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

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

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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.

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

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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.

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(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

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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.

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

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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