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Opinion Article

OPINION ARTICLE

Revisiting Mitochondrial Bioenergetics: Experimental Considerations for Biological Interpretation

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Mitochondria exist within almost every cell of the human body, and the function of these small organelles influences diverse processes, including metabolic homeostasis, apoptosis, endoplasmic reticulum stress, and redox balance. Mitochondria are required for aerobic ATP production and have an immense capacity to adapt to cellular stress. As a result, these organelles have direct implications in diverse physiological situations across the health spectrum. Indeed, increases in mitochondrial content represent the cornerstone of training adaptations in skeletal muscle,[1] while maladaptive mitochondrial responses have been directly linked to the development of insulin resistance, aging, cancer, and various neurological diseases. Despite the necessity of unraveling the regulation of mitochondrial bioenergetics, until recently methodological limitations have hindered our progress in understanding mitochondrial biology.

Mitochondrial function is directly influenced by the provision of substrates (fatty acids and carbohydrates), a process that is highly regulated by blood flow/delivery, membrane transport, and intracellular metabolism/enzymatic flux. Historical assessments of mitochondrial function have been focused on determining the maximal capacity of the oxidative phosphorylation system (saturating/nonlimiting substrates) in isolated mitochondria, which removes regulation from other intracellular processes. The experiments conducted by Chance and Williams in 1955[2] represent a major advancement in our understanding of biochemical properties of mitochondria, providing the ability to directly examine the redox state of mitochondria, mitochondrial leak respiration, coupling efficiency (P/O ratios), and maximal oxidative phosphorylation. The biological importance of oxidative capacity was solidified by Holloszy's landmark findings delineating that increases in mitochondrial content and function are characteristic of exercise training adaptations,[1] and that mitochondrial respiratory capacity directly correlates with maximal aerobic capacity, an observation with links to exercise performance and all-cause mortality. While the importance of these observations cannot be understated, the experimental approach to studying mitochondria has remained relatively unaltered in the past 70 years, limiting our understanding of these dynamic organelles.

While molecular approaches to increase/decrease mitochondrial content have strengthened the relationship between this organelle and cellular homeostasis, mitochondrial oxidative capacity and maximal reactive oxygen species (ROS) production are not altered in several "pathological" conditions, including Type 2 diabetes and aging. These data suggest possible external regulation exists that is not reflected with historical methodological approaches examining maximal mitochondrial capacity. In this respect, it has become apparent in recent years that submaximal mitochondrial respiration may be more reflective of biology, a parameter also directly affected by mitochondrial content. As ADP is a key regulator of oxidative phosphorylation and ROS production, and concentrations of free ADP can rapidly change in response to cellular stresses such as exercise, determining mitochondrial ADP sensitivity has particular biological relevance. This thought process of performing sequential ADP titrations to form a Michaelis–Menten kinetic curve of mitochondrial substrate sensitivity arose as early as 1955[2]; however, many methodological challenges hindered early biological interpretations. For instance, in isolated liver mitochondria, Chance and Williams[2] determined an apparent ADP K_m (concentration of ADP required to half-maximally drive respiration) of 30 μM,[2] and similar reports were later evident in human skeletal muscle isolated mitochondria. These findings were perplexing as the concentration of free ADP in resting skeletal muscle is ∼20 μM, suggesting that mitochondrial respiration at rest would be ∼50% of maximal capacity. In vivo, respiration rates at rest are only 1%–2% of maximal capacity, representing a situation of low oxygen consumption; therefore, these original

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estimates of mitochondrial ADP sensitivity did not display biological relevance and overestimated respiration nearly 50-fold. Clearly, methodological challenges existed that limited biological understanding of submaximal respiratory kinetics, and further experimental considerations were warranted.

The development of the permeabilized muscle fiber technique in the late 1980s[3] was a powerful step to rectify these discrepancies. However, reports in permeabilized fibers from human skeletal muscle indicated an apparent K_m of ~120–150 μM ADP,[4] and while orders of magnitude higher than that of isolated mitochondria, still appeared to overestimate mitochondrial ADP affinity and would predict ~10-fold higher respiration rates than in vivo estimates. In 2011, it was identified that this was a direct consequence of spontaneous, temperature-dependent muscle contraction of permeabilized fibers during experimental protocols that could be prevented with a myosin II-ATPase inhibitor.[5] This finding had profound implications for the development of experimental protocols to understand the true interactive nature of mitochondrial bioenergetics in response to various physiological concentrations of substrates. As a result, there has been a paradigm shift in the past 5 years with the understanding that it is important to utilize submaximal "environments" for interrogating the relationship between mitochondrial bioenergetics and cellular homeostasis.

Since this finding, submaximal ADP-supported mitochondrial respiration and/or ROS emission have been reported to be altered following diverse physiological situations, including, but not limited to, acute exercise, chronic exercise training, single-leg immobilization, blood flow restriction, aging, the development of HFD-induced insulin resistance, and between males and females.[6–8] Moreover, more contemporary approaches in isolated mitochondria have been developed to assess mitochondrial bioenergetics, ROS emission, energy transfer, and enzymatic flux across a range of substrates and ATP-free energies, rather than excessive ADP.[9] Similarly, using submaximal substrate concentrations, including ketones, pyruvate, glutamate, and the CPT-I specific substrates/inhibitors palmitoyl-CoA, L-carnitine, and malonyl-CoA,[7,10] has revealed a complex interaction in biology that is dependent on the intracellular environment mitochondria are exposed to. These findings have clear implications for numerous avenues of future research in mitochondrial biology.

Overall, while literature historically examined maximal mitochondrial capacity as an indication of mitochondrial function, it is now apparent that additional levels of regulation exist. The development and progression of the isolated mitochondria and permeabilized muscle fiber techniques allow for interpretation of biological responses to submaximal and physiological concentrations of substrates within mitochondria. This conceptual shift in experimental design will undoubtedly be applied to various situations across the health spectrum in the future, improving our understanding of the true interactive nature of mitochondrial bioenergetics and biology.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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