An energetics perspective on geroscience: mitochondrial protonmotive force and aging

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Abstract Mitochondria are organelles that provide energy to cells through ATP production. Mitochondrial dysfunction has long been postulated to mediate cellular declines that drive biological aging. Many well-characterized hallmarks of aging may involve underlying energetic defects that stem from loss of mitochondrial function with age. Why and how mitochondrial function declines with age is an open question and one that has been difficult to answer. Mitochondria are powered by an electrochemical gradient across the inner mitochondrial membrane known as the protonmotive force (PMF). This gradient decreases with age in several experimental models. However, it is unclear if a diminished PMF is a cause or a consequence of aging. Herein, we briefly review and define mitochondrial function, we summarize how PMF changes with age in several models, and we highlight recent studies that implicate PMF in aging biology. We also identify barriers that must be addressed for the field to progress. Emerging technology permits more precise in vivo study of mitochondria that will allow better understanding of cause and effect in metabolic models of aging. Once cause and effect can be discerned more precisely, energetics

approaches to combat aging may be developed to prevent or reverse functional decline.

Keywords Membrane potential · Metabolism · AMPK · mTOR · Autophagy

Introduction

Mitochondria have long been thought to play a central role in biological aging. The mitochondrial free radical theory of aging, developed as a variation on Harman's free radical theory of aging [1], posits that biological aging is driven largely by reactive oxygen species produced in mitochondria as a by-product of oxidative metabolism [2]. Although it now seems clear that the mitochondrial free radical theory of aging is not sufficient to explain many aspects of aging, there is growing consensus that mitochondria can also impact aging through multiple non-free radical mechanisms.

Mitochondrial dysfunction is recognized as one of the nine “hallmarks of aging” [3], and a case can be made that mitochondrial dysfunction may causally contribute to each of the other aging hallmarks [4, 5] (Fig. 1). Mitochondrial reactive oxygen species (ROS) produced as a by-product of oxidative metabolism likely represent an important source of damage within cells, both to mitochondria themselves and to other macromolecules and cellular components [6–8]. Damage to proteins arising from ROS likely contributes to loss of proteostasis and damage to nuclear DNA, which can give rise to genome instability, telomere dysfunction, and cell

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senescence [9–12]. Dysregulation of mitochondrial metabolism has been associated with declining stem cell function, epigenetic alterations, and dysregulated nutrient sensing during aging [13–15]. Mitochondria also play a central role in apoptotic cell death, which when perturbed can exacerbate many aging phenotypes [16]. More recently, mitochondrial DNA has been shown to induce a potent inflammatory response during aging which likely contributes to the well described phenomenon of “inflammaging” [17–19]. These interactions are by no means a one-way street, with several other aging hallmarks also contributing to age-associated mitochondrial dysregulation, but they do illustrate the importance of mitochondrial function for preserving youthful cellular, tissue, and organismal health.

One challenge the field has faced in attempting to define the multitude of ways that mitochondria are important during aging has been the limitations of available technologies for studying mitochondrial structure and function in living systems. Recently developed methods have allowed more precise observation and control over mitochondria in their natural state in living tissue or in whole organisms [20–25]. One important step forward has been the development of *in vivo* imaging technology. Microscopic advances have allowed interrogation of mitochondrial structure within tissues [26]. This has revealed that mitochondria are more diverse than the stereotypical bean-shaped organelles found in textbooks. Mitochondria can form networks or reticula that take on strikingly different morphologies in different cell types [27–30]. In addition, mitochondria and associated metabolic parameters can now be imaged and experimentally perturbed in living mammalian tissues [21, 31]. New understanding of how mitochondria differ between tissues has also expanded metabolic research to consider context when studying metabolism. For example, isolation of tissue-specific mitochondria is now possible and has revealed that mitochondrial genomes are passed on in different amounts across tissues [32]. It is now clear that metabolic study of one tissue or cell type cannot necessarily be applied to a different tissue or cell type. Recent years have seen a paradigm shift away from considering mitochondria as simple powerhouses as researchers in many fields have begun to recognize mitochondria as signaling organelles important for diverse aspects of cellular physiology [33].

Here we approach geroscience from an energetics perspective where we consider the mitochondrial protonmotive force (PMF) as a potential driver of

biological aging and a potential target for intervention. We describe the function of the PMF, evidence for its role in aging and disease, and new technologies that can be applied to experimentally manipulate PMF in cells and animals.

Mitochondrial PMF

Mitochondria are powered by an electrochemical gradient termed protonmotive force (PMF). The PMF is a proton gradient across the mitochondrial inner membrane (IM). This proton gradient is made up of two connected potential energies: an electrical membrane potential ($\Delta\psi_m$) and a pH differential (ΔpH). Mitochondria remove electrons from cellular metabolites and shuttle them through an electron transport chain (ETC) located in the IM. As electrons are passed through oxidation-reduction reactions, some energy from each transfer is used to pump protons across the IM from the matrix to the intermembrane space (Fig. 2). Proton translocation by the ETC establishes PMF that is then used by ATP synthase, also located in the IM, to make ATP for cells to use. In addition to this energetic function, mitochondria also regulate calcium signaling, apoptosis, redox homeostasis, and stress resistance signaling [34–36]. Here, we define mitochondrial function as respiration (electron flux and oxygen consumption), energetics (ATP production and availability), and the resultant signaling outputs dependent on respiration and energetics (Fig. 2). Each of these aspects of mitochondrial function is independent, but often works together. Importantly, each aspect is ultimately governed by the PMF. The collapse of PMF will eventually halt all mitochondrial function and cause cell death.

A popular analogy for mitochondrial function in the bioenergetics field is the “proton circuit” [37, 38]. Electron flux through the ETC is analogous to amperage in an electrical circuit; electron flow through the ETC is like electron flow through the wires of a circuit. The PMF is analogous to voltage, a potential energy that powers the circuit. Both components are intrinsic to the circuit and can be observed separately. This analogy holds true for mitochondria as well; respiration and PMF work together, and they can be studied either together or independently, depending on the methods used to study them. This not only is a useful analogy, but it also serves as a warning for experimental interpretation of mitochondrial experiments. If only one

Fig. 1 Mitochondrial contribution to hallmarks of aging. Mitochondria interact with each of the “hallmarks of aging,” and underlying mitochondrial dysfunction likely contributes to cellular dysfunctions associated with each hallmark. These complicated interactions suggest that targeting mitochondrial function directly and specifically may improve health as organisms age

parameter (either respiration or PMF) is measured or perturbed in a study, interpretation cannot be applied to the unmeasured component. Similarly, studying mitochondria in isolation from the *in vivo*, physiologic context may produce confounding results, just like isolated components of a circuit cannot fully describe a city’s power grid. This nuance can cause confusion when trying to interpret the literature from different model systems or under different metabolic conditions. Like the circuit analogy, we will consider mitochondrial PMF like batteries in that respiration charges up a voltage to provide energy.

A nuanced principle in metabolic research is the experimental separation of respiration and PMF. For example, one study genetically inhibited mitochondria in cells which resulted in decreased PMF, respiration, and mitochondrial signaling and in increased cell death [39]. This study then independently rescued either respiration or PMF through genetic regulators. The authors found that PMF rescue led to different outcomes than respiratory rescue. Rescued PMF preserved cell viability, redox homeostasis, and restored normal mitochondrial signaling through AMP-activated protein kinase (AMPK), a cellular energy-sensor [40]. Rescued

Fig. 2 Mitochondrial function schematic. A mitochondrion is pictured with a magnified inner membrane (IM). The electron transport chain (ETC) in the IM passes electrons, consumes oxygen, and pumps protons (H^+) from the mitochondrial matrix to the intermembrane space (IMS). This is the respiration aspect of mitochondrial function. Proton pumping by the ETC establishes

protonmotive force (PMF), an electrochemical gradient across the IM (represented by + and -). ATP synthase uses the PMF to make ATP by allowing proton reentry into the matrix. ATP production provides energy for the cell and will be referred to as energetics. Downstream of the ETC, PMF, and ATP production are diverse signaling outputs that coordinate cellular physiology

respiration also preserved cell viability but did not restore AMPK signaling [39]. This concisely demonstrates that PMF and respiration are separable in a cellular context and that mitochondrial signaling outputs can be different depending on which mitochondrial function is perturbed. However, it remains difficult to specifically manipulate PMF acutely and reversibly in vivo, which limits progress in understanding physiologic mitochondrial functions. Regardless, this principle of separate but related mitochondrial functions may be particularly important in aging systems where multiple processes become dysregulated in cells and tissues and the charge of the mitochondrial battery declines with age.

PMF and aging

Many lifespan-extending interventions seem to implicate mitochondrial function. Mutations in, or RNAi knockdown of, electron transport chain components, for example, have long been known to extend lifespan in *Caenorhabditis elegans* [41–43]. This counter-intuitive example illustrates the complexity of mitochondrial biology, as reduced mitochondrial function can, in some cases, induce a protective response through a process referred to as mitohormesis [44]. Although the precise mechanisms underlying this protective response remain unclear and somewhat controversial [45, 46], examples of mitohormesis-associated lifespan extension have been observed in budding yeast [47, 48], nematode worms [49, 50], fruit flies [51], and mice [51–53], with suggestive evidence for such a mechanism in humans as well [54]. It is worth also noting, however, that a mouse model with deficient respiration was neither long-lived nor short-lived and had no obvious deleterious phenotypes [55]. Thus, the relationship between mitochondrial respiration and lifespan is complex, and the role of mitohormesis in mammalian aging remains to be fully elucidated.

In contrast to mitohormesis, most longevity- and health span-promoting interventions are associated with preservation of mitochondrial function during aging. Exercise is a classic example in humans where enhanced mitochondrial biogenesis and function are thought to promote preservation of muscle function [56, 57], reduced age-associated disease incidence [58, 59], and increased life expectancy [60, 61]. Another example from pre-clinical studies of aging is reduced signaling

through the mechanistic target of rapamycin (mTOR) pathway. Genetic or pharmacologic inhibition of mTOR increases lifespan and functional measures of health during aging in yeast, worms, fruit flies, and mice [62, 63]. Experimental evidence suggests that inhibition of mTOR enhances mitochondrial function during aging through multiple mechanisms, including maintaining mitochondrial protein quality control, increasing degradation of damaged mitochondria via autophagy, and regulation of expression of nuclear encoded mitochondrial proteins both transcriptionally and translationally [64, 65]. Intriguingly, inhibition of mTOR with the drug rapamycin can also rescue severe mitochondrial dysfunction in animal models and cell culture [66–70] with initial indications of efficacy in human patients [71, 72].

Several lines of evidence converge to suggest that regulation of PMF specifically may play an important role in linking mitochondrial function to health during aging. In budding yeast, for example, an early indicator of biological age is decreased PMF [73]. Decreased PMF is also a well-known feature of cells from old animals [74], cells used as models for diseases of aging [75], and replicatively aged cells in culture [38, 75]. Further, PMF and respiration decreases in mammalian tissues in different models of aging and diseases of aging [75, 76]. Importantly, measures of both PMF and respiration decline in mitochondria isolated from old mice, supporting a model of declining PMF with age [77]. Causation between declining PMF and biological aging has yet to be confirmed, however. Thus far, the literature supports a model of mitochondrial decline as an early, potentially causative factor in functional decline with age (Fig. 3a). Therefore, developing methods to target PMF specifically may be a preventive strategy to combat aged pathophysiology.

PMF and energy sensing

Organisms can sense and communicate nutrient availability throughout cells and tissues. Perturbing nutrient or energy sensing has powerful effects on aging. Caloric restriction, which can be defined as reducing calories without malnutrition, is by far the most well-studied intervention for slowing aging across many different species [78, 79]. Some beneficial effects of calorie restriction may be mediated through changes in PMF. In rodents, fasting was able to slightly ameliorate metabolic decline that occurs in middle aged brains, indicating that modulation of nutrient signaling involves

Fig. 3 An energetics perspective on aging. Mitochondrial PMF is represented as battery charge level. **a** The maximum potential energy or battery charge of mitochondria decreases with age in many models. **b** A hypothetical age prevention therapy may target

and preserve mitochondrial PMF to promote cellular health and longevity. **c** A hypothetical rejuvenation intervention may target lost PMF therapeutically to restore lost battery charge to reverse cellular dysfunction associated with age

mitochondrial function directly [76]. Further, mitochondria from calorie-restricted old rats had preserved PMF and respiration levels [80, 81]. In *C. elegans*, increased lifespan triggered by fasting requires functional mitochondrial dynamics (fission and fusion) and respiration [82]. Also in *C. elegans*, lifespan extension from mTOR inhibition requires the energy sensor, AMPK [83]. How PMF regulates the interplay between mTOR, AMPK, mitochondrial dynamics, and fasting is still unclear.

The lifespan-extending drugs metformin and rapamycin are thought to mimic conditions of calorie restriction, possibly through mitochondrial signals [65, 84–86]. Metformin targets both AMPK and mitochondrial respiration, and rapamycin specifically targets mTOR. In a study using both metformin and rapamycin, toxic effects of metformin in old *C. elegans* were ameliorated by rapamycin [87]. Metformin toxicity was likely ameliorated by preserved PMF via rapamycin, as demonstrated in cell models [87, 88]. This shows that rapamycin may have some of its beneficial effects through stabilization of PMF. Overall, these studies indicate that the effects of true or mimicked calorie

restriction may function through mitochondrial PMF regulation of energy sensing. Testing the effects of calorie restriction and mimetic drugs on the PMF will be important to better understand the molecular mechanisms of fasting and longevity.

PMF and proteostasis

Protein homeostasis, or proteostasis, is a balance between protein production and clearance of damaged or misfolded proteins [89, 90]. One link between mitochondrial PMF and proteostasis is through lysosomal function. Lysosomes maintain an acidic internal environment for proteolytic activities to support proteostasis [91, 92]. An early indicator of biological aging is decreased PMF that is associated with decreased lysosomal acidity [73]. Pharmacologic or genetic rescue of lysosomal acidity rescued mitochondrial PMF in yeast [73]. PMF maintenance is thought to depend on availability of iron sulfur clusters, essential components of the mitochondrial ETC [93]. Lysosomes regulate metal ion availability, including iron for iron sulfur cluster

assembly in mitochondria [94, 95]. This linked regulation could explain the mechanism of rescue between lysosomal and mitochondrial functions; functional lysosomes provide iron sulfur clusters for functional ETC activity and PMF generation. This link makes it a likely assumption that dysfunction in one organelle will translate to dysfunction in the other. Moreover, functional interaction between lysosomes and mitochondria may be mediated through signaling outputs of the PMF.

Mitochondria are also recycled like other organelles and proteins in cells. Mitochondria continually undergo fission and fusion in vivo for various purposes [96, 97]. It is thought that mitochondrial fission acts to separate dysfunctional mitochondria, or mitochondria with low PMF, away from healthy mitochondria. The dysfunctional mitochondria can then be degraded and recycled by lysosomes through mitochondrial autophagy (mitophagy) [98–100]. Mitophagy may become dysfunctional with age, allowing damaged mitochondria to accumulate and cause cellular dysfunction. Causation has been difficult to establish between dysfunctional mitophagy and persistence of mitochondria with low PMF. However, recent evidence suggested that direct and specific PMF dissipation in cells caused initiation of mitophagy [25]. In addition, in a study that linked beneficial autophagy to longevity, permeabilizing the mitochondrial IM switched autophagy to a damaging force and shortened lifespan in *C. elegans* [101]. Permeabilizing the IM in vivo would result in dissipated PMF. This study showed that beneficial effects of autophagy on lifespan are dependent on sufficient PMF. This again supports the hypothesis that maintained PMF is beneficial for healthy aging and connected to proteostasis. It will be important to test precisely how changes in PMF are linked to proteostasis, mitophagy, and lysosomal acidity in vivo.

PMF and stem cell function

Stem cells have regenerative potential that decline with age in many tissues, and restoration of stem cell function has been associated with reversal of age-related functional declines [102]. Mitochondria are directly involved in stem cell function through their role in providing the raw material and energy for growth. In *Drosophila*, old stem cell mitochondria had decreased ATP levels and decreased PMF. Glycolytic metabolism was concurrently upregulated in these old stem cells with low PMF [103]. It remains unclear whether low PMF

occurred as a signal for the metabolic switch to glycolysis or if the metabolic switch itself resulted in the low PMF. This uncertainty is representative of many metabolic studies that are limited by the experimental tools available.

In aging mouse or human hematopoietic stem cells, PMF can vary across cell populations [104]. In such variable stem cell populations, cells with relatively higher mitochondrial PMF had transcriptional profiles similar to younger cells [105]. Stem cells with lower PMF had lower transcriptional rates and reduced DNA repair responses, indicating an older biological phenotype. When PMF was preserved by feeding animals the mitochondrial targeted antioxidant, mitoQ [106], youthful stem cell phenotypes were reestablished [105]. This work highlights how low PMF is associated with aged biology, and that intervening to increase PMF in vivo can reverse aged phenotypes. While the mechanism of mitoQ PMF preservation is unclear, this study provided some evidence for the causal nature of PMF changes in stem cell metabolism and aging. Testing how PMF affects stem cell functions will require precise control over mitochondrial function in vivo.

An energetics perspective on aging

The battery power of mitochondrial function declines with age in many models across species and tissues (Fig. 3a). Here, we outline an emerging geroscience hypothesis based on literature suggesting that preserving or boosting mitochondrial PMF may act to prevent or reverse age-associated dysfunction. This idea builds upon previous goals of broadly targeting mitochondria for anti-aging therapy [107]. Intervening before mitochondrial function significantly declines could prevent the loss of mitochondrial function and PMF that may drive cellular aging (Fig. 3b). Similarly, rejuvenating mitochondrial PMF in aged organisms may act to restore mitochondrial functions and reverse aging phenotypes (Fig. 3c). To test these hypotheses, precise control of PMF and mitochondrial functions is necessary in vivo to produce meaningful physiologic results. The work described herein began to test these hypotheses, and the results thus far have informed our proposed concept of an energetics perspective on aging.

Testing how PMF affects aging

It seems clear that determining cause and effect between PMF changes and physiology will drive progress in understanding disease. It is also clear, however, that determination of cause and effect is difficult and sometimes impossible given limitations of experimental methods. A recent study addressed this difficulty and coined the phrase “causal metabolism.” The study described a method to fundamentally alter metabolism in living mice and asked what the effects were [108]. Expressing an enzyme to artificially decrease the NADH/NAD⁺ ratio in vivo revealed changes in metabolism as a direct result of the changed redox ratio [108]. The NADH/NAD⁺ ratio governs electron metabolism and metabolic signaling through many signaling networks in cells and subcellular compartments [109]. Oxidizing NADH to NAD⁺ directly in live animals changed metabolism through affecting one variable. The resultant physiologic observations could then be attributed directly to the changed NADH/NAD⁺ ratio upstream. This intervention improves upon classical approaches of observing many aspects of metabolism that are associated with each other in different disease models. Therefore, this study is a great model for causal metabolism studies in aging. Manipulation of single metabolic variables with proper experimental controls should be a standard of mitochondrial research in aging models. First characterizing which aspects of mitochondrial function (ETC flux, ATP/ADP levels, PMF) are affected is required for clear interpretations. Once these effects are characterized for a specific intervention, in vivo application can be used to test what aspects of mitochondrial function cause physiologic changes that may be associated with aging.

Causal metabolic studies that achieve some or all of these standards are emerging in invertebrate models. Traditional genetic interventions to change metabolism were applied in *C. elegans*, and both respiration and PMF were monitored to show how an unfolded protein response coordinated mitochondrial biogenesis [110]. This showed that mitochondrial function, protein expression and stress resistance signaling programs depend on one another for proper function. The reverse situation—changing the PMF and monitoring physiologic responses—is now possible in *C. elegans* as well, through optogenetics. Using light-sensitive proton pumps targeted to mitochondria allowed precise control of PMF in isolation from other metabolic processes

[111]. Optogenetic tools like these were developed to both increase or decrease mitochondrial PMF in live animals using light [22, 23]. Elevating PMF in vivo revealed a causal connection between PMF and energy sensing through AMPK. When PMF was artificially increased in living *C. elegans*, starvation-induced AMPK activation was reversed [22]. Similarly, optogenetic dissipation of PMF resulted in activation of AMPK and could modulate starvation behavior [23]. Boosting PMF simulated a fed state and deactivated AMPK signaling, and dissipated PMF activated AMPK and signaled a starved state. These results confirmed a causal link between PMF and energy sensing through AMPK. Therefore, it is likely that changing levels of PMF experimentally would affect aging physiology through the effects on AMPK and nutrient sensing signaling. Determining cause and effect between PMF changes that happen with age and cellular dysfunctions will provide much needed clarity and unity between models of metabolism in aging. Controlling PMF in vivo could be used to reverse decline of PMF that happens in (at least some) tissues with age. Increasing PMF in old animals and monitoring dysfunction associated with specific hallmarks of aging will directly test whether lost PMF causes physiologic dysfunction for that hallmark. Ultimately, artificially increasing PMF in animals and monitoring lifespan would test if the observed decline in PMF with age is causing functional decline and mortality.

In addition to PMF intervention, control over oxidative stress and ROS production is now also amenable to aging research questions. ROS can be produced or scavenged specifically in mitochondria and physiologic responses can be easily monitored [112–114]. One study has already causally implicated mitochondrial ROS with telomere dysfunction in the nucleus [113]. Using these new reagents allows control over mitochondrial redox signaling, and lifespan can be monitored to determine the causal effects of mitochondrial function on aging in a more precise way than has been possible previously. This experimental system can begin to test what levels and types of mitochondrial ROS production are beneficial versus detrimental.

These causal metabolism approaches could be used to answer questions brought up in earlier examples reviewed here, like “does decreased PMF in stem cells cause a switch to glycolytic metabolism?”, and “does increased PMF prevent initiation of mitophagy?” Answering these questions will provide directionality in

models that will streamline future investigations and focus hypotheses. Eventually, these approaches and knowledge generated in invertebrates could be applied to mammalian models. Many rodent studies use observational techniques like genomics, proteomics, and metabolomics [65, 115, 116] to observe metabolic changes that impact physiology. These observations could be paired with causal metabolism techniques and optogenetics to enhance the interpretation of these studies.

Further, these techniques can be used tissue specifically. *Drosophila* and *C. elegans* could be used to identify causal effects of PMF changes or ROS production in specific tissues to inform more targeted mammalian approaches. Some of this work has already begun in *C. elegans*, where neuronal AMPK was shown to respond to PMF changes [23]. Moreover, neuronal mTOR inhibition resulted in AMPK-mediated lifespan extension that involved preservation of healthy mitochondrial structure [83]. Overall, using invertebrate models will aid in identifying targets for adapting new interventions in mammals. This will advance our understanding of how mitochondrial PMF causes physiologic change that can be precisely targeted to prevent or reverse the decline that occurs with age in humans.

Integrating an energetics perspective on aging with other models

While we propose that PMF maintenance may support healthy longevity, this model must be reconciled with previous findings suggesting a seemingly opposite interpretation. In studies using isolated mitochondria from long-lived animals such as the naked mole rat, for example, decreased PMF and intrinsically increased basal respiration have been observed [117, 118]. The same is true for bats [119], pigeons [120], and some other animals that are the same size as mice but longer lived. Further, pharmacologic PMF dissipation using protonophores to collapse electrochemical gradients has also been reported to extend lifespan in *C. elegans* and in female Swiss Webster outbred albino mice [121, 122]. However, these observations are still compatible with the model that preserved PMF during aging may support enhanced longevity and health span. First, it is important to recognize that correlations between decreased PMF and longevity come almost exclusively from laboratory-isolated mitochondria, cells, or tissues. Naked mole rat cells, in particular, require substantially

different culture conditions compared to mouse cells, which will likely have an impact on measurements of mitochondrial structure and function. Also, correlations between in vitro PMF measurements and any physiologic output (such as lifespan) should be cautiously interpreted due to methodological considerations [38]. Therefore, whether differences in PMF are actually present in vivo in these long-lived animals remains unknown. The use of protonophores to extend lifespan is particularly interesting but could conceivably act through mitohormesis, as described above, and even potentially result in an overall preservation of PMF into old age. Consistent with this, treatment of mice with protonophores enhanced mitochondrial biogenesis [123], which could lead to more functional mitochondria and preserved PMF.

Another mechanism by which a protonophore could promote longevity is through reduced oxidative stress [117]. Decreased PMF reduces mitochondrial oxidant overproduction in isolated mitochondria [124–126], and the longer-lived mice treated with a protonophore showed some markers of decreased oxidative damage [121]. The relationship between oxidative stress and longevity has been mixed [127], however, and direct in vivo evidence for decreased oxidative stress causing increased longevity is still lacking. Long-lived naked mole rats, for example, show signs of high levels of systemic oxidative damage [128]. Moreover, the free radical theory of aging has not been universally supported experimentally, with studies showing that redox status remains unchanged across mammalian tissues with age [115], and that in some cases increased oxidation is associated with longevity [129]. This underlines a central theme of mitochondrial research—outcomes can seem opposite depending on experimental methods and models. To address these disparate results, experiments that test cause versus effect between PMF and lifespan are necessary to bring clarity and mechanistic understanding.

One recent study illustrates how the seemingly inconsistent evidence for increased or decreased PMF promoting aging can be reconciled. In a naturally aged rodent model, mitochondrial matrix pH in cardiomyocytes was measured under different conditions as a proxy for PMF levels. Old cardiomyocytes had decreased PMF and dysfunctional metabolism [130]. One known mediator of PMF and respiration is the adenine nucleotide transporter (ANT) [131, 132]. The ANT transports ADP into mitochondria for

conversion to ATP, and it transports ATP out of mitochondria to be used to do work in the cell. In addition, the ANT can allow protons to reenter the mitochondrial matrix resulting in decreased PMF [132]. In this study, the ANT was pharmacologically inhibited to preserve PMF in the old cells. Upon ANT inhibition, respiratory dysfunction was reversed, PMF was stabilized, and youthful physiology was restored in the old cardiomyocytes [130]. Part of the proposed mechanism involved stabilized ETC flux and redox state. The authors reasoned that if ETC flux is uninhibited and oxidative stress is low, then increasing PMF with age is beneficial to cell physiology and is still compatible with models of decreasing PMF to decrease oxidative stress.

These data again demonstrate how the PMF can signal independently from other mitochondrial functions such as respiration efficiency and redox signaling. Importantly, the data show that if redox status is maintained, preserved PMF is beneficial, as long as it is opposing a natural decline. On the other hand, if metabolism is pathologically increased and oxidative stress occurs, then decreasing PMF would be beneficial to decrease mitochondrial oxidant production [133]. Ultimately, when both electron flux and PMF are maintained, aging phenotypes can be delayed [130]. These phenomena demonstrate the importance of tissue, cell, organ, and time specific studies to understand precisely when, where, and how mitochondrial function is impaired. These seemingly opposed models of PMF regulation and longevity can be reconciled through precise methods, experimental design, and interpretation. Applying general metabolic results from isolated systems to broad whole-organism models may not be as informative because of this dichotomy in results from PMF regulation and aging.

Conclusions

In much the same way that portable batteries must be replaced after repeated charge/drain cycles, mitochondria decline in function with age. Restoring mitochondrial function, like replacing a battery, can restore cells' ability to do work and may have a positive impact on human health. Some ways to "replace the battery" of mitochondrial function may include stimulating mitophagy and mitochondrial dynamics, and modulating energy-sensing signaling, possibly through interventions such as calorie restriction. To learn how to

effectively use these approaches to maintain mitochondrial health, it will be important to precisely answer when, where, and how PMF is regulated and if boosting PMF in vivo will affect aging biology and lifespan. Therefore, testing whether PMF changes are a cause or a consequence of physiologic dysfunction is a priority for metabolic models of aging. Further, it will be exciting to test whether PMF levels can be an early indicator of aging dysfunction. This analysis will depend on development of reliable measures of PMF and mitochondrial function in whole organisms, and eventually in humans [134, 135]. This may include identifying appropriate tissues to monitor and non-invasive methods to assess in situ PMF. Considering the impact of bioenergetics on hallmarks of aging may also reveal novel mechanisms of regulating hallmarks of health [136]. These newly described hallmarks also likely depend on cellular energetics, and deeper understanding of mitochondria will help to integrate hallmarks of aging and health with molecular underpinnings that can be targeted therapeutically.

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Declarations

Conflict of interest The authors declare no competing interests.

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