reductase, ubiquinone cytochrome *c* reductase, and cytochrome *c* oxidase) in EC compared to LDL or control. The abundance of mitochondria-associated ROS and the release of ROS from EC were significantly increased following glyLDL or oxLDL treatment. The findings suggest that glyLDL attenuates activities of key enzymes in multiple mitochondrial ETC, decreases mitochondrial oxygen consumption and mitochondrial membrane potential, and increases ROS generation from mitochondria in EC, which potentially contributes to mitochondrial dysfunction and vascular disorders in diabetic patients.

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4P.14 Cell biological consequences of isolated complex I deficiency in a KO mouse model

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In humans, deficiency of complex I (CI or NADH:ubiquinone oxidoreductase) is associated with a wide range of progressive neurological disorders that are characterized by an early onset and a short devastating course. CI resides in the mitochondrial inner membrane (MIM), where it channels electrons from food-derived NADH into the electron transport chain (ETC). Together with three other complexes (CII-CIV) and the ATP-generating enzyme F_OF_1-ATPase (CV), CI constitutes the mitochondrial oxidative phosphorylation system (OXPHOS). CI is a large (about 1 MDa) multi-subunit complex that consists of 45 different subunits of which 7 are encoded by the mitochondrial DNA (mtDNA) and the remainder by the nuclear DNA (nDNA). To study the pathophysiology of CI dysfunction, we use a novel knockout (KO) mouse model lacking an nDNA-encoded CI subunit (NDUFS4), which is a mutational hotspot in human CI deficiency. For this purpose we generated mouse embryonic fibroblast (MEF) cell lines from wild type (WT) and KO animals. Analysis of mitochondrial fractions revealed a greatly reduced CI activity in KO MEFs (about 5% of WT), whereas other activities (CII, CIII, CIV, CV and citrate synthase) were normal. At the protein level, MEFs from KO animals lacked fully assembled CI but instead contained a CI subcomplex of about 850 kDa. Reintroduction of the NDUFS4 subunit in KO cells led to reappearance of the fully assembled CI holocomplex. Functionally, KO MEFs contained mitochondria with a hyperpolarized membrane potential, probably due to reverse-mode action of CV. When cultured in glucose-containing (GLU) medium, reactive oxygen species (ROS) levels in WT and KO cells were identical. However, when a galactose (GAL) medium was used, KO MEFs displayed increased ROS levels whereas WT MEFs did not. In KO MEFs, complementation of the *NDUFS4* gene defect fully normalized the increased ROS levels. NADP(H) levels in KO MEFs were higher than in WT MEFs, whereas cytosolic resting [ATP] (determined with the novel fluorescent ATP-sensor 'ATeam') where the same. Currently, experiments are in progress to determine the effects of NDUFS4 knockout on cytosolic calcium handling and expression of energy-sensing proteins. Moreover, established antioxidants and nutraceuticals are tested for their beneficial action on WT and KO MEFs.

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4P.15 Molecular investigation of riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency (RR-MAD) patients

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Riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency (RR-MAD) is a lipid storage myopathy characterised by muscle weakness, decreased β-oxidation and muscle carnitine, altered urinary organic acids and plasma acyl-carnitine. We studied a cohort of 11 RR-MAD patients, performing a battery of biochemical and molecular tests to better characterise the metabolic disturbance of this disease and to explore the pathophysiological events [1–3]. Biochemical alterations of pre-therapy patient muscles included: (i) reduction in β-oxidation and in flavin-dependent enzymes (short and medium-chain acyl coenzyme A dehydrogenases, complexes I and II); (ii) reduction of FMN and FAD concentrations, (iii) increased activity of mitochondrial FAD pyrophosphatase [1, 3], thus suggesting an altered regulation of mitochondrial flavin cofactor homeostasis [4]. Proteomic investigation of muscle mitochondria in one RR-MAD patient [3] revealed decrease or absence of several proteins. All these deficiencies were completely rescued after riboflavin treatment. Proteomic data indicate that the enzymatic defects are multiple, coordinated, riboflavin-responsive and more extensive than previously described. In patients with RR-MAD the molecular defect is still unknown. Recently it was described that RR-MAD is associated with defects in electron transfer flavoprotein dehydrogenase (*ETFDH)* gene in a large proportion of cases. Therefore we performed molecular investigation by sequencing the *ETFDH* gene to identify the defects in this group of RR-MAD patients. The genomic sequence of each exon of *ETFDH,* including intron/exon boundaries has been PCR-amplified for 30 cycles. The PCR products have been purified and directly sequenced.

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