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.

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S8.6 Role of peroxisomes in cell calcium homeostasis

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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.

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

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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/2×10^7 cells) whereas HL-60 cells have nearly twice the O_2 consumption (27 nM/min/2×10^7 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 (cytaa_3), 17±2 pmol/2×10^7cells, compared to ML-1 cells which have 31±4 pmol/2×10^7 cells, even though HL-60 cells have a higher O_2 consumption. At baseline, both cyt*c* and cytaa_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 cytaa_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 cytaa_3 being highly oxidized in ML-1 cells also sensitizes them to the pro-apoptotic effects of lonidamine.

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

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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.

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

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

energy by uncoupling of respiration from ATP synthesis. The physiological function of UCP2 is still unknown. Despite its high homology with UCP1, UCP2 does not seem to have a role in adaptive thermogenesis while mounting evidence implicates UCP2 in the protection against oxidative stress. UCP2 is expressed in several mammalian tissues including white adipose tissue. Leptin, a hormone involved in the control of energy balance, increases lipolysis and fatty acid oxidation in white adipose tissue and simultaneously up-regulates UCP2. The aim of the present study is to investigate whether in white adipose tissue UCP2 serves as an energy dissipatory mechanism which facilitates fatty acid oxidation or prevents oxidative damage. Metformin (dimethylbiguanidine) is a drug widely used to treat type 2 non-insulin dependent diabetes mellitus. We show that metformin raises the UCP2 levels in white adipose tissue of mice and in 3T3-L1 adipocytes. Up-regulation of UCP2 correlates with a higher superoxide dismutase activity, lower aconitase and high levels of reactive oxygen species while lipolysis is not yet induced. These evidence strongly suggest that UCP2 is not directly involved in fatty acid metabolism and reinforces their role in the defence against oxidative stress.

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S8.10 Brain mitochondrial bioenergetics is differentially affected by anesthetics fentanil and remifentanil

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Mitochondria have been proposed to be implicated in cellular effects of anesthetics. The purpose of our study was to investigate effects of fentanil and remifentanil on the bioenergetics of isolated rat brain mitochondria. Mitochondria were isolated and respiratory rates, respiratory control ratio (RCR), ADP/O ratio, mitochondrial membrane potential ($\Delta\Psi_m$) and mitochondrial respiratory complex activities (II, III, IV and V) were determined. Increased concentrations of fentanil and remifentanil resulted in compromised respiratory mitochondrial function in a dose-dependent decrease in RCR and uncoupling of oxidative phosphorylation evidenced by the decreasing ADP/O values. The $\Delta\Psi$ generated by respiration and mitochondrial complexes II, III, IV and V activities significantly decreased with concentration of fentanil ranging from 4 to 20 μg/mL. Similar effects were observed at higher concentrations of remifentanil (10 and/or 20 μg/mL). In conclusion our study demonstrated that fentanil and remifentanil exerted significant and differential effects on the mitochondrial bioenergetics: remifentanil slightly affects the mitochondrial bioenergetic functions in contrast with the deleterious effects of fentanil.

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S8.11 Photobiomodulation of flavonoid effects on human T cells mitochondrial network state

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The goal of studies was to reveal flavonoid induced changes in human acute T leukemic Jurkat cells mitochondrial reticulum size and state, as well as soft laser irradiation influence thereon. Apoptosis induction and cell cycle progression of flavonoid treated, irradiated and/or non-irradiated T leukemia lymphoblasts, were additionally monitored. Analysis of JC-1 red/green fluorescence intensity ratios, PI, and of MitoTracker Green signals, collected by flow cytometry and/or by confocal microscopy allowed us to determine mitochondrial membrane potentials, mitochondrial membrane depolarization/hyperpolarization related apoptosis induction/cell cycle blockade/progression, and mitochondrial network weight. Natural flavonoids epigallocatechine gallate and quercetin induced changes in sizes and polarization degrees of cell subpopulations of high and low mitochondrial membrane polarization, and in the mitochondrial reticulum volume, in a dose and exposure-time dependent manner. Low concentrations of flavonoids caused no or mild effects, reversible in time, while alterations of mitochondrial reticulum size and state, induced by high concentrations of flavonoids, were correlated with apoptosis induction and cell cycle blockade. 680 nm far-red and 830 nm infrared laser lights promoted/reversed the flavonoid induced changes in a dose, wavelength and irradiation regime dependent manner.

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S8.12 Opposite effects of action potential on spatial patterns of photosynthesis and extracellular pH in a plant cell

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Characean cells exposed to illumination arrange their plasmamembrane H^+ fluxes and photosynthesis in coordinated spatial patterns that facilitate the acquisition of inorganic carbon from the aquatic environment. The limited availability of CO_2 in alkaline bands accounts for lower effective quantum yield of photosystem II ($\Delta F/Fm'$) in chloroplasts of these bands compared to acidic zones. In this work we studied the effect of electrically triggered action potential (AP) on the spatial distribution of photosynthetic parameters ($\Delta F/Fm'$ and non-photochemical quenching, NPQ) and extracellular pH with fluorescence imaging and pH microsensors. In the resting cell at a range of light intensities, the periodic profile of extracellular pH is parallel to the profile of NPQ and antiparallel to that of $\Delta F/Fm'$. The principal discovery of this study is that, after triggering AP, the pH banding disappeared temporarily, whereas the effective quantum yield and NPQ patterns became more contrast.