Open peer review and authors' responses

The ABC of hypoxia – what is the norm

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Reviewer 1

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*Only major points from review and responses included.

Reviewer 1

Oxygen is the terminal acceptor in the mitochondrial electron transport chain. Besides being part of mitochondrial physiology, oxygen also influences cellular physiology by the formation of reactive oxygen species (ROS). In the past years, some reviews have emphasized the roles played by O_2 in mammalian physiology. Our understanding of the role of oxygen in physiology and biochemistry must be confronted with its tissue availability. In this context, the paper from Chris Donnelly and collaborators reviews normoxia (reference), hyperoxia (high oxygen supply), and hypoxia (low oxygen environment), integrating deviations from these reference points caused by three different mechanisms: 1. Ambient alterations, 2. Biological O_2 demand, and 3. Critical oxygen pressure.

Particularly considering the high burden of hypoxia in human physiology and pathology, the article is very effective in emphasizing the need for a better understanding of O_2 availability for a better design and interpretation of in vitro and in vivo experimental studies. It critically discusses the definitions of normoxia as a reference for hypoxia and functional normoxia. Finally, it sheds light on the causes of deviations from normoxia, giving special attention to the application of hyperoxic conditions in experimental studies of cultured cells and isolated mitochondria. The text is very well written, the topic is interesting, and worth merit. However, the authors must address some points.

Authors

First and foremost, we thank Dr Facundo for their careful reading and comments on our manuscript. We agree with Dr Facundo that certain areas require improvement in

our manuscript to communicate our messages effectively with the readership. We have addressed all comments in the manuscript and in our point-by-point responses below.

Reviewer 1

The authors bring the concept of functional hypoxia, affirming that is restricted to very low intracellular pO2 they call 'oxygen control region' as described in Chance 1965 (properly referenced in the text). Knowing that authors could show (if possible) in Figure 1 the "oxygen control region" published by chance. Additionally, this reviewer thinks that the authors could improve the understanding of the term "oxygen control region" by also showing it in Figure 2.

Authors

We thank Dr Facundo for this comment and suggestion. Rather than amending the Figure to include the trace from Figure 5 in Chance 1965 we have decided to include a description in the legend of Figure 1 and Figure 2 to clarify that "the oxygen control region described by Chance (1965) and referred to in the main text is from the p_c to anoxia" and clarifying as much as possible this concept for the reader.

Reviewer 1

The review needs a better introduction/definition of ambient normoxia (section 2.1.A.) to give the reader the reference of the force (ambient normoxia) that dictates the O2 transport from the environment to the various compartments of an organism. The authors could take into account the variability of "normoxic oxygen" in air, lungs, blood, and tissues (such as the brain). These observations would strengthen the notion that "biological compartmental pO2 is a function of aerobic metabolic activity and O2 transport from the environment to the various compartments of an organism", as written by the authors in section "B".

Authors

We have included another Figure (Figure 2) giving examples of biological compartmental normoxia for some tissues, referencing this Figure in section 2.B.

Reviewer 1

In section 2.2 (causes of deviation from normoxia), the authors bring a very elegant point of view on categories (static) and causes of deviations (dynamic) from normoxia. The first paragraph (also in table 1) defines the static variables in capital letters (A, B, and C) and the dynamic variables in lowercase letters. Firstly, I would suggest changing the separation of dynamic variables in numbers (1, 2, and 3). Changing it would also impact the abstract and other sections. If the authors insist on lowercase letters, please correct the second column of Table 1.

Authors

Thank you for the suggestion. After deliberation we have decided to change the theme to ABC and the deviations **Δ**A, **Δ**B and **Δ**C. We chose this so as to emphasize that the reference points ABC and deviations **Δ**A, **Δ**B and **Δ**C are tightly linked. We have duly updated the second column of Table 1.

Reviewer 1

Section 2.5 (named as Partial pressure and concentration of oxygen) brings interesting basic concepts of normoxia and oxygen distribution in air and water (serum). Given the nature of the discussion in this section, I would suggest moving it to the initial parts of the paper. Maybe, the authors could merge this section with section 2.1.A. "Ambient Normoxia".

Authors

Having extensively considered this suggestion, we decided not to move this section closer to the beginning of the paper. Our reasoning is that our main message is ABC not partial pressure and concentration. We therefore prioritized having the systematic definitions of normoxia in section 2.1. That said, although this is a secondary message, we agree with Dr Facundo that this is an important and complimentary topic to the ABC. We have therefore, thoroughly rewritten section 2.5 considering the comments from both reviewers.