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Is mitochondrial reactive oxygen species production proportional to oxygen consumption? A theoretical consideration

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Abstract :

It has been assumed that at the whole organismal level, the mitochondrial reactive oxygen species (ROS) production is proportional to the oxygen consumption. Recently, a number of researchers have challenged this assumption, based on the observation that the ROS production per unit oxygen consumed in the resting state of mitochondrial respiration is much higher than that in the active state. Here, we develop a simple model to investigate the validity of the assumption and the challenge of it. The model highlights the significance of the time budget that mitochondria operate in the different respiration states. The model suggests that under three physiologically possible conditions, the difference in ROS production per unit oxygen consumed between the respiration states does not upset the proportionality between the whole animal ROS production and oxygen consumption. The model also shows that mitochondrial uncoupling generally enhances the proportionality.

Keywords : mitochondria, respiration states, theoretical model, , uncoupling, variation

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Introduction

Mitochondria are the major site for both ATP and reactive oxygen species (ROS) production. When produced in excess for antioxidants to keep the steady state concentration in balance, ROS cause oxidative damage to cellular lipids, proteins and DNA, and so become a major contributing factor for oxidative damage and aging [1-5], although some studies have shown extension of lifespan by mildly increasing ROS concentration, perhaps through hermetic mechanisms [6].

Mitochondrial ROS production rate can vary significantly both between and within individuals [7-9]. The "rate of living theory of aging" and its modern version, "the oxidative stress theory of aging", assume a proportional relationship between ROS production and oxygen consumption at the whole organismal level [5,10-12]. This assumption has been challenged by a number of researchers (e.g., [1,13,14]). The challengers noticed the significant difference in the rates of ROS production per unit oxygen consumed between the resting and the active states of mitochondrial respiration, i.e. the minimal and maximal rates of Complex IV activity. ROS production depends on the redox state of the electron transfer chain (ETC) and proton motive force (PMF) across the inner mitochondrial membrane created by the pumping out of protons by the mitochondrial respiratory chain complexes [13,15]. PMF is positively correlated with membrane potential and the gradient of proton concentration [16]. In the resting respiration rate (State 4, the non-phosphorylating state) of the mitochondria, the PMF and membrane potential are high compared to the active state (State 3, the phosphorylating state). This condition causes a high rate of ROS production [17]. In contrast, during periods of active respiration when ATP is being synthesized at a high rate, the elevated oxygen consumption and decreased oxygen partial pressure cause a reduction in the rate of ROS production (as described in Fig. 1 in [17]).

Another factor that affects the difference in ROS production between the resting and active states is mitochondrial uncoupling. During oxidative phosphorylation, the leakage of protons across the mitochondrial inner membrane leads to uncoupling, in which protons bypass the ATP synthase molecule and so shortcut the coupling of substrate oxidation to the phosphorylation of ADP to produce ATP [18]. By reducing PMF, the uncoupling process decreases the rate of ROS production [18]. However, the uncoupling-induced reductions of ROS production are different in the resting and the active states. In the resting state, where PMF is high, the production of ROS is extremely sensitive to the strength of the membrane potential, i.e., a slight uncoupling, which causes a slight reduction in potential, causes a substantial reduction in ROS production. In contrast, in the active state, where PMF is low, the ROS production is not as sensitive to the membrane potential as in the resting state, so in the active state the same degree of uncoupling causes relatively little reduction in the ROS production [19].

Due to the concerted effects of these factors, the ROS production per unit oxygen consumed (denoted as *ROS*/*Oxy* hereafter) is substantially different between the resting and the active. It is noteworthy that for one unit of oxygen consumed, ROS production in the resting state can be as much as 10 times higher than in the active state [13,19], i.e., from the resting state to the active states there can be no or even negative correlation between the ROS production and oxygen consumption. Because of these observations, some researchers claimed that "live fast and die young," the notion underlying the "rate of living theory of aging" [20] is wrong, and should be abandoned [1,13]. (Note: This statement mainly applies for mitochondrial contributions, because mitochondria are not the only source of ROS. They consume 90% of the oxygen uptake by animals. In this study, we do not consider the other 10% non-mitochondrial oxygen consumption, because it is not linked to variation in mitochondrial function.)

In this essay, we test the validity of the claim that whole organismal ROS production rate is proportional to oxygen consumption rate by a simple theoretical model, which is tautological, but allows evaluation of how ROS production varies with oxygen consumption under different levels of mitochondrial activity. We also discuss how mitochondrial uncoupling affects the proportionality at the whole organismal level.

To test the validity, we will compare the ratios of whole organismal ROS production per unit oxygen consumption between two hypothetical animals. This comparison can be applied to individuals of the same species with different body sizes due to individual variation, or individuals of the different species within a taxon, such as different mammalian species. In both cases, animals' mass-specific oxygen consumption (mass-specific metabolic rate) generally decreases with body size [21,22]. The conventional rate of living theory suggests, and data agree, that within a taxon, the mass-specific lifetime energy expenditure of organisms is independent on body mass [20,23-25]. Thus, with a few exceptions, larger animals have lower mass-specific metabolic rate but longer lifespan than smaller ones. According to the most widely accepted modern theory of aging, the free radical theory, the free radicals, such as ROS, are the major driving force of aging. Many researchers in the field (e.g., Barja and co-workers) have shown that the rate of mitochondrial ROS production rate (mtROS) is the "critical factor" for aging [1], and "long-lived animals would not need to maintain high antioxidant enzyme levels, ……, because they would produce mtROS at a low pace." Meanwhile, empirical data have shown that the whole animal ROS production also has strong negative correlation with body size (e.g.,[1]). Based on these theories and observations, it is proposed that at the whole organismal level, the ROS production is proportional to the oxygen consumption. However, the challengers of the rate of living theory have suggested that they are not correlated with each other, because in the

resting state the mitochondrial ROS production rate is as large as 10 time lower than that in the active state.

What the challengers of the theory focused on is the comparison between the active and resting states. Across the respiration states, the ROS production, indeed, has weak or even no correlation with oxygen consumption rate. But the rate of living theory considers the comparison between different animals. It is unclear if, and under what condition, the disproportionality between the respiration states affects the relationship between ROS production and oxygen consumption at the whole organismal level. We will employ a simple theoretical model to investigate this question.

It is important to note that this is a conceptual model. Our purpose is to investigate whether the great difference in *ROS*/*Oxy* between the active and the resting states would break the proportionality between the ROS production and oxygen consumption at the whole organismal level. The model does not aim to simulate experiments, or fit empirical data to obtain values of certain parameters, but makes important conceptual predictions. Thus, the model does not include detailed physiological and biochemical mechanisms of mitochondrial respiration. Although simple, it offers a departure point for future theoretical models that include complex and physiologically realistic mechanisms.

The rate of oxygen consumption is regulated by the ATP requirements of the cells, which depend on the activity of the animal. We presume the resting state of mitochondria in our model to be nearly in (but never equal to) respiration state 4; in the true state 4 condition ATP synthesis ceases completely, but this only occurs during assays of isolated mitochondria. The resting state in our model refers to the *in vivo* state, where ATP synthesis is low but not zero. The active state is similar to respiration state 3, in which ATP synthesis rate is high and *ROS*/*Oxy* is low,

compared to state 4. In reality, mitochondria are somewhere along a continuous function between two states within an organism. In this simple conceptual model, we only chose two extreme states, because the difference in *ROS*/*Oxy* between these two extremes is the largest, and the variation of the proportionality between the whole organismal ROS production and oxygen consumption in the medium states will be bracketed by the two extreme states.

Modeling development and Results

We now present the key assumptions, together with definitions of the parameters and variables.

1. *Level of mitochondrial activity*: One of the most important parameters in our model is the probability *k* of a mitochondrion operating at the active state. This parameter can be interpreted in two ways. Averaging over all the mitochondria in an animal, *k* is the proportion of time that a single mitochondrion is operating in the active state. Alternatively, *k* can be considered as the fraction of the total mitochondria in an animal that are operating in the active state during a given period. These two interpretations are equivalent. The current general consensus is that mitochondria *in vivo* spend a high proportion of their time actively producing ATP [4], but the exact value of *k* is unknown. Thus, in our model, we vary *k* from 0 to 100%.

At the whole organism level, the oxygen consumption rate at maximal rates of exercise has been found to be 3-20 times greater than that at the resting state [26]. We assume that this ratio of maximal to resting rates of oxygen consumption is of similar magnitude at the mitochondrial level, and use "*g*" to denote it; we set *g* to be 5.0 in our calculation.

2. *Difference in ROS production per unit oxygen consumed (ROS/Oxy) between two states*: ROS production is highly variable, having been found to depend on PMF, ADP

availability, substrate concentrations, oxygen partial pressure, and whether the measurement is conducted in isolated mitochondria or *in vivo* [4,15]. The *in vivo* values of *ROS* are currently little known due to technical limitations in measuring ROS production in living animals, and extrapolation of absolute rates of ROS production by isolated mitochondria to the *in vivo* situation is problematic [4]. However, our goal here is to compare *ROS*/*Oxy* between different respiration states, and for this goal it is not necessary to know the absolute values of ROS production. What is important is the difference (the ratio) in it between two respiration states. We denote the ratio of *ROS*/*Oxy* in the active state and that in the resting state as *h*. Some studies on isolated mitochondria suggested that *ROS*/*Oxy* in State 4 can be 10-fold of that in State 3, i.e., the value of *h* is about 0.1 [13,19]. Other studies showed smaller differences between the two states. In isolated mitochondria from mud clam, *ROS*/*Oxy* is twice as high as in State 4 than in that in State 4 ($h = 0.5$) [27]. Another study on mitochondria from rat skeleton muscle showed a roughly 4-fold difference ($h = 0.25$) [28]. Our interest here is to study whether the assumption of "the rate of living" hypothesis—the proportionality between the whole organismal ROS production and oxygen consumption [10,12]—still holds when considering the difference between the resting and active states. Thus, we set *h* to vary between 0.005 and 0.5, so that the *ROS*/*Oxy* in the resting state is 2 (=1/0.5) to 200 (= 1/0.005) times greater than that in the active state.

We now consider an animal. An average mitochondrion of the animal that operates in the resting state consumes *C* units of oxygen per unit time. The oxygen consumption rate of the average mitochondrion in the active state is *g* times higher, so the oxygen consumption in the active state is $g \times C$. Recalling our first assumption, during a given period, the fractions of mitochondria in this animal operating in the resting and the active states are $1 - k$ and k,

respectively, so the whole animal's total oxygen consumption ($O_{2,\text{whole org}}$) is the weighted sum of the oxygen consumptions in the two states: $O_{2,\text{whole org}} = (1-k) \times C + k \times g \times C$. In the resting state, we set the value of *ROS*/*Oxy* to be *R*, and the value in the active state is therefore *h*×*R*. Note, *R* and *h*×*R* are values of per unit oxygen consumed. So, for *C* units of oxygen consumed, the ROS produced in the resting state is *R*×*C*, and that in the active state is *h*×*R*×*C*. Again, the fractions of mitochondria operating in the resting and the active states are $1-k$ and *k*, respectively. So the total ROS produced by all the mitochondria (the weighted sum of the resting and active states) is $ROS_{\text{whole org}} = (1-k) \times R \times C + k \times g \times h \times R \times C$.

Thus, at the whole organismal level, the ratio of the total ROS production and the total oxygen consumption, denoted as *F* (=$ROS_{\text{whole org}}/O_{2,\text{whole org}}$), can be estimated as

$$F = \frac{(1-k) \times R \times C + k \times g \times h \times R \times C}{(1-k) \times C + k \times g \times C} = R \times \frac{(1-k) + k \times g \times h}{(1-k) + k \times g}$$

Here, *R* is set to be the value of *ROS*/*Oxy* in the resting state, which is a constant with an arbitrary unit. As explained above, our goal is not to estimate the absolute value of *F* and compare it to empirical data. Thus, we set the constant *R* to be 1.0 for estimating the relative values. The equation above then reduces to:

$$F = \frac{(1-k)+k \times g \times h}{(1-k)+k \times g} \qquad \text{Eq. 1}$$

With *g* being a constant, the whole animal ROS production per unit oxygen consumption (*F*) only depends on two parameters, *h*, the ratio of ROS per unit oxygen in the active state to that in the resting state; and *k*, the fraction of the time that mitochondria operate in the active

state. We explore the consequences of variation in these two parameters. It is straightforward to see from Eq. 1 that F increases with h, and decreases with k (Fig.1).

Showing that the whole organismal $ROS_{\text{whole org}}/O_{2,\text{whole org}}$ (the value of F) decreases with activity level (k) and increases with the ratio of ROS/Oxy between the respiration states (h) is not the goal of this study, because even without a quantitative model, like ours, researchers in this field can easily reach the same but qualitative conclusion. As stated in the Introduction, our goal is to investigate if, and under what conditions, the disproportionality between the respiration states affect the relationship between the whole animal ROS production and oxygen consumption, and verify the validity of the assumption of the rate of living and oxidative stress theories.

To reach this goal, we need to compare the values of F ($=ROS_{\text{whole org}}/O_{2,\text{whole org}}$) of different animals. If the animals have the same F, then the proportionality holds, i.e., as the whole animal oxygen consumption increases, the whole animal ROS production increases proportionally. In this case, the ratio of F's of two animals is equal to 1.0. If this ratio is close to 1.0, then the variation in F between animals is insignificant, and the whole animal ROS production is roughly proportional to the whole animal oxygen consumption. In contrast, a ratio that is far away from 1.0 indicates that for the same amount of oxygen consumption, one animal produces more ROS than the other animal, and the assumption of "rate of living" hypothesis does not hold.

To estimate the ratio of F's of animals, first, we set the F of an animal with a k of 0.7 as our reference value (note: k varies between 0 and 1. As a reference, it can be set at any value); And then we calculate the ratio of F's of animals with k = 0.1, 0.3, and 0.5 relative to this reference value, i.e., $F_{\text{animal1,2,3}}/F_{\text{reference animal}}$, while h varies from 0.005 to 0.5. Second, we vary k

211 from 0.0 to 1.0, and set the F of an animal with a h of 0.1 as the reference value; we then
212 calculate the ratio of F's of animals with h = 0.005, 0.01, and 0.5 relative to this reference value.
213 Figure 2A shows that if the value of *ROS*/*Oxy* in the active state is 200 times smaller than
214 that in the resting state (h = 0.005, the left ends of the curves in Fig. 2A), then the ratio of F of
215 one animal to the reference ranges from 2-fold (k = 0.5 versus k = 0.7) up to 8-fold (k = 0.1
216 versus k = 0.7). For interpretation of this result, consider two animals, in both of which the
217 difference in *ROS*/*Oxy* between the resting and the active state is 200-fold (i.e. h = 0.005). If the
218 mitochondria of one animal spend 10% of their time in the active state (i.e. k = 0.1), and those of
219 the other animal spend 70% of their time in the active state (k = 0.7), then the first animal
220 produces eight times more ROS per unit oxygen consumed than does the second animal. This is
221 because the mitochondria of the first animal spend most of their time (90%) in the resting state,
222 in which *ROS*/*Oxy* is much higher (200 times) than that in the active state. However, these are
223 extreme values for both h and the difference in k between the two animals. The value of
224 *ROS*/*Oxy* in the resting state is unlikely to be 200 times higher than that in the active state, and
225 the fraction of time spent in the active state of one animal is unlikely to be 7 times smaller than
226 the other animal (k = 0.1 versus k = 0.7).
227 The ratio of F's decreases both as h increases and as the difference in k between two
228 animals decreases. For a more realistic physiological setting, where h = 0.1 (a value obtained
229 from empirical study of isolated mitochondria [19]), and the two animals have similar values for
230 k (e.g. k = 0.5 versus k = 0.7), the ratio of F of one animal to the reference value is greatly
231 reduced from 8-fold to 1.46-fold. Moreover, the real value of h can be even larger than 0.1.
232 Studies on mitochondria isolated from mud clam [27] and rat [28] found that the ROS per unit
233 oxygen in the resting state is 2- and 4-fold of that in the active state, respectively (h = 0.5 and

234 0.25). Once *h* is above 0.1, the ratio is very close to 1.0, as indicated by shallow gradients for the
235 curves in Fig. 2A. Thus, with these realistic physiological parameters, we consider that ROS-
236 oxygen proportionality at the whole organismal level generally holds.

237 Figure 2B shows the ratio of *F*'s for three pairs of animals with different *h* values (*h* =
238 0.005, 0.01, 0.05 versus *h* = 0.1), as *k* varies. The ratio of *F*'s is almost independent on *h* at low-
239 medium values of *k*: for $k < 0.7$, the ratio of *F*'s only ranges from 1.0 to 1.5-fold, even when
240 comparing two animals with very different values of *h* (e.g. *h* = 0.005 versus 0.1; black curve in
241 Fig. 2B). However, the effect of the difference in *h*'s between animals on the ratio of *F*'s
242 becomes increasingly important, as *k* approaches 1.0.

243

244 **Discussion**

245 *Four conditions for the whole organismal proportionality*

246 Our model suggests that, different from the claims by the challengers of the "rate of
247 living" hypothesis (e.g., [1]), the difference in *ROS*/*Oxy* between the resting and the active states
248 ($h \neq 1$) does not necessarily cause the disproportionality between the whole animal ROS
249 production and oxygen consumption. It depends on the values of *k* (the probability of a
250 mitochondrion operating in the active state, which equivalent to the fraction of time it is in this
251 state) and *h* (the difference in *ROS*/*Oxy* between the respiration states) with *k* playing a more
252 important role.

253 The blue curve in Fig. 2A shows that if two animals have the same *h* value that is larger
254 than 0.05, and the *k* values of theirs are similar (*k* = 0.5 versus *k* = 0.7), the ratio of *F*'s between
255 the two animals is smaller than 1.5, even if the *ROS*/*Oxy* in the active state is 20 times lower than
256 that in the resting state (*h* = 0.05). When *h* = 0.5 (a value found in some empirical studies), the

ratio of *F*'s is insignificantly different than 1.0. So, in the case of the blue curve in Fig.1, the whole organismal ROS production is virtually proportional to oxygen consumption for a wide range of *h* values. This means that *ROS*/*Oxy* can be very different between respiration states, but the whole organismal proportionality still holds.

Thus, the first condition for the whole organismal proportionality is that animals under comparison have the same *h* value that is larger than 0.05 and similar values of *k*. The smallest value of *h* found in the empirical studies is 0.1. Also, empirical data suggest that the variation of *h* between animals is small. For example, *h* of mud clam is 0.5 [27] and *h* of rat muscle is 0.25 [28], i.e., 2-fold difference between two species from very different taxon groups. So, we assume that the difference in *h* of the animals from the same taxon is insignificant. Thus, regarding the first condition, the values of *k* are the dominating factor. Excluding the extreme comparisons, such as extreme active versus sedentary individual animals or animal in torpor versus pregnant animals, the condition of "similar *k*" is physiologically realistic, especially for the animals of the same species, which live in the same niche, and have similar level of energy demand. Moreover, it has been found that animals within a taxon, such as mammals or birds, generally have similar field active scope (the ratio of field and resting metabolic rate) [21], indicating that they have similar relative activity level. Thus, it is reasonable to assume that they have similar *k* values too.

The first condition is sufficient but necessary, as the red curve in Fig.2A shows the second condition for the proportionality. If the *k* values of animals are not similar (e.g., $k = 0.3$ v.s. 0.7), as long as the *h* value is large enough (>0.5), the ratio of *F*'s is still insignificantly close to 1.0. However, this condition may not be realistic, because, as far as we are concerned, the largest empirical value of *h* was found to be 0.5 [27].

279 The blue curve in Figure 2B suggests the third condition for the proportionality, which is
280 similar to the first condition. If two animals have the same *k* values, and similar *h* values (e.g., *h*
281 = 0.05 v.s. 0.1 in the blue curve), the ratio of *F*'s is close to 1.0. As we discussed above, this
282 condition is physiologically possible, especially for animals of the same species.
283 The third condition is also sufficient but necessary, as the red and black curves in Fig 2B
284 suggest the fourth condition. The curves show that even if the *h* values of animals under
285 comparison is very different (such as 20-fold, $h = 0.005$ v.s. 0.1, the black curve), for a large
286 range of *k*, from 0 to ~ 0.6, the ratio of *F*'s between two animals is still close to 1.0 (<1.5). Thus,
287 the fourth condition is that the animals have the same value of *k* that is smaller than 0.6. This is
288 also a sufficient but not necessary condition. Very few, if any, empirical studies have
289 investigated how mitochondria allocate their time between operating in the resting versus the
290 active state (the *k* value), and how this varies with physiological demands or environmental
291 conditions. Recalling that *k* is the proportion of time that a single mitochondrion operates in the
292 active state, averaging over all the mitochondria in an animal during a given period, or
293 equivalently the fraction of the total mitochondria in an animal that operates in the active state
294 during a given period. Although no empirical data is available for verification, it is possible that
295 for animals that are not under continuous high energy demands, such as lactating, during any
296 given period an average mitochondrion does not allocate more than 60% of its time in the active
297 state, or no more than 60% of the total mitochondria operating in the active state ($k < 0.6$). We
298 call for future research to investigate this question.
299 Together, these four conditions highlight the importance of the parameter *k*: As long as
300 the animals under comparison have similar *k* values, but do not have to be the same, which are
301 lower than a certain value (our model suggests the value to be 0.6), then no matter how different

the *ROS*/*Oxy* between the active and resting states is (how large the *h* value is) , even if it is as large as 200-fold ($h = 0.005$), the whole organismal proportionality virtually holds, opposite of the suggestion from the challengers of the rate of living theory. Again, it is worth to note that very small value of *h*, such as 0.005 is physiologically unrealistic, because the smallest *h* value found in empirical study is $h = 0.1$ (10-fold difference between the states) [13,19]. Thus, the *k* values of animals under comparison and how close they are the critical factors of the whole organismal proportionality.

It is possible that for a given energy demand, the time budget of mitochondria deviates from that which would minimize ROS production due to other constraints or tradeoffs. For instance, minimizing ROS is unlikely to be of prime importance in semelparous species during their single breeding season, since their fitness is unaffected by any oxidative damage that would only have effects over the long term. It is also worth mentioning that high levels of exercise would shift mitochondria towards the active state, but also increase ROS production defenses at the same time. Quantitative studies on the arms race between the positive and negative effects of exercise, however, remain to be performed.

The effects of mitochondrial uncoupling on the proportionality

It has been shown across a diversity of organisms (including snail, lizard, rat and horse [18]) that the degree of uncoupling, the fraction of oxygen consumption spent on offsetting the proton leak, ranges from 15-25% (in the mitochondria of cells from snail hepatopancreas) to 35~50% (in the mitochondria of rat muscle) with an average of 20%. These values are the averages over the mitochondria operating at different states; the level of uncoupling is usually lower in the active state than in the resting state [18].

Uncoupling may affect the proportionality of the whole animal ROS production and oxygen consumption through two different mechanisms. First, as explained in the Introduction section, uncoupling reduces ROS production by reducing membrane potential. However, the uncoupling-induced reductions in ROS production are different in the resting and the active states. ROS production is more sensitive to membrane potential in the resting state than it is in the active state. So, the same degree of uncoupling in the active state causes relatively less reduction in ROS production, compared to that in the resting state [19]. *ROS*/*Oxy* in the active state is lower than that in the resting state [13,19,27,28], and uncoupling reduces the *ROS*/*Oxy* difference between the two states. Recalling that in our model a declination of the *ROS*/*Oxy* difference between two respiration states is indicated by an increasing *h*, thus uncoupling makes the value of *h* larger. Fig. 2A shows that the variation in *F* across animals (the ratio of *F*) decreases as *h* increases. Thus, our theoretical model suggests that, if everything else kept the same, the mitochondrial uncoupling reduces the variation in *F* across animals, and therefore strengthens the proportionality between the whole organismal *ROS*/*Oxy*.

Second, since uncoupling reduces the ATP synthesis rate, it is possible, although we are not aware of empirical evidence, that to meet the ATP demand of animals, mitochondria may spend more time in the active state, where the ATP synthesis rate is high. This means that uncoupling may increase the value of *k*. However, increasing *k* may not necessarily affect the whole organismal proportionality. Fig. 2A shows that if two animals have the same value of *h*, the ratio of *F's* of them increases as the *difference* in their *k*'s increases (instead of *k* itself). It is possible that the same degree of uncoupling in two animals, especially animals of the same species, increases their *k*'s to the same degree, so that the difference in their *k*'s keeps unchanged. In this case, the ratio of *F*'s is not affected by uncoupling.

Nonetheless, if two animals have different h values, Fig. 2B shows that an increase in k does cause an increase in the ratio of F's. It is important to note, however, the curves shown in Fig. 2B include the cases, where the differences in h's between two animals are very large, h = 0.005 v.s. 0.1 (20-fold), and h = 0.01 v.s. 0.1 (10-fold), which are unrealistic, especially for the animals of the same species. More likely, the difference in h's is much smaller than those values, and uncoupling may not enlarge the difference greatly. So, a more physiologically realistic curve with a smaller difference in h will be around or even below the blue curve in Fig. 2B (h = 0.05 v.s. 0.1, 2-fold difference). In such a curve, even a large k (>0.7) does not offset the proportionality too much.

Conclusion

The assumption of proportionality between ROS production and oxygen consumption at the whole organismal level is one of the fundamental pillars of the rate of living theory and the oxidative stress theory, and plays important roles in the study of aging, such as developing theoretical models [5,29,30], and interpreting the results of experiments [7,31]. Thus, the utility of this model lies in its contribution to conceptually clarifying this controversial issue in the field. Our model considers only two extreme mitochondrial respiration states. A quantitative model that aims to mimic the real mitochondrial respiration, and simulate experiments would consider continuous states of mitochondrial respiration between the two extremes. Moreover, our model assumes static states. For example, the two key parameters in the model are fixed constants during a given period. In reality, they vary with animal's ATP demand, activity level, and other factors, such as aging. So, a more realistic model would consider the dynamic state functions of time, which will lead to first or second order differentials.

Our model suggests that the variation in whole animal ROS production per unit of oxygen consumption across individual animals depends on two parameters, the fraction of the time that mitochondria operate in the active state (the *k* value) and the difference in ROS production per unit oxygen consumed between the active and that in the resting state (the *h* value), with the former affecting the variation more heavily than the latter. The model suggests that under four conditions, three of which are physiologically possible, the difference between the respiration states (the *h* values) does not upset the proportionality between whole animal ROS production and oxygen consumption. Finally. the model suggests that in general the mitochondrial uncoupling makes the correlation between ROS production and oxygen consumption more proportional.

The authors declare no conflict of interest.

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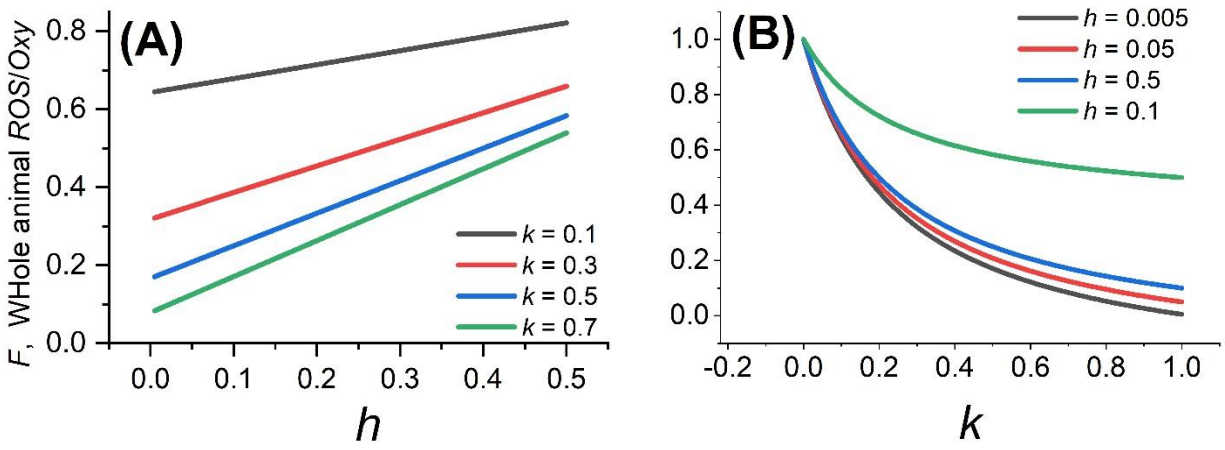

Figure 1. The whole organismal ROS production per unit oxygen consumed, *F*, as a function of *h* (Fig.1A), and *k* (Fig.1B).

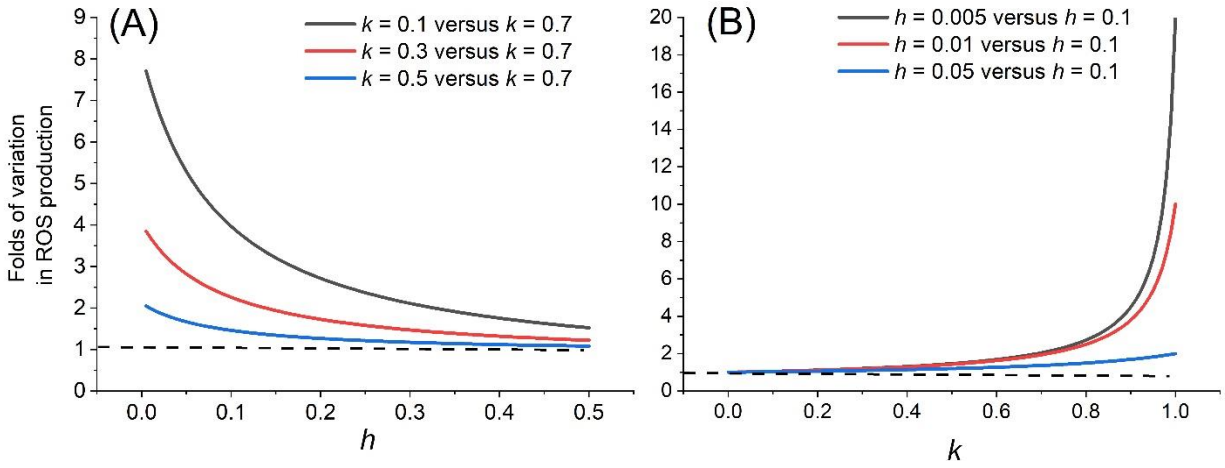

Figure 2. The ratio of *F*'s (the whole organismal ROS production per unit of oxygen consumed) between two animals. (A) The ratio in three pairs of animals as a function of *h*. In each pair, two animals have different values of *k*; **(B)** The ratio in three pairs of animals as a function of *k*. In each pair, two animals have different values of *h*. Curves illustrate the ratios of *F*'s of two animals with different parameter values, e.g., the black line in panel (A) expresses the *F* ratio of an animal with $k = 0.1$ relative to the one with $k = 0.7$. The dashed horizontal lines indicate $F = 1.0$ (perfect proportionality). See text for further explanation.