Respiration without oxygen - reactions driving CO2 emission in anoxia

H. Vellama[I]

[I]Department of Physiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia, Tartu, Estonia

While measuring oxygen (O) consumption to estimate mitochondrial electron transport chain (ETC) activity is well established with standardized protocols, the evaluation of citric acid cycle (CAC) activity by monitoring CO has received comparatively less attention. Although tightly interconnected, the ETC and CAC also fulfill separate functions; therefore, monitoring both is essential for understanding how these pathways are coupled under various pathological conditions. To address this, we developed a method for simultaneously measuring O consumption and carbon dioxide (CO) production in adherent cell lines. As CO production primarily reflects CAC flux, this approach allows for a dynamic estimate of its activity, enabling real-time assessment of redox coupling between the ETC and CAC. Using this method, we observed persistent CO production in HKC8 and HepG2 cells under anoxic conditions, suggesting residual CAC activity independent of oxygen availability. Using specific substrates and inhibitors, we further explored the reactions contributing to anoxic CO production and their role in sustaining cellular metabolism in the absence of oxygen. Finally, employing 13C-labeled glucose and glutamine, we demonstrate real-time flux changes from these carbon sources to CO , highlighting their contributions to CAC activity under dynamic conditions.