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

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Introduction

- The stage of differentiation of primary human airway epithelial cells (HAEC) represents a potential state of altered vulnerability to the adverse effects of environmental exposures.
- Morphologically and functionally, HAEC induced to differentiate by culturing at an air liquid interface (HAEC-ALI) on a semi-permeable membrane support represent the most relevant *in vitro* model of the human airway.
- The mitochondrial bioenergetic profile of HAEC-ALI during differentiation and its susceptibility to environmental oxidative stress have not been investigated.
- The mitochondrial stress test carried out on the Seahorse extracellular flux analyzer provides a high temporal resolution assessment of mitochondrial respiration.

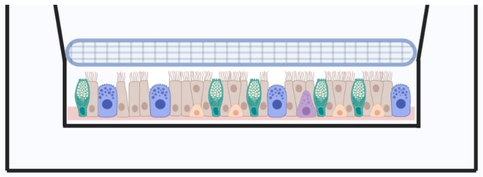

Figure 1. Primary HAEC in Seahorse XF24 Islet Capture Microplate (Agilent Technologies) under a mesh screen.

Methods

- Primary HAEC obtained by bronchial brushing of a healthy adult volunteer following a protocol approved by the Biomedical Institutional Review Board of the University of North Carolina at Chapel Hill, were expanded to passage three in bronchial epithelial growth medium.
- HAEC were cultured on 6.5 mm Corning Transwell (Corning, NY) membranes inserted into 24-well culture plates and ALI culture was initiated 48 hours later by removing the apical medium.
- HAEC-ALI cultures at weeks 0, 1, 2, 3, and 4 of ALI were assayed using the Seahorse XFe24 Analyzer (Agilent Technologies, Santa Clara, CA).
- HAEC-ALI cultures on their membrane supports were detached from the Transwell cup and placed at the bottom of a Seahorse XF24 Islet Capture Microplate (Agilent Technologies) under a mesh screen.
- HAEC-ALI were then analyzed using the Seahorse mitochondrial stress test, in which the cellular oxygen consumption rate (OCR) was monitored continuously at baseline and following exposure to 0-15 mM cinnamaldehyde (CA), with sequential addition of the complex V inhibitor oligomycin, the protonophore FCCP, and the complex I and III inhibitors rotenone and antimycin A.

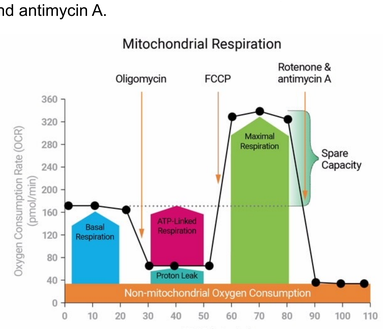

Figure 2. Agilent Seahorse XF Cell Mito Stress Test Profile. Showing the key parameters of mitochondrial function: basal respiration, ATP turnover, proton leak, maximal respiration and spare respiratory capacity.

Objective

Examine the effect of the stage of cellular differentiation on mitochondrial respiration in HAEC-ALI at baseline and during oxidative stress

The most pronounced effects of differentiation can be seen in ATP Production, Proton Leak, and Non-Mitochondrial Respiration

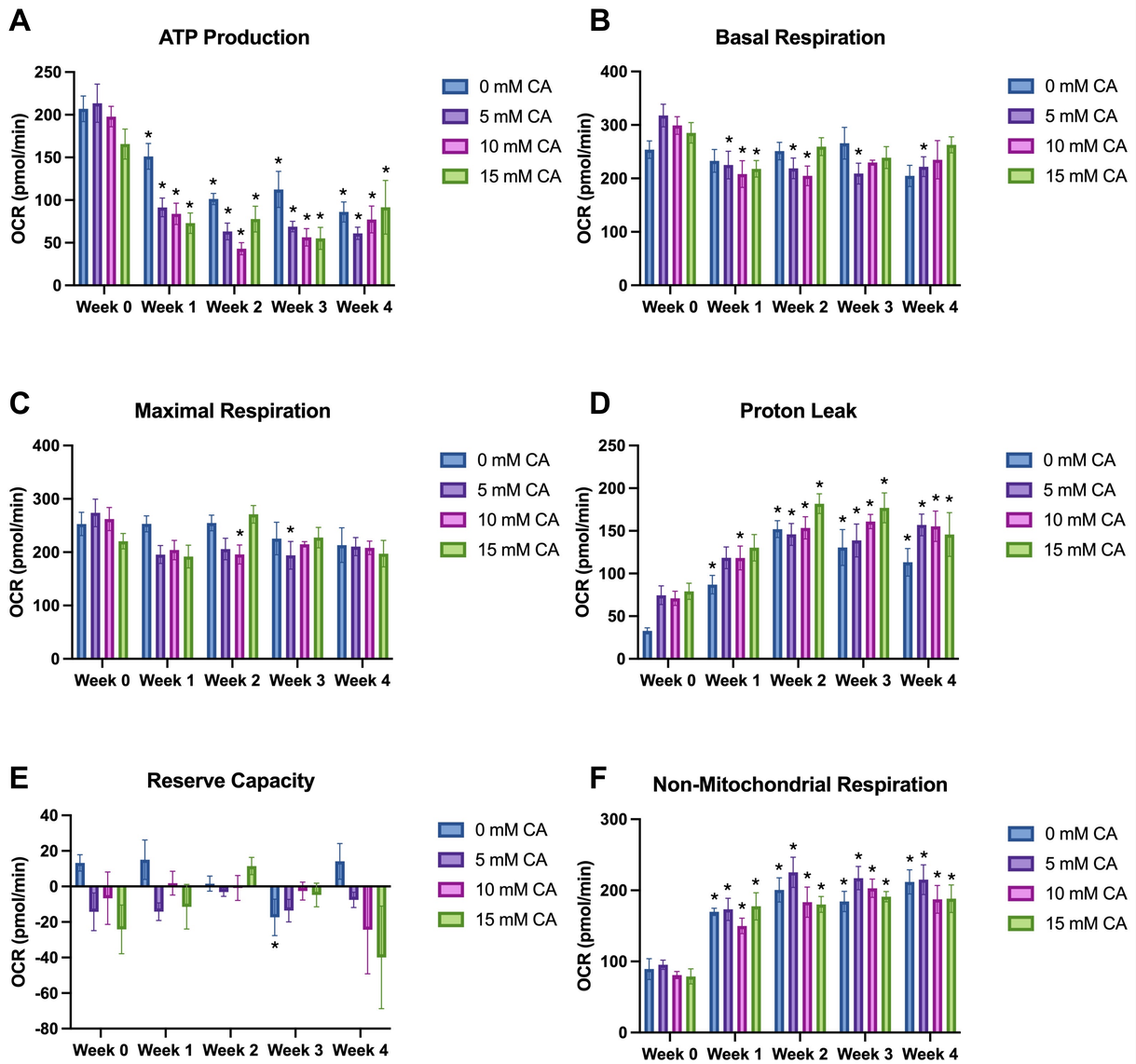

Figure 3. Mito Stress Test Bioenergetic Parameters. HAEC-ALI were exposed to 0, 5, 10, and 15 mM concentrations of cinnamaldehyde (CA) at weeks 0, 1, 2, 3, and 4 of differentiation, and changes in mitochondrial OCR were evaluated using a Seahorse bioanalyzer (n=5-6). ATP production (A), basal respiration (B), maximal respiration (C), proton leak (D), reserve capacity (E), and non-mitochondrial respiration (F). Shown are Mean +/-, SEM, n=3 separate experiments in which each condition was analyzed in duplicate. Significant differences from week 0 at ALI were determined by two-way ANOVA and Dunnett's multiple comparisons test. Significance represented as * P<0.05.

OCR changes as HAEC-ALI at baseline differentiate

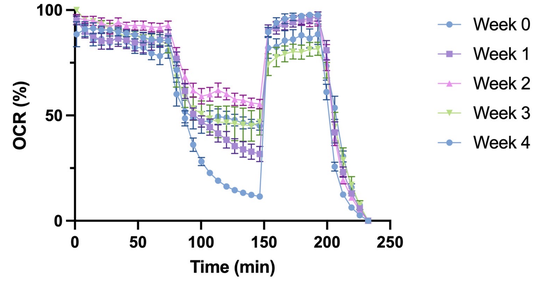

Figure 4. Baseline HAEC-ALI Mitochondrial Stress Test. Changes in mitochondrial OCR in HAEC-ALI at weeks 0, 1, 2, 3, and 4 of differentiation were evaluated using a Seahorse bioanalyzer (n=5-6). OCR values are normalized to baseline respiration.

ATP Production is compromised as HAEC-ALI differentiate

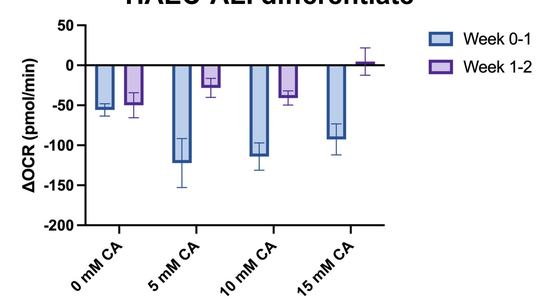

Figure 5. ATP Production in change in OCR from weeks 0-2. ATP Production is compromised as HAEC-ALI differentiate during the first two weeks of differentiation.

Summary & Conclusions

- HAEC-ALI undergoing differentiation demonstrate a significant decrease in baseline mitochondrial respiration that is accompanied by increases in non-mitochondrial respiration and proton leak.
- The effect of electrophilic oxidative stress induced by cinnamaldehyde on mitochondrial respiration was most pronounced on ATP production, potentiating the decrease associated with differentiation.
- Ongoing studies will correlate these findings with markers of differentiation, including the development of cilia, ciliary beat frequency and transepithelial resistance.
- These findings suggest profound changes in HAEC-ALI undergoing differentiation, demonstrating a potential change in susceptibility to environmental exposures.

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