolism. The main methods used were confocal microscopy and respirometry with the Oxygraph.

We show the formation of a regular arrangement of the mitochondria and cytoskeletal alterations in parallel to the maturation of energy transfer systems of mitochondrial metabolism. The affinity for ADP of mitochondrial respiration decreased while localization of mitochondria became progressively more organized within the cellular interior. At birth the K_m' for ADP was relatively low ($75.0 \pm 4.5 \mu\text{M}$; 3-day old rat cardiomyocytes), and then steadily increased to the adult level ($317 \pm 30 \mu\text{M}$; 84 days). This reflects the formation of increased diffusion restrictions for ADP and is a manifestation of higher regulation of energy transfer. Simultaneously, the creatine kinase system is becoming progressively more capacitive in mediating the feedback regulation between ATP consumption in the cytosol with production by mitochondria. These functional alterations are accompanied by changes in structural organization of mitochondria both relative to myofibrils and to cytoskeletal proteins like αIV and βII tubulin - these proteins could participate in the regulation of voltage dependant anion channel (VDAC) permeability for adenine nucleotides. Functional interactions between mitochondria and components of the cytoskeleton in cardiac cells are necessary prerequisites for highly organized metabolic networks characteristic of adult cardiomyocytes.

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B2-04 Regulation of oxidative phosphorylation during work transitions in different tissues results from its kinetic properties.

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The regulation of oxidative phosphorylation (OXPHOS) during work transitions in skeletal muscle, heart and other tissues is still not well understood. Different computer models of this process have been developed that are characterized by various kinetic properties. In the present theoretical study it is shown that models belonging to one group [1-3], which assume an approximately uniform distribution of metabolic control over oxygen consumption (J_{O_2}) among particular oxidative phosphorylation complexes, C (CI, CIII, CIV, ATP synthase, ATP/ADP carrier, phosphate carrier), predict that all OXPHOS complexes are directly activated in parallel with ATP usage and NADH supply by some external cytosolic factor/mechanism during low-to-high work transitions in skeletal muscle and heart ('each-step-activation' mechanism) [1,3,4]. A direct activation during work transitions of the ATP supply block in general was first proposed in relation to skeletal muscle by Peter Hochachka [5,6]. Models belonging to another group [6,7], which assume that among OXPHOS complexes CIII keeps almost all of the metabolic control over J_{O_2} (the control of other complexes is close to zero) and that it is strongly activated by inorganic phosphate (P_i), predict that an increase in P_i is the main mechanism responsible for OXPHOS activation (feedback-activation mechanism) [7,8]. It is demonstrated that computer models based on the each-step activation mechanism reproduce experimental data much better than models assuming the feedback-activation mechanism. Experimental studies revealed an approximately equal distribution of control over J_{O_2} among OXPHOS complexes. They predict that different each-step activation intensities generate different (slopes of) the $J_{O_2}/[ADP]$ relationships encountered in different muscles. These models enable a good homeostasis of not only ADP and PCr, but also of P_i and NADH during large increases in J_{O_2} . Finally, they do not imply a very high value of proton leak at low work in heart and at rest in skeletal muscle.

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B2-05 Comparative mitochondrial physiology in blood feeding insect vectors and parasites.

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Hematophagy poses a challenge to blood-feeding organisms since products of blood digestion can exert cellular deleterious effects. Mitochondria perform multiple roles in cell biology acting as the site of aerobic energy transducing pathways, and also an important source of reactive oxygen species (ROS), modulating redox metabolism. Our research group is currently investigating basic aspects of mitochondrial physiology in some hematophagous organisms that cause or transmit tropical diseases. The models currently investigated include the blood fluke *Schistosoma mansoni*, the mosquito *Aedes aegypti* and the kissing bug *Rhodnius prolixus*. In *Aedes* flight muscle mitochondria, we observed that blood intake caused a transient reduction in both oxygen consumption and hydrogen peroxide generation, which were parallel to blood digestion process, regardless the mitochondrial metabolic state. In the kissing bug *Rhodnius*, a transient reduction on flight muscle oxygen consumption was observed along the blood digestion cycle, without affecting hydrogen peroxide generation. Interestingly in *Rhodnius*, both parameters were profoundly affected by aging, causing a 75% inhibition of respiration and hydrogen peroxide production in the OXPHOS state in old insects. Maximum hydrogen peroxide production was also specifically affected by blood intake but not plasma. Sexual differences in oxygen consumption in *Aedes* flight muscle were also identified, being significantly higher in females than in males, regardless of the substrate utilized. In this model, pyruvate and proline were the preferential substrates utilized to sustain oxygen consumption. Finally, in the blood fluke *Schistosoma*, where adult female worms digest ten times more blood than males, sexual differences in terms of respiratory capacity were also reported, where male worms exhibit higher mitochondrial oxygen consumption rates than females, regardless the substrates utilized. Curiously, in female worms, hydrogen peroxide production was higher than in males, whereas females were more resistant to oxidative challenge promoted by menadione than males. Concluding, the blood-feeding habit promotes functional and structural remodeling in mitochondria from hematophagous organisms, which may represent an important adaptation to this exquisite dietary source.

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B2-06 Ancestral function of Letm1 as determined in the evolutionary diverged pathogen *Trypanosoma brucei*.

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Leucine zipper EF-hand containing transmembrane protein 1 (Letm1) is evolutionarily conserved in diverse eukaryotic lineages having energized mitochondria, from opisthokonts, comprising metazoa and fungi, to plastid containing plants and apicomplexans. Its deletion from the human genome has been implicated in causing the seizure symptoms of Wolf-Hirschhorn syndrome. Research in predominantly opisthokont model systems has resulted in disparate roles of Letm1 such as a cation/proton antiporter, translocating either potassium or calcium across the inner membrane (mtIM), and anchoring of mitochondrial ribosomes to facilitate mtIM incorporation of hydrophobic respiratory system subunits.

We have undertaken functional analysis of Letm1 in the highly evolutionarily diverged *Trypanosoma brucei*, member of the excavate order Kinetoplastida, taking advantage of the existence of *in vitro* cultured forms bearing mitochondria with different physiological states, including a petite mutant-like organelle. RNAi-interference in all of these cell types results in swelling that can be mitigated by the ionophore nigericin, suggesting that it plays a role in the efflux of the cation from the matrix. An observed effect on mitochondrial translation is a downstream effect of potassium accumulation in the organelle.

By comparison of our results with those from other systems, we conclude that the basal function of Letm1 is to regulate potassium efflux from the matrix to maintain mitochondrial volume. The protein shows remarkable conservation of function not only across wide evolutionary distances, but also in different mitochondrial states. *T. brucei* is an appealing model for study of mitochondrial biology due to its amenability to reverse genetics, existence of *in vitro* cultures bearing different mitochondria and its diverged position in the eukaryotic tree of life, bringing another perspective for comparative biochemical approaches.



B2-07 The role of mitochondrial ATP-dependent potassium channel in the adaptation of organisms to stress.

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It is known that different people and animals within the same species have different resistance to the stress damage. It is believed that these differences are largely genetically determined or acquired during the life of the differences in the activity of the stress system implementing the organism's response to stress-factor. Our objective was to determine the role of mitochondrial ATP-dependent potassium channel (mtK_{ATP}), which plays a key role in protecting the myocardium from ischemia [1], in protecting of the organism against some types of stress and adaptation to it.

The work was carried out on heart and liver mitochondria of rats of different genetic lines. To perform the first part of the study we selected purebred white rats having high and low resistance to acute hypoxic stress. Part of the low resistant animals were then artificially adapted to acute hypobaric hypoxia. The second part of the work was carried out on rats Wistar and August lines, having different resistance to the stress damage of

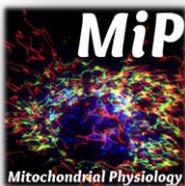
the circulatory system [2] and to hypoxia [3] (August rats are resistant to stress influences, Wistar rats are less resistant).

When using outbred rats we have shown that the rate of ATP-dependent potassium transport was higher (by 15% in liver mitochondria and 30% in heart mitochondria) in mitochondria of high resistant to hypoxia rats compared with a low resistance. In adapted to oxygen deficiency rats rate of ATP-dependent potassium transport in mitochondria of both tissues is increased by 45-50%. It is accompanied by a decrease in the amount of K^+ in the mitochondria of both tissues by an average of 30%, which may indicate the activation also K^+/H^+ -antiporter in mitochondria. Thus, the potassium cycle is activated in mitochondria. The same effect was observed in the study of potassium transport in mitochondria of rats Wistar and August lines. In more resistant to stress *August* line rats potassium uptake into the mitochondria via mtK_{ATP} and potassium amount were significantly higher compared to Wistar line rats.

A high rate of mitochondrial potassium transport led to a marked reduction (by 25-30%) of the rate of generation of H_2O_2 in the mitochondria. We presume that the effect of reducing the formation of reactive oxygen species in the mitochondria as a result of the activation mtK_{ATP} may mediate the positive effect of hypoxic training as well as genetic resistance to oxidative stress.

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B2-08 Potassium controls rat mitochondria function: *In vivo* and *in vitro* considerations.

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The intracellular potassium concentration, $[K^+]$, varies depending on tissue type and is about 60 mM in kidney proximal tubular cells whereas it is approximately 120 mM in cardiac myocytes. Intracellular $[K^+]$ may therefore affect mitochondria function.

By measuring the respiratory function and swelling of mitochondria isolated from kidney cortex and heart at $[K^+]$ ranging from 15 mM to 146 mM we showed that $[K^+]$ controls mitochondria function in the heart. Increasing $[K^+]$ resulted in increased resting state respiration and decreased respiratory control ratio (RCR) in kidney cortex mitochondria. The reduced RCR corresponded with mitochondria swelling, indicating failing mitochondria. The effects of K^+ in kidney mitochondria was prevented by inhibitors of ATP-sensitive K^+ -channels and voltage-gated K^+ -channels (glibenclamide and by 4-aminopyridine, respectively). On the contrary, RCR in heart mitochondria increased with increasing $[K^+]$ and was unaffected by K^+ channel inhibitors.

These results demonstrate $[K^+]$ control of mitochondria function in rat kidney and heart.

C1

MiPathology: Diabetes and obesity - general



C1-01 Building the mitochondrial medicine, need to define mtDNA variations and its function.

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Mitochondrion is at the center of life phenomena; it supplies energy, controls tasks of the cell, i.e., signaling, differentiation, growth, and death. We found the evidences that mitochondrial dysfunction is causally related to diabetes mellitus, metabolic syndrome and lately atherosclerosis [1,2] and its genome (mtDNA) variations confer a genetic susceptibility to these states [3], we were reconstructing pathogenic mechanisms. We found poor mitochondrial biogenesis during development, be it fetal or after the birth, might be behind so-called thrifty phenotype hypothesis [4], and environmental pollutants could cause those diseases by damaging mitochondrion [5,6]. This scheme could easily explain why diabetes and obesity are associated with enhanced cancer risk.

I reasoned that defining function of different mtDNA should be a cornerstone to build a coherent mitochondrion centered disease model, as mitochondrion is also controlled by nuclear genes. It is better if it is defined quantitatively, as it could be linked to whole body in quantitative terms, applying metabolic scale law. However our efforts were not successful so far. In this presentation, I will explain why comparative mitochondrial physiologic studies are essential.

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C1-02 Glucose tolerance and skeletal muscle coenzyme Q(10).

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Q(10) content, mitochondrial density, and mitochondrial oxidative phosphorylation (OXPHOS) capacity were measured in simvastatin-treated patients ($N=10$) and in well-matched control subjects ($N=9$).

A prevalent side effect of statin therapy is muscle pain, and yet the basic mechanism behind it remains unknown. We hypothesize that a statin-induced reduction in muscle Q(10) may attenuate mitochondrial OXPHOS capacity, which may be an underlying mechanism.

Plasma glucose and insulin concentrations were measured during an oral glucose tolerance test. Mitochondrial OXPHOS capacity was measured in permeabilized muscle fibers by high-resolution respirometry in a cross-sectional design. Mitochondrial content (estimated by citrate synthase [CS] activity, cardiolipin content, and voltage-dependent anion channel [VDAC] content) as well as Q(10) content was determined.

Simvastatin-treated patients had an impaired glucose tolerance and displayed a decreased insulin sensitivity index. Regarding mitochondrial studies, Q(10) content was reduced ($P=0.05$), whereas mitochondrial content was similar between the groups. OXPHOS capacity was comparable between groups when Complex I- and Complex II-linked substrates were used alone, but when Complex I + II-linked substrates were used (eliciting convergent electron input into the Q intersection [maximal *ex vivo* OXPHOS capacity]), a decreased ($P<0.01$) capacity was observed in the patients compared with the control subjects.

These simvastatin-treated patients were glucose intolerant. A decreased Q(10) content was accompanied by a decreased maximal OXPHOS capacity in the simvastatin-treated patients. It is plausible that this finding partly explains the muscle pain and exercise intolerance that many patients experience with their statin treatment.



C1-03 Calcium regulation of metabolism in adipocytes.

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Calcium (Ca^{2+}) is an important intracellular signalling molecule in mammalian tissues, and has been associated with the regulation of diverse processes, including contraction, secretion, autophagy, ion pumping, and the activation of metabolic enzymes. Mitochondria maintain a calcium gradient between the matrix and the cytoplasm, via the actions of specific transporters in the inner mitochondrial membrane. Under conditions of metabolic dysfunction (such as insulin resistance), mitochondrial calcium homeostasis is often altered (for a recent review, see [1]).

With the recent identification of the mitochondrial calcium uniporter (MCU) and associated regulatory proteins, as well as the use of targeted aequorin probes, we are now able to investigate the role of mitochondrial Ca^{2+} in the regulation of whole-cell adipocyte metabolism [2,3]. Preliminary data suggest that the induction of insulin resistance (by treatment with 100 nM insulin for 24 h) in cultured adipocytes alters the expression of key components of the uniporter (+75% MCUa and +42% MCUB compared to control, $P<0.05$), and increases Ca^{2+} uptake into the mitochondria (+76%, $P<0.05$). Similarly, feeding mice a high-fat-high-sugar diet alters the expression of these genes in visceral white adipose tissue (+54% MCUa and +127% MCUB compared to chow-fed control, $P<0.05$). Additionally, we saw that direct manipulation of mitochondrial calcium by overexpression of MCUB in cultured adipocytes was able to alter a number of metabolic parameters, including mitochondrial membrane potential, NADH dynamics, and

the rate of glucose oxidation. The mechanism and significance of these metabolic alterations is currently under investigation.

We theorise that mitochondrial calcium plays an important role in the orchestration of metabolic homeostasis in adipocytes. As such, the MCU may represent an interesting node of metabolic regulation - and therefore therapeutic potential - in times of metabolic dysregulation.

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C1-04 Role of mitochondrial function for the onset and progression of kidney disease.

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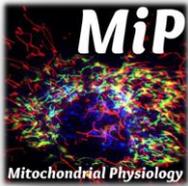
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Diabetes is closely associated with increased oxidative stress, especially from the mitochondria. A mechanism to reduce increased mitochondria superoxide production is to reduce the mitochondrial membrane potential by releasing protons across the mitochondrial membrane. This phenomenon is referred to as mitochondrial uncoupling since oxygen is consumed independently of ATP being produced, and can be mediated by uncoupling proteins (UCPs). However, increased oxygen consumption is potentially detrimental for the kidney since it can cause tissue hypoxia. Therefore, this thesis aimed to investigate the role of mitochondria uncoupling for development of diabetic nephropathy.

UCP-2 is the only isoform expressed in the kidney, and localized to the tubular segment performing the majority of tubular electrolyte transport. Streptozotocin-induced diabetic rats have increased UCP-2 protein expression which correlates to increased non-transport dependent oxygen in isolated proximal tubular cells. These effects are prevented by intense insulin treatment to the diabetic animals demonstrating a pivotal role of hyperglycemia. Importantly, elevated UCP-2 protein expression increases mitochondria uncoupling in mitochondria isolated from diabetic kidneys. Mitochondria uncoupling and altered morphology was also evident in kidneys from db/db-mice, a model of type-2 diabetes, together with proteinuria and glomerular hyperfiltration which are both clinical manifestations of diabetic nephropathy. Treatment with the mitochondrial targeting antioxidant coenzyme Q10 prevents mitochondrial uncoupling as well as morphological and functional alterations in these kidneys. Acute knockdown of UCP-2 paradoxically increases mitochondrial uncoupling via a mechanism involving the adenosine nucleotide transporter. Increased uncoupling via adenosine nucleotide transporter decreases mitochondrial membrane potential and kidney oxidative stress but does not affect glomerular filtration rate, renal blood flow, total kidney oxygen consumption or intrarenal tissue oxygen tension. The role of increased mitochondrial oxygen consumption *per se* has been demonstrated by administering the chemical uncoupler dinitrophenol to otherwise healthy rats. Importantly, increased mitochondrial oxygen consumption results in kidney tissue hypoxia, proteinuria and increased staining of the tubular injury marker vimentin demonstrating a crucial role of increased oxygen consumption and the resulting kidney tissue hypoxia for the development of nephropathy.

Taken together, these observations demonstrate an important and pivotal role of mitochondrial uncoupling for the development of chronic kidney disease.



C1-05 The effects of Angiotensin II on mitochondrial respiration: A role of normoglycemia versus hyperglycemia.

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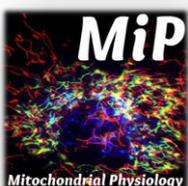
Exaggerated activation of the renin angiotensin aldosterone system (RAAS) is a key feature in diseases such as hypertension, diabetes and chronic kidney damage. Angiotensin II (Ang II) can activate type 1 receptors (AT₁R), resulting in increased blood pressure and formation of reactive oxygen species via activation of the NADPH oxidase, or type 2 (AT₂R) receptors, normally counter-acting AT₁R-activation. Recently, an intracellular RAAS was demonstrated with AT₁R and AT₂R expressed in nucleus and mitochondria [1]. AT₁R activation in nucleus increases production of inflammatory cytokines, but the function of mitochondrial AT₁R is unknown. AT₂R in both nucleus and mitochondria is coupled to increased nitric oxide (NO) production and subsequently decreased mitochondrial respiration. Interestingly, diabetes is associated with both mitochondrial dysfunction [2] and increased intracellular Ang II concentration [3]. Therefore, the present study investigated the role of Ang II in kidney cortex mitochondria isolated from control and streptozotocin-induced diabetic rats.

Mitochondrial respiration was recorded after addition of Ang II (1 μM), candesartan (AT₁R antagonist, 100 nM), Ang II + candesartan, Ang II + PD-123319 (AT₂R antagonist; 3 μM) or in combination. Mitochondria isolated from diabetic animals were also incubated with L-NAME (nitric oxide synthase inhibitor).

Ang II decreased oxygen consumption in mitochondria from both control (-16.9±1.0%) and diabetic animals (-15.0±0.0%). AT₁R inhibition did not affect the response to Ang II, whereas AT₂R inhibition abolished the response in mitochondria from control animals. However, AT₂R inhibition resulted in increased oxygen consumption in mitochondria from diabetic animals (+10.4±1.0%), but combined AT₁R and AT₂R inhibition did not exert any effect in either of the groups. Furthermore, mitochondria from diabetics displayed increased oxygen consumption in response to Ang II in the presence of nitric oxide synthase inhibition (+10.9±2.4%).

In conclusion, Ang II regulates mitochondrial respiration via AT₂R-mediated NO release in both control and diabetic animals. AT₁R does not regulate mitochondrial function in control animals, but can regulate mitochondrial function in diabetic animals in the absence of AT₂R. Taken together, these results demonstrate a role of Ang II in regulation of kidney mitochondria function.

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C1-06 Intermittent fasting improves oxidative stress but not metabolic disturbances and atherosclerosis in hypercholesterolemic mice.

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Different regimens of food restriction have been associated with protection against obesity, diabetes and cardiovascular diseases. Food (caloric) restriction can favorably

modulate cell and body redox state. In this study, we hypothesized that food restriction could bring benefits for the atherosclerosis-prone hypercholesterolemic LDL receptor-deficient mice.

Two-month-old mice were submitted to an intermittent fasting (IF) regimen (fasting every other day) over a 3-month period that resulted in an overall 20% reduction in food intake. As expected, IF ameliorated two oxidative stress markers, the susceptibility of VLDL to oxidation (increased lag time to oxidation by 25%) and the rate of liver mitochondrial ROS generation (decreased in DCF oxidation by 43%). Unexpectedly, the IF mice had epididymal and carcass fat depots significantly enlarged. Accordingly, plasma levels of leptin were 50% higher in IF mice. In addition, the IF mice presented increased total (37%), VLDL (3-fold) and LDL (50%) cholesterol plasma levels. Glucose homeostasis was also disturbed by IF with elevation of glycemia (40%), insulinemia (50%), glucose intolerance and insulin resistance in IF mice. The systemic inflammatory markers TNF- α and C-reactive protein were elevated and spontaneous atherosclerosis development was markedly increased (3-fold).

Although IF caused some benefits on systemic and tissue redox state, this type of food restriction induced obesity and diabetes and worsened atherosclerosis in these LDL receptor-deficient mice. Thus, IF is not beneficial in the context of genetic hypercholesterolemia.

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C1-07 Regulation of mitochondrial permeability transition by Sirt3-catalyzed cyclophilin D deacetylation and its relevance for ventricular dysfunction in metabolic syndrome.

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Metabolic syndrome (MS) can be defined as a group of signs that increases the risk of cardiovascular diseases (CVD). These signs include central obesity, hypertriglyceridemia and hypertension. We are interested in the mechanisms that trigger ventricular dysfunction in a MS murine model, as a way to understand how mitochondrial function fails in CVD. The sustained opening of the mitochondrial permeability transition pore (PTP) is a major event in the onset of irreversible myocardial injury. Several mitochondrial proteins modulate the PTP, including the cyclophilin D (CyD), the adenine nucleotide translocator (ANT) and the SIRTUINS. In this regard, SIRT-3 has emerged recently as a pivotal mediator of mitochondrial metabolism and PTP inducer in a knockout murine model. However, the precise role of SIRT-3 in a pathophysiologic context remains indefinable. Male Wistar rats with sucrose-induced MS were subjected to cardiac echocardiography and *ex-vivo* contraction measurements at 6 and 12 months. Respiratory activity, calcium retention capacity and deacetylation profile were investigated in isolated mitochondria. The expression of SIRT-3, ANT and CyD was evaluated by qPCR or western blot. We observed differences in the E/A ratio (control 1.07 ± 0.01 vs. MS 0.85 ± 0.06 , $P < 0.029$) and the ventricular deceleration time (0.029 ± 0.002 vs. 0.034 ± 0.003 , $P < 0.04$) indicating an abnormal lusitropism. No significant differences were found in respiratory activity and respiratory control from isolated mitochondria. Nevertheless, calcium retention in MS mitochondria was reduced (0.83 ± 0.03 vs 0.65 ± 0.04 Abs.min⁻¹, $P < 0.004$) indicating premature PTP opening. Proneness in PTP opening was associated with lower expression (60% $p < 0.001$) of SIRT-3. These changes correlated with the MS heart's mitochondrial acetylation profile. It appears that metabolic changes inherent to MS promote alterations in the expression of SIRT-3 and correlates with PTP opening sensibility.

C2**MiPathology: Tissue differences in diabetes – mouse and rat models****C2-01 Comparative study of brown and white adipose tissue mitochondria in mice upon cold acclimation.**

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The ability of certain white adipose tissue depots to extensively increase relative expression levels of the gene for the uncoupling protein 1 (UCP1) has recently attracted considerable attention as potential anti-obesity treatment tool. The white adipose tissues and cells that display the ability to express UCP1 are presently referred as brite or beige [1]. To date, studies of brite/beige adipose tissue have mostly focused on mRNA measurements, and the metabolic relevance of such expression may be questioned: Is UCP1 able to implement thermogenesis in brite/beige-fat mitochondria?

To examine the thermogenic properties of brite/beige adipose tissue and qualitatively relate these to those in classical brown adipose tissue, we exposed mice to thermoneutrality (30 °C; a thermogenically non-recruited state) or to cold conditions (4 °C) for about a month, i.e. until a new acclimation state had been achieved (a recruited state). We then dissected out the interscapular brown adipose tissue depot (IBAT) and the inguinal white adipose tissue depot (ingWAT) from these animals and isolated mitochondria in parallel from the dissected depots.

The mitochondrial preparation from non-recruited ingWAT was qualitatively different from IBAT mitochondria. The IBAT mitochondria showed UCP1-dependent thermogenesis (oxygen consumption) with canonical GDP sensitivity, high FCCP response and low phosphorylation capacity, whereas non-recruited ingWAT mitochondria exhibited no UCP1 activity and remarkable low oxidative capacity. In ingWAT mitochondria isolated from cold-acclimated mice, UCP1 protein levels increased almost to those of brown-fat mitochondria. The recruited ingWAT mitochondria showed UCP1-dependent oxygen consumption with both lipid or carbohydrate substrates and loss of thermogenesis in UCP1-KO mice. The levels of representative subunits of the respiratory Complexes CI-IV per mg mitochondrial protein were remarkably similar between the IBAT and ingWAT under recruited condition. Also phosphorylation capacity was similarly low in IBAT and ingWAT. Among many similarities there were several differences: The level of carnitine palmitoyl transferase I was significantly lower in ingWAT as compared to IBAT, and the level of glycerol-3 phosphate supported oxygen consumption was less than 50% of the level in IBAT. This may have implication concerning the necessity for reducing equivalent transfer in ingWAT. The difference between obesogenic C57Bl/6 and obesity-resistant 129Sv mouse strains was paralleled by a lower thermogenic potential in C57Bl/6 mitochondria.

Thus, recruited brite/beige-fat mitochondria-derived thermogenesis within inguinal white adipose tissue could be locally significant, especially in utilization of carbohydrate-derived substrates.

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C2-02 Sex differences in murine mitochondrial oxidative capacity following a 24 week high-fat diet.

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High-fat diets have been reported to affect skeletal muscle, white adipose tissue (WAT), and liver mitochondrial abundance and respiratory function in rodents [1,2]. However, few data exist that have examined sex differences in mitochondrial respiratory function in response to a high-fat diet in these metabolic tissues. We therefore examined whether there were differences in mitochondrial respiratory capacity between male and female Balbc mice following a 24 week of high-fat diet. High-resolution respirometry was utilized to perform a multiple substrate-inhibitor titration (SUIT) protocol to assess mitochondrial respiratory function in permeabilized skeletal muscle, liver, and WAT from male and female Balbc mice [3,4]. The SUIT protocol investigated uncoupled (LEAK) respiration (glutamate and malate), oxygen phosphorylation (OXPHOS) capacity (OXPHOS_{GM}: glutamate, malate plus ADP; OXPHOS_{GMS}: glutamate, malate, succinate, plus ADP), and electron transfer system (ETS) capacity (glutamate, malate, succinate, ADP, plus FCCP). Tibialis anterior, soleus, and liver samples were initially mechanically permeabilized with the Shredder SG3 followed by chemical permeabilization with digitonin to ensure complete permeabilization. WAT was minced and chemically permeabilized with digitonin. Overall, there were no significant differences between males and females for the amount of weight gained following the 24 week high-fat diet. In the tibialis anterior, the males had higher LEAK respiration ($P<0.01$), OXPHOS_{GM} capacity ($P<0.05$), and OXPHOS_{GMS} capacity ($P<0.05$) than the females. In the soleus, the males had higher LEAK respiration ($P<0.01$) than the females. In the WAT, the males had higher OXPHOS_{GMS} ($P<0.05$) and ETS capacity ($P<0.05$) than the females. In the liver, no significant differences were observed between males and females. In conclusion, in response to a high-fat diet males compared to females had higher skeletal muscle LEAK respiration in the tibialis anterior and soleus, higher OXPHOS capacity in the tibialis anterior and WAT, and higher ETS capacity in WAT.

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C2-03 Metabolic flexibility before and after lifestyle intervention in a diet-induced type 2 diabetes animal model.

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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. Nowadays, there is still a controversy whether mitochondrial dysfunction plays a causative role on type 2 diabetes etiology [2,3,4].

Our current interest is to assess the metabolic adaptations occurring on an animal model of diet-induced type 2 diabetes and the effect of a lifestyle intervention programme in reverting them. For this purpose we are studying three different experimental 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.

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 2 diabetic-like state.

This communication will be presenting how mitochondrial function is modulated by the different experimental conditions. Mitochondrial function is being evaluated in liver, hypothalamus, glycolytic and oxidative skeletal muscle, and white and brown adipose tissues.

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C2-04 With type 2 diabetes mitochondrial dysfunction develops earlier in liver than in skeletal muscle.

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Impaired mitochondrial function is implicated in the development of type 2 diabetes mellitus (T2DM). This was investigated in mitochondria isolated from skeletal muscle and liver of the Gato-Kakizaki (GK) rat, which spontaneously develops T2DM with age. The early and the manifest stage of T2DM was studied in 6- and 16 weeks old GK rats, respectively.

In GK16 compared to GK6 animals, a decrease in OXPHOS capacity with palmitoyl carnitine (Pal) as substrate was observed in muscle. Yet, an increase was seen in liver. To test the Complex II contribution to OXPHOS capacity, succinate was added together with Pal. In liver mitochondria this resulted in a ~50% smaller respiratory increase in the GK6 group compared with control and no respiratory increase at all in the GK16 animals. Yet, no difference between groups was seen in skeletal muscle mitochondria. RCR and P/O ratio was increased ($P < 0.05$) in liver, but unchanged in muscle in both GK groups. We observed increased lipid peroxidation and decreased Akt phosphorylation in liver with the progression of T2DM, but no change in muscle.

During the progression of T2DM in GK rats, liver mitochondria are affected differently and earlier than muscle mitochondria. Succinate dehydrogenase flux in the presence of fatty acids was severely reduced in liver but only marginally affected in muscle mitochondria during manifest T2DM. The observations support the notion that T2DM pathogenesis is initiated in the liver and only later muscle mitochondria are affected.

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C2-05 Mitochondrial respiration in heart and soleus of *ob/ob* mice.

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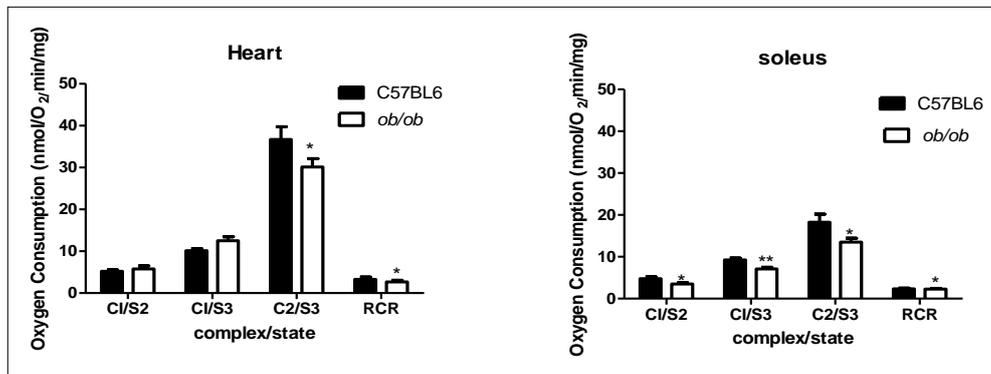
In the West, obesity and type II diabetes (DM2) have reached epidemic proportions. The pathophysiological mechanisms behind these conditions are multifactorial, however recent evidence suggests that altered energy metabolism plays a key role in their development, with impaired cardiac and/or skeletal muscle respiratory capacity strongly implicated.

In this study, we aimed to compare mitochondrial respiratory function in the heart and skeletal muscle of 16 week old obese *ob/ob* mice and lean C57BL6 controls ($N=8$ per group). Muscle fibre bundles prepared from heart and soleus were permeabilised with saponin, and respirometry performed using Clark-type O_2 electrodes, with a substrate-inhibitor titration used to determine ETS function. Additionally, palmitoyl-carnitine+malate and pyruvate+malate were used to assess fat and carbohydrate oxidation, respectively.

In the hearts of *ob/ob* mice, LEAK respiration, (L) and OXPHOS capacity (P) via Complex I (CI) were the same as in lean controls (Fig. 1); however in soleus, *ob/ob* mice L through CI was 19% lower than in controls ($p < 0.05$), whilst P was 30% lower ($p < 0.01$). In both tissues, CII respiration rates were lower in *ob/ob* mice than in controls, by 18% in heart ($p < 0.05$) and 35% in soleus ($p < 0.01$). With pyruvate, cardiac L was 31% higher in *ob/ob* mice than controls ($p < 0.01$), whilst maximal respiration was

the same. In contrast, pyruvate respiration in the soleus of *ob/ob* mice was impaired, with *L* 18% lower ($p < 0.01$) and *P* 47% lower ($p < 0.001$) than in controls. With palmitoyl-carnitine, respiration rates were higher in both tissues of *ob/ob* mice than in controls, with cardiac *L* and *P* 51% ($p < 0.01$) and 28% higher ($p < 0.05$), respectively, and soleus *L* and *P* 38% ($p < 0.05$) and 79% higher ($p < 0.01$), respectively.

In conclusion, whilst fatty acid oxidation was elevated in both heart and soleus of *ob/ob* mice, compared with controls, there were soleus-specific defects in CI respiration, which may be a marker of oxidative stress. Our findings may suggest that skeletal muscle mitochondrial dysfunction in *ob/ob* mice precedes that of heart. **Figure 1:**



C2-06 Tissue specific changes in respiratory substrate kinetics in the ZDF rat and in response to resveratrol supplementation.

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Mitochondrial dysfunction has been implicated in both high-fat diet induced skeletal muscle insulin resistance and diastolic heart failure. However there is considerable controversy regarding these concepts, as mitochondrial function has traditionally been assessed in the presence of saturating substrates (ADP and reducing equivalents), conditions which may not reflect the *in vivo* situation.

Therefore, we investigated respiratory substrate kinetics in the heart and skeletal muscle in a model of type 2 diabetes, the ZDF rat. In addition, we determined the ability of the polyphenolic compound resveratrol to recover potentially impaired kinetic profiles in these tissues. Intriguingly, maximal respiration was not altered in either muscle in the ZDF rat, however tissue specific differences were observed in respiratory kinetics. Specifically, in skeletal muscle, submaximal ADP-stimulated respiration rates were lower ($P < 0.05$) in ZDF rats, which coincided with decreased adenine nucleotide translocase 2 (ANT2) protein content. This decrease in submaximal ADP-stimulated respiration occurred in the absence of a decrease in electron transfer system function. Treating ZDF rats with resveratrol improved skeletal muscle insulin resistance and this was associated with increased submaximal ADP-stimulated respiration rates as well as an increase in ANT2 protein content. These results coincided with a greater ability of ADP to attenuate mitochondrial ROS emission and an improvement in cellular redox balance. In the heart, unlike skeletal muscle ADP kinetics were not altered by either genotype or resveratrol supplementation. In contrast, while P-CoA sensitivity was impaired (higher K_m') in the heart of ZDF rats, resveratrol normalized P-CoA kinetics, which coincided with a recovered diastolic function and myocardial lipid profile (TAG, DAG and ceramide species). Altogether, these data suggest that mitochondrial dysfunction is present in the skeletal muscle and heart of type 2 diabetic animals, and resveratrol improves bioenergetics by altering respiratory kinetics in a tissue specific manner.



C2-07 Early mitochondrial dysfunction associated with type 2 diabetes mellitus in heart and skeletal muscle.

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As a consequence of the rising overall obesity and aging of the population, insulin resistance (IR) and type 2 diabetes mellitus (T2DM) are becoming more prevalent in developing countries. Although evidence has been accumulating that T2DM is accompanied by mitochondrial dysfunction in muscles, the link between the mitochondrial dysfunction and the pathogenesis of T2DM remains unclear [1]. T2DM is a complex disease that causes various changes in the body metabolism. The difficulty is to identify mitochondrial defects involved in the cause of the disease rather than those that occur as consequences of the disease. Our study aims at understanding the role of mitochondrial metabolism in the muscle during the early stages of T2DM.

The fructose-fed rat was used as the animal model of pre-diabetes. After only 6 weeks of a fructose-enriched diet (10% fructose added to the drinking water), rats showed clear signs of IR (glucose tolerance test). Mitochondrial respiration was measured in permeabilized muscle fibers using high-resolution respirometry (Oroboros Instruments, Austria) in the cardiac muscle and in two skeletal muscles, one oxidative (soleus) and one glycolytic (extensor digitorum longus, EDL). A wide range of substrates were used in order to cover the carbohydrate metabolism and the fatty acid oxidation. Acylcarnitine profiles were also measured in each tissue.

The mitochondrial content was preserved in skeletal muscle as shown by the lack of difference of citrate synthase (CS) activity and Complex IV respiration. In the heart, CS activity was slightly, but significantly, reduced in the fructose-fed rats. The soleus muscle shows a decrease in Complex II respiration (succinate+rotenone) whereas the EDL muscle shows reduced Complex I and CI+II respiration in the presence of pyruvate but not when glutamate is used as a substrate. There is no defect in fatty acid oxidation with the soleus muscle in fructose-fed animals. The EDL muscle, however, shows a defect located in the long-chain acyl-CoA dehydrogenase (LCAD). Surprisingly, the mitochondrial dysfunction in the heart mirrors the mitochondrial dysfunction in the EDL muscle, rather than in the soleus.

Our results highlight important early mitochondrial dysfunction associated with T2DM. The defects are clearly specific to the type of muscle studied. Furthermore, our study also points to the importance of differences between human and animal models because the role of LCAD in fatty acid oxidation is significant in rodent muscles but not in most human tissues [2].

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C2-08 Some mitophagy markers in liver and skeletal muscle in Goto Kakizaki rats.

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Diabetes is the epidemic of the XXI century, it is estimated that worldwide 3.2 million deaths are attributable to diabetes every year. It has been described that the severity of insulin resistance in skeletal muscle in type 2 diabetes and obesity is associated with alterations in mitochondrial function [1]. The Goto-Kakizaki (GK) rat is a non-obese Wistar substrain which develops type 2 diabetes mellitus early in life. These rats have been used by the scientific community as it is one of the models to study the relationship between mitochondria and type 2 diabetes mellitus.

The present study was focused on evaluation of the contribution of mitophagy/autophagy in the development of type 2 diabetes in the two most important metabolic tissues in Goto Kakizaki rats. Liver and skeletal muscle tissues were obtained from 1-year old adult male Goto Kakizaki rats and Wistar rats (as controls) of the same age. The samples were analyzed by Western blot. LC3b protein expression and total ubiquitinated protein were used as markers to evaluate mitophagy. The results showed different profiles depending on the tissue. In liver, we observed a ratio LC3bI/LC3bII significantly higher in Goto Kakizaki than Wistar rats, indicating less autophagy activation in the liver of Goto Kakizaki rats. In contrast, the levels of total ubiquitinated protein did not differ significantly. In the case of skeletal muscle, LC3bI/LC3bII levels in Goto Kakizaki were significantly lower than in Wistar rats, indicating increased mitophagy activation in Goto Kakizaki muscle, while the levels of total ubiquitinated protein were not significantly different.

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C3

MiPathology: Ageing, neurodegeneration, inherited



C3-01 Mitochondrial membranes of the longest-lived metazoan (*Arctica islandica*) are lipoxidation-resistant.

Munro Daniel, Blier PU

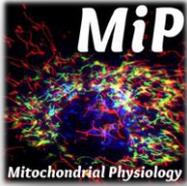
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Reactive oxygen species (ROS) initiate chain reactions of lipid oxidation (lipoxidation) within biological membranes, generating detrimental breakdown products (reactive aldehydes) acting as secondary ROS. These additional ROS further contribute to the mutation load on mtDNA, and hence senescence of post mitotic tissues [1]. A membrane's susceptibility to lipoxidation is proportional to its content in highly oxidizable PUFA and can be estimated by calculating the peroxidation index (PI) value following lipid analysis. Past researches on mammals and birds revealed inverse correlations between the PI value and the species' longevity for skeletal muscle total phospholipids and liver mitochondria phospholipids [2].

The longest-lived metazoan, the mud clam *Arctica islandica* (maximum reported longevity = 507 y) offers an opportunity for a robust test of the relationship outside the traditional homeothermic taxons characterized by a much conserved physiology. Indeed bivalves experience daily to annual changes in factors known to affect membrane lipid composition in invertebrates such as temperature, salinity, pressure and p_{O_2} which raises the question of the relative importance of adapting lipid in relation to longevity in these organisms. We compared *A. islandica* with four other sympatric bivalve molluscs greatly differing in longevity (*Mya arenaria* = 28, *Spisula solidissima* = 37, *Mactromeris polynyma* = 92, and *Mercenaria mercenaria* = 106 y). Using the gill tissue, we measured the PI value for mitochondrial membrane as well as for other membranes contained in cellular debris devoid of mitochondria.

We found a significant negative relationship between the PI value and longevity for both biological fractions (mitochondria: $R^2 = 0.855$, $P = 0.024$; cellular debris: $R^2 = 0.924$, $P = 0.009$) with particularly low values for *A. islandica*. Furthermore, the best fit model for the relationship was exponential for mitochondria while it was linear for other cell membranes. These results suggest a generalized association between longevity and membrane PI among animals. They further suggest a relatively greater importance in adjusting the lipid composition of the mitochondrial membrane over that of other cellular membranes.

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C3-02 Structure and function of aged mouse mitochondria.

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Mitochondria are the energy-converting cell organelles. Their dysfunction has been described as a cause or symptom of ageing. Mutations of mitochondrial DNA lead to respiratory system defects, possibly causing an increased release of reactive oxygen species. Affected cells accumulate, resulting in tissues with mosaic respiratory system deficiency that impairs the efficiency of affected organs [1].

We have previously found dramatic morphological changes in mitochondria of the fast-ageing model organism *Podospora anserina* [2]. Here, we investigated the morphology and function of aged mammalian mitochondria. We used mouse models that would reveal changes in mitochondrial structure and function in dependence of organismal age. Mitochondrial morphology was examined by electron cryo-tomography, and function was assessed by respirometry in parallel experiments. Thus, in contrast to electron microscopy of resin-embedded samples, we were able to obtain three-dimensional volumes of mitochondria at higher resolution in a close-to-native state, and to correlate our findings to respiratory activity.

We analysed isolated mitochondria from heart, kidney and liver tissue of young and aged mice. Interestingly, we observed clear tissue-specific differences. Whereas heart mitochondria were functionally and structurally unchanged in aged animals, mitochondria from old mouse kidney and liver showed increased populations with altered cristae morphology. Furthermore, we analysed samples from the mtDNA mutator mouse, an established ageing model. In these animals, the highly mutated mitochondrial DNA caused a wide range of structural defects that were accompanied by loss of function.

In summary, mouse mitochondria show subtle, tissue-dependent age-related changes, the molecular causes of which remain to be elaborated. In contrast to a fungal ageing model, a drastic rearrangement of the mitochondrial inner membrane was not observed.

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C3-03 The effects of methylmalonic acid on α -ketoglutarate supported oxidation in isolated brain, heart and liver mitochondria.

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Methylmalonic acid (Mma) is a common intermediate in many catabolic processes. Its accumulation is associated with neurological symptoms. In methylmalonic acidemia mitochondrial dysfunction can be observed. In this study the effects of Mma were tested in isolated brain, heart and liver mitochondria. Oxygen consumption of isolated mitochondria was measured by Clark electrode, ATP synthesis was estimated by coupled enzyme assay, mitochondrial membrane potential was measured by safranin fluorescence. It was found that in the presence of Mma α -ketoglutarate (α KG) oxidation was significantly increased in isolated mitochondria. The oxidation of Mma is reflected in the increased ATP production and membrane hyperpolarization. This phenomenon could be explained by: (i) The mitochondrial transport of α KG was increased by Mma; (ii) The added Mma was itself activated and oxidized; (iii) α -ketoglutarate dehydrogenase (α KGDH) activity was increased by Mma. The effect of Mma on isolated α KGDH was tested and found that the enzyme activity was inhibited by Mma. Our results are in good agreement with that of Melo et al (2012). According to their interpretation the stimulating effect of Mma can be attributed to the stimulation of α KG transport. Our recent experiments however showed that this stimulation does not occur in liver mitochondria but it is pronounced in mitochondria isolated from the brain and heart, respectively. One of the differences between these types of mitochondria is that heart and brain are tissues able to oxidize ketone bodies. We interpret our results that a reaction analogous to the ketone body activation may activate methylmalonate to methylmalonyl-CoA. This reaction occurs in the heart and brain, but not in the liver mitochondria, respectively. This hypothesis is supported by the Mma-induced changes in P/O ratio. In brain and heart mitochondria in the presence of Mma, P/O ratio of α KG oxidation decreases. This finding is in agreement with the hypothesis that acetoacetate:succinyl-CoA transferase can transfer CoA and activates Mma to Mma-CoA.

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C3-04 *TigarB* causes mitochondrial dysfunction and neuronal loss in PINK1 deficiency.

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Loss of function mutations in PINK1 typically lead to early onset Parkinson's Disease (EOPD). Zebrafish (*Danio rerio*) are emerging as a powerful new vertebrate model to study neurodegenerative diseases. We used a *pink1* mutant (*pink1*^{-/-}) zebrafish line with a premature stop mutation (Y431*) in the PINK1 kinase domain to identify molecular mechanisms leading to mitochondrial dysfunction and loss of dopaminergic neurons in PINK1 deficiency.

The effect of PINK1 deficiency on the number of dopaminergic neurons, mitochondrial function and morphology was assessed in both zebrafish embryos and adults. Genome-wide gene expression studies were undertaken to identify novel pathogenic mechanisms. Functional experiments were carried out to further investigate the effect of PINK1 deficiency on early neurodevelopmental mechanisms and microglial activation.

PINK1 deficiency results in progressive loss of dopaminergic neurons as well as early impairment of mitochondrial function and morphology in *Danio rerio*. Expression of *TigarB*, the zebrafish orthologue of the human, TP53-induced glycolysis and apoptosis regulator *TIGAR*, was markedly increased in *pink1*^{-/-} larvae. Antisense-mediated inactivation of *TigarB* gave rise to complete normalisation of mitochondrial function with resulting rescue of dopaminergic neurons in *pink1*^{-/-} larvae. There was also marked microglial activation in *pink1*^{-/-} larvae but depletion of microglia failed to rescue the dopaminergic neuron loss, arguing against microglial activation being a key factor in the pathogenesis.

pink1^{-/-} zebrafish are the first vertebrate model of PINK1 deficiency with progressive loss of dopaminergic neurons. Our study also identifies TIGAR as a promising novel target for disease-modifying therapy in PINK1-related PD.



C3-05 α -Synuclein mediated alterations in mitochondrial oxidative phosphorylation system: Implications in the pathogenesis of Parkinson's disease.

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α -Synucleinopathy and mitochondrial dysfunction are important elements of sporadic Parkinson's disease (PD) pathogenesis [1]. It is, however, not clear whether the accumulated α -synuclein in degenerating dopaminergic neurons in PD causes mitochondrial injury and subsequent cell death. Our earlier study has shown that α -synuclein causes functional impairment of rat brain mitochondria incubated *in vitro* [2].

Mitochondrial membrane potential was measured using the carbocyanine dye JC1, and the phosphorylation capacity determined spectrophotometrically from inorganic phosphate utilization [2,3]. The respiratory functions of mitochondria in isolated preparations and within intact cells were analyzed by high-resolution respirometry. α -Synuclein accumulation within SHSY5Y cells was induced by lactacystin treatment and detected by immunoblotting. The transfection of SHSY5Y cells with α synuclein specific SiRNA was carried out using the lipofectamine kit (Invitrogen).

Our results show that α -synuclein causes a loss of membrane potential and phosphorylation capacity with alterations in respiratory parameters in isolated rat brain mitochondria. Some of these effects were inhibited very significantly by cyclosporine (1 μ M). When SHSY5Y cells were exposed to 5 μ M lactacystin for 24 h, α -synuclein accumulation occurred intracellularly as detected by immunoblotting experiments. Further, lactacystin treatment of SHSY5Y cells also leads to mitochondrial dysfunction and cell death concomitant with α synuclein accumulation. To confirm the involvement of α synuclein in lactacystin induced mitochondrial dysfunction, the effects of cyclosporine and the gene silencing of α -synuclein with specific SiRNA on these phenomena are being investigated.

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C3-06 Platelets, amyloid beta and mitochondrial respiration.

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Platelets or thrombocytes are the key players in hemostasis and contain >90% of the circulating amyloid precursor protein, which upon proteolytic cleavage by β -secretase BACE1 (β -site APP cleaving enzyme 1) and the presenilin-containing γ -secretase complex produces amyloid beta peptide (A β). A β , a major mediator of neuronal death, is also cytotoxic to cerebral endothelial cells, vascular smooth muscle cells and can accumulate in the cerebral blood vessels leading to cerebral amyloid angiopathy. In this report we have evaluated the effect of A β active fragment containing amino acid sequence 25-35 (A β ₂₅₋₃₅) on platelet activity and mitochondrial respiration. Exogenous A β ₂₅₋₃₅ was found

to induce platelet aggregation, PAC-1-binding and P-selectin exposure in washed human platelets, which was comparable to that induced by other physiological agonists. $A\beta_{25-35}$ also induced ATP release indicative of secretion from platelet dense granules. High-resolution respirometry (HRR) showed that $A\beta_{25-35}$ elicited sharp rise in mitochondrial respiration in intact platelets. Thus, $A\beta$ induced platelet activation that was associated with an increase in oxygen consumption rate.



C3-07 Bioenergetics of permeabilized and intact nerve cell terminals from ApoE deficient mice and wild type mice.

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Reductions in ATP production by oxidative phosphorylation and glycolysis have been observed in many neurological diseases including Alzheimer's disease (AD) [1]. One of the known risk factors for the development of AD is the inheritance of a specific genotype of a lipid transporting protein known as apolipoprotein E (ApoE). How ApoE contributes to the development of AD is poorly understood [2]. The purpose of this study was to determine if the lack of ApoE leads to mitochondrial dysfunctions. Mitochondrial bioenergetics were investigated in mice that lack ApoE (ApoE KO) and in wild-type mice. Since AD develops with age, mitochondrial performance was studied in both old (12-22 months old) and young mice (4-6 months old).

We isolated nerve cell terminals (synaptosomes) from wild-type (C57BL/6J) and ApoE KO (ApoE^{tn1Unc}) mice using Percoll gradient centrifugation. Oxygen consumption of permeabilized and nonpermeabilized synaptosomes was measured using the OROBOROS Oxygraph-2k at 37 °C. ROUTINE respiration of nonpermeabilized synaptosomes was significantly higher in young mice than in old mice (0.183 ± 0.02 vs. 0.136 ± 0.02 pmol O_2 s⁻¹ μ g protein⁻¹, $n=6$, $P<0.001$). Respiration increased after addition of FCCP to 0.90 ± 0.15 pmol O_2 s⁻¹ μ g protein⁻¹ in young mice which was significantly higher in comparison to old mice (0.65 ± 0.12 pmol O_2 s⁻¹ μ g protein⁻¹, $n=6$, $P=0.008$). In the presence of the F_0F_1 -ATPase inhibitor, oligomycin, oxygen consumption significantly dropped to 0.09 ± 0.02 and 0.06 ± 0.01 pmol O_2 s⁻¹ μ g protein⁻¹ in old and young mice, respectively ($n=6$, $P=0.002$). However, oxygen flux did not differ significantly between the two genotypes (2-way ANOVA, $P>0.05$). Oxygen consumption of permeabilized synaptosomes from young wild-type mice in the presence of malate, glutamate, and pyruvate was 0.32 ± 0.03 pmol O_2 s⁻¹ μ g protein⁻¹ and increased to 2.50 ± 0.2 pmol O_2 s⁻¹ μ g protein⁻¹ in the presence of saturating levels of ADP. The addition of succinate increased oxygen flux by approximately 40% under these conditions and successive FCCP titrations induced an additional increase in oxygen flux of about 15%. Surprisingly, oxygen flux in permeabilized synaptosomes did not differ significantly among genotypes or age groups (2-way ANOVA, $P>0.05$, $n=3$). Our preliminary results suggest that ApoE deficiency does not impact mitochondrial function. Significantly reduced mitochondrial activity has been previously demonstrated in aged rodents [3]. Interestingly, our study only revealed reductions in oxygen utilization of glycolytic poised synaptosomes from aged mice.

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C3-08 Similar alterations in mitochondrial proteome of brain and skeletal muscle in two transgenic mouse models for Huntington's disease.

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Several observations indicate that mitochondrial dysfunction plays an important role in Huntington's disease (HD) pathogenesis. HD patients lose significantly body weight despite normal or increased food intake and impairment of ATP synthesis occurs even in pre-motor manifest HD expansion mutation carriers. Until now the molecular mechanisms linking mutant huntingtin to mitochondrial dysfunction are not known. To get a more detailed picture of mitochondrial changes in HD, mitochondria of brain and skeletal muscle from two different mouse models for HD (R6/2 mice and HdhQ-knock in mouse) were isolated and the mitochondrial proteomes of the HD mice and respective controls were compared using a 2D-DIGE (2-dimensional differential in-gel electrophoresis) approach.

After optimizing the isolation of mitochondria using differential centrifugation, the purity of the mitochondrial fraction was confirmed by Western Blot. Mitochondrial lysates were then used for proteome analysis. In mitochondria of R6/2 mouse brains mitochondrial proteins of the citric acid cycle, the amino acid degradation pathway, mitochondrial fusion and heat shock proteins were increased. Surprisingly, only a minority of selected mitochondrial proteins were downregulated in the R6/2 mice. Alterations of mitochondrial proteome in skeletal muscle included an upregulation of several proteins of the citric acid cycle, the electron transfer system, ATP synthesis, fatty acid metabolism, amino acid degradation and heat shock proteins. The mitochondrial proteome alterations in the knock-in mouse model were similar, although the total number of differently expressed mitochondrial proteins was lower. In contrast, when comparing the amount of certain differentially expressed mitochondrial proteins by Western Blot using whole tissue lysates of brain or skeletal muscle, respectively, downregulation of these proteins was observed.

In summary, these results show similar changes in the proteome of purified mitochondria in brain and skeletal muscle of two different mouse models for HD. Unexpectedly, the majority of differentially expressed mitochondrial proteins in the R6/2 and HdhQ mice were upregulated. Whereas these upregulations for some mitochondrial proteins in whole tissue lysates could not be confirmed by Western blot, suggesting that a possible overall lower number of mitochondria is compensated by an upregulation of several metabolic pathways, reflecting at least in part compensatory changes. Currently high-resolution respirometry measurements are performed on samples from brain and skeletal muscle of the 2 HD mouse models to study possible alterations of respiratory system function and respiratory capacities due to the observed changes in the mitochondrial proteome.



C3-09 The cardiolipin defect linked to a tafazzin mutation in Barth syndrome disturbs mitochondrial metabolism, alters apoptosis and autophagic fluxes but does not affect cell cycle progression.

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Cardiolipin (CL) is a key phospholipid involved in ATP generation. Since progression through the cell cycle requires ATP, we examined the regulation of CL synthesis during S-phase in human cells and investigated whether CL or CL synthesis was required to support nucleotide synthesis in S-phase. HeLa cells were made quiescent by serum depletion for 24 h. The addition of serum resulted in a substantial stimulation of [*methyl-3H*]thymidine incorporation into cells as compared to serum-starved cells by 8 h, confirming entry into the S-phase. CL mass was unaltered at 8 h, but increased 2-fold 16 h after addition of as compared to serum-starved cells. The increase in CL mass upon entry into S-phase resulted from an increase in the activity and the expression of CL *de novo* biosynthetic and remodelling enzymes and this paralleled the increase in mitochondrial mass. Barth syndrome lymphoblasts or HeLa cells in which tafazzin was knocked down exhibit reduced CL (with acyl chain changes), a massive accumulation of monolysoCL and abnormal mitochondria. However, altered mitochondrial respiration appeared to be compensated for, at the cellular level, by an increase in mitochondrial mass as ascertained by citrate synthase activity and electron microscopy. These events are also described on Barth syndrome-derived lymphoblasts where we have shown that cardiolipin is required for apoptosis in the type II mitochondria dependent response to Fas stimulation, since apoptosis is completely abolished in the Barth syndrome-derived cells or the equivalent HeLa cell model used. Further, in Barth syndrome-derived lymphoblasts and an equivalent HeLa cell model, we have shown that cardiolipin is required for apoptosis in the type II mitochondria dependent response to Fas stimulation [2]. These results provide the missing link between receptors at the plasma membrane and the mitochondria. Barth syndrome-derived lymphoblasts also exhibit a subtle increase in ROS production. We also show in these cells an increase in autophagic fluxes. However, these cells showed similar [*methyl-3H*]thymidine incorporation into cells upon serum addition to serum-starved cells as compared with cells from normal aged-matched controls. The cells passed through the cell cycle in a very similar fashion perhaps because the global level of ATP is similar to normal cells even though individual mitochondria may differ in their functional activities.

These results indicate that *de novo* CL biosynthesis is up-regulated via elevated activity and expression of CL biosynthetic genes and this accounts for the doubling of CL seen during S-phase. However, normal *de novo* CL biosynthesis or CL itself is not essential to support nucleotide synthesis during entry into S-phase of the human cell cycle.

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C4

MiPathology: Inflammation, ischemia-reperfusion, cancer



C4-01 Regulatory role of mitochondrial ROS upon inflammation.

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Mitochondrial reactive oxygen species (mtROS), considered earlier as a harmful by-product of mitochondrial respiration, have been recognized as an important component of intracellular signalling cascades. This study aimed to clarify the impact of mtROS on cellular dysfunction induced by inflammation.

We used an *in vivo* model of systemic inflammatory response induced by LPS in rats and an *in vitro* model of hepatocytes cultured with inflammatory mediators (IM) that were obtained by incubation of white blood cells with LPS. MitoTempo, a mitochondria targeted antioxidant, was used to clarify the effects of mtROS on mitochondrial function, the ER stress response, and cell damage (ALT, AST). The maximal concentration of mitoTEMPO, which does not disturb mitochondrial function, was determined as 500 nmol/l, and was therefore used in the experiments. The levels of ROS and nitric oxide (NO) were determined using spin and fluorescent probes. Gene expression was examined by RT-PCR and Western Blot analysis. Respiratory function of mitochondria was determined by high-resolution respirometry.

In vivo, LPS increased the levels of liver dysfunction markers (ALT and AST) and the levels of NO-Hb complexes in blood, but changes in the respiratory function of mitochondria were very modest. MitoTEMPO significantly reduced the levels of ALT, AST and Hb-NO in animals receiving LPS. A possible functional connection between mtROS, NO and hepatocyte dysfunction was examined in *in vitro* experiments. Using mitoTEMPO and a specific inhibitor of NO synthase, we showed that hepatocytes exposed to IM build up a feed-forward mtROS-iNOS cycle which drops mitochondrial potential due to oxidative damage of mitochondrial membranes (for details see MiP2013 abstract by Paier-Pourani). Simultaneously, mtROS upregulated genes involved in inflammatory response and downregulated genes involved in unfolded protein response (GRP78).

Our data suggest that inflammation mediated elevation of mtROS in liver cells reduce membrane potential without significant impairment of mitochondrial function. In addition ROS amplify inflammatory gene expression, and inhibit unfolded protein response and thereby prevent the recovery of major liver functions. Further studies are required to clarify pathophysiological relevance of these three pathways.



C4-02 Iron-mediated injury of mitochondria is attenuated by nitrite.

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Recently nitrite has been shown to protect various organs from ischemia-reperfusion injury, in particular ameliorates mitochondrial dysfunction. The mechanisms of protection, however, are still unclear. We aimed to investigate the impact of nitrite on mitochondrial damage mediated by free iron, a strong promoter of oxidative stress in an *in vitro* model of hypoxia/reoxygenation.

Isolated rat liver mitochondria were subjected to 15 min of hypoxia followed by 15 min of reoxygenation (H/R). Respiratory activity of mitochondria was monitored in an Oxygraph-2k (OROBOROS Instruments). Dinitrosyl-iron-complexes (DNIC) and reactive oxygen species (ROS) were detected by electron paramagnetic resonance spectroscopy (EPR), the latter by using CMH, a hydroxylamine spin probe. Lipid peroxidation was assessed spectrophotometrically by determination of thiobarbituric acid reactive substances (TBARS).

H/R induced mitochondrial damage resulting in decreased respiration rates. Mitochondrial damage occurred predominantly during reoxygenation, accompanied by elevated ROS and TBARS levels. Exogenous Fe aggravated these changes in a concentration-dependent manner. Cytochrome c completely restored respiration with succinate but not with glutamate, suggesting two sites of damage in mitochondria, namely outer mitochondrial membrane and Complex I. Nitrite protected mitochondrial respiration and reduced TBARS levels but not ROS levels. This protective effect was accompanied by the formation of chemically inactive Fe-NO complexes. Iron chelators (desferoxamine, EDTA) protected mitochondria similar to nitrite manner.

Our data suggest that Fe directly, without intermediate ROS formation, induces damage to the outer mitochondrial membrane via activation of lipid peroxidation. Nitrite is reduced during hypoxia to NO, which scavenges free iron to form inactive NO-Fe complexes and thus, preventing damage to the outer mitochondrial membrane and cytochrome c release. The involvement of Complex I in the mechanisms of nitrite-mediated cytoprotection has to be further investigated.



C4-03 Mitochondrial response to heart and brain ischemia.

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Stroke and heart infarction are the most common causes of death in the world. Ischemic injury is multidimensional and is thought to involve mitochondrial damage leading to apoptotic or necrotic cell death. In this study, we provide comparative analysis of early events in development of ischemia-induced mitochondrial damages in brain and heart using various models of global ischemia.

We compared three models of cerebral ischemia in piglets – unilateral and bilateral carotid artery occlusion as well as bilateral occlusion with hypotension for 3 h. These surgical procedures produced different effects on microcirculation which were accompanied by the gradual decline in the activity of mitochondrial oxidative phosphorylation. We found that LEAK respiration (measured in the presence of pyruvate plus malate but without ADP) was not affected by ischemia in any experimental model. The OXPHOS capacity with pyruvate+malate as substrates decreased by 20% and 80% compared to the control level after bilateral carotid artery occlusion and bilateral carotid artery occlusion plus hypotension, respectively, resulting in the decrease of respiratory control index. OXPHOS capacity with succinate as substrate remained constant after unilateral carotid artery occlusion or bilateral carotid artery occlusion but decreased by 50% after bilateral carotid artery occlusion and hypotension. No loss of cytochrome c from mitochondria was observed in any model of cerebral ischemia. This suggests that damage to Complex I of the mitochondrial respiratory system is the primary target of ischemic insult and may lead to subsequent delayed neuronal death in piglet model of global cerebral ischemia.

In the *in vitro* model of rat global brain ischemia, mitochondrial OXPHOS activity with pyruvate plus malate and to a lesser extent with succinate was decreased after 30 min ischemia. OXPHOS activity with both substrates decreased further during 60-120 min period. This inhibition was not reversed in the presence of added cytochrome c suggesting that inhibition of OXPHOS was not caused by the loss of cytochrome c. Measurements of mitochondrial content of cytochrome c confirmed this as there was no change in cytochrome c levels during 30-120 min ischemic period. These findings are in contrast to reported previously for heart ischemia where it was found that the loss of cytochrome c from mitochondria is the earliest event in ischemic mitochondrial damage leading to caspase activation and cell death [1].

In conclusion, our data suggest that brain and heart ischemia causes mitochondrial damage, however, the mechanisms involved are different: ischemic damage to heart mitochondria is primarily related to opening of mitochondrial permeability transition pore and the release of cytochrome c, whereas during brain ischemia the earliest event is inhibition of Complex I.

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C4-04 The protective effect of NO against ischemia induced brain mitochondrial injury.

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Our research group has previously reported that NO can protect heart mitochondria from ischemia-induced mitochondrial permeability transition pore-related release of cytochrome c and subsequent cell death [1]. In this study we sought to determine whether preconditioning with NO can decrease brain mitochondrial sensitivity to calcium and protect against ischemia-induced mitochondrial damages as mediated by activation of protein kinase G.

Nitric oxide donor (NOC-18) (50 μM) was infused into the *vena cava* for 5 min and induced ischemia by keeping isolated brain in a hypoxic chamber for 90 min. To test if the protective effect of NO on brain mitochondria during 90 min ischemia was due to PKG activation before inducing ischemia in *vena cava*, we infused the PKG inhibitor KT5823 (1 μM) and NOC-18.

We found that preconditioning of the brain with NO donor increased the resistance of subsequently isolated mitochondria to calcium-induced opening of the mitochondrial permeability transition pore (PTP). This was assayed by measuring the extramitochondrial Ca^{2+} concentration with Calcium Green-5N. In mitochondria isolated from ischemic brain pre-treated with NO, 44% more calcium was necessary to cause PTP opening compared to ischemic brain mitochondria. Pre-treatment of brain in the ischemic group with PKG inhibitor and NO donor resulted in no statistically significant differences to controls. Similarly, pre-treatment with NO had no effect on mitochondrial respiration.

Ischemia-induced release of lactate dehydrogenase (LDH) indicates that cells died by necrosis. Pre-treatment of brain with NOC-18 abolished LDH release by 33% compared to ischemia. KT5823 applied together with NOC-18 restored necrosis back to a level similar to that induced by ischemia without NOC-18, indicating that PKG mediates the protective action of NOC-18.

These findings suggest that NO increases mitochondria sensitivity to calcium ions and protects brain mitochondria from ischemia induced PTP and necrotic cell death in a PKG depending manner.

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C4-05 The accumulation of long chain acyl-carnitines is a major cause of mitochondrial damage during ischemia.

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Elevated fatty acid (FA) levels are associated with the increased risk for cardiovascular diseases. Although it has been shown that excessive exogenous FA cause mitochondrial dysfunction in isolated mitochondria and could play a detrimental role in myocardial ischemia-reperfusion injury. Our aim was to study the cardiac mitochondrial damage in relation to accumulation of FA derivatives during ischemia-reperfusion.

Male Wistar rats were used for the experiments. The effects of palmitoyl-coenzyme A and palmitoyl-carnitine on mitochondrial respiration were determined. The isolated rat heart ischemia-reperfusion experiment was performed based on Langendorff technique. Hearts were perfused with modified Krebs-Henseleit buffer solution containing 9,10-^[3H]palmitate before or after left anterior descending coronary artery occlusion. Mitochondria were isolated from the non-risk area and the area at risk to measure radiolabeled FA. In addition, acyl-coenzyme A and acyl-carnitines (C2-C18) levels were determined in whole homogenate and mitochondrial fraction by UPLC MS/MS.

Both palmitoyl-coenzyme A and palmitoyl-carnitine inhibited mitochondrial OXPHOS capacity in a dose-dependent manner. The IC₅₀ values for palmitoyl-coenzyme A and palmitoyl-carnitine were $4.7 \pm 0.7 \mu\text{M}$ and $16 \pm 2 \mu\text{M}$, respectively.

After ischemia, cardiac 9,10-^[3H]palmitate concentration was 3-fold higher in the area at risk compared to the non-risk area. After reperfusion, cardiac 9,10-^[3H]palmitate concentration was decreased by 19%. Meanwhile, the 9,10-^[3H]palmitate concentration was 3.4 times higher in mitochondria isolated from the area at risk. There was no difference in 9,10-^[3H]palmitate concentration in mitochondria isolated after ischemia or after reperfusion. No accumulation of 9,10-^[3H]palmitate in cardiac tissue and mitochondria were observed during reperfusion. These results indicate that FAs accumulate during ischemia, but not during reperfusion. Moreover, the accumulated FAs are not metabolized during reperfusion.

After ischemia, the cardiac concentrations of medium (C6-C12) and long chain (C14-C18) acyl-carnitines and long chain (C14-C18) acyl-coenzymes A in the area at risk were $17 \pm 3 \mu\text{M}$, $896 \pm 140 \mu\text{M}$ and $10 \pm 2 \mu\text{M}$, respectively. The levels were increased 2-3 fold compared to the non-risk area. In cardiac mitochondria isolated from the area at risk only concentrations of long-chain acyl-carnitines and acyl-coenzyme A were significantly increased ($144 \pm 12 \mu\text{M}$ vs. $59 \pm 12 \mu\text{M}$ and $29 \pm 3 \mu\text{M}$ vs. $14 \pm 2 \mu\text{M}$). In cardiac tissue and mitochondria isolated from the area at risk, the concentration of palmitoyl-carnitine was 112 and 8 folds higher than concentration of palmitoyl-coenzyme A, respectively.

Our results demonstrate that long chain acyl-coenzymes A are more toxic to mitochondria than long chain acyl-carnitines. Nonetheless, due to higher concentrations of acyl-carnitines compare to acyl-coenzymes A, the accumulation of long chain acyl-carnitines is the major cause of cardiac mitochondrial damage.



C4-06 Reverse carboxylation glutaminolysis in breast cancer cells.

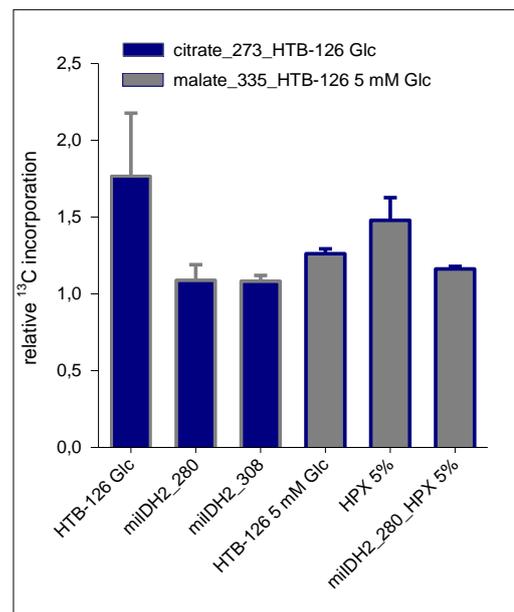
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The sustained high rate of tumor cell proliferation results in aglycemia, initiating the revival of OXPHOS in conjunction with the promotion of glutaminolysis [1], which proceeds either as OXPHOS-involved, encompassing forward Krebs cycle truncated after citrate synthase; or as reductive carboxylation ("anoxic") glutaminolysis (RCG), using the only two enzymes of Krebs cycle acting in the reversed mode – isocitrate dehydrogenase isoform-2 (IDH2) and reversed aconitase. In 2011, it became clear that RCG determines and accelerates tumorigenesis in grade 2 and 3 gliomas, secondary glioblastomas (less frequently in primary glioblastomas [2]) and acute myeloid leukemia. Reverse reaction of heterozygous mutants of IDH2 and cytosolic IDH1 produces from 2-oxoglutarate the oncometabolite D-2-hydroxyglutarate [3], which further promotes neoplasia, e.g. by competitive inhibition of histone demethylation, leading to genome-wide alternations in the methylation of histones and DNA.

We have provided evidence that RCG exists in breast carcinoma cells, compromising the Krebs cycle metabolite flux, using IDH2 silencing by miRNA/shRNA expression. Respiration of intact cells and maximal respiration of HTB-126 Glc cells with stably silenced IDH2 increased, indicating substrate (e.g. glutamine) utilization towards the Krebs cycle and NADH synthesis. Increased mitochondrial coupling and decreased uncoupling in miIDH2 cell lines implies an increased respiratory flux used for ATP synthesis, and decreased proton leak, respectively, both indicating higher effectivity of mitochondrial substrate utilization by inactivation of the reductive carboxylation pathway. ATP levels in cell lysates were concomitantly elevated upon IDH2 silencing, and were drastically decreased upon glutamine withdrawal demonstrating increased glutamine involvement in NADH synthesis and ATP production rather than its utilization in reductive carboxylation pathway when IDH2 was inactivated.

We have also confirmed reductive carboxylation flux by GC-MS studies, where ¹³C incorporation from 1-¹³C-glutamine into citrate and malate was decreased with IDH2 silencing compared to controls. Noteworthy, ¹³C incorporation into malate is only possible after ACL and MDH reactions in cytosol after preceding citrate export from mitochondria. We have found a substantial RCG increase under hypoxia. RCG was also elevated in the presence of OXPHOS blockers in SH-SY5Y neuroblastoma cells.



Supported by grants no. P301/12/P381 from Czech Science Foundation and by GAUK 426411 from Grant Agency of Charles University.

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C4-07 Response of cancer cells to mitochondrial DNA damage.

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Human mitochondrial DNA (mtDNA) is present in each cell in hundreds to thousands of copies representing the autonomous hereditary unit of our genome and encoding 13 core polypeptides of the oxidative phosphorylation complexes together with 22 tRNAs and 2 rRNAs. It is stored in the form of DNA-protein bodies called nucleoids which are regularly distributed in the mitochondrial reticulum. This redundancy together with the proximity to free radical-producing respiratory complexes makes mtDNA highly prone to accumulation of mutations which eventually lead to cellular bioenergetics failure. Therefore, mtDNA quality and quantity is under tight control of multiple independent pathways, e.g. macroautophagy (mitophagy).

This study aimed at investigating the role of mitophagy in the response of cancer cells to DNA intercalating agents, which are routinely used for cancer treatment and are known to preferentially bind to mtDNA [1].

Three different cancer cell lines (HepG2, HeLa, Flp) were treated with mtDNA intercalators ethidium bromide (EtBr). The composition and distribution of nucleoids was followed with SIM and FPALM nanoscopy or regular confocal microscopy. Moreover, mitochondrial bioenergetics was assessed using Oroboros Oxygraph-2k. mtDNA copy number and gene expression were analyzed with Q-PCR and mitochondrial fluorescence *in situ* hybridization [2].

EtBr treatment resulted in inhibition of mtDNA transcription and replication and in a significant drop of cellular respiratory capacity. Moreover, it caused reorganization of nucleoids into large clusters stored in separate mitochondrial cisternae and relatively depleted of mtSSB protein. Interestingly, the mitophagy marker LC3BII was significantly upregulated while mtDNA copy number per cell decreased dramatically. However, subsequent studies with autophagy inhibitors wortmannin and chloroquin revealed that nucleoid clusters were protected from mitophagy. These results suggest that cancer cells respond to global mtDNA damage by forming novel structures – nucleoid clusters, which apparently protect cells against complete loss of mtDNA under conditions of extreme genotoxic stress. The relevance of this process for human physiology and medicine will be discussed.

Supported by grants no. P305/12/P388 from Czech Science Foundation and by GAUK 418811 from Grant Agency of Charles University.

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C5

MiPathology: Diagnosis



C5-01 Comparative biochemistry of tumorigenesis: role of mitochondria in the metastatic process.

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The classic bioenergetic phenotype of cancer cells of enhanced glycolysis was described by Otto Warburg approximately 90 years ago. However, the Warburg hypothesis does not necessarily imply mitochondrial dysfunction. Current thinking envisages tumor cells as compliant to an oxygen gradient within the tumor mass. Those cells on the periphery utilize oxygen whereas those found in hypoxic regions display metabolic symbiosis with the adjacent stromal cells [1]. Essentially metabolic reprogramming means up-regulation of pathways that increase the rate of ATP production, synthesis of lipids and redox balance. The process of carcinogenesis is guided by gene expression regulation that promote these metabolic changes in a different and complex way for each cancer cell, thus, the energy metabolism of cancer cells is very heterogeneous. For example, not all tumor cells display a high glycolytic flux as proposed by Warburg. Progression to metastasis appears to require mitochondrial function, a hypothesis that is compatible with the results obtained by our group.

In order to show this we resorted an experimental model of murine melanoma cells. A melanocyte cell line was subjected to several cycles of adhesion impediment, producing stable cell lines exhibiting phenotypes representing a progression from non-tumorigenic to metastatic cells. These were: non-tumorigenic cells melan-a (ma); non-tumorigenic cell line 4C (obtained after 4 cycles of adherence abrogation); non-metastatic 4C11- and metastatic 4C11+ melanoma cell lines. The metabolic profile of each of these different cell lines was investigated by evaluating enzyme activities and expression of members of the glycolytic and oxidative pathways [2].

Our results showed that only metastatic cell line (4C11+) released the highest amounts of lactate and exhibited high LDH activity related to glutamine catabolism. In contrast, high-resolution respirometry (HRR) showed that 4C11+ intact cells had increased (2.8-fold) oxidative metabolism, with enhanced (2.6-fold) oxygen flux coupled to ATP synthesis when compared to the other pre-malignant stages. Moreover, in 4C11+ cells, we observed an increase in succinate dehydrogenase (Complex II) activity confirmed by HRR in permeabilized cells. We did not observe an increase in mitochondrial content, mitochondrial biogenesis, but we observed an increase (2-fold) in fission process. These results suggest enhanced OXPHOS. This was thought to be associated to metastasis, a condition which would benefit from unrestricted supply of oxygen.

Detailed analysis of patterns in this and other models of tumor progression may reveal whether the modulation of the oxidative metabolism is a feature of the metastatic process. To test this hypothesis we produced 4C11+ Rho 0 cells. Preliminary results showed a decreased in proliferation.

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C5-02 Importance of mitochondrial haplotypes in the expression of metabolic phenotypes under different conditions.

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Differential expression of genes mediated by environmental parameters has the potential to influence the organismal phenotype. Considering the central importance of mitochondria in physiological processes such as senescence and life history traits, we hypothesize that expression of mitochondrial genes, under different environmental conditions, should be under strong evolutionary constraints. Integrity of mitochondrial functions requires a synergistic interaction between the mitochondrial and nuclear genomes with proteins produced from mtDNA genes interacting with proteins imported from nuclear encoded genes to produce a functional mitochondrial electron transfer system (ETS). Using permeabilized fibers of *Drosophila* expressing different mtDNA haplotypes in a homogenous nuclear background, we investigated the effect of several conditions such as aging, temperature and diet on mitochondrial functions. We showed that different sets of conditions may trigger the expression of a particular phenotype caused by mtDNA divergences. This is of paramount importance to understand the influence of the environment on mitochondrial evolution in a wide variety of species including humans and has the potential to provide a cohesive picture of the underlying mechanisms of co-evolved ETS Complexes.



C5-03 The challenge of understanding myopathies in horses using permeabilized muscle cells.

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In horses, myopathic syndromes are common but most often their aetiology and pathogenesis are unknown. Recently, the cause of Type 1 polysaccharide storage myopathy (PSSM) and the one of atypical myopathy (AM) have been discovered. Type 1 PSSM is caused by a glycogen synthase (*GYS1*) mutation [1] and is characterized by exercise-induced muscle damage. The frequently fatal syndrome of AM results from the ingestion of the toxin hypoglycin A contained in seeds of some *Acer* species [2]. The metabolite of hypoglycin A inhibits several acyl-CoA dehydrogenases leading to a multiple acyl-CoA dehydrogenases deficiency (MADD) phenotype. The mitochondrial respiration has never been determined in myopathic horses despite the potential role of mitochondria in the pathogenesis of inherited and acquired equine myopathies.

High-resolution respirometry (HRR) and multiple substrate-uncoupler-inhibitor titration (SUIT) protocols were applied to study mitochondrial respiration in *triceps brachii* of horses suffering from PSSM ($N = 5$), exertional rhabdomyolysis (ER) from unknown origin ($N = 3$), and from AM ($N = 15$). Respirometric parameters of myopathic horses were compared to those of healthy controls with specific fitness level according to the one of the group investigated [3].

With the exception of one PSSM horse (*i.e.* Horse PSSM+ in Fig. 1), all myopathic horses showed a severe decrease in muscle respiratory capacity. Flux control ratios, *FCR*, and substrate control ratios, *SCR* indicate an increase of CI-linked respiratory capacity as a common feature to PSSM and ER horses whereas AM was characterized by a greater contribution of CII respiration on maximal OXPHOS capacity. This study shows that the

understanding equine myopathies with HRR necessitates substrates supply to selected segments of the ETS and determination of maximal OXPHOS and ETS capacities.

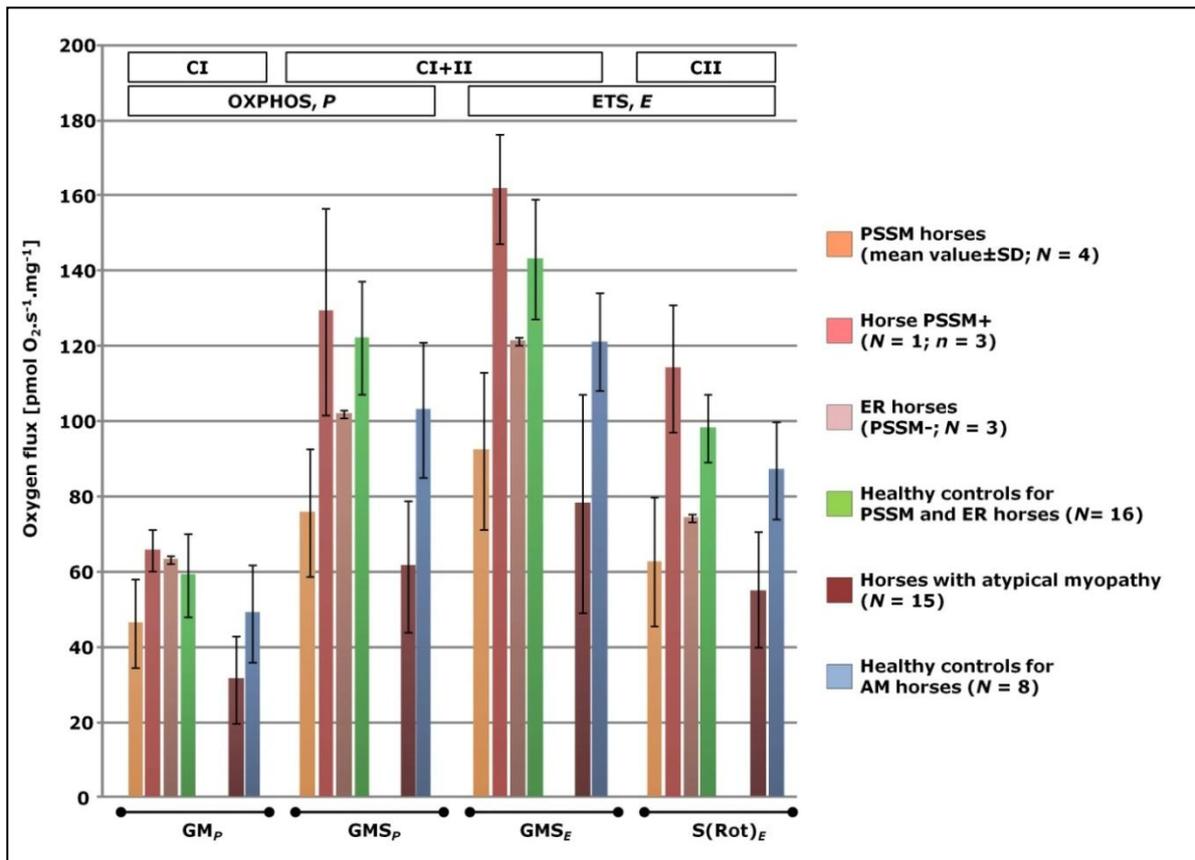


Figure 1: OXPHOS (*P*) and ETS (*E*) capacity (Oxygen flux [$\text{pmol O}_2 \cdot \text{s}^{-1} \cdot \text{mg}^{-1}$ wet weight]) with CI- (GM_p : glutamate+malate), CI+II- (GMS_p and GMS_E : glutamate+malate+succinate), and CII-substrate (S(Rot)_E : succinate+rotenone); *N*: number of horses (2 or 3 runs per horse); *n* = number of runs for this horse. See text for other abbreviations.

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C5-04 Mitochondrial bioenergetic parameters, reactive oxygen species production and the status of antioxidant defense system can be used to differentiate mitochondrial defects studied in the fibroblasts from patients with various mitochondrial disorders.

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Defects in the mitochondrial respiratory system are often associated with mitochondrial dysfunction and increased reactive oxygen species (ROS) production within the cell. The aim of our studies was to determine the differences in the mitochondrial bioenergetic parameters, ROS production and antioxidant enzymes status profiles between different types of mitochondrial defects.

Fibroblasts derived from patients with defined mitochondrial disorders (mutations in the genes of subunits Complex I, SCO2, SURF1, MTATP6, SERAC1, TAZ and tRNA^{Leu}) have been studied. Bioenergetic parameters, ROS production and the level of individual antioxidant enzymes have been estimated. Finally, a multiparameter statistical analysis has been performed.

Anomalies in the bioenergetic parameters, modification of the antioxidant enzymes levels as well as enhancement of intracellular ROS confirmed the occurrence of oxidative stress in the fibroblasts. Principal component analysis showed that individual defects were grouped in separate clusters. This indicates that mitochondrial defects in the patients' fibroblasts are characterized by a unique profile of important parameters of cellular bioenergetics and ROS homeostasis as well as that the different molecular background has a unique impact on the mitochondrial and antioxidant defense system dysfunctional pattern.

This approach may open new possibility to use the proposed set of mitochondrial parameters and comparative analysis in the studies essential for distinguishing the molecular background of mitochondrial defect.

Supported by the Statutory Founding from Nencki Institute of Experimental Biology, MNiSW nr W100/HFSC/2011 and Internal Projects of CMHI 125/2012.



C5-05 Comparative studies of reactive oxygen species production and the level of antioxidant defense system in the fibroblasts derived from patients with defined mitochondrial disorders.

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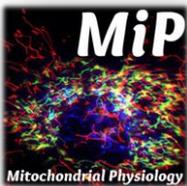
One of the most important mitochondrial energy-providing reactions is carried out by the oxidative phosphorylation system (OXPHOS) in the mitochondrial respiratory system. OXPHOS disorders vary from fatal encephalomyopathies of early childhood, for example Leigh syndrome, to severe diseases of adulthood, like Alzheimer's disease or Parkinson's disease [1]. Mitochondrial respiratory deficiencies are often associated with increased reactive oxygen species (ROS) production.

The aim of the study was to determine the differences in mitochondrial bioenergetic parameters, ROS production and antioxidant enzyme levels between known mitochondrial defects. Fibroblasts derived from patients (mutations: *MTND*, *SCO2*, *SURF1*, *MTATP6*, *SERAC1*, *TAZ* and tRNA^{Leu}) with various defined mitochondrial disorders

were used as a model of a self-propelling intracellular oxidative stress. Additionally we measured the level of p66Shc phosphorylation (associated with increased ROS production). In order to decrease the global and p66Shc-related ROS generation, we tested idebenone (demonstrating antioxidant capacities) and hispidin (kinase inhibitor).

Characterization of bioenergetic parameters and ROS production showed that fibroblasts from patients demonstrate an increased ROS production and attenuated respiratory activity and have an increased status of Ser36-p66Shc phosphorylation. After treatment with hispidin or idebenone, decreased ROS production was observed in comparison with untreated cells. This could imply the involvement of p66Shc in ROS production related to the mitochondrial respiratory system. Principal component analysis of the data indicates that the particular molecular background has a unique impact on the mitochondrial dysfunctional pattern.

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C5-06 The use of lymphocytes for diagnostics of mitochondrial oxidative phosphorylation disorders.

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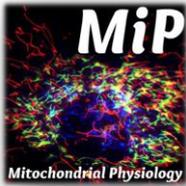
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Mitochondrial diseases belong to most severe inherited metabolic diseases affecting the pediatric population. Diagnostics of a substantial part of mitochondrial diseases with unknown genetic cause relies on clinical symptoms and biochemical analysis of energetic function and content of individual mitochondrial proteins in patient tissues – mainly in bioptic samples of skeletal muscle and cell cultures of skin fibroblasts. However, due to their invasive nature, the biopsies are often refused by the parents of the patient. Therefore, we tested the diagnostic suitability of easily obtainable patient material – lymphocytes isolated from peripheral blood.

High-resolution respirometry enables sensitive analysis of the mitochondrial oxidative phosphorylation system in isolated lymphocytes. Substrate-inhibitor measurements in digitonin-permeabilized lymphocytes provide a complex evaluation of individual respiratory complexes, coupled ATP synthesis and kinetic parameters of mitochondrial respiratory enzymes. We employed this approach in a large cohort of 48 children including mostly subjects with suspected mitochondrial disease, previously diagnosed patients with OXPHOS disorders, and controls. In combination with cytofluorometric detection of mitochondrial membrane potential and protein analysis by SDS and native electrophoreses it was possible to diagnose specific defects of Complexes I, CIV and CV using small amounts of peripheral blood within 1-2 days. Importantly, functional manifestations of mitochondrial disorders caused by *SURF1* [1] and *TMEM70* [2] mutations in lymphocytes recapitulate previous findings in fibroblasts.

The noninvasiveness, reliability and speed of such an approach demonstrate the high potential of isolated lymphocytes for diagnostics of oxidative phosphorylation disorders in patients with suspected mitochondrial disease.

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C5-07 Oxidative damage of proteins and the status of antioxidant enzymes in autopsy material from the central nervous system of patients with diagnosed or highly probable mitochondrial diseases.

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Mitochondrial diseases occur as a result of mutations in the nuclear or mitochondrial genome. Such impairment is often associated with dysfunctions in proteins which are part of the respiratory chain. First of all, our aim was to study whether the time elapsed from death until the dissection has significant impact on the amount of detected protein damage and thus affects or falsifies the results.

To address this problem, the material (brains) was obtained from mice. After sacrificing, the animals were stored under the same conditions as in the procedures dealing with the remains of patients. Dissection material was collected in a similar time regime, to what is done when obtaining autopsy material from the deceased patients.

The results demonstrated no significant alterations in the profiles of successive time point analyses in studied proteins levels and damage.

This work should allow the future use of autopsy material in the study of oxidative damage, which can help and direct further research toward the diagnosis of mitochondrial diseases, which in the vast majority is accompanied by elevated levels of reactive oxygen species.

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C5-08 Effects of mechanical ventilation after blunt chest trauma on diaphragmatic mitochondrial respiration in chronically cigarette smoke exposed mice - a clinically relevant model?

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Previous studies clearly indicate that diaphragm disuse during mechanical ventilation (MV) rapidly leads to atrophy of this muscle [1]. On the biochemical level, this MV related diaphragmatic weakness is due to an increased production of reactive oxygen species (ROS) and impaired mitochondrial respiration [2,3]. The dysfunction of the diaphragm is a major cause of difficult weaning from MV. This issue is a leading problem in critical care medicine, and a particularly severe challenge in patients with pre-existing pulmonary disorders like chronic obstructive pulmonary disease (COPD). However, no data are available yet on the effects of MV on diaphragmatic function especially in subjects with pulmonary conditions resembling human COPD. Therefore, our aim was to measure mitochondrial respiratory activity in diaphragm and heart in chronically cigarette smoke exposed (CSE) mice, i.e. in a COPD-model, after a standardized blunt chest trauma [4] and 5 hours of mechanical ventilation.

Maximum mitochondrial respiration in small, homogenized biopsies of heart and diaphragm was measured in the FCCP-induced uncoupled state under combined stimulation of the respiratory system with Complex I (pyruvate, malate and glutamate) and Complex II (succinate) substrates using an Oxygraph-2k (Oroboros Instruments,

Austria). We studied 4 groups of animals (controls vs. CSE with or without trauma and MV, $N = 5-8$).

Only in the diaphragm of CSE mice mitochondrial respiration already decreased after the short period of MV (Figure 1).

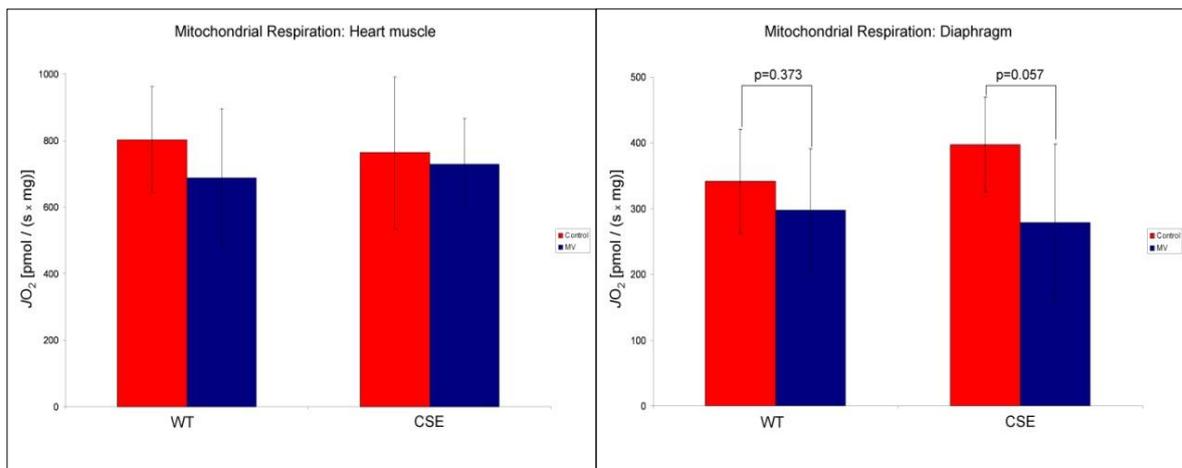
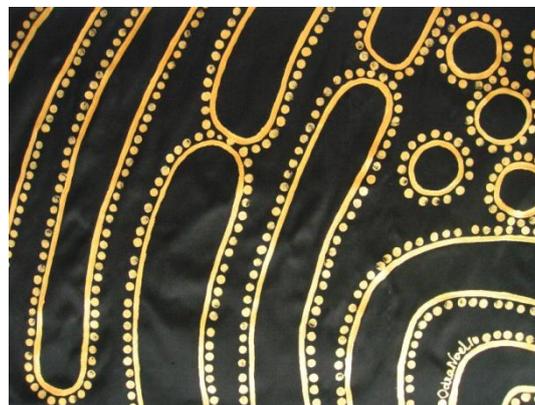


Figure 1: Maximum mitochondrial respiration in homogenised diaphragm (left) and heart biopsies (right; means \pm SD). Control (left bars) vs. MV (right bars) in wildtype (WT) and cigarette smoke exposed (CSE) mice.

In contrast to healthy individuals, in subjects suffering from chronic pulmonary diseases and airway obstruction similar to human COPD diaphragmatic mitochondrial dysfunction already results from short periods of MV and inactivity of the respiratory muscles.

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C6

MiPathology: Treatment



C6-01 SkQ1, the first mitochondria-targeted medicine available in drugstores.

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"One can qualify himself as a physiologist only if he succeeded in normalizing a physiological process damaged by a pathology" (IP Pavlov)

In this group, a concept was put forward considering mitochondrial reactive oxygen species (mROS) as key intermediates of programmed aging of organism. As a consequence of such a concept, it was suggested that aging program can be retarded (or even switched off) by mitochondria-targeted antioxidants [5]. To this end, 10-(6'-plastoquinonyl) decyltriphenyl phosphonium cation (SkQ1) was synthesized. It was shown that SkQ1 (i) is good penetrant for model and mitochondrial membranes, (ii) has very high affinity to membranes, (iii) is reduced by center *i* of Complex III of mitochondrial respiratory chain in the inner leaflet of the inner mitochondrial membrane, (vi) electrophoretically accumulates in this leaflet, being driven by mitochondrial membrane potential, (v) prevents peroxidation of mitochondrial cardiolipin by mROS, (vi) arrests the ROS-induced apoptosis and necrosis, (vii) prolongs the lifespan of various organisms (from fungi and plants to mammals), (viii) retards development of many traits of age-related diseases [1-7]. In particular, it was found that drops of SkQ1 instilled to eyes of rats prevent aging of tear glands, an effect leading to cure of such a disease as the dry eye syndrome which is usually assumed to be incurable and can result in uveitis. Clinical trials of drops of 250 nM SkQ1 solution called "Visomitin" showed that the three-week treatment (3 drops per day) completely cure the dry eye syndrome in 60% patients. The following favorable changes were shown: an increase in amount of tears, stability of tear film, acuity of vision as well as disappearance of inflammation in the eye tissues [7]. Drops of Visomitin are available in drugstores of Moscow and other places of Russia since July, 2012. By May 12, 2013, about 50 000 samples of the SkQ1 drops were sold and no claims concerning an unfavorable side effect were sent to the producers. Clinical trials of Visomitin as potential medicine to treat two other age-related eye diseases, namely cataract and glaucoma, were completed. For one of them (cataract), results are already available. In particular, it was found that acuity of vision was increased in 80.5% cataract patients ≥ 70 years.

Preclinical trials of SkQ1 in treatment of the dry eye syndrome and uveitis originally performed in Russia and are now confirmed in three laboratories in the USA (Ora Inc., Andover; Toxikon Corp., Minneapolis; Comparative Biosciences Inc., Sunny Vale). In the next future, clinical trials of Visomitin will start in the USA.

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C6-02 Developing novel treatment strategies for mitochondrial disease.

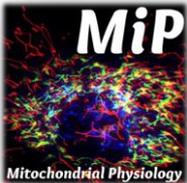
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The OXPHOS system consists of 5 multi-subunit Complexes (CI to CV) that contain 92 different structural proteins encoded by the nuclear (nDNA) and mitochondrial DNA (mtDNA). Biogenesis of a functional OXPHOS system further requires the assistance of nDNA-encoded OXPHOS assembly factors (chaperones), of which 35 are currently identified. OXPHOS and mitochondrial dysfunction are not only associated with relatively rare monogenic mitochondrial disorders but also observed during more common pathologic conditions, such as Alzheimer's, Huntington's and Parkinson's disease, cancer, cardiac disease, diabetes, epilepsy, and obesity. In addition, a progressive decline in the expression of mitochondrial genes is observed during normal human aging and mitochondrial function is inhibited by environmental toxins and frequently used drugs. Mutations in OXPHOS structural genes are associated with neurodegenerative diseases including Leigh Syndrome, which is probably the most classical OXPHOS disease during early childhood. Our research focuses on gaining a quantitative mechanistic understanding of mitochondrial (patho)physiology at the (sub)cellular level in OXPHOS disorders. To this end, the following scientific questions are addressed: (i) How are mitochondrial (ultra)structure and cell metabolic function connected during mitochondrial (dys)function, (ii) How do cells adapt to mitochondrial dysfunction? (iii) How can mitochondrial dysfunction be mitigated at the cellular and organismal level? To this end, protein-based and chemical fluorescent reporter molecules are introduced in healthy and patient-derived primary cells, as well as established cell lines to allow analysis of mechanistic aspects. (Patho)physiology is then investigated using biochemical and molecular cloning techniques, high-resolution respirometry, state-of-the-art quantitative (sub)cellular life cell microscopy, single-molecule spectroscopy, mathematical modelling, image processing, data mining and machine learning techniques. Using this strategy, we have developed a collection of novel small molecules that are currently investigated in animal models of mitochondrial deficiency.

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C6-03 Expression of *Ciona intestinalis* alternative oxidase in mouse.

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The primary objective of this study was to examine the consequences of expressing alternative mitochondrial respiratory enzymes under conditions of mitochondrial dysfunction *in vivo*. Multiple evidence indicates that *Ciona intestinalis* alternative oxidase (AOX) can safely be expressed in mammalian cells, where it is efficiently targeted to mitochondria and enzymatically active under conditions where the final enzyme of the electron transfer system, cytochrome c oxidase (COX), is inhibited either chemically and/or genetically [1-3].

AOX was targeted to the ROSA26 locus where it is expressed under the control of the strong and ubiquitous CAG promoter. We analyzed the general expression pattern by Western blots, consequences of AOX expression on complex assembly by two-dimensional blue native/SDS-PAGE and respiration of isolated mitochondria.

ROSA26^{AOX} mice are viable and have no obvious phenotype or alteration in physiological parameters. AOX is expressed in most tissues with highest level in heart, skeletal muscle, lung and liver while expression in the brain is virtually absent. Where expressed, AOX exists as a monomer but also forms higher assemblies. The majority of AOX is freely diffusible and not integrated into supercomplexes. Functionally, AOX expression supports azide- and antimycin A-resistant respiration. However, the degree of mitochondrial coupling in AOX-supported respiration depends on the applied substrates and inhibitors of the respiratory complexes.

Therefore, AOX expressing mice represent a valuable and versatile tool to study mitochondrial dysfunction *in vivo*. The ROSA26^{AOX} mouse will help to rescue disease models of mitochondrial dysfunction thereby deciphering molecular mechanisms and aiding in the eventual development of therapies. In addition, exploiting alternative respiratory enzymes is a novel approach to studying mitochondrial respiration and its control mechanisms.

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C6-04 Hypothetical *Trypanosoma* protein helps to anchor the F₁-ATPase moiety to the mitochondrial membrane.

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Trypanosoma brucei is a parasitic flagellate that causes devastating diseases of humans and livestock. The infective form dwells in the glucose rich environment of mammalian blood and generates energy solely via glycolysis. In consequence, the bloodstream stage single mitochondrion is highly reduced lacking key Krebs cycle enzymes and traditional cytochrome mediated respiratory chain. Interestingly, the essential mitochondrial membrane potential ($\Delta\psi_{mt}$) is maintained by hydrolytic activity of the unique F_oF₁-ATPase, which contains several trypanosoma specific subunits of unknown function [1].

We determined that one of the largest novel subunits, Tb2930 (43 kDa), is membrane-bound and localizes into monomeric and multimeric assemblies of the F_oF₁-ATPase. RNAi silencing of Tb2930 led to a significant decrease of $\Delta\psi_{mt}$ and consequently to *T. brucei* growth inhibition, indicating that the F_oF₁-ATPase is not functioning properly even though its structural integrity seems to be almost unchanged. To further explore the function of this protein, we employed naturally occurring trypanosoma strain that lacks mitochondrial mtDNA (dyskinetoplasmic, Dk) including subunit *a*, an essential component of the F_o-moiety and proton pore. These Dk cells maintain the $\Delta\psi_{mt}$ by electrogenic exchange of ATP⁴⁻/ADP³⁻ by the ATP/ADP carrier (AAC) and hydrolytic activity of the soluble F₁-ATPase [2]. So far, it has been assumed that only the F₁-moiety subunits are present and will be essential for these parasites. Interestingly, glycerol gradient sedimentation and native electrophoresis of Dk mitochondria revealed the presence of high molecular weight ATPase complexes that correspond to the bloodstream stage monomeric and multimeric F_oF₁-ATPase. Furthermore, the Tb2930 subunit is expressed in Dk cells and co-sediments with these high molecular weight membrane bound complexes. The RNAi study demonstrated that Tb2930 subunit is essential for Dk trypanosoma cells and crucial for maintaining $\Delta\psi_{mt}$. Importantly, upon ablation of Tb2930 we observed a shift of the F_oF₁-ATPase complexes to the lower S-values on glycerol gradient, where the free F₁-ATPase sediments, indicating changes in the structural integrity of the Dk F_oF₁-ATPase. In conclusion, we propose that Tb2930 is responsible for connecting the Dk F₁-ATPase to the mitochondrial membrane in the absence of subunit *a* of the F_o-moiety, thus increasing the efficiency of the functional association between F₁-ATPase and AAC.

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C6-05 LEA protein ameliorates inhibition of mitochondrial respiration in *Drosophila melanogaster* Kc167 cells and isolated mitochondria.

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Although severe water loss is often detrimental to life, some exceptional animals have developed mechanisms to survive water loss to 0.02–0.05 g H₂O/g dry mass (anhydrobiosis). How the structural and functional integrity of the mitochondrion is maintained during desiccation and rehydration is currently not understood. The brine shrimp *Artemia franciscana* serves as an important model organism for anhydrobiosis. Desiccation tolerance in this animal is correlated with the accumulation of large amounts of highly hydrophilic macromolecules termed Late Embryogenesis Abundant (LEA) proteins. Most LEA proteins are intrinsically disordered in solution and thought to stabilize other proteins and membranes during desiccation [1]. Two mitochondrial targeted LEA proteins, *AfrLEA3m* and *AfLEA1.3*, have been described in *A. franciscana* and may help to maintain the integrity and functionality of the organelle when water is scarce [2, 3].

In order to evaluate the impact of *AfLEA1.3* on water-stress tolerance of mitochondria the protein was transgenically expressed in Kc167 cells from the desiccation intolerant fruit fly *Drosophila melanogaster* [4]. A protein construct composed of *AfLEA1.3* and green fluorescent protein was found to accumulate within mitochondria. Expression of *AfLEA1.3* in non-permeabilized cells reduced mitochondrial proton leak by 20–30% in presence and absence of hyperosmotic stress. Oxygen consumption of permeabilized cells in presence of mixed substrates and ADP was 18% less inhibited by increasing concentrations of NaCl in cells expressing *AfLEA1.3* compared to control cells. Mitochondria isolated from wild-type and *AfLEA1.3* expressing cells showed a high respiratory control ratio (RCR) of 11.3 ± 1.4 . Oxygen consumption in presence of ADP and substrates that supply NADH to Complex I were significantly reduced after freezing and thawing and this reduction was significantly greater in mitochondria from control cells (RCR = 7.3 ± 1.0) than in cells expressing *AfLEA1.3* (RCR = 10.1 ± 0.7 , $P < 0.05$).

Expression of *AfLEA1.3* improved functions of mitochondria from *D. melanogaster* in several models of water stress. Protection was observed at hydration states in which *AfLEA1.3* most likely lacks extensive secondary structure and the protein was most likely intrinsically disordered. *AfLEA1.3* thus can operate outside the classic structure function paradigm. However, the observed effects afforded by the hydrated *AfLEA1.3* protein do not preclude even greater stabilization of biological structure and function in the dried state.

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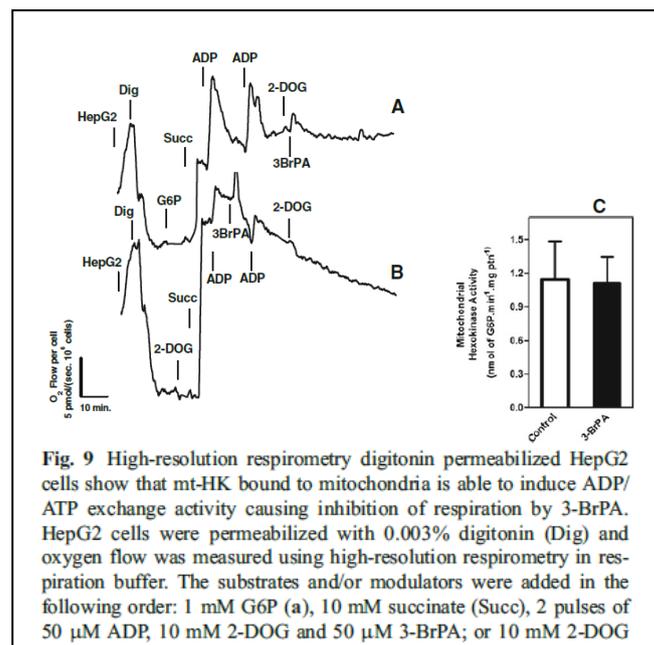


C6-06 Effects of antitumor alkylating agent 3-bromopyruvate on energy transducing pathways in hepatoma HepG2, liver mitochondria and SERCA: Is there any role for mitochondrial hexokinase activity?

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It was proposed that the alkylating agent 3-bromopyruvate (3-BrPA) could act as an antitumor agent in different cell lines of hepatocellular carcinoma mainly by targeting the mitochondrial hexokinase type 2 that is overexpressed in many tumor cells. Several revisions of drug therapy for cancer treatment have taken this as the main mechanism of action. Despite the potent negative effects of 3-BrPA on cell viability of the tumors, the analogue of pyruvate/lactate alkyl is oxidizing, as expected, other enzymes in energy transducing metabolism in tumor cells. However, little attention has been given to these side effects of 3-BrPA on tumor mitochondria, glycolysis and calcium pump SERCA. A dataset of high-resolution respirometry, analysis of flux, recovery of enzyme activities and metabolomics evaluated by our group in human hepatoma HepG2, isolated liver mitochondria and activities measured of calcium transport in sarcoplasmic reticulum vesicles mediated by SERCA, point to different metabolic targets with significant implications for the mechanism of cell death in tumor. Among the enzymes as targets we list the main ones: monocarboxylate transporter (MCT), glyceraldehyde dehydrogenase (GA3PDH); 3-phosphoglycerate kinase (3PGK); succinate dehydrogenase (SDH); pyruvate dehydrogenase (PDH); glutamate dehydrogenase (GDH); malate dehydrogenase (MDH) and SERCA 2a. Interestingly, mt-HK 1 and 2 are not significantly inhibited by 3-BrPA, but on the contrary contribute to depletion of the cytosolic ATP pool of the ATP-consuming path of glycolysis. Importantly, the mt-HK acts by modulating the rate of oxidative phosphorylation putting succinate dehydrogenase in a state of greater reactivity and inhibition by 3-BrPA. Given these observations we postulate that the mitochondrial hexokinase is not the primary molecular target of tumor cells but a potent depletory agent of cellular ATP and modulator of succinate dehydrogenase inhibition in mitochondrial supported respiration and inducer of permeability transition pore formation involved in cell death in tumor cells.





C6-07 Lithocholic acid delays aging in yeast and exhibits an anti-tumor effect in human cells by altering mitochondrial composition, structure and function.

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We use the yeast *Saccharomyces cerevisiae* as a model to study the molecular mechanisms by which age-related changes in mitochondrial membrane lipids regulate longevity [1-8].

We found that an exogenously added bile acid called lithocholic acid (LCA) extends yeast chronological lifespan and accumulates in the inner mitochondrial membrane (IMM). To define the mechanism by which LCA extends yeast longevity, we 1) examined how LCA influences mitochondrial proteome and lipidome; 2) investigated the effect of LCA on the composition and stoichiometry of respiratory complexes and supercomplexes in the IMM; 3) assessed how LCA affects mitochondrial oxygen consumption, membrane potential and reactive oxygen species (ROS); 4) examined how LCA influences mitochondrial morphology and how it affects the chronology of events characteristic of age-related, mitochondria-controlled apoptosis; and 5) investigated the effect of LCA on the lifespans of long- and short-lived mutants lacking individual components of the mitochondrial fission and fusion machines.

Our findings imply that LCA delays yeast aging by increasing the level of phosphatidylserine (PS) and decreasing the levels of phosphatidylethanolamine (PE) and cardiolipin in the IMM. By altering the abundance of these lipid species, LCA greatly expands mitochondrial membrane cristae. In addition, LCA enhances the positive effect of PS and weakens the negative effect of PE on membrane protein machines whose activity they modulate – thereby 1) stimulating protein machines driving mitochondrial respiration, the maintenance of mitochondrial membrane potential and ROS homeostasis, and mitochondrial fusion; and 2) inhibiting protein machines promoting mitochondrial fission and mitochondria-controlled apoptosis.

We found that LCA also functions as a potent and selective anti-tumor compound in cultured human neuroblastoma, glioma and breast cancer cells by modulating mitochondrial processes playing essential roles in both cancer and aging. The effects of LCA on these processes seen in cancer cell cultures are opposite of those observed in non-cancerous human cells and in chronologically aged, quiescent yeast.

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C6-08 Studies of bioenergetics alterations in breast cancer lines induced by histone deacetylase inhibitors.

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Tumor cells are characterized by different bioenergetic phenotypes compared to normal cells. This phenotype is influenced by changes in the microenvironment and tumor progression to metastasis. Classically, solid tumors display enhancement of glycolysis even when the oxygen tension is normal (aerobic glycolysis). However, recent results have suggested that mitochondrial function is important for tumor development control [1]. In our experiments we have taken advantage of the pleiotropic effects of histone deacetylase inhibitors (HDACis) to probe these changes.

The literature highlighting the various effects caused by histone deacetylase inhibitors (HDACis), which induces accumulation of acetylated substrates, generating pleiotropic effects, as cell cycle arrest, differentiation and cell death [2]. Little is known about HDACis effects in the energy metabolism modulation. Recent studies have shown that HDACis are able to modulate the glycolytic metabolism and mitochondrial function from highly glycolytic lung tumor cells [3].

In this context we have investigated how sodium butyrate (NaB), a histone deacetylase inhibitor that alters the energy metabolism in breast tumor cell lines at different stages of tumorigenicity: MCF-10A (non-tumorigenic), MCF-7 (tumorigenic, non-metastatic), T47D (tumorigenic, metastatic) and MDA-MB-231 (tumorigenic, highly-metastatic, invasive and aggressive).

We observed that NaB treatment induced an attenuation of glycolysis, reflected by a decrease in lactate release in MCF-7 and T47D lines. Furthermore, the treatment induced an increase in routine, leak and ETS respiration in T47D and MDA-MB-231, while no change was observed in MCF-10A AND MCF-7. Interestingly, we observed an increase in ROX respiration of T47D and MDA-MB-231, suggest that NaB can be inducing others oxidases activities.

These distinct alterations after treatment with NaB suggest a relevant question: are there different effects depending on cell metabolism with others cancer drugs? Taken together, these results demonstrate that cell's bioenergetic profile has an impact on the effect mediated by HDACi and show the importance of tumor metabolic characterization before drug treatments.

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C6-09 *Drosophila Trap1* protects against mitochondrial dysfunction in a *PINK1/parkin* model of Parkinson's disease.

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Mitochondrial dysfunction caused by protein aggregation has been shown to have an important role in neurological diseases, such as Parkinson's disease (PD). Mitochondria have evolved at least two levels of defense mechanisms that ensure their integrity and the viability of their host cell. First, molecular quality control, through the upregulation of mitochondrial chaperones and proteases, guarantees the clearance of damaged proteins. Second, organellar quality control ensures the clearance of defective mitochondria through their selective autophagy. Studies in *Drosophila* have highlighted mitochondrial dysfunction linked with the loss of the PTEN-induced putative kinase 1 (*Pink1*) as a mechanism of PD pathogenesis. The mitochondrial chaperone TNF receptor-associated protein 1 (*Trap1*) was recently reported to be a cellular substrate for the *Pink1* kinase. We characterized *Drosophila Trap1* null mutants and described the genetic analysis of *Trap1* function with *Pink1* and *parkin*. We showed that loss of *Trap1* resulted in a decrease in mitochondrial function and increased sensitivity to stress, and that its upregulation in neurons of *Pink1* mutant flies rescued mitochondrial impairment. Additionally, the expression of *Trap1* was able to partially rescue mitochondrial impairment in *parkin* mutant flies; and conversely, expression of *parkin* rescued mitochondrial impairment in *Trap1* mutants. We conclude that *Trap1* works downstream of *Pink1* and in parallel with *parkin* in *Drosophila*, and that enhancing its function may ameliorate mitochondrial dysfunction and rescue neurodegeneration in PD.





C6-10 The inhibitor of L-carnitine biosynthesis protects brain mitochondria against anoxia-reoxygenation injury.

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¹Riga Stradins University, Latvia; ²Latvian Institute of Organic Synthesis, Riga, Latvia; ³University of Latvia, Riga, Latvia. - k.volska@inbox.lv

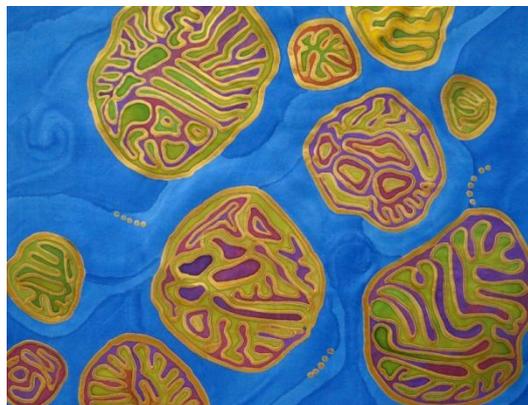
L-carnitine takes part in the regulation of cellular energy metabolism. Recently it has been shown that mildronate, an inhibitor of L-carnitine biosynthesis, improves the neurological outcome after ischemic damage of brain tissue [1]. The aim of the present study was to investigate the effects of mildronate treatment on brain mitochondrial function using an *in vitro* model of anoxia-reoxygenation.

Wistar rats were treated daily with mildronate (per os; 100 mg/kg) for 14 days. Control animals received water. The mitochondrial respiration measurements were performed in isolated brain mitochondria with a Clark-type oxygen sensor. OXPHOS capacity was measured using ADP and various substrates to evaluate respiration of all respiratory complexes. In order to investigate anoxia-reoxygenation damage, brain mitochondria were subjected to 5 min anoxia, followed by 5 min reoxygenation. In parallel, isolated mitochondria were treated under the same conditions but without 5 min anoxia to obtain control (normoxic) measurements. Respiratory parameters were determined: LEAK respiration in the absence of ADP (L_N); OXPHOS capacity (P); LEAK respiration after phosphorylation of ADP to ATP (L_T); respiratory control ratio (P/L_T , RCR).

Under normoxic conditions, mildronate treatment did not affect L_N and P . However, L_T was increased by 30%, resulting in a 28% decreased RCR. Anoxia-reoxygenation induced a significant 2.8-fold decrease in P and a 1.6-fold increase L_T . These effects of anoxia-reoxygenation resulted in 4-fold reduction of the RCR. The mildronate treatment significantly diminished the anoxia-reoxygenation-induced decrease in P and increase in L_T by 20% and 36%, respectively. After anoxia-reoxygenation the RCR was almost 2 times higher in the mildronate treated group compared to controls.

These results demonstrate that mildronate treatment induces uncoupling preconditioning-like effect and improves tolerance against anoxia-reoxygenation.

1. Svalbe B, Zvejniece L, Vavers E, Pugovics O, Muceniece R, Liepinsh E, Dambrova M (2011) Mildronate treatment improves functional recovery following middle cerebral artery occlusion in rats. Behav Brain Res 222: 26-32.





C6-11 Cyclization of potassium in rat liver mitochondria in the function of the mitochondrial ATP-dependent potassium channel. Its possible role in cardioprotection.

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The mitochondrial ATP-dependent potassium channel (mtK_{ATP}) plays a key role in protecting the myocardium during ischemia [1]. In our laboratory it was shown that the stimulation of mtK_{ATP} activates the output potassium system in mitochondria in exchange for protons. The activation of this cycle should lead to mild uncoupling, indicated by a slight decrease in membrane potential. Reduced potential across the inner mt-membrane by 10% leads to a decrease in the formation of peroxides by 70% [2]. A possible result of active cyclization of potassium in mitochondria, in our opinion, is the reduction in oxidative stress and, consequently, the recovery of the energy balance in the ischemic myocardium.

To record cyclization of potassium in the mitochondria, we used a spectrophotometric method. Log potassium ions was determined by the rate of swelling of mitochondria in hypotonic medium. The kinetics of swelling was recorded by changing the optical density of the suspension of mitochondria at a wavelength of 520 nm for 30-40 min. The swelling rate was calculated from the change in light scattering per unit time. Under these conditions, there was swelling and shrinking of organelles that was manifested in the form of two or three waves. This probably reflects synchronized input and output of potassium in some organelles. The study of the influence of the degree of coupling of mitochondria in the cyclization of potassium showed that over time the oxidation of free organelles increases and respiratory control was reduced. Thus, the cyclization of potassium in mitochondria depends on their coupling. We studied the influence of modulators of mtK_{ATP} on the system cyclization of potassium in the mitochondria to determine whether the channel is involved in the observed fluctuations of the potassium ion. The addition of ATP-Mg²⁺ at physiological concentration of 1 mM led to a decrease in the rate of swelling of organelles reflecting volatile input potassium ion. When 5 mM of 4-aminopyridine was added to the incubation medium of mitochondrial suspensions, a swelling-inhibiting effect was observed as with the addition of ATP. One of the activators of the mtK_{ATP} channel is ADP. In our experiments 500 μM ADP activates entry of potassium ions into the mitochondria by 85%. It is known that sildenafil exhibits cardioprotective effects on the whole organism, and a specific inhibitor of mtK_{ATP} 5-hydroxydecanoate removes this effect [3]. We made the assumption that sildenafil has an impact on mtK_{ATP}, being its activator. We found that sildenafil in concentration of 125 μM accelerates the entry of potassium ions into the mitochondria of rat liver by 30-40%, and also partially removes the inhibitory effect of ATP-Mg²⁺. Thus, the cardioprotective effect of sildenafil may be associated with activation of the mtK_{ATP} channel.

Supported by RFBR №10-04-00920-a; DPNNiT №4.3010.2011, RFBR №12-04-32187-mol_a.

1. Garlid K, Paucek P, Yarov-Yarovoy V, Murray H, Darbenzio R, D'Alonzo A, Lodge N, Smith M, Grover G (1997) Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K⁺ channels. Possible mechanism of cardioprotection. *Circ Res* 81: 1072-1082.
2. Korshunov SS, Skulachev VP, Starkov AA (1997) High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett* 416: 15-18.
3. Ockaili R, Salloum F, Hawkins J, Kukreja R (2002) Sildenafil (Viagra) induces powerful cardioprotective effect via opening of mitochondrial K_{ATP} channels in rabbits. *Am J Physiol Heart Circ Physiol* 283: 1263-1269.

www.mitophysiology.org/?MiPcalendar

MiPsummer time



MiPsummer 2014

7th MiPsummer School on Mitochondrial Physiology
University College of London
London, UK

Organizer:

Michael Duchen (London, UK)

Email: m.duchen@ucl.ac.uk



MiPsummer 2015

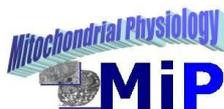
8th MiPsummer School on Mitochondrial Physiology
East Carolina University, Brody School of Medicine
Greenville, NC, USA

Organizer:

Darrel Neufer (Greenville, NC, USA)

Email: neuferp@ecu.edu

The next world-summits on Mitochondrial Physiology



MiP2014

10th Conference on Mitochondrial Physiology
Obergurgl, Austria

See page 94



MiP2015

11th Conference on Mitochondrial Physiology
Prague, Czech Republic

Organizer:

Petr Pecina (Prague, CZ)

Email: petr.pecina@gmail.com

www.bioblast.at/index.php/MiP2014



MiP2014 - a point/counterpoint meeting

10th Conference on Mitochondrial Physiology
08 to 12 Sep 2014, Obergurgl, Austria

In each of the 12 sessions a point/counterpoint introduction will be given by invited keynote speakers to provide a balanced overview and promote discussions. Short oral presentations are selected from abstracts, including student presentations. Rapid platform statements will introduce posters which contribute to a specific session. Posters are up throughout the meeting, and poster discussions are integrated into the platform sessions.

A. Mitochondrial respiratory control

- A1. mtDNA, mt-phenotype and disease – is there biochemical and molecular-genetic evidence to explain observed associations?
- A2. Fission and fusion, mitochondrial structure and function – is there a tight connection?
- A3. Mitochondrial density and normalization of respiration – is there a general biochemical or molecular marker?
- A4. Biochemical reserve capacity, respiratory control ratios and coupling efficiency – are conventional concepts inconsistent?

B. Experimental protocols in mitochondrial biochemistry

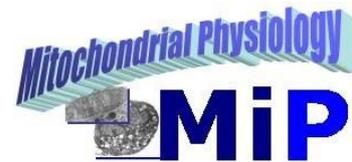
- B1. Incubation media for mt-preparations and experimental temperature in functional studies of mammalian mitochondria – guided by concept or tradition?
- B2. Intracellular oxygen pressure versus oxygen regimes in studies of ROS production and respiration – are mitochondria sources or sinks of ROS?
- B3. Quantitative measurement of mt-membrane potential – potentiometric/fluorometric.
- B4. Instrumental platforms in mitochondrial physiology – industrial presentations.

C. Towards quality assurance

- C1. Do blood cells versus muscle biopsies provide valid models for diagnosis of mitochondrial pathologies?
- C2. Integrity of mitochondrial preparations – is a consensus possible on quality control criteria?
- C3. From availability and quality control of chemicals to mitochondrial respiratory protocols – who controls?
- C4. Standards for the diagnosis of mitochondrial pathologies – a challenge for collaboration with mitochondrial organizations?

http://www.bioblast.at/index.php/MitoGlobal_Organizations

Time	Monday Sep 08	Tuesday Sep 09	Wednesday Sep 10	Thursday Sep 11	Friday Sep 12
07:00		Breakfast	Breakfast	Breakfast	Breakfast
08:30	A	Session A1	Session B1	Session C1	D
10:00	R	Coffee	Coffee	Coffee	E
10:30	R	Session A2	Session B2	Session C2	A
12:00	I	Lunch	Lunch	Lunch	P
13:30	V	Walks and talks	Excursion	Session C3	R
15:30	A	Session A3	Session B3	Session C4	T
17:00	L	Coffee	Coffee	Coffee	U
17:30	Welcome reception	Session A4	Session B4	Conclusions	R
19:00	Dinner	Dinner	Dinner	Dinner	E
20:30	Opening	Programme committee meeting Round tables / Posters	Project groups Posters	Social evening	Postconference Workshop



Becoming a MiPmember

Operating as an informal group since MiP2003 in Schroecken, the MiP*society* was formally established at MiP2011 in Bordeaux as an international non-government organization with its legal base in Innsbruck, Austria. The MiP*society* organizes annual summer schools and biannual conferences, bringing together international leading scientists and young researchers in the rapidly expanding field of Mitochondrial Physiology. MiP*members* benefit from

1. Earliest information on MiP*events*
2. MiP*circulares*
3. Reduction of registration fees at MiP*events*

MiP in the **Mitochondrial Global Network: MitoGlobal** aims at providing a WorldWide information platform for scientific mitochondrial organizations and mitochondrial research consortia.

Two options for MiPmembership

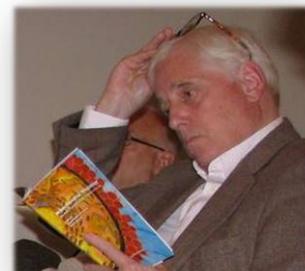
- **MiPmember** bi-annual fee of EUR 70.- (student fee EUR 30.-)
- **MiPlifetime-member** EUR 350.-

Application

Please, submit your application form (available for download at our website www.mitophysiology.org) to join as a MiP*member*. Your application will be confirmed by the Executive MiP*committee* by Email.

Honorary Gentle Science Member of the MiP*society*

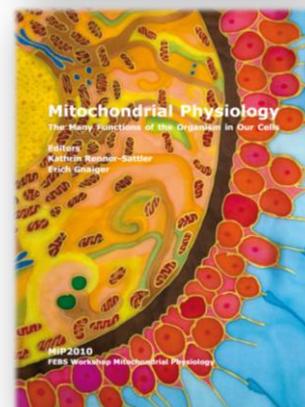
Nobel laureate Professor Sir John Walker (Cambridge MBU, UK; Nobel prize 1997 in chemistry) has joined the MiP*society* as the first '**Honorary Gentle Science Member**' of the Mitochondrial Physiology Society, following his presentation of 'The ATP Synthase' at the MiP*summer* School 2012 on July 10 in Trinity Hall, Cambridge, UK.



Contact

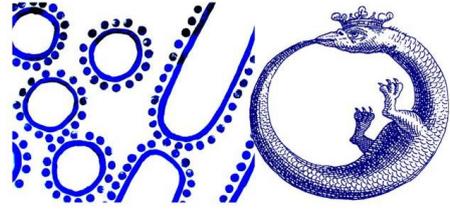
Mitochondrial Physiology Society - MiPs

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MiP*Art* by Odra Noel

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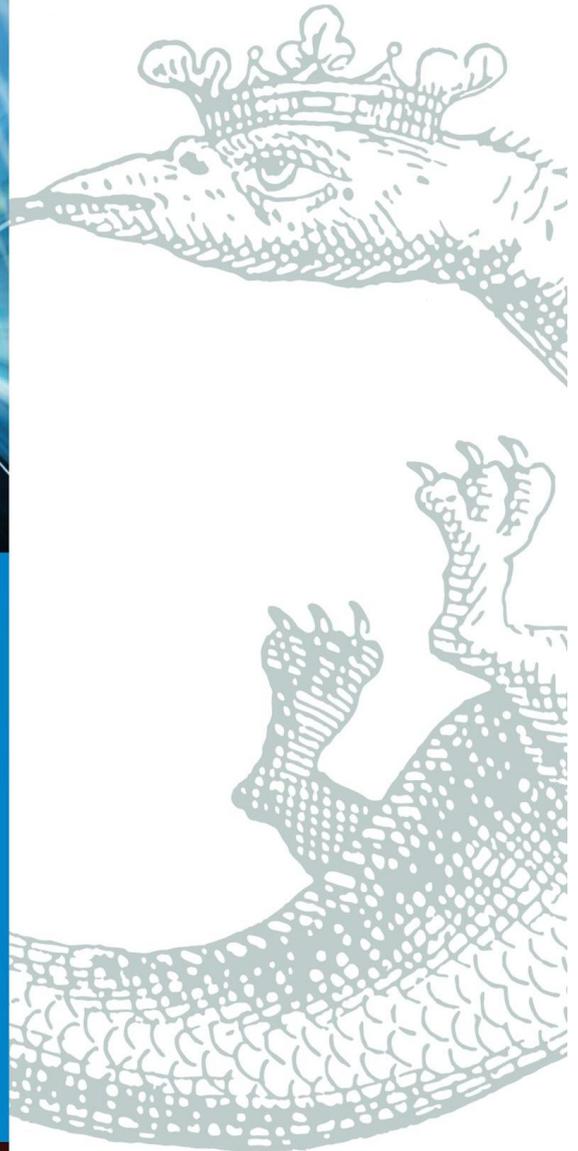
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