Title:

Role for mitochondria on the response of highly proliferative and invasive bladder cancer cells to the combined inhibition of mTOR and SIRT1

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Abstract:

Bladder cancer (BC) has a high incidence and recurrence rate. In addition, patients have a poor survival expectancy. Multiple signaling pathways that interact with mitochondria are involved in events related to tumor aggressiveness and growth. Thus, molecular classification and characterization of the tumor is pivotal to predict clinical outcomes, responses to chemotherapy and develop novel treatments. There are several targets towards personalized medicine, including mitochondrial DNA, mitochondrial metabolic enzymes and cellular signaling proteins. Among those, the mammalian target of rapamycin (mTOR) and NAD-dependent deacetylase sirtuin-1 (SIRT1) are known to independently mediate some cancer related features and mitochondria functioning. Herein we aimed to characterize how the activation or inhibition of SIRT1 and/or mTOR modulate the metabolic and bioenergetics profiles of highly proliferative and invasive stage IV BC cells. For that purpose, TCCSUP cells (BC stage IV) were cultured during 24 h in a normal media or supplemented with EX527 (SIRT1 inhibitor); YK-3-237 (SIRT1 activator) and Rapamycin (mTOR inhibitor), as well as in a combined treatment of EX527+Rapamycin. In addition to cytotoxicity and migration tests, we determined the metabolic profile (metabolic fluxes, ^{1}H-NMR), expression of membrane transporters (GLUT3 and MCT1/MCT4, qPCR), mitochondrial potential (JC-1 fluorescence), intact cell respirometry (Clark-type electrode) and mitochondrial copy number (qPCR) of the cells from each experimental group. Our results show that mTOR inhibition alone or in combination with SIRT1 activation decreased cell density in BC cells. In addition, mitochondrial potential of BC cells was repressed after exposure to the combined treatment of mTOR inhibition with SIRT1 activation/inhibition. In parallel with this,

BC cells presented mitochondrial proton leak stimulation, with increased acetate consumption and decreased lactate production after the combined treatment with SIRT1/mTOR inhibitors. Interestingly, inhibition of mTOR alone upregulated the levels of transporters such as GLUT3 and MCT4, but there was no direct action on the levels of metabolites transported by these transporters. On the other hand, activation of SIRT1 downregulated the levels of MCT1 but again, it did not affect lactate levels in the extracellular medium. Overall, our results show that the combined inhibition of mTOR and SIRT1 in highly proliferative and invasive BC cells affects mitochondria physiology, which may elicit positive effects on the treatment of bladder cancer. Nevertheless, although our data shows promising results in the response of highly proliferative and invasive BC cells to the combined treatment with combined mTOR and SIRT1 inhibitors, this is a first assessment of the metabolic and bioenergetics profile of these cells. Further studies will be needed to unveil the molecular mechanisms by which mitochondria mediates the positive response of highly proliferative and invasive BC cells to the combined inhibition of mTOR and SIRT1.

Acknowledgements:

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High fat-load induces cardioprotection in hearts from obese mice

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An ischemic insult is associated with increased circulating fatty acids (FA) due to an adrenergic activation of adipose tissue lipolysis. Therefore, hearts will not only be challenged by hypoxia, but also by an acute FA-load, which has been shown to induce adverse cardiac effects such as mitochondrial dysfunction, oxidative stress, oxygen wasting and inefficiency. Although obesity is a contributing factor to the development of type 2 diabetes and heart failure it remains unclear if and how obesity-associated chronic hyperlipidemia affects the cardiac response to an acute FA-load. Thus, we have examined the effect of high FA on hearts from a murine model of obesity.

Diet-induced obesity (DIO) was obtained by feeding 5-wk old male C57BL/6J mice obesogenic diet for 20 wks. Age-matched chow-fed mice were included as controls (CON). *Ex vivo* left ventricular (LV) function (working heart perfusions, n=8-11) and ischemic susceptibility (LV post-ischemic functional recovery and infarct size, Langendorff perfusions, n=12-15) were examined in hearts exposed to normal (0.35 mM) or high (1.8 mM) palmitate levels. We also assessed myocardial O_2 consumption (MVO_2), FA oxidation and mechanical efficiency (n=12-15), as well as myocardial ROS content (DHE tissue staining) and mitochondrial respiration (high-resolution respirometry, n=6-8).

DIO mice demonstrated elevated plasma FA levels (0.37±0.03 vs 0.58±0.04 mM, $p<0.01$) and insulin resistance (4.4 fold higher HOMA-IR). They also developed diastolic dysfunction with only a mild systolic dysfunction. High FA perfusion did not alter LV function in neither CON nor DIO hearts. However, elevated FA decreased mechanical efficiency (due to increased MVO_2, 28±2 vs 37±2 µmol/min/g, $p<0.01$), induced oxidative stress and reduced mitochondrial OXPHOS rate and coupling (RCR) in CON hearts. These FA-mediated changes were not found in DIO hearts. Furthermore, in contrast to CON hearts (where ischemic tolerance was not altered by the FA-load), DIO hearts exposed to high FA levels showed increased functional recovery (53±5 vs 36±5 % Rate-Pressure-Product, $p<0.01$) and decreased infarct size (47±2 vs 62±5%, $p<0.02$). This cardioprotective effect was corroborated in hearts from obese, type 2 diabetic (*db/db)* mice (54±6 vs 36±5 % recovery of RPP, $p<0.05$, and 55±5 vs72±2 % infarction, $p<0.01$, respectively).

This study shows that hearts from obese/diabetic mice are resistant to the adverse effects an acute FA-load. Although dyslipidemia plays a role in the development of obesity/diabetes-mediated heart failure, we suggest that these hearts undergo adaptive changes where elevated FA levels exert cardioprotection.

MiP School – Tromso Bergen 2018 – Abstract

Concomitant respiration and ATP production measurements to analyse P»/O_2 ratios at physiological normoxia

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Most of the studies regarding mitochondrial respiration are performed in hyperoxic conditions in comparison to the physiological O_2 concentrations in the cell. However, there are evidences that mitochondrial phosphorylation efficiency increases in lower partial O_2 pressures (pO_2) [1]. The mitochondrial respiration, and especially coupling control ratios, have been broadly used as parameters to assess mitochondrial function and efficiency. Nevertheless, those methods allow to analyse the O_2 consumed coupled to the phosphorylation by ATP synthase in OXPHOS state, but not the ATP production itself. A method to measure ATP production using a Mg^{2+}-sensible fluorescent probe (Magnesium Green, MgG), exploiting the differential binding of ADP and ATP to Mg^{2+} has been developed and can be used concomitantly to respiration assays in the Oroboros O2k FluoRespirometer [2,3]. Therefore, we aim to further develop the use of these two methods combined to analyse P»/O_2 ratios in different samples, and especially in physiological normoxic conditions. This will allow to explore the contribution of different pathway control states (e.g. NADH, succinate and glycerophosphate pathways) at physiological intracellular normoxia.

In this context, we intend to use mouse heart isolated mitochondria to first analyse whether the P»/O_2 ratios differ when measured at physiological normoxia and at the conditions typically used in most of these assays, performed at ambient O_2 levels that lead to hyperoxic conditions. Physiological normoxia will be obtained by injecting N_2 in the chambers of the O2k FluoRespirometer in order to set-up the oxygen partial pressure desired.

By assessing mitochondrial O_2 consumption under physiological normoxia in parallel with ATP production using MgG, as well as membrane potential using safranin, and ROS production using the probe for H_2O_2 Amplex UltraRed, we expect to provide a more comprehensive view of the mitochondria physiology. This can be further applied to study the impact of ischemia-reperfusion injury on cardiac muscle mitochondria, which occurs in diseases such as myocardial infarction and also in transplantation, having a great impact in public health.

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Title:

Mitochondrion at the crosstalk between metabolic disease and dysfunction in male fertility?

Authors:

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Abstract:

Male fertility parameters have declined worldwide in recent decades [1], whilst the prevalence of metabolic diseases has increased . Lifestyle, particularly poor food habits linked to high-fat diets, are regarded as a leading cause for the present trend [2], but the biochemical link between diet, metabolic disease and poor male fertility potential is not known yet [3]. Diet reversion (DR) is the first prospective treatment for metabolic disease, but it is not clear to what extent it is effective in recovering male fertility parameters.

In this study we describe the effect of diet reversion in the metabolic and reproductive function of mice. To achieve this, 3 groups of 12 mice were fed with different diets (CTRL – standard mucedola; HFD – high-fat diet and HFD_t – high-fat diet for 60 days, then replaced by standard mucedola). Animals were kept in environmental challenging cages for 200 days after weaning, with unrestricted water and food supply. Mice weight, and food and water intake were monitored during the experiment. Metabolic stress tests (IPGTT and IPITT) were performed a week before sacrifice. After sacrifice, several tissues were collected and weighted. Testicular tissue was used for metabolomics analysis by ^{1}H-NMR. Sperm was obtained from epididymis, and mice fertility determined.

Our results show that DR was effective in recovering the body weight and metabolic function in mice: HFD_t mice quickly approximate CTRL mice weight after diet replacement, and both groups perform similarly during the metabolic stress tests. However, HFD and HFD_t mice had significantly lower sperm viability and motility than CTRL mice, depicting a limited effect of DR in reproductive function. Metabolomics analysis revealed an altered lipid and energy metabolism in HFD mice, coupled to increased oxidative stress (Figure 2). Notably, HFD_t mice

presented a significant increase in testicular succinate content, compared to other groups. This accumulation may explain the unique characteristics presented by this group, and elicit a mitochondrial involvement in the changes promoted by DR [4].

Overall our data suggest that high-fat diet compromises male fertility and DR has limited impact on the affected parameters. Thus, weight gain may cause permanent damage on male reproductive tract. Testicular metabolic changes promoted by DR prospect the role of mitochondria as the “missing link” between high-fat diet and male reproductive dysfunction.

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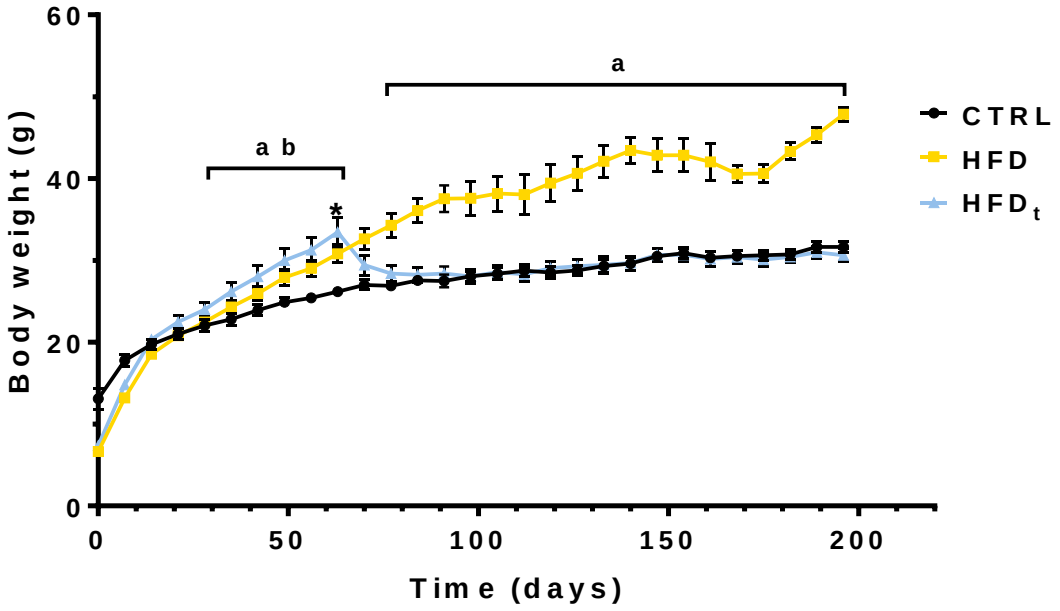

Figure 1: Mice body weight (g) throughout the experiment. Weight of each mice was measured every 7 days. Results are expressed as mean (g) ± SEM (n = 12

for each group). Results were tested by one-way ANOVA with Tukey's Multicomparison test. Statistical significance was considered when $p \leq 0.05$. a) CTRL vs. HFD; b) CTRL vs. HFD_t; * Diet Reversion of the HFD_t mice.

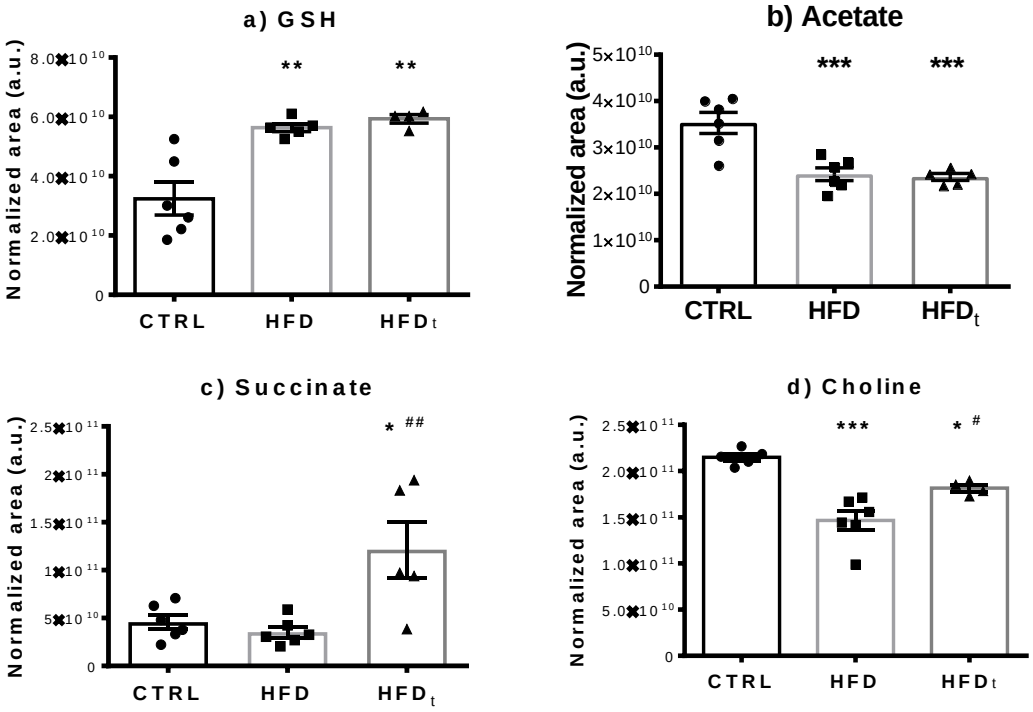

Figure 2: Absolute quantification of a) GSH – Reduced glutathione, b) acetate, c) succinate and d) choline using ^{1}H-NMR. Results are expressed as mean ± SEM (n = 6 for each condition). Results were tested by one-way ANOVA with Tukey's Multicomparison test. * Significantly different results relative to control ($p < 0.05$). $^{\#}$ Significantly different results relative to HFD ($p < 0.05$).

Analysis of mitochondrial function in iron deficiency anemia and iron overload conditions

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Iron is an essential co-factor for a variety of metabolic processes and mitochondria are the main sites of iron utilization as they need iron for mitochondrial respiration [1,2]. Iron deficiency negatively affects cellular energy metabolism and iron overload increases the production of reactive oxygen species (ROS) through the Fenton reaction [2]. Therefore, the consequences of iron deficiency anemia and iron overload conditions on mitochondrial function need to be investigated.

Iron deficiency anemia and iron overload will be investigated in different mouse models with dietary and genetic predisposition to these diseases. Mitochondrial respiratory capacity will be investigated by means of high resolution respirometry (Oroboros, Innsbruck) in isolated mitochondria from liver, heart and brain. In addition, the usage of AmplexUltraRed will provide knowledge about the effects of the two pathologies on mitochondrial respiratory capacity and ROS production [3].

The subsequent comparison of the effects on mitochondrial respiration and ROS production will eventually show individual characteristics caused by the individual disease origin (dietary vs. genetic iron deficiency anemia and dietary vs. genetic iron overload) of the pathologies. As we showed before, dietary iron overload leads to an impairment in mitochondrial respiratory capacity partly due to iron-mediated oxidative stress within mitochondria [2]. However, little is known about the effects of iron deficiency anemia on mitochondrial respiration and their possible implications with the patients' symptoms like weakness and fatigue. Therefore, it is crucial to further identify the effects and underlying causes of these two pathological conditions in order to be able to provide improved treatment options for patients.

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Phenotyping mitochondrial metabolism in Barrett's metaplasia-dysplasia-adenocarcinoma sequence: respiratory capacity, extracelular proton flux and ROS production

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With an increasing global incidence of cancer, prevention and therapy is one of the major public health challenges of the 21st century. According to the data of the Global Cancer Observatory, approximately 18,1 million of new cases of cancer are expected in 2018. Esophageal cancer (EC) accounts for 3,2% of the total number of new diagnosed cases.[1] Its severity is reflected in the fact that it is responsible for more than half a million deaths per year and has a 5-year survival rate of only 18%. The main causes for the devastating influence of esophageal cancer are the lack of convenient biological markers and effective treatments. Metabolism of cancer cells is highly adaptable and expresses great plasticity when it comes to shifting the metabolic pathways with one ultimate goal: to survive and to spread. Numerous advantages are ascribed to such changes, the generation of the energy for life and building blocks for growth being the main one.[2,3] Recently, dysregulated pH emerged as a key player as a survival mechanism in mammalian cancer cells and it is also conferring to them properties of resistance to chemotherapy and invasiveness.[4,5] Whether it is a cause or consequence, this hallmark of most cancers contributes to the metabolic shift and to changes in their microenvironment. Also, the alteration in ROS production has been described as beneficial for cancer cell genesis and survivability. ROS are vital for various cellular processes in both healthy and cancer cells but can also cause oxidative stress in excessive amounts, leading to genetic and functional damage. It is supposed that limiting the Oxidative phosphorylation in cancer cells have protective role in the sense of limiting the amount of ROS generated. [3,6]

Our plan is to establish SUIT protocols for functional analysis of respiratory capacities, extracellular proton flux and ROS production in cell lines and human tissue samples of esophageal mucosa and to investigate the influence of different extracellular pH values on the above mentioned parameters. Following human cell lines will be included: radioresistant SCC-25, radiosensitive SCC-090 and HGF cell lines as control.

Experiments are going to be performed using High-Resolution FluoroRespirometry in order to measure mitochondrial respiration, ROS production rate and extracellular proton flux production. Esophageal cell culture models will be used and compared with fresh human esophageal biopsies representing non-cancerous cells/tissue threat different succeeding stages in the Barrett's metaplasia-dysplasia-adenocarcinoma-sequence. Before starting experiments with experimental cell lines and samples of human tissue, training with HEK239 cells, quality control and proficiency test must be accomplished a proof of competence, skills and research quality. This study is directed towards discovery of new tools for diagnosis and potential targets for a new therapeutic approach in the treatment of esophageal cancer.

Key words: cancer, mitochondrial physiology, ROS, pH, esophagus, human tissue

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1. OXPHOS efficiency in skeletal and cardiac muscles: Proton leaks, ROS and glutathione redox

Oxidative phosphorylation (OXPHOS) is estimated to account for roughly 90% of cellular ATP production, though this can vary between cell types, metabolic conditions, and disease states. The efficiency of ATP production by OXPHOS is highly variable, and efficiency can be expressed as units ATP produced per unit of substrate oxidized, or as units ATP produced per unit of oxygen consumed. Efficiency of OXPHOS is affected by site of electron entry into the electron transport system. It also is greatly affected by proton leak uncoupling, and other routes of proton return to the mitochondrial matrix. Proton leak has poorly understood mechanisms, but includes the uncoupling proteins and the adenine nucleotide translocator (ANT). Beyond decreasing the efficiency of OXPHOS, proton leak decreases emission of reactive oxygen species (ROS) from the electron transport system by lowering protonmotive force. High levels of ROS can cause damage to cellular lipids, proteins, DNA and RNA. However low levels of ROS are important in cell signaling processes. Glutathione is the major non-protein antioxidant in cells and in mitochondria. It also plays important roles in the post-translational modification of protein thiols, and this can protect the proteins from further damage, and can modify the function of the proteins. The latter concepts will be covered in this lecture, and experimental findings from studies of skeletal and cardiac muscles will be presented.

2. Deacetylation acceleration of BAT thermogenesis

In some small mammals, the activation of brown adipose tissue (BAT) can cause a doubling of resting metabolic rate. This remarkable increase in whole body oxygen consumption and thermogenesis relies heavily on uncoupling protein-1 (UCP1) which is expressed in BAT and in beige adipose tissue. The capacity of BAT for such high rates of oxygen consumption and thermogenesis stems from 1) a dense mitochondrial network in BAT, 2) a very high amount of UCP1 per unit mitochondrial protein (10%), and 3) extensive capillary networks and arteriovenous anastomoses that rapidly efflux heat into the blood circulation. When BAT is activated the major sources of fuel are fatty acids, which are oxidized by beta-oxidation, and the ensuing reducing equivalents/electrons then drive electron transport system (ETS) activity. These oxidative reactions are responsible for the actual thermogenesis. Importantly, fatty acids also activate UCP1 protein, and this is thought to occur through interfering with purine nucleotide inhibition of UCP1. When UCP1 is active, it allows protons to leak back into the mitochondrial matrix; this stimulates ETS activity because it removes the back-pressure of proton motive force, that would otherwise prevent ETS activity. In other words, it removes the brakes on the system, and uncoupled respiration can occur at very high rates.

Despite the recent excitement emanating from the discovery that BAT is present in adult humans, there is only a rudimentary understanding of the mechanisms that turn BAT on and off. Recent developments in the area of BAT have focused on 1) characterizing the amount of active BAT in adult humans, 2) brown fat adipogenesis and the common cellular origins of brown adipocytes and muscle cells, 3) the mechanisms through which beige adipose tissue develops and is activated, and 4) the role of redox and reactive oxygen species (ROS) in the activation of UCP1 in BAT. One aspect of BAT physiology that is not well understood is the role of deacetylation control of BAT mitochondrial energetics and thermogenesis. Indeed, the role

of post-translational control of BAT proteins in general is poorly understood. We reasoned, given that fatty acid oxidation is exceptionally high in active BAT, it would be informative to examine the impact of mitochondrial protein acetylation in active *versus* inactive BAT in the presence and absence of the mitochondrial deacetylase, SIRT3. It is well known that the acetylation of mitochondrial proteins can be particularly high when rates of fatty acid oxidation are high, due to the high rates of acetyl CoA production. Acetylation status of BAT mitochondrial proteins was investigated using label-free acetylomics. We quantified over 2000 acetylation sites, and our findings from acetylome profiling in BAT mitochondria, and corresponding functional assays will be described.

Two populations of muscle mitochondria: heart and skeletal muscle

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Based on morphology of cardiac muscle cells, mitochondria are located under the sarcolemma, between the myofibrils, and in the central perinuclear region. Using concepts of cellular enzymology, protein yields, transmission electron microscopy (TEM) by Drs. Bernard Tandler and Hisashi Fujioka, high-resolution scanning electron microscopy (HRSEM) with Dr. Alessandro Riva, and functional studies with Dr. June Palmer, methods were devised to remove the sarcolemma from heart tissue producing a skinned myofibril releasing of a specific population of mitochondria, dubbed subsarcolemmal mitochondria (SSM). The resultant myofibril pellet was then subjected to protease treatment to disrupt the fibrils, leading to liberation of mitochondria from the interfibrillar space (IFM). The overall recovery of mitochondria was 70 -80% with only 5% released into the supernatants and the remaining ~20% entrapped in the tissue debris consisting largely of digested myofibrils. The content of mitochondrial enzymes and activity of oxidative phosphorylation is @150% in IFM compared to the SSM. Of the multiple examples of pathophysiological changes found only in one population, we will focus on the cardiomyopathy in the Syrian Hamster (only IFM affected), diabetes mellitus in the rat (only SSM affected), and aging in the rat heart (only the IFM involved). Because of the location of the IFM and t-tubule system in proximity of the intercalated discs, the involvement and interaction of these with Ca2+ signaling in cardiac excitation-contraction coupling (E-C) has been explored.

In skeletal muscle there is controversy as to whether the mitochondria exist as a reticulum or as distinct entities. There are more studies published using the two populations of skeletal muscle mitochondria than from heart. We were unable to prepare purified SSM from skeletal muscle as judged by transmission electron microscopy, because of the presence of vesicles of undetermined origin. Attempts to remove these contaminants were unsuccessful (unpublished with Dr. Linda Brady). When we switched from using the Polytron homogenizer to disrupt the sarcolemma in muscle tissue to using proteolytic enzymes (collagenase for heart and dispase for skeletal muscle) to expose the sarcolemma so that a gentle physical force could be applied (Potter-Elvehjem homogenizer) to disrupt that plasma membrane, we observed a remarkable diminution in the presence of vesicles in the SSM. Nicola Lai modified the skeletal muscle procedure to maximize SSM and IFM recovery (overall ~80%) while preserving mitochondrial integrity, function, and structure. We will discuss the skeletal muscle mitochondrial observations in a model of pacing-induced heart failure in a dog model. The decrease in respiratory rates of skeletal muscle SSM are neither relieved upon collapsing the mitochondrial potential with an uncoupler nor increased in the presence of maximal ADP concentrations, showing a defect in the ETC. In contrast, respiratory rates of skeletal muscle IFM from HF were relieved with the uncoupler and partially improved in the presence of maximal ADP concentrations. These IFM alterations in the phosphorylation apparatus were detected with a decreased amount of ANT isoform 2 and increased amount of isoform 1. The IFM dysfunction may be explained by this shift in ANT isoforms.

In conclusion, the study of the two populations of muscle mitochondria is necessary to truly understand the presence and type of dysfunction.

Mitochondrial function during ischemia/reperfusion injury

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Multiple studies have documented that myocardial ischemia results in progressive damage to the mitochondrial electron transport chain. Oxidative phosphorylation has been used to track the sequence and localize the area of damage. In early ischemia, glutamate oxidation is decreased and appears due to a defect in complex I, as well as in complex V and the adenine nucleotide translocase. These defects are resolved with reperfusion. Subsequently, a decrease in succinate oxidation occurs with prolonged ischemia suggesting a defect distal to complex I, which persists with reperfusion. The subsarcolemmal mitochondria (SSM) located under the sarcolemma are affected earlier in ischemia that those mitochondria between the myofibrils (interfibrillar, IFM). In rabbit heart with prolonged ischemia, we have identified a defect in cytochrome *c* oxidase, a loss of the phospholipid, cardiolipin, as well as of cytochrome *c* from the SSM only. Reperfusion did not further damage the mitochondria as these defects persist, but do not progress. Blockade of the electron transport chain with the irreversible inhibitor, rotenone, modulated the ischemic damage with preservation of both cardiolipin and cytochrome *c* content of the SSM while improving the oxidation through complex IV.

In the isolated buffer-perfused rat heart, global ischemia decreases oxidative phosphorylation and damages the distal electron transport chain, with decreased complex III activity, cytochrome *c* content, and respiration through complex IV in both SSM and IFM. The defect in complex III has been identified as a functional loss of the iron-sulfur center of the Rieske iron-sulfur protein (ISP) without loss of the subunit peptide. In the aged rat heart, oxidative function is decreased in the IFM only with the defect localized to complex III at Qo site. At the onset of reperfusion in the aged heart, IFM contain two tandem defect in Complex III, which provides a mechanism for the enhance oxidant production and reperfusion damage. Resolution of the aging complex II defect in the aged heart resolves the combined defect and the treated-aged heart now behaves like a young heart.

High-Resolution FluoRespirometry: new perspectives for the study of bioenergetics in algae and plants.

Maria Huete-Ortega, Timea Komlodi, Erich Gnaiger

Abstract:

Bioenergetics is the study of energy transformations in cells, mitochondria, and chloroplasts as a basis to perform biochemical work. In chloroplasts solar energy is acquired and assimilated through photosynthesis to synthesize the organic matter required for growth, which is associated with production of oxygen, while mitochondrial respiration consumes oxygen to transform organic matter into the chemical energy that fuels cellular activity. Energetic coupling between chloroplasts and mitochondria has been described. For example, mitochondrial physiology is involved in mitigating light stress in the photosynthetic pathway or chloroplast-generated reducing equivalents replace photophosphorylation as a source of ATP in the mitochondria. Therefore, a good functionality and cross-talk between both organelles is necessary to maintain metabolic integrity in plant cells. High-Resolution FluoRespirometry (HRFR) is widely used to assess mitochondrial respiration and other bioenergetic parameters of isolated mitochondria, cultured cells, tissue preparations and human biopsies. In the biomedical field of mitochondrial physiology and its clinical applications, studies with HRFR relate to degenerative diseases and life style-linked preventive medicine. My current research aims to extend the experimental options of the Oroboros O2k developed by Oroboros Instruments for HRFR to the study of mitochondrial respiration, photosynthesis and other bioenergetic parameters in algae and plants, including the development of new technology such as using a three-electrode system [1,2] inserted into the Oroboros Q2k to evaluate the redox state of the Coenzyme Q.

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Oscillations in mitochondrial ROS production during the early cell cycles in Xenopus embryos

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Abstract

The ability to repair and regenerate tissues is an essential process for the survivability and development of the organisms. Amphibians excel on these processes and are invaluable models to study the molecular and cellular mechanisms underlying scar free wound healing and tissue regeneration. Among these, we have used the african clawed frog, *Xenopus,* as an animal model to study the role of reactive oxygen species (ROS) during the early embryonic development and appendage regeneration. Both embryonic development and tissue repair/regeneration require cell proliferation, which relies on the synchronized mechanisms that regulate the cell cycle [1]. The mitochondrion is the powerhouse of the cell but it is also involved in other processes such as cellular signaling and calcium buffering. However, the roles of mtROS during early vertebrate development have remained largely unknown. For this reason, our main aim is to understand how the mitochondria, metabolism and ROS are regulated during early development and tissue regeneration. We have recently shown, using transgenic *Xenopus* frog embryos expressing the genetically encoded ROS indicator HyPer, that mtROS is increased after fertilization and that it oscillates during each cell division. When we exposed the embryos to mitochondrial inhibitors we observed that complex II is the primary source of ROS *in vivo* and that the inhibitors differentially affect the oscillatory patterns of ROS production. Furthermore, in order to identify the

source of mtROS in the electron transfer system, we performed a study of mitochondrial function in a cell-free system (i.e. egg extract) combining High-Resolution FluoRespirometry, hydrogen peroxide production and membrane potential [2]. Our study reveals that the succinate dehydrogenase complex (CII), specifically the flavoprotein in the SdhA subunit, is the major source of mtROS when the mitochondria are fuelled by succinate. Also, we have found that the calcium acts upstream of ROS production from the mitochondria. Finally, we have discovered that the ATP levels also oscillate during the cell cycle and our results suggest that cellular metabolism alternates between aerobic glycolysis (Warburg Effect) and oxidative phosphorylation in association with the cell cycle. Our results highlight an entanglement between calcium, metabolism and ROS but further work is required to understand how these processes are related to the cell cycle and its relevance for the early development and tissue regeneration.

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AMPK deficiency elicits changes in OXPHOS in heart mitochondria.

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AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme, which is a sensor and regulator of the energetic state of the cell. Its activation results, among others, in increase of glucose uptake, mitochondrial fatty acid oxidation and mitochondria biogenesis. The role of AMPK for mitochondrial function in cardiac tissue has not been deeply investigated.

The purpose of this study was to assess whether the loss of AMPK in heart induces any alterations in mitochondrial physiology and whether potential changes are gender specific.

Heart-specific double (α1 and α2 subunits) AMPK deletion was obtained in a model of a conditional tamoxifen-inducible KO mouse. Experiments were carried out with heart mitochondria, isolated from control and KO mice, both male and female. Mitochondrial respiration was measured with a Clark-type electrode, with substrates for Complex I (glutamate, malate), II (succinate), IV (TMPD), as well as with palmitoyl carnitine and DNP as uncoupling agent. Enzymatic activities of complex I and carnitine palmitoyl transferase 1 and 2 were assessed by a spectrophotometric assay. In addition, markers of mitochondrial density, such as citrate synthase activity have been analyzed. Our results show that

AMPK-deficiency results in defects in OXPHOS and altered mitochondrial mass, and that some of these changes are gender-specific. The data indicate that loss of cardiac AMPK has some consequences already observed for skeletal muscle, but also additional effects. In particular, they reveal sex-specific responses possibly related to gender-specific metabolic differences in the heart.

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Dietary and pharmacological treatment of diet-induced obese mice – impact on mitochondrial function

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Aims: To determine the impact of (i) dietary supplementation with Calanus oil (a novel marine oil extracted from the marine copepod *Calanus finmarchicus*) and (ii) infusion of exenatide (a GLP-1 anaog) on mitochondrial respiration in various organs of obese mice, including adipose tissue, liver, heart and skeletal muscle.

Methods: Six weeks old female mice (C57bl/6J) received a high-fat diet (HFD, 45% energy from fat) for an initial period of 12 weeks. Thereafter they were separated in 3 subgroups which were fed with either HFD, HFD supplemented with 2% Calanus oil (HFD+Cal), or HFD combined with administration (via mini-osmotic pumps) of 10 µg/kg/day exenatide (HFD+Ex). Mitochondrial respiration was measured in homogenates of liver, heart, and intra-abdominal white adipose tissue (WAT), as well as in permeabilized fibers of skeletal muscle.

Results: Preliminary data indicate that respiration was reduced in liver, WAT and skeletal muscle of (HFD+ Cal) mice compared to the non-supplemented HFD group, while it was increased in cardiac tissue. Exenatide also seemed to reduce respiration in skeletal muscle and heart, while increasing respiration in liver and WAT. These data will be presented and discussed during the training school.

Conclusion: Dietary supplementation with Calanus oil, as well as administration of exenatide, impacts on mitochondrial respiration in major organs of mice fed a high-fat diet. The data need, however, further interpretation and statistical evaluation.

Cardioprotective flavoniods as a natural modulators of mitoBKCa channel.

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Potassium channels such as K_{ATP}, BK_{Ca} or Kv1.3 have been found in the inner mitochondrial membranes of various cells. It is believed that potassium channels regulate the mitochondrial membrane potential, matrix volume, respiration, and Ca^{2+} ion homeostasis. There are hypotheses that mitochondrial BK_{Ca} channels play an important role in ischemic preconditioning. It was also shown that mitoBK_{Ca} channels are potential targets for some flavonoids in the anti-ischemic strategies.

Our pervious study, showed functional properties of the mitoBK_{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 reported in glioma, skeletal muscle, brain and cardiac.

In the current study, single channel activity of the mitoBK_{Ca} channel was measured with patch-clamp technique of the mitoplasts isolated from EA.hy 926 endothelial cell line. We have shown data describing regulation of the mitoBK_{Ca} channel by different cardioprotective flavonoids (luteolin, quercitin and cyanidin). Cellular breathing tests using the oximeter were also carried out.

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Cytoplasmic hybrid cells as a model to characterize the OXPHOS system: control sample with glutamate metabolism defect.

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Mitochondrial diseases are heterogenous multisystem organ disorders which can be caused by alterations in both nuclear and mitochondrial DNA (mtDNA). Defects in the oxidative phosphorylation system (OXPHOS) are presented in many cases. Cytoplasmic hybrid (cybrid) cells have the same nuclear background so they are a good model to investigate the influence of mtDNA alterations on cell function [1]. We have been performing molecular diagnostics of the mtDNA for patients with suspected mitochondrial diseases for several years now and recently we started to use cybrid cell models to evaluate the effect of the mtDNA mutations on the cell functions. To evaluate the OXPHOS system functionality of the mutant cell lines, we compare them to the control lines harbouring the same haplogroup. Thus, cells with mutation m.9185T>C in the gene *MT-ATP6* belonging to the haplogroup U4d were compared with the two control lines belonging to haplogroups U5b and U5a. During measurements U5a line showed unexpected results for the control line.

Cybrid cell lines were developed using osteosarcoma derived rho zero cells and platelets from patients and healthy individuals. Healthy donors were chosen due to their belonging to the mtDNA haplogroups. All created cell lines were checked for the suspected haplogroup or mutation. Mitochondrial functionality was determined by high resolution respirometry using Oroboros O2k. Respiratory chain complex I – IV and additionally complex I+III and II+III activities were measured spectrophotometrically in isolated mitochondria as previously described [2] with modifications.

Till now we have made 4 cybrid cell lines harbouring pathogenic mutations and 15 control cell lines with several clones for each. Up to date respirometry measurements have been performed for two mutant and three control cell lines. Surprisingly the clone 1 of U5a cell line showed no effect in O2 flux after addition of glutamate and succinate, though the addition of rotenone induced a significant inhibition of respiration. Additional measurements of respirometry in another clone of this cell line (clone 2) showed declined effect after addition of glutamate, but overreaction after addition of succinate. Respiratory chain complex activities for both clones of the U5a line were within reference intervals, however they showed higher citrate synthase activity compared to the other control lines, thereby, the ratio of complex activities to citrate synthase activity for complexes I, II, IV and II+III were impaired in comparison to references. Full length mtDNA sequencing for the U5a line revealed no pathogenic mutations.

Respirometry results of the control cybrid cell line harbouring U5a haplogroup suggest possible defect in electron transfer system (more likely glutamate metabolism). Further investigations are necessary to specify the causative mechanism. Our results also remind that all control samples should be checked well before including in experiments and sometimes they can reveal unexpected findings.

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Fads2 Exacerbates Myocardial Ischemia-Reperfusion Injury in Mice: Role of Mitochondria

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Common *FADS2* haplotypes associated with hyperactivity of its gene product, delta-6-desaturase (D6D), predict cardiovascular morbidity and mortality in humans[1, 2]. D6D is the rate-limiting enzyme in essential polyunsaturated fatty acid (PUFA) metabolism, but its role in the pathogenesis of cardiovascular disease is unclear.

To investigate this, we generated mice with global (CMV promoter) transgenic overexpression of *Fads2* (*Fads2*-TG). *Fads2*-TG mice exhibit consistent moderate D6D overexpression at the mRNA, protein, and activity levels, including ~60-80% increase in D6D protein and mRNA in liver, and expected increases in D6D product/ precursor ratios in tissue phospholipids compared to FVB wild-type (WT) controls. In particular, *Fads2*-TG mice exhibited higher omega-6 arachidonic acid (20:4n6) levels relative to its precursor linoleic acid (18:2n6) in cardiac phospholipids. *Fads2*-TG mice also develop mild glucose intolerance and aortic stiffening, but exhibited no overt cardiac pathology. To investigate cardiac tolerance to ischemia-reperfusion (I/R) stress, hearts from *Fads2*-TG and WT animals were perfused in Langendorff mode *ex vivo* and assessed for myocardial infarct size by TTC staining. Subsequent experiments investigated OXPHOS capacity, H_2O_2 release, and Ca^{2+}-tolerance of isolated cardiac mitochondria.

Fads2-TG hearts exhibited a 60% higher infarct size compared to WT (WT: 25 ± 3%, *Fads2*-TG 40 ± 3%) following a 45/120 minute I/R protocol. *Fads2*-TG mitochondria also exhibited a 40% higher Ca^{2+}-induced swelling over WT (FVB-WT: 10.8 ± 0.3%, *Fads2*-TG 15.1 ± 0.3%) after a 200uM Ca^{2+} incubation. OXPHOS-linked respiration and H_2O_2 release from isolated *Fads2*-TG and WT mitochondria were measured by high-resolution respirometry/fluorometry (Oroboros Instruments) supported by saturating Complex I+II substrates and ADP, under basal and Ca^{2+}-stimualted conditions (25 uM Ca^{2+} titrations every two minutes up to 350 uM). *Fads2*-TG mitochondria lost OXPHOS capacity at a lower [Ca^{2+}] and had a greater rate of decline than WT (Figure 1). *Fads2*-TG mitochondria also released more H_2O_2 over time and a higher rate of H_2O_2 release than WT (Figure 2). Phospholipid analysis of cardiac mitochondria isolated before and after I/R demonstrated a greater depletion of archidonic acid from mitochondria phospholipid-membranes in *Fads2*-TG compared to WT. Therefore, we next investigated the effects of blocking arachidonic acid hydrolysis from mitochondrial phospholpids during Ca^{2+}-overload by incubating mitochondria with the phospholipase A_2 inhibitor bromoenol lactone (BEL). We found that BEL reduced Ca^{2+}-induced swelling in *Fads2*-TG and WT mitochondria to below WT control levels.

Our results indicate that hyperactivity of D6D reduces myocardial tolerance to ischemia, and suggest a possible role of mitochondrial arachidonic acid signaling on mitochondrial response to Ca^{2+}-overload. This is consistent with emerging evidence for a potentially deleterious role of mitochondrial arachidonic acid metabolism on cardiac I/R tolerance.

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Figure 1

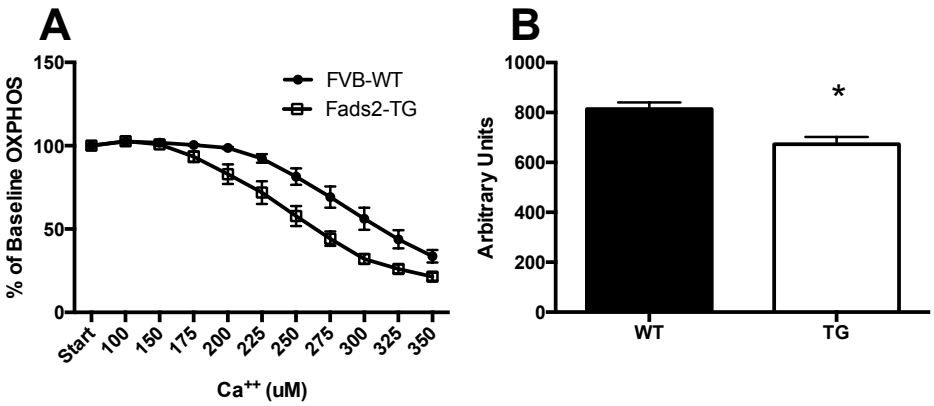

Figure 1: Delta-6-Desaturase Hyperactivity Decreases Mitochondrial Tolerance to Calcium Titrations. A) Data expressed as percent of state 3 OXPHOS baseline after serial titration of calcium. In response to calcium titrations, *Fads2*-TG mitochondria decrease OXPHOS rate at a lower calcium concentration than FVB-WT controls. B) Area under the curve for data presented in panel A; Student's T-Test shows decrease in *Fads2*-TG compared to FVB-WT, pvalue <0.05.

Figure 2

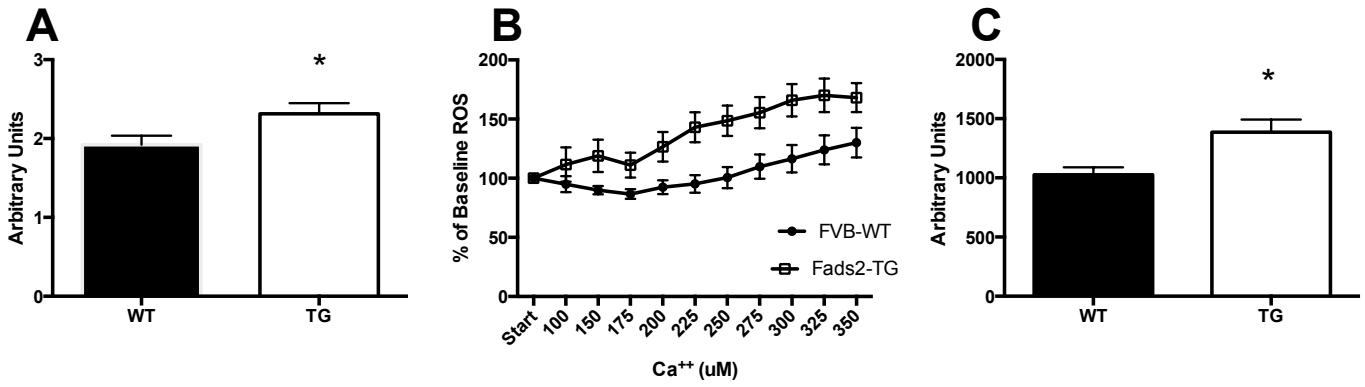

Figure 2: Delta-6-Desaturase Hyperactivity Increases ROS production with Calcium Titrations. A) Area under the curve for resorufin fluorescence over the course of the calcium titrations; Student's T-Test shows increase in *Fads2*-TG compared to FVB-WT, pvalue <0.05. B) Data expressed as percent of resorufin production in state 3 OXPHOS baseline after serial titration of calcium. In response to calcium titrations, *Fads2*-TG mitochondria had increased ROS compared to FVB-WT controls. C) Area under the curve for data presented in panel C; Student's T-Test shows increase in *Fads2*-TG compared to FVB-WT, pvalue <0.05.

Cardiolipin Synthesis in Brown and Beige Fat Mitochondria Is Essential for Systemic Energy Homeostasis

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Activation of energy expenditure in thermogenic fat is a promising strategy to improve metabolic health, yet the dynamic processes that evoke this response are poorly understood. Here we show that synthesis of the mitochondrial phospholipid cardiolipin is indispensable for stimulating and sustaining thermogenic fat function. Cardiolipin biosynthesis is robustly induced in brown and beige adipose upon cold exposure. Mimicking this response through overexpression of cardiolipin synthase (Crls1) enhances energy consumption in mouse and human adipocytes. Crls1 deficiency in thermogenic adipocytes diminishes inducible mitochondrial uncoupling and elicits a nuclear transcriptional response through endoplasmic reticulum stress-mediated retrograde communication. Cardiolipin depletion in brown and beige fat abolishes adipose thermogenesis and glucose uptake, which renders animals insulin resistant. We further identify a rare human CRLS1 variant associated with insulin resistance and show that adipose CRLS1 levels positively correlate with insulin sensitivity. Thus, adipose cardiolipin has a powerful impact on organismal energy homeostasis through thermogenic fat bioenergetics.

Keywords: cardiolipin, CRLS1, phospholipidslipid, metabolism, mitochondria, brown adipose, beige adipose, thermogenesis, insulin resistance, CHOP-10

Cardiac fatty acid oxidation: from in vitro to in vivo

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Fatty acid oxidation (FAO) plays an essential role in heart bioenergetics and provides 60-95% of necessary ATP for normal heart function. Thus, impairment in FAO results in significant disturbances in cardiac function.

Before being oxidized fatty acids are activated to acyl-CoAs via long-chain acylCoA synthase. Before entering the β-oxidation pathway, acyl-CoAs have to be transported into the mitochondria. In contrast to medium- and short- chain acyl-CoAs, long-chain acyl-CoAs cannot directly pass the inner mitochondrial membrane, and their mitochondrial uptake is mediated by the carnitine-dependent transport system, which is rate-limiting step in FAO. As the first step, acyl-CoAs are converted to respective acylcarnitines by carnitine palmitoyltransferase I (CPT I). Formed acylcarnitines are subsequently transported into the mitochondria, where they are converted back to the respective acyl-CoAs by carnitine palmitoyltransferase II (CPT II) in the mitochondrial matrix. Moreover, the measurements of fatty acid oxidation in mitochondria are highly dependent on addition of co-factors like malate, carnitine and BSA.

Overall, different activated fatty acids can be used to characterize various steps in FAO processes in cardiac mitochondria. However, it is important to interpretate results with caution, taking into account FAO biochemical pathway.

Long-chain fatty acids are inert molecules, but their intermediates, acyl-CoAs and acylcarnitines, could actively participate in the regulation of cardiac mitochondrial bioenergetics. Interestingly that long-chain acylcarnitines, but not long-chain acyl-CoAs, are able to reduce the pyruvate oxidation rate in mitochondria ad induce insulin resistance [1,2]. The accumulation of long-chain fatty acids activated intermediates, is observed in the ischemic myocardium after acute ischemia-reperfusion. Both long-chain acyl-CoAs and acylcarntines decreased OXPHOS-dependent mitochondrial respiration in dose-dependent manner. However, the content of long-chain acyl-CoAs was up to 50-fold lower than the measured acylcarnitine content in ischemic mitochondria. Our results demonstrate that long-chain acylcarnitines inhibit oxidative phosphorylation in cardiac mitochondria, thus, inducing mitochondrial membrane hyperpolarization and stimulating the production of reactive oxygen species, which can lead to the cell death [3]. The increase in long-chain acylcarnitine content induced an increase in infarct size, while pharmacological reduction of long-chain acylcarnitine content decreases ischemia-reperfusion induced mitochondrial dysfunction and significantly decreases infarct size [3,4].

Overall, present results demonstrate that long-chain acylcarnitines, but not long-chain acyl-CoAs, orchestrate mitochondrial energy metabolism pattern and determine ischemia-reperfusion induced damage in cardiac mitochondria.

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Mitochondrial Dynamics of Human Sertoli Cells Under the Effect of Leptin and Ghrelin

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Degradation of lifestyle habits is the major cause for the increase of obesity. This disease is characterized by an accumulation of adipose tissue in the body and is associated with other metabolic conditions, such as diabetes. The rising in the number of individuals with obesity becomes a worldwide problem that is increasing even in undeveloped countries. Some reports provide evidence for the fact that an incidence of metabolic diseases is associated with a decrease in sperm quality and birth rates. The effects of body energy balance in the reproductive function are evident, however the mechanisms behind this remain to be elucidated. In the organism, food intake and energy homeostasis are under the hormonal control of leptin-ghrelin axis, and the levels of these hormones are a reflex of the energy status of the subject. In the testis, the somatic Sertoli cells are the major checkpoint for spermatogenesis, and also an important hormonal target. Since, the effects of leptin and ghrelin in Sertoli cells are still unclear, we aim to disclose the effects of these hormones in the mitochondrial dynamics of human Sertoli cells (hSCs).

Clonetics™ hSCs and hSCs from testicular biopsies were exposed to leptin (0, 5, 25, 50 ng/mL) and ghrelin (0, 20, 100 and 500 pM) during 24h, respectively. The concentrations were chosen mimicking the levels reported in obese, normal weight, and severely undernourished or morbidly obese individuals. Protein expression of mitochondrial complexes was assessed by western blot in hSCs exposed to both hormones. We also evaluated mitochondrial membrane potential by JC-1 assay in hSCs exposed to leptin. The expression of genes involved in mitochondrial biogenesis was also measured by qualitative PCR.

Human SCs exposed to 5 and 50 ng/mL of leptin presented a decrease in protein levels of complex II compared with non-exposed cells. Transcript levels of Sirtuin 1 presented an increase in hSCs exposed to 50 ng/mL of leptin when compared with the other conditions. Ghrelin was able to modulate the protein expression of complex I, III and V in hSCs exposed to this hormone. Regarding the mitochondrial membrane potential, hSCs exposed to 100 pM of ghrelin showed a decrease in JC-1 ratio when compared with non-exposed cells. Finally, hSCs exposed to 500 pM of ghrelin presented an increase in JC-1 ratio when compared with cells exposed to 100 pM.

Leptin and ghrelin modulate mitochondrial complexes of hSCs, as well as mitochondrial dynamics. An alteration in mitochondrial dynamics of hSCs can be reflected in pivotal changes on spermatogenesis. Nevertheless, the role of leptin and ghrelin in mitochondria of hSCs remains to be elucidated and further experiments will be necessary.

Sweetheart: Cardiac consequences of fetal exposure to maternal gestational diabetes on the offspring mitochondrial function.

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Gestational diabetes mellitus (GDM) is defined as a state of glucose intolerance and hyperglycemia with first onset during pregnancy. The prevalence of GDM has been steadily increasing over the last 20 years being considered one of the most common complications of pregnancy, by affecting 5%–10% of pregnant women. Offspring of mothers with GDM are more prone to develop metabolic disorders, such as obesity, type 2 diabetes and, Cardiovascular diseases (CVD). Despite some results suggesting that exercised pregnant women gave birth to offspring with increased cardiac function, metabolic capacity and resistance (10), it is still unclear whether exercise during pregnancy affects fetal cellular signaling pathways, including mitochondrial bioenergetics, leading to a more resistant mitochondrial phenotypes in adulthood.

Using a rodent model of gestational diabetes, our objective is to demonstrate that diabetic pregnant mothers subjected to a protocol of voluntary physical activity (VPA) have offspring with a more robust mitochondrial function when compared with offspring from sedentary pregnant mothers.

Our preliminary results attested that the new model showed pronounced impact in the body weight gain and glucose tolerance when Sprague-Dawley females become pregnant. The absence of effects on non-pregnant Sprague-Dawley females reinforces our conviction that the proposed protocol mimics adequately the metabolic disturbances characteristic of GDM. We observed increased litter sizes in rats exposed to HFHS (C 12, HFHS 16) and at weaning, we detected a significant increase in the body weight of the offspring of the female rats fed with the HFHS diet, comparing to the offspring of mothers fed with the control diet during pregnancy. We will also present recently acquired data regarding the cardiac mitochondrial bioenergetics of the offspring of GDM with and without VPA.

The understanding of mitochondrial cardiac metabolism from the offspring of diabetic mothers is critical for appreciating the consequences of diabetes during gestation. We propose that VPA could be an invaluable tool for preventing the alterations the fetus is subjected to, and

specially protect the cardiac function when gestating inside a diabetic mother.

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OXPHOS Protocols: Understanding the Patterns

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For the mitochondrial physiologist, the substrate-uncoupler-inhibitor titration (SUIT) protocol represents a flexible method for the assessment of several facets of mitochondrial respiratory function, often within a single protocol. For example, protocols can provide information on absolute and relative capacity for the oxidation of particular substrates (e.g. fatty acid derivatives) or the function of the electron transfer system (ETS) and its associated pathways. SUIT protocols can also address questions relating to coupling efficiency and ideally include quality control steps to validate the integrity of a mitochondrial preparation. It is vital to select the most appropriate protocol when designing experiments, with this determined by the scientific question as well as the tissue studied and mitochondrial preparation used. In this session, I will discuss issues to be aware of when selecting an OXPHOS protocol and interpreting the results. We will touch on complementary analytical techniques that can aid interpretation of SUIT data, and I will open a discussion on some current issues relating to protocol design. During the session, we will refer to the MitoPedia SUIT library[1], a recent innovation by Oroboros Instruments to support users, with the opportunity to provide feedback on this developing resource.

1. http://www.bioblast.at/index.php/MitoPedia:_SUIT

Alpha-tomatine as a novel membrane-permeabilizing agent for mitochondrial respiration measurements

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In mitochondrial respiration measurements, digitonin is a widely used substance for the permeabilization of the plasma membrane. Digitonin is a mixture of several different saponins, naturally varying in their composition. Therefore, the effective concentration of each digitonin batch must be determined individually. We recently identified the secondary plant metabolite α-tomatine to exhibit the same permeabilizing properties as digitonin. Furthermore, it can not only permeabilize the plasma membrane but also the outer mitochondrial membrane.

In this study, we used α-tomatine to selectively permeabilize the cellular and mitochondrial membrane of HEK-293 cells in respirometric measurements. The permeabilization of the outer mitochondrial membrane was combined with an excess of reduced cytochrome C to directly modulate the activity of complex IV. In standardized substrate-uncoupler-inhibitor-titration protocols, complex IV activity is usually influenced by changes in substrate availability, the substrate oxidation capacity of different enzymes and the size of the ubiquinone and cytochrome C pool.
We demonstrate that our assay bypasses these limitations. Thus, the maximal activity of complex IV within the inner mitochondrial membrane can be assessed more specifically. In conclusion, the use of α-tomatine allows a more precise determination of factors responsible for changes in state 3 respiration and to increase our basic understanding of the role of complex IV in the respiratory chain.

Obesity-induced mitochondrial hepatic changes during pregnancy

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Pregnancy represents a unique maternal metabolic challenge [1]. Liver disease in pregnancy occurs in 3-10% of cases causing maternal morbidity and mortality [2]. High maternal BMI exacerbates metabolic and hepatic complications. Mitochondrial substrate oxidation supports the maternal and fetal metabolic demands. Mitochondrial defects have been associated with maternal and fetal complications [3]. A better understanding of MO induced maternal physiological changes is needed to prevent adverse outcomes during pregnancy. Our aim was to characterize liver mitochondrial profile and redox network in term pregnant MO ewes.

Rambouillet:Columbia ewes consumed either an obesogenic (MO: 150% of NRC requirements; n=8), or control diet (C: 100% NRC; n=10) from 60 days prior to conception and through pregnancy. Maternal livers were removed at 0.9 gestation for right lobe measurements. Mitochondrial and antioxidant defense system proteins were determined by Western blot. Mitochondrial respiratory chain complex activities were determined in isolated fractions. Using whole liver tissue we determined catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase activities by spectrophotometry and reduced and oxidized glutathione. Lipid peroxidation was assessed by fluorometry by malondialdehyde (MDA) formation. Data expressed as mean±SE and comparison between groups performed by Mann-Whitney test, P-value<0.05 as significant.

In MO mothers we found increased maternal hepatic MDA indicating greater lipid peroxidation and decreased reduced glutathione, indicating imbalance of endogenous antioxidant defenses. Despite unchanged MO mtDNA copy number, content of proteins implicated in mitochondrial metabolism was altered, with decreased succinate dehydrogenase complex subunit B, increased VDAC1, cyclophilin D and cytochrome c. Complex I activity was decreased in MO-livers.

MO in pregnancy alters maternal hepatic mitochondrial biology impairing redox state, eventually predisposing mothers to metabolic diseases including non-alcoholic fatty liver disease.

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Intracellular energy-transfer networks in health and disease – the results of oxygraphic studies

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Compartmentalization of high-energy phosphate carriers between intracellular micro-compartments is a phenomenon that ensures efficient energy use. To connect these sites, creatine kinase (CK) and adenylate kinase (AK) energy-transfer networks, which are functionally coupled to oxidative phosphorylation (OXPHOS), could serve as important regulators of cellular energy fluxes [1]. However, for most tissues the intracellular diffusion restrictions for energy metabolites and accompanying micro-compartmentalization together with energy transport circuits is a relatively unexplored and undervalued area in cellular bioenergetics.

Selective permeabilization of cellular outer membrane and high-resolution respirometry can be used to study functional coupling between CK or AK pathways and OXPHOS in different cells and tissues. Using different oxygraphy protocols the ability of creatine or AMP to stimulate OXPHOS through CK and AK reactions, respectively, is easily observable and quantifiable. Additionally, functional coupling between hexokinase and mitochondria can be investigated by monitoring the effect of glucose on respiration [2].

The results of our recent study show that the decline in the heart muscle performance is not caused by the changes in the respiratory chain complexes activity but mainly by the decrease in the energy transfer efficiency, especially by the CK pathway. During aging, decline in the CK pathway is the first detectable sign of the alterations in bioenergetics metabolism in 1-year-old (middle-aged model) rat cardiomyocytes while the alterations in the AK pathway are not significant [3].

In wolframin1 gene knockout (Wfs1KO) mice (a model of diabetes and endoplasmatic reticulum stress) in the oxidative heart muscle the AMP ability to stimulate respiration is higher, while the creatine activated respiration is lower than in control animals. However, in the glycolytic *m. rectus femoris* the activity of AK pathway shows a slight decrease in comparison to the control. In the oxidative heart muscle of Wfs1KO mice the glucose stimulated respiration is significantly higher than in the control group. These results indicate to significant changes in energy metabolism of adult mice heart and skeletal muscle cells accompanied with Wfs1 deficiency.

In conclusion, systemic functional analysis of changes in cellular phosphotransfer networks may help to explain many pathogenic mechanisms in numerous diseases.

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Role of succinate in prostate cancer cells: uptake and mitochondrial respiratory function

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Succinate dehydrogenase (SDH, mitochondrial Complex II) links the oxidation of succinate and FAD to fumarate and $FADH_2$ in the tricarboxylic acid (TCA) cycle to electron transfer (ET) from $FADH_2$ to ubiquinone in the ET system. Changes in ET capacity through the succinate pathway affect TCA cycle function and cell respiration [1]. In addition, succinate transmits oncogenic signals from mitochondria to the cytosol by stabilization of hypoxia inducible factor 1α. This, in turn, stimulates the expression of genes involved in angiogenesis and anaerobic metabolism [2], finally enabling tumour progression and metastasis. Succinate uptake is enhanced in various cancer cells and its mitochondrial utilisation is increased in permeabilized prostate cancer cells [3].

To decipher the pathophysiological role of succinate in prostate cancer, we tested the plasma membrane permeability for succinate and utilization of external succinate by mitochondria in terms of succinate pathway capacity and kinetic properties in prostate cancer (multiple metastatic origins) and control cell lines. Respiration in RWPE-1 (prostate; noncancerous), LNCaP (prostate; lymph node metastasis) and DU145 (prostate; brain metastasis) cells was measured using High-Resolution FluoRespirometry (O2k, Oroboros Instruments) and substrate-uncoupler-inhibitor titration (SUIT) protocols developed specifically for the study. To assess succinate utilization in intact cells independent of a plasma membrane succinate transporter, we applied novel plasma membrane-permeable succinate prodrugs (pS) [4].

In LNCaP cells, transport of external succinate is enhanced through the plasma membrane as compared to the other cell lines, while pS exerted similar effects in all cell lines, suggesting an important regulatory role of the transport mechanism. Furthermore, in LNCaP cells, mitochondria utilize succinate with higher affinity than control cells. Importantly, kinetic measurements demonstrated the most pronounced difference in the affinities in the physiological intracellular succinate concentration range (< 100 µM), underlining its pathophysiological role.

Our results indicate a "succinate-phenotype" in LNCaP, with enhanced transport and utilization. As such, succinate is a potential mitochondrial metabolic biomarker in prostate cancer cells. We propose a model in which succinate does not only play a role in the signalling but has a central role in the maintenance of mitochondrial respiration as a fuel substrate.

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Mitochondrial kinases: key players in respiratory control and energy transfer

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Mitochondrial isoforms of nucleoside diphosphate kinase (NDPK-D or NME4) and creatine kinase (mtCK) are phylogenetically unrelated but share important structural and functional properties. They are both located in the intermembrane/cristae space and use mitochondrially generated ATP to phosphorylate their specific substrates, NDPs or creatine, thus regenerating ADP within the mitochondria. Both enzymes functionally interact with inner membrane adenylate translocator, thus allowing for privileged exchange (channeling) of these metabolites. We will discuss the molecular basis of this metabolite channeling and its functional consequences beyond the simple maintenance of proper nucleotide pools: stimulation of respiration, “energy export”, and regulation of ROS production, permeability transition and mitochondrial shape.

Detailed information can be found in our recent reviews on these proteins:

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Effect of metformin treatment on blood platelet bioenergetics and platelet function in STZ-diabetic and non-diabetic rats

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Metformin is a potent anti-hyperglycaemic agent that effectively decreases diabetes-related cardiovascular complications [1], however, the mechanism responsible for this beneficial effect is still unknown. *In vitro* studies have shown that metformin used in high concentrations can inhibit complex I of the mitochondrial chain and reduce mitochondrial membrane potential in blood platelets [2], as it does in liver or muscle cells [3]. Since blood platelet activation and aggregation are highly energy-depended processes [4], a mild limitation of respiratory chain by metformin could potentially reduce platelet response to stimulating agents, and - in consequence, reduce the risk of cardiovascular complications. Our aim was to evaluate *in vivo* the effect of metformin on blood platelet mitochondrial respiration and on blood platelet activation and aggregation.

For this purpose sixty 2-month-old Sprague–Dawley rats were randomly allocated into healthy and diabetic groups. In order to induce diabetes the animals were intraperitoneally injected with streptozotocin (STZ) at a dose of 65 mg/kg b.w., while the control group received only a vehicle (0.1 mol/l citrate buffer). After 1-month of confirmed diabetes, animals were divided into four groups: (1) healthy (non-diabetic) rats – not treated with metformin; (2) healthy animals that were given metformin in a dose of 50 mg/kg b.w./day for 4 months; (3) untreated diabetic rats; (4) diabetic rats treated with metformin at a dose of 50 mg/kg b.w./day for 4 months. Animals that survived the experiment were anaesthesized and the blood was collected from the abdominal aorta onto 3.2% sodium citrate. Blood platelets were isolated using a multiple-stage centrifugation procedure. The mitochondrial respiration parameters in intact and permeabilized blood platelets were monitored with the use of high resolution respirometry (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria). Collagen-stimulated platelet aggregation was monitored with the use Multiplate® analyzer (Roche Diagnostics, Mannheim, Germany). Platelet activation and reactivity to physiological agonists (collagen, thrombin or ADP) were evaluated by the flow cytometric determination of the expressions of platelet surface activation marker (CD62P). Blood samples were examined with the automated haematology analyzer (ABX Micros 60, HORIBA ABX SAS, France).

Four-month treatment with metformin resulted in a small, but statistically significant ($p < 0.05$) decrease in blood glucose concentration in STZ-diabetic animals compared to non-treated diabetic group (501 [478 – 509.5] mg/dl *vs.* 473.5 [463.75 – 485.5] mg/dl, respectively). However, our results indicate that the treatment with metformin at dose 50 mg/kg b.w./day for 4 months is not sufficient to modify platelet mitochondria respiration or collagen-stimulated platelet aggregation in control and STZ-diabetic rats. Since, the mechanism of metformin action is underlied by its accumulation in cells, we conclude that probably a higher dose is needed to observed beneficial effects in STZ-diabetic rats, including better glycemic control.

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Subchronic *in vivo* study for the evaluation of hepatic mitochondrial toxicity induced by silver nanoparticles

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Manufactured nanomaterials have been of extreme importance due to the beneficial physicochemical properties they possess compared to bulk parental materials. However, the properties that make them so attractive are also the same that can cause harm both to humans and environment. Among all used nanoparticles, silver nanoparticles (AgNPs) have the highest level of commercialization. The main toxicological concern is the fact that AgNPs preferentially accumulate in mitochondria. Since mitochondria have an essential bioenergetic function, impairment of mitochondria by nanoparticles may have drastic consequences on cellular function.

Sprague–Dawley rats were weekly intraperitoneally injected with either 10- or 75-nm AgNPs, with or without a previous injection of the antioxidant N-acetylcysteine (NAC), for 4 weeks. Animals were sacrificed and various parameters (including mitochondrial bioenergetics) from liver, heart and kidney were evaluated

No significant differences were found in typical hepatic injury serum markers, showing that AgNPs toxicity is a silent phenomenon. However, mitochondria isolated from animals exposed to a very low dose of AgNPs showed a significant increase on reactive oxygen species (ROS) generation. Moreover, AgNPs caused impairment of rat liver mitochondrial function, mainly due to alterations of mitochondrial membrane permeability leading to an uncoupling effect on the oxidative phosphorylation system. AgNPs also compromised the electron transfer along the electron transport chain by affecting the activity of complexes II and IV of the respiratory chain and interfered with the mitochondrial permeability transition (MPT) induction. We found that most of the effects caused by AgNPs exposure were prevented by pretreatment with N-acetylcysteine (NAC), which highlights the crucial role of oxidative stress in AgNPs' toxicity.

In summary, our results indicate that the liver is a target of AgNPs exposure leading to alteration in hepatic mitochondria functions. Thus, the mitochondrial toxicity may have a central role in the toxicity resulting from exposure to silver nanoparticles. The efficiency of NAC administration against AgNPs toxicity suggests that ROS are involved in the mitochondrial toxicity caused by AgNPs treatment.

Intracellular complement factor 3 in the heart – a link to metabolism?

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The complement system has traditionally been investigated systemically. The proteins involved in complement activation are produced in the liver and are released into the circulation in order to eliminate invading pathogens [1]. However, recent discoveries show that the complement system can be activated intracellularly in T-cells and is linked to cell metabolism and survival through mTOR [2]. In this project, we investigate intracellular complement activation in the heart.

Isolated hearts of adult wildtype (WT) and Intracellular component 3 knock-out (C3KO) C57BL/6 male mice were *ex vivo* retrogradely perfused at constant pressure (70 mmHg). Left ventricular pressure was monitored through a fluid-filled balloon placed in the left ventricle. The experimental set-up: 20 minutes stabilization, 35 minutes global ischemia, and 60 minutes reperfusion. Infarct size was measured with 1% TTC staining and Lactate dehydrogenase (LDH) release was measured from the collected coronary perfusates. mRNA and protein expression was measured with qPCR and western blotting.

After 35 minutes of ischemia, C3KO hearts had significantly higher Left Ventricular End-Diastolic pressure (LVEDP) compared to WT hearts. C3KO hearts also exhibited significant larger infarct size and LDH release compared to WT hearts. Additionally, in WT hearts subjected to *ex vivo* ischemia-reperfusion, we found an increase in C3 mRNA expression compared to WT hearts briefly flushed with PBS. We confirmed the presence of intracellular C3 in isolated cardiomyocytes, cardiac fibroblasts, and hearts subjected to *ex vivo* ischemia-reperfusion with western blotting, which was not present in the C3KO.

Intracellular C3 is present in cardiac cells and was showed to be protective in *ex vivo* ischemia-reperfusion injury. The role of intracellular C3 and its potential connection to mitochondria will be part of future experiments.

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MitoTEMPO amolarietes hyperglycemia induced mitochodrial damage in cardiomyocytes

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Reactive oxygen species (ROS) are generated in a wide range of normal physiological conditions. However, ROS production increases in many pathological conditions including cardiovascular diseases, autoimmune diseases and aging. Hyperglycemia and hyperinsulinemia are major contributing factors to oxidative stress increase in mitochondria, which contributes toward the pathogenesis of heart diseases. Experimental studies demonstrated that treatment with MitoTEMPO in hyperglycemic and/or hyperinsulinemic animals improved the cardiac function. Moreover, it has been also shown that an improvement of mitochondrial antioxidant capacity with MitoTEMPO could prevent insulin resistance and preserve vascular and cardiac dysfunctions in animals with either metabolic syndrome or diabetes as well as in aged-animals. MitoTEMPO is a mitochondria-targeted antioxidant and prevents mitochondrial permeability transition pore opening, necrosis and mitochondrial apoptosis after ATP depletion recovery. The mito-TEMPO is a nitroxide conjugated with a triphenylphosphonium moiety that is mitochondria-targeted. Nitroxides are known to be superoxide dismutase (SOD)-mimetics, mito-TEMPO may act as a mitochondrial superoxide scavenger and to protect mitochondria from the hypergiycemia-induced oxidative damage. We used high sucrose diet induced metabolic syndrome (MetS) rats or high glucose incubated H9c2 cells for the experiments. Intracellular free Zn^{2+}, mitochondrial superoxide (MitSOX), mitochondrial membrane potential (MMP) are measured from isolated cardiomyocytes. MetS caused increased intracellar free Zn^{2+} and MitSOX and depolarization of MMP. Incubation MetS cardiomyoxytes with MitoTempo for 2 hours prevented increase of intracellular free Zn^{2+} and MitSOX production and protected MMP depolarization. Determine the role of MitoTempo on sarco(endo)plasmicreticulum/ mitochondria coupling, we measured mitofusin-protein Mfn-1/2, a mitochondrial fission protein, Fis-1 in isolated cardiomyocytes. Our data demonstrated that MitoTempo prevented mitochondrial fission in MetS rats. Overall, recent data suggest that new therapeutic approaches directly targeting the mitochondria are very important in pathological conditions, particularly under insulin resistance, for heart dysfunction (This project supported by TUBITAK SBAG…..).

Exome Analysis sheds light on Mitochondrial Disorders

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The Malta BioBank (BBMRI.mt) [1], participated in the collaborative BBMRI-Large Prospective Cohort (BBMRI-LPC) whole exome sequencing (WES) call, jointly organised by BBMRI-LPC, EuroBioBank, RD-Connect and Centro Nacional de Análisis Genómico (CNAG-CRG). The main objective was to identify the molecular pathology of 50 genetically undiagnosed patients with mitochondrial disorders by WES (MITOMUTWES).

50 patient samples were identified from the RD-Connect sample catalogue [2]. These were located in the Malta BioBank and Hacettepe University, Turkey, both of which form part of the EuroBioBank rare disease network. The Maltese cohort included 13 probands (7 children and 6 adults) and 2 unaffected relatives. WES was carried out at CNAG-CRG. Phenotypic information of each patient was recorded on the RD-Connect PhenoTips instance [3]. Bio-informatics analysis was undertaken using the RD-Connect Genome-Phenome Analysis Platform [4].

A critical analysis of rare nuclear and mitochondrial (MT) gene mutations was performed. 10 patients were carriers for more than one rare variant with no clear candidates. 3 cases were found to have: i) a mis-sense mutation c.308C>T (rs749249430) in NDUFAF3 that caused Mitochondrial Complex 1 deficiency (MC1d); ii) a splice donor and two mis-sense variants: c.207+2T>G (rs782792601), c.206A>G (rs781909386) and c.205A>G (rs782503581) in NDUFB11 that affected the exon-splice site and is also thought to cause MC1d; iii) the MT variant m.3243A>G mutation (rs199474657) that caused Mitochondrial myopathy, Encephalopathy, Lactic Acidosis and Stroke-like episodes (MELAS), and another variant m.4336T>C (rs41456348) that caused the splice site and sensinueronal deafness and migraine.

Whole exome sequencing served to establish a genetic diagnosis in 3 of the 13 Maltese rare disease patients. A combined genomic and transcriptomic approach will be used to identify candidate modifier genes that may play a crucial role in the development and / or modification of the clinical phenotype. This integrative analysis will characterise the effect of these gene mutations on mitochondrial function.

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Marko Vendelin

1. Restrictions to intracellular diffusion of ATP and ADP in cardiomyocytes

In this talk, I will review the evidence of compartmentalization in the heart muscle cell. On the example of ATP and ADP diffusion, I will show how the system can be studied and which experimental approaches have been applied to analyze the movements of molecules in the cardiac cell. The creatine kinase system and its role in energy transfer between mitochondria, actomyosin and EC coupling system will be discussed.

2. Modeling actomyosin contraction in the heart muscle

In this talk, I will give an introduction to the models of cross-bridge cycle. With the simple examples, challenges in the modeling of this system will be explained. Theoretical background on how to link generation of mechanical force to ATP hydrolysis will be given.

3. Introducing a platform for primary kinetics data analysis

Typical respiration kinetics study involves many parts with the data analysis playing a large role, as in many other studies. In respiration kinetics data analysis, there are several steps that lead from the measurement data to the desired readout. Here, we present the software package that has been designed for processing kinetics data with the simplicity of the secure access to the data as one of the main focuses of the package. While originally developed for respiration kinetics experiments, we have extended the software to support other experiments, such as patch clamp experiments on cardiomyocytes. The developed software has an extendable plugin system which allows to support multiple hardware providers and experimental protocols.

Title:

Development of analytical assays for the detection and quantification of reactive oxygen and nitrogen species in an animal model of type 1 diabetes - ROS formation involving mitochondrial and NADPH oxidase

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Abstract:

Oxidative stress is a major trigger of endothelial dysfunction and cardiovascular disease [1-3]. Mitochondria and NADPH oxidase contribute to formation of ROS [4,5] and accurate determination of reactive oxygen and nitrogen species (ROS & RNS), especially nitric oxide, superoxide and peroxynitrite, is of great importance for the evaluation of disease mechanisms and the potential targets for drug therapy [6,7]. Our objective is to develop assays to assess mitochondrial, cytosolic and membrane ROS and RNS formation for spatial characterization of the oxidative stress burden in order to identify relevant ROS sources for future therapeutic targeting.

We are working with HPLC methods such as DHE for superoxide production [8-10], Amplex red for hydrogen peroxide detection [8], as well as microplate assays of L-012 chemiluminescence for measuring oxidative burst in in whole blood, cells and tissues [11-14]. Peroxynitrite is indirectly detected by measuring concentration of protein-bound 3-nitrotyrosine (3-NT) by the semi-quantitative method DOT-blot and ELISA kits but also UHPLC with electrochemical detection [15,16], which is the new superior method we are currently working on and could be potentially applied to nitrated mitochondrial proteins as well. MitoSOX-DHE is another method that I currently try to establish in order to detect superoxide production in mitochondria [17]. One of the models of cardiovascular disease we often work with is diabetes, where we use C57Bl/6 mice and streptozotocin (STZ) induced diabetes [18].

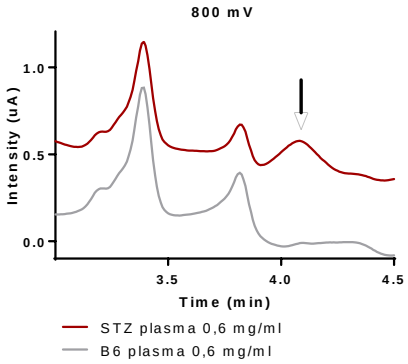

***Figure 1**. HPLC-Coularray chormatografic profile at 800 mV of mouse plasma of control and STZ treated animals.*

Detection of 3-NT in plasma of control and STZ group clearly shows that the diabetic animals had more 3-NT formation (control < STZ). Although differences can be seen in the chromatographic peaks (Figure 1.), there is a need for optimizing the method for more precise and sensitive quantification of 3-NT. The method of Coularray detection offers high sensitivity and specificity and should be adapted for detecting 3-NT not only in plasma and heart tissues but also in isolated mitochondria and platelets in the future.

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Aging-related accumulation of mitochondrial DNA deletions in skeletal muscle occurs preferentially in Type IIb fibers – high mitophagic flux protects Type I fibers

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During aging, skeletal muscle accumulates mitochondrial DNA (mtDNA) deletions, leading to severe mitochondrial dysfunction in individual muscle segments. It is unclear if and how such a mosaic plays a role in the development of age-related sarcopenia and muscle weakness. To study this, we generated mice with accelerated accumulation of mtDNA deletions specifically in muscle by expressing a dominant-negative variant of the mitochondrial TWINKLE helicase (R26-K320E-Twinkle$^{loxP/+1,2}$ x MLC1f-Cre: K320E-Twinkle^{skm}). Mice showed age-dependent accumulation of mtDNA deletions (8 -15 fold) and a concomitant increase in the proportion of cytochrome c oxidase deficient fibers, especially in fast-glycolytic, type IIb fiber-rich muscles like tibialis anterior and gastrocnemius (>10% in cross-sections at 24 months), while type I fiber-rich soleus muscle was spared. This is reminiscent of human muscle at old age and in patients with mitochondrial diseases. Despite this obviously severe mitochondrial myopathy, no signs of motor impairment could be observed, neither *in vivo* nor *ex vivo*, emphasizing the important involvement of other contributors such as motoneuron and muscle stem cell defects in sarcopenia. RNAseq data confirm the previously described induction of the FGF21 and Mthfd2 genes, but also showed remodeling of the AMPK, cAMP, MAPK, hypoxia and insulin signaling pathways. These changes, together with upregulation of amino acid transport, amino acid, protein and glycogen biosynthetic processes strongly suggest a generally increased turnover. Analysis of LC3, p62 and Lamp1 *in situ* shows that type I fiber-rich muscles have a higher autophagic and mitophagic rate than type IIb fiber-rich gastrocnemius and tibialis anterior, which may transiently protect the affected myofibers from the detrimental effects of increased accumulation of mtDNA deletions.

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Metabolic flexibility and mitochondrial function in the diabetic heart

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Optimal mitochondrial function is crucial for cardiac function, and mitochondrial ATP production is linked to contractile activity ('energetic-contraction coupling'), as well as the excitation by calcium ('excitation-energetic coupling'). Maintaining cardiac energy homeostasis requires optimal substrate sensing, transport, storage, and utilization; the interplay between these factors is coined 'metabolic flexibility'. Cardiac metabolic flexibility is required to optimally integrate energy production and demand in order to maintain energy homeostasis and cardiac contractility. The major energy-providing substrates of the heart include triglycerides, long-chain fatty acids, glucose and glycogen, while lactate, ketone bodies and a variety of branched-chain amino acids are contributing substrates.

Patients with Type 2 Diabetes Mellitus (T2DM) and insulin resistance often suffer from circulating hypertriglyceridemia and hyperglycemia. This increased free fatty acid availability leads to increased FA uptake and reduced insulin-mediated myocardial glucose uptake in the heart in patients with uncomplicated T2DM without overt cardiomyopathy. This preferential supply of FFAs to the myocardium in early and late diabetes shifts myocardial substrate metabolism towards almost exclusively FA oxidation for cardiac ATP production, causing metabolic inflexibility. Cellular FFA accumulation can result in cellular oxidative stress, and we showed that cytosolic and mitochondrial calcium homeostasis is impaired in the diabetic heart. We also observed in various animal models of diabetes (pigs, rats, mice) that particularly NADH-coupled mitochondrial OXPHOS was more vulnerable to be affected in the diabetic heart, whereas succinate/rotenone-coupled respiration was less affected. During my talk, I will also highlight some recent work on compounds that worsen mitochondrial dysfunction and cause further cardiac contractile dysfunction in mice with T2DM.

Bioenergetic characterization of skin fibroblasts from patients with Congenital disorders of glycosylation

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Congenital disorders of glycosylation (CDG) are a fast growing group of rare inherited diseases caused by abnormal protein and lipid glycosylation. Recently published studies and our preliminary data indicated possible interconnection between glycosylation defects and mitochondrial function abnormalities.
Aim of this study was to analyze mitochondrial respiration and glycolysis in fibroblast cell lines from patients with Congenital disorders of glycosylation (specifically patients with: ALG8-CDG, PGM1-CDG, PMM2-CDG, Man1B1-CDG, RFT1-CDG, SLC10A7-CDG, ATP6AP1-CDG, NUS1-CDG) and compare them with control fibroblast cell lines. Measurements were performed by using Oxygraph-2k (Oroboros) and Seahorse XFe24 Bioanalyzer (Agilent).
Our preliminary results showed abnormal mitochondrial respiration in most of fibroblast cell lines from the patients with CDG with various respiration patterns in individual CDG type. Decreased Complex II dependent respiration on Oxygraph-2k and decreased oxidative phosphorylation value were found in fibroblasts derived from Man1B1-CDG patient. Slightly diminished basal respiration and decreased maximal respiration to nearly 50% in Man1B1-CDG line compared to controls on Seahorse Bioanalyzer was shown as well. Glycolytic function was decreased almost at all fibroblast cell lines from patients with CDG (except ATP6AP1-CDG) in comparison with the control cell lines.
These results indicate secondary functional abnormalities in mitochondria and glycolytic dysfunction due to a breakdown of the glycosylation pathway. The study of mitochondrial metabolism in congenital disorders of glycosylation may contribute to the elucidation of pathomechanisms in unclear metabolic diseases.

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