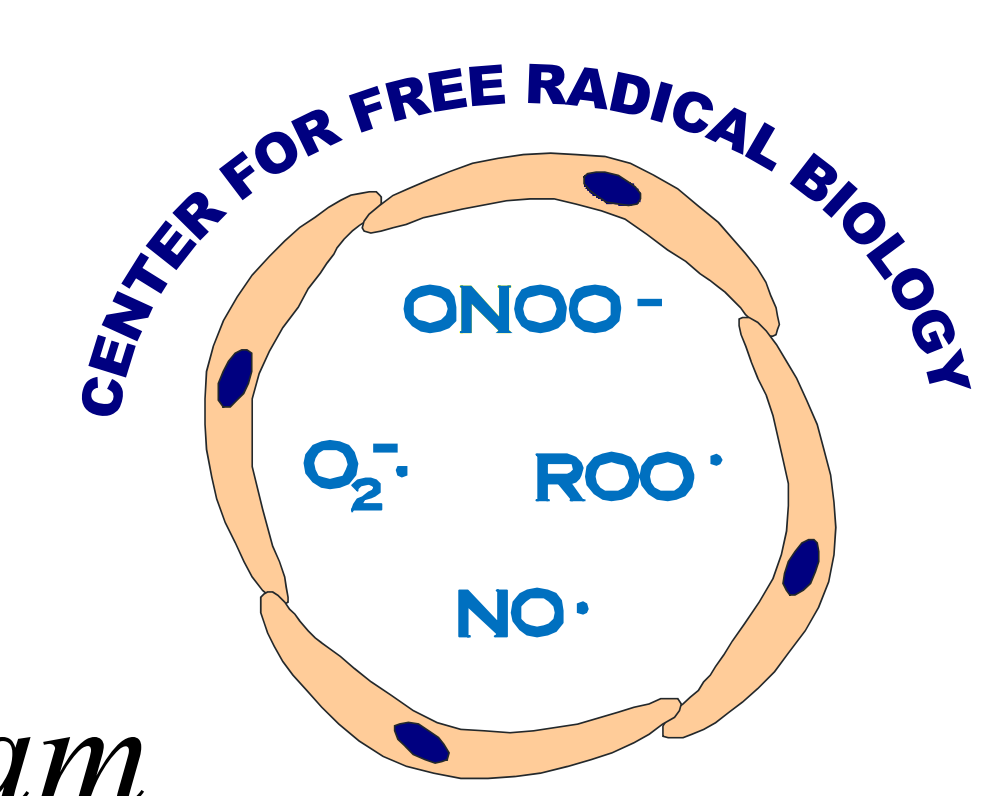




Bioenergetics changes induced by hydrogen peroxide

exposure in endothelial cells

Gloria A. Benavides¹, Brian P. Dranka¹, Brian Benoit², and Victor M. Darley-Usmar¹
¹Center for Free Radical Biology, Department of Pathology, University of Alabama at Birmingham
and ²Seahorse Bioscience.

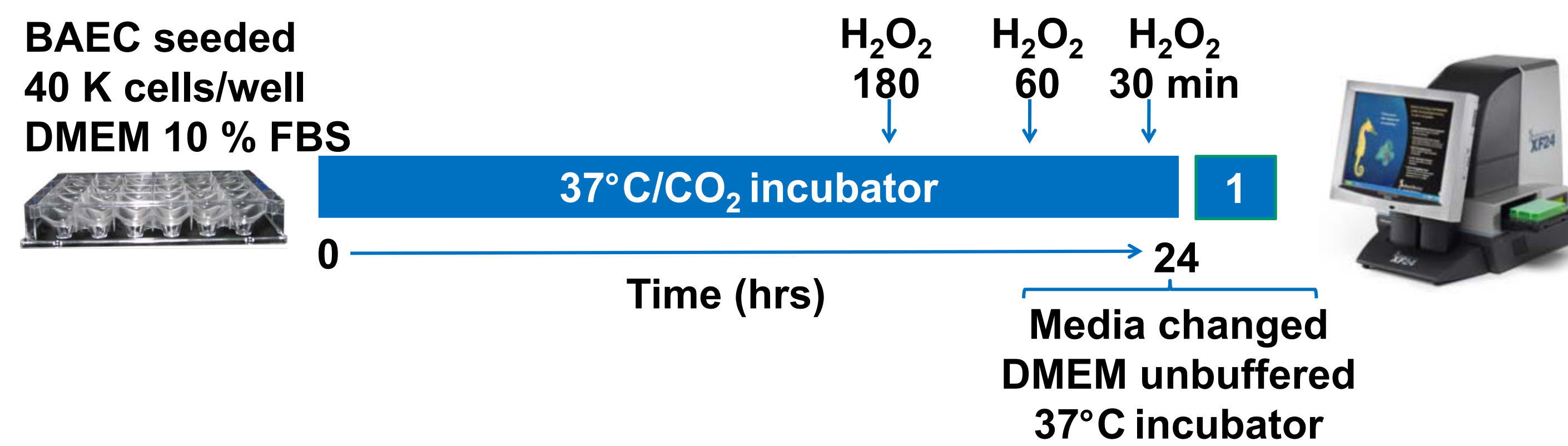


Introduction

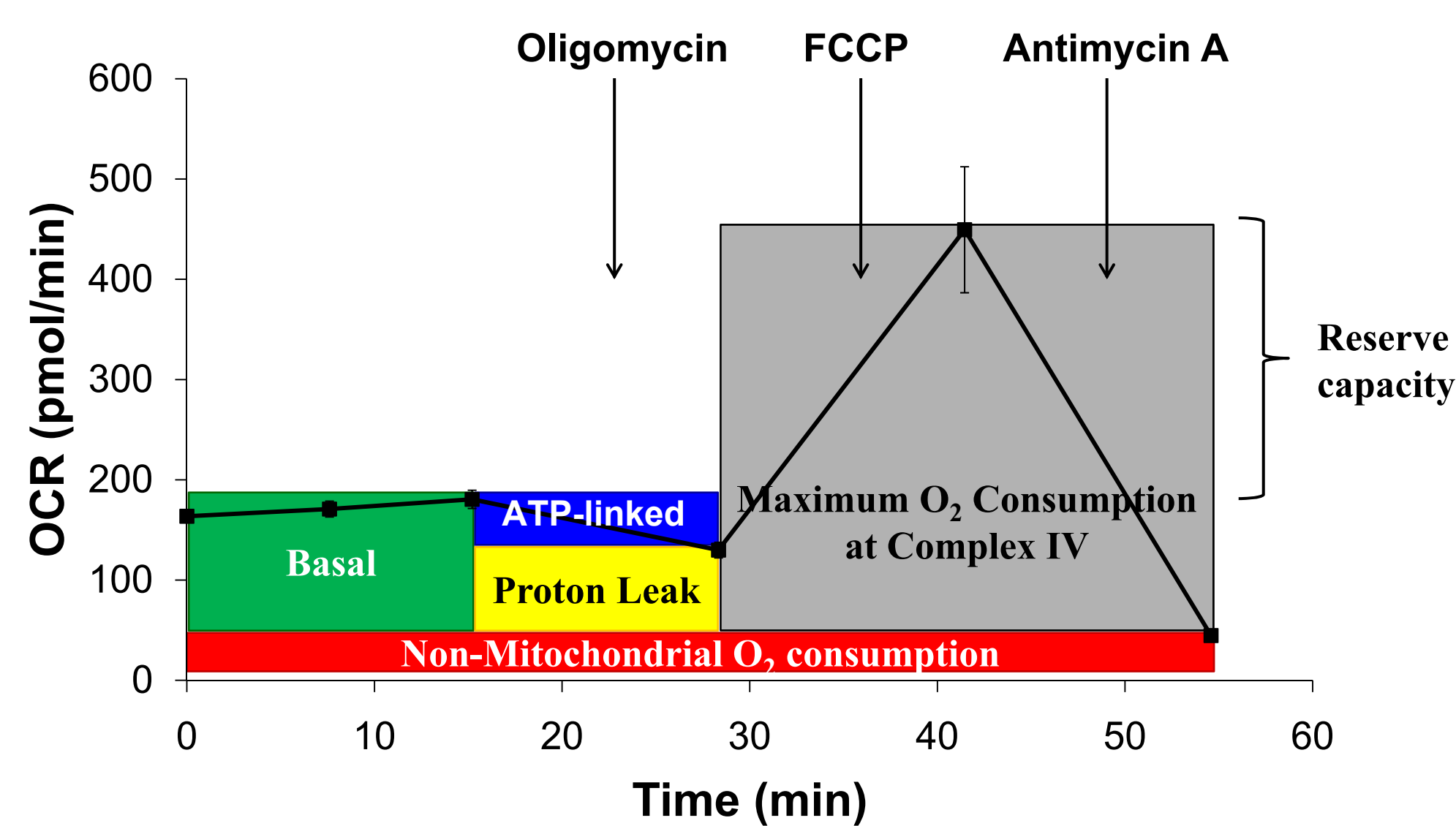
- Increased reactive oxygen species (ROS) generation underlies many pathologies of the cardiovascular system including ischemia/reperfusion injury, atherosclerosis, and the peripheral vascular complications of diabetes.
- While mitochondrial bioenergetic pathways have been reported to be compromised in these pathologies, little is known about how mitochondria function in response to oxidative stress in intact endothelial cells.
- In the present study, we determined the effects of bolus doses of hydrogen peroxide (H₂O₂) and intracellular H₂O₂ production by 2,3-dimethoxy-1,4-napthoquinone (DMNQ) on mitochondrial respiration of adherent cells using a Seahorse Bioscience XF24 Extracellular Flux Analyzer, as well as the effects on a kinase important in controlling cellular energy metabolism (AMPK).

Methods

Standard Protocol



Measurement of Mitochondrial Function by the extracellular flux analyzer



- Protein concentration was determined using the Bradford assay.
- Viability was evaluated using the MTT assay.
- SDS/PAGE and Western blot. Lysates were obtained by adding 10 μL of Laemli Buffer into each well. Two replicate wells were combined to run samples on SDS-PAGE. After transfer to nitrocellulose membranes, these were probed using a phospho-specific and total antibodies against AMPK.

Results

Bioenergetic profile of BAEC treated with H₂O₂ (Dose-response)

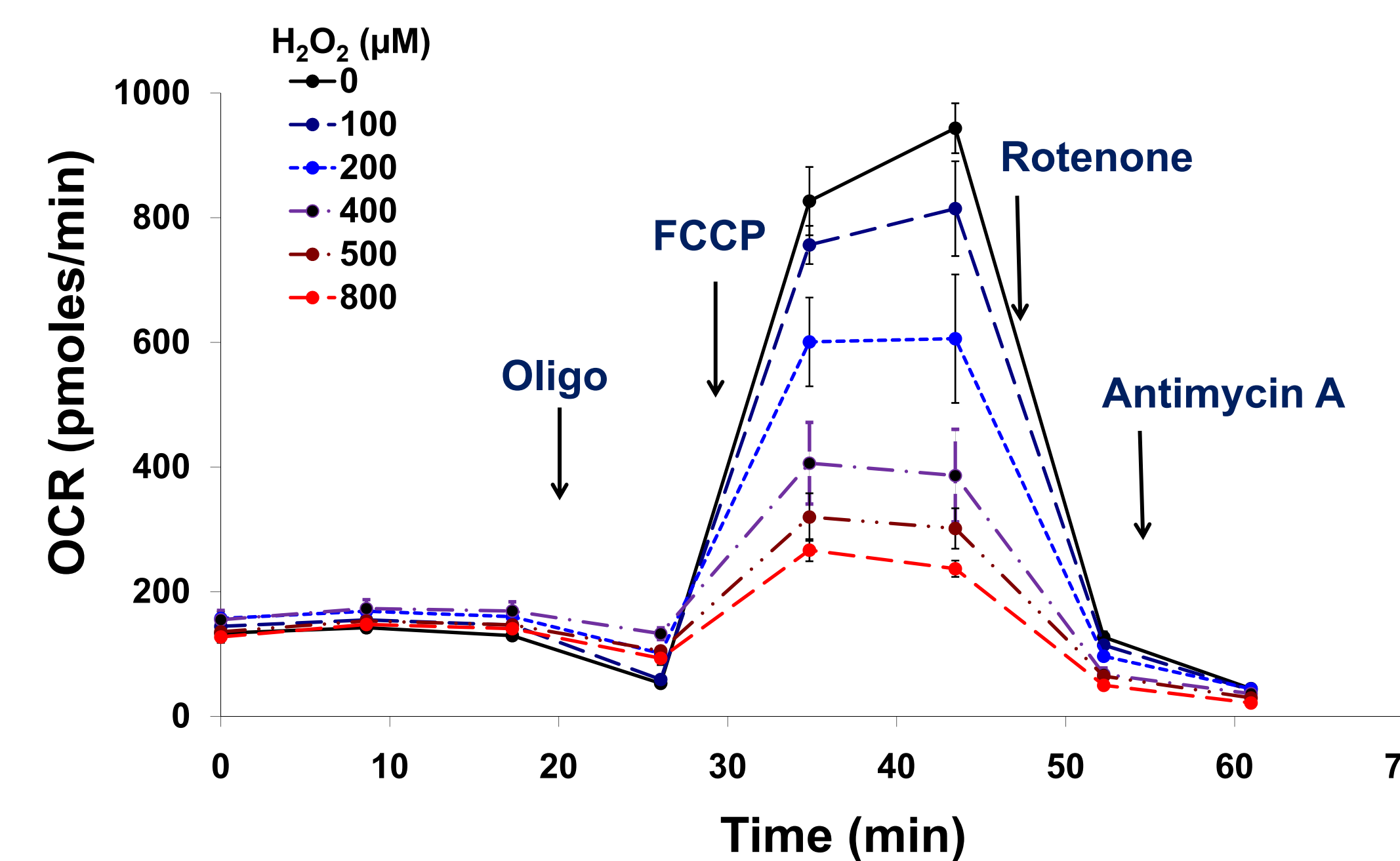


Fig. 1. Oxygen consumption rate (OCR) of BAEC treated with inhibitors of mitochondrial respiration. BAEC were pretreated with H₂O₂ (0 to 800 μM) for 30 minutes previous to XF24 Analyzer. Results are mean ± SEM, n≥3 per group.

Cell viability

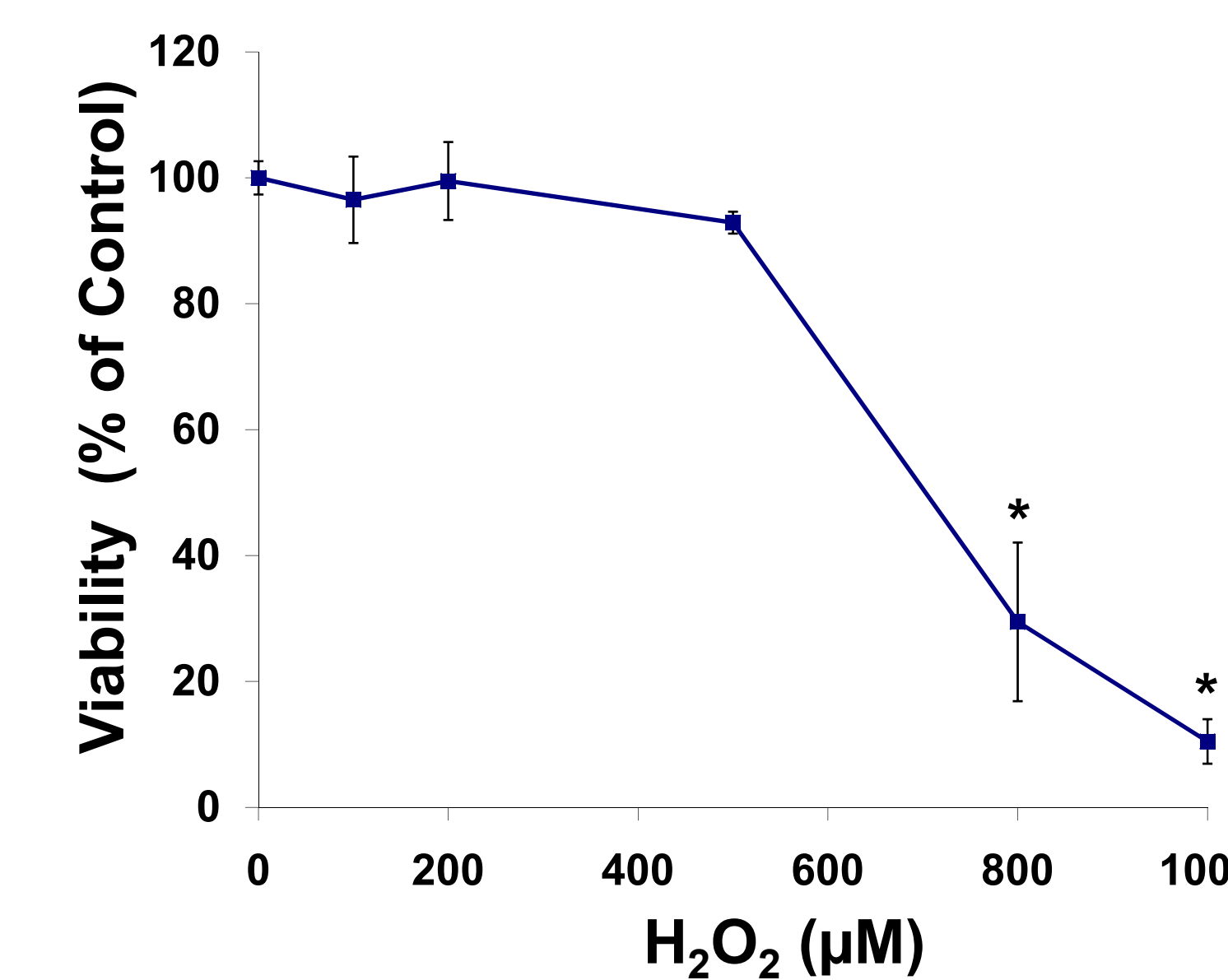


Fig 2. Viability of BAEC treated with H₂O₂. Cells were treated with indicated concentrations of H₂O₂ for 3 hrs. Viability was determined using MTT assay. Results are mean ± SEM, n≥3 per group, *, p < 0.001.

Effects of DMNQ on Mitochondrial Function

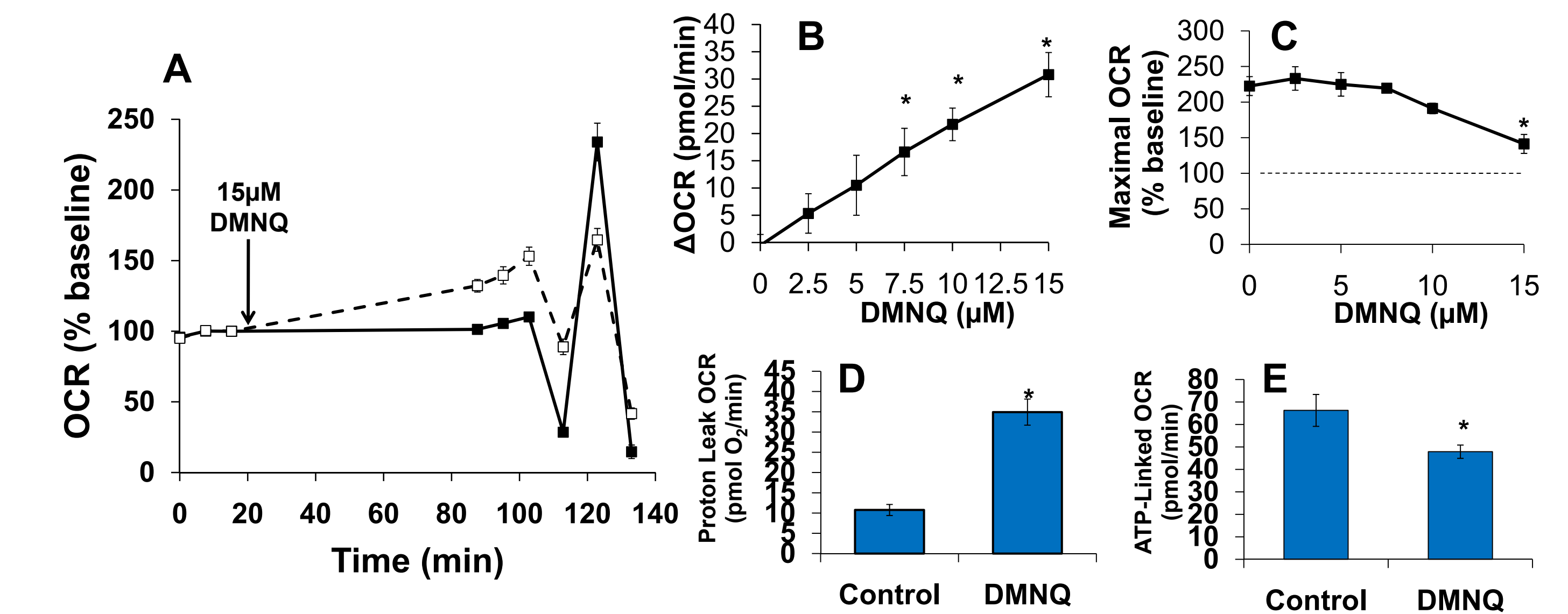


Fig. 4. OCR of BAEC treated with DMNQ. A. Representative trace of OCR of BAEC injected at the time shown with 15 μM DMNQ for 1 h. B. DMNQ increases Basal OCR in a dose-response manner. C. Decrease in maximal OCR by DMNQ. D. Proton leak increased and E. ATP-Linked decreased after 15 μM DMNQ. Results are mean ± SEM, n≥3 per group, *, p ≤ 0.05.

Effects of bolus doses of H₂O₂ on Mitochondrial Function

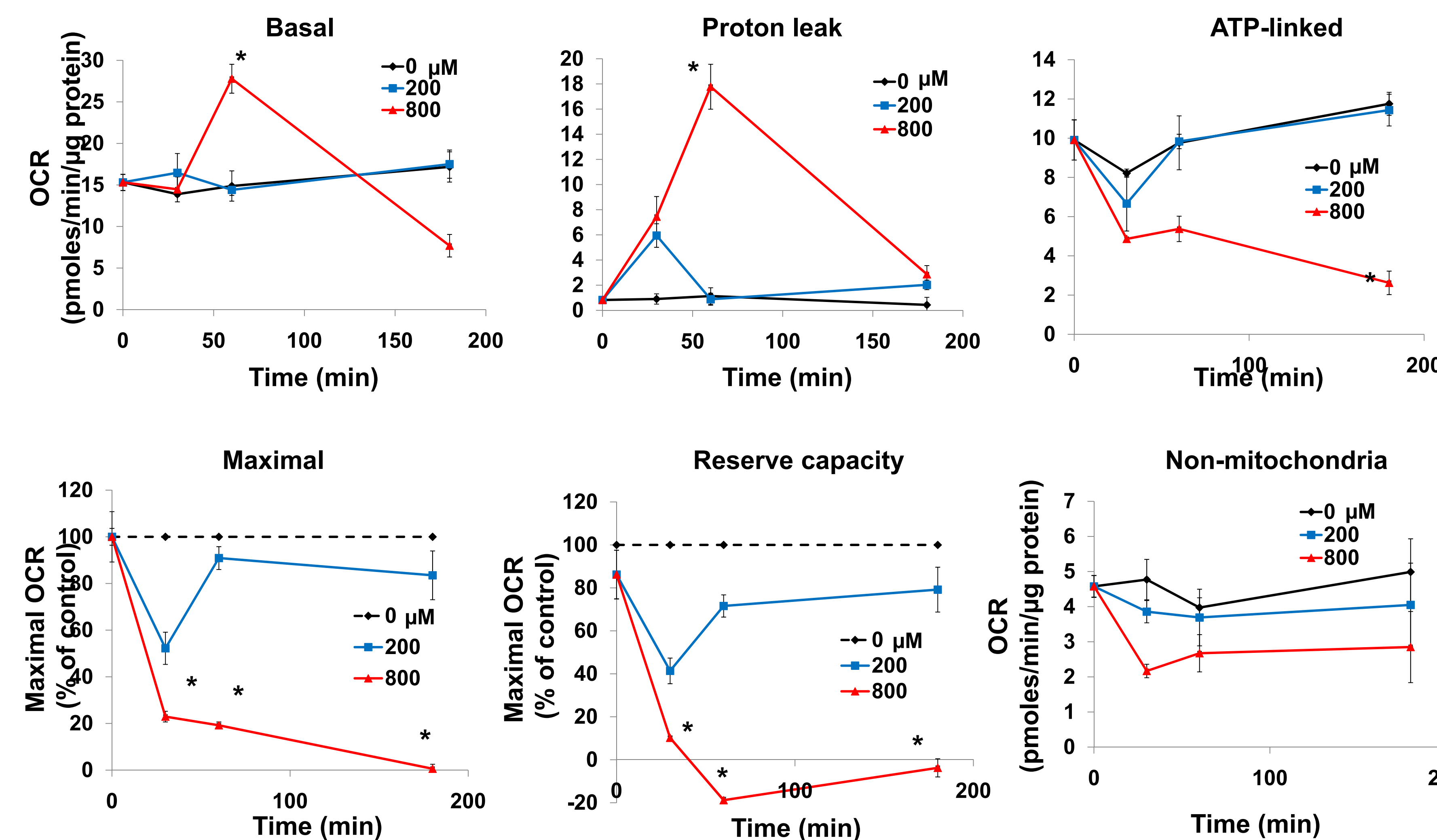


Fig. 3. Mitochondrial function analysis of BAEC treated with H₂O₂ (0, 200, and 800 μM) for 0, 30, 60, and 180 min. Six parameters of mitochondrial function as describe in figure 1 were calculated. Results are mean ± SEM, n≥3 per group, *, p ≤ 0.05.

Phosphorylation of AMPK by H₂O₂

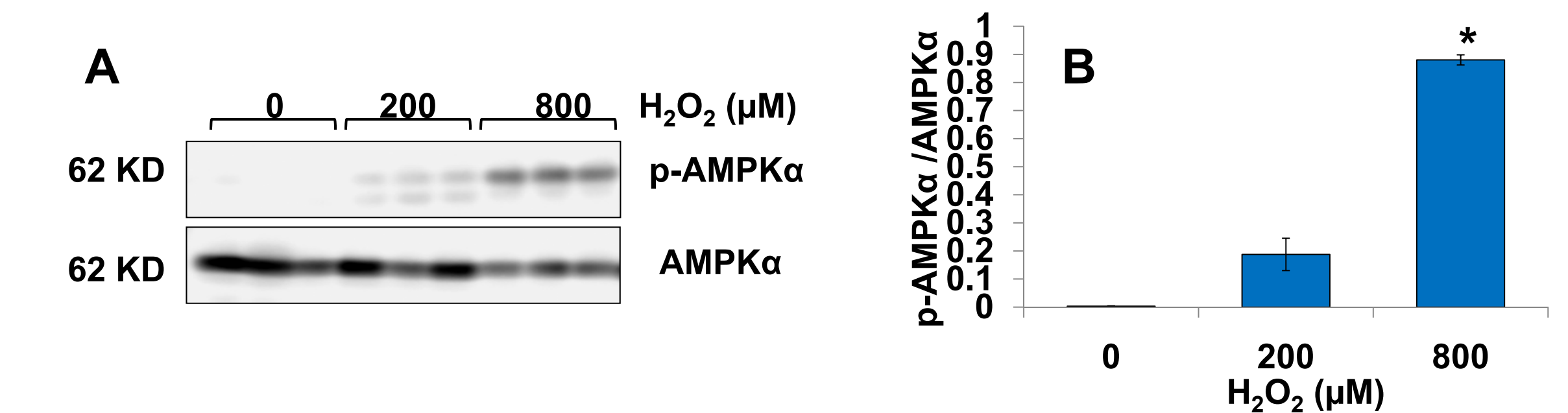


Fig. 5 Effect of H₂O₂ on AMPK Phosphorylation. BAEC seeded at 40 K cells/well were treated with H₂O₂ at 200 and 800 μM for 3 h. A. Cells were harvested and probed for AMPK. B. Blots were quantified as a function of total AMPK. Data shown are means± SEM, n=3 per group, *, p < 0.001.

Summary

- In bovine aortic endothelial cells (BAEC), we found that up to 500 μM H₂O₂ was not cytotoxic, however AMPKα was activated in a dose dependent manner and mitochondrial respiration was altered.
- The maximal and reserve capacities were decreased in a dose dependent manner following H₂O₂ treatment, indicating a loss in maximal respiratory capacity at Complex IV.
- Taken together these results demonstrate a decrease in the bioenergetics of intact cells by the oxidant, hydrogen peroxide which we hypothesize involves activation of the AMP kinase pathway.

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