dependent on the presence of free fatty acid. Conclusions: by activating processes enhancing fatty acid oxidation T2 could protect skeletal muscle against lipotoxicity.

doi:10.1016/j.bbabio.2008.05.189

### S8/2 In situ oxidative phosphorylation, oxidative stress, and mitochondrial morphology of INS-1E and HEP-G2 cells

Petr Ježek, Lydie Hlavatá-Plecitá
*Department 75, Institute of Physiology, Academy of Sciences, Prague, Czech Republic*
*E-mail:* jezek@biomed.cas.cz

We have used 4Pi microscopy 3D imaging (~250 nm lateral and ~100 nm axial resolution) to demonstrate that cells relying on intensive oxidative phosphorylation contain in fact a single mitochondrion, a slightly branched dense mitochondrial reticulum filling the substantial cell volume. Unlike conventional confocal microscopy, resolving apparently tubules of ~800 nm diameter, we clearly show an average tubule diameter of 262 nm in insulinoma INS-1E cells and 284 m in hepatocellular carcinoma HEP-G2 cells cultivated at 5 mM glucose. Moreover, mitochondrial reticulum shapes resulting from fission induction by decreasing OXPHOS cannot originate from a sole fission but must originate also from concomitant fusion, since e.g. uncoupling led to rings, obviously arisen from fusion of two ends of short segments, while uncoupling at an inhibited respiratory chain led to rings with closed outlets, i.e. to vessel type objects, where fusion must be even more prominent. HEP-G2 cells cultivated at 25 mM glucose exhibited thicker tubules but also lower matrix-released superoxide production, the un-dismuted surplus ($J_m$) confocally indicated by MitoSOX. Rotenone caused a 5-fold $J_m$ increase, completely attenuated by uncoupling and by MitoQ. A hydrophobic amiloride that acts on the ND5 subunit and inhibits Complex I $H^+$ pumping enhanced $J_m$ and even countered the attenuating effect of FCCP, but not that of MitoQ.

Supported by grants NR/9183-3; IAA500110701.

doi:10.1016/j.bbabio.2008.05.190

### S8/3 Mitochondrial respiratory physiology: Convergent electron transport system and flux control of oxidative phosphorylation in intact and permeabilized cells

Erich Gnaiger
*Medical University of Innsbruck, Department of General and Transplant Surgery, D. Swarovski Research Laboratory, 6020 Innsbruck, Austria*
*E-mail:* erich.gnaiger@i-med.ac.at

Oxidative phosphorylation (OXPHOS) is a key element of bioenergetics, extensively studied to resolve mechanisms of energy transduction and respiratory control in the electron transport system (ETS). Electrons flow to oxygen from Complex I or II with three or two coupling sites. The functional design of the ETS was studied in permeabilized NIH3T3 fibroblasts by high-resolution respirometry with multiple substrate-uncoupler-inhibitor titration protocols. Compared to *ETS* capacity in intact cells, conventional State 3 respiration in permeabilized cells was only 0.38±0.06 with ADP and glutamate +malate. *ETS* capacities were identical in intact and permeabilized uncoupled cells, however, with convergent electron flow to the Q-junction from glutamate+malate+succinate through Complexes I and II (CI+II e-input). Coupled *OXPHOS* flux was 0.50±0.09 of *ETS* capacity, reflecting control of the phosphorylation system over *OXPHOS*. Convergent CI+II e-input provides the relevant basis for quantifying enzymatic thresholds and excess capacities of individual steps of *OXPHOS*, and for evaluation of mitochondrial defects. Convergent CI+II e-input corresponds to operation of the tricarboxylic acid cycle and mitochondrial substrate supply *in vivo* and yields novel insights into the physiological diversity of mitochondria from various tissues. Multiple substrate-uncoupler-inhibitor titration protocols extend the diagnostic potential of mitochondrial physiology in health and disease.

doi:10.1016/j.bbabio.2008.05.191

## (S8) Mitochondria and cell physiology symposium abstracts (poster and raised abstracts)

### S8.4 Mitochondrial superoxide generation is diminished during glucose-stimulated insulin secretion in INS-1E cells

Tomáš Špaček, Lydie Plecitá-Hlavatá, Petr Ježek
*Department 75, Institute of Physiology, Academy of Sciences, Prague, Czech Republic*
*E-mail:* spacek@biomed.cas.cz

One of the unique features of β-cells lies in their relatively low expression of antioxidant enzymes. It makes them liable to oxidative damage — one of etiologies for type 2 diabetes development. Using matrix-localized MitoSOX, we have monitored excessive superoxide production released to the mitochondrial matrix ($J_m$) in insulinoma INS-1E cells before and after glucose addition, i.e. under glucose-stimulated insulin secretion (GSIS) conditions. Independently of the original glucose level (cells cultivated at 11 mM or 3 mM glucose) $J_m$ substantially decreased upon glucose addition. % decrease in $J_m$ was linearly dependent on the incremental glucose in mM. $J_m$ was also suppressed by an uncoupler or a fatty acid, showing attenuating effects of mild uncoupling. Since previously we have demonstrated increasing ATP synthesis (OXPHOS) with increasing glucose added to glucose-depleted INS-1E cells, saturating above 12 to 15 mM glucose, our data indicate that increasing OXPHOS and concomitantly increasing $H^+$ backflow across the $F_O$ part of ATP synthase attenuates mitochondrial superoxide production including that on Complex I. We conclude that GSIS does not induce oxidative stress in mitochondrial matrix *in situ* but actually attenuates superoxide production established at mild starvation. Supported by grants NR/9183 - 3; IAA500110701.

doi:10.1016/j.bbabio.2008.05.192

### S8.5 Regulation of oxidative phosphorylation in response to graded uncoupling towards the limit of electron transport capacity

Patrick Subarsky[a,b], Erich Gnaiger[a]
[a]*Department of General and Transplant Surgery, D. Swarovski Research Laboratory, Innsbruck Medical University, Austria*
[b]*Department of Medical Biophysics, University of Toronto, Canada*
*E-mail:* p.subarsky@utoronto.ca

Mitochondrial oxygen consumption is divided between the support of ADP phosphorylation and *LEAK* (including proton leak through the inner membrane and proton slip in the respiratory complexes). The aim of our study was to determine the distribution of oxygen consumption between the two processes in intact cells (32D, myeloblast-like). Electron transport capacity ($E$) was defined as the maximum respiration under conditions of optimal FCCP concentration (76.2±12.9 pmol $O_2$ $s^{-1}$ per $10^6$ cells). Cell respiration ($R$) under

physiological control of routine ATP demand was 0.41 of *E*. Similarly, the oligomycin-inhibited respiration (*L*; representing *LEAK*) which was 0.25 of *R*. *LEAK* was increased from an *L/E* ratio of 0.09 by stepwise additions of FCCP. The corresponding stress-induced compensation of cell respiration was measured and the contribution to phosphorylating activity (net*R*) was calculated as *R*−*L*. Complete maintenance of phosphorylating activity would be indicated by an unchanged net*R*, whereas we observed only a partial compensation reflected by a significant decline of net*R*/*E*. Our results show that even at high *L/E* ratios, respiratory activity can support ADP phosphorylation, albeit with some loss in capacity. This model of uncoupling injury is further evaluated in the pathophysiological context of simultaneously diminished electron transport capacity.

doi:10.1016/j.bbabio.2008.05.193

### S8.6 Role of peroxisomes in cell calcium homeostasis

Francesco M. Lasorsa[a], Paolo Pinton[b], Pasquale Scarcia[a], Luigi Palmieri[a], Rosario Rizzuto[b], Ferdinando Palmieri[a]
[a]*Department of Pharmaco-Biology, University of Bari and CNR Institute of Biomembranes and Bioenergetics, Bari, Italy*
[b]*Department of Experimental and Diagnostic Medicine, University of Ferrara, Italy*
*E-mail:* fpalm@farmbiol.uniba.it

The ability of peroxisomes to handle $Ca^{2+}$ and be involved in cell signalling pathways has been investigated for the first time. We generated two novel peroxisomally targeted $Ca^{2+}$-sensitive aequorins, peroxAEQwt and peroxAEQmut, for low and high [$Ca^{2+}$] measurements, respectively. By dynamic monitoring of $Ca^{2+}$ concentration, we showed that a large transient $Ca^{2+}$ increase (up to ~100 μM) occurs in peroxisomes of agonist-stimulated cells. Furthermore, $Ca^{2+}$ is stably maintained in peroxisomal lumen during resting at concentrations ~20-fold higher than in cytosol. Peroxisomal $Ca^{2+}$ uptake is sensitive to ionophores and reagents that dissipate electrochemical gradients across biological membranes, thus unravelling is an unexpected bioenergetic framework across the peroxisomal membrane where $H^+$- and $Na^+$-gradients appear to sustain the $Ca^{2+}$ flux towards the peroxisomal matrix. Peroxisomal $Ca^{2+}$ homeostasis displays unique characteristics when compared with those of other subcellular compartments. It is suggested that yet unidentified $Ca^{2+}$-transporting systems exist in the peroxisomal membrane and that $Ca^{2+}$ can play an important role in regulating peroxisomal metabolism.

doi:10.1016/j.bbabio.2008.05.194

### S8.7 Cellular metabolic profile and lonidamine-induced cytochrome *c* release

Maureen O. Ripple, Michelle Abajian, Roger Springett
*Department of Radiology, Dartmouth College, Hanover, NH, USA*
*E-mail:* maureen.ripple@dartmouth.edu

Lonidamine, an agent which induces apoptosis via the intrinsic pathway, causes cytochrome *c* (cyt*c*) release in some leukemia cell lines (ML-1) but not others (HL-60 and Jurkat). ML-1 cells are highly glycolytic and have a low basal rate of $O_2$ consumption (14 nM/min/$2\times10^7$ cells) whereas HL-60 cells have nearly twice the $O_2$ consumption (27 nM/min/$2\times10^7$ cells). We have developed an optical system to measure the concentration and oxidation state of electron transport chain (ETC) cytochromes in living cells in real time. HL-60 cells have a low content of cytochrome oxidase (cyt$aa_3$), 17±2 pmol/$2\times10^7$cells, compared to ML-1 cells which have 31±4 pmol/$2\times10^7$ cells, even though HL-60 cells have a higher $O_2$ consumption. At baseline, both cyt*c* and cyt$aa_3$ are highly oxidized in ML-1 cells, 91.0±1.5 and 92.9±1.5% respectively, compared to the more normal profile of 62.0±1.9 and 76.2±1.8% in HL-60 cells. The metabolic profile of the Jurkats is similar to that of the HL-60 cells. In all three cell lines, lonidamine causes an immediate decrease in oxygen consumption and an oxidation of cyt*c* and cyt$aa_3$ consistent with an inhibition upstream of the ETC. However, cyt*c* was only released from the mitochondria in ML-1 cells. We hypothesize that the metabolic perturbations that lead to cyt*c* and cyt$aa_3$ being highly oxidized in ML-1 cells also sensitizes them to the pro-apoptotic effects of lonidamine.

doi:10.1016/j.bbabio.2008.05.195

### S8.8 Native low-density lipoproteins cause mitochondrial dysfunction in human proximal tubular cells: Multiple players involved

Annamaria D'Aprile[a], Claudia Piccoli[a], Eustacchio Montemurno[a], Laura Calabresi[b], Rosella Scrima[a], Giovanni Quarato, Maria Ripoli[a], Domenico Boffoli[a], Loreto Gesualdo[a], Nazzareno Capitanio[a]
[a]*Department of Biomedical Science, University of Foggia, Foggia, Italy*
[b]*Center E. Grossi Paoletti, Department of Pharmacological Sciences, University of Milano, Milano, Italy*
*E-mail:* n.cap@unifg.it

The effects caused by non-oxidised native low-density lipoproteins (nLDL) have been poorly examined in extra-endothelial tissues. In this study we investigated the consequences of nLDL-treatment of human proximal tubular cells (HK2) on the oxidative metabolism. It is shown that nLDL caused a time- and dose-dependent increase of cellular ROS production. This was completely abrogated by specific inhibition of NADPH oxidase (NOX). Moreover, mitochondria of nLDL-treated HK2 displayed a marked decrease of membrane potential, enhanced accumulation of $Ca^{2+}$ and loss of cytochrome *c*. These effects were prevented by ruthenium red and cyclosporine A. Notably, all the observed changes caused by nLDL treatment were prevented by EGTA (chelating extracellular $Ca^{2+}$) and by AACOCF3 (inhibiting the cytoplasmic phospholipase A2-(cPLA2)). Noteworthy, ROS detection by the mitochondrial-specific probe (MitoSox) suggested also direct participation of mitochondria in the nLDL-induced redox unbalance in HK2. However, mitochondrial ROS production was abrogated by extra-cellular added SOD/catalase. Overall, the results presented show that nLDL cause in renal cells a marked change in the intracellular redox state by a mechanism that initially involving $Ca^{2+}$-dependent cPLA2 and NOX further propagates by redox-signaling to mitochondria provoking broader cell-harming outcomes. These observations may help in defining the pathogenesis of hyperlipidemia-associated renal damage and to individuate previously unappreciated potential therapeutic targets.

doi:10.1016/j.bbabio.2008.05.196

### S8.9 Metformin causes oxidative stress and up-regulates expression of UCP2 in white adipocytes

Andrea Anedda, Eduardo Rial, Mar Gonzalez-Barroso
*Centro de Investigaciones Biológicas, CSIC, Madrid, Spain*
*E-mail:* andrea.a@cib.csic.es

The uncoupling proteins (UCPs) are transporters of mitochondrial inner membrane whose postulated function is to dissipate