

Assessing mitochondrial quality in nontraditional animal models

Daniel Munro^a, Pierre U. Blier^b, and Matthew E. Pamerter^{a,c}

^aDepartment of Biology, University of Ottawa, Ottawa, ON, Canada; ^bDépartement de Biologie, Université du Québec à Rimouski, Rimouski, Canada; ^cOttawa Institute of Systems Biology, Ottawa, ON, Canada

Corresponding author: **Matthew E. Pamerter** (email: mpamerter@uottawa.ca)

Abstract

Studying mitochondrial respiratory function is critical to understanding the plasticity of cellular energetics. Mitochondrial respiratory function is usually studied *ex vivo* and requires isolation of high-quality mitochondrial preparations. However, mitochondria are usually damaged by this process, and the degree of damage is typically assessed by measuring respiratory control ratios (RCRs) and the change in respiration rate mediated by exogenous cytochrome C addition to isolated mitochondrial preparations. The standard thresholds in the field for determining whether isolated mitochondria are “acceptable” are based on studies in common laboratory rodents and are entirely arbitrary. However, zoologists are increasingly employing nonstandard animal models in the study of mitochondrial respiratory function across a wide variety of fields and there is growing evidence that the canonical thresholds of acceptable RCRs and the cytochrome C response that have been used to identify healthy mitochondrial preparations may not be useful in other species. In this commentary, we discuss the limitations of using RCRs and cytochrome C-mediated changes in mitochondrial respiration in the study of nontraditional laboratory species and propose alternative means of evaluating mitochondrial integrity that may permit more accurate assessment of the quality of mitochondria isolated, and thus better support comparison of data across species and studies.

Key words: cytochrome C, inner mitochondrial membrane, respiratory control ratio, nontraditional laboratory species

Studying mitochondria in isolation

Mitochondria are a remarkable example of the maxim that form follows function. Traditionally, mitochondria have often been thought of as individual bean-shaped organelles; however, modern studies have demonstrated that mitochondria instead often comprise flowing ribbon-like structures that weave throughout the cytoplasm, contacting and interacting with other organelles and the plasma membrane (Frey et al. 2002), and often connecting to each other along long string-like structures (often termed mitochondria on a string), or via nanotunnels, which are a relatively recent and poorly understood form of intra-mitochondrial connection (Vincent et al. 2017). Indeed, it is now clear that mitochondrial architecture demonstrates a high degree of organization of membranes, while mitochondrial cristae have a plastic structure that changes according to the needs of the cell (Cogliati et al. 2016; Cheng et al. 2021b). Probing their architectural details and how they are modulated or disrupted during periods of acclimation to different environments and/or in response to various stress or disease states is thus crucial to understanding how cells function (Galli and Richards 2014; Pamerter 2014, 2016; Tyumentsev et al. 2018; Hickey et al. 2024), and may also be determinants of development, animal performance, and life history (Heine and Hood 2020).

Studying mitochondrial respiratory function in isolation poses a significant challenge because conducting such experiments *ex vivo* requires the isolation of high-quality mitochondria, but techniques to isolate mitochondria comprise harsh cycles of cellular disruption via homogenization and repeated centrifugation to fragment cells and separate mitochondria from other cellular constituents (Liao et al. 2020). These isolation steps also usually result in the loss of a significant portion of the mitochondria in a given tissue and can lead to preferential loss of particular subpopulations of mitochondria between different tissue types in a given organ (e.g., sarcolemma vs. myofibrillar mitochondria in heart (Adhihetty et al. 2005)). Until recently, the “optimal” outcome of standard approaches to isolate mitochondria has been a pool of usually round or oval-shaped structures containing intact membranes and cristae, which have been traditionally viewed as “intact” mitochondria, but which are more likely reconstituted sections of larger ribboned mitochondrial structures (Picard et al. 2011). However, mitochondrial respiratory function is highly dependent on the maintenance of the ultrastructural integrity of mitochondria, and particularly of both the integrity of the inner mitochondrial membrane (IMM) and the enzymatic matrix components that generate a protonmotive force, which drives aerobic oxidative phosphorylation (OXPHOS) (Frey and Mannella 2000). It is

Box 1. Key terminology in mitochondrial respiratory physiology.

Term	Definition
Proton motive force	A form of potential energy in the form of an electrochemical proton gradient that is used by cells to drive processes such as ATP synthesis and ion transport across membrane.
Coupled vs. uncoupled respiration	Coupled respiration occurs when the proton gradient (the proton motive force) is used to power ATP synthesis. Uncoupled respiration occurs when protons are allowed to leak back across the mitochondrial membrane without powering ATP phosphorylation.
Phosphorylating vs. nonphosphorylating respiration	Phosphorylating respiration is essentially coupled respiration and occurs when the proton motive force is harnessed to phosphorylate ATP. Nonphosphorylating respiration is essentially uncoupled respiration and occurs when the proton motive force is allowed to dissipate to generate heat or dissipate reactive oxygen species generation.
State 3 respiration	Aerobic ADP-stimulated respiration state that occurs when ADP is added to isolated mitochondria for conversion to ATP. Reflects coupling of OXPHOS to ATP production and is a measure of a tissues ability to produce ATP aerobically.
State 4 respiration	Aerobic mitochondrial respiration that occurs in the presence of respiratory substrates and oxygen but without ADP to support ATP phosphorylation.

unclear whether the respiratory functional capacity of isolated mitochondrial components accurately reflects that of in vivo intact mitochondria. Nonetheless, these structures are presently the only approach to allow researchers to explore many mitochondrial functions, but their use requires means of validating the quality of the preparation under study.

Traditional assessments of mitochondrial quality in traditional laboratory rodents

High-magnification imaging of mitochondria (using transmission electron microscopy or similar approaches) is a gold-standard in assessing the quality of mitochondria both in vivo and in vitro (Neikirk et al. 2023). However, this is expensive and time-consuming and requires samples to be fixed and sliced before imaging. Thus, this is not useful in assessing mitochondrial health and functional viability of live mitochondrial preparations in real-time. Instead, researchers rely on indicators of mitochondrial integrity to determine whether mitochondria are functionally intact. Specifically, the respiratory control ratio (RCR) and the relative increase in respiration following addition of cytochrome C during phosphorylating states of respiration (state 3, or OXPHOS, see Box 1) are usually considered proxies of the quality of mitochondrial isolates (Gnaiger et al. 1998; Munro and Pamerter 2019; Gnaiger 2020). These benchmarks have been developed using carefully curated laboratory rodents of matched age, life history, and sex, and in the context of medical research, and may thus not be universally applicable in all rodents, let alone across animal species in the wild in general.

It is important to note that these are independent measures of mitochondrial quality that do not necessarily influence each other. Specifically, the RCR presents a ratio of phosphorylating to nonphosphorylating respiration. This ratio will decrease proportionally to the loss of matrix NADH/NADPH-producing enzymes fuelling phosphorylating

respiration and/or to the increase in nonphosphorylating state not limited by the integrity of the IMM (i.e., induced by increased proton conductance across the inner membrane). As such, the RCR can be viewed as a test of the integrity of the IMM. Importantly, the RCR is also known to vary across different species, mitochondrial subcomplex assemblies, and environmental contexts, as a function of the capacity of the mitochondrial electron transport system (ETS). This variability in the capacity of the ETS is precisely the subject of study (of interest) among many ecology and evolutionary biology researchers examining the interplay between life history, metabolic rate, and physiological performance (Koch et al. 2021). Clearly, an experimental variable is not suitable for simultaneous use as a quality control checkpoint.

The cytochrome C test provides a measure of the integrity of the outer mitochondrial membrane (OMM), as disruption of this membrane allows this electron carrier to leak to the medium, such that its exogenous addition recapitulates a normal respiration rate. Thus, it is important for a study to report both RCR and cytochrome C respiration rates to reasonably and comprehensively evaluate the quality of ex vivo mitochondrial preparations.

According to the current standards of mitochondrial physiology research, an RCR of at least 4, and a maximal increase in respiration of ~15% or less following addition of exogenous cytochrome C are viewed as minimum thresholds for concluding that mitochondria are sufficiently preserved after isolation. Notably, and although these values are generally accepted in the field of mitochondrial physiology, these thresholds are entirely arbitrary: they are often referenced but rarely attached to a citation. Indeed, there seems to be no seminal study suggesting objective criteria for establishing these accepted values. Furthermore, many studies simply do not report these values, or report only one or the other, which can make it challenging to assess the quality of the mitochondrial preparation, and limits comparison across studies conducted in different laboratories and by different researchers.

Traditional assessments of mitochondrial quality in nontraditional model species

Despite the above issues, these measures of mitochondrial quality have been regularly applied to nontraditional model species by zoologists, including to field-caught mammals, reptiles, fish, amphibians, invertebrates, etc. Unfortunately, many such studies either do not report mitochondrial quality control values or report them inconsistently. For example Galli et al. explored the function of anoxia-tolerant freshwater turtle heart (*Trachemys scripta*, (Schoepff, 1792)) tissue and reported RCR values for isolated cardiac mitochondria and cytochrome C values for cardiac fibres, but did not report the complementary values for each preparation (Galli et al. 2013). Similarly, Bundgaard et al. have studied mitochondrial function across representative bird, mammal, amphibian, reptile, and fish species, but typically do not report either RCR or cytochrome C values, other than stating that cytochrome C values do not exceed 10% (Bundgaard et al. 2020, 2023). Jorgensen et al. explored the relationship between mitochondrial function and cold adaptation in six species of *Drosophila* Fallen, 1823 and incorporated a cytochrome C test into their experimental protocol but did not report RCRs. We are not innocent of similar oversights, and some of our previous publications have not included one or both of these quality control metrics (e.g., Pamerter et al. 2016). Indeed, it is often difficult to find quantitative presentations of these measurements in studies of any species.

Assessing mitochondrial quality in nontraditional rodent models: RCRs

The use of nontraditional rodent models and of multiple species comparisons in the study of mitochondrial respiratory function is increasing rapidly, in part due to the increasing portability of specialized high resolution respirometry equipment suitable for experimentation with fresh mitochondrial tissue in remote locations (e.g., the Oroboros O2k system). In addition, while zoologists have long valued the study of nontraditional animal models, biomedical scientists are increasingly applying broader comparative approaches to medical research questions, and the study of such nontraditional animals is accelerating rapidly. For example, hibernating species are increasingly studied as models of hypometabolism with a view towards learning how to survive long-term space travel (Cerri et al. 2021); various subterranean species are emerging as major research models in the fields of cancer and gerontology (Pamerter and Cheng 2022; Kadamani et al. 2024) etc. As these animals are studied increasingly broadly, experiments evaluating mitochondrial physiology are also expanding to many of these new study species (Munro and Pamerter 2019). This raises an important question regarding the evaluation of mitochondrial quality: do the arbitrary thresholds with which researchers judge the quality of mitochondrial preparations in typical laboratory rats and mice also apply to nontraditional animal models in the field? To our knowledge, there is no study attempting to evaluate their relevance in wild species, despite large differ-

ences in life histories, as well as in the architecture, super-complex assemblies, ratios of complexes, and therefore, flux control of the ETS between species adapted to varying physiological niches.

Beyond the dearth of information regarding how well these traditional thresholds of determining mitochondrial quality apply to nontraditional models, there are also impacts of physiological adaptations in some such species that likely undermine these measures. For example, the calculation of RCRs is exquisitely sensitive to proton permeability of the IMM by simple virtue of the fact that state 2 (LEAK, see Box 1) respiration is the denominator in its calculation. Simply put, for any constant state 3 (OXPHOS) value, the RCR will tend toward an infinite value as the value of the LEAK state decreases towards zero. This is important because heterothermic species may have less “leaky” mitochondrial membranes, as passive leak of ions across the mitochondrial membrane represents a loss of energy from the ETS/OXPHOS system, and at least some species adapted to use hypometabolic strategies have tighter membranes to reduce energy loss (e.g., in naked mole-rat brain: Pamerter et al. 2018).

Indeed, the basal whole animal metabolic rate of mammals is also associated with the permeability of its IMM (Speakman et al. 2004). Depending on the tissue being studied, mitochondrial ion transport and associated processes may account for up to ~40% of cellular energy usage (Schneider et al. 1990; Buttgerit et al. 1991; Buttgerit and Brand 1995; Rolfe and Brown 1997). Reduced membrane permeability and/or a lower basal metabolic rate would thus both tend to drive the leak state towards zero, and thereby greatly inflate the resulting RCR calculation (or overly sensitize this calculation to small differences and/or errors in measurements of this low state 2 measurement).

This is important for many nontraditional rodent species because mice (Linnaeus, 1758) and rats (Linnaeus, 1758) are homeotherms, whereas many other rodents are heterothermic and adapt their metabolic needs to suit their environment and energy availability. Indeed, heterotherms, and particularly subterranean and hibernating rodents, typically have lower basal metabolic rates than homeotherms, and/or use behavioural and physiological strategies of hypometabolism when in environments with limited resources (e.g., of food, heat, or oxygen availability) (Storey 1997; Staples 2016; Sumnera 2019; Pamerter 2022). Furthermore, the leakiness of membranes is inherently impacted by physiological temperatures; in heterothermic mammals, the fluidity of membranes changes with changing body temperatures during torpor and/or hibernation (Hulbert and Else 1999), or following exposure to chronic hypoxia (Farhat et al. 2020; Farhat and Weber 2021). Similarly, changes in body temperature directly impacts membrane fluidity in ectotherms (Cooper et al. 2014; Chung and Schulte 2020), and thus mitochondrial membrane leak should be expected to vary with environmental thermal niche in such species.

Taken together, membrane permeability is an adjustable trait that will necessarily impact the leak state, and therefore the RCR. As a result, the information that may be obtained regarding mitochondrial quality from measuring RCRs may be biased by unrelated changes in membrane structures in

response to a wide range of physiological and environmental variables.

Assessing mitochondrial quality in nontraditional rodent models: the cytochrome C response

The respiratory response of a mitochondrial preparation to the addition of cytochrome C is often presented as solely reflecting the integrity of the OMM; a disrupted membrane would allow cytochrome C to leak during preparation steps, and replenishing pools of this electron carrier from exogenous sources would thus markedly recapitulate the normal flow of electrons (high percent of OXPHOS increase). This interpretation, however, completely disregards any natural interspecific variation in the degree of flux control exerted at the level of the cytochrome C electron carrier within the ETS. Indeed, cytochrome C is not synthesized *de novo* inside the intermembrane space but must instead be imported by proteins located in the OMM, which may or may not remain fully active after mitochondrial isolation. Furthermore, an increase in respiration following addition of cytochrome C may instead result from opening of the mitochondrial cristae organizing system, a component of the IMM, and may thus not reflect changes in OMM permeability at all (Hickey et al. 2024). Moreover, there is little basis to expect consistency in the relationship between RCRs or the response to the addition of cytochrome C as reliable proxies for quality control in studies across wide ranges of taxon, particularly because the cytochrome C compounds used in most laboratory studies of mitochondrial respiration are derived from horse, and given that the number of variants in cytochrome C structure and function increase considerably with increasing phylogenetic differences between species (Margoliash and Fitch 1968).

For species with important flux control at the level of the shuttling of electrons from complex III to complex IV of the ETS, cytochrome C addition is more likely to result in increases in OXPHOS respiration, regardless of whether there is damage to the OMM. To our knowledge, there are no studies in comparative mitochondrial physiology addressing the flux control exerted at the level of cytochrome C electron shuttling across species, if this is linked to a reorganization of the ETS associated with the tolerance to environmental stressors (such as routine exposure to hypoxia), or if this is associated with greater mitochondrial respiratory responses to exogenous addition of cytochrome C during OXPHOS. However, there is evidence that cytochrome C control of the ETS may be modulated by phosphorylation/dephosphorylation processes such that the phosphorylation state is then dependent on a functional mechanism of regulation, and more importantly, will dictate the impact of exogenous cytochrome C addition on OXPHOS (Kalpage et al. 2020). It is also important to consider that measurements of mitochondrial respiration are performed under saturating concentrations of molecular O₂, which removes any O₂-limitation at the level of cytochrome C oxidase (Burke et al. 1997; Gnaiger and Kuznetsov 2002), likely amplifying the importance of diverging control of respiration by cytochrome C across species. Thus, studies have

identified considerable heterogeneity in the cellular management of cytochrome C and sensitivity of OXPHOS to cytochrome C, which calls into question the use of this system to assay OMM damage across highly divergent species.

Other variables impacting “normal” RCRs in laboratory-raised and wild-caught animals

When using RCR values to compare the integrity of mitochondrial preparations from different studies it is important to consider the large number of factors that can impact the RCR. For example, RCR values are dependent upon the respiratory substrate used in a given experiment, such that higher RCR values are typically found with NADH-supported respiration than with succinate (Tomar et al. 2022). Similarly, different tissues typically express variable RCRs, with higher RCRs expected in brain and cardiac and skeletal muscle, and lower values obtained from liver or adipose tissue (Rolfe et al. 1994). Even within a given tissue, there are sometimes differences in RCR values based on cell types or groupings. For example, subsarcolemmal or intermyofibrillar mitochondria from heart have divergent RCR values, despite originating in the same tissue (Lombardi et al. 2000; Iossa et al. 2004). Values may also vary across strains of the same species. For example, sex impacts mitochondrial respiration differently in different common strains of mice (Norheim et al. 2019). These factors are under the control of the experimenter and can be planned around in both laboratory and field experiments and accounted for when comparing values from other species in the literature. However, many other aspects of physiology and life history can impact RCR values and are challenging to consider in field studies.

Indeed, working with wild-caught animals in the field requires the researcher to accept numerous unknowns about the life history of an organism, many of which may impact RCRs. For example, it is often not possible to ascertain the age, health status, reproductive stage, or even sex of a wild-caught animal, all of which may impact mitochondrial respiration and RCRs (Porter et al. 1996, 2015; Brand et al. 2003; Iossa et al. 2004; Klinge 2008, 2020; Chen et al. 2009; Gaignard et al. 2017; Norheim et al. 2019; Boel et al. 2020; Melanie et al. 2020; Andrieux et al. 2021; Barbe et al. 2023; Sartori et al. 2024). Furthermore, these factors often interact with each other and have variable impacts across different tissues. For example, in birds, RCRs tend to decline with allometry in skeletal muscle but not cardiac mitochondria (Barbe et al. 2023). Taken together, these variables highlight the difficulties in using a standard “cut-off” to assess acceptable RCR values.

Additional considerations—physiological temperatures

Another common issue that often arises in the study of wild animals is that there are often small variations in the

resting body temperature of different species. For example, NMRs have a body temperature of $\sim 32^\circ\text{C}$, whereas the body temperature of social species of African mole-rats is around 35°C , and that of standard laboratory rodents is $\sim 37^\circ\text{C}$. This is an important consideration, and many researchers choose to conduct experiments at a common temperature (usually the more anthropomorphic temperature of 37°C). However, we argue that this is not appropriate. Certainly, mitochondrial enzymes of animals (ectotherms or endotherms) adapt to specific body temperatures to maintain their rate of activity. The causes of such adaptation includes genetic changes to support a given ecological niche, but in ectotherms, this also include a permanent or temporary change in the lipid microenvironment of the enzymes to maintain their fluidity inside a rigidifying membrane layer with decreasing temperatures (Munro et al. 2015). For this reason, it must not be assumed that measuring mitochondrial respiratory function at a lower temperature would result in lower respiratory rate per se if this were done with a species adapted to this lower temperature. For example, in a previous study, our group measured higher rate for certain mitochondrial functions in mitochondria from the marine clam *Arctica islandica* (Linnaeus, 1767) at 10°C (Munro et al. 2023), than we obtained with rat skeletal mitochondria at 37°C (Munro et al. 2019). Others have found similar results (Lemieux et al. 2017). Indeed, mitochondrion are very sensitive to changes in temperature but are not necessarily impacted by temperature in a predictable manner (Blier et al. 2014). Therefore, we think that comparing mitochondria of species with naturally divergent body temperatures must be done using each species' natural temperature.

A case study

As a case in point, we recently studied cardiac mitochondrial respiratory function in isolated mitochondria from 10 rodent species (Munro et al. 2025), including mice and rats, an insectivorous subterranean rodent with a relatively high metabolic demand (star-nosed moles *Condylura cristata* (Linnaeus, 1758)), and 7 species of hypoxia-tolerant African mole-rats (see Fig. 1 legend for species). Apart from mice, rats, and naked mole-rats *Heterocephalus glaber* Rüppell, 1842, all the animals used in this study were wild-caught and thus represent an uncontrolled and unpredictable range of ages and life-histories (see supplementary materials section for methodological details). Notably, the species used in our study are relatively similar in size, ranging in mass from $\sim 50\text{ g}$ to $\sim 2\text{ kg}$, a range of sizes across which whole animal mass is not expected to significantly impact mitochondrial function or RCRs (Boel et al. 2023).

In this study, we used standard rodent heart mitochondria isolation methods (developed specifically in rat heart). When analyzing our results, we noticed highly variable values for both RCR and cytochrome *C* tests but decided to present the results as if mitochondrial preparations of all species were adequately healthy because (1) the values for the rat were quite good with respect to those previously reported for this benchmark species (we obtained an RCR of 7.9 and a 5% increase in respiration rate after addition of cytochrome *C* to

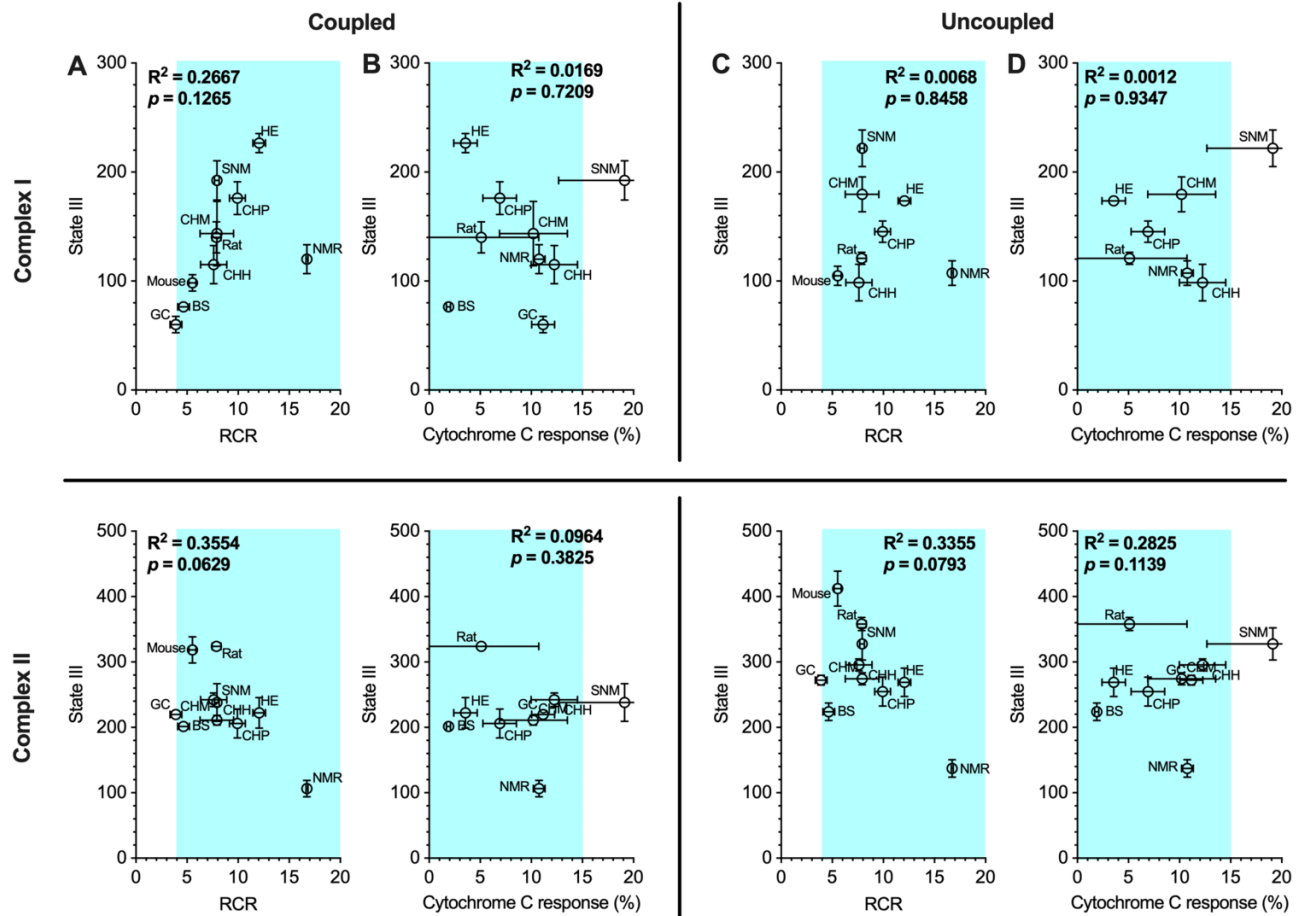
rat cardiac mitochondria) (Gostimskaya and Galkin 2010), (2) mitochondrial isolations were performed using the same protocol, by the same well-versed experimenter (D. Munro), and with the same instruments for all species, and (3) respiration rates were robust and repeatable across each species.

However, we recognized that the protocol that we used may not be optimal for every species that we included in the study. Therefore, we (1) expected some degree of variability in the RCR and % cytochrome *C* increase variables, and (2), because these two measures are supposedly representative of damage to mitochondrial membranes, we expected some degree of correlation whereby lower RCR values and/or greater increases in respiration after addition of cytochrome *C* would be associated with damaged mitochondria and lower respiration rates. Surprisingly, this is not what we found; despite a huge degree of variability across these two markers, there were no clear relationships between these variables and respiration rate across the range of species, for either coupled or uncoupled mitochondria, or with mitochondria respiring through complex I or complex II (Fig. 1).

Furthermore, there was no clear association between “good” RCR values and “good” cytochrome *C* responses within species that would concomitantly suggest a good mitochondrial preparation. For example, it is unlikely that the IMM could have been affected by the isolation protocol in the case of the African mole-rat *Bathyergus suillus* (Schreber, 1782); a relatively poor RCR of 4.66 ± 0.57 , when the OMM seemed remarkably intact with a $1.90\% \pm 0.19$ increase in respiration after addition of cytochrome *C*. As another good example, we considered naked mole-rats (Ruppell, 1842), which have very low basal metabolic rates, a possible requirement for a fossorial species thriving on root vegetable of low caloric value that needs extensive digging from the underground. Accordingly, the LEAK state of our naked mole-rat mitochondrial preparation was very low when measured in the Oroboros chambers in the presence of malate or malate/glutamate, despite OXPHOS and noncoupled states being perfectly comparable to that of other species after addition of ADP and the uncoupler carbonyl cyanide *m*-chlorophenyl hydrazone. The resulting RCR values are therefore very high in naked mole-rats (16.74 ± 0.91); however, this was not associated with a particularly low value for the cytochrome *C* addition test ($10.77\% \pm 0.57$ increase during OXPHOS_{GM}).

In evaluating the above data, we considered the cytochrome *C* and RCR responses from each experiment and also the quality and reproducibility of the experimental data across animals and experiments. Ultimately, we opted to include data from experimental preparation with values in both tests that exceeded the “standard” thresholds for acceptability, but to also openly publish our quality control data to allow the reader to evaluate the results for themselves. The impact of this choice is that populations of subquality mitochondria may have been included in our analysis and thus impacted our conclusions. However, the value of this data from rarely studied and hard to access species is high and we could not exclude the possibility that variation in RCR was a natural phenomenon occurring due to adaptation instead of a poor experimental preparation.

Fig. 1. Relationship between the respiratory control ratio (RCR; A and C) or the effect of exogenous cytochrome C addition (B and D) on coupled (left panels) or uncoupled (right panels) cardiac mitochondrial respiration through complex I (upper panels) or complex II (lower panels) across 10 rodent species. Blue boxes indicate the standard range of accepted but arbitrary values for these parameters. Abbreviations: NMR—naked mole-rat *Heterocephalus glaber*/HE—*Heliophobius emini*/CHP—*Cryptomys hottentotus pretoriae*/CHM—*Cryptomys hottentotus mahali*/CHH—*Cryptomys hottentotus hottentotus*/GC—*Georchus capensis*/BS—*Bathyergerus suillus*/SNM—star-nosed mole *Condylura cristata*.



Solutions and future directions

Our experience suggests that the standard methods of evaluating the quality of mitochondrial preparations may not be useful when working with diverse animal species, and particularly when working with wild-caught specimens with unknowable life histories and/or when the RCR itself is the subject of study. Instead, we propose that arbitrary thresholds of “acceptable” values from RCR and cytochrome C tests should be discarded by the field and instead these values should only be used to evaluate the quality and consistency of preparations within the same species/strain and tissue and not extrapolated to any other species. For example, a researcher studying cardiac function in a particular mouse strain should compare the RCR and cytochrome C test values to results from other studies using the same strain and tissue (assuming they have controlled for substrate used, age, sex, etc.), but a researcher studying cardiac function in a wild-caught African mole-rat should not evaluate the quality of their mitochondrial preparation by comparison to standards derived from studies in mice.

Clearly, alternative methods of quality-control are necessary. This could be accomplished, for example, by a comparison of respirometry and imagery with mitochondrial preparations (Picard et al. 2011), although this is expensive and challenging in the field. Alternatively, comparing the ratio of matrix bound to membrane bound mitochondria-specific enzymes between the original tissue and the mitochondrial isolate would indicate the degree of loss of matrix content, indicating damage to both membranes during isolations steps. Functional measurements can also be used. For example, under certain conditions, measuring or estimating the oxidative phosphorylation efficiency ratio (ATP/O₂) of a given preparation, such as by addition of small, nonsaturating quantities of ADP, or in combining respiration measurement with fluorescent measures of mitochondrial ATP production (Cheng et al. 2021a). Similarly, measurements of mitochondrial membrane potential can be taken to provide a functional assay of IMM integrity, because an intact IMM is required to sustain the proton-motive force that underlies mitochondrial membrane potential (Komlodi et al. 2018).

Alternatively, an increasingly popular approach is to use permeabilized tissue preparations in place of isolated mitochondria (Dawid et al. 2020). Although this approach has its own limitations and issues, which are mostly associated with ensuring that substrates and uncouplers can access the mitochondria through the cell membrane and/or in bulky tissues, it offers the benefit of a relatively intact preparation with mitochondria remaining resident within the cell and presumably better retaining their in situ structure and connections. As a result, data obtained from such preparation may be more physiologically relevant than data from isolated mitochondria. Of course, while this preparation may yield healthier mitochondria, it also requires proper quality control approaches and is subject to the same concerns as those detailed above. There are also additional concerns that are specific to the use of permeabilized preparations. For example, saponin used to permeabilized the cell membrane may also permeabilize the mitochondrial membranes to some degree. In addition, it may be necessary to evaluate these parameters in the presence of an inhibitor of either the ATPase or nucleotide transferase because a certain degree of low level OXPHOS usually persists in intact cells because it is impossible to entirely remove ADP.

Taken together, it is apparent that the current reliance of the field on measurements of RCRs and cytochrome C-mediated respiration are not suitable means of assessing the quality of isolated mitochondria outside of traditional laboratory rodents. This commentary is intended to spark discussion in the field regarding (1) the limitations and pitfalls when using existing and standard methods of mitochondrial quality control, and (2) to highlight that the “optimal” values for these assays originate from experiments in common laboratory rodents and that these thresholds may not apply to the wide range of nontraditional model organisms currently being investigated. We hope that a broader discussion of these issues will lead to a less dogmatic interpretation of the quality of mitochondrial preparations from diverse animal models with variable life-histories.

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No original data are associated with this manuscript.

Author information

Author ORCIDs

Pierre U. Blier <https://orcid.org/0000-0003-1881-2421>

Matthew E. Pamenter <https://orcid.org/0000-0003-4035-9555>

Author contributions

Conceptualization: DM, PUB, MEP

Data curation: DM

Formal analysis: DM, MEP

Funding acquisition: DM, PUB, MEP

Investigation: DM

Methodology: DM, PUB

Project administration: DM, MEP

Resources: DM, MEP

Software: DM

Supervision: MEP

Validation: DM, MEP

Visualization: DM

Writing – original draft: DM, PUB, MEP

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