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