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EDITORIAL

Revisiting mitochondrial dysfunction in aging biology research

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Abstract

Aging is characterized by a persistent decline in function across cells, tissues, and organisms. Given the well-known role of mitochondria in ATP generation, age-related changes in mitochondrial function have been extensively studied in the context of aging and age-related diseases. Although mitochondria are extremely multifunctional, aging research primarily focuses on their bioenergetic role, often assessed through respirometry measurements. Using isolated mitochondrial enzymes, intact mitochondria, intact cells, and permeabilized cells and tissues, researchers have investigated age-related changes in mitochondrial respiratory function in various model species. Findings from these studies remain largely inconsistent, with reports indicating either a decline or no change in mitochondrial respiratory function during aging processes. These variations may depend on factors such as choice of substrates, tissue origin of mitochondria, sex, species, and experimental conditions, making it difficult to establish universal conclusions. Additionally, methodological limitations and inappropriate techniques have further complicated interpretations in the aging field. To advance our understanding, we encourage researchers to acknowledge the intrinsic dynamic nature of mitochondria and their fundamental differences across tissues. Employing an integrated approach that concurrently measures multiple markers of mitochondrial health and bioenergetic status is critical to the comprehensive study of age-related changes in mitochondrial function.

Keywords: Aging, mitochondrial dysfunction, respiration, mitochondrial membrane potential

Aging is a persistent decline in an organism's age-specific fitness components, associated with and perhaps caused by a progressive reduction in Hamilton's forces of natural selection [1]. In gerontology and geroscience literature it is routinely characterized as a complex biological phenomenon that leads to a decline in physiological function [2]. In 1955, Harman [3] proposed the free radical theory of aging (FRTA), suggesting that free radicals produced during aerobic respiration cause cumulative oxidative damage, leading to aging and death. Although this theory received some initial support between the 1960s and 1980s [4], it has been heavily criticized by both empirical studies and theoretical models in recent decades [5]. Given the predominant role of mitochondria in ATP generation via oxidative phosphorylation and the perception of (mostly mitochondrially derived) reactive oxygen species (ROS) as byproducts of this process, FRTA has evolved into the mitochondrial theory of aging [6,7]. After decades of research, growing evidence suggests that while mitochondria are not the sole cause of aging, declines in mitochondrial function are a fundamental hallmark of aging [2]. More importantly, given that mitochondria play a central role in cellular metabolism (which interconnects all aging hallmarks [8]), mitochondrial dysfunction is also a key regulator of other fundamental aging processes.

The terms "mitochondrial function" and "mitochondrial dysfunction" are widely used in aging studies [9]. Researchers often use this terminology to refer to the bioenergetic function of mitochondria, given their well-known role in ATP generation through oxidative phosphorylation. However, mitochondria serve multiple physiological functions beyond ATP production, including inter-organelle communication, macromolecule biosynthesis and degradation, genome stability maintenance, protein dynamics, and ion transport [10]. A decline in any of these functions—many of which have been documented in aging research—can be termed "mitochondrial dysfunction". For example, due to the lack of protective histones and the limited efficiency of mitochondrial DNA (mtDNA) repair

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mechanisms compared to nuclear DNA repair, mutations and deletions in mtDNA may contribute to aging phenotypes [11]. The ability of mitochondria to maintain genomic stability is often independent of their bioenergetic functions. As a result, the commonly used terms "mitochondrial function" and "mitochondrial dysfunction" can be misleading. We encourage researchers to better specify the particular mitochondrial (dys)function they are referring to in their publications.

Even within the domain of mitochondrial bioenergetics, mitochondria control a complex network of fundamental processes beyond ATP generation, including (i) regulation of cellular redox status (primarily through ROS), (ii) maintenance of the antioxidant glutathione in a reduced state, (iii) calcium homeostasis, (iv) production, regulation, and transport of tricarboxylic acid (TCA) cycle metabolites, and (v) both programmed and necrotic cell death. However, here we will focus on the respiratory function of mitochondria during aging.

In the context of aging biology, mitochondrial respiratory function has been extensively studied using approaches including isolated mitochondrial enzymes or submitochondrial particles, isolated intact mitochondria, permeabilized cells and tissues, and intact cells. However, many of these studies focus on age-related diseases [12], making it difficult to separate the effects of aging from disease conditions [13]. Studies using isolated mitochondrial enzymes—while not in complete agreement—largely report a decline in the activity of mitochondrial enzymes such as citrate synthase, mitochondrial electron transport chain complexes, and cytochrome c with aging [14]. However, enzymatic activity assays often reflect mitochondrial quantity rather than quality in tissues [15]. Consequently, age-related declines in mitochondrial respiratory enzyme activity may largely be attributed to a reduction in mitochondrial number rather than intrinsic mitochondrial respiratory dysfunction.

Compared to enzyme-based studies, investigations using isolated mitochondria to assess age-related changes have yielded inconsistent results. Because skeletal muscle is one of the few readily available tissues in human aging research, it remains the most studied tissue for mitochondrial respiration across aging models. Both age-related declines and no significant changes in skeletal muscle mitochondrial respiration have been reported [13, 16-18]. Similar trends have been observed in other tissues, including the brain, liver, spleen, intestine, kidney cortex, heart, and blood cells in humans and laboratory rodents [13]. Notably, most of these studies used isolated mitochondria with excessive substrates and inhibitors to investigate age-related changes, which may not reflect physiologically relevant mitochondrial respiration. In fact, comparative studies suggest that mitochondrial isolation procedures may not always align with *in situ* results. Conversely, when using intact or permeabilized cells and tissues, only small or no age-related changes in mitochondrial respiration have been documented across various tissues and cell types. A recent large-scale analysis of age-related mitochondrial respiration found that age effects on mitochondrial function can be largely tissue- and sex-specific [19]. In this study, aging significantly affected mitochondrial activity in the brain, adipose tissue, skeletal muscle, eyes, and gastrointestinal tract, while tissues such as the kidney cortex remained largely resilient. It is important to note that these respirometry analyses were performed on mitochondria-enriched lysates derived from frozen tissues, which primarily measure maximal mitochondrial respiration rather than physiologically relevant levels. Interestingly, direct enzymatic activity assays of mitochondrial complexes did not reveal significant age-related changes in various mouse tissues. Taken together, findings on age-related mitochondrial respiratory changes remain inconclusive and may depend on factors such as substrate availability, tissue type, sex, species, and experimental conditions.

Beyond respiration, proton motive force (Δp) is another key indicator of mitochondrial ATP generation. The proton circuit serves as the sole link between the respiratory chain and ATP synthase. Although Δp consists of both the pH gradient (ΔpH) and the mitochondrial membrane potential (Δψ), Δψ is the dominant component and is most commonly measured. Due to technical challenges in accurately measuring Δψ in biological systems, limited research has focused on age-related changes in Δψ. The most common method for measuring Δψ involves fluorescent probes such as rhodamine 123 and tetramethylrhodamine methyl ester (TMRM) in isolated mitochondria or intact cells. Reports generally indicate that mitochondria from aged animals exhibit lower Δψ compared to younger counterparts in isolated cells such as lymphocytes and hepatocytes as well as whole organisms such as yeast and *C. elegans* ([20], but see [21]). However, a closer examination of these reports reveals inconsistencies, with some studies reporting Δψ values that are not only incompatible with their respiration data but also physiologically implausible [22]. These discrepancies may result from incorrect probe usage in quench mode or interference from plasma membrane potential [22]. Using tetraphenylphosphonium (TPP) electrodes to monitor Δψ avoids many of the issues associated with fluorescent probes. Studies employing TPP electrodes also report age-related declines in Δψ [23, 24], but these changes may be substrate-specific [23], and dramatic Δψ reductions observed in isolated mitochondria may not be physiologically relevant [22].

In conclusion, the age-related decline in mitochondrial respiratory function is widely recognized in aging research, often characterized by reduced respiration rates and/or decreased coupling capacity [respiratory control ratio (RCR) = state 3 respiration/state 4 respiration]. Interestingly, mild mitochondrial uncoupling, which is indicated by higher state 4 respiration and lower RCR, has been suggested to delay aging processes [25]. Maintaining mitochondrial respiratory homeostasis is crucial for cellular function, as aging can disrupt mitochondrial respiration, and vice versa. However, these age-related changes may be highly substrate-, tissue-, sex-, species-, and condition-specific. Therefore, using integrated approaches to study-

ing mitochondrial bioenergetics, such as metabolic control analysis, may be better suited for aging research, as they allow quantification of how small perturbations in one bioenergetic parameter affect the entire network of linked parameters. Given this, we suggest it is essential to examine respiration, membrane potential, proton leak, and ATP demand within an integrated framework using appropriate methodologies when studying links between mitochondrial function and aging.

Declarations

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