mitochondria, and nucleus. A comprehensive analysis of acute intracellular H_2O_2 signals in hCMEC/D3, subjected to hypoxia/reoxygenation and glucose deprivation, was conducted. Our results revealed that up to 2 hours of hypoxia exposure at 1 kPa did not induce H_2O_2 increase in any cell compartment within CMEC cells. However, during 3-6 hours of hypoxia exposure, a substantial H_2O_2 increase was specifically identified in the cytosol. Following 24 hours of reoxygenation at 18 kPa, H_2O_2 concentrations reverted to normal basal levels. Notably, elevated H_2O_2 levels were solely detected in the cytosol, not in the mitochondria under any conditions, indicating mitochondria's resilience to hypoxic conditions even after reoxygenation. Examination of glucose variations demonstrated that high glucose (11 mM Glc) and low glucose (Glc deprivation, 5 mM) treatments for 3 hours did not induce H_2O_2 level changes. In contrast, in different cellular compartments, subjected to 3 hours of glucose starvation, exhibited a marked increase in H_2O_2 in the cytosol and nucleus, while the mitochondria remained unaffected. In conclusion, hypoxia does not induce significant H_2O_2 changes yet glucose starvation leads to a marked increase in H_2O_2 levels. This study unveils nuanced dynamics in ROS responses, providing valuable insights for potential therapeutic interventions in stroke-related oxidative damage.

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PP II_E113

REDOX AND LIPIDOMIC ANALYSIS OF HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS (HCMEC/D3) ADAPTED TO PHYSIOLOGICAL OXYGEN LEVELS

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The majority of studies with endothelial and other cell types are conducted in standard culture incubators gassed with 5% CO_2 and room air (18 kPa O_2), well known to induce oxidative stress. Cells *in vivo* experience significantly lower O_2 levels, with brain endothelial cells exposed to ~3-7 kPa. Alterations in lipid metabolism mediated by oxidative stress play a key role in ischemic stroke and also are involved in signal transduction. This study aimed to compare the lipidomic profile and redox phenotype of human brain microvascular endothelial cells line (hCMEC/D3) adapted long-term (5 days) to hyperoxia (18 kPa O_2) or physiological normoxia (5 kPa O_2). Redox stress was significantly increased in hCMEC/D3 cells adapted to 18 kPa compared to 5 kPa O_2, as evidenced by elevated basal intracellular GSH (Fig. 1A) and sulforaphane upregulation of NRF2 regulated NQO1 expression. Lipidomic profiling was performed using Laser Desorption - Rapid Evaporative Ionisation Mass Spectrometry, a novel ambient ionization method developed for rapid, sample-preparation-free analysis of complex biological samples. Samples were fixed, washed to remove media and cells were directly analyzed. Preliminary multivariate statistical analysis revealed 87 spectral features as significantly different between hyperoxia (18 kPa) and physiological normoxia (5 kPa). As shown in Fig. 1C, these features are tentatively annotated as glycerophospholipids, specifically phosphatidylglycerol O-36:1, Phosphatidylinositol 38:4 and 38:3. In ongoing further data analysis we are linking these characteristic shifts with specific metabolic pathways. Our study highlights the critical importance of adapting cells *in vitro* to physiological O_2 levels and provides the first insights into shifts in the complex lipidome of cells exposed to different levels of oxidative stress.

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PERICYTES ARE IMPLICATED IN NO-REFLOW AFTER CEREBRAL ISCHEMIA

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Incomplete reperfusion of the microvasculature ("no-reflow") damages salvageable brain tissue after ischemic stroke. Using longitudinal in vivo 2-photon single-cell imaging we show 87% of pericytes, perivascular cells regulating microvascular flow, constrict during cerebral ischemia, and remain constricted post-reperfusion. Moreover, we reveal ischemic pericytes are fundamentally implicated in capillary no-reflow by limiting and arresting blood flow within the first 24 hours post-stroke. Despite sustaining acute membrane damage, we observe over half of all cortical pericytes survive ischemia and respond to vasoactive stimuli, upregulate unique transcriptomic profiles and replicate. Finally, we demonstrate delayed recovery of capillary diameter by ischemic pericytes after reperfusion predicts vessel reconstruction in the sub-acute phase of stroke and that pharmacological inhibition of Rho-kinase dilates constricted pericytes thereby increasing cerebral blood flow. Cumulatively, these findings demonstrate surviving cortical pericytes remain both viable and are promising therapeutic targets to counteract no-reflow after ischemic stroke.

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PP II_E115

THE RELATIONSHIP BETWEEN MITOCHONDRIAL OXIDATIVE STRESS AND HUNTINGTON'S DISEASE PROGRESSION

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Huntington's Disease (HD) is an autosomal dominant neurodegenerative disease characterized by the accumulation of mutant Huntingtin (mHtt) protein, which interferes with cellular physiological processes. Although the involvement of complex processes such as excitotoxicity, mitochondrial dysfunction, oxidative stress, transcriptional dysregulation, astrocytic dysfunction, and neuroinflammation in the development of the disease is known, the underlying molecular mechanisms remain largely elusive. Numerous studies have indicated the significant role of oxidative stress in HD pathogenesis. However, due to the limited temporal resolution and invasive nature of current analysis methods, there is still insufficient knowledge about the dynamic regulation of oxidative stress. In our study, we visualized real-time oxidative stress and mitochondrial dynamics at the single-mitochondrion level in striatal neurons using an in vitro HD model with the H_2O_2 biosensor HyPerRed. We showed that mitochondrial trafficking was interrupted, and mitochondrial H_2O_2 increased during the mutant Htt protein aggregation. Time-lapse imaging revealed that the subcellular increase in mitochondrial H_2O_2 levels, dependent on mutant Htt aggregation, was correlated with the disruption of intracellular transport. Moreover, we observed oxidative stress increase at the single organelle level with high spatial and temporal resolution for the first time through genetically encoded biosensors during the formation of Huntington's aggregates. This approach provides a new perspective on understanding the role of oxidative stress in the pathogenesis of neurodegeneration in greater detail and sheds light on potential treatments that can halt or reverse disease progression.

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PP II_E116

NOVEL MIRNA TARGETS AND CHARACTERISATION OF OPTIC NERVE FUNCTION IN A MOUSE MODEL OF HYPOPERFUSION

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Background: Understanding biomarkers of vascular dysfunction as mediators of cognitive decline is essential for dementia research. A promising avenue of