Editors' comments and author responses

Manuscript: The extraordinary energy metabolism of the bloodstream *Trypanosoma brucei* forms: a critical review and hypothesis

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Editors: Erich Gnaiger, Sabine Schmitt

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Manuscript edited 2022-11-22: *Only major points included.*

Editors

In the abstract, mitochondrial membrane potential is only a partial component of the protonmotive force. Suggest protonmotive force.

Authors

Changed to mitochondrial protonmotive force.

Editors

Page 5 the sentence "For example, *T. cruzi* epimastigotes have fully functional TCA cycle, ETS and OXPHOS, which maintains *pmF* to allow mitochondrial ATP production." - OXPHOS does not fit here. During OXPHOS, the *pmF* is used but it is only the ETS which really builds up the *pmF*. Of course, the substrates for this come from the TCA cycle and it is therefore also needed, but not OXPHOS. Suggestion: "For example, in *T. cruzi* epimastigotes, a functional TCA cycle and ETS maintain the *pmF* to allow mitochondrial ATP production by oxidative phosphorylation."

Authors

Accepted.

Editors

Since AOX is the abbreviation for alternative oxidase, TAO should be changed to TAOX in the figures

Authors

We do not agree with this change as TAO is the acronym used for trypanosome alternative oxidase by the literature (https://pubmed.ncbi.nlm.nih.gov/?term=trypanosoma+%22alternative+oxidase%22+TAO+NOT+review&sort=date&size=200), not TAOX. Thus, we did not change this.

Editors

This section must be re-written to avoid confusion between temperature change, steady-state temperature, and heat flux. The molar enthalpy change of a defined chemical process is independent of the size of the organelle, cell, or organism. The total enthalpy change depends on the rate of the chemical process. Control of the rate of a chemical process is the most important mechanism to control heat dissipation, far more important than anything considered in this section. At the same rate of O_2 consumption, the rate of heat dissipation is lower in coupled mitochondria only, if ATP accumulates (in contrast to ATP turnover in the cell) or if - at steady-state of ATP/ADP levels and temperature - anabolic endproducts accumulate (more generally: changes of state occur, such as position in the gravitational field, increase of temperature of the system) which lower the enthalpy change of the total process. For a proper thermochemical analysis, see

1. Von Stockar U, Gustafsson L, Larsson C, Marison I, Tissot P, Gnaiger E (1993) Thermodynamic considerations in constructing energy balances for cellular growth. Biochim Biophys Acta 1183:221-40.
2. Gnaiger E, Méndez G, Hand SC (2000) High phosphorylation efficiency and depression of uncoupled respiration in mitochondria under hypoxia. Proc Natl Acad Sci U S A 97:11080-5. https://doi.org/10.1073/pnas.97.20.11080
3. Gnaiger E, Kemp RB (1990) Anaerobic metabolism in aerobic mammalian cells: information from the ratio of calorimetric heat flux and respirometric oxygen flux. Biochim Biophys Acta 1016:328-32.

This topic warrants a critical review of the entire, frequently misleading literature, but this seems to be beyond the scope of the present manuscript.

Authors

We agree with your comment that a detailed thermodynamic discussion of heat flux is beyond the scope of our manuscript. What we want in this discussion is to raise the possibility that the non-conservative nature of energy transduction provided by TAO in bloodstream forms of *T. brucei* could have a "thermogenic role" considering the robust evidence that uncoupling increases heat dissipation at the same respiration rate (doi: 10.1073/pnas.97.20.11080). With this in mind, we then included your suggestions and revised this paragraph as following:

"The non-conservative energy flux through the GPSh-TAO respiratory system in BSF raises a central thermodynamic question: does BSF dissipate energy by increasing the heat flux? If so, are there benefits by increasing the heat flux for a unicellular organism, or is it an unavoidable consequence of this particular "metabolic design"? A simple answer for these intriguing questions remains open and has not yet been directly addressed. Temperature regulation in unicellular eukaryotes is considered to be unlikely because of their microscopic size and the fast heat diffusion from cells to the environment (Jarmuszkiewicz et al 2010). However, we think that evidence collected to date suggests that at least part of the chemical energy made available via GPSh-TAO can be diverted to increase the heat flux, which consequently could be translated to an increase in temperature within mitochondria. Indeed, increased heat flux was already quantified by calorimetry not only in intact brown adipocytes, but even in isolated BAT mitochondria (Ricquier et al 1979; Bokhari et al 2021; De Meis et al 2012). Also, a recently developed temperature-sensitive fluorescent probe (MitoThermo Yellow, MTY) allows the assessment of mitochondrial temperature (Arai et al

2015). Although MTY was originally designed to sense intracellular temperature changes due to alterations in the extracellular milieu, MTY fluorescence can be used to quantify temperature increase/decrease by mitochondrial metabolism (Chrétien et al 2018). This was elegantly demonstrated by ectopically expressing AOX in human embryonic kidney 293 cells which caused no apparent effects on respiration and temperature when cells respire through CIV activity. However, when CIV-dependent respiration was blocked, both processes were preserved strongly indicating that the energy made available by the electron short-circuit provided by AOX is engaged in heat dissipation (Chrétien et al 2018). However, we must keep in mind that the rates of chemical reactions govern the changes in the molar enthalpy and thus heat flux. Since respiratory rates in BSF are much lower than in PCF, it is quite likely that the molar enthalpy change in BSF are not affected (or even reduced) despite its non-conservative nature of energy transduction. However, without a direct assessment of mitochondrial heat flux by calorimetry parallel to oxygen consumption in T. brucei life forms, the possibility of GPSh-TAO could increase heat flux remains open."