



Menthol pod-based e-cigarettes induces mitochondrial dysfunction in lung epithelial cells



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INTRODUCTION

- E-cigarette use is on the rise in western populations resulting in a ban, by the FDA, on flavored e-cigarette pods except for menthol and tobacco
- Certain states, like New York, have also placed regulation on Menthol flavored e-cigarettes
- JUUL Labs now only sells Menthol, Classic Tobacco, and Virginia Tobacco pod flavors
- JUUL Pods are composed of PG/VG, nicotine, benzoic acid, and flavors
- E-cigarettes and flavoring chemicals have been shown to induce mitophagy and mitochondrial dysfunction

HYPOTHESIS

Exposure to pod-based menthol and tobacco flavored aerosols will result in alterations in mitochondrial bioenergetics and electron transport chain (ETC) protein levels

METHODS

- Beas2b were grown in DMEM:F12 complete media with 5% FBS, 15mM HEPES, and 1% pen/strep
- Cells were serum deprived to 0% FBS and exposed to 66 puffs of JUUL Menthol 5% nicotine or JUUL Virginia Tobacco 5% nicotine vapors or air
- 20,000 cells were plated in 6 wells with 2 blank wells in a seahorse cell culture miniplate and exposed, immediately or 24 hours post final exposure Cell Mito Stress Test was performed using Seahorse XFP
- 300,000 cells were plated in each well of a 6 well plate and exposed, immediately or 24 hours post final exposure Western Blot was performed using cell protein and probed using total OXPHOS antibody

RESULTS

Figure 1: Mitochondrial Bioenergetics are Altered Immediately After JUUL Menthol Exposure

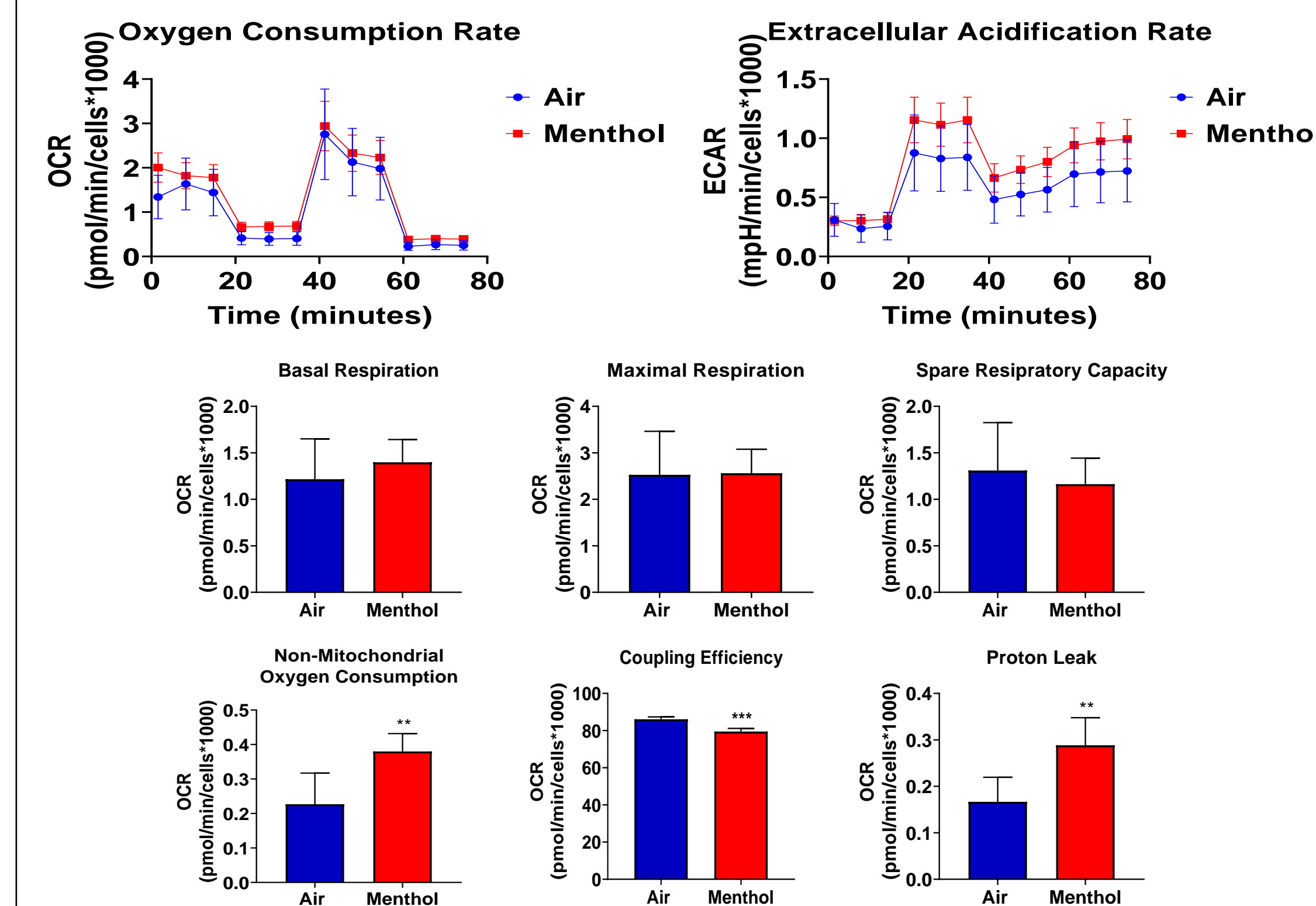


Figure 2: Mitochondrial Respiration is Altered at 24 Hours After JUUL Menthol Exposure

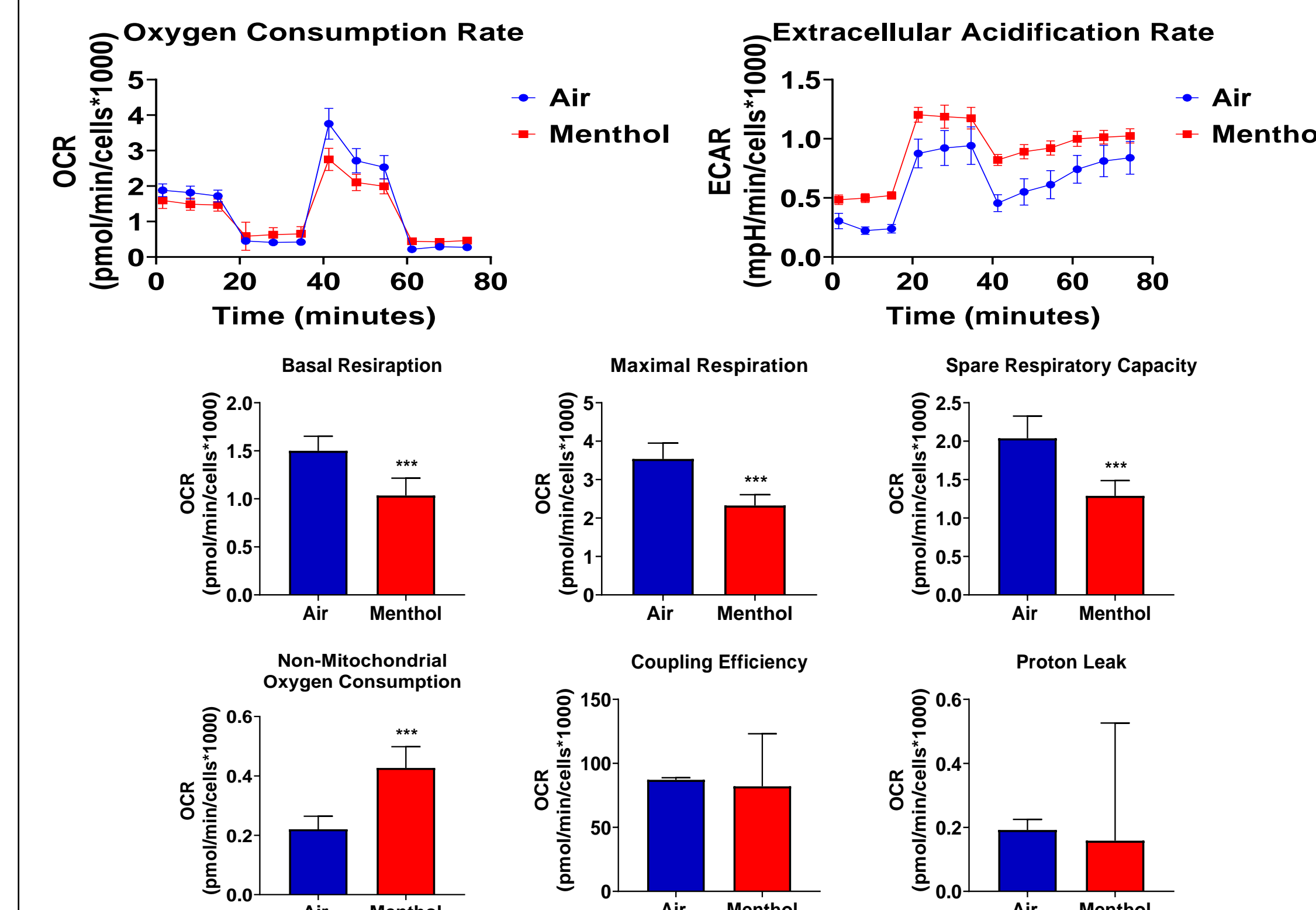


Figure 3: ETC Proteins are Altered at Both Time Points After JUUL Menthol Exposure

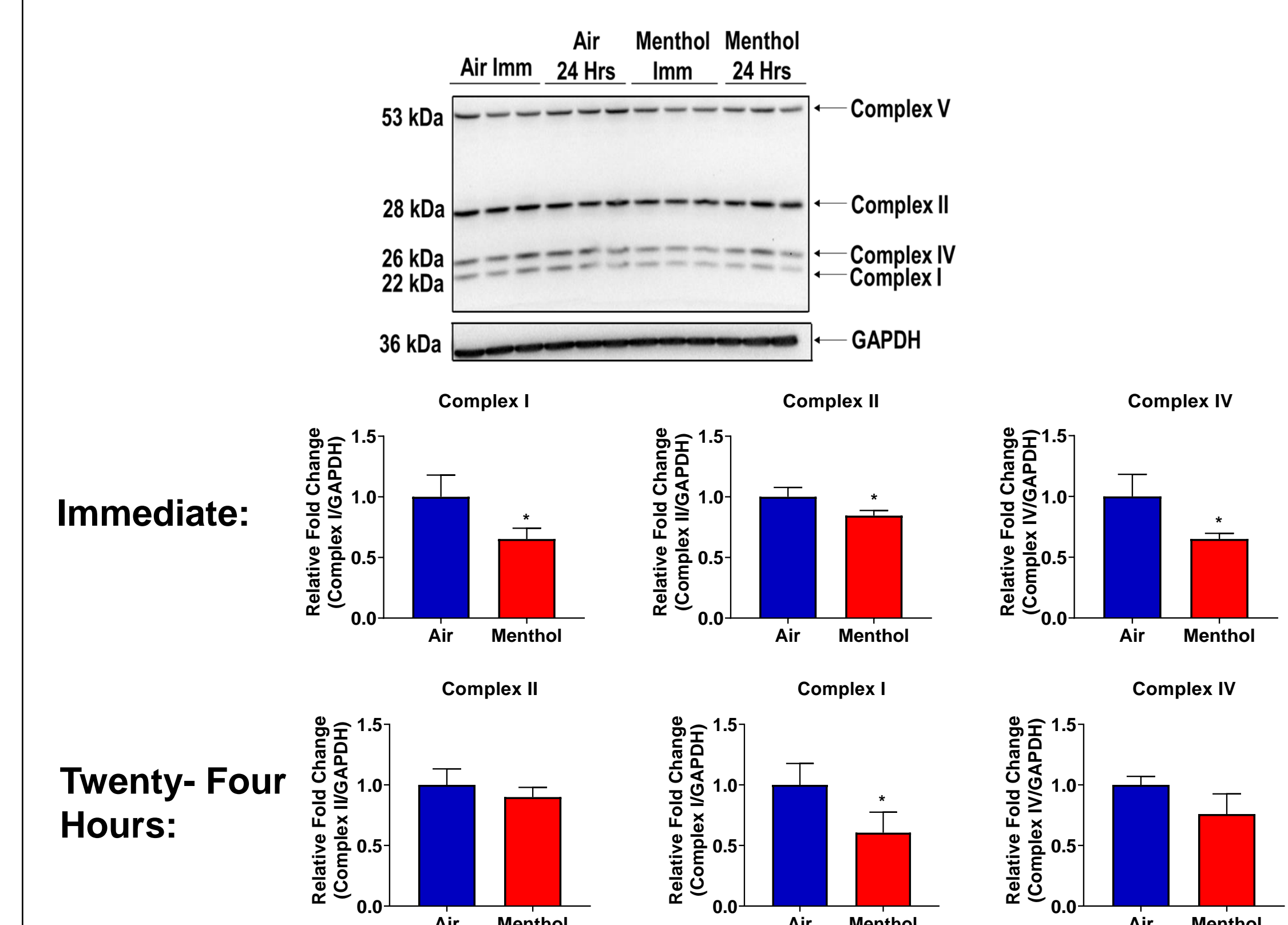


Figure 4: Mitochondrial Respiration isn't Altered Immediately After JUUL Virginia Tobacco Exposure

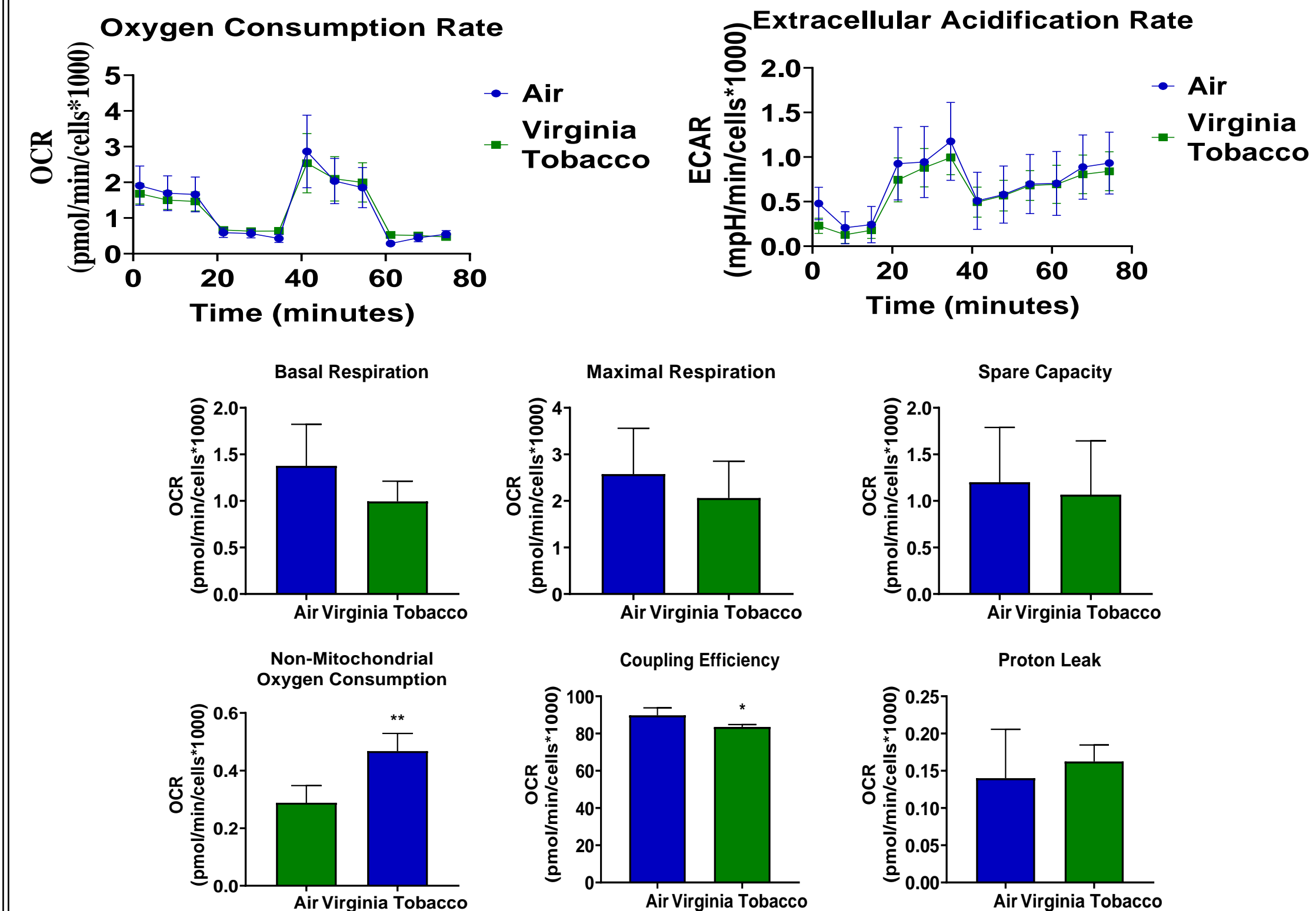


Figure 5: Mitochondrial Respiration isn't Altered 24 Hours After JUUL Virginia Tobacco Exposure

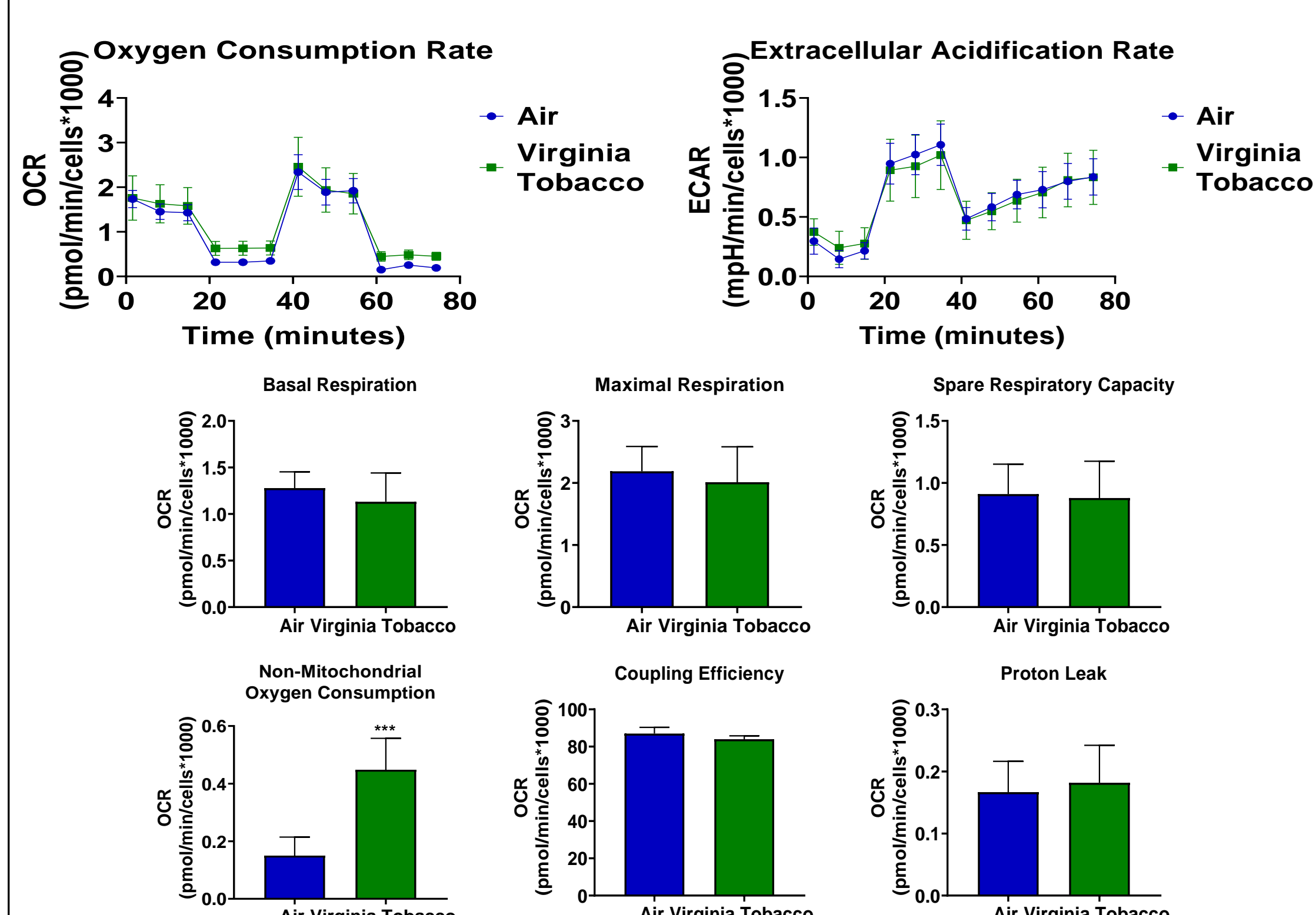
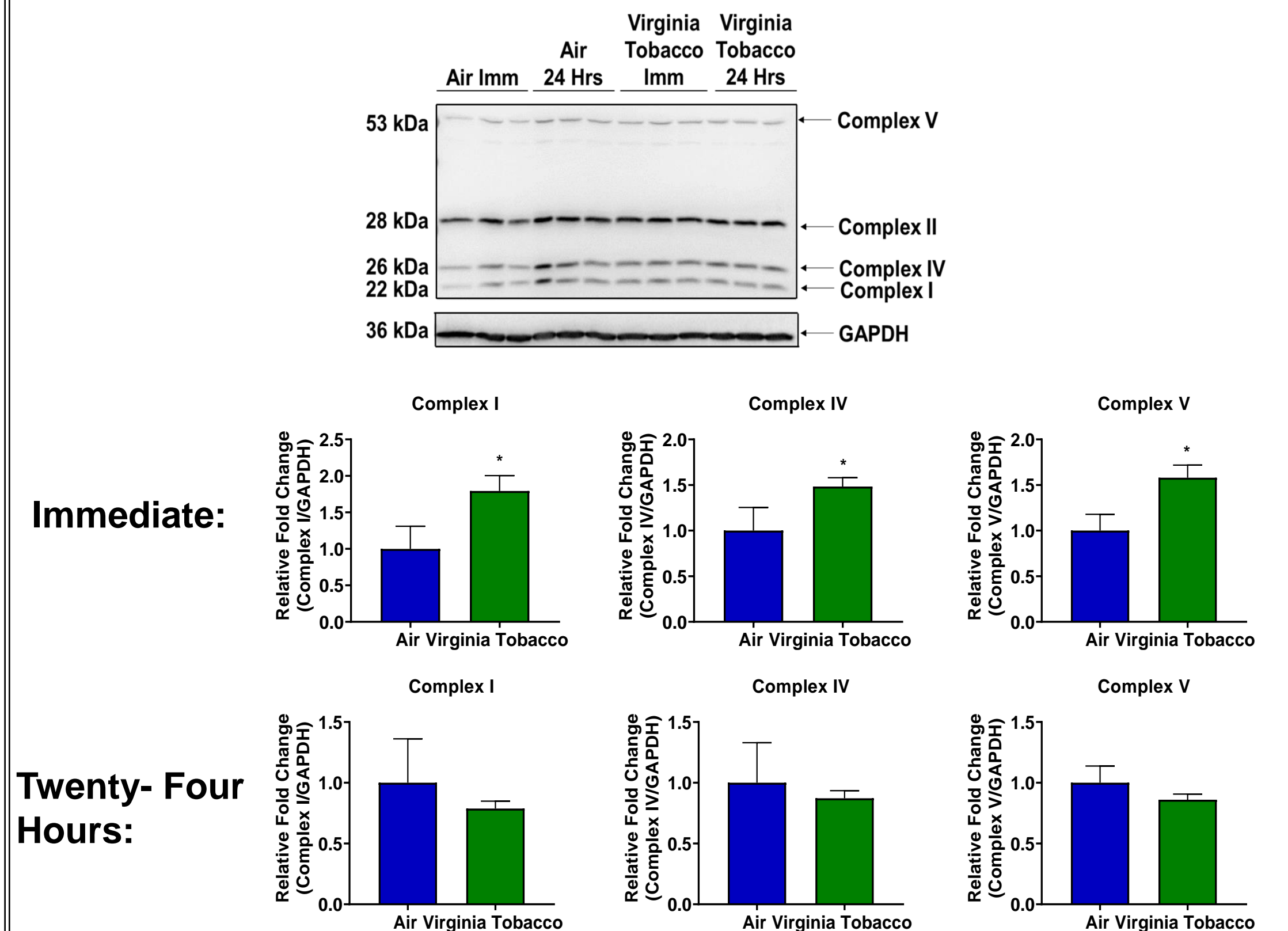


Figure 6: ETC Proteins are Altered at Immediately After JUUL Virginia Tobacco Exposure



SUMMARY

- Immediately after final JUUL Menthol exposure resulted in a significant increase in non-mitochondrial oxygen consumption and proton leak, while significantly decreasing coupling efficiency
- 24 hours after final JUUL Menthol exposure resulted in a significant increase in non-mitochondrial respiration while significantly decreasing basal respiration, maximal respiration, and spare respiratory capacity
- JUUL Menthol exposure decreased electron transport chain proteins
- JUUL Virginia Tobacco exposure did result in a significant increase in non-mitochondrial oxygen consumption at both time points but did not alter mitochondrial respiration at either time points
- JUUL Virginia Tobacco exposure resulted in a significant increase in certain ETC proteins at the immediate

CONCLUSION

- Non-mitochondrial oxygen consumption is increased in JUUL Menthol and JUUL Virginia Tobacco exposed cells
- JUUL Menthol exposure may induce a shift towards glycolysis due to an increase in extracellular acidification rate, a measure of glycolysis in the cell mito stress test
- JUUL Menthol and not JUUL Virginia Tobacco exposure results in decrease in mitochondrial respiration with a decrease in basal respiration at the twenty-four hour time point and a decrease in complex I, II, and IV at the immediate time point and complex I at the twenty-four hour time points

ACKNOWLEDGEMENTS

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