



HAL
open science

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

Chen Hou, Neil B. Metcalfe, Karine Salin

► **To cite this version:**

Chen Hou, Neil B. Metcalfe, Karine Salin. Is mitochondrial reactive oxygen species production proportional to oxygen consumption? A theoretical consideration. *BioEssays*, 2021, 43 (4), pp.2000165. 10.1002/bies.202000165 . hal-04203335

HAL Id: hal-04203335

<https://hal.science/hal-04203335>

Submitted on 13 May 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Hou, C., Metcalfe, N. B. and Salin, K. (2021) Is mitochondrial reactive oxygen species production proportional to oxygen consumption? A theoretical consideration. *BioEssays*, 43(4), 2000165. (doi: [10.1002/bies.202000165](https://doi.org/10.1002/bies.202000165)).

There may be differences between this version and the published version. You are advised to consult the publisher's version if you wish to cite from it.

This is the peer reviewed version of the following article:
Hou, C., Metcalfe, N. B. and Salin, K. (2021) Is mitochondrial reactive oxygen species production proportional to oxygen consumption? A theoretical consideration. *BioEssays*, 43(4), 2000165, which has been published in final form at [10.1002/bies.202000165](https://doi.org/10.1002/bies.202000165). This article may be used for non-commercial purposes in accordance with [Wiley Terms and Conditions for Self-Archiving](#).

<http://eprints.gla.ac.uk/234339/>

Deposited on: 16 April 2021

24 between the respiration states does not upset the proportionality between the whole animal ROS
25 production and oxygen consumption. The model also shows that mitochondrial uncoupling
26 generally enhances the proportionality.

27

28 **Key words:** mitochondria, variation, respiration states, uncoupling, theoretical model

29

30 **Introduction**

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

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

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

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

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

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

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

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

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

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

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

127

128 **Modeling development and Results**

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

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

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

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

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

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

168 respectively, so the whole animal's total oxygen consumption ($O_{2,whole\ org}$) is the weighted sum
 169 of the oxygen consumptions in the two states: $O_{2,whole\ org} = (1 - k) \times C + k \times g \times C$. In the
 170 resting state, we set the value of ROS/Oxy to be R , and the value in the active state is therefore
 171 $h \times R$. Note, R and $h \times R$ are values of per unit oxygen consumed. So, for C units of oxygen
 172 consumed, the ROS produced in the resting state is $R \times C$, and that in the active state is $h \times R \times C$.
 173 Again, the fractions of mitochondria operating in the resting and the active states are $1 - k$ and k ,
 174 respectively. So the total ROS produced by all the mitochondria (the weighted sum of the resting
 175 and active states) is $ROS_{whole\ org} = (1 - k) \times R \times C + k \times g \times h \times R \times C$.

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

$$178 \quad F = \frac{(1-k) \times R \times C + k \times g \times h \times R \times C}{(1-k) \times C + k \times g \times C}$$

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

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

$$183 \quad F = \frac{(1-k) + k \times g \times h}{(1-k) + k \times g} \quad \text{Eq. 1}$$

184

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

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

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

198 To reach this goal, we need to compare the values of F ($=ROS_{\text{whole org}}/O_{2,\text{whole org}}$) of
199 different animals. If the animals have the same F , then the proportionality holds, i.e., as the
200 whole animal oxygen consumption increases, the whole animal ROS production increases
201 proportionally. In this case, the ratio of F 's of two animals is equal to 1.0. If this ratio is close to
202 1.0, then the variation in F between animals is insignificant, and the whole animal ROS
203 production is roughly proportional to the whole animal oxygen consumption. In contrast, a ratio
204 that is far away from 1.0 indicates that for the same amount of oxygen consumption, one animal
205 produces more ROS than the other animal, and the assumption of “rate of living” hypothesis
206 does not hold.

207 To estimate the ratio of F 's of animals, first, we set the F of an animal with a k of 0.7 as
208 our reference value (note: k varies between 0 and 1. As a reference, it can be set at any value);
209 And then we calculate the ratio of F 's of animals with $k = 0.1, 0.3, \text{ and } 0.5$ relative to this
210 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

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

261 Thus, the first condition for the whole organismal proportionality is that animals under
262 comparison have the same h value that is larger than 0.05 and similar values of k . The smallest
263 value of h found in the empirical studies is 0.1. Also, empirical data suggest that the variation of
264 h between animals is small. For example, h of mud clam is 0.5 [27] and h of rat muscle is 0.25
265 [28], i.e., 2-fold difference between two species from very different taxon groups. So, we assume
266 that the difference in h of the animals from the same taxon is insignificant. Thus, regarding the
267 first condition, the values of k are the dominating factor. Excluding the extreme comparisons,
268 such as extreme active versus sedentary individual animals or animal in torpor versus pregnant
269 animals, the condition of "similar k " is physiologically realistic, especially for the animals of the
270 same species, which live in the same niche, and have similar level of energy demand. Moreover,
271 it has been found that animals within a taxon, such as mammals or birds, generally have similar
272 field active scope (the ratio of field and resting metabolic rate) [21], indicating that they have
273 similar relative activity level. Thus, it is reasonable to assume that they have similar k values too.

274 The first condition is sufficient but necessary, as the red curve in Fig.2A shows the
275 second condition for the proportionality. If the k values of animals are not similar (e.g., $k = 0.3$
276 v.s. 0.7), as long as the h value is large enough (>0.5), the ratio of F 's is still insignificantly close
277 to 1.0. However, this condition may not be realistic, because, as far as we are concerned, the
278 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

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

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

317 *The effects of mitochondrial uncoupling on the proportionality*

318 It has been shown across a diversity of organisms (including snail, lizard, rat and horse
319 [18]) that the degree of uncoupling, the fraction of oxygen consumption spent on offsetting the
320 proton leak, ranges from 15-25% (in the mitochondria of cells from snail hepatopancreas) to
321 35~50% (in the mitochondria of rat muscle) with an average of 20%. These values are the
322 averages over the mitochondria operating at different states; the level of uncoupling is usually
323 lower in the active state than in the resting state [18].

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

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

347 Nonetheless, if two animals have different h values, Fig. 2B shows that an increase in k
348 does cause an increase in the ratio of F 's. It is important to note, however, the curves shown in
349 Fig. 2B include the cases, where the differences in h 's between two animals are very large, $h =$
350 0.005 v.s. 0.1 (20-fold), and $h = 0.01$ v.s. 0.1 (10-fold), which are unrealistic, especially for the
351 animals of the same species. More likely, the difference in h 's is much smaller than those values,
352 and uncoupling may not enlarge the difference greatly. So, a more physiologically realistic curve
353 with a smaller difference in h will be around or even below the blue curve in Fig. 2B ($h = 0.05$
354 v.s. 0.1 , 2-fold difference). In such a curve, even a large k (>0.7) does not offset the
355 proportionality too much.

356

357 **Conclusion**

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

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

380

381 The authors declare no conflict of interest.

382 **References:**

- 383 1 **Barja G.** 2013. Updating the Mitochondrial Free Radical Theory of Aging: An Integrated View,
384 Key Aspects, and Confounding Concepts. *Antioxidants & redox signaling* **19**: 1420-45.
- 385 2 **Sohal RS, Orr WC.** 2012. The redox stress hypothesis of aging. *Free Radical Biology and Medicine*
386 **52**: 539-55.
- 387 3 **Metcalfe NB, Alonso-Alvarez C.** 2010. Oxidative stress as a life-history constraint: the role of
388 reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology* **24**:
389 984-96.
- 390 4 **Murphy M.** 2009. How mitochondria produce reactive oxygen species. *Biochem J* **417**: 1-13.
- 391 5 **Hou C, Amunugama K.** 2015. On the complex relationship between energy expenditure and
392 longevity: Reconciling the contradictory empirical results with a simple theoretical model.
393 *Mechanisms of Ageing and Development* **149**: 50-64.
- 394 6 **Ristow M, Zarse K.** 2010. How increased oxidative stress promotes longevity and metabolic
395 health: The concept of mitochondrial hormesis (mitohormesis). *Experimental Gerontology* **45**:
396 410-8.
- 397 7 **Salin K, Auer SK, Rey B, Selman C, et al.** 2015. Variation in the link between oxygen
398 consumption and ATP production, and its relevance for animal performance.
- 399 8 **Munro D, Treberg JR.** 2017. A radical shift in perspective: mitochondria as regulators of reactive
400 oxygen species. *Journal of Experimental Biology* **220**: 1170-80.
- 401 9 **Salin K, Villasevil EM, Anderson GJ, Auer SK, et al.** 2018. Decreased mitochondrial metabolic
402 requirements in fasting animals carry an oxidative cost. *Functional ecology* **32**: 2149-57.

403 10 **Sohal RS, Mockett RJ, Orr WC.** 2002. Mechanisms of aging: an appraisal of the oxidative stress
404 hypothesis. *Free Radical Biology and Medicine* **33**: 575-86.

405 11 **Speakman JR, Talbot DA, Selman C, Snart S, et al.** 2004. Uncoupled and surviving: individual
406 mice with high metabolism have greater mitochondrial uncoupling and live longer. *Aging Cell* **3**:
407 87-95.

408 12 **Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA.** 2007. Life and Death: Metabolic Rate,
409 Membrane Composition, and Life Span of Animals. *Physiological Reviews* **87**: 1175-213.

410 13 **Nicholls DG.** 2004. Mitochondrial membrane potential and aging. *Aging Cell* **3**: 35-40.

411 14 **Speakman JR, Garratt M.** 2014. Oxidative stress as a cost of reproduction: beyond the simplistic
412 trade-off model. *Bioessays* **36**: 93-106.

413 15 **Kadenbach B.** 2003. Intrinsic and extrinsic uncoupling of oxidative phosphorylation. *Biochimica*
414 *et Biophysica Acta (BBA)-Bioenergetics* **1604**: 77-94.

415 16 **Dzbek J, Korzeniewski B.** 2008. Control over the contribution of the mitochondrial membrane
416 potential ($\Delta\psi$) and proton gradient ($\Delta\mu\text{H}^+$) to the protonmotive force ($\Delta\mu$). In silico
417 studies. *J Biol Chem* **283**: 33232-9.

418 17 **Barja G.** 2007. Mitochondrial Oxygen Consumption and Reactive Oxygen Species Production are
419 Independently Modulated: Implications for Aging Studies. *Rejuvenation Research* **10**: 215-24.

420 18 **Brand MD.** 2000. Uncoupling to survive? The role of mitochondrial inefficiency in ageing.
421 *Experimental Gerontology* **35**: 811-20.

422 19 **Korshunov SS, Skulachev VP, Starkov AA.** 1997. High protonic potential actuates a mechanism
423 of production of reactive oxygen species in mitochondria. *FEBS Letters* **416**: 15-8.

424 20 **Pearl R.** 1928. *The rate of living*: Alfred A. Knopf, New York.

425 21 **Nagy KA, Girard IA, Brown TK.** 1999. Energetics of free-ranging mammals reptiles and birds.
426 *Annual Review of Nutrition* **19**: 247-77.

427 22 **Savage VM, Gillooly JF, Woodruff WH, West GB, et al.** 2004. The predominance of quarter-
428 power scaling in biology. *Functional Ecology* **18**: 257-82.

429 23 **Rubner M.** 1908. *Das Problem der Lebensdauer und seine Beziehungen zum Wachstum und*
430 *Ernahrung* Munich: Oldenbourg.

431 24 **McCoy MW, Gillooly JF.** 2008. Predicting natural mortality rates of plants and animals. *Ecology*
432 *Letters* **11**: 710-6.

433 25 **Speakman JR.** 2005. Body size, energy metabolism and lifespan. *Journal of Experimental Biology*
434 **208**: 1717-30.

435 26 **Weibel ER, Hoppeler H.** 2005. Exercise-induced maximal metabolic rate scales with muscle
436 aerobic capacity. *Journal of Experimental Biology* **208**: 1635-44.

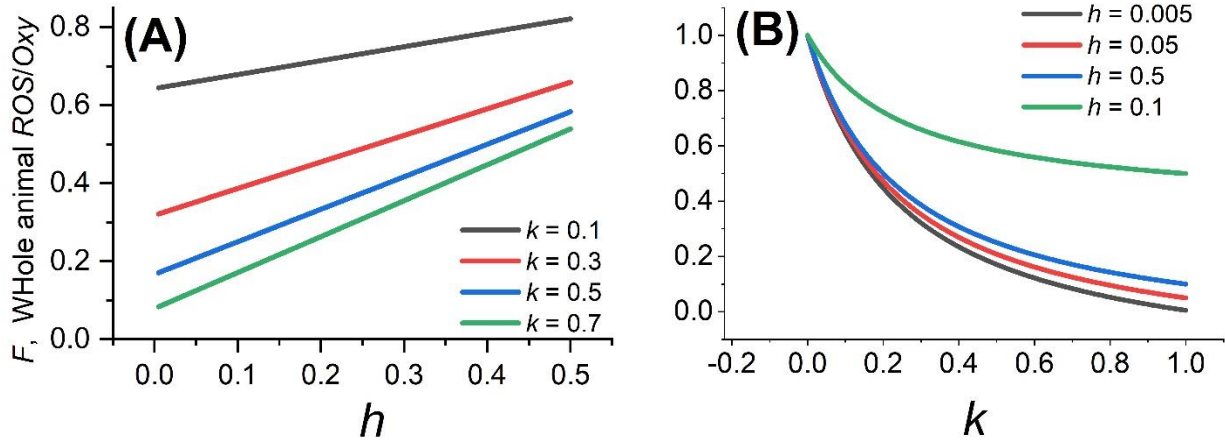
437 27 **Abele D, Heise K, Pörtner H-O, Puntarulo S.** 2002. Temperature-dependence of mitochondrial
438 function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*.
439 *Journal of Experimental Biology* **205**: 1831-41.

440 28 **Venditti P, Bari A, Di Stefano L, Di Meo S.** 2009. Effect of T3 on metabolic response and
441 oxidative stress in skeletal muscle from sedentary and trained rats. *Free Radical Biology and*
442 *Medicine* **46**: 360-6.

443 29 **Hou C.** 2013. The energy trade-off between growth and longevity. *Mechanisms of Ageing and*
444 *Development* **134**: 373-80.

445 30 **Drenos F, Kirkwood TBL.** 2005. Modelling the disposable soma theory of ageing. *Mechanisms of*
446 *Ageing and Development* **126**: 99-103.

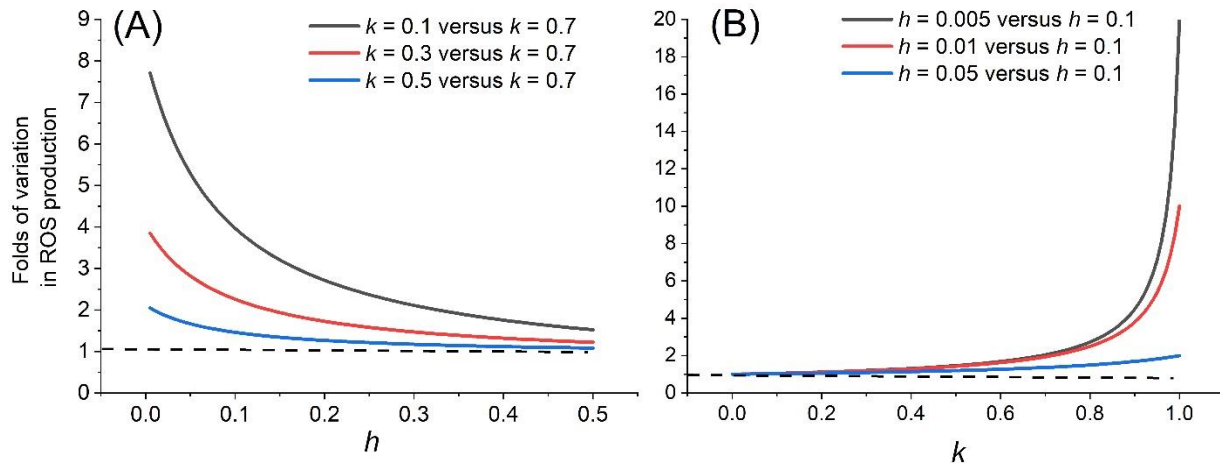
447 31 **Metcalfe NB, Monaghan P.** 2013. Does reproduction cause oxidative stress? An open question.
448 *Trends in Ecology & Evolution* **28**: 347-50.



450

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

453



454

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