Session 8: Molecular Bioenergetics

Lectures

L8.1

Impact of temperature and training on skeletal muscle mitochondrial bioenergetics

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The impact of an endurance training (ET) on the bioenergetics of muscle mitochondria under hypothermia (25ºC), normothermia (35ºC), and hyperthermia (42ºC) will be discussed by the example of rat skeletal mitochondria. In skeletal muscles, ET leads to considerable elevations of mitochondrial biogenesis and oxidative capacity, which are accompanied by several qualitative temperature-dependent changes at the mitochondrial bioenergetics level. In skeletal muscle mitochondria, ET (i) augments the phosphorylation rate and coenzyme Q (Q) level, (ii) significantly increases reactive oxygen species (ROS) production (and Q reduction level) in non-phosphorylating mitochondria, (III) decreases ROS production (and Q reduction level) in phosphorylating mitochondria, and (iv) upregulates mitochondrial capacity to oxidize the lipid-derived fuels (palmitoylcarnitine and glycerol-3-phosphate). The ET-induced decrease in mitochondrial uncoupling, including uncoupling protein 3 (UCP3)-mediated proton leak, may explain the increased ROS production (in non-phosphorylating mitochondria) and enhanced oxidative phosphorylation efficiency. Moreover, ET magnifies the hyperthermia-induced increase in oxidative capacity and attenuates the hyperthermia-induced decline in oxidative phosphorylation efficiency and ROS formation of non-phosphorylating mitochondria. Thus, ET increases the bioenergetic efficiency of skeletal muscle mitochondria. The ET-induced quantitative and qualitative changes in muscle mitochondria may be important for maintaining muscle cell energy homeostasis, muscle cell signalling, and exercise performance, especially at hyperthermia.

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Acknowledgements:
This work was supported by the National Science Centre, Poland (2016/21/B/NZ3/00333).

L8.2

Warming-up of the chloroplast

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Sunlight is a primary source of energy driving living organisms and photosynthesis is practically a sole process converting energy of electromagnetic radiation to the forms which can be used in biochemical reactions. In plants, the photosynthetic apparatus is localized in chloroplasts comprising of thylakoid membranes hosting functional pigment-protein complexes. Most of the photosynthetic complexes are specialized to absorb light and to transfer electronic excitations towards the reaction centers. Efficient excitation energy transfer assures minimum losses, particularly important under conditions of low light intensity. To our great surprise, we have observed pronounced quenching of excitation energy in chloroplasts exposed to very low light intensity [1]. Such a mechanism could be anticipated to operate at very high light intensities, in order to protect the photosynthetic apparatus against photo-destruction, but it is hard to find a good reason to rationalize its operation at low light intensities. Concepts regarding such an intriguing observation will be presented and discussed. One of them is based upon "warming-up" of the chloroplast, *via* thermal deactivation of excited pigments, necessary to achieve optimal conditions for biochemical reactions.

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L8.3

Mitochondrial regulation of mitochondrial potassium channels

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Lecture will present the most interesting issues regarding function, regulation and pharmacology of the mitochondrial potassium channels. There are eight potassium channels known to contribute to the potassium permeability of the inner mitochondrial membrane: ATP-regulated channel, calcium-regulated channels of large (large, intermediate and small conductance), voltage-regulated Kv1.3 and Kv7.4 channels, two-pore-domain TASK-3 channel and SLO2 channel. The primary function of the mitochondrial potassium channels is regulation of the mitochondrial membrane potential. Additionally, mitochondrial potassium channels alter cellular respiration, regulation of the mitochondrial volume and ROS synthesis. The focus of the presentation will be on mitochondrial specific regulation of mitochondrial potassium channels such as interaction with respiratory chain and stretch regulation of mitochondrial channels.

Acknowledgements:
This study was supported by a grant 2015/17/B/NZ1/02496 (to AS), 2015/18/E/NZ1/00737 (to BK), 2016/21/B/NZ1/02769 (to PB) and 2015/19/B/NZ1/02794 (to PK) from the National Science Centre, Poland

Oral presentations

O8.1

Single-channel properties of the ROMK2-pore-forming subunit of the mitochondrial ATP-regulated potassium channel

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The increased flux of potassium ions into mitochondrial matrix through the ATP-sensitive potassium (mitoK$_{ATP}$) channel has been implicated in the mechanism of cytoprotection. However, details of this phenomenon remain unclear. Recently, it has been suggested that the mitochondrial-targeted isoform of ROMK protein create a pore forming subunit of mitoK$_{ATP}$ channel in the inner membrane of heart mitochondria. In our research, we focused on biophysical and pharmacological properties of ROMK as mitoK$_{ATP}$ channel. For the first time, we detect single-channel activity and describe pharmacology of the mitoK$_{ATP}$ channel formed by a splice variant of the renal outer medullary potassium channel (ROMK2) protein in H9c2 cells. Using the mitoplast patch-clamping methods, we observed a potassium channel with a mean conductance of 94 pS in symmetric 150/150 mM KCl. The activity of the channel was inhibited by ATP/Mg^{2+} and 5-hydroxydecanoic acid and partially inhibited by glibenclamide. The channel was also activated by the potassium channel opener diazoxide. The channel was also blocked by Tertiapin Q, a known inhibitor of the ROMK-type channels. Additionally, using assay based on proteinase K we describe the topology of the channel in the inner mitochondrial membrane. We conclude that the observed activity of the channel formed by the ROMK2 protein corresponds to the electrophysiological and pharmacological properties of the mitoK$_{ATP}$ channel. Our data suggest that both the C- and N- termini of the protein are localized in the mitochondrial matrix.

Acknowledgements:
This work was supported by the Polish National Science Centre grants No.2015/18/E/NZ1/00737 and 2015/17/B/NZ1/02496 and the Nencki Institute of Experimental Biology.

O8.2

Evaluation of mitochondrial energetic status and oxidative stress during tardigrade anhydrobiosis

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Many tardigrade species are able to survive complete dehydration and this phenomenon is called anhydrobiosis. During dehydration tardigrades curl up into a little ball called a tun which can be regarded as a consequence of cytoprotective mechanisms triggered at cellular and molecular levels. Anhydrobiosis can be therefore defined as an organized state and as such it requires some form of energy supply. This imposes mitochondria involvement in successful anhydrobiosis. We decided to estimate mitochondrial coupling in active and anhydrobiotic tardigrades by application of the fluorophore (TMRM) transported into mitochondria in the presence of the mitochondrial inner membrane potential. We also studied the levels of mitochondrial ROS production by the application of the MitoSOX Red fluorescent dye as a mitochondrial superoxide indicator. The resulting fluorescence were analyzed under fluorescence microscopy. We observed that the presence of functional mitochondria in tuns correlates with successful recovery to the active stage. We were also able to detect differences in MitoSOX fluorescence between the active stage and the tun stage. These results indicate the important role of mitochondial activity and related ROS production in the mechanisms responsible for successful anhydrobiosis. The applied fluorescent dyes seem to be useful tools in this kind of studies.

Acknowledgements:
The work was supported by the research grant of National Science Centre, Poland, NCN 2016/21/B/NZ4/00131.

O8.3

Dietary oxidized phospholipids: digestion, absorption and metabolism

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The results of numerous epidemiological studies indicate that the type, quality and intake of food-delivered lipids contribute to the prevention or promotion of diet related and metabolic diseases such as: type 2 diabetes, obesity or atherosclerosis. Among the food-delivered lipid compounds, phospholipids (PLs) attract increasing attention due to their high nutritional value and functional properties. PLs, especially those containing essential unsaturated fatty acids (FAs) exhibit a number of key biological activities. At the same time, polyunsaturated FAs incorporated in the structure of natural occurring PLs are particularly susceptible to oxidation. Oxidized phospholipids (OxPLs) delivered with food and products of their digestion can be potentially toxic molecules for to the epithelial cells of digestive tract. Accumulation of OxPLs can lead to gut pathologies as a result of cell membrane modifications as well as DNA and protein damage. In addition, previous research suggested that lipid hydroperoxides, which were not hydrolyzed during intestinal digestion, may be released to the blood stream after absorption by enterocytes and thereby contribute to the pathogenesis of atherosclerosis. Additionally, OxPLs may lead to pathological conditions in cells by aberrant regulation of numerous genes implicated in cell proliferation, differentiation, cellular stress, inflammation and lipid metabolism. Among transcription factors activated by OxPLs are such important gene expression regulators as PPARs, Nrf-2 or ATF4.

O8.4

Coenzyme Q and ROS production in mitochondria

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Acanthamoeba castellanii is a small non-photosynthesizing amoeba. These free-living organism is frequently used in studying of mitochondrial respiratory chain. Important component of electron carrier is mitochondrial coenzyme Q (Q). It is a mobile component of the mitochondrial electron transport chain and participates in aerobic respiration producing energy in the form of ATP. Coenzyme Q takes part in mitochondrial reactive oxygen species (ROS) production, contributing to oxidative stress and damaging mitochondria and cells. On the other hand, Q consists of an antioxidant property that protects the cells from harmful ROS and may be able to prevent the growth of cancer cells. The aim of our study was to understand the relationship between respiratory rate, membrane potential, ROS formation, and Q reduction level in isolated *A. castellanii* mitochondria. These mitochondrial parameters were measured under various conditions, i.e., when substrate succinate dehydrogenases (Q-reducing pathway) and different QH_2-oxidizing pathways (the cytochrome pathway and/or alternative oxidase) were engaged under phosphorylating, uncoupling, and non-phosphorylating conditions. The rates of Q-reducing and QH_2-oxidizing pathways were titrated progressively by substrate availability and/or inhibitors of respective respiratory chain components. Our results indicate that membranous Q reduction level is directly proportional to ROS formation within a defined respiratory path-dependent range. These studies are important because disorders related to Q and ROS production play an important role in oxidative stress, aging and the development of many pathological diseases including cancer therapy.

Acknowledgements:
This work was supported by the National Science Centre, Poland (2016/21/B/NZ3/00333).

Posters

P8.1

The impact of chronic hypoxia on aerobic metabolism of human endothelial EA.hy926 cells

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The aim of this study was to determine the impact of chronic hypoxia conditions on aerobic metabolism of human umbilical vein endothelial cells, EA.hy926 cell line. The impact of hypoxia on mitochondrial respiratory function in endothelial cells (ECs) and isolated mitochondria were assessed in ECs cultured for 6 days at 1% and 20% oxygen tension. In ECs exposure to 1% O_2 provoked a shift from aerobic towards anaerobic catabolic metabolism. Chronic hypoxia did not alter ECs viability and mitochondrial biogenesis. Even though, chronic hypoxia increased fermentation in ECs. Under chronic hypoxia conditions, mitochondrial respiration during oxidation of carbohydrate, fatty acid and glucogenic amino acid were lowered. Oppositely, ketogenic amino acid oxidation were elevated. Chronic hypoxia caused elevated reactive oxygen species (ROS) production (intracellular and mitochondrial). Nevertheless, antioxidant defence (superoxide dismutases SOD1 and SOD2, and uncoupling protein 2, UCP2) were not escalated. Additionally, UCP2 activity and expression were declined. In mitochondria of hypoxic ECs, elevated expression and activity of complex II, and declined expression and activity of complex I were observed. Our results point out an important role of mitochondria in response to metabolic adaptations of ECs related to hypoxia.

Acknowledgements:
This work was supported by the National Science Center Grants, Poland (2012/07/N/NZ3/00495 and 2016/21/B/NZ3/00333).

P8.2

The relationship between standard reduction potential and thermodynamic constants of antioxidant compounds – creation of Antioxidant Power Series

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The Antioxidant Power Series (APS) is proposed as a way to organize antioxidants important for human health based on determinations of their reduction potentials, in relation to glutathione – the main physiological antioxidant.
In our study, the values of reduction potentials of antioxidants were determined by potentiometric titration. All measurements were carried out using an Ag/AgCl|3M KCl reference electrode and a Pt working electrode at 295.15 K, 298.15 K, 310.15 K, 314.15 K, 318.15 K in phosphate buffered saline (pH=7.4). On the basis of titration curves received, the inflection points were read by the non-linear regression method using the proposed sigmoidal, 5-parameter model (with determination coefficient r^2 almost equal to 0.999). The results obtained allowed calculation the values of standard reduction potentials (E^0) and thermodynamic data for oxidation reactions: standard Gibbs free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0).
Investigated antioxidants could be divided into two groups, based on the obtained thermodynamic data: straight line and parabola-shaped of dependence $E^0=f(T)$ with minimum for the temperature near 310.15 K (37°C). Parabolic shape means that the reaction of oxidation at minimum point is the least spontaneous (the highest ΔG^0), which may have great importance in biological processes.

Acknowledgement:
This work was supported by the National Science Centre in the framework of programme „Maestro 6" (application no: 2014/14/A/ST4/00640).

P8.3

Tryptophan and taurine deficiency affects neuroactive amino acid pool in cerebellum of rats

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Tryptophan serves as the precursor for several neurotransmitters and metabolic regulators, which have major importance for interpreting symptoms of dietary tryptophan deficiency. Symptoms of tryptophan deficiency include reduced feed intake, impaired development of the central nervous system, and some pellagra-like signs, including dermatitis and sudden weight loss. Several lines of research suggest that gamma-aminobutyric acid (GABA) and glutamate may be involved in development of some wasting eating disorder.
The aim of the study was to assess the levels of neuroactive amino acids (glutamate, GABA, glutamine, aspartate, etc) in cerebellum of rats undergone tryptophan deficient diet (T-DD) and combined tryptophan-taurine deficient diet (TT-DD).
To simulate a tryptophan deficiency in rats, they fed only maize for 35 days. Combined deficiency of tryptophan and taurine was caused by consumption of the 3% beta-alanine solution by the T-DD animals as the sole source of fluid for 21 days. Both T-DD and TT-DD rats were exposed to intragastrical administration of tryptophan at the dose of 80 mg/kg bw/day in the last 7 days of the experiment. After decapitation of rats, the levels of GABA, glutamate, and some other neuroactive amino acids were measured in the cerebellum homogenates by HPLC. The activity of the glutamate decarboxylase (GAD) was determined by the spectrofluorimetric method.
Prolonged non-tryptophan diet conducted to a reliable increase in the levels of GABA, aspartate, and asparagine and decrease in the glutamine content in the cerebellum of rats. Combined tryptophan and taurine deficiency was followed by significant increase in the levels of glutamine, and alanine compared to the T-DD group. Introduction of tryptophan against T-DD retained a high concentration of GABA in the cerebellum without changing the activity of GAD. The level of glutamine was lower than in the control group. Administration of tryptophan to the TT-DD rats accelerated the synthesis of GABA from glutamate and increase concentration of both inhibitory amino acids. The glutamine and asparagine levels also increased in relation to the TT-DD group.
The results obtained suggest that both tryptophan and taurine dietary deficiencies conducted to activation of GABA synthesis from glutamate and glutamine. Introduction of tryptophan against these states accelerate accumulation of the GABA and glycine in cerebellum due to unknown mechanism, which requires further studies.

P8.4

Cardioprotective flavoniods as natural modulators of mitochondrial potassium channels

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Potassium channels such as K_{ATP}, BK_{Ca} or Kv1.3 have been found in the inner mitochondrial membranes of different cells types. It is considered that potassium channels regulate the mitochondrial membrane potential, respiration, matrix volume and calcium homeostasis. There are hypothesis that mitochondrial e.g. BK_{Ca} channels play an important role in ischemic preconditioning. It was also shown that mitochondrial potassium channels are potential targets for some flavonoids in the anti-ischemic strategies.
Our pervious study showed functional properties of the BK_{Ca} channel in mitochondria of endothelial cells (EA.hy 926). Large conductance (270 pS), voltage dependence, a high open-state probability at positive potentials, sensitivity to Ca^{2+}, NS1619 (a BK_{Ca} channel opener) and paxilline (BK_{Ca} channel inhibitor) indicate similarity to the mammalian BK_{Ca} channel. Previously, these channel was also discovered in glioma, brain, skeletal muscle and cardiac.
In the current study, single channel activity of the mitoB-K_{Ca} channel was measured after patch-clamp of the mitoplasts isolated from endothelial cell line (EA.hy 926). We show detailed data describing regulation of the mitoBK$_{Ca}$ channel by the cardioprotective flavonoids (luteolin, quercitin and cyanidin). We have observed that opening probability of the channel increased from 0.15 in the control conditions (100 mM Ca^{2+}) to 0.26 after application of cyaniding in micromolar concentration range.

Acknowledgements:
This study was supported by a grant 2016/21/B/NZ1/02769 from the National Science Centre, Poland.
Work implemented as a part of Operational Project Knowledge Education Development 2014-2020 cofinanced by European Social Fund (for Aleksandra Sęk).

P8.5

Identification of potassium channels in the mitochondria of human bronchial epithelial cells

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Mitochondria have been recognized for their multifunctional roles in energy transduction, ion transport, signaling and cell death. It has been observed that potassium flux through the inner mitochondrial membrane regulates synthesis of the reactive oxygen species, affects the mitochondrial volume and changes both the mitochondrial membrane potential and the transport of calcium ions into the mitochondria. Additionally, it has been shown that activation of mitochondrial potassium channels (e.g. mitoBK$_{Ca}$) protects against cell death during myocardial infarction or cerebral hypoxia.
Our studies using the patch-clamp technique proves the presence of two different potassium channels in the inner mitochondrial membrane of human bronchial epithelial cell line (16HBE14o-). We identified the activity of rectifying potassium channel and large-conductance Ca^{2+}-regulated potassium channel (mitoBK$_{Ca}$ channel). Using reverse transcriptase-PCR, we detect mRNA transcript for KCNJI (ROMK) channel as a molecular component of the mitoK$_{ATP}$ channel. Moreover, the protein of ROMK was also observed by Western Blot analysis. Additionally, it has been confirmed the presence of α-subunit and modulatory β-subunits of BK channel proteins (Western Blot analysis) and genes expression (reverse transcriptase-PCR analysis).
We believe that our findings of the potassium channels of epithelial mitochondria, it will help us better understand of their role in global protective mechanisms.

Acknowledgements:
This study was supported by a grant 2016/21/B/NZ1/02769 from the National Science Centre, Poland and partially by the Nencki Institute of Experimental Biology. Work implemented as a part of Operational Project Knowledge Education Development 2014-2020 cofinanced by European Social Fund; Project number POWER.03.02.00-00-I007/16-00 (Aleksandra Sęk).

P8.6

BK-DEC splice variant forms a functional BK_{Ca} channel in the inner mitochondrial membrane

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Ischemia of brain or heart tissue is the one of the most common causes of death worldwide. In the inner mitochondrial membrane several potassium channels have been identified whose activation lead to cytoprotection during ischemic event. It was found that activation of mitochondrial large conductance calcium activated potassium channel (mitoBK$_{Ca}$) preserves brain and heart muscle cells. Recently, the molecular identity of the mitoBK$_{Ca}$ channel was described. A BK-DEC splice variant of BK$_{Ca}$-type channels α subunit has been demonstrated to localize in mitochondria. However it is not known whether this isoform is able to form a functional channel in mitochondria.
In our study we used HEK293T cells transfected with cDNA encoding BK-DEC splice variant. Electrophysiological recordings with use of mitoplast isolated from transfected cells revealed presence of the large conductance and voltage dependent ion channel. This type of channel was not present in mitoplasts isolated from untransfected cells. We found that recorded channel showed all basic pharmacological properties typical for the mitoBK$_{Ca}$ channels described previously. The channel was Ca^{2+} sensitive, its activity was stimulated by potassium channel opener NS1619 and inhibited by paxilline, well known mitoBK$_{Ca}$ channel inhibitor. Additionally, kinetics and conductance of observed channel were very similar to the mitoBK$_{Ca}$ channel. Based on collected data we conclude that BK-DEC splice variant forms a functional channel in the inner mitochondrial membrane of HEK293T cells.

Acknowledgements:
This work was supported by the Polish National Science Centre grant No.2015/18/E/NZ1/00737 and the Nencki Institute of Experimental Biology.

P8.7

The phenotype of the yeast *Saccharomyces cerevisiae* double mutants depleted of the copper-and zinc-containing superoxide dismutase (CuZnSOD) and voltage dependent anion channel (VDAC) encoding genes

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Superoxide dismutase (SOD) is regarded as a fundamental defense against superoxide anion generated mainly by mitochondrial respiration. Two forms of SOD have been identified in eukaryotic cells: a manganese-containing enzyme (MnSOD or SOD2) located in the mitochondrial matrix and a copper-and zinc-containing enzyme (CuZnSOD or SOD1) located in different cell types in various cellular compartments including the intermembrane space of mitochondria. In the yeast *Saccharomyces cerevisiae* cells CuZnSOD accounts for 90–95% of the total superoxide dismutase activity that makes the cells useful model in studies of CuZnSOD role in etiology of some diseases, e.g. neurodegenerative and autoimmunological ones. The voltage dependent anion channel (VDAC), the mitochondrial outer membrane protein, is regarded as pivotal for mitochondria functioning. VDAC functions as a major channel allowing metabolites and ions to pass between mitochondria and the cytosol. The channel may exist as isoforms encoded by separate genes, displaying different channel-forming activities and probably playing different roles. We have reported that CuZnSOD is important for yeast VDAC1 activity but the effect was not determined for yeast VDAC2 which we have recently shown to form a channel. Thus we decided to check the impact of the simultaneous absence of CuZnSOD and the VDAC isoforms on yeast cell phenotype as well as the functionality of human VDAC isoforms expressed in the obtained Δ*sod1* mutants.

P8.8

Flavonoid-induced changes in oxygen consumption and mitochondrial membrane potential in isolated endothelial mitochondria

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Many flavonoids have been shown to be cardioprotective. These natural substances show antihypertensive and antiatherosclerotic effects. They prevent platelet aggregation and progression of endothelial dysfunction. Some of these effects can be assigned to the antioxidant properties of flavonoids. However, numerous studies suggest that the key role in flavonoid-induced protective effects is played by the subtle changes in K^+ permeability of the inner mitochondrial membrane. In this study, we have examined the influence of several cardioprotective flavonoids on endothelial mitochondria. In mitochondria isolated from EA.hy926 cells, the rate of succinate-sustained non-phosphorylating respiration was enhanced in response to naringenin and quercetin, but not cyanidin. What is interesting, the effect was partially abrogated by iberiotoxin, a mitochondrial large-conductance calcium-activated potassium channel (mitoBK_{Ca}) blocker. Naringenin and quercetin induced a modest (~ 1mV) depolarization of mitochondrial membrane potential that was determined with a TPP^+-specific electrode. Again, the value of membrane potential was partially reestablished after treatment with iberiotoxin. The influence of Ca^{2+} on the flavonoid effect has been also examined. Thus, our results imply that flavonoids modulate oxygen consumption and membrane potential of endothelial mitochondria via their interaction with mitochondrial BK_{Ca} channel.

Acknowledgements:
This study was supported by a grant 2016/21/B/NZ1/02769 from the National Science Centre, Poland.

P8.9

Role of mitochondrial alternative oxidase in successful anhydrobiosis of the tardigrade *Milnesium tardigradum*

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Tardigrades are water dwelling, eight legged, segmented invertebrates which have the ability to withstand almost complete drying (dehydration) due to tun formation. This adaptation for periodic drying occurs naturally in their environment and is known as anhydrobiosis. At present, the recovery from the tun stage to the active stage is the only attainable evidence of successful anhydrobiosis. Nevertheless it is clear that the capability to recover is triggered by and based on cellular processes. Accordingly, it is known that tardigrade tuns show tolerance to high concentrations of potassium cyanide that imposes the role of mitochondrial alternative oxidase (AOX). The mitochondrial enzyme, known to protect cells under stress conditions, is present in plant, fungi and protist mitochondria but sparsely in animal ones. Genome/transcriptome analysis of three tardigrade species, including *Milnesium tardigradum* (our model organism) have shown the presence of functional *AOX* gene. Therefore we checked BHAM effect (AOX inhibitor) on tun formation and recovery to the active stage. Under the applied dehydration conditions and BHAM not toxic concentrations, BHAM appeared to delay recovery from the tun stage to active life. The obtained results suggest important role of mitochondria in successful anhydrobiosis and point to AOX as putative marker for estimation of anhydrobiosis success.

Acknowledgements:
The work was supported by the research grant of National Science Centre, Poland, NCN 2016/21/B/NZ4/00131.

P8.10

Influence of *Acanthamoeba castellanii* UCP protein expressed in yeast on viability of SOD1- and SOD2-deficient yeast under oxidative stress

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Mitochondrial uncoupling proteins (UCPs) are the inner mitochondrial membrane transporters. They mediate proton leak into mitochondrial matrix that uncouple electron transport in respiratory chain from ATP synthesis. Therefore, UCPs are energy-dissipating proteins decreasing reactive oxygen species production and affecting energy cellular metabolism. We analysed the impact of *A. castellanii* uncoupling protein (AcUCP) that was expressed heterologously in superoxide dismutase (SOD1 and SOD2)-deficient *Saccharomyces cerevisiae* cells. Because *S. cerevisiae* do not possess UCP, they can be used to confirm functionally AcUCP gene's product presence. We transformed SOD1- and SOD2-deficient yeast strains with the pYES2+AcUCP or empty pYES2 vectors. SOD knockout yeast were sensitive to oxidative stress that affected their growth. The growth of SOD1-deficient yeast was much more decreased compared to SOD2- deficient yeast. The abundance of AcUCP protein in yeast cells was confirmed by a mass spectrometry analysis. Moreover, we analysed yeast cell viability under oxidative stress conditions, i.e., in different concentrations of hydrogen peroxide added for 24h. In both SOD deficient yeast, especially in SOD1-deficient yeast, the AcUCP presence increased considerably their viability. Our results indicate that amoeba AcUCP may complement an antioxidative function of yeast mitochondrial superoxide dismutases.

Acknowledgements:
This work was supported by the National Science Centre, Poland (2016/21/B/NZ3/00333).

P8.11

The role of bleomycin hydrolase in mitochondria functionality

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Bleomycin hydrolase (BH) is a cytoplasmic, highly conserved cysteine protease known of its protective role against homocysteine thiolactone toxicity. The name of the protease comes from its ability to inactivate the glycopeptide bleomycin applied in cancer chemotherapy. It has been also shown that BH modulates expression of brain proteins in a mouse model of Alzheimer's disease, however the exact molecular mechanism of this interaction remains veiled. BH is also present in the yeast *Saccharomyces cerevisiae* often applied as a model in studies of pathogenesis of human diseases. Interestingly, we observed that the yeast *Saccharomyces cerevisiae* BH depleted mutant cells (D*bh*) are unable to grow on media containing non-fermentable carbon source but grow on media containing fermentable carbon source although distinctly slower that the isogenic wild type cells. This observation suggested some problems with mitochondria functionality. Accordingly, our measurements of oxygen uptake rate enabling determination of mitochondria coupling status both in intact cells and isolated mitochondria indicated at serious problems with the mitochondrial respiratory chain functionality. The possible explanation is a putative regulatory function of the BH but to explain its molecular background further research is needed.

Acknowledgements:
Financed from the project number 2014/15/B/NZ2/01079, granted by the Polish National Science Centre.

P8.12

Metabolic markers of active and anhydrobiotic tardigrades – preliminary results

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Anhydrobiosis is known as a desiccation tolerance that denotes the ability to survive almost complete dehydration without sustaining damages and has been reported for invertebrates like rotifers, nematodes or tardigrades. Tardigrades are considered the most indestructible animals on our planet. The analysis of available data suggests that anhydrobiosis success requires proper carbohydrate and lipid metabolism. Therefore we decided to compare metabolic profiles of active and anhydrobiotic tardigrades (so called "tuns") to find metabolic marker(s) allowing discrimination between active and anhydrobiotic tardigrades. In the study we used active and anhydrobiotic specimens of terrestrial tardigrade – *Echiniscus testudo* (Doyère, 1840). To follow putative alterations of metabolism we applied a metabolomic approach, i.e. untargeted metabolomic profiling based on gas chromatography-mass spectrometry (GC-MS). The settled methodology allowed us for detection of different metabolites and determination of metabolic differences between the active and tun stages. The detected unscrambled metabolites represented mainly amino acids, monosaccharides, carboxylic acids, membrane lipids and some products of the tricarboxylic acid (TCA) cycle. The approach applied for tardigrade species differing in anhydrobiosis capability should provide metabolic marker(s) of successful anhydrobiosis.

Acknowledgements:
The work was supported by the research grant of National Science Centre, Poland, NCN 2016/21/B/NZ4/00131.

P8.13

Mitochondrial stress response in *parkin* mutant fibroblasts derived from patients with Parkinson's disease

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The *parkin* gene is one of the most frequently mutated genes in familial cases of Parkinson's disease. Its protein product belongs to E3-ubiquitine ligase family, and due to its enzymatic activity mediates numerous cell processes, including mitochondrial turnover and dynamics. It is believed that dysfunction of mitochondria is tightly connected with pathophysiology of Parkinson's disease. Therefore, we investigated mitochondrial stress response in fibroblasts derived from patients with clinically diagnosed Parkinson's disease carrying mutations in the *parkin* gene. Mitochondrial properties were estimated by measurements of reactive oxygen species production level, mitochondrial membrane potential, mitochondrial mass, and levels of main proteins involved in mitochondrial turnover and dynamics.

P8.14

Mitochondrial dynamics and function in bronchial epithelial cells after long-term exposure to total particulate matter from a candidate modified-risk tobacco product and reference cigarette

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Smoking cessation remains the most effective approach for minimizing the risk of smoking-related diseases. However, reduction of harmful constituents by heating rather than combusting tobacco, without modifying the amount of nicotine, is a promising new paradigm in harm reduction. Such approach was applied in Tobacco Heating System 2.2 (THS 2.2).

In this study we compared the effects of chronic (12 weeks) treatment of BEAS-2B bronchial epithelial cells with THS 2.2 aerosol (7.5, 37.5 and 150 mg/ml) or with total particulate matter (TPM) from 3R4F reference cigarette smoke (7.5 mg/ml) on oxidative stress, mitochondrial morphology and function.

Treatments with 7.5 mg/ml TPM from 3R4F or with 150 mg/ml of THS2.2 led to alterations of mitochondrial network morphology and to changes in the levels of proteins regulating mitochondrial fusion and fission (decreased mitofusins levels and Drp1 phosphorylation). 7.5 mg/ml TPM from 3R4F and 150 mg/ml THS2.2 aerosol had opposing effects on respiration rates (respectively, increase and decrease in basal respiration rates). Both treatments resulted also in increased protein oxidative damage, however cytosolic ROS levels were not changed by any of the treatment.

Together, the findings indicate that 3R4F TPM had a stronger effect on oxidative phosphorylation, and proteins involved in oxidative stress than TPM from the candidate modified-risk tobacco product THS2.2.

Acknowledgements:
The research was sponsored by Philip Morris International.

P8.15

Mutation of genes participated in mitochondrial quality control in PD patients

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Parkinson's disease (PD) is associated (5 - 10%) with mutations in many genes, like *PRKN* and *PINK1* found to participate in the quality control of mitochondrial function. Parkin, encoded by *PRKN* is an E3 Ub ligase. Mutations in *PRKN* genes cause early-onset PD and regulate mitochondrial homeostasis in collaboration with another early onset-PD gene product, PINK1 which is a serine/threonine protein kinase. Loss of transmembrane potential in damaged mitochondria leads to the accumulation and activation of PINK1 on the mitochondrial outer membrane. The activated PINK1 recruits Parkin to the mitochondria, stimulates Parkin E3 activity, promoting mitophagy. Tom70 TOM receptor subunit is crucial for PINK1 import into the mitochondria. In this study we investigated the mutation in *PINK1*, *PRKN* and genes encoding TOM receptor subunits: Tom70 and Tom20 in the 30 PD patients. For each studied genes we obtained rare and yet unknown heterozygote polimorphism located in the functional domains. In the case of TOM70 the SNP was found in the 3'UTR, found as a binding side for miRNA involved in gene regulation.

P8.16

Lipophilic antioxidants in blood plasma HIV-infected patients treated by antiretroviral drugs

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Numerous data on hepatotoxic effects of various drugs suggest that liver disease medications are one of the most important problems of modern hepatology. Drug-induced liver injury (DILI) associated with HIV treatment has represented an one of the most important side effect since the beginning of the highly active antiretroviral therapy (HAART) era. The lack of standard definition and specific markers makes assessment of DILI very challenging. Several clinical syndromes of DILI have been described over the years; but the pathogenic mechanisms are not fully understood. Mitochondrial oxidative damage contributes to a wide range of pathologies. One therapeutic strategy to treat these disorders is targeting antioxidants to mitochondria. The concentration of Coenzymes Q (CoQ) and alpha-tocopherol, are two highly lipophilic antioxidants which can be dissolved only in lipid layers or attached to cell protein structures, were measured in 132 HIV-infected patients, 20% of which are not receiving HAART.

Results. Increased concentration of CoQ and α-tocopherol, and the positive correlation between them ($R=0.47$; $p<0.0001$) in the blood plasma of patients with symptoms of DILI is one of hepatocyte protective mechanism against side-effect of drugs used in the treatment of HIV infection. CoQ is a mitochondrial targeted antioxidant that plays an essential role in the normal function of the electron transport chain. Was determined activation of mitochondrial antioxidant defense components, confirmed an increase in the plasma content CoQ by 25.3% from baseline, is proof of the interest of mitochondrial metabolism in the leveling of the destructive effects of lipid peroxidation in the mitochondria and the cell as a whole.