
Bioblast
2012
Editors
Erich Gnaiger
Barbara Meißner
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Mitochondrial Oroboros by Odra Noel


Mitochondria


ORORBOROS INSTRUMENTS
high-resolution respirometry
O2k-Fluorometer
ORORBOROS INSTRUMENTS
high-resolution respirometry
Human Heart – a Paradox?

World Health Worries

By Odra Noel

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

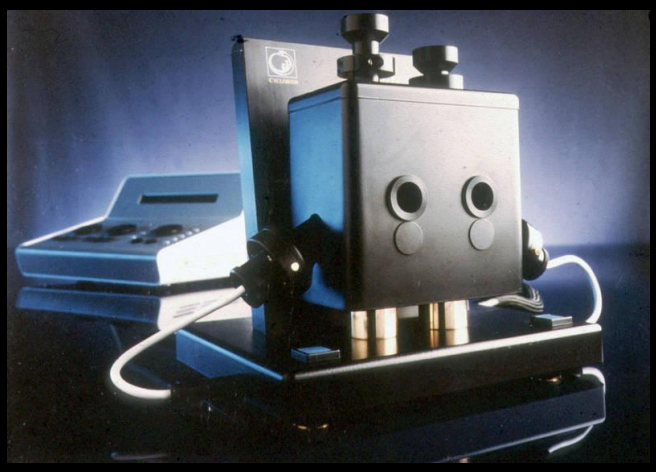
Most developed and developing countries have cardiovascular diseases as their main cause of death. North America struggles with obesity (adipose tissue), with the harmful effects of alcohol (hepatic tissue) represented in Alaska. Europe, with its ageing population, has a heavy load of neurodegenerative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

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

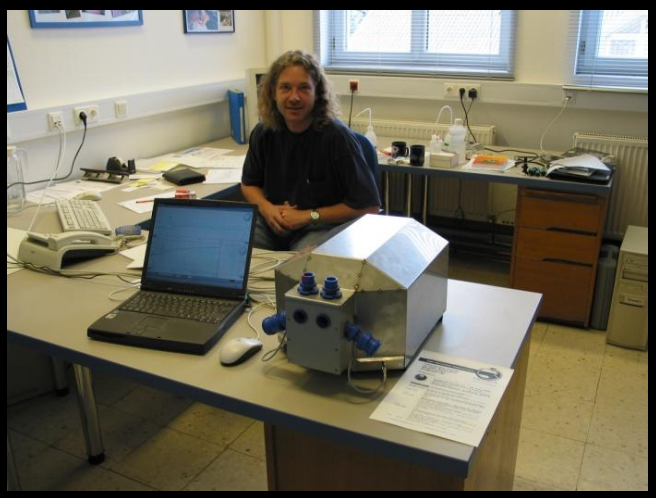
Based on data on causes of death and burden of disease (World Health Organisation, public website. Data from 2008, accessed October 2012).


HAUT · MEDOC

OROBOROS - historic impressions

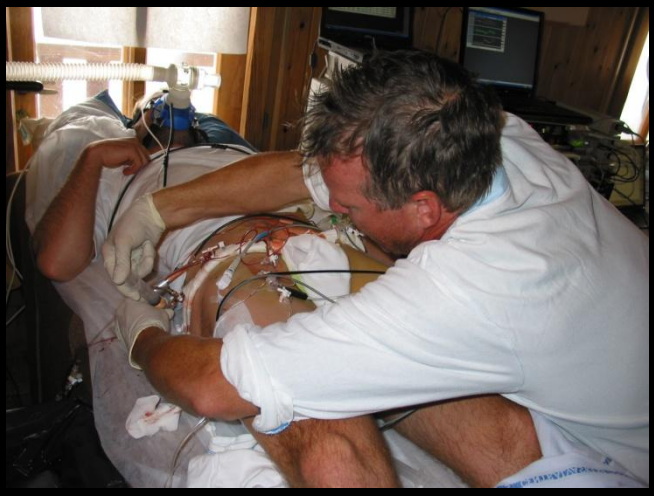


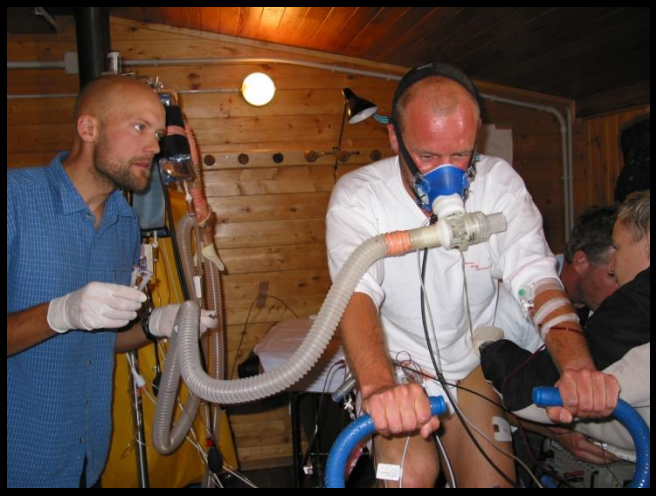
The very beginning of the OROBOROS O2k. *Left:* The first generation instrument for high-resolution respirometry (Paar Oxygraph, 1992). *Right:* Development of the Oxygraph-2k (WGT Elektronik, competent O2k-partner since 2001).



Left: Serial production of the TIP2k (2004). *Right:* Philipp Gradl, WGT Elektronik with the prototype of the O2k (2002).

Left: Erich Gnaiger, UMDF Meeting and O2k-Workshop (San Diego, IOC40 2007). *Right:* Kathrin Renner: 'Wine respiration' with pre-prototype O2k (IOC21 2002).





MitoCom Tyrol

K-Regio

www.bioblast.at

Life Style, Aging, and Disease

Mitochondrial stress → *Healthcare*

- Athletic vs. sedentary life style
- Exercise and caloric balance
- Hypoxia, ischemia-reperfusion
- Microorganisms and infection

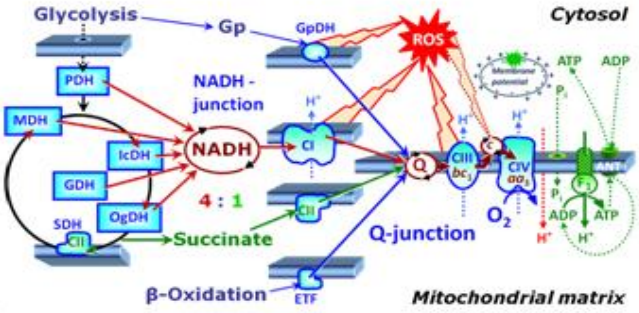
Mitochondrial Competence

MitoScore → *Tyrol – a Heart for Sport*

Mitochondrial Medicine

Mitochondrial physiology has become one of the hot topics in research aimed at finding solutions to the present challenges in our health care system.

Bioblasts and the Power of Life



MiPArt by Odra Noel

High-Resolution Respirometry

K-Regio MitoCom Tyrol

This project is funded by the Tyrolean Government and the European Regional Development Fund (ERDF) and is subject to the regulations of EU law as well as to the Directive of the Tyrolean Government on the Funding of Science, Research and Development.

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C. Lass-Flörl, Div. Hygiene and Medical Microbiology; M. Krismer, Dept. Orthopaedic Surgery, Medical University of Innsbruck; M. Burtscher, Inst. Sport Science, University of Innsbruck, Austria; OROBOROS INSTRUMENTS, Innsbruck and WGT-Elektronik, Kolsass, Austria.

www.bioblast.at/index.php/MitoCom

Houska Award 2011

Light in the Powerhouse of the Cell – *MitoCom Tyrol*

In 2012, Dr. Erich Gnaiger (Medical University of Innsbruck, D. Swarovski Research Laboratory), the O2k-Team (OROBOROS INSTRUMENTS, Innsbruck) and partner Philipp Gradl (WGT-Elektronik, Kolsass) received the Houska Award 2011, the largest privately endowed research award in Austria worth 120,000 €.

The winning project 'Light in the powerhouse of the cell' (*MitoCom Tyrol*) aims at developing, evaluating and applying a new high-resolution instrument, the O2k-Fluorometer, which is based on the OROBOROS Oxygraph-2k. The new instrument is being developed within the framework of the K-Regio project *MitoCom Tyrol* and has great market potential.

The award ceremony in Vienna, April 2012.
From right to left: Dr. Erich Hampl (B&C Stiftung), Doz. Dr. David Harrison, Mag. Katharina Stelzl, Dr. Mario Fasching, Mona Fontana-Ayoub, A.Univ.-Prof. Dr. Erich Gnaiger, Mag. Georg Bauthen, Mag. Andrea Gnaiger, Mag. Katrin Stecher, Mag. Lydia Staudacher, Dr. Wolfgang Hofer (B&C Stiftung), Dr. Andrea Eigentler, DI (FH) Barbara Meissner.

K-Regio Project *MitoCom Tyrol*

The O2k-Fluorometer and Mitochondrial Physiology

Applicant: Medical University of Innsbruck
A.Univ.-Prof. Dr. Erich Gnaiger

Project duration: 2011-05-01 to 2014-04-30

Partners:
OROBOROS INSTRUMENTS GmbH
WGT-Elektronik GmbH & Co KG
Leopold-Franzens-University Innsbruck
Medical University of Innsbruck (lead partner)

Aim of the project

Preservation, gain or loss of ***mitochondrial competence*** is playing an increasingly important role in therapeutic and preventive medicine. Exercise and caloric balance rank among the most efficient measures for reducing various age-related health risks and degenerative diseases. Evaluation of mitochondrial competence requires improved methods and concepts for the diagnosis of mitochondrial functions and mitochondrial injuries. Mitochondrial competence represents a major current challenge for biomedical research and development.

The aim of the present project *MitoCom Tyrol* is the development, evaluation and application of a new high-resolution instrument, the O2k-Fluorometer. High-resolution respirometry (HRR) is based on the O2k – the OROBOROS Oxygraph-2k developed and extended since 2001 by OROBOROS INSTRUMENTS and WGT-Elektronik. The integration of optical devices (fluorometry, spectrophotometry) into the O2k establishes the potential for analysing various diagnostically significant cellular functions. These are measured simultaneously with mitochondrial respiration. In particular, HRR is combined with the fluorometric detection of reactive oxygen species (ROS; oxidative stress) and mitochondrial membrane potential using established fluorescent dyes.

In a joint effort to helping mitochondrial patients and making a difference to society, the regional extension of *MitoCom Tyrol* emphasizes international mitochondrial networking for exchange of expertise and standardization of diagnostic approaches.

This project is funded by the Tyrolean Government and the European Regional Development Fund (ERDF) and is subject to the regulations of EU law as well as to the Directive of the Tyrolean Government on the Funding of Science, Research and Development.

Milestones

The first O2k-Fluorometry-Workshop was held in March 2012. The first O2k-Fluorescence LED2-Module is available since April 2012. 50 modules have already been sold, indicating a continously increasing demand.

www.bioblast.at/index.php/O2k-Fluorescence_LED2-Module

www.bioblast.at/index.php/K-Regio_MitoCom_Tyrol

Support

K-Regio Project *MitoCom Tyrol*

The project Open Innovation (OROBOROS INSTRUMENTS, Barbara Meissner) is co-financed by the Standortagentur Tirol.

Tourismusverband Innsbruck und seine Feriendörfer

Announcement

The Next World-Summits on Mitochondrial Physiology:

MiP2013

9th Conference on Mitochondrial Physiology
10 years MiP*society*

September 23 to 27, 2013
Innsbruck and Obergurgl, Austria

Organizer:
Erich Gnaiger (Innsbruck, AT)
Email: erich.gnaiger@mitophysiology.org

MiP*summer* School

MiP*summer* 2013

6th MiP*summer* School on Mitochondrial Physiology

August 26 to 30, 2013
Copenhagen, Denmark

Organizer:
Flemming Dela (Copenhagen, DK)

Version 2: 2013-01-15

Bioblast 2012

Conference on Mitochondrial Competence

A Mitochondrial Festival in the Spirit of Gentle Science

Editors
Erich Gnaiger
Barbara Meißner
Verena Laner

Mitochondr Physiol Network 17.12

OROBOROS MiPNet Publications (2012)

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Table of Contents

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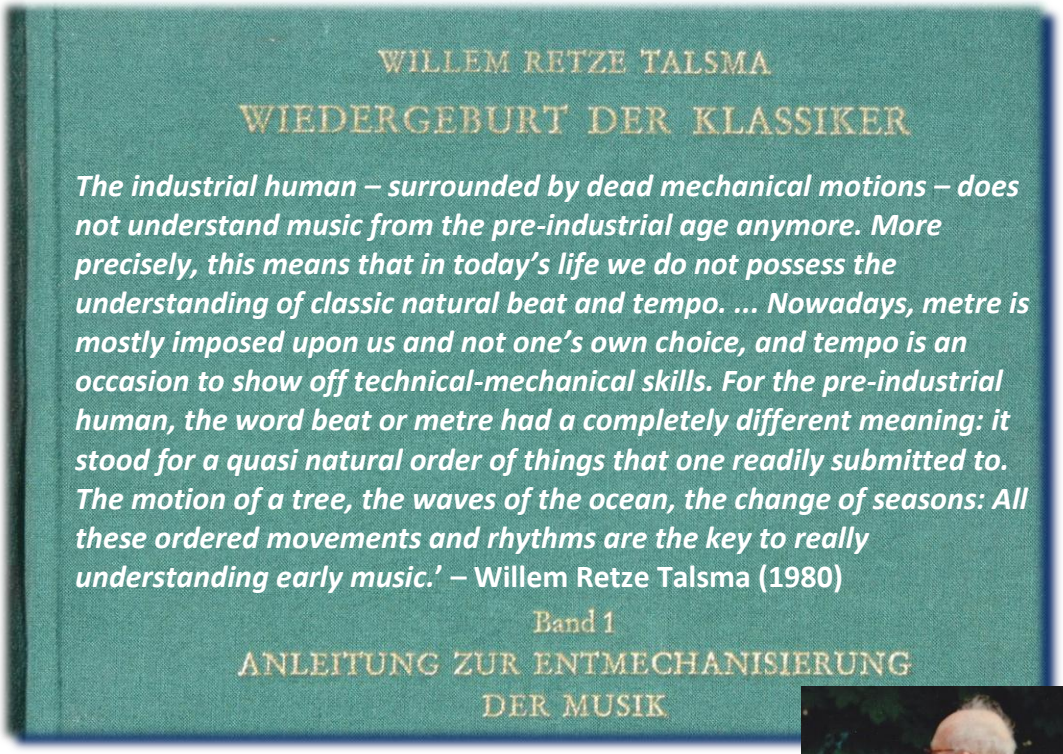
Glass artist: Bernd Weinmayer, Photo: Philipp Gradl, Layout: The OROBOROS Team

Preface: Tempo giusto

Tempo giusto in classical music and the speed of life. Power, efficiency and time – a progressive return to *mitochondrial lifestyle activity*.

Uwe Kliemt[1], Erich Gnaiger[2]
[1]*Pianist and TIME-researcher, Kaudiekskamp 4a, 22395 Hamburg, Germany;* [2]*OROBOROS INSTRUMENTS, Austria.* - *uk@tempogiusto.de*



300 years ago (1712) the first steam engine - converting the energy of steam to mechanical work - was built by Newcomen and Calley and applied in mines of England. Various applications and developments in the field of thermodynamics, particularly the steam engine, were major forces in boosting the Industrial Revolution at the time of Wolfgang Mozart (1756-1791) and Ludwig van Beethoven (1770-1827) [1]. The concomitant acceleration of energy fluxes has been shaping our industrial society ever since and penetrates our lives even as far as to the most fundamental aspects of our culture, a unique development in the history of humankind. Engines and electrical gadgets are not only urging us on more and more, by overexploiting global resources we are also contributing to global warming. *In today's fast moving world, we are rich in energy, but lacking time* [2]. The speed shaping our daily lives is ever increasing, yet does not win us any extra time. But what good does all the time in the world if it is bereft of energy resources and biological diversity, and if future humankind is crumbling to dust under the burden of economic hyperthermia?

Salvador Dali (1970) in Swarovski Crystal Worlds. Photo by Mario Katzmayr.

1. Increase of speed = distance/time

Distances become longer. Time spent underway remains constant or is prolonged at accelerated speed. Energy requirements escalate for automobilised transport. Speed is uncoupled from muscle activity. Daily ***mitochondrial lifestyle activity*** decreases, except if we take time for sport. Time to change to a mitochondrial lifestyle.

2. Increase of speed = number/time or amount/time (rate or

flow)

More letters are sent more frequently (text messages, Emails). The time needed for answers or deletions increases.

3. Increase of rhythm and tempo = cycles/time (frequency)

Images are moving ever faster (TV) and we stare longer and longer.

4. Increase of power (performance) = energy/time

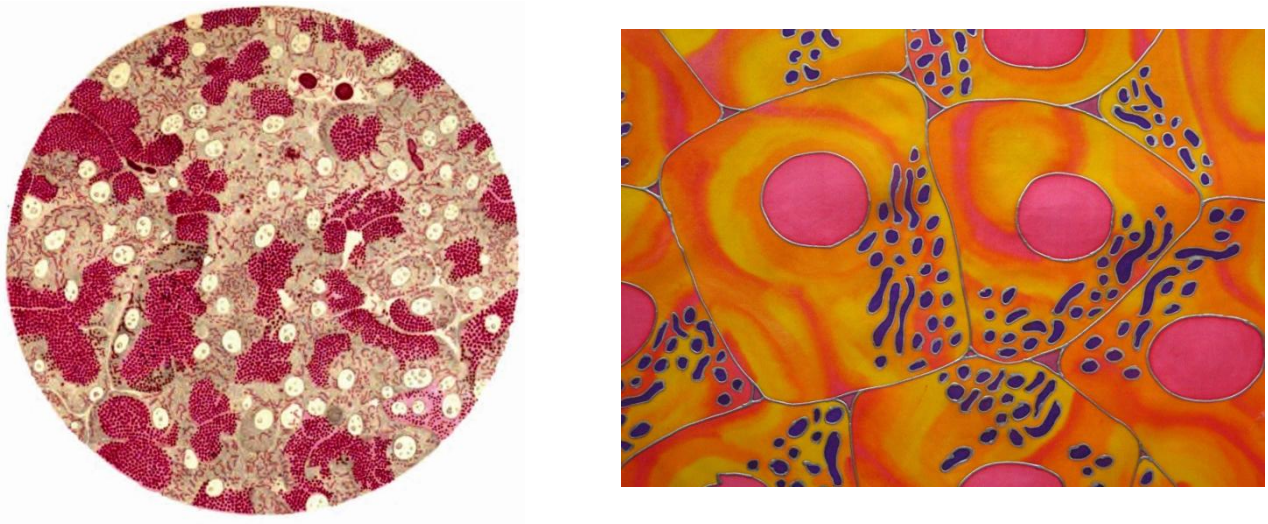
There is an increasing waste of energy and we complain about the constantly lacking time. Power versus efficiency: Excessive speed is waste of energy - is excessive efficiency waste of time? Have we lost an appropriate tempo? Is acceleration part of the insanity of our time? Is there any time left for reflection? Tempo Giusto is the appropriate tempo in music and the Re-birth of Classics: a revolutionary concept for reflection.

Tempo giusto in *MiPArt*

Mitochondrial physiology (MiP) is taking up the topic 'tempo' in the context of the speed of energy turnover in the living cell. Tempo and efficiency of cell respiration - biological combustion - are regulated by evolutionary optimization on the basis of ergodynamic laws of nature and are attuned to various vital functions – from explosively using muscular energy during the race for prey to moderately restricting energy turnover during hypoxia and anoxia [2]. Tempo Giusto is not a guide to deceleration, but rather a re-discovery of the rhythms between tension and relaxation, of the heartbeat between calm and motion, and of the sensuality and vitality of music performed in an appropriate tempo [1,3].

We invite all participants of Bioblast 2012 to join our Conference on *Mitochondrial Competence* in the spirit of Gentle Science. Richard Altmann's *bioblasts* [4] are beautiful, yet bioblasts raise *World Health Worries* (Odra Noel) as displayed in *MiPArt*. Experience *Tempo giusto* in concert with Uwe Kliemt in Swarovski Crystal Worlds on 2012-12-12.

1. Willem Retze Talsma (1980) Die Wiedergeburt der Klassiker. Anleitung zur Entmechanisierung der Musik. – Rebirth of Classics. A guide to the de-mechanisation of music. Wort und Welt Verlag Innsbruck.
2. Gnaiger E (1993) Efficiency and power strategies under hypoxia. Is low efficiency at high glycolytic ATP production a paradox? In: Surviving Hypoxia: Mechanisms of Control and Adaptation. Hochachka PW, Lutz PL, Sick T, Rosenthal M, Van den Thillart G (eds) CRC Press, Boca Raton, Ann Arbor, London, Tokyo: 77-109.
3. http://www.mipart.at/?MiPArt-Tempogiusto - German/English translation from *MiPArt* Tempo giusto by Verena Marte.
4. Altmann R (1894) Die Elementarorganismen und ihre Beziehungen zu den Zellen. Zweite vermehrte Auflage (The Elementary Organisms and Their Relationships to the Cells. Second Extended Edition). Verlag Von Veit & Comp, Leipzig. 160 pp, 34 Tafeln.



Bioblasts. *Left:* Richard Altmann (1894). *Right:* Odra Noel (2010) Homage to pioneers – Altmanns bioblast.

Abstracts Bioblast 2012

Gnaiger 2012 Abstract Bioblast

Gentle Science and the Bioblast Conference.

Erich Gnaiger
OROBOROS INSTRUMENTS, Innsbruck, Austria; and D. Swarovski Research Laboratory, Department of Visceral, Transplant and Thoracic Surgery, Medical University of Innsbruck, Austria.
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The concept of Gentle Science has been a driving force for more than 20 years of continuing development of high-resolution respirometry, which was initiated in a FWF project (1989). Our first generation *Oxygraph* was commercially available in 1992 (Fig. 1A). A series of applications was presented at a BTK conference in 1994 [1]. A full description of the instrument, method and concept of high-resolution respirometry was published in 1995 [2], when the positive feedback from early users had confirmed the validity of our approach [3]. The concept of O2k-MultiSensor extensions was implemented in the second generation Oxygraph-2k, developed in close cooperation with Phlipp Gradl (WGT-Elektronik) and distributed since 2002 (Fig. 1B).

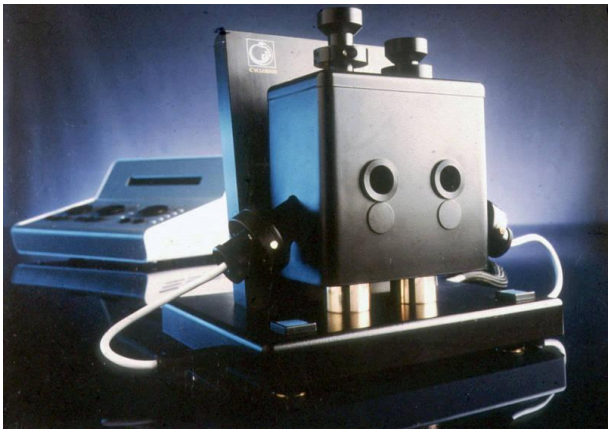

Figure 1: OROBOROS high-resolution respirometry. The first generation OROBOROS *Oxygraph* was developed in projects headed by Erich Gnaiger (FWF Austria, FFF Austria, Cyclobios; Haller et al 1994) and not more than 60 instruments were produced by Anton Paar (Graz, Austria). OROBOROS INSTRUMENTS still provides service for the few systems which remain in operation until today.

The strategy of OROBOROS INSTRUMENTS emphasizes the close link between university and company, teaching and training with Open Access to our published [4] and unpublished scientific background information, detailed descriptions of O2k-Protocols on our website, and direct communication with our customers and O2k-users (Mitochondrial Physiology Network).

Figure 2: In 2012, the O2k-Fluorescence LED2-Module was added to the O2k-MultiSensor concept based on the O2k-Core, developed in cooperation with WGT-Elektronik (Philipp Gradl) in the framework of the K-Regio Project *MitoCom Tyrol*.

Conforming to the OROBOROS logo we are proud to receive highly positive O2k-Feedback, but also provide help with O2k-Troubleshooting in open discussions. Standardization of methods and protocols is an important yet elusive topic for the development of mitochondrial physiology and functional diagnosis in mitochondrial medicine. Our O2k-Workshops contribute to a more general understanding of the rationale for standardized protocols, while the Bioblast wiki website (launched in

2010) may help to develop a more consistent terminology in mitochondrial physiology. Several projects have taken a long time for completion from experiment to publication [4,5]. It is satisfactory if results stand the test of time [5].

Since a 2007 workshop in Schroecken and the first MiPsummer school 2007, advanced SUIT protocols for sequential activation particularly of CI-, CI+II- and CII-linked respiration are explained in the 'Blue Book'. Following a 2nd electronic edition, the third edition is presented at Bioblast 2012 [5], complementary to peer-reviewed publications [4]. It is not in the spirit of Gentle Science, however, to receive enthusiastic feedback by Emails and then read in a publication (2008) about "*protocols developed by us*" without citing the original work. In this regard the present 3rd edition should be more successful.

More than 800 publications refer to the OROBOROS Oxygraph-2k, reflecting the increasing interest in mitochondrial physiology. The O2k-Team thanks all users for their joint contributions to high quality science, and is proud to be part of a global network that makes a contribution to a new mitochondrial medicine.

The Bioblast Conference should allow open exchange of results and ideas in an environment inspired by art, music and friendship. Mitochondrial Gentle Science is an illusion worthwhile to maintain.

Contribution to K-Regio Project *MitoCom Tyrol.*

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3. Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. Respir Physiol 128: 277-297.
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5. Gnaiger E, Méndez G, Hand SC (2000) High phosphorylation efficiency and depression of uncoupled respiration in mitochondria under hypoxia. Proc Natl Acad Sci USA 97: 11080-11085.
6. Gnaiger E (2012) Mitochondrial Pathways and Respiratory Control. An Introduction to OXPHOS Analysis. 3rd ed. Mitochondr Physiol Network 17.18. OROBOROS MiPNet Publications, Innsbruck: 64 pp.

Supplementary references: Gnaiger 2012 Abstract Bioblast-Gentle Science

Leo 2012 Abstract Bioblast

Happiness and mitochrondrial activity.

Hannes Leo, www.cbased.com - leo@cbased.at

Happiness is probably the ultimate goal in live although many of our fellow human beings are (voluntarily or involuntarily) fully submerged in the collection and consumption of worldly goods and services. Likewise, policy makers are predominately focussing on economic growth as measured by the Gross Domestic Product (GDP). Increasing unease with these *obsessions* has stimulated happiness research, which is mostly based on surveys. The nexus to mitochondrial processes has so far been neglected, although the link might be immediate and straightforward. Consequently, some of the basic insights of happiness research and its links to mitochondrial activities will be highlighted.

Mitochrondrial lifestyle activity and happiness.

Erich Gnaiger, *MitoCom Tyrol*

The link fits the spirit of a conference on *mitochondrial competence* for multiple reasons. It is the link to an inspiring extension of conventional economic values beyond financial measures of evaluation, with a focus on the quality of life and 'happiness'.

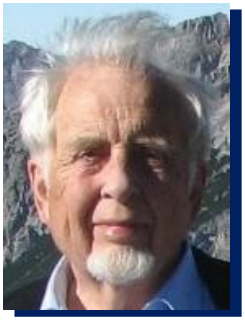
Bioblast 2012 takes place in Innsbruck in the heart of the Alps. The high recreational value of the famous mountainous landscape of the Tyrol paves the way for many locals and visiting guests to engage in outdoor *mitochondrial lifestyle activities*. These activities have directly visible effects on the population, with significantly lower BMI in alpine

regions compared to lowland regions, in areas with high educational standards compared to less favoured provinces. The *mitochondrial lifestyle activities* have invisible and hence largely unrecognized effects on the microscopic powerhouses in our cells – the bioblasts. Mitochondria may be seen as internal organisms established as a firmly enclosed minority in our body, but only when counting mass or volume rather than numbers. These permanent guests are in motion within their intracellular environment provided by the host (the major part of our body). Body locomotion and other forms of physical activity critically depend on mitochondrial energy transformation, yet exercise is in turn required for the proliferation and rejuvenation of the mitochondrial population. Mitochondrial rejuvenation is not sufficient, but necessary for positive emotions maintained in populations with an increasing life expectancy world-wide.

Bioblast 2012 is organized within the framework of the K-Regio project *MitoCom Tyrol*. The 'light in the powerhouse of the cells' relates to the optical approach for extending the analytical potential of high-resolution respirometry in OXPHOS analysis. In addition, a new light should improve the visibility of mitochondria and public awareness. Perspectives are summarized under the header 'Tyrol – a heart for sport' to convey the importance of mitochondrial health in *optimization medicine* as a decisive economical factor of the future health care system.

Bioblast 2012 is a celebration of the 20-years experience in connecting science and economy for the development of high-resolution respirometry, with continuing optimization and innovation (O2k-Fluorometer), in cooperation with our partner WGT-Elektronik. OROBOROS INSTRUMENTS is responsible for the worldwide distribution of the Oxygraph-2k. Mitochondrial function and happiness are of primary importance for our corporate identity and corporate social responsibility (see Top 10 Reasons). Happy O2k-users are highly satisfied with the unique performance of the O2k and our scientific standard. We want to maintain an outstandingly high quality as the basis of successful dissemination - this is our mitochondrial competence. We want mitochondrial scientists to be happy with the O2k.

1. From Bioenergetics to Mitochondrial Medicine



Garlid KD 2012 Abstract Bioblast

Bioenergetics: a physiological overview.

Keith D Garlid
Dept. Biology, Portland State Univ., Portland, OR, USA. - garlid@pdx.edu

The chemiosmotic theory, for which Peter Mitchell was awarded the Nobel Prize in Chemistry, was presented as a hypothesis far in advance of experimental evidence, and it stands as a glorious monument to the scientific method. Mitchell [1] proposed that nature uses protonic batteries to drive ATP synthesis and that biological energy conservation is essentially a problem in membrane transport. This was summarized in four postulates: 1) The electron transport system is vectorially oriented so that the energy of electron transport drives ejection of protons from the matrix, creating a proton electrochemical potential gradient. 2) The F_1F_O ATPase is also vectorially oriented so that the energy of ATP hydrolysis drives ejection of protons from the matrix. Because it is reversible, protons driven inward through the enzyme by the protonmotive force will cause ATP synthesis. 3) Ion leaks would short-circuit the protonmotive batteries, so the inner membrane must have a low diffusive permeability to ions in general and to protons in particular. 4) Cation leaks are compensated by electroneutral cation/proton antiporters, and low permeability for substrate anions is compensated by electroneutral anion exchange porters. Each of these postulates was, at the time, a radical departure from conventional wisdom. Postulates 3 and 4 form the basis for one aspect of mitochondrial physiology, but mitochondrial physiology is a rich and varied field, and includes cellular processes such as autophagy, fission/fusion, and apoptosis. In a brief talk, it will be necessary to focus on one aspect, and I will review recent progress in understanding the K^+ cycle and its role in the cell.

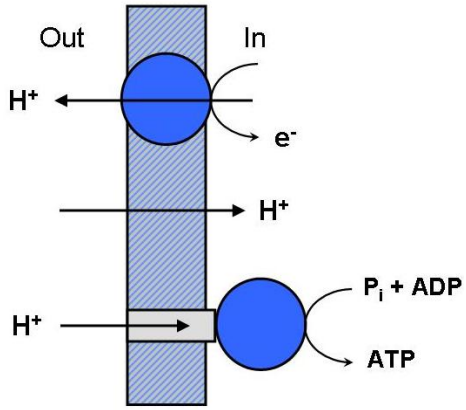
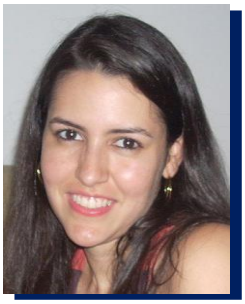


Figure 1: Coupling of electron transport with ATP synthesis. Protons are ejected electrogenically by the electron transport chain, generating a protonmotive force. This drives protons back through the ATPsynthase, leading to ATP production. Some of the energy is dissipated by electrophoretic back-diffusion, primarily of K^+ and H^+. Also necessary for ATP synthesis are the adenine nucleotide translocase, which catalyzes electrophoretic ATP/ADP exchange, and the phosphate transporter, which catalyzes electroneutral phosphate uptake. A considerable amount of mitochondrial physiology, including volume homeostasis, Ca^{2+} regulation, regulation of ROS production, and intramitochondrial signaling, is governed by inner membrane cation porters for Na^+, K^+ and Ca^{2+}.

1. Mitchell P (2008) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biological Reviews 41: 445-501.



Amoedo 2012 Abstract Bioblast

Mitochondrial function and bioenergetics during malignant transformation and metastasis.

Nivea Dias Amoedo[1], Rodrigues MF[1], Pereira SAS[1], Melo FH[2], Jasiulionis MG[2], Galina A[1], Rumjanek FD[1]
[1]Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [2]Departamento de Farmacologia, Universidade Federal de São Paulo, São Paulo, Brazil. - nivea_amoedo@yahoo.com.br

The classic bioenergetic phenotype of cancer cells, an enhanced glycolysis with concomitant production of lactate even in high oxygen tension, was described by Otto Warburg approximately 90 years ago. However, the Warburg hypothesis does not necessarily imply mitochondrial dysfunction. Current thinking considers tumor cells as adapted to an oxygen gradient within the tumor mass. Those cells exposed to oxygen will utilize the gas and those found in hypoxic regions of the tumor will adjust by means of metabolic symbiosis. Tumor cells exhibit accelerated growth as well as metastasis, two major events that must be supported by sufficient energy supply. This can be translated as metabolic reprogramming that up-regulates pathways that ultimately increase the rate of ATP production, synthesis of lipids and redox balance. According to *Smolková et al.* the process of carcinogenesis is guided by waves of gene expression that promote these metabolic changes. The energy metabolism of cancer cells is very heterogeneous. Indeed not all tumor cells display a high glycolytic flux as proposed by Warburg. Similarly, not all cancer cells grow fast and show intense anabolism. Furthermore, progression to metastasis appears to require mitochondrial function, a hypothesis that is compatible with the results obtained by our group. In order to show this, we resorted to a murine model of melanoma. In this model a melanocyte cell line was subjected to several cycles of adhesion impediment, which produced stable cell lines exhibiting phenotypes corresponding to a progression from non-tumorigenic to metastatic cells. The different stages of malignant transformation were as follows: Non-tumorigenic cells melan-a (**ma**) (original murine melanocytes); non-tumorigenic cell line **4C** (obtained after 4 cycles of adherence abrogation); non-metastatic **4C11-** and metastatic **4C11+** melanoma cell lines, which were obtained by diluting the cells from the spheroids of the **4C** cell line. The metabolic profile of each of these different cell lines was investigated by evaluating enzyme activities and expression of members of the glycolytic and oxidative pathways.

Our results showed that metastatic cell line (**4C11+**) released the highest amounts of lactate and exhibited high LDH activity, typical of the Warburg effect. In contrast, results obtained with high-resolution respirometry with **4C11+** intact cells showed an increased oxidative metabolism, with enhanced rates of oxygen consumption coupled to ATP synthesis when compared to the other pre-malignant stages. Moreover we observed an increase in succinate dehydrogenase (Complex II) activity in these cells. Concomitantly, we detected an increase in activity of the electron transport system (ETS capacity). We did not observe an increase in mitochondrial content, mitochondrial biogenesis, nor alterations in fusion and fission process. These results suggest enhanced OXPHOS. This was thought to be associated to metastasis, a condition which would benefit from unrestricted supply of oxygen. Detailed analysis of patterns in this and other models of tumor progression may reveal whether the modulation of the oxidative metabolism is a feature of the metastatic process.

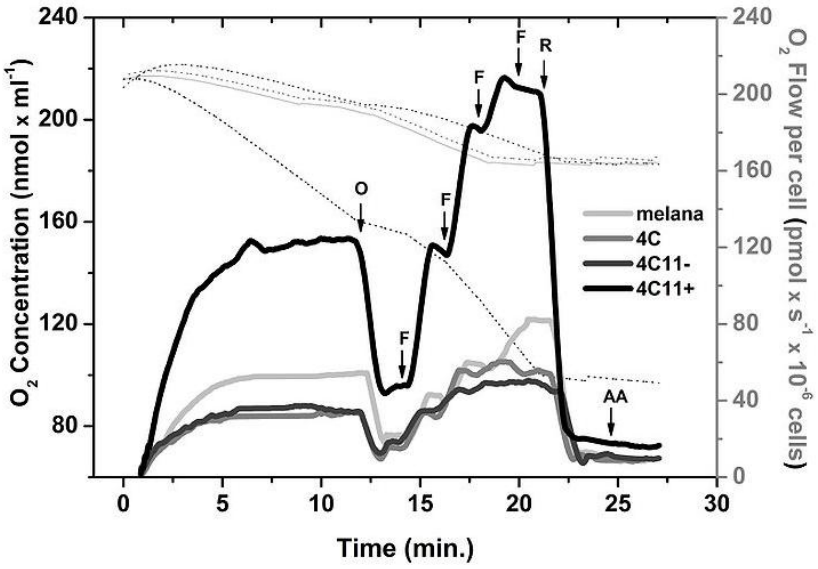


Figure 1: High-resolution respirometry showed an increase in oxidative metabolism in metastatic cell line. Representative assay of oxygen concentration (dashed line) and oxygen flow (solid line) of melanoma cells (ma - non-tumorigenic cells melan-a (original murine melanocytes); non-tumorigenic cell line **4C** (obtained after 4 cycles of adherence abrogation); non-metastatic **4C11-** and metastatic **4C11+** melanoma cell lines, obtained by diluting the cells from the spheroids of **4C** cell line) as picomoles of O_2 per second per 106 cells. This experiment was performed using an Oroboros O2k instrument. During the assay, intact cells were maintained in RPMI medium with glucose and glutamine without fetal bovine serum. The modulators of respiration were added in the following order: oligomycin (O; Omy), FCCP (F), Rotenone (R; Rot) and Antimycin A (AA; Ama).

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Tronstad 2012 Abstract Bioblast

Mitochondrial morphology and biogenesis in cellular stress.

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Mitochondria are major producers of ATP and they are important contributors in cellular processes of adaptation, stress, and survival/death. In this sense they represent centers of crosstalk between metabolism and signaling. Depending on the cellular context, mitochondrial activities are regulated by elements of metabolism/bioenergetics, biomass and structural organization. Although evidence has been provided that mitochondrial shape and function are interconnected, more quantitative single cell studies are required. Within cells, mitochondrial morphology varies from spherical individual organelles to interconnected filamentous networks. Mitochondrial architecture is controlled by fission and fusion processes. Furthermore, recent findings have given new insights into the roles of mitochondrial biogenesis in cellular responses to energy stress, mutations and aging. Clearly, the mechanisms directing mitochondrial biogenesis and function are diverse, and involve multiple key factors regulating essential properties in the viable cell [1,2]. In the present project, we are addressing the roles and mechanisms of mitochondrial participation in cellular adaptation and stress tolerance. A part of this has been to develop a protocol allowing 3D analysis of multiple mitochondrial parameters, and compare this with the data obtained

from 2D image analysis [3]. Here we will present a protocol enabling analysis of mitochondrial properties such as number, volume, surface area, sphericity and network related descriptors (e.g. branching points) using z-stacks acquired by standard confocal microscopy. Moreover, we will discuss data showing diverse cellular responses to "mitochondrial boosters". The metabolic phenotype of a cell, including mitochondrial respiration, may represent an important determinant of stress tolerance. Using new tools to study these phenomena is therefore important in order to learn more about mitochondrial physiology.

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Wojtczak 2012 Abstract Bioblast

A novel putative function of uncoupling proteins.

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Apart from the long known uncoupling protein of the mammalian brown adipose tissue, responsible for the thermogenic function of this tissue and designated as uncoupling protein-1 (UCP1), a number of analogous proteins have been identified during last two decades at minute quantities in some other mammalian tissues as well as in birds, insects, plants, fungi and protists. Although they are classified as uncoupling proteins (UCPs), their precise function and importance are debated. We summarize here our recent studies [1, 2], which show that UCP3 in rat heart and skeletal muscle mitochondria and UCP2 in mouse brain mitochondria can function as transporters of the superoxide anion, thus contributing to the extrusion of this noxious oxygen free radical from the mitochondrial inner compartment to the intermembrane space and further on to the cytosol.

Generation of reactive oxygen species (ROS) by mitochondria incubated with respiratory substrates is highly increased by antimycin A, inhibitor of complex III of the respiratory chain. In addition, ROS generation in the presence of complex I substrates is increased by rotenone. We found that this high ROS release to the incubation medium by mitochondria from rat heart and skeletal muscles and from mouse brain was decreased by purine nucleoside di- and tri-phosphates GDP and GTP, known inhibitors of UCP. Among two components of ROS that were determined in the incubation medium, hydrogen peroxide and superoxide anion radical, only the latter was decreased by GDP or GTP. In contrast, intramitochondrial level of ROS, as assessed by inactivation of the matrix enzyme aconitase, was increased by GDP and GTP. No or little effect on the release of ROS was exerted by carboxyatractyloside, specific inhibitor of the mitochondrial adenine nucleotide transporter. Much lower effect of GDP or GTP on the release of ROS was observed in mitochondria of brains isolated from transgenic mice deprived of UCP2.

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Harper 2012 Abstract Bioblast

Metabolic effects of microRNA-133 and its antagomir in mice.

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The unequivocal identification of functional brown adipose tissue (BAT) in adult humans (reviewed in [1]) has led to a resurgence of interest in this unique tissue and its potential in novel anti-obesity therapies [2-3]. Unlike white adipose tissue, BAT is rich with mitochondria. BAT is capable of remarkably high rates of uncoupled respiration due to the activity of uncoupling protein-1 (UCP-1). The cellular origins of brown adipocytes are not well understood, but the transcription factor Prdm16 is necessary and sufficient in establishing brown adipocyte lineage [4]. *In vitro* loss-of-function of Prdm16 induces myogenic differentiation of committed preadipocytes from BAT, while gain-of-function induces committed myoblasts to differentiate into brown adipocytes [4]. Satellite cells are adult skeletal muscle stem cells, located beneath the basal lamina of muscle, and when activated, they proliferate and differentiate into multi-nucleated muscle cells. In this study we show that brown adipocyte determination of satellite cells is controlled by miR-133.

Loss-of-function of miR-133 during muscle regeneration in mice commits satellite cells to the brown adipocyte lineage, causing local uncoupled respiration, increased glucose uptake and whole body thermogenesis, as well as decreased diet–induced obesity.

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4. Harper 2012 Abstract Bioblast

Garlid AO 2012 Abstract Bioblast

Na,K-ATPase signaling to mitochondria.

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Binding of cardiac glycosides to the Na,K-ATPase has two effects: increased contractility (inotropy) and cardioprotection against ischemia-reperfusion injury [1]. Cardioprotection is mediated by caveolar vesicles (signalosomes) that bud off the plasma membrane, move to mitochondria, and use a terminal kinase to phosphorylate an outer membrane protein. This activates inner membrane PKCεs, causing opening of the mitochondrial ATP-sensitive K^+ channel (mtK_{ATP}) and inhibition of the mitochondrial permeability transition (mtPT). These events are assayed *in vitro* using purified signalosomes from treated hearts and mitochondria from untreated hearts [2].

The terminal kinase of GPCR signalosomes is PKG [2]; however PKG does not mediate cardioprotection by ouabain, so we set out to determine the terminal kinases used by the ouabain signalosome. Rat hearts perfused with ouabain yielded a signalosome fraction that was caveolar in nature and enriched with caveolins 1 and 3, Src, PKCε and the α-1-subunit of the Na^+,K^+-ATPase. Electron microscopy of purified signalosomes revealed vesicles approximately 140 nM in diameter that were found by immunogold labeling to be decorated with caveolin-3. Ouabain signalosomes from heart opened mtK_{ATP} in

mitochondria isolated from untreated hearts and liver. The terminal kinases of the ouabain signalosome are Src and PKCε, which together phosphorylated an endogenous outer membrane p38MAPK. We conclude (1) that ouabain cardioprotection utilizes the signalosome mechanism; (2) that the terminal kinases acting on mitochondria are Src and PKCε, (3) that this is a general mechanism of cell signaling, given that signalosomes from rat heart open mtK_{ATP} in rat liver mitochondria. (4) that ouabain cardioprotection acts via a mitochondrial p38 MAPK.

Digitalis has been used in the treatment of heart failure since 1785. It was thought for many years that its efficacy was due to its positive inotropic effect. This may not be the case. Cardioprotection in both rat and rabbit is seen with concentrations of ouabain that have no inotropic effect.

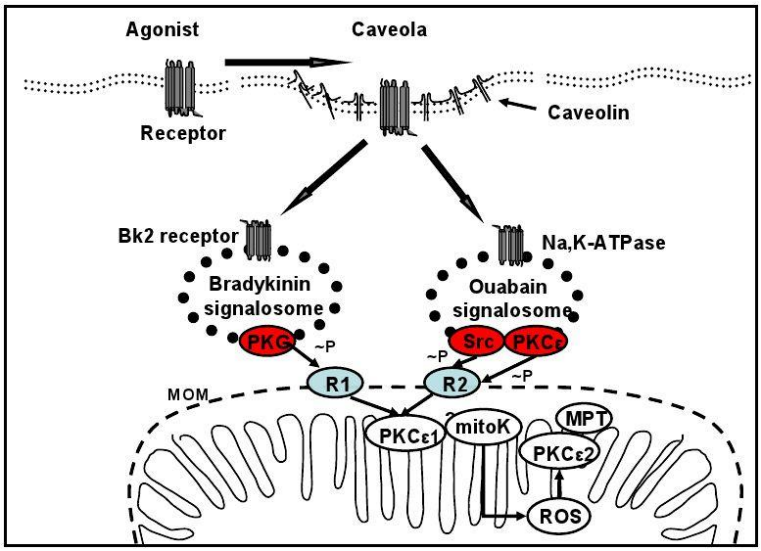

Figure 1: Signaling to mitochondria via signalosome. Upon binding to their respective receptors, bradykinin and ouabain trigger formation of caveolar signaling platforms that are encapsulated in signalosomes. Signalosomes bind to and phosphorylate mitochondrial outer membrane (MOM) receptors via specific terminal kinases. This causes the signal to be transmitted across the MOM and intermembrane space, to PKCε1 on the mitochondrial inner membrane and trigger the intramitochondrial signaling pathway, leading to activation of PKCε2 and inhibition of MPT. The terminal kinase of the bradykinin signalosome is PKG, which phosphorylates a MOM receptor (R1) of unknown identity. The terminal kinases of the ouabain signalosome are Src and PKCe acting in concert to phosphorylate a MOM p38MAPK.

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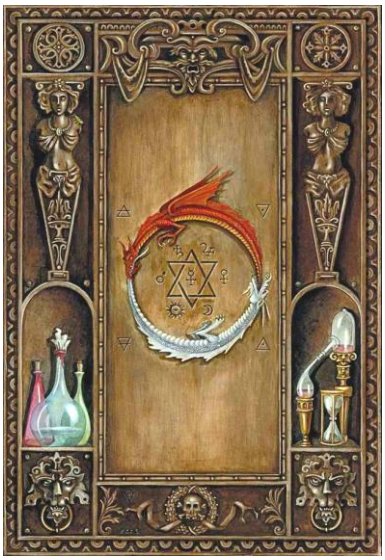
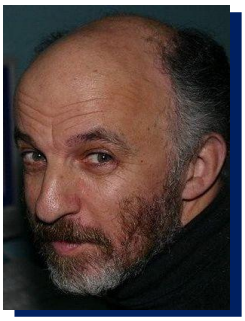


2. Mitochondrial Testing



Duchen 2012 Abstract Bioblast

Separation of NADPH and NADH fluorescence emission in live cells using fluorescence lifetime imaging microscopy.

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Measurements of fluorescence intensity of endogenous NADH and NADPH have been used to monitor cellular metabolic state for ~50 years [1]. As NADH and NADPH are fluorescent and NAD+ and NADP+ are not, the fluorescence signal gives a unique measure of the redox state of the NADH:NADPH pool, reflecting the balance of substrate supply and respiratory rate at the level of the single cell. An additional dimension of information is available through measurements of fluorescence lifetime (the mean dwell time of an electron in an excited state), as lifetime measurements are exquisitely sensitive to the microenvironment of a fluorophore. Thus, the fluorescence lifetime of NADH in solution (~0.4ns) increases more than 5-fold when NADH is enzyme bound, with a precise lifetime governed by the enzyme to which the cofactor is bound (giving a mean lifetime of ~2.7ns). It is also apparent that lifetimes vary depending on cellular metabolic state, but the biochemical basis for those variations in lifetime has not been clarified (e.g.[2]). We have used fluorescence lifetime imaging (FLIM) to explore changes in NADH and NADPH lifetimes in cells in culture under a variety of carefully controlled metabolic conditions. Lifetimes were measured using a Becker and Hickl system coupled to a Zeiss META NLO microscope using a Coherent Chameleon pulsed laser tuned to 700 nm, with emission measured between 420-480 nm.

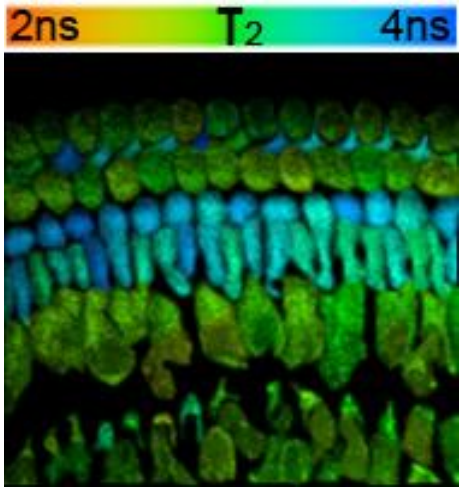

Figure 1: The image shows the distribution of long (enzyme bound) lifetimes (tau 2) of NADH; NADPH acquired from a rat cochlear explant culture. Excitation was at 700nm, emission acquired between 420-480nm. Lifetimes are colour coded between 2-4ns graded from red to blue, as indicated. Note the layer of cells with lifetimes close to 4ns, in contrast to the majority in which the mean long lifetime is close to 3ns.

We have found that changes in relative glycolytic or oxidative metabolism do not change lifetimes, but do change the relative weighting of the lifetimes that arise from free and enzyme bound NADH. Further, we found that enzyme bound NADH and NADPH, two species which are functionally distinct but spectrally identical, show distinct lifetimes, allowing separation of NADH and NADPH pools in cells and tissues. Thus, in HEK cells stably overexpressing NAD kinase (NADK), in which NADPH levels are elevated about 15 fold [3], the fluorescence lifetime of free NAD(P)H was unchanged at ~0.4ns, but the fluorescence lifetime arising from enzyme-bound NADPH was greatly increased to 3.9 ± 0.4 ns compared to 3.0 ±0.2 ns measured in HEK cells in which NADK was stably knocked-down. FLIM studies in complex preparations (brain slices, cochlear explant cultures, renal slices etc) revealed individual cell types enriched in NADPH. Thus, FLIM imaging of cochlear explant cultures from postnatal day 2-3 rats revealed a subset of

supporting cells showing an equivalent long lifetime (mean 3.6ns), suggesting high levels of NADPH in these cells (Fig 1). The long lifetime signal was reduced by exposure to the drug Epigallocatechin-3-gallate (EGCG) which displaces NADPH from its binding sites, supporting this conclusion. These data suggest that FLIM provides a unique and novel approach to separate NADH and NADPH signals, at last providing an approach to address specific changes in NADPH and NADH and their contributions to physiology in living cells and tissues.

This work is supported by a PhD studentship to TB funded by the EPSRC and CoMPLEX at UCL.

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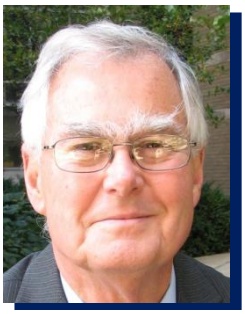

Hoppel 2012 Abstract Bioblast

Mitochondrial functional testing – muscle biopsy, isolated mitochondria and others.

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Mitochondrial functional testing revolves around the key role played by ATP in energy metabolism. The mitochondrial process involves substrate translocation, metabolism with production of reducing equivalents, $NADH^+$, $FADH^+$, oxidation through a series of oxido-reductases (complexes) ultimately to form water, pumping protons from the matrix side to the intermembrane space, the selective impermeability of the inner membrane to protons, and the flow of protons through Complex V leading to the production of ATP from ADP, Pi, and protons. Integration of this mitochondrial function can be assessed (measured) as oxidative phosphorylation in freshly isolated mitochondria.

We isolate mitochondria from biopsies of skeletal muscle (vastus lateralis) and rarely liver from patients suspected of a mitochondrial disease. The patients have a clinical presentation and laboratory findings that suggest a mitochondrial disease. Frequently, mtDNA from whole blood has been sequenced with no known pathological mutations detected. The activity of the electron transport chain is determined in the fresh muscle and in the isolated mitochondria.

The rate of ATP production is directly determined using an amino acid and a fatty acid as substrates. The adenine nucleotide translocase and Complex V (ATPase) are measured in the isolated mitochondria. Proteomic analysis is done using Blue Native Gel Electrophoresis to quantify supercomplexes and unincorporated complexes. Quantitative phospholipid analysis is done with characterization of cardiolipin molecular species on the isolated mitochondria.

Integrated mitochondrial function is measured as oxidative phosphorylation using 19 different substrates to probe various metabolic pathways and distinct entry points of reducing equivalents into the oxidative machinery. Six different fatty acid substrates are used to assess the metabolic pathways in fatty acid oxidation. Mitochondrial membrane integrity is directly measured using NADH oxidation. The use of oxidative phosphorylation, skeletal muscle and isolated mitochondrial ETC activities provide critical information when ETC defects are accompanied with mitochondrial proliferation; i.e., a five-fold increase in mitochondrial content can move skeletal muscle ETC activity into the low normal range, whereas the defect is clear with isolated mitochondria. We believe we are identifying a new group of disorders, which have defects in oxidative phosphorylation without accompanying defects in the ETC. The amount and distribution of

supercomplexes (respirosomes) is abnormal in some of these patients; alterations are still being explored in others.

Votion 2012 Abstract Bioblast

The cause of atypical myopathy in grazing European horses revealed.

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Atypical myopathy (AM) is a frequently fatal pasture myopathy that emerges in Europe. More than one thousand European cases have been communicated to the AM Alert Group (AMAG) since autumn 2006. This seasonal condition kills 75% of affected horses within 72 hours with signs resulting from acute degeneration in postural and respiratory muscles.

From epidemiological studies performed on European cases [1] and by elucidating the pathophysiological mechanism [2], using several samples collected through the AMAG network, the assumption of a toxin of environmental origin that would alter the energy metabolism has been hypothetised. Indeed, affected horses have acquired deficiencies in multiple acyl-CoA dehydrogenases resulting, among others, from defects in several mitochondrial dehydrogenases [2].

Recently, it was shown that Seasonal Pasture Myopathy (SPM) in the US was caused by the toxic amino acid hypoglycin A present in the seeds of box elder trees (*Acer negundo*) [3]. Once ingested, hypoglycin A is metabolized into methylenecyclopropyl acetic acid (MCPA) that disrupts energetic metabolism leading to the biochemical derangements seen in both, SPM and AM.

In a preliminary study, the mitochondrial respiration in cultured equine skeletal myoblasts was monitored with high-resolution respirometry with or without addition of serum of AM-affected horses. We observed a dose-dependent inhibition of the mitochondrial respiration (up to the full inhibition) which was not induced by serum of healthy controls but that was similar to the one obtain with MCPA.

Hypoglycin A may be contained in seeds of *Acer pseudoplatanus* (maple tree; *Aceraceae*) that was consistently present in pastures of affected horses and currently, sera from European cases are being analyzed to search for MCPA-conjugates in blood. We should know soon if AM is due to the same toxin than SPM in the US.

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Hepple 2012 Abstract Bioblast

Is latent mitochondrial dysfunction in aging muscle exposed through mitochondrial isolation?

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Mitochondrial alterations are strongly implicated in aging, particularly that of post-mitotic tissues like skeletal muscle. To date the majority of studies have examined this issue using mechanically isolated mitochondria. Given the fragmentation of the native mitochondrial architecture induced by this approach, and the stress this imposes on the organelle (e.g., potentiating ROS emission and sensitivity to an apoptotic challenge [1]), it is important to consider whether mitochondrial isolation affects the assessment of alterations in mitochondrial function in aging skeletal muscle.

To this end, we compared alterations in mitochondrial function (respiration, reactive oxygen species [ROS] emission, and function of the mitochondrial permeability transition pore [mPTP]) in aging skeletal muscle between isolated mitochondria and saponin-permeabilized myofibers, the latter representing a method that preserves mitochondrial architecture. Strikingly, we observed that routine mechanical isolation of mitochondria profoundly exaggerated the impact of aging on all indices of mitochondrial function, whereas permeabilized myofibers from aged muscles had no change in respiratory capacity, and relatively modest alterations in ROS emission and mPTP function [2].

In addition to having important implications for our understanding of the severity of mitochondrial dysfunction in aging skeletal muscle, our results also suggest that vulnerabilities in aging mitochondria from skeletal muscle may be at least partially compensated for by the mitochondrial network structure *in vivo* and these vulnerabilities become unmasked following organelle isolation. The implications of these findings for our understanding of aging on mitochondrial function will be discussed.

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Labieniec-Watala 2012 Abstract Bioblast

Seasonal fluctuations affect the physiology of laboratory animals – facts and artifacts from the perspective of heart mitochondrial respiratory response to tested compounds.

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All living organisms are subject to biorhythmic energetic fluctuations. Usually, it is associated with a pathological background relevant to aging or diseases. However, it can be also linked to the compromising of the survival under unfavorable environmental conditions, such as hypoxia, hypothermia or the lack of food. Recently, several pieces of evidence have been accumulated, showing that seasonal variations can play an essential role in the metabolism, behavior and activity of the laboratory animals. These observations are consistent data (originating from both *in vitro* and *in vivo* experiments) demonstrating that mitochondria isolated from rats in the autumn-winter period are characterized by the "respiratory depression", while the mitochondria derived from

spring-summer animals seem to be less sensitive to tested compounds and characterized by more efficient respiratory capacity. The objective of our presentation is to show the effect of seasonality on mitochondrial bioenergetics and - in consequence, the further interpretation of the study outcomes.

Using rat heart mitochondria as a model, and PAMAM dendrimer G3 and methylglyoksal (MG) as the tested chemicals, we have revealed significant differences in the mitochondrial response when carrying out our experiments and acquiring data throughout the year (September – June). The obtained data give a possibility to assume that lower respiratory capacity of mitochondria derived from the autumn-winter period is likely to result from suppressed energy metabolism and metabolic depression. Mitochondrial parameters, such as RCR and ADP/O, were significantly increased for animals derived in spring administered with the tested compounds.

These data support the reasoning that in spring period mitochondria are in a better condition compared to those tested in the autumn/winter period. Therefore, it is noteworthy to remark that in order to obtain the reliable conclusions, the aspect of seasonal changes in laboratory animals should be taken into consideration at the experimental design of the study lasting a few months and spanning for a longer period including different seasons.

Finally, it is important to emphasize that there is a high probability that some outcomes derived merely from the *in vitro* experiments may never have their absolute relevance to clinical studies.

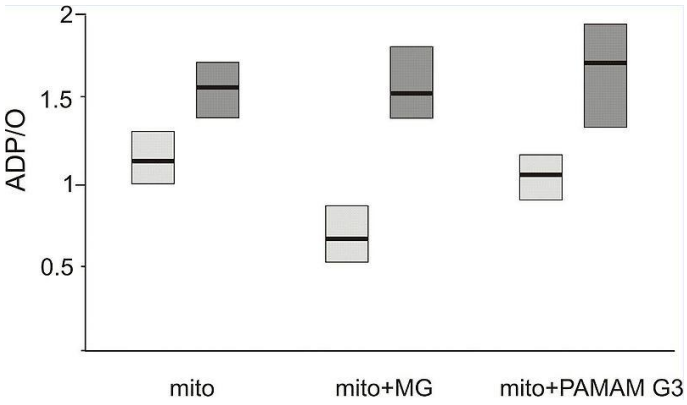

Figure 1: ADP/O of Wistar rat heart mitochondria *in vitro* exposed to methyloglyoxal (MG, [500 µM]) or PAMAM dendrimer G3 [100 µM]. Data, collected in autumn (light boxes) or spring (dark boxes), presented as median (solid horizontal line) and interquartile range (lower and upper quartile, boxes), n=18. Statistical differences estimated by two-ANOVA followed by post-hoc Tukey test for multiple comparisons. $p<0.01$, mito (in autumn) vs. mito (in spring) $p<0.001$, *mito+PAMAM G3* (in autumn) vs. *mito+PAMAM G3* (in spring) $p<0.0001$ *mito+MG* vs. (in autumn) vs. *mito+MG* (in spring).

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Eigentler 2012 Abstract Bioblast

The PBI-Shredder - an auxiliary HRR-tool for the preparation of tissue homogenates for diagnosis of mitochondrial respiratory function.

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Two well established methods of tissue preparation in studies of mitochondrial respiratory function are isolated mitochondria (Imt) and permeabilized tissue or cells (P_{tic}) including permeabilized muscle fibres (Pfi). Comparison of Imt and Pfi demonstrates some

advantages of Pfi since less tissue is required, the mitochondrial morphology is not fragmented and all mitochondrial populations are present [1-3]. On the other hand, homogenized tissue (Tho) provides different advantages as the preparation is faster and no detergents like saponin are required [4]. Smaller amounts of tissue are needed compared to Imt, and Tho are well suited for the study of mitochondrial respiration [4,5].

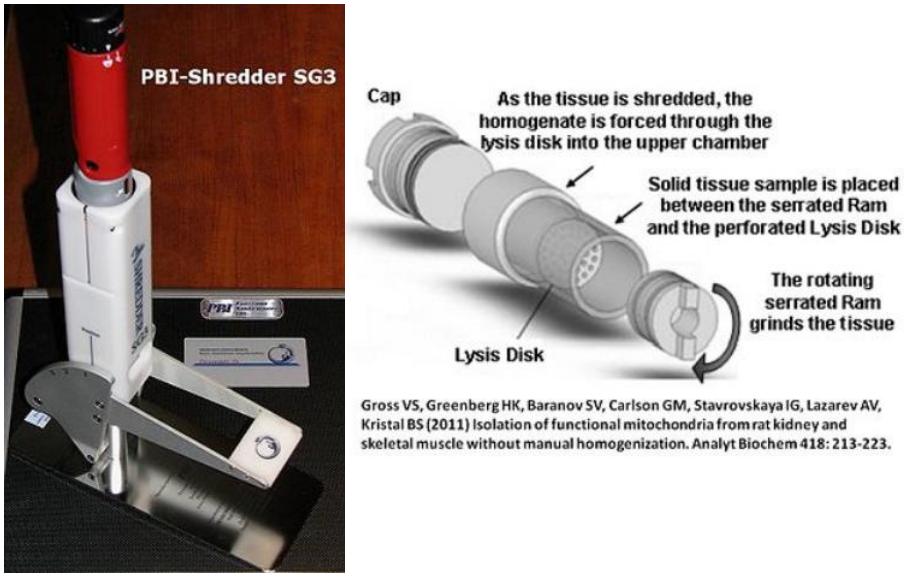

Figure 1. PBI Shredder SG3 for tissue homogenate preparation. Left: Heavy duty high torque SG3 driver with convertible handle, SG3 base with 3 position force setting lever. Right: Shredder-Tube including a Lysis Disk, serrated Shredder-Ram and Shredder-Screw Cap [6].

Furthermore, Tho have various advantages in O2k-Fluorometry compared to Pfi: (1) Whereas all preparations can be applied if the fluorophore is dissolved in the incubation medium (e.g. Amplex UltraRed), the use of Pfi is not possible in the O2k-chamber if the fluorophore binds to the tissue or mitochondria (safranin). (2) Hyperoxia is necessary when working with Pfi to avoid diffusion limitation and hypoxic conditions within the fibre, which is problematic for ROS production. Oxygen limitation is less pronounced in Tho. (3) With Pfi, variability between chambers is high due to tissue heterogeneity, which restricts comparability when different protocols are applied in parallel in different O2k-chambers. With Tho variability between chambers is restricted to instrumental reproducibility, the degree of homogenization and reproducibility of pipetting subsamples from the homogenate.

A high-quality preparation of Tho represents an optimum compromise for a variety of respirometric and fluorometric studies. The PBI-Shredder is an auxiliary HRR-Tool providing a standardized approach to prepare homogenates of various tissues (e.g. heart, liver, brain) with high reproducibility of mitochondrial yield and mitochondrial function as evaluated with HRR.

Contribution to K-Regio Project *MitoCom Tyrol.*

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Fasching 2012 Abstract Bioblast

O2k-Fluorometry - a *MitoCom* project.

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High-resolution respirometry, HRR [1] is extended by O2k-MultiSensor techniques allowing the simultaneous measurement of oxygen consumption and one additional parameter within a single chamber, including mitochondrial membrane potential, pH, Ca^{2+}, or NO concentration [2,3]. Combining optical measurements with HRR increased the analytical scope of OXPHOS analysis. In particular, fluorometric methods are available for a wide range of analytical parameters of major interest in mitochochondrial physiology: H_2O_2 production, mitochondrial membrane potential, intracellular pH, Ca^{2+}, Mg^{2+}, and NADH levels, and ATP production.

The simultaneous measurement of additional parameters in a single respirometric chamber under strictly identical conditions offers important advantages: (i) Respirometric performance provides a quality control of cells or mitochondrial preparations; (ii) overtitration of uncouplers, incomplete action of inhibitors, or non-saturating substrate concentrations are evaluated by the simultaneous response of multiple parameters; (iii) side effects of TPP^+ or fluorophores (inhibition, dyscoupling) are detected by respiration and artifacts are, therefore, excluded; (iv) the direct relationship between the different parameters eliminates the variability induced by of normalization for a separately determined mitochondrial marker; and (v) additional information is obtained for limited amounts of sample. In addition, there are practical and economical advantages, saving handling time and money. The glass chamber of the OROBOROS Oxygraph-2k (O2k) respirometer allows transmitting optical signals through the chamber wall. This extends O2k-MultiSensor applications, obtaining the optical signal in addition to the signals of the oxygen sensor and of other electrodes inserted through the stopper. The O2k-Fluorescence LED2-Module is an add-on module for the O2k. Optical sensors are inserted through the front window of the O2k-glass chambers, for measurement of hydrogen peroxide production (Amplex Ultrared), ATP production (Magnesium green), mt-membrane potential (Safranin), Ca^{2+} (Calcium green), and numerous other applications open for O2k-user innovation, with high flexibility gained by application of different LEDs and optical filters for the LED and photodiode.

Figure 1. Fuorescence-Sensor: LED with a specified wavelength, a photodiode, and a filter-cap attached with a specific optical filter for the LED and/or photodiode. Each Fluorescence-Sensor, Green and Blue, is equipped with a removable filter-cap for exchange of optical filters, which is possible independently for optical pathways from the LED and to the photodiode.

In the development of the O2k-Fluorescence LED2-Module, filter sets were optimized to record a fluorescence signal free from absorption artifacts. The nature of the observed drift was studied and performance parameters compared with a traditional spectrofluorometers. We describe an application for simultaneous HRR measurement of oxygen consumption, using Amplex UltraRed®.

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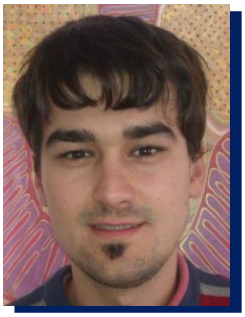

Horvath 2012 Abstract Bioblast

Respiratory substrate effects on mitochondrial membrane potential - experiments with mouse cardiac muscle homogenate.

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Mitochondria play a key role in the pathogenesis of different diseases including the pathologies of the heart. According to the World Health Organization, chronic diseases are responsible for 63% of all deaths in the world, with cardiovascular disease as the leading cause of death. New findings of the cardiac muscle mitochondria functions can be further used to develop new therapeutic strategies.

The most important parameters of heart mitochondrial activity are oxidative phosphorylation capacity and mitochondrial membrane potential. With these two properties of mitochondria we can determine the coupling state of respiration and the impact of the mitochondrial substrate on the membrane potential [1]. The Fluorescence module for the Oxygraph-2k (Oroboros Instruments, Innsbruck) combines optical measurement with high-resolution respirometry and with this new technology it is possible to detect changes in both parameters simultaneously.

The experiments were carried out in mouse heart homogenate. The measurements were performed using the O2k. We detect the oxygen-consumption with a polarographic oxygen sensor and the membrane potential changes with safranin fluorescence dye at 480 nm. The experiments were carried out following modified SUIT protocols [2]. We investigated Complex I and II and a combination of CI+II in different coupling control states.

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Stoeger 2012 Abstract Bioblast

Cell respiration and hydrogen peroxide production with yeast as a model.

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The present experimental results are part of a scientific project necessary to obtain the High School diploma. The aim of the project was to extend the use of Baker´s yeast as a simple model for cell respiration [1] and to develop a didactic demonstration experiment with the OROBOROS Oxygraph-2k (O2k) for introducing mitochondrial physiology at school. Cell respiration and H_2O_2 production of Baker´s yeast were measured with the O2k and the O2k-Fluorescence LED2-Module. Baker's yeast is an anhydrobiotic organism which is well known for its persistence without water for decades. When rehydrated, it can rapidly restore active metabolism within minutes [2]. In addition, dried baker's yeast has a high level of viability when rehydrated at 30 to 40 °C [2,3]. Commercially available

freeze-dried baker's yeast (5 mg dry weight) was rehydrated in K-P buffer (100 mM, pH 7.2) at 37 °C. Vortexing for 3 min at 2,200 rpm was necessary to obtain a homogenous cell suspension, immediately prior to adding a subsample of 100 µl into the 2 ml Oxygraph-2k chamber (0.25 mg/ml final cell density; 37 °C).

For the detection of H_2O_2 production rate 10 µl Amplex Ultrared (AmR) and 4 µl horseradish peroxidase (HRP) were added at the beginning. After addition of the cells into the O2k-chamber, endogenous ROUTINE respiration (e) was observed (Fig. 1). Then glucose (Glc, 20 mM) and ethanol (EtOH, 2%) were titrated to stimulate respiration which was observed until anoxia was reached. Reoxygenation was achieved with pure O_2 and oxygen concentration was again allowed to dedline to anoxia. Another step of reoxygenation to approximately 300 µM oxygen was conducted, resulting in the GlcEtOH-reox state. Respiration was observed until O_2 concentration reached 80 µM and then AntimycinA (Ama, inhibitor of Complex III) was added to inhibit respiration. Finally residual oxygen consumption (ROX) was observed for several minutes at 80 µM O_2 and after reoxygenation at 300 µM O_2.

Stimulation of respiration by addition of Glucose and EtOH was observed (from 829 to 1,065 pmol $O_2 \cdot s^{-1} \cdot mg^{-1}$). The maximum of respiration was obtained after reoxygenation (1,815 pmol $O_2 \cdot s^{-1} \cdot mg^{-1}$). ROX was as low as 2% of maximum respiration. H_2O_2 production showed a pronounced dependence on oxygen concentration.

The simple handling of yeast cells and the O2k makes this model suitable for application in school lessons to demonstrate respiration of cells as well as inhibition of respiration and H_2O_2 production in real time.

Contribution to K-Regio Project *MitoCom Tyrol.*

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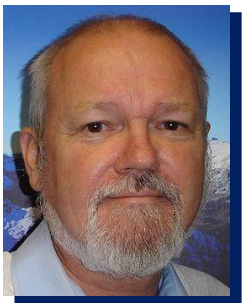

Harrison 2012 Abstract Bioblast

O2k-Spectrophotometry – A *MitoCom* Project.

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The principal thrust of the technical development of the O2k high-resolution respirometer within the *MitoCom* project is towards the O2k-Fluorometer. However, simultaneously, a parallel project is working towards the integration of spectrophotometry into the O2k in order to measure the redox state of cytochromes – principally cytochrome *c* and *aa*3, but also cytochrome b.

The first approach to measuring cytochrome absorption spectra in the O2k respirometer used a specially designed stopper that incorporated two lightguide fibres: one used to conduct light from an external lamp into the cuvette, the other to collect the emergent light scattered by the medium [1].

However, the development of multiple parameter sensors that can be inserted through the conventional stoppers led to the concept of using the chamber window as the optical port-hole for both fluorometry and spectrophotometry [2] instead of the special optical stopper (which could not be used in conjunction with other probes). The initial spectrophotometric approach was to use a pair of lightguides: the first to transmit light through the chamber window and the second to receive the light scattered back from the medium. A series of experiments was carried out in order to determine the optimal configuration and spacing for the lightguides. In order to carry out comparisons, it was necessary to develop analytical methods in order to compare the signal-to-noise (S/N) ratios of the difference spectra (reduced minus oxidised cytochrome absorption spectra) obtained using the different configurations and the results will be presented.

The latest approach involves incorporation of a white light emitting diode (LED) into the O2k itself, in addition to the standard illuminating LED. Initial results using this configuration demonstrate an order-of-magnitude increase in S/N ratio compared with the previous configurations [3]. Work is continuing in order to determine the optimal spectral characteristics for the LED.

Contribution to K-Regio project *MitoCom Tyrol.*

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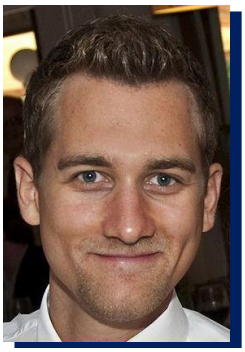

Kiss 2012 Abstract Bioblast

The negative impact of alpha-ketoglutarate dehydrogenase complex deficiency on matrix substrate-level phosphorylation.

Gergely Kiss[1], Csaba Konrad[1], Judit Doczi[1], Anatoly A Starkov[2], Hibiki Kawamata[2], Giovanni Manfredi[2], Steven F Zhang[2], Gary E Gibson[3], M Flint Beal[2], Vera Adam-Vizi[1], Christos Chinopoulos[1,2]

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Objectives: Provision of succinyl-CoA by the alpha-ketoglutarate dehydrogenase complex (KGDHC) is essential for generation of matrix ATP (or GTP) by substrate-level phosphorylation catalyzed by succinyl-CoA ligase. A decline in KGDHC activity has been associated with neurodegeneration.

Methods: Mitochondrial phosphorylation was investigated in tissues of transgenic mice with deficiencies in KGDHC subunits.

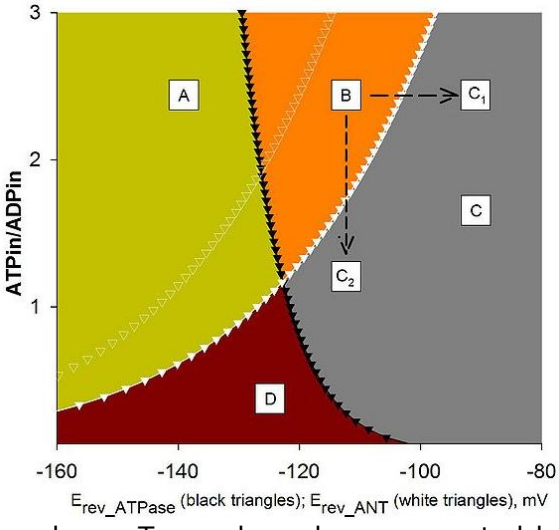

Figure 1: Computational estimation of the reversal potential of adenine nucleotide translocase (Erev_ANT) and reversal potential of F_0-F_1ATPase (Erev_ATPase). A: ATPase forward, ANT forward; B: ATP reverse, ANT forward; C, C1, C2: ATPase reverse, ANT reverse; D: ATPase forward, ANT reverse. Black solid triangles represent Erev_ATPase; white solid triangles represent Erev_ANT. Values were computed for [ATP]out = 1.2 mM, [ADP]out = 10 µM, Pin = 0.01 M, n = 3.7 (2.7 plus 1 for the electrogenic ATP4-/ADP3- exchange of the ANT), pHi = 7.38, and pHo = 7.25. White open triangles represent Erev_ANT values computed for [ATP]out = 1.4 mM, and all other parameters as above. Traces have been computed by Erev estimator; the software and instructions on how to use it can be downloaded here.

Results: We demonstrate ATP consumption in respiration-impaired isolated and in situ neuronal somal mitochondria from transgenic mice with a deficiency of either dihydrolipoyl succinyltransferase (DLST) or dihydrolipoyl dehydrogenase (DLD) exhibiting a 20-48% decrease in KGDHC activity. Import of ATP into the matrix of mitochondria from transgenic mice was attributed to a shift in the reversal potential of the adenine

nucleotide translocase towards more negative values due to diminished matrix substrate-level phosphorylation, causing the translocase to reverse prematurely. Immunoreactivity of all three subunits of succinyl-CoA ligase and maximal enzymatic activity were unaffected in transgenic mice as compared to wild-type littermates. Therefore, decreased matrix substrate-level phosphorylation was due to diminished provision of succinyl-CoA. These results were further corroborated by the finding that mitochondria from wild-type mice respiring on substrates supporting substrate-level phosphorylation exhibited ~30% higher ADP-ATP exchange rates compared to those obtained from DLST+/- or DLD+/- littermates.

Conclusions: We propose that KGDHC-associated pathologies are subserved by the inability of respiration-impaired mitochondria to rely on "in-house" mitochondrial ATP reserves.

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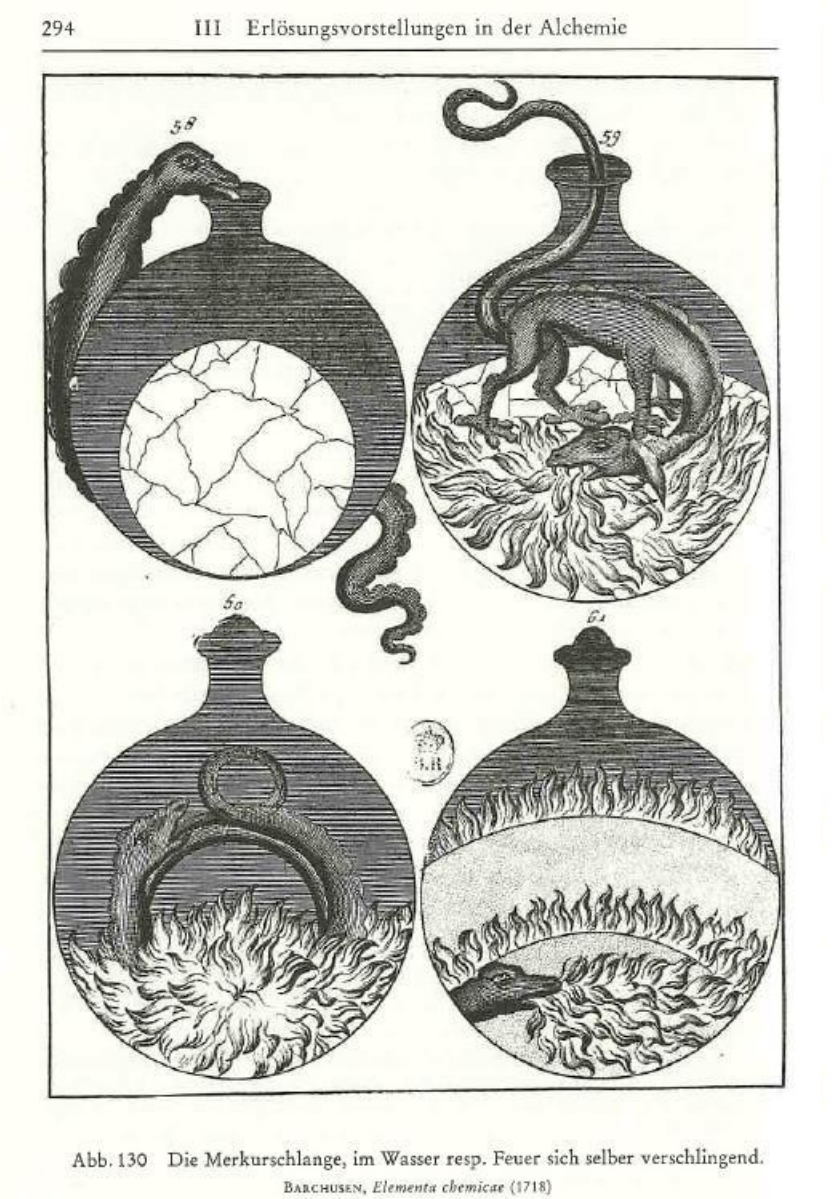

294 III Erlösungsvorstellungen in der Alchemie

Abb. 130 Die Merkurschlange, im Wasser resp. Feuer sich selber verschlingend.
BARCHUSEN, *Elementa chemicae* (1718)

3. Muscle Activity and Mitochondrial Health

Burtscher 2012 Abstract Bioblast

Strategies for improving exercise tolerance: targeting the skeletal muscles and/or the brain

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The individual level of exercise tolerance is closely associated with mortality and quality of life. Thus, to maintain or improve exercise tolerance is one of the most important goals in the elderly or those suffering from various diseases. Exercise training has been evidenced as the most effective way to achieve this goal. Exercise tolerance is related to the ability to use a high percentage of the individual maximum oxygen uptake which is thought predominantly to result from chronic adaptations in skeletal muscle. These adaptations include the increase of key enzyme activities of the mitochondrial electron transport chain and an associated increase in mitochondrial protein accumulation and increased capillary supply. The resulting improvements in performance seem mainly due to a higher rate of fat oxidation and a concomitant reduction in glycolytic flux, and a tighter control of the acid-base status. However, in certain cases, the rapid development of fatigue which cannot simply be explained by metabolic aspects of working muscles affects exercise performance. Recent studies suggest that exercise increases not only muscle but also brain mitochondrial biogenesis thereby likely contributing to reduced fatigue and improved exercise performance [1]. Beside exercise training, repeated passive exposures to short-term hypoxia (interval hypoxia) have also been demonstrated to increase exercise tolerance, e.g. in patients suffering from respiratory or heart diseases [2]. Similar to exercise, transient hypoxia with and without exercise may be capable to stimulate mitochondrial biogenesis in the skeletal muscle as well as the brain [3], thereby contributing to the observed changes in muscle metabolism and the rating of perceived exertion after interval hypoxia [2]. Our observations, derived from measurements of cerebral and muscle oxygenation by NIRS, indicate that beneficial effects on exercise tolerance following exercise training might rather be due to reduced peripheral fatigue and following interval hypoxia due to diminished central fatigue.

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Jacobs 2012 Abstract Bioblast

Mitochondria express enhanced quality as well as quantity in parallel with aerobic fitness across recreationally active individuals up to elite athletes.

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Differences in skeletal muscle respiratory capacity parallel that of aerobic fitness. It is unknown whether mitochondrial content, alone, can fully account for these differences in skeletal muscle respiratory capacity. The aim of the present study was to examine quantitative and qualitative mitochondrial characteristics across four different groups (n = 6 each), separated by cardiorespiratory fitness. High-resolution respirometry was performed on muscle samples to compare respiratory capacity and efficiency in active (AT), well-trained (WT), highly-trained (HT), and elite (ET) individuals. Maximal exercise capacity (ml O_2 min^{-1} kg^{-1}) differed across all groups with mean ± SD values of 51 ± 4, 64 ± 5, 71 ± 2, and 77 ± 3, respectively. Mitochondrial content assessed by citrate synthase activity was higher in ET compared to AT and WT (29 ± 7 versus 19 ± 4 and 16 ± 4 nmol min^{-1} mg ww^{-1}, respectively). When normalizing mitochondrial respiration to content, the respiratory capacities during maximal fatty acid oxidation (P = 0.003), OXPHOS capacity (P = 0.021), and total electron transport system capacity (P = 0.008) varied between groups.

The coupling efficiency of β-oxidation, however, was not affected by level of fitness. These data demonstrate the quantitative and qualitative differences that exist in skeletal muscle mitochondrial respiratory capacity and efficiency across individuals that differ in aerobic capacity. Mitochondrial-specific respiration capacities during β-oxidation, maximal oxidative phosphorylation, and electron transport system capacity all improve in parallel with aerobic capacity, independent of mitochondrial content in human skeletal muscle.

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Le 2012 Abstract Bioblast

Two-week acclimatization to 5200m markedly alters substrate utilization and increases OXPHOS efficiency, with no effect on respiratory capacity in skeletal muscle mitochondria.

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[1]Cell and Molecular Biology, Colorado State University, Fort Collins, CO, USA; [2]Health and Exercise Science, Colorado State University, Fort Collins, CO, USA;[3] University of Oregon, Eugene, OR, USA;[4]Altitude Research Center, University of Colorado Denver, Aurora, CO, USA;[5]Medical University of Innsbruck and OROBOROS INSTRUMENTS, Innsbruck, Austria.- catherinele@me.com

Previous research examining the effects of high-altitude hypoxia on muscle mitochondrial function has been primarily limited to morphological and biochemical analyses [1]. The

aim of this study was to comprehensively examine the effects of short-term acclimatization to 5200m on skeletal muscle mitochondrial respiratory function. Vastus lateralis samples were obtained from 15 healthy subjects (8 male, 7 female 21 ± 2 yrs of age) from Eugene, OR prior to and following 16 days atop Mt. Chacaltaya in the Bolivian Andes. Mitochondrial respiratory function was determined in permeabilized muscle fibers by high-resolution respirometry using a variety of substrate, uncoupler, and inhibitor combinations. Respirometric analysis revealed marked elevations in phosphorylation rates with all substrates (maximal complex I+II) following altitude acclimatization, with a 31% increased reliance on CI vs. CII respiration (S-Rot) and no significant effect on total ETS capacity/mg of tissue (FCCP uncoupled rate). This resulted in a 39% improvement in OXPHOS efficiency with all substrates ($P<0.001$). Strikingly, OXPHOS efficiency (P/E) with palmitoylcarnitine + malate was increased by 79% ($P<3.78E-06$), with less robust improvements with pyruvate + malate. Interestingly, there was nearly a 100% decreased contribution of glutamate oxidation in the presence of saturating supply of both fat and carbohydrate substrates, and a 56% reduction when combined with pyruvate alone ($P<0.001$ and $P<0.05$, respectively).

Taken together, these data indicate that 16 days of exposure to 5200m markedly alters muscle mitochondrial substrate utilization and improves OXPHOS efficiency without eliciting any significant effect on muscle respiratory capacity.

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Carraro 2012 Abstract Bioblast

Muscle training in ageing, denervation and beyond.

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During the last decade we contributed to research on rehabilitation of aging complications studying mechanisms and effects of physical exercise induced by Functional Electrical Stimulation (FES) in the special case of Spinal Cord Injury patients affected by complete injury of the Conus Cauda, a syndrome where the denervated leg muscles are fully disconnected from the nervous system. Denervated human muscles become unexcitable with commercial electrical stimulators and undergo ultra structural disorganization within a few months from SCI, while severe atrophy with nuclear clumping (Fig. 1) and fibro-fatty degeneration appear within 3 and 6 years, respectively [1-4]. To counteract these progressive changes a novel therapy concept for paraplegic patients with complete lower motor neuron denervation of the lower extremity was developed in Vienna: home-based functional electrical stimulation of long-term denervated muscles (h-b FES). New electrodes and a safe stimulator for h-b FES have been designed to reverse severe atrophy by delivering high-intensity (up to 2.4 J) and long-duration impulses (up to 150 ms) able to elicit contractions of denervated skeletal muscle fibers in absence of nerves [5,6]. At the same time, specific clinical assessments and training strategies were developed at the Wilhelminenspital Wien, Austria [7], based on sound evidence from animal experiments [8]. Main results [9-11] of a clinical study on patients which completed the 2-year h-b FES training are: (1) Significant increase of muscle mass and of myofiber size, with striking improvements of the ultra-structural organization; (2) recovery of tetanic contractility with significant increase in muscle force output during electrical stimulation; (3) capacity to perform FES-assisted stand-up and stepping-in-place exercise (Figure 2). The study demonstrated that h-b FES of permanent denervated muscle is an effective home therapy that results in rescue of muscle mass, function and perfusion Additional benefits, important for the patients, are

the improved cosmetic appearance of the legs and the enhanced cushioning effect for seating. We are now extending our studies to application of h-b FES to the larger cohort of elderly. In order to assess the effects of exercise on aging rehabilitation, we are analyzing by morphometric light and electron microscopy and molecular biology quadriceps muscle biopsies from young (23 years) [12] and senior male subjects: sedentary elderly and senior sportsmen (a peculiar group of subjects that performed life-long sport activities) with a mean age of 70 years. The group of sedentary seniors was also exercised for 10 weeks with two different types of training (leg press or electrical stimulation) and the analyses performed before and after the training period. Preliminary results confirm the effectiveness of h-b FES.

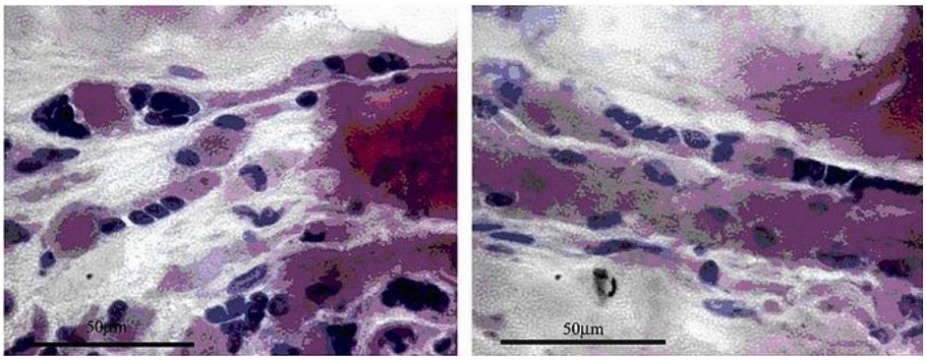

Fig. 1: Four-year denervated human muscle showing severely atrophic myofibers in which contractile apparatus is almost absent, but nuclear clumps are frequent (left panel). Clumps of nuclei are better identified in transvers sections (right panel).

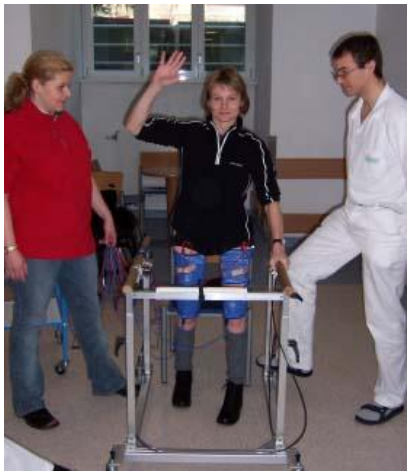

Fig. 2: Stand-up exercise by h-b FES. Note that the blue large electrodes fully cover the thigh muscles.

Based on our recent observation of the presence of a subclinical myopathy in patients affected with newly diagnosed colorectal cancer [13], we are now extending our approaches to oncologic rehabilitation. The factors associated with a subclinical myopathy at this stage of disease are unknown. A comprehensive study on the potential molecular mechanisms that are responsible for this cancer-associated myopathy could possibly provide new diagnostic and prognostic markers and new therapeutic targets to prevent the severe loss of muscle tissue which characterizes late-onset cancer cachexia.

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[4 – 13] Carraro 2012 Abstract Bioblast

Fischer 2012 Abstract Bioblast

Myofacial pain syndrome and myofascial trigger points.

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A recent study in the European Union found that musculoskeletal disorders account for 49% of all absences from work and 60% of permanent disability at an estimated cost of €240 billion [1]. However, treatment of chronic musculoskeletal pain is limited in its efficacy in part because the neurobiological mechanisms responsible for pain have not been fully elucidated. Myofacial pain syndrome is characterized by the presence of myofascial trigger points (MTrPs), which are defined as hyperirritable nodules located within a taut band of skeletal muscle [2]. While active MTrPs generate local and referred spontaneous pain, latent MTrPs can only elicit such pain when palpated; nevertheless, both types appear to be responsible for motor dysfunction associated with range of motion and stiffness issues.

Recent cytokine findings in MTrPs in the context of ischemia and local pH changes suggest that mitochondria may be dysfunctional and play a key role in the pathogenesis [3]. Looking at mitochondrial function in MTrPs may help to determine what pharmacological and/or other manual therapy strategies might be useful to treat myofascial pain. It will be important to identify key biochemical entities to target future research.

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Holloway 2012 Abstract Bioblast

Over-expressing mitofusin-2 in healthy mature mammalian skeletal muscle does not alter mitochondrial bioenergetics.

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The role of mitofusin-2 (MFN-2) in regulating mitochondrial dynamics has been well-characterized in lower order eukaryotic cell lines through the complete ablation of MFN-2 protein. However, to support the contractile function of mature skeletal muscle, the subcellular architecture and constituent proteins of this tissue differ substantially from simpler cellular organisms. Such differences may also impact the role of MFN-2 in mature mammalian muscle, and it is unclear if minor fluctuations in MFN-2 - as observed in response to physiological perturbations - have a functional consequence.

Therefore, we have transiently transfected MFN-2 cDNA into rat tibialis anterior muscle to determine the effect of physiolgically relevant increases in MFN-2 protein on mitochondrial bioenergetics. Permeabilized muscle fibres generated from muscle following MFN-2-transfection were used for functional assessments of mitochondrial bioenergetics. In addition, we have further established a novel method for selecting fibre bundles from this region that are positively transfected, and using this approach transient transfection increased MFN-2 protein ~2.3 fold in selected muscle fibres. However, this did not alter maximal rates of oxygen consumption or the sensitivity for ADP-stimulated respiration.

In addition, MFN-2 over-expression did not alter rates of H_2O2 emission. Altogether, and contrary to evidence from lower order eukaryotic cells, our results indicate that over-expressing MFN-2 in healthy muscle does not influence mitochondrial bioenergetics in mature mammalian skeletal muscle.

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Pesta 2012 Abstract Bioblast

Prevention of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotides targeted to *mIndy*.

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INDY as part of the SLC13 protein family is a high-affinity di- and tricarboxylate plasma membrane transporter involved in citrate import. In Drosophila, genetic deletion of INDY alters energy metabolism and extends lifespan. Mice lacking INDY are protected from both diet induced and age associated insulin resistance 1. In the present work, we use anti-sense oligonucleotides (ASOs) to study the effects of a constitutional knock down in the liver of the mitochondrial Indy protein (mINDY) of rats on energy and glucose metabolism assessed by a hyperinsulinemic-euglycemic clamp (HEC). Rats were fed a high fat diet (safflower diet) for 4 weeks. The treatment group (n=15) was injected 2 times per week with Indy ASO, the control group (n=15) with the same volume of saline. After 4 weeks of treatment, mINDY mRNA was reduced by 91% ($p<0.001$) in the treatment group. No changes in body weight between control and INDY ASO group were observed after treatment [388 ± 7.8 vs. 377 ± 5.1 g]. Hepatic triglycerides were reduced by 55% in mINDY ASO treated rats [57.7 ± 8.1 vs. 31.2 ± 4.1 mg/g liver, $p<0.001$].

Basal glucose turnover [5.9 ± 0.6 vs. 8.4 ± 0.8 mg/(kg-min), $p<0.05$] as well as insulin stimulated glucose turnover [30.7 ± 1.8 vs. 34.7 ± 1.2 mg/(kg-min), $p<0.1$] and suppression of hepatic glucose production during HEC [19.7 vs. 61.6%, $p<0.05$] were higher in the mINDY ASO treated group. Peripheral glucose uptake was not different in muscle and white adipose tissue. Conclusion: Taken together these data suggest that knockdown of hepatic mINDY by ASO may be a novel therapeutic approach for the treatment of non-alcoholic fatty liver disease and hepatic insulin resistance.

Supported by an Erwin Schrödinger Fellowship, FWF, Austria.

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4. Melatonin and Mitochondria in Ageing

Acuna-Castroviejo 2012 Abstract Bioblast

Melatonin and the quality of life: a magic antioxidant with mitochondrial action.

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The first relationship between melatonin and mitochondria came from histological studies showing changes in mitochondrial density and morphology after pinealectomy or melatonin administration to experimental animals. After the discovery of the antioxidant activity of melatonin in 1993, the possibility that melatonin exerts its effects on the mitochondria, the main ROS-producing organelle, was hypothesized. The first experiments demonstrated a highly efficient ability of melatonin to counteract the mitochondrial oxidative stress *in vitro* and *in vivo*. In parallel, melatonin increases the respiratory chain activity, reduces the oxygen consumption, and increases the ATP production. In some of these experiments we could demonstrated that the mitochondria take up melatonin in a time- and concentration-dependent manner. To further analyze the ability of melatonin to prevent and/or counteract mitochondrial dysfunction, different experimental models of aging and disease, including sepsis, Parkinson's disease, and Alzheimer's disease, was evaluated. In all of them, melatonin administration restored the full bioenergetic capacity of the mitochondria, restoring or even increasing their ATP production. Along this time, it was showed that most of the tissues and organs produce melatonin independently of the pineal gland. An important feature of this extrapineal source of melatonin is that its synthetizing enzymes, AANAT and ASMT, are inducibles, i.e., the cells produce melatonin when they require it for protective purposes. This melatonin does not exit to the extracellular fluid. To further analyze the dynamics of the extrapineal melatonin, we recently studied the subcellular distribution of the indoleamine in liver and brain. These studies showed that melatonin is produced in considerably higher amounts in these tissues than in the pineal gland, it is not uniformly distributed in the cell, and is mainly located in the membrane, mitochondria and nucleus. Interestingly, membrane content of melatonin increased in a dose-dependent manner after administration of melatonin to rats, but the content of the indoleamine in the nucleus and mitochondria is saturated.

There is evidence of the ability of mitochondria (and chloroplasts) to synthetize melatonin, which explains the high levels of the indoleamine in this organelle. The phylogenetic origins of the mitochondria, and the presence of melatonin in ancient one cell organisms, speak in favor of a melatonin-mitochondrion connection along the evolution, and the role of melatonin in mitochondrial homeostasis.

Supported by grants # P10-CTS-5784 and PI08-1664.

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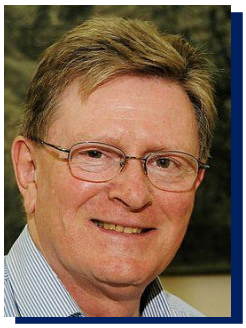

Breitenbach 2012 Abstract Bioblast

Roles of mitochondria in aging and in energy metabolism.

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A yeast deletion mutant in the gene AFO1, coding for a mitochondrial ribosomal protein, confers respiratory deficiency, resistance to several oxidants, a 60% increased replicative lifespan, and very surprisingly, rapid growth on glucose and a highly efficient energy metabolism. The gene defect induces loss of the mitochondrial genome. We showed that the effect of the mutation on lifespan is independent of the retrograde response, and defines a longevity signaling mechanism from the mitochondria to the nuclear/cytoplasmic gene expression system which depends on the presence of an intact TOR1 gene and glucose as a carbon source. The mutant displays an extraordinarily low level of oxygen radicals. We show that this mutation grows rapidly and produces ethanol and biomass on glucose with a kinetics comparable to wild type, in stark contrast to a *bona fide* ethidium bromide induced rho-zero strain, which grows slowly.

The growth phenotypes were shown to be the same in two quite different genetic backgrounds, one of them completely prototrophic. Transcriptome and metabolic analysis of wild type and mutant confirms metabolic similarity of the two strains and points to futile metabolic cycles in the *bona fide* rho-zero strain, which could be reponsible for slow growth of the rho-zero strain. Taken together, the phenotype of the mutant points to the fact that slow growth of rho-zero strains is not caused by slow and inefficient production of ATP, as is often maintained in the textbooks, but is rather a metabolic regulatory phenomenon. It is the intention of this contribution to aid understanding of the role of oxidative stress response and the mitochondria in the mother cell-specific aging process.

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Albertini 2012 Abstract Bioblast

Cystathionine beta synthase modulates senescence of human endothelial cells.

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Availability of methionine is known to modulate the rate of aging in model organisms, best illustrated by the observation that dietary methionine restriction extends the lifespan of rodents. However, the underlying mechanisms are incompletely

understood. In eukaryotic cells, methionine can be converted to cysteine through the reverse transsulfuration pathway thereby modulating intracellular methionine availability. Whereas previous results obtained in yeast and fruit flies suggest that alterations in the reverse transsulfuration pathway modulate the rate of aging, it is not known whether this function is conserved in evolution. Here we show that depletion of cystathionine beta synthase (CBS), a rate limiting enzyme in the reverse transsulfuration pathway, induces premature senescence in human endothelial cells. We found that CBS depletion induces mild mitochondrial dysfunction and increases the sensitivity of endothelial cells to homocysteine, a known inducer of endothelial cell senescence and an established risk factor for vascular disease. Our finding that CBS deficiency induces endothelial cell senescence in vitro, involving both mitochondrial dysfunction and increased susceptibility of the cells to homocysteine, suggests a new mechanism linking CBS deficiency to vascular aging and disease.

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Koziel 2012 Abstract Bioblast

NADPH oxidase Nox4 induces mitochondrial dysfunction in human endothelial cells.

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Oxygen radicals produced by NADPH oxidases play an important role in regulating cell proliferation, survival and differentiation [1]. NADPH oxidase 4 (Nox4) induces cellular senescence in human endothelial cells [2]; however mechanisms of senescence induction remained elusive. Here we show that Nox4 induces mitochondrial dysfunction in human endothelial cells.

Nox4 depletion induced alterations in mitochondrial morphology, stabilized mitochondrial membrane potential, and decreased mitochondrial production of free radicals. Importantly, respiratory activity decreased with extended passaging in control cells but was maintained at high level in Nox4-depleted cells, suggesting that mitochondrial energy production is compromised by Nox4.

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Doerrier 2012 Abstract Bioblast

Treatment with melatonin prevents myocardial mitochondrial dysfunction in experimental sepsis in mice.

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Sepsis is a systemic inflammatory response syndrome of an organism against an infection. Sepsis is the major cause of hospitalization in the Intensive Care Unit of

developed countries, and shows a high mortality and morbidity. Previous studies showed that sepsis induces a significant increase of mitochondrial iNOS isoform (i-mtNOS), with a consequent increase in nitric oxide (NO•) levels. NO• can react with the superoxide anion ($O2•^-$) generating the highly toxic peroxynitrites ($ONOO^-$) that, in turn, irreversibly impair all complex of the respiratory chain (RC). Thus, during sepsis, the activity of the respiratory complexes decreased significantly, favoring the electron leak and, hence, the formation of reactive oxygen species (ROS). Melatonin (aMT) is a potent-free radical scavenger with antioxidant and anti-inflammatory properties. Melatonin counteracts the damage by oxidative stress in sepsis through iNOS/i-mtNOS inhibition, increases the mitochondrial respiratory chain activity, decreasing ROS production, stabilization of mitochondrial membranes, decreasing the lipid peroxidation and stimulating the expression and/or activity of some antioxidants systems. In the present study, we evaluated the involvement of mitochondrial dysfunction in the development of experimental sepsis in mice and the protective role of melatonin. Studies were performed in heart muscle of male C57BL/6 mice (3 months), and sepsis was induced by cecal ligation and puncture (CLP). Mitochondrial respiration was assessed with permeabilized fibres from mouse myocardium by high-resolution respirometry at 37° C.

Our results showed a significant decrease in the OXPHOS capacities with different substrates combinations in septic mice. Electron transfer system (ETS) capacity decreased also with the development of the disease. Together, the data suggest a severe mitochondrial dysfunction during sepsis. Melatonin treatment significantly improved the bioenergetic failure, restoring the normal mitochondrial physiology in heart from septic mice.

Supported in part by grants # RD06/0013/0008, P07-CTS-03135, and P10-CTS-5784

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5. *MiPArt* and Science Communication

Zipperle 2012 Abstract Bioblast

From the bench to the public: visualization of purinergic signalling as a model for contemporary science communication.

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The fundament for the essential exchange between lab bench and society is science communication on a level that is both, well-founded and attractive. However, many researchers hesitate at the point where the presentation of their data should also fulfil aesthetic requirements. In fact, an artistically and educationally valuable processing of data by no means contradicts the demands to scientific publications. The transformation of raw data into graphics takes place in every publication in the form of figures. Although those who do research are always entitled to a clear and understandable presentation of their data, aesthetics and comprehensibility often get lost in the striving for a minimalist layout. Within this project at the interface between art and science we aim to creating an example how raw data can be transformed in a generally accessible medium that does justice to science, art and comprehensibility. In contrast to common promotion practice the following milestones from science to communication are performed by one investigator:

(1) Cell culture and life cell imaging: Investigations on the role of autocrine purinergic signalling in the neutrophil immune response.

(2) Mining and processing of acquired data: Movement tracking and data conversion. Results from cell culture assays and microscopy are converted into importable data sets via table handling and ImageJ.

(3) 3D Modelling: Simulation of neutrophil morphology, physiology and behaviour by implementation of raw data. Following the modelling on the basis of microscopic appearance, data sets are incorporated in the texturing and rigging in a 3D application. After post-production the animated movie is published on a web 2.0 knowledge base.

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Meissner 2012 Abstract Bioblast

Open Innovation.

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Innovation is the industrial realization of a new idea – be it a new product, production process or service. Any innovation is only useful if there is a market, i.e. consumers that are interested in the application of the new goods. Many innovations in science are marketed within a highly specialized user group. A successful strategy to gaining strength in such a market is *open innovation*: complementing feedforward to the

customers by explicitly opening the system for feedback from the users. OROBOROS INSTRUMENTS implements this notion with the company logo: the Oroboros. The feedback process of the dragon feeding on its tail can be translated into a successful scientific and economic strategy. A study by van de Vrande in 2009 [1] shows that SMEs (small and medium-sized enterprises) which are willing to share internal knowledge manage to find alternative pathways to markets – making them in the long run *healthier*.

Tools engaged by OROBOROS INSTRUMENTS for giving and receiving feedback are (i) the highly valued O2k-Workshops, attaining a cooperation of the O2k-Team with invited guest tutors – international experts in the field of mitochondrial physiology - and the participants - ranging from undergraduate students to full professors [2]. (ii) Organization of and participation in international conferences and summer schools with high-level person-to-person scientific interactions [3]. (iii) The Wiki-based webpage Bioblast is intended to assist not only the users of the Oxygraph-2k, but is open to the community interested in learning about the complexity of the powerhouses of our cells. Discussions are openly shared world-wide. Questions of our customers relate to scientific topics and to instrumental troubleshooting. By active co-operation our scientific and technical team gains knowledge on how to improve present standards achieved in the applications of high-resolution respirometry, how the available technologies can be optimized and which innovations may meet upcoming demands.

In November 2012 the Bioblast Wiki has been accessed >100,000 times and >870 O2k-Publications are listed on our website [2]. Currently, 233 leading research teams from 36 countries have joined as MiPNet Reference laboratories for HRR with open contact information [3]. The Bioblast wiki, however, is not (yet) used actively for various reasons that provide divergent scenarios for the future. Either the users regard the wiki as a top-down service to be maintained by OROBOROS and made available to passive users. Potentially, however, a collaborative approach will develop gradually by active participation of a MitoPedia community. In particular, we invite the leading scientists in the field to share their expertise for accurate definitions of terms, aiming at a consistent terminology [4]. This extends the concept of Open Innovation to facilitate communication, to open up the partially arcane field of bioenergetics and mitochondrial physiology: Open Science Communication, for helping the specialists and reaching out to a wider scientific and social community collaborating in the development of a modern mitochondrial and optimization medicine.

Co-financed by the *Standortagentur Tirol:* InnovationsassistentIn.

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6. Evolution, Adaptation and Mitochondrial Dysfunction

Lane 2012 Abstract Bioblast

The elementary organism: chemiosmotic LUCA.

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Living cells maintain low entropy through high enthalpy expenditure ($\Delta G = \Delta H - T\Delta S$). Prokaryotes turnover about 50-55 billion ATPs per division, 10-100 times cell mass. At the origin of life, energy demands must have been even higher, as early catalysts were necessarily less specific than evolutionarily refined enzymes. Yet this high energy requirement for the origin of life is frequently overlooked. Similarly, energy conservation as ion gradients across membranes is universal, yet the origin of membrane bioenergetics remains obscure. Alkaline hydrothermal vents suggest possible solutions to both problems, by providing sustained far-from-equilibrium conditions, in which hot alkaline fluids rich in H_2 interface with acidic oceans saturated in CO_2; as well as natural proton gradients across catalytic Fe(Ni)S mineral walls, giving an electrical potential of ~150-300 mV. This is remarkably similar in polarity and magnitude to the membrane potential of modern autotrophs. But harnessing geochemical proton gradients raises problems of its own: (i) how could natural proton gradients be harnessed in the absence of coupling proteins; and (ii) later, why and how would cells dependent on natural proton gradients begin pumping ions to generate their own gradient? The energy metabolism of anaerobes living in hydrothermal environments today – methanogens and acetogens – offers clues to the origin of membrane bioenergetics. Such cells use proton gradients to reduce the FeS protein ferredoxin (via the energy-converting hydrogenase, Ech), which in turn reduces CO_2 to acetyl CoA. The pH-dependent reduction potential of ferredoxin and FeS minerals suggests that proton gradients could have driven primordial CO_2 reduction in a similar way. Lowering the barrier to CO_2 reduction could in principle drive accumulation of organics, leading to organic protocells occupying the micropores of alkaline vents. Such cells would benefit from tighter membrane coupling, as focusing of proton flux through membrane proteins such as Ech and ATP synthase would enhance ferredoxin reduction and ATP synthesis. However, the steadily decreasing permeability of organic membranes to small ions must have threatened a bioenergetic crisis, as proton flux is no longer neutralised by hydrothermal flux, necessitating active pumping. This crisis could have been averted by the evolution of a H^+/Na^+ antiporter, which transduces geochemical proton flux into a Na^+ gradient, improving coupling, and potentially explaining the Na^+/H^+ promiscuity of ancient bioenergetic proteins. We propose that promiscuous H^+/Na^+ bioenergetics fuelled the transition to the first free-living cells, accounting for the ubiquity of chemiosmotic coupling and the deep divergence of bacteria and archaea [1].

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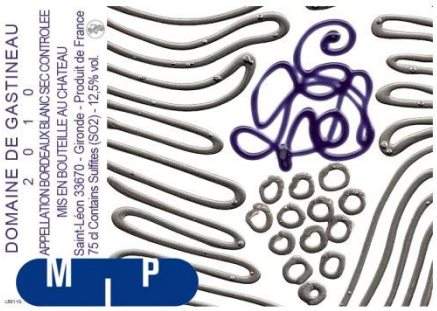

Brown 2012 Abstract Bioblast

Low selective pressure on our mitochondrial genome may explain its rapid evolution, poor adaption and our aging.

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There is a much lower effective selective pressure on our mitochondrial genome relative to nuclear-encoded mitochondrial genes because the mitochondrial genome: 1) is present at high copy numbers per cells, 2) does not undergo recombination; 3) is selected in females only, and 4) is not selected by the sperm race. In addition, the mitochondrial genome may mutate at a higher rate. The large mismatch between mitochondrial and nuclear genes in the ability of evolution to select beneficial and eliminate detrimental variants might be a cause of the rapid evolution and poor adaption of mitochondrial genes. As mammals and humans became larger, brainier, with more skills to pass on and delayed sexual-maturity, and decreased extrinsic causes of death, there would have been selection pressure to delay ageing and age-related disease. But that selection pressure would have had relatively little effect on the mitochondrial relative to nuclear genome, causing mitochondria to be a major contributor to aging.

Koopman 2012 Abstract Bioblast

Cellular consequences of mitochondrial dysfunction.

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To function normally, human cells require energy in the form of ATP that is generated by a variety of metabolic pathways. In most cell types ATP is primarily produced by the mitochondrial oxidative phosphorylation (OXPHOS) system. The latter consists of 5 multi-subunit complexes (complex I to complex V) that contain 92 different structural proteins encoded by the nuclear (nDNA) and mitochondrial DNA (mtDNA). Biogenesis of a functional OXPHOS system further requires the assistance of nDNA-encoded OXPHOS assembly factors (chaperones), of which 35 are currently identified. Importantly, mitochondria do not only generate ATP but also play key roles in other important cellular processes, such as adaptive thermogenesis, ion homeostasis, innate immune responses, production of reactive oxygen species (ROS), and programmed cell death (apoptosis). Mitochondrial dysfunction is not only associated with relatively rare monogenic mitochondrial disorders but is also observed during more common pathologic conditions, such as Alzheimer's disease, Parkinson's disease, cancer, cardiac disease, diabetes, epilepsy, Huntington's disease, and obesity. In addition, mitochondrial function is inhibited by environmental toxins and frequently used drugs. Also during normal human aging, a progressive decline in the expression of mitochondrial genes is observed.

Mutations in OXPHOS structural genes are associated with neurodegenerative diseases including Leigh syndrome, which is probably the most classical OXPHOS disease during early childhood. During the last decade analysis of cells from patients with monogenic mitochondrial diseases has considerably advanced our general understanding of the cellular (patho)physiology of mitochondrial (dys)function.

Here it will be summarized how these insights were obtained and how they can contribute to the rational design of intervention strategies for mitochondrial dysfunction. To this end, our on-going research on human mitochondrial complex I deficiency will be presented as an example of mitochondrial medicine.

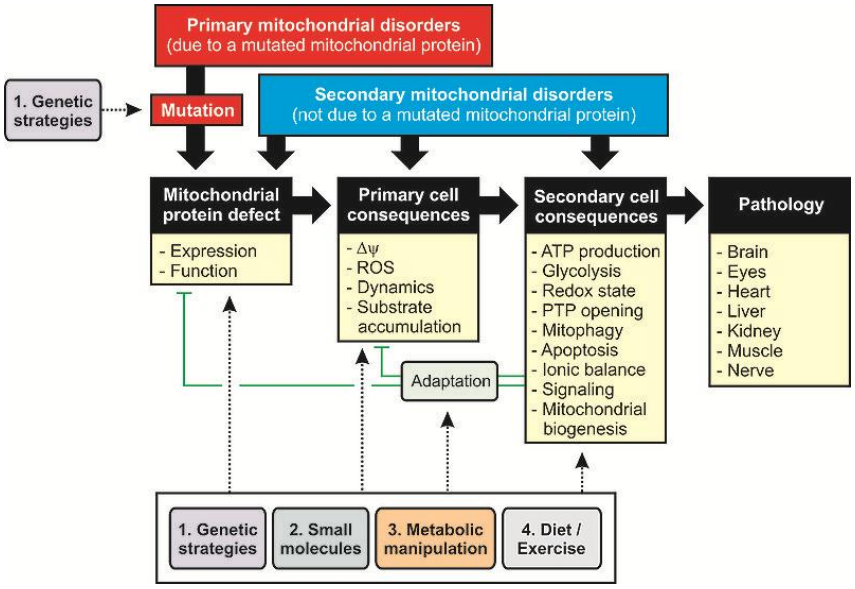

Figure 1: Mitochondrial disorders can be of the primary or secondary category. At the cellular level, the mitochondrial protein mutation has primary and secondary consequences ultimately leading to pathology. Several cellular feedback mechanisms exist that (partially) counterbalance (green line) the effects of the mitochondrial dysfunction. Possible intervention strategies are indicated by dotted lines (see Koopman et al, NEJM, 2012 for details).

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Mark 2012 Abstract Bioblast

Ocean warming and acidification – when fish mitochondria turn sour.

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Ongoing ocean warming and acidification have been found to particularly affect polar marine ecosystems. However, few data exist about the ability of Antarctic fish to respond to environmental change. While whole animal and transcriptomic data on the effects of ocean acidification and warming exist for fish, the acclimation capacities of the subcellular, organelle levels have been poorly studied in this respect. Diffusion of CO_2 into the bloodstream and into the intracellular milieu in aquatic water breathing organisms leads to an acidification of body fluids. Fish can regulate extracellular and intracellular pH by actively accumulating bicarbonate to compensate for the acidification of the extracellular and intracellular milieu. However, chronically elevated bicarbonate levels may interfere with a variety of metabolic processes. For example, bicarbonate is known to strongly interact with mitochondrial metabolism, among others it competitively inhibits citrate oxidation, ultimately influencing ETS capacities. We therefore studied the capacities for mitochondrial acclimation towards elevated PCO_2 and bicarbonate levels in fish that were incubated at different water PCO_2.

We studied ETS capacities in permeabilised heart fibres in fish-MiR06, modified to contain different levels of [HCO_3^-], and enzymatic activies of citrate synthase and cytochrome c oxidase. We found a strong influence of bicarbonate on mitochondrial metabolism and a compensatory increase of mitochondrial capacities after hypercapnia

acclimation. Our findings illustrate the importance of adjusting bicarbonate levels to represent intracellular conditions in fish and other aquatic organisms.

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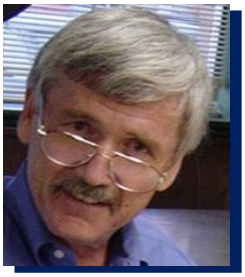

Hand 2012 Abstract Bioblast

Respiration in embryo lysates reveals diminished Complex I activity and inhibition of the phosphorylation system during diapause in Artemia franciscana.

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Encysted embryos of *Artemia franciscana* undergo a dramatic respiratory depression upon release from the adult female as they enter a state of hypometabolism termed diapause [1]. Strategic enzymes involved in trehalose catabolism are inhibited during diapause, namely trehalase, hexokinase, pyruvate kinase and pyruvate dehydrogenase [2]. Trehalose is the sole source of fuel in the embryos, and hence downregulation of trehalose catabolism results in severe limitation of metabolic fuel available to the mitochondrion. We now report that such metabolic depression is heightened by inhibitions within the mitochondrion. Respiration studies of embryo lysates document a depression of oxidative phosphorylation during diapause (compared to post-diapause) in the case where substrates for respiratory Complex I (pyruvate+malate) are used as the fuel source in the OXPHOS state (Fig. 1).

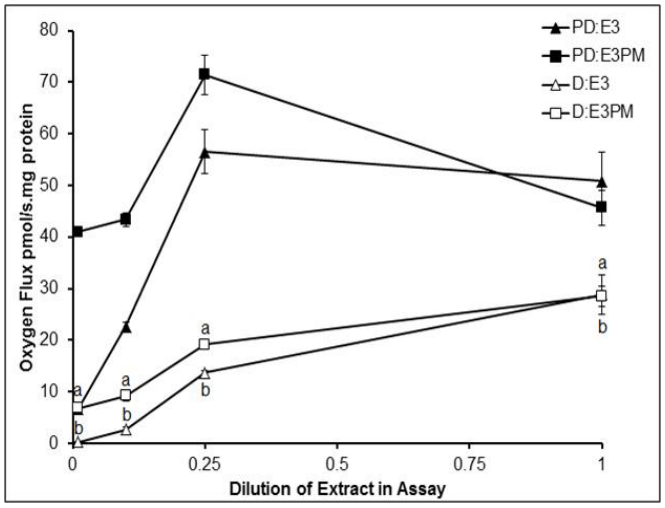

Figure 1: Oxygen flux stimulated by saturating ADP with endogenous substrates alone (e_P) and by ADP with exogenous pyruvate+malate (ePM_P) as a function of dilution of lysates prepared from diapause (D) and post-diapause (PD) embryos. Values are expressed as means ± standard deviation, N=4 (post-diapause), N=5 (diapause). Values for ePM_P and e_P measured in diapause lysates were significantly lower (as indicated by 'a' and 'b' respectively) at all dilutions when compared to corresponding oxygen fluxes in post-diapause lysates ($P \leq 0.0001$).

Reduced respiration through Complex I is supported by Western blot analysis that demonstrates pyruvate dehydrogenase becomes phosphorylated during entrance into diapause [2]. When substrates for Complexes I+II (pyruvate+malate+succinate) are added simultaneously, the increased electron flow through the electron transfer system (*E*, non-coupled) allows the detection of respiratory inhibition by the phosphorylation system during diapause, as judged by the lower OXPHOS capacity (*P*, coupled) and correspondingly lower *P/E* coupling control ratios.

The inhibition is eliminated as the diapause extract is diluted, a result consistent with the presence of a diffusible inhibitor. Similar patterns are seen with Complex II substrate alone (succinate), which supports a much higher OXPHOS capacity than that observed

through Complex I alone. The nature of the potential inhibitor is unknown at present, but one candidate could be long-chain acyl CoA esters that are known to inhibit the ANT [3] from either side of the inner mitochondrial membrane.

Taken together, restriction of glycolytic carbon to the mitochondrion appears to be the primary mechanism for metabolic arrest in *A. franciscana* embryos during diapause, which is accentuated by inhibition of Complex I and the phosphorylation system.

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Chicco 2012 Abstract Bioblast

Substrate-specific impairment of cardiac mitochondrial respiration in Taz-deficient mice: insight into the pathogenesis of Barth Syndrome.

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Barth syndrome (BTHS) is an X-linked cardioskeletal myopathy resulting from a mutation in the Tafazzin (Taz) gene encoding a mitochondrial transacylase required for the remodeling of cardiolipin (CL). CL is an inner membrane phospholipid essential for the function of several mitochondrial proteins, but it remains unclear how Taz deficiency or aberrant CL remodeling lead to mitochondrial dysfunction and cardiomyopathy. In this study, the cardiac mitochondrial phenotype of a new Taz shRNA mouse model of BTHS was characterized. High resolution respirometery revealed 40-50% lower OXPHOS rates in Taz vs. wild-type (WT) mitochondria using pyruvate and palmitoylcarnitine (PC) as substrates ($P < 0.001$). Succinate respiration was also lower in Taz, but only by 13% ($P = 0.07$), suggesting a possible defect in Complex I and/or NADH generation from pyruvate and PC oxidation. Interestingly, glutamate respiration was 46% greater in Taz vs. WT ($P < 0.05$), and reached OXPHOS rates equal to that obtained with pyruvate and PC in WT mitochondria. Analysis of the Taz mitochondrial proteome revealed deficiencies in enzymes involved in beta-oxidation, pyruvate transport, amino acid catabolism, complex I, and the TCA cycle. However, malate dehydrogenase, the primary source of NADH from glutamate oxidation, was elevated 40% in Taz vs. WT mice ($P < 0.05$).

Cardiac metabolomic profiling revealed an accumulation of substrates congruent with observed mitochondrial enzyme deficiencies. Mitochondrial ROS release and sensitivity to Ca^{2+}-induced permeability transition (MPT) were both reduced in Taz vs. WT mitochondria.

Taken together, these data suggest that Taz deficiency selectively impairs carbohydrate and lipid oxidation, and argues against a significant role of respiratory chain dysfunction, ROS production, or MPT in the pathogenesis of Barth syndrome.

Funding: American Heart Association and the Barth Syndrome Foundation

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Cervinkova 2012 Abstract Bioblast

Evaluation of mitochondrial functions – an important tool for experimental hepatology.

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Liver plays an essential role in intermediary and energy metabolism; besides other important functions hepatocytes are responsible for biotransformation of the majority of endogenous and exogenous substances including most of drugs. The majority of toxic agents act either fully or partially via oxidative stress, the liver, specifically the mitochondria in hepatocytes, being the main target. Maintenance of mitochondrial function is essential for the survival and normal performance of hepatocytes, which have a high energy requirement. Liver has amazing capacity to regenerate after toxic liver injury or partial hepatectomy; resulting proliferative processes represent a great demand for energy. Therefore, understanding of the role of mitochondria in hepatocytes is of fundamental importance. Our research group has been working for many years in the field of experimental hepatology, namely in:

1. Liver regeneration induced by partial hepatectomy or toxic injury *in vivo*.
2. Study of the mechanisms responsible for toxic liver injury *in vivo* and *in vitro*.
3. Study of liver regeneration and toxic injury in the terrain of NAFLD (non-alcoholic fatty liver disease) which became the most common chronic disease of the liver.

In our projects we analyse mitochondrial function using several basic models: hepatocytes cultured *in vitro*; mitochondria in permeabilised hepatocytes; tissue homogenates; and isolated mitochondria. For measurement of oxygen uptake we are using the High-Resolution Oxygraph-2K, for evaluation of mitochondrial membrane potential (MMP) tetraphenylphosphonium (TPP^+) electrode or accumulation of Rhodamin123; JC-1 a fluorescence probe is used for visualisation of the MMP. Using these methods we contributed to the knowledge about role and changes in liver mitochondria under various pathological conditions.

There is another important outcome of our work. As a university department an essential part of our activities is education. We have participated in two European projects within last 3 years; HEPIN - Hepatology Institute in Hradec Kralove and PhysiSciNet (Physiology Science Network). In frame of these projects we have organised twice a year Bioenergetic Workshops, so we became a training base for young researchers from the Czech Republic being interested in bioenergetics.

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Watala 2012 Abstract Bioblast

Oxygen consumption in resting and activated blood platelets: platelet mitochondria and cyclooxygenases as compounding targets for high-resolution respirometry.

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Background and objectives: Little is known about the efficiency of mitochondrial respiration in blood platelets under pathological conditions, and it is even more so considering platelets in atherothrombotic states. Under conditions of accelerated arachidonate-prostanoid pathway intraplatelet oxygen is consumed not only by mitochondria, but also by membrane-associated cyclooxygenases [1]. Hence, mitochondria and cyclooxygenases in blood platelets originating from experimentally-diabetic animals constituted two major targets in our studies.

Diabetes impairs platelet prostanoid metabolism, but only slightly affects platelet mitochondria: Diabetic platelets demonstrate enhanced COX-1-mediated eicosanoid metabolism. The increased oxygen consumption in blood platelets from diabetic animals has been attributed to the increased activity of COX-1, while changes in the maximum consumption of oxygen or RCR ratio were very subtle. Interestingly, the activation of platelet COX-1 concerned both cyclooxygenase and peroxidase domain of the enzyme. The pathophysiological implications of such an increased COX-1 activity are very straightforward: elevated synthesis of thrombogenic thromboxane and its contribution to atherothrombosis.

Severe diabetic hyperglycaemia affects platelet COX-1 and endothelial COX-2: The increased COX activity in diabetes may directly result from non-enzymatic modification by sugars and - consequently the advanced changes in protein structure in diabetes. Platelet COX-1 incubated *in vitro* with excessive glucose or methylglyoxal demonstrated enhanced activity of cyclooxygenase and peroxidase subunits of COX-1, respectively. Such modifications did not abolish the subsequent acetylation of the enzyme (implications for ASA therapy?). Otherwise, COX-2 present in endothelium, demonstrated reduced activity when non-enzymatically modified with glucose, 1,6 bisphosphate fructose or methyl glyoxal.

COX-2-dependent blood vessel vasodilation in diabetes: Dysfunctional, non-enzymatically modified diabetic endothelium demonstrates decreased NO production, but is able to compensate this deficit by enhanced synthesis of COX-2 derived vasodilatory prostaglandins (PGI2 and PGE2). This effect is of special importance when considering the application of selective COX-2 inhibitors known as 'coxibs' in patients with cardiovascular disease.

Non-invasive assay of blood platelet functioning – a new perspective to monitor (patho)physiology of intact circulating platelets: The prevailing majority of experiments on blood platelets reactivity are conducted with the use of *in vitro* methods. The platelets' susceptibility to activation upon blood collection, as well as in the course of sample preparation, largely contributes to possible artifactual observations encountered when using these methods. Therefore, validation of methods for *in vivo* testing on blood platelets reactivity may become a challenging rationale [2]. In our hands, the agonist-induced platelet aggregation in a bloodstream is monitored in a microcirculation with the use of a non-invasive Laser Doppler Flowmetry.

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Ali 2012 Abstract Bioblast

Interrogating Egyptian mitochondria!

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Many neurodegenerative, cardiovascular, cancer, and psychological disorders are known to involve mitochondrial dysfunction and deregulated levels of reactive oxygen species. Aging is associated with a sustained increase in superoxide radical levels, which is associated with a progressive decline in cognitive function and increased prevalence of neurodegeneration. Mitochondria were identified as one source of oxidant production in brain during aging, but several recent studies suggest that an alternative, extra-mitochondrial source of superoxide may also be important to aging-associated pathologic phenotype. In this presentation I will briefly discuss some of our contributions to the field including our studies on the dynamics of mitochondrial superoxide production, and our recent discovery that the superoxide-producing enzyme NADPH-oxidase-2 (Nox2) is induced during aging and remain constitutively active in neurons and synaptosomes from aged brain and from schizophrenia mouse model.

Finally, I'll present our progress in establishing the Center for Aging and Associated Diseases (CAAD) as part of the newly founded Zewail City of Science and Technology, the Egyptian National Project of Scientific Renaissance. In CAAD, we are focusing primarily on establishing a state-of-the-art facility to study diseases in relevance to the Egyptian people. In one of CAAD's core facilities, the Oroboros® O2k-MultiSensor system will be combined with the Seahorse XF24 and the Magnettech MS400 EPR spectrometer to study mitochondrial dynamics in the context of aging, metabolic, cardiovascular, and neurodegenerative diseases.

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Konrad 2012 Abstract Bioblast

Absence of Ca^{2+}-induced mitochondrial permeability transition but presence of bongkrekate-sensitive nucleotide exchange in *C. crangon* and *P. serratus*.

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Mitochondria from the embryos of brine shrimp (*Artemia franciscana*) do not undergo Ca^{2+}-induced permeability transition in the presence of a profound Ca^{2+} uptake capacity [1]. Furthermore, this crustacean is the only organism known to exhibit bongkrekateinsensitive mitochondrial adenine nucleotide exchange, prompting the conjecture that refractoriness to bongkrekate and absence of Ca^{2+}-induced permeability transition are somehow related phenomena [2]. Here we report that mitochondria

isolated from two other crustaceans, brown shrimp (*Crangon crangon*) and common prawn (*Palaemon serratus*) exhibited bongkrekate-sensitive mitochondrial adenine nucleotide transport, but lacked a Ca^{2+}-induced permeability transition. Ca^{2+} uptake capacity was robust in the absence of adenine nucleotides in both crustaceans, unaffected by either bongkrekate or cyclosporin A. Transmission electron microscopy images of Ca^{2+}-loaded mitochondria showed needle-like formations of electron-dense material strikingly similar to those observed in mitochondria from the hepatopancreas of blue crab (*Callinectes sapidus*) [3] and the embryos of *Artemia franciscana* [2].

Alignment analysis of the partial coding sequences of the adenine nucleotide translocase (ANT) expressed in *Crangon crangon* and *Palaemon serratus*versus the complete sequence expressed in *Artemia franciscana* reappraised the possibility of the 208-214 amino acid region for conferring sensitivity to bongkrekate. However, our findings suggest that the ability to undergo Ca^{2+}-induced mitochondrial permeability transition and the sensitivity of adenine nucleotide translocase to bongkrekate are not necessarily related phenomena.

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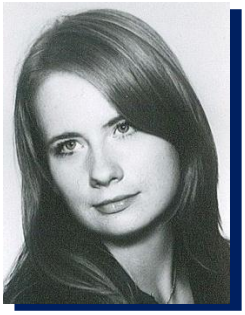

Dudzinska 2012 Abstract Bioblast

The influence of the extract from *Rubus parvifolius* leaves (EL17) on expression and activity of apyrase (CD 39) on Human Umbilical Vein Endothelial Cells (HUVEC).

Dominika Dudzińska, Bogusława Luzak, Lidia Stańczyk, Joanna Rywaniak, Cezary Watała

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Human Endothelial Cells possess a lot of mechanisms which enable them prevention against thrombosis and plaque formation. One of such preventive systems is antigen CD39 which has the ability to decompose ATP and ADP. This process results in decreased aggregation of blood platelets activated via purinergic pathway. Polyphenols, plant secondary metabolites, are widely known for their antioxidative and cardioprotective activities. Blackberry fruits, which are the rich source of polyphenolic compounds, were proved in the *in vivo* studies to possess antithrombotic properties. We examined the influence of polyphenolic extract derived from *Rubus parvifolius* leaves (EL17), on the expression and ADPase activity of CD39 in HUVECs and on blood platelet aggregation. Platelet aggregation was determined in the whole blood obtained from healthy individuals. Whole blood samples were incubated with EL17 at the concentration of 7.5 and 15 µg/ml and agonized with 6.4 µg/ml ADP.

To assess the effect on CD39 expression, HUVECs were incubated for 24 h with EL17 (at the concentration of 1, 5, 10 or 15 µg gallic acid per ml), then labeled witch anti-CD39 antibodies, washed (two times) and measured by flow cytometry. To estimate CD39 ADPase activity HUVECs were incubated with EL17 for 24 h and the samples were assayed using Malachite Green method.

Platelet aggregation, upon the incubation with 15 µg/ml EL17, was inhibited by 19% (132 ± 53 AU vs. 165 ± 52 AU in control without EL17). Moreover, the expression of CD

39 increased significantly after incubation with EL17 (up to 30% and 50% after the treatment with 10 and 15 µg/ml EL17, respectively). The ADPase activity in the presence of EL17 remained at the same level as in control. To conclude, the extract from *Rubus parvifolius* leaves, exerts beneficial influence on platelet reactivity via purinergic pathway and may be considered as a potential antiplatelet modulator in antiplatelet therapy and prevention of cardiovascular diseases.

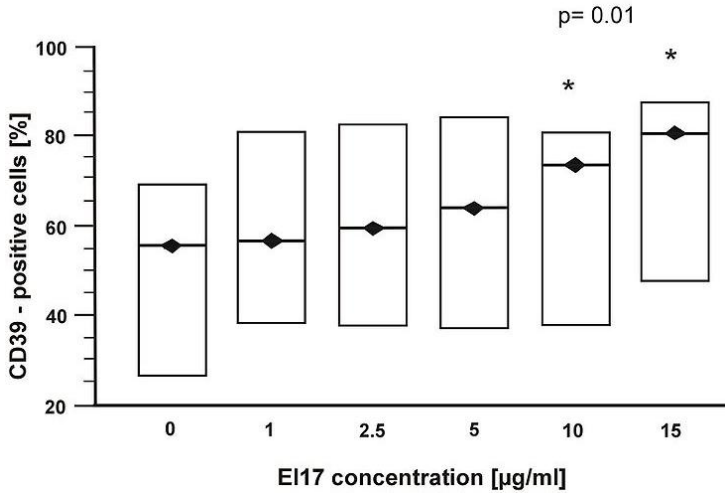

Figure 1: Effect of EL17 on CD39 expression on HUVEC's surface. Data shown as median and interquartile range (n=14). At higher concentration EL17 increased CD39 expression.

Supported by project 'Production of polyphenol extracts of plant origin with antiplatelet and cardioprotective properties – FLAWOPIRYNA', co-financed by European Union from European Regional Development Fund within the frames Innovative Economy Operational Programme.

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7. Discussion: Global Mitochondrial Network

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

The Global Mitochondrial Network (GMN) aims at providing a WorldWide information platform for scientific mitochondrial organizations and mitochondrial research consortia.

- GMN Organizations: Organizations with a focus on mitochondrial research and medicine, particularly mitochondrial societies.
- GMN Centres: Scientific and medical centres or research consortia focussing on mitochondrial research and medicine.
- GMN Events

Pesta 2012 Abstract Bioblast-GMN

Global Mitochondrial Network - From a young investigator's perspective.

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The Global Mitochondrial Network (GMN) aims at providing a WorldWide information platform for scientific mitochondrial organizations and mitochondrial research consortia. An important by-product of such a networking initiative could be the development of a *competence platform*, a platform where work groups come up with their core competences and techniques. These can generally be derived form already existing webpages and publications, but could be structured to be easily accessible by other investigators. Like this, visibility of concentrated mitochondrial competence from the Americas, Europe, Asia, Australia, and Africa would be apparent and accessible for the scientific community. From a young investigator's perspective, this could bring considerable benefits to the fields:

- Visibility of the field with respect to the scientific community; when applying for scientific funding worldwide, partners would be at hand (e.g. within the European Union, partners from non-European countries are mostly welcome as associated or third countries).
- Partners for possible cooperation would be chosen based on scientific excellence, which would gradually increase scientific quality in a research environment which has long gone global.
- This initiative would also be of great value for young investigators to foster exchange of ideas and students (e.g. transfer of ideas via master/PhD students).

This platform could be a way for the community to move closer by exchanging ideas and know-how but at the same time it would open up the field to a new dimension of collaboration, also for investigators from different fields. Funding on an international level will gain importance; this platform could be a first step to join forces.

Supported by an Erwin Schrödinger Fellowship, FWF, Austria.

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

Mutschler 2012 Abstract Bioblast

Regenerative mitochondrial medicine – from basic research to medical practice.

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Where mitochondria are damaged and energy production is reduced, there are resulting deseases and malfunctioning of tissue and organs. The most important tool in regenerative mitochondrial medicine during treatment of secondary mitochondropathia is to avoid the causing noxes. Further, very important parts in regenerative mitochondrial medicine are orthomolecular therapy, nutritional therapy and microbiological intestinal therapy as well as treating the milieu. The latest novelty is focussing on the amygdala for neurostress reduction.

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8. Neuronal Mitochondrial Function

Chinopoulos 2012 Abstract Bioblast

Exclusive neuronal expression of SUCLA2 in the human brain.

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SUCLA2 encodes for the ADP-forming β-subunit (A-SUCL-β) of succinyl CoA ligase, an enzyme of the citric acid cycle [1]. Mutations in SUCLA2 lead to a mitochondrial disorder associated with mitochondrial DNA depletion [2]. This mitochondrial disorder manifests as neonatal encephalomyopathy exhibiting dystonia, deafness and pronounced lesions in the basal ganglia [3]. Despite that a SUCLA2 gene defect results in distinct brain pathology, precise localization of the encoded protein has never been investigated.

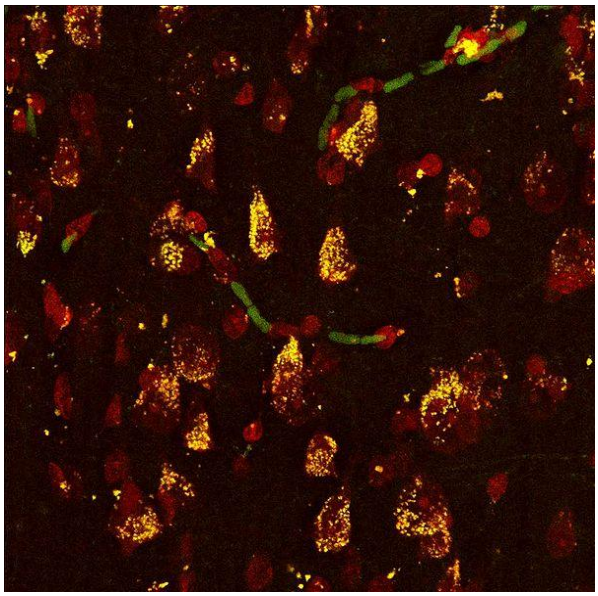

Fig. 1: Co-localization of fluorescent Nissl staining the cytosol of neurons (red) and the ADP-forming β-subunit (A-SUCL-β) of succinyl CoA ligase (green-->yellow) in human temporal cortex.

Here we show that the immunoreactivity of A-SUCL-β in the human cerebral cortex was present exclusively in neurons, identified by their morphology and visualized by double labelling with a fluorescent Nissl dye. The A-SUCL-β immunoreactivity co-localized >99% with that of the d-subunit of the mitochondrial F_0-F_1 ATP synthase. Specificity of the anti-A-SUCL-β antiserum was verified by the absence of labelling in fibroblasts from a patient with a complete deletion of SUCLA2. A-SUCL-β immunoreactivity was absent in glial cells, identified by antibodies directed against the glial markers GFAP and S100. Our work establishes that SUCLA2 is expressed exclusively in neurons in the human cerebral cortex (Fig. 1). Therefore, all encephalopathic features of the disease emerging by mutations in this gene originate solely from the neuronal cell population.

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Sumbalova 2012 Abstract Bioblast

Evaluation of mitochondrial respiration and membrane potential in mouse brain homogenate.

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Experimental and diagnostic studies require precise evaluation of mitochondrial function in small samples. Using tissue homogenates instead of isolated mitochondria would require minimal amount of tissue and time for preparation and therefore could be widely used for diagnostic purposes. The aim of our study was to compare mitochondrial function in 3 preparations from mouse brain: crude homogenate (Hmt), supernatant after low-speed centrifugation (Smt), and isolated mitochondria (Imt). Mitochondrial respiration, JO_2, and membrane potential, ΔΨ were measured simultaneously at 37°C in Oxygraph-2k MultiSensor system with DatLab software equipped with an ion selective electrode system (Oroboros Instruments, Innsbruck, Austria), in MiRO6 and tetraphenylphosphonium (TPP^+) concentration 1 and 1.5 µM. Coupling and substrate control states [1] were established in 6 different SUIT protocols. In calculation of ΔΨ, mitochondrial protein was normalized to citrate synthase (CS) activity. The value of 11 µl/mg for K'out and K'in constants describing external and internal binding of TPP^+, and 1 µl/mg for mitochondrial volume, were applied for all preparations. The signal of the TPP^+ electrode was corrected for side effects of titrated chemicals [2].

Mitochondria in all preparations were well coupled, without significant damage of outer membrane, respiration and ΔΨ were stable for 4-5 hours. JO_2/CS were similar in all preparations, only State 4 respiration was slightly lower in Imt (-21 % vs Hmt).The ratio of respiration stimulated by 0.25 mM and 2.25 mM ADP in Hmt reached 88% of the value in other preparations, RCR (CI) was significantly higher (+50%) in Smt in comparison with Hmt and Imt. Absolute values of calculated ΔΨ were lowest in Imt. The differences in ΔΨ between respiratory states, ΔΔΨ, were systematicaly highest in Imt and lowest in Hmt. Variation of K'out would reduce differences in ΔΨ and ΔΔΨ between preparations, however, exact determination of K'out is problematic. High sensitivity and stability of Oroboros TPP^+ electrode enables determination of mitochondrial respiration and membrane potential simultaneously in Oxygraph-2k MultiSensor system. Homogenates could be a good alternative to Imt for determination of mitochondrial function in small samples.

Supported by FEMtech (NMVIT, Austria)

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Bouillaud 2012 Abstract Bioblast

Sulfide a remarkable mitochondrial substrate.

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Sulfide (H_2S gas, HS^- anion) shows the same toxicity as cyanide, NO or CO for mitochondrial complex IV (cytochrome oxidase). NO, CO and H_2S are considered as

gasotransmitters of physiological relevance. While signaling is expected to occur by different pathways, the question of involvement of mitochondria remains sometimes unresolved and particularly because some experiments used concentrations relevant to bioenergetics. Cellular metabolism generates low amount of sulfide and the activity of bacteria in the colonic lumen exposes the colonic wall to extracellular concentrations of sulfide [#60µM], large enough to inhibit cellular respiration. Therefore, the question of sulfide disposal needs to be adressed.

Mitochondria themselves appear as best candidate to explain sulfide disposal: a *Sulfide Oxidation Unit* (SOU) oxidizes sulfide into thiosulfate in many cell types in culture and in mitochondria from liver, heart or kidney. When sulfide is infused to mitochondria or cells at rates that stay within their sulfide oxidation capacities they oxidize it and maintain a low (<500nM with cells) external concentration of sulfide well behind the toxic level (IC50 10-20µM with cells). Notably, SOU activity could not be detected in brain mitochondria or neuroblastoma cells making them intolerant to sulfide. SOU is constituted by a sulfide quinone reductase (SQR) associated with a sulfur transferase and a dioxygenase. Present knowledge indicates that two molecules of H_2S and one of oxygen (O_2) are consumed by SOU to deliver two electrons to quinone. Then the stoichiometry for mitochondrial respiration based on sulfide oxidation is (1+0.5) O_2 / 2 H_2S = 0.75 and for the same rate of electron transfer three times more oxygen are needed than with NADH/$FADH_2$ coenzymes. In consequence, infusing sulfide immediately and significantly increases oxygen consumption of respiring cells/mitochondria. Mitochondria show a high affinity for sulfide and its oxidation usually takes priority over ongoing mitochondrial oxidation processes. Something that is mandatory to ensure efficient sulfide disposal in presence of largely greater intracellular concentrations of other substrates. Extracellular sulfide is therefore a remarkable substrate: it could be used at nanomolar concentrations, its gaseous nature allows fast transfer to mitochondria without transporters, it is directly usable to reduce quinone without metabolic processing (including for example initial ATP consuming steps). This metabolic role of sulfide and its reductant properties contrast sharply with the other two gazotransmitters and particularly with NO which is pro-oxidant. There are still uncertainties about the lowest concentration of sulfide that SQR would *detect* but, in physiological conditions, the overlap between bioenergetically relevant and signaling concentrations appears even more likely with sulfide than with NO or CO. Colonocytes are adapted to high sulfide exposure. In these cells *reverse bioenergetic reactions* including reverse electron flux in complex I are taking place to ensure continuation of a fast sulfide oxidation even if complex IV is inhibited.

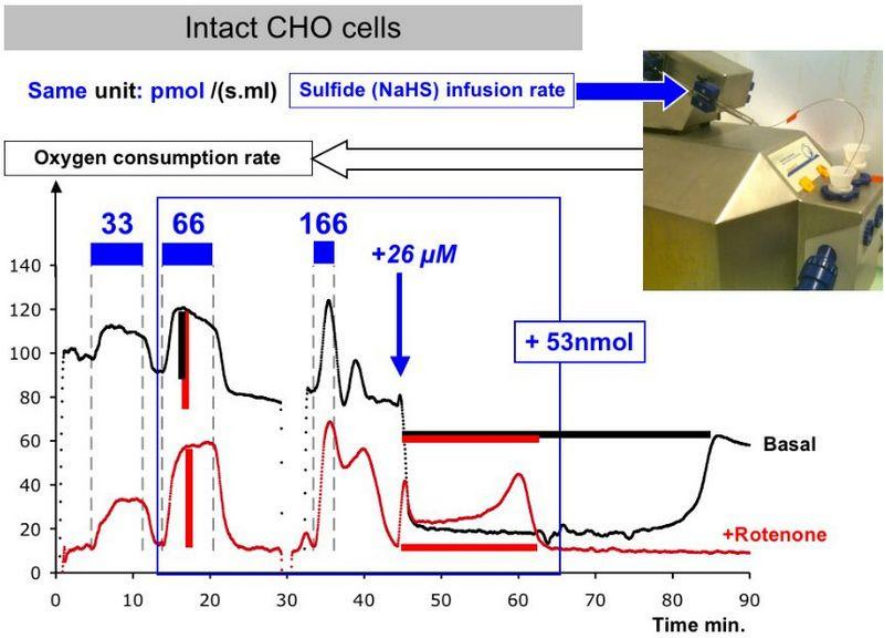

Figure 1: The oxygen consumption (JO_2) of CHO cells in suspension is recorded, the basal rate (black) and in presence of rotenone inhibition of mitochondrial complex I (red) are shown. Sulfide additions are indicated in blue. Addition of the same amount of sulfide (+53nmol in 2ml) has different consequences when it is infused (blue bars) at a rate that matches with the sulfide oxidation capacities of cells (66), exceeds it (166) or added as a single injection (+26µM). Note that the maximal sulfide flux (66) compares well with the

initial basal rate of JO_2 (100). The increase in JO_2 is figured with vertical bars (red/black) with the red repeated side to the black for comparison. The ratio (increase in JO_2) / (Sulfide injection rate) provides an indication of the stoichiometry Oxygen/ sulfide. This value is close to the theorical value of 0.75 in presence of rotenone but close to 0.5 for basal. This suggests that electrons from sulfide replaced electrons previously provided to quinone by complex I (priority of sulfide oxidation over other substrates). In contrast, when inhibition by sulfide takes place (+26µM) the length of the horizontal red/black bars (= time to oxidize sulfide) indicates that in these conditions the complex I becomes an opponent to sulfide oxidation probably because of the change in its reduction state.

Calzia 2012 Abstract Bioblast

Interactions between sulfide metabolism and oxygen concentration.

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Even in higher species sulfide is quickly metabolised and thus degraded by an ancient metabolic pathway linked to the mitochondrial respiratory chain [1]. Since this metabolic process is strictly aerobic, the efficiency of sulfide metabolism is expected to decrease at low oxygen. This mechanism may explain how sulfide acts as an oxygen sensor within cells [2]. Therefore, in a series of preliminary experiments we quantified the sulfide metabolism in a stable cell line derived from alveolar macrophages (AMJ2-C11), which previously proved to efficiently metabolise sulfide under aerobic conditions [3], at O_2 partial pressures approaching hypoxia from 0.8 KPa down to 0.4 and 0.1 KPa.

Methods: All measurements were conducted using an O2k-Oxygraph together with a TIP2k titration pump (Oroboros Instruments, Austria). For each O_2-partial pressure we performed 6 – 8 separate experiments. Before performing the experiments we recorded several transitions to anoxia in order to quantify the un-inhibited respiratory capacity close to anoxia [4] under the O_2-partial pressures of 0.1, 0.4, and 0.8 KPa respectively. The inhibition of mitochondrial respiration was quantified in terms of the total amount of sulfide required to reduce the routine oxygen flux (JO_2) to 50% by means of a continuous sulfide injection at a rate of 10 nM/s. A second titration pump was used to simultaneously maintain the oxygen concentration rate at the predefined level adopting a previously described technique [5].

Results: Figure 1 shows representative records of 2 anoxic transition experiments in AMJ2-C11 cells; the JO_2 was 74%, 92%, and 96% of maximum JO_2 [JO_{2max}] at 0.1 KPa, 0.4 KPa, and 0.8 KPa. The total amount of sulfide required for achieving a 50%-inhibition of mitochondrilal respiration was about 10% at 0.1 KPa when compared to that needed at 0.8 KPa. (0.2 ± 0.03 µmol at 0.1 KPa vs. 1.30 ± 0.4 µmol at 0.4 KPa, and 2.2 ± 1.1 µmol at 0.8 KPa) [see Figure 2].

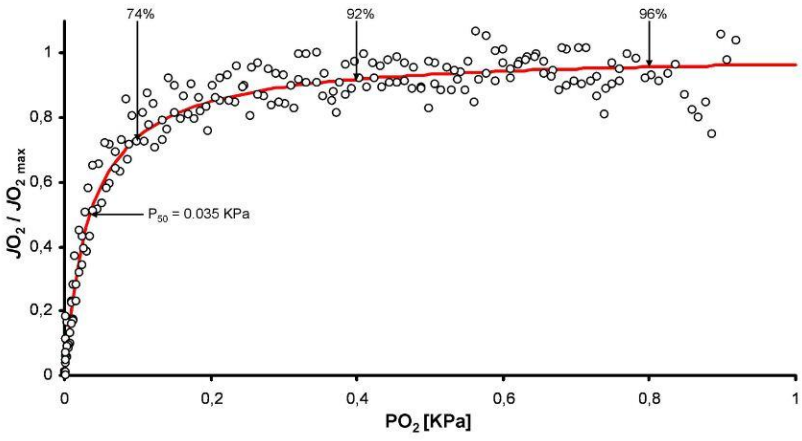

Figure 1: The figure shows original data on JO_2/JO_{2max} from 2 representative anoxic transition experiments conducted with AMJ2-C11 cells (open dots). As indicated by the vertical arrows, the sulfide titrations have been performed under O_2-partial pressures of 0.1, 0.4, and 0.8 KPa, corresponding to a JO_2 of 74%, 92%, and 96% of JO_{2max} respectively.

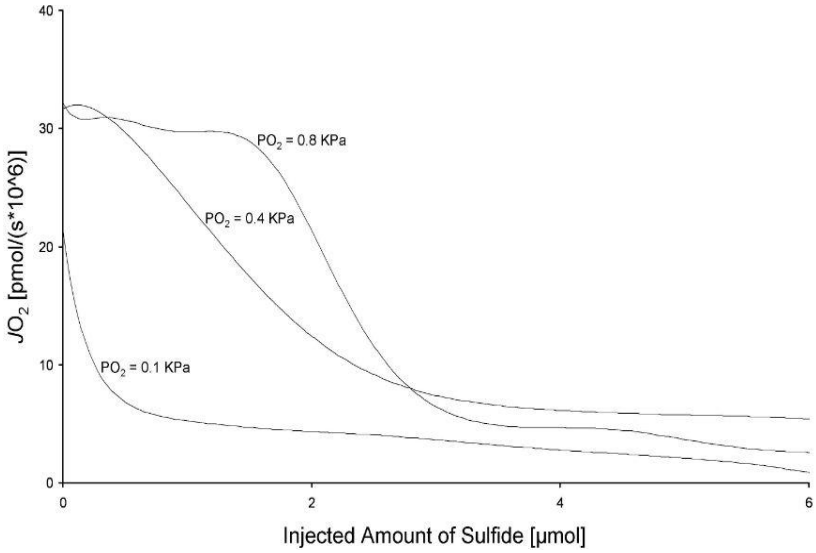

Figure 2: The figure plots the injected amount of sulfide against the JO_2 at all 3 partial pressures.

Discussion: These results confirm a much less efficient degradation of sulfide at the lower oxygen level suggesting potential interactions between sulfide and cell metabolism when approaching the anoxic condition. In theory, sulfide concentration should increase and thus potentially inhibit mitochondrial respiration at low oxygen, provided that the production rate is sufficiently high and remains constant under these conditions, and that the excess sulfide is not chemically bound by proteins (*sulfhydration*). On the other hand, the inhibition of mitochondrial respiration should in turn decrease the oxygen consumption of the cell, thus potentially decelerating or even impeding a further decrease of the oxygen concentration. So far, however, whether and how sulfide interacts with metabolism at low oxygen remains the subject of future investigations.

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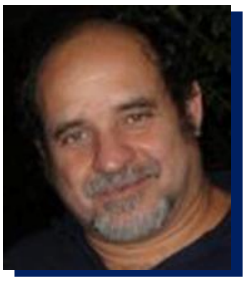

Galina 2012 Abstract Bioblast

Nitric oxide inhibits succinate dehydrogenase-driven oxygen consumption in potato tuber mitochondria in an oxygen tension-independent manner.

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NO (nitric oxide) is described as an inhibitor of plant and mammalian respiratory chains owing to its high affinity for COX (cytochrome c oxidase), which hinders the reduction of oxygen to water [1]. In the present study we show that in plant mitochondria NO may interfere with other respiratory complexes as well [2]. We analysed oxygen consumption supported by complex I and/or complex II and/or external NADH dehydrogenase in Percoll isolated potato tuber (*Solanum tuberosum*) mitochondria. When mitochondrial respiration was stimulated by succinate, adding the NO donors SNAP (S-nitroso-N-acetylDL-penicillamine) or DETA-NONOate caused a 70% reduction in oxygen consumption rate in state 3 (stimulated with 1 mM of ADP). This inhibition was followed

by a significant increase in the Km value of SDH (succinate dehydrogenase) for succinate (K of 0.77+/- 0.19 to 34.3 +/- 5.9 mM, in presence of NO). When mitochondrial respiration was stimulated by external NADH dehydrogenase or complex I, NO had no effect on respiration.

NO itself and DETA-NONOate had similar effects to SNAP. No significant inhibition of respiration was observed in the absence of ADP. More importantly, SNAP inhibited PTM (potato tuber mitochondria) respiration independently of oxygen tensions, indicating a different kinetic mechanism from that observed in mammalian mitochondria. We also observed, in an FAD reduction assay, that SNAP blocked the intrinsic SDH electron flow in much the same way as TTFA (thenoyltrifluoroacetone), a non0competitive SDHinhibitor.

We suggest that NOinhibits SDH in its ubiquinone site or its Fe–S centres. These data indicate that SDH has an alternative site of NO action in plant mitochondria [3].

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Iyer 2012 Abstract Bioblast

Gentle Science in the real world of mitochondrial physiology and genetics.

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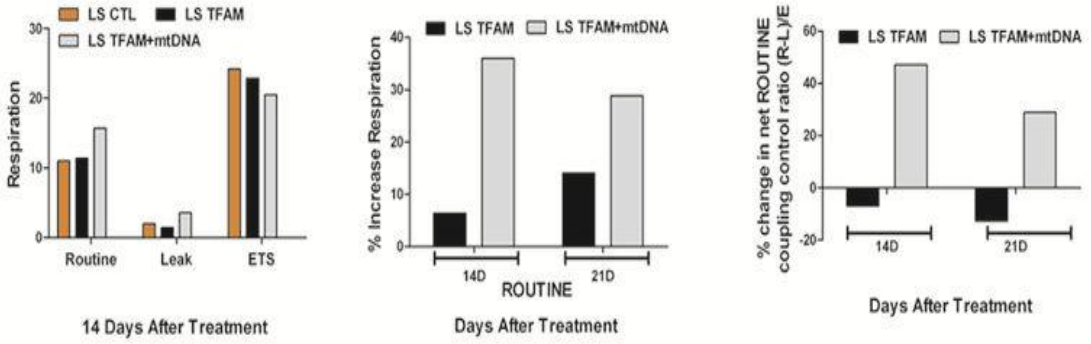
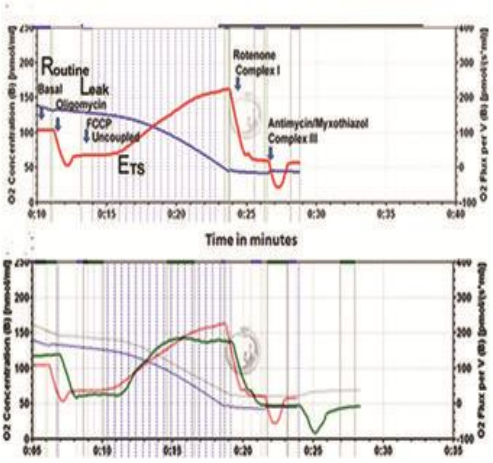
The business of science is often a rat-race as we are searching for solutions in isolated closed environments, with the goal of reaching the top in our respective research fields. But, as Mahatma Gandhi said, "*There is more to life than increasing its speed;"* and "*In a gentle way, we can shape the world*." When we apply these guiding principles to our research endeavors towards generating solutions for incurable diseases, it leads to open research environments which could eventually become more collaborative, more global, and gently shape the world. Thus, Gentle Science aims to create a bridge between science and spirituality to better understand our own lives and to live in harmony within ourselves and with the universe around us. It is important that we practice the tenets of Gentle Science in the real world of mitochondrial physiology and genetic disorders. Our Gentle Science [1] approaches have been enriched by our interactions with educators, scientists, clinicians, entrepreneurs and the patient community suffering from incurable mitochondrial disorders.

Many of these disorders represent a large group of diseases with heterogeneous clinical and pathological expressions characterized by irrevocable damage and improper functions of specialized metabolically active cell types. Examples include classical mitochondriopathies, Leber's hereditary Optic Neuropathy (LHON), Leigh's syndrome (LS), Amyotrophic Lateral Sclerosis (ALS), Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke (MELAS) syndrome, that affect children and adults. Mitochondrial DNA (MtDNA) mutations and deletions are often found in these disorders and contribute to a decline in mitochondrial energy function and diminished vigor in these disorders.

We have developed a novel approach that uses a recombinant mitochondrial transcription factor A protein (rhTFAM) for external manipulation of the mitochondrial genome present inside cells. In the context of developing relevant cell-based models for targeted drug discovery, we have used this approach to introduce, replicate, and transcribe pathogenic mtDNA in human neural progenitor stem cells, while maintaining

multipotency and successful differentiation into neuronal lineage in the short term [2]. In parallel, we also used healthy mtDNA complexed with rhTFAM to transduce into the mitochondria of two classic mitochondrial diseases, as cell models for proof-of-principle studies toward conducting mitochondrial gene therapy in the future. We introduced healthy mtDNA first into the cytoplasmic hybrid (cybrid) cells containing platelets from an LHON patient and, subsequently, into primary skin fibroblasts obtained from an LS patient [3]. We showed that using healthy donor mtDNA circles complexed with rhTFAM improved respiration (Figure 1) and biogenesis in LS and LHON disease cell lines caused by different pathogenic mtDNA point mutations.





Cells	Respiration			Coupling Control Ratios		
	Routine	Leak	ETS	R/E	L/E	(R-L)/E
LH CTL 2wk	11.01	1.97	24.20	0.45	0.08	0.37
LH Tfam 2wk	11.35	1.46	22.86	0.50	0.06	0.43
LH Tfam+mtDNA 2wk	15.70	3.60	20.51	0.77	0.18	0.59

Figure 1: Mitochondrial gene therapy improves respiration in Leigh's patient fibroblasts carrying T8993G mutation (see Iyer et al., Human Gene Therapy, 2012 for details).

Results from these ongoing studies will contribute to (a) patient- and cell- specific stem cell models for drug testing and (b) therapeutic approaches for improving respiration in patients suffering from these incurable mitochondrial disorders.

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Burtscher 2012 Abstract Bioblast

Mitochondrial function in the mouse hippocampus.

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Epilepsy is one of the most common neurological diseases featuring a prevalence of 1-2%. A high percentage of patients is refractory to antiepileptic medication, especially in mesial temporal lobe epilepsy (mTLE). Epilepsy is characterized by seizures, in which a lot of glutamate is released leading to excitotoxicity and neuronal loss. Seizure related alterations in neurons are often associated with damaged mitochondria and with impaired functions of distinct complexes of the electron transport chain in human patients and animal models. However, mitochondrial alterations during the development of epilepsy (epileptogenesis) are not well characterized and it is not yet known, whether mitochondrial alterations are cause or consequence of epileptogenesis. Answers to these questions are important to learn more about the neurochemical processes underlying epileptogenesis and to assess implications on the development of antiepilept(ogen)ic medication.

Therefore, we are in the process of developing protocols to analyze different mitochondrial parameters using the Oxygraph-2K (Oroboros Instruments, Innsbruck) in hippocampal tissue - which is strongly affected in mTLE - of mice. We apply the kainic acid model of TLE in mice. Injection of kainic acid into the hippocampal CA1 region results in *Status epilepticus*, a subsequent silent phase and ultimately recurrent seizures.

We want to study the activities of electron transport chain (ETC) complexes I, II and IV across different time points of these phases of epileptogenesis.

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Chinopoulos C 2012 Abstract Bioblast

Properties of the mitochondrial permeability transition in human cells lacking the adenine nucleotide translocase isoform 1.

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In pathological conditions, mitochondria recruit proteins to the inner membrane to form a pore that disrupts membrane integrity. This pore, termed *permeability transition pore* (PTP) is of a sufficient size to allow the passage of solutes and water since the mitochondrial matrix is hyperosmotic to the cytosol that may also result in rupture of the outer membrane [1]. For long, the adenine nucleotide translocase (ANT) was suspected to be a structural component of the pore; however, recent experiments showed that

mitochondria obtained from ANT knock out mice were still capable of demonstrating pore formation; it was therefore, inferred that ANT modulates the opening of the mitochondrial permeability transition pore, but it is not a structural element *per se* [2].

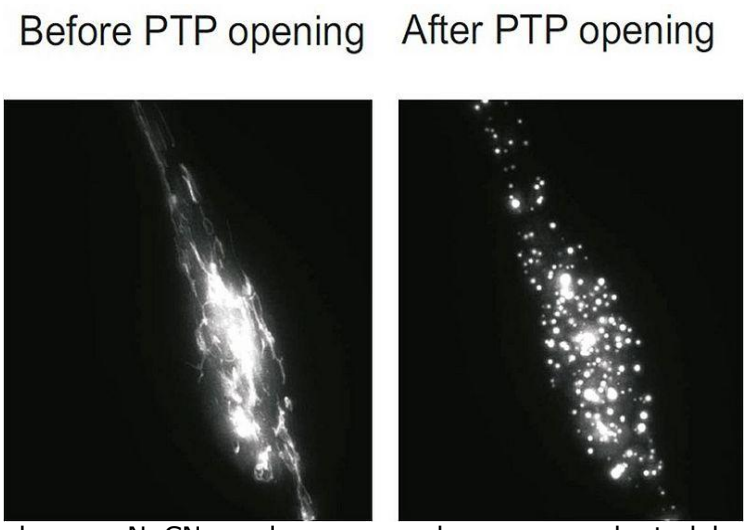

Figure 1: mito-DsRed2 targeting of fibroblast mitochondria reveals *in situ* permeability transition.

Hereby we investigated the properties of *in situ* permeability transition in human fibroblasts obtained from a patient with a complete absence of ANT isoform 1. *In situ* fibroblast mitochondria of the patient and a disease-free related individual were visualized by mito-DsRed2 targeting and challenged by calcimycin. The effects of glucose, NaCN, and an uncoupler were evaluated by measuring mitochondrial volume using the thinness ratio technique. The absence of ANT1 abolished the hastening of the swelling of *in situ* mitochondria by glucose deprivation and NaCN co-application, upon calcimycin exposure.

As expected, the extent of swelling quantitated by the thinness ratio technique was the same for both cell types. The impact of a lack of an electrochemical gradient substantiated by absence of glucose and presence of cyanide predisposing to pore opening is therefore mediated by ANT1.

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20 Years High-Resolution Respirometry – OROBOROS INSTRUMENTS and WGT

The production of the OROBOROS Oxygraph-2k was preceeded by instrumental and methodological developments [1,2], particularly of the Twin-Flow Microrespirometer [3] for simultaneous applications with microcalorimetry in an open-flow system (Fig. 1). This Twin-Flow Microrespirometer was distributed on a small scale, but particularly applied in international cooperations (e.g. [4]).

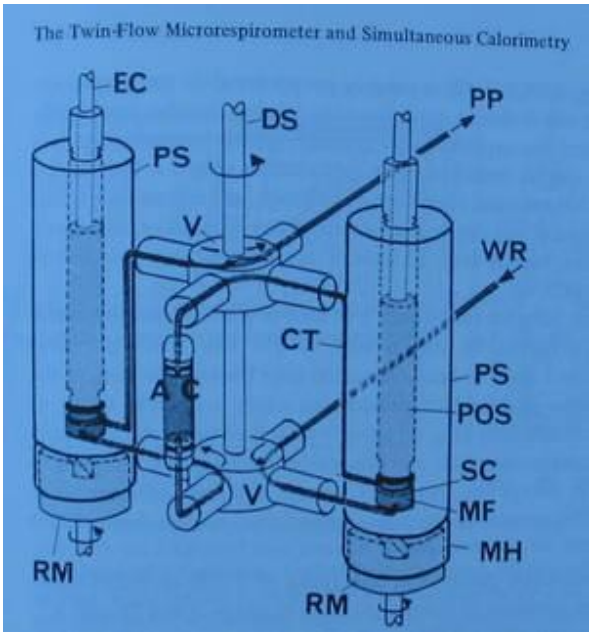

Figure 1: The Twin-Flow principle: The twin-flow microrespirometer and simultaneous calorimetry [3].

1989: The prototype of the Oxygraph was developed in the frame of a FWF Project "Thermodynamics and respiratory control in aerobic and anoxic mitochondria" (E. Gnaiger, principal investigator).

1992: The first series of the OROBOROS® Oxygraph was completed (initially "Cyclobios Oxygraph" with Paar; Graz).

1994: OROBOROS® was founded by Erich Gnaiger, taking over the world-wide distribution of the Oxygraph. At that time, Prof. R. Margreiter initiated with the assistance of Erich Gnaiger the D. Swarovski Research Laboratory at the Department of Transplant Surgery. The Oxygraph provided the fundamental instrument for an extensive series of applications in hypoxia [5-8] and cold storage ischemia-reperfusion injury. These studies established a first level of bioenergetic protocols for biomedical applications of high-resolution respirometry. With completion of the development of DatLab 2 Dr. Michael Reck), the term "high-resolution respirometry" was coined and a new standard in software-support was set. About 60 Oxygraphs were produced until **1998** and distributed in 17 countries.

2000: OROBOROS INSTRUMENTS Corp.

2001: Philipp Gradl (WGT Elektronik; Kolsass) became the competent partner for development and production of the next generation OROBOROS Oxygraph-2k, O2k. Lukas Gradl (ssn - software security networks; Innsbruck) took over the software development, generating the refined Windows™ version of DatLab 3. All developments and production of the Oxygraph-2k take place within 20 km of Innsbruck.

August 2003: The first of 100 instruments of Series B were completed, and applied in a series of workshops on high-resolution respirometry. The O2k Series B provided the basis for integration of the Titration-Injection microPump (TIP2k) and modular expansion to the O2k-MultiSensor MiPNetAnalyzer.

2004: 10 years OROBOROS INSTRUMENTS was celebrated and a major development was completed to improve the design of the polarographic oxygen sensor (OroboPOS), which is available since November 2004. Developments of the O2k-MultiSensor MiPNetAnalyzer benefit from user innovation. Based on the expertise and cooperation within MiPNet, various electrochemical sensors (NO, pH, TPP^+, Ca^{2+}, K^+) and optical sensors (light scattering, fluorescence probes), can be integrated into the Oxygraph-2k

and supported by the multichannel-software DatLab 4 (Windows™). Since December 2004, DatLab 4 and the O2k-MultiSensor MiPNetAnalyzer are available, including the Titration-Injection microPump TIP2k and input channels for high-power amplification of ion-selective electrodes.

June 2006: The O2k #100 was sold (Series B). Since January **2007**, the OROBOROS INSTRUMENTS Corporation (GmbH) replaced the previous private company in the world-wide distribution and service of the OROBOROS Oxygraph-2k and accessories.

2009: OROBOROS INSTRUMENTS celebrated the 50th International Course on High-Resolution Respirometry and the 1st O2k-MultiSensor Workshop (April 2009, Schröcken).

2010: The Bioblast Wiki was launched: Together with MitoPedia, OroboPedia was started by OROBOROS INSTRUMENTS on 2010-07-12 to provide short definitions of terms, abbreviations and symbols frequently used in the context of high-resolution respirometry and mitochondrial physiology. Annual sales increased to 60 O2k per year in 2010 and 2011. The innovative collaboration between the Medical University of Innsbruck (D. Swarovski Research Laboratory), OROBOROS INSTRUMENTS and WGT-Elektronik was recognized by granting the Cluster Life Sciences Award 2010 for the O2k, at which occasion the grant application was initiated for the K-Regio project *MitoCom Tyrol*.

2011: Production of O2k-Series E and launch of K-Regio project *MitoCom on* May 01 [9-12].

January 2012: OROBOROS startet with the new concept of the O2k-Core. The first O2k-Fluorescence Workshop was held in March 2012. Up to date 430 instruments of the new O2k-generation were sold to 37 countries. In April 2012, the O2k-Team received the Houska Award (120,000.- €; Vienna, B&C).

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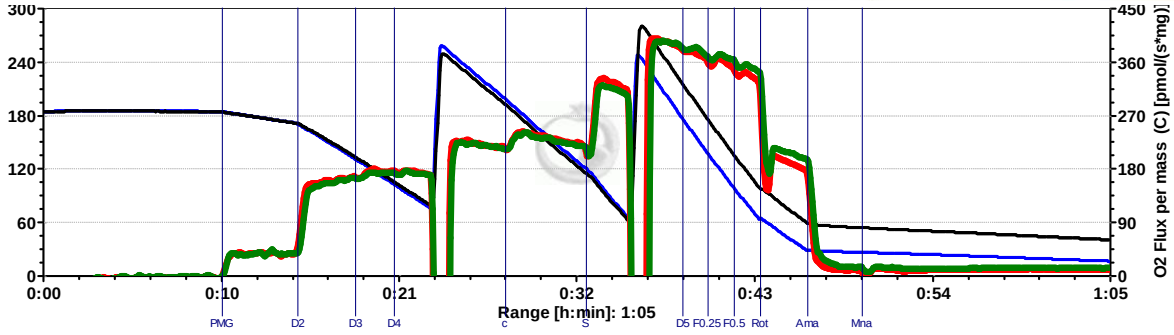
Mitochondrial Physiology Network 17.15: 1-6 (2012)

Version 1: 2012-12-05

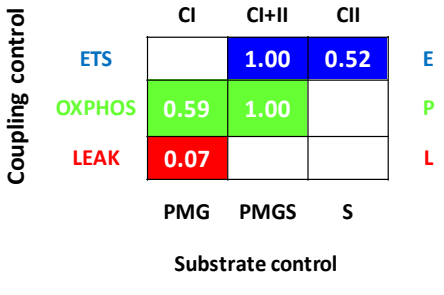
Tissue Homogenates for Diagnosis of Mitochondrial Respiratory Function: Mouse Heart, Brain and Liver

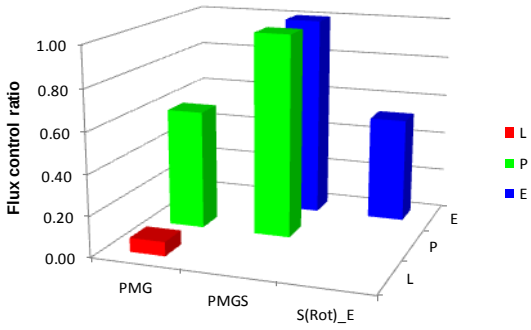
Andrea Eigentler, Mona Fontana-Ayoub, Erich Gnaiger

>> *Updated version*: MiPNet17.15



SUIT protocol with homogenate of mouse myocardium. Overlay of O2k-traces of two chambers. Oxygen concentration [µM] (black and blue line) and oxygen flux per mass [pmol·s^{-1}·mg^{-1}] (red and green line). MiR05Cr, 37 °C.





Coupling control and substrate control ratios for mouse heart homogenate, calculated as mean of chamber C and chamber D.

OROBOROS INSTRUMENTS
high-resolution respirometry

O2k-Protocols

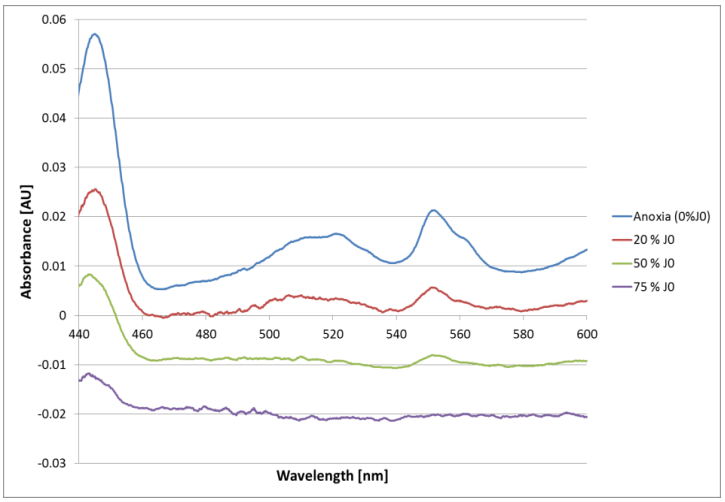
Mitochondrial Physiology Network 17.16: 1-4 (2012)

Version 1: 2012-12-05

O2k-Spectrophotometry – A *MitoCom* Project.

David K Harrison[1], Mario Fasching[1], Erich Gnaiger[1,2]

>> *Updated version*: MiPNet17.16



Average cytochrome difference spectra at 0%, 20%, 50% and 75% J_0. The spectra have been offset for clarity.

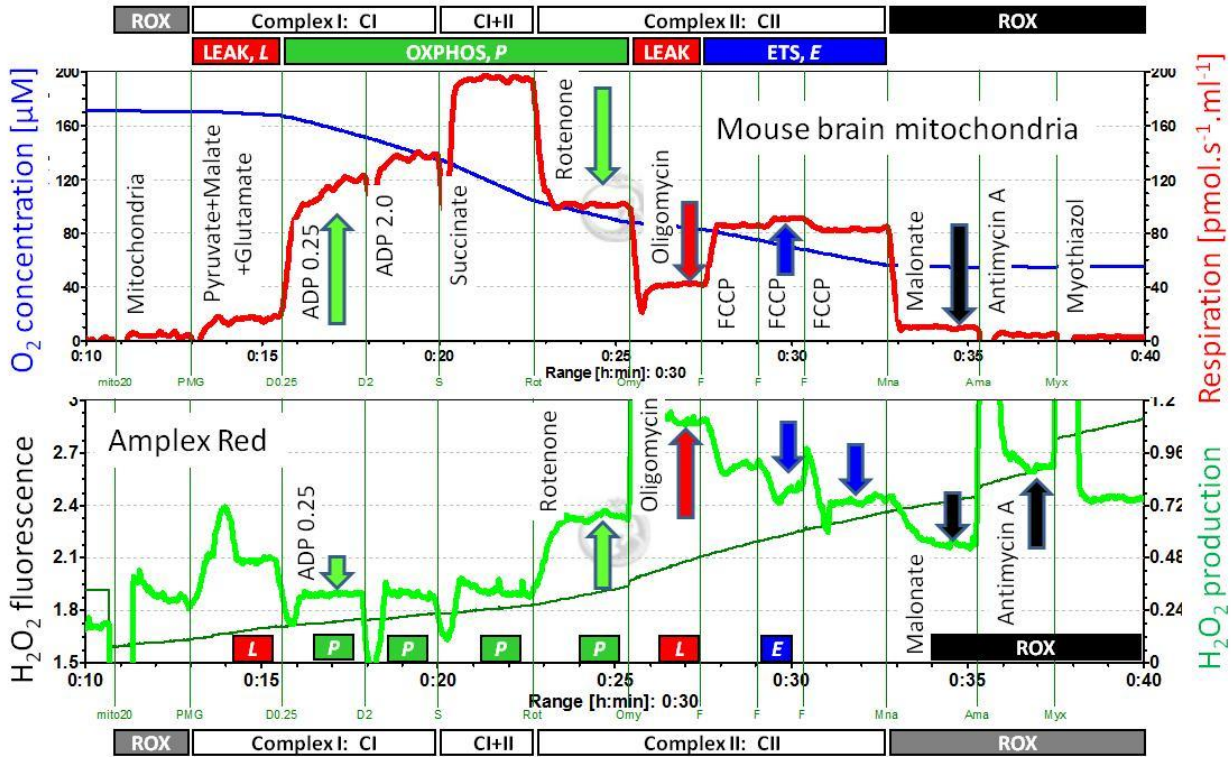
Mitochondrial Physiology Network 17.17: 1-4 (2012)

Version 1: 2012-12-05

O2k-Fluorometry – a *MitoCom* Project

Mario Fasching[1], Zuzana Sumbalova[2], Erich Gnaiger[1,3]

>> *Updated version*: MiPNet17.17



Simultaneous measurement of respiration (top) CI-linked in the LEAK state followed by ADP titration, and substrate control in the OXPHOS state from CI, CI+II- to CII-linked states, sequential oligomycin and FCCP titration and inhibition to the ROX state. H_2O_2 production is independent of respiratory rate and is a function of metabolic state, decreasing with stimulation by ADP, but increasing with inhibition by rotenone, oligomycin and antimycin A.

Mitochondrial Physiology Network 14.10: 1-4 (2012)

Version 5: 2012-11-29

Top 10 Reasons for OROBOROS INSTRUMENTS

Erich Gnaiger

>> *Updated version*: MiPNet14.10