Alterations in Mitochondrial Respiration in Human Airway Epithelial Cell Cultures as a Function of Time at Air-Liquid Interface

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The biochemical pathways involved in the utilization of energy are fundamental determinants that govern every aspect of the interaction of the cell with its environment and are increasingly recognized as having pivotal regulatory roles in the cellular response and adaptation to environmental exposures. Morphologically and functionally, fully-differentiated human airway epithelial cells (HAEC) cultured at an air-liquid interface (ALI) on a semi-permeable membrane support represent the most relevant *in vitro* model of the human airway. The dependence of HAEC on glycolysis and mitochondrial respiration during differentiation at ALI has not been examined. In this study, we used extracellular flux analyses (Seahorse) to obtain a high temporal resolution metabolic profile of HAEC cultures as a function of time at ALI. Bronchial biopsies were obtained from healthy adult volunteers using an IRB-approved protocol. HAEC cultures at days 0, 7, 14, 21 and 28 of ALI were assayed using the Seahorse mitochondrial stress test, in which the cellular oxygen consumption rate (OCR) was monitored continuously at baseline and following sequential addition of complex V inhibitor oligomycin, the protonophore FCCP, and the complex I and III inhibitors rotenone and antimycin A. The results showed a significant and progressive decrease in baseline ATP production and spare ATP production capacity in HAEC during differentiation days 0-14, stabilizing thereafter, consistent with the notion that HAEC differentiation is accompanied by a decrease in energy utilization. Cinnamaldehyde, an electrophilic phenylpropanoid compound commonly used as a flavorant in foods and electronic cigarettes was previously reported to suppress mitochondrial respiration in HAEC-ALI. Differentiation at ALI may potentiate the inhibitory effect of cinnamaldehyde on HAEC-ALI. These results reveal alterations in the metabolic profile of HAEC as a function of time at ALI, which may be associated with the promotion of metabolic quiescence in differentiating cells. These findings have implications for the assessment of the risk of adverse effects induced by exposure to ambient air pollutants in health and disease. Funded by NIEHS 1R25ES031870-01.