Use of a Novel Live Cell Oxygen Consumption Rate Monitor to Assess Effects of Oxidized Phosphatidylcholine on Mitochondrial Function in Lung Cells

Katarina Kowatsch[1,2], Azadeh Dalvand[1,2], Jignesh Vaghasiya[1,2], Andrew J. Halayko[1,2]

[1]Department of Physiology and Pathophysiology, University of Manitoba, Winnipeg, MB [2]Biology of Breathing Group, Children's Hospital Research Institute of Manitoba, Winnipeg, MB.

INTRODUCTION

- **Asthma:**
  - Most common childhood chronic disease globally and the primary reason for childhood emergency room visits in Canada
  - Many features linked to reactive oxygen species produced endogenously and after environmental exposures
- Mitochondrial dysfunction and oxidative stress are linked with asthma pathophysiology
- Reactive oxygen species can damage airway phospholipids generating proinflammatory oxidized phosphatidylcholines (OxPCs), which are also elevated in patients with asthma
- Previous studies: **mitochondrial metabolism compromised by OxPCs dose-dependently**
- **RESIPHER:** novel oxygen consumption rate (**OCR**) reader for continual live monitoring of cells in culture

AIM

- With the RESIPHER, characterize baseline metabolism of five primary and matched human telomerase reverse transcriptase (hTERT) human airway smooth muscle (HASM) from asthmatic donors using sustained real-time measurement of OCR
- Use the RESIPHER to measure OCR changes in a human lung epithelial carcinoma cell line after exposure to varying concentrations of OxPCs

METHODS

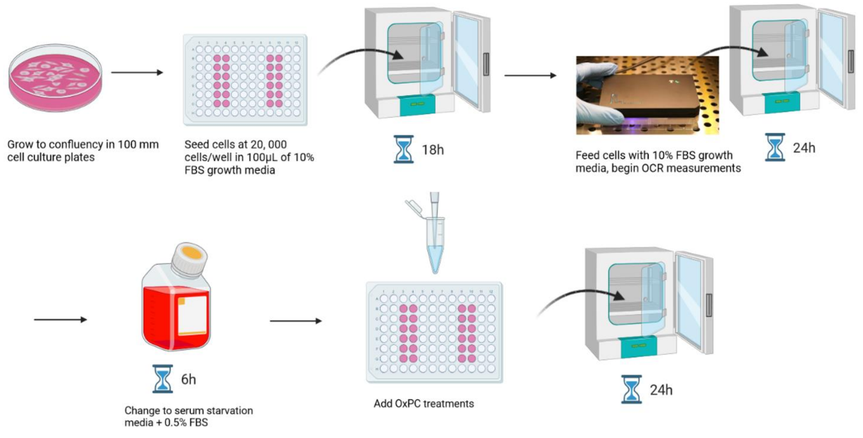

Figure 1. Protocol used to characterize baseline metabolism and responses to OxPC exposure. FBS: fetal bovine serum. Created in Biorender.com. RESIPHER image from Lucid Scientific (2021).

RESULTS

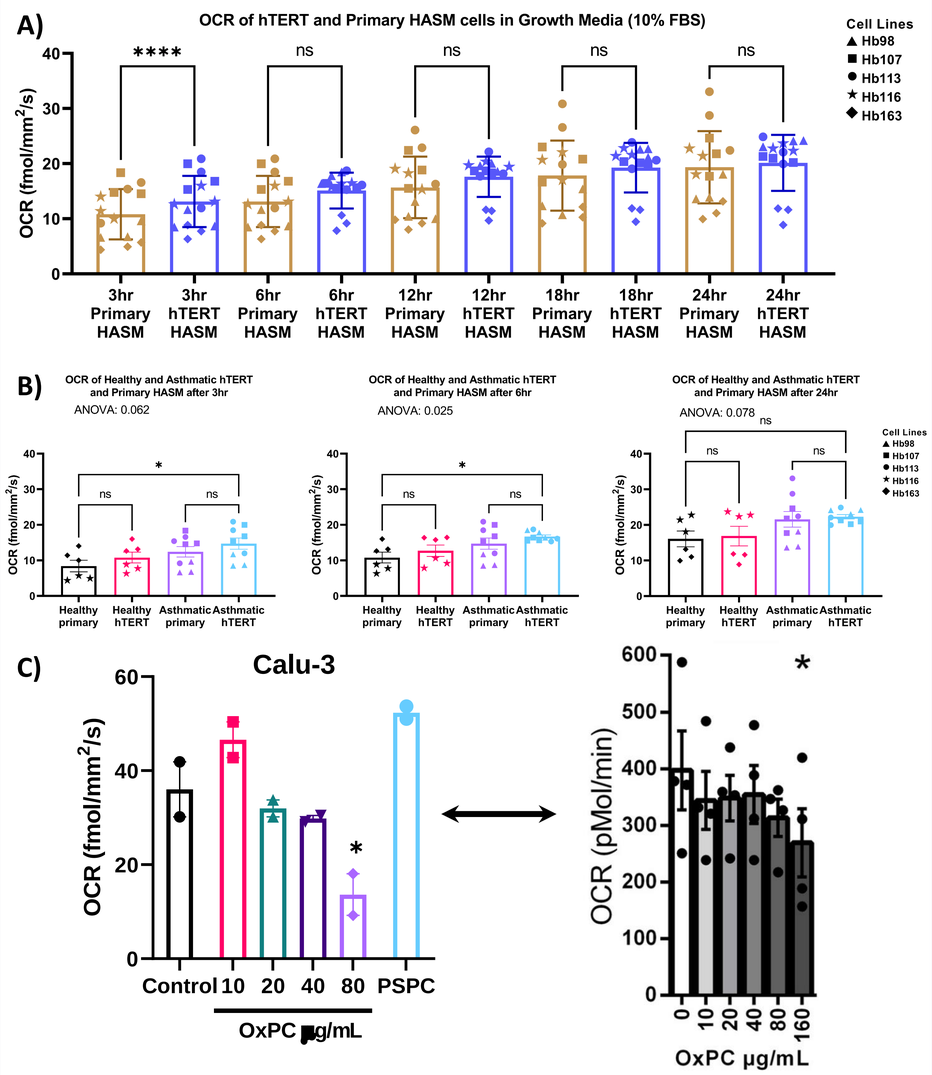

Figure 2. A) OCR of matched primary human airway smooth muscle (HASM) and hTERT HASM in 10% FBS media (24 hours). **B)** Comparison of OCR of healthy donor matched primary and hTERT HASM (n=2) and asthmatic donor matched primary and hTERT HASM (n=3) in 10% FBS media (24 hours). **C)** OCR of Calu-3 human epithelial lung carcinoma cell line following 24 hours exposure to OxPCs (0, 10, 20, 40, 80 µg/mL) or non-oxidizable phosphocholine (PSPC 80 µg/mL), comparison of current results from RESIPHER (left) with published data using Seahorse XF Analyzer (right). (Pascoe et al. (Am J Physiol: Lung, 2021). OCR values analyzed by one-way ANOVA with Tukey correction for multiple comparisons.

RESULTS SUMMARY

- No significant difference in OCR for primary versus hTERT-HASM, nor healthy versus asthmatic-HASM from 6-to-24 hours in growth media
  - ***Initial period of increased hTERT-HASM OCR was evident at 3-hours (p<0.001)***
- Calu-3: dose-dependent decrease in OCR in response to OxPC (20µg/mL: p<0.05, 80µg/mL: p<0.001) compared to cells in OxPC-deficient medium. PSPC treatment had no effect on OCR.

CONCLUSION

- OCR under growth conditions was similar for primary and hTERT HASM cells from healthy and asthmatic donors
- OCR is decreased by OxPC exposure in human airway epithelial cells, confirming an effect of OxPCs on mitochondrial metabolic function

SIGNIFICANCE

- The RESIPHER live cell OCR monitor is a sensitive and cost-effective tool to assess mitochondrial biology in cultured cells

REFERENCES

Pascoe, C. D., Roy, N., Turner-Brannen, E., Schultz, A., Vaghasiya, J., Ravandi, A., Halayko, A. J., & West, A. R. (2021). Oxidized phosphatidylcholines induce multiple functional defects in airway epithelial cells. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *321*(4), L703–L717. https://doi.org/10.1152/ajplung.00539.2020

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