November 6, 2017

Dear Dr. Gnaiger:

Thank you for this timely effort. I have worked/discussed with Dr. Buettner on suggestions for this manuscript, from here at The University of Iowa. I offer additional suggestions on the 2017-11-01(14) version as posted (http://www.mitoeagle.org/index.php/MitoEAGLE_preprint_2017-09-21) . Hopefully, the below listed comments will aid in your manuscript preparation and enhance its content and editing.

1. Lines 179 – 180: would be better as "Mitochondria are the oxygen-consuming, electrochemical generators that evolved from endosymbiotic bacteria …"
2. Lines 323-326: Lists are best delineated by a colon (:) to start the list and semicolon to separate elements of the list. This better communicates to readers that each element is a separate and important idea or concept. Strong punctuation brings this across well. It also allows the use of comas (,) within elements of the list without confusion.
3. Line 452, Caption of Fig.4: There should be a period after Fig.2. This is a complete, imperative sentence.
4. Line 487: which should be that.
5. Lines 502-503: love the alliteration it's to the point and concise. Science needs more poetry!
6. 517-518: P-L and E-P there should be a space on each side of the minus sign, P – L and E – P (figure has the spaces).
7. 528—529: See should begin with uppercase S, and the sentence should end with a period.
8. 533: need a colon after on: keeps consistent with comment 2
9. 536, 537, 542, 549: need spaces (E – L) and E – P
10. 574: there should be a period after below below.). This is a complete sentence.
11. 587: I would have insert the word rate "… to ATP at a rate of coupled oxygen …"
12. 595-596: again insert "rate" a decline in the rate of oxygen consumption
13. 618: again a sentence (See Mitchell 2011.),
14. In the footnotes of Table 4, some constants are presented. These are the current values, but they will be changing in 2019. We just as well quote them. See https://www.nature.com/news/new-definitions-of-scientific-units-are-on-the-horizon-1.22837 There are digits for which we will never have the precision to need, but we hope this work stands the test of time far beyond 2019.
15. 738: of: (Comment 2)
16. 739: phosphorylation; (Comment 2)
17. 749-750: Again, the text in the parentheses is used in a full complete sentence. Upper case "For" and period 2017.). are needed. (Comment 3)
18. 891: the multiplication. Is not placed correctly.
19. 915: it would be better to have a space on each side of the + (makes clear this is a formula not a new algebraic expression or constant)
20. 1288: need a space at end of sentence.
21. 1306, 1308; multiplication symbol need better placement.

There may be other edits parallel to those I present above to improve the manuscript and make it more grammatically consistent and clearer.

I think a short section on specification of dose of biochemical tools, such as oligomycin, might be appropriate. The simple concept(s) below can be universally employed across the full scope of biology: this will aid in the design and interpretation of experiments and enhance published data's longevity and usefulness to future generations of scientist. I offer the following suggestion, *Section 4.6:*

4.6 Specification of dose of biochemical additions

Many biochemical tools are used to dissect mitochondrial function. These are generally reported as nominal concentration, *i.e.,* initial concentration as mol L^{-1}. A particular concentration for a reagent can be chosen in light of K_m for a chemical process, *e.g.* ADP and P_i. Other reagents are chosen to inhibit or alter some process, *e.g.* biochemical tools such as oligomycin and carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP). The amount of these tools in an experimental incubation is selected to maximize effect, yet not lead to unacceptable off-target consequences that would adversely affect the data being sought. Specifying the amount of substance in an incubation as nominal concentration can be ambiguous (Doskey *et al. 2015)*. Whereas specifying dose/exposure in mol $cell^{-1}$, *i.e*. ((nominal moles (or mass) of xenobiotic)/(number of cells or, as appropriate, mass of material) with parallels to Section 4.3, *vide supra*). This approach to specification of dose/exposure provides a scalable parameter that can be used to design experiments, help interpret a wide variety of experimental results, and provide absolute information that allows researchers worldwide to make the most use of published data (Doskey *et al. 2015)*.

Doskey CM, van 't Erve TJ, Wagner BA, Buettner GR. (2015) Moles of a substance per cell is a highly informative dosing metric in cell culture. *PLoS ONE* **10(7):** e0132572. PMID: 26172833 http://dx.doi.org/doi:10.1371/journal.pone.0132572 ***Open Access*** PMCID: PMC4501792

End 4.6

We look forward to the final manuscript being published as it has a broad and timely appeal to many. Our team would like to reference this work in future publications. It is not clear where it this work will be submitted and potentially published.

Please consider submitting this work to a peer-reviewed open access journal that is indexed by The *Web of Science* and *PubMed*. ***PubMed*** is especially important!! If the journal is indexed by both, then of course Google Scholar, Scopus, Chem Abstracts, and many others will index the work. This approach to dissemination will ensure the widest possible audience for this work.

Thank you for your attention, Brett A. Wagner, The University of Iowa