

Dear reviewers, dear editor,

We would like to thank you a lot for your suggestions on our paper. They improved further the quality of our manuscript. We followed carefully all of your suggestions and responded to them below. The main changes are colored in red in the new version of the manuscript.

We hope it is now ready for being recommended in PCI!

Kind regards,

F. Noël, C. Karamaoun, J.A. Dempsey and B. Mauroy.

Review by [Elad Noor](#), 2021-04-27 06:11

Although I haven't seen the original version of the manuscript, it seems that the text organization and clarity has significantly improved, and that all of the previous reviewers' comments have been addressed. In addition, table 3 (the authors wrote in the reply that they added table 2, but I assume they meant 3) is indeed very helpful and clearly demonstrates that the approach is successful. Although I am not an expert on this topic, it seems very convincing that this model and the results are an important step in understanding the allometric scaling laws and filling in some of the existing gaps.

I only have a few minor comments:

- When the mass parameter (M) is introduced, add that it is always given in kg (this is important for the allometric scaling laws, where these units are not mentioned).

Indeed! We added the unit where M is introduced (page 2) and in the legend of Table 1 (page 4).

- I did find it a bit difficult to compare the fractions (predicted) to the decimal point values (observed) in table 1, e.g. when the predicted value was $-1/12$. Perhaps one could add a decimal value next to the fraction (in parentheses)?

Thanks for this remark, we followed your advice and added the decimal values in parenthesis.

- Placing footnotes among the reference list is new to me, and a bit un intuitive. I'm also curious if this wouldn't break some of the automatic cross-referencing tools that exist in journals and aggregators. This is not a critical point, but I can suggest moving them to an appendix with multiple subsections (or a table with numbered bulletpoints), and keeping the citation list clean. Also, it should be clear if a reference is to a paper or to a footnote while reading the main text.

As suggested, we replaced the footnotes in the bibliography by a new appendix with subsections.

- There still remain quite a few grammatical errors, here is a partial list:
 - Page 3: "see equations 3" -> remove "s"
 - Page 3: "the dependance to the metabolic rate" -> "the dependence on the metabolic rate". The word "dependence" is writted as "depend<a>nce", in at least one other occurance.
 - Page 6: "This functional constrain writes in our model ..." -> I'm not sure what this means.

- Page 6: "corresponds to the mean need **in** oxygen of a mammal..." -> "corresponds to the mean oxygen demand for a mammal of mass M"
- Page 6: "the approach is not able to predict the allometric laws at other regimes than BMR" -> "at regimes other than BMR".
- Page 7: there is still a reference to "**the** carbon dioxide".
- Page 10: footnote 40 contains broken references - [?]

Thanks a lot for your help. We corrected the errors mentioned and reread carefully the paper.

Review by [Megumi Inoue](#), 2021-04-20 06:12

I found the concept and research question of the manuscript very interesting, and would like to recommend it for publication with some comments. I am interested in lung branch formation in humans, and while I have found mathematical models that predict branching patterns, I had yet to read an article describing the transition zone between the bronchial and alveolar zones and its predictive location across the mammalian species. As a disclaimer, I am not able to fully follow the mathematical derivations and vet for the models presented.

The authors propose a generalized model across mammals to predict the location of a 'transition zone' between convective and diffusive transport areas in the lung. While several models have proposed predictions of branching structures, models have not been applied to predict where this transition occurs taking into account the energetic cost of ventilation according to mass and metabolic rates across mammalian species. In the manuscript, two models were developed for separate purposes: (1) to estimate O₂ capture in lungs and (2) to estimate ventilation costs. Authors conclude that allometric scaling laws across species for breathing rates and tidal volumes minimize mechanical energy used. This study adds to the work that argues that mammalian tidal volumes and breathing rates are dependent upon a select number of geometrical characteristics.

The introduction clearly explains the two functional zones of the lung bronchial tree and respiratory zones, and what has been found in recent literature regarding the transition zone between the two in humans. The authors build up to the question of expanding across mammalian species in a natural and cohesive manner. The potential for the impact of such a model is explained -- a successful model would be a powerful tool to predict breathing rates at maximal exercise of various mammals, for example. I would be curious to know how we might see applications in obtaining accurate predictions for this. Who would want to use it and why?

Actually, the ventilation parameters at intermediate regimes are difficult to obtain, making the study of the metabolism of mammals at these regimes difficult to analyse [Speakman et al. 1998]. Hence, a clear biophysical understanding of the origin of these scaling laws could allow to extend ventilation-related analyses performed for one mammal species to another mammal species. This could improve the pertinence of using animal models [Matute-bello et al. 2008, Rocco et al. 2020], or, on the contrary, of using human data, richer in the literature, to understand other mammals' metabolism [Haverkamp et al. 2005].

My main concern is with the oversimplification of branching patterns in the lungs. Although this is addressed in discussion, authors may want to consider bringing it up sooner. Lung branching in the mouse is well known to have three modes of branching: domain, planar, and orthogonal branching as described by Metzger et al. 2008, with two phases of a stereotypic pattern layout followed by a series of bifurcations. Lung branching has been described as 'volume filling' -- which is not best described by continuous rounds of bifurcations at the same angle. Early stereotypic branching allows for a final pattern with many alveoli present close to the trachea. This may suggest a larger respiratory zone reserve

than the one considered here. Figure 1 describes a branching pattern with alveoli present only on the outer surfaces. I would also be interested in seeing what branching generations the model predicts in well-studied species (ie. it is known that mice have 13-17 branching generations, and humans 17-23).

Actually, the schematics of the branching of our model (Figure 1) is not really representative of the 3D distribution of airways and alveoli, as we do not account for space localization of the airways in our model. Hence, angles have no meanings in our model, only length and diameters of the airways are accounted for. This is a reasonable assumption considering the physical models used for oxygen transport. Hence, the schematics in Figure 1 should be viewed as an “unfolded tree” and should be viewed as representative of the variations in length and diameters only and not as an indication of spacial occupation.

Indeed, our model accounts only for the property of a branching pattern and not for the specificity of the branching of mammals’ species, such as the mouse. Although it is a limitation of our model, our results indicate that the property of branching might well be a main determinant of the ventilation parameters, and that more specific features might play a secondary role. It is also important to keep in mind that allometric laws are nevertheless “crude” laws and such lung’s geometrical specificities might explain existing drifts of the experimental data from the allometric law.

To make these points clearer, we added:

- In the description of the geometrical model, page 3: “This model accounts for the branching pattern of the lung and for the lengths and diameters of the airways, but not for their spatial distribution. Actually, properties such as branching angles and orientations of the branching planes are not relevant in the model of oxygen transport developed in this work. Moreover, some mammals species have specific branching pattern [Raabe et al. 1976, Maina et al. 2001, Metzger et al. 2008], but we only retain in our model the core property of the mammals lungs to have a tree-like structure.”
- In the legend of Figure 1 the following sentence: “The model for the physics of oxygen transport depends only on the lengths and diameters of the airways but not on their spatial distribution in the lungs. Hence, branching angles and branching planes orientations are not accounted for in this work.”

Our model predicts a total of about 13 generations for a mouse of 25 g and of about 23 generations for a human of 70 kg. We added these example on page 4, after relating the number of generations to the mass:

“Hence, the number of airways generations $N=G+H$ predicted is about 13 for a 30 g mouse and about 23 for a 70 kg human, in agreement with physiological data [Gomes et al. 2002, Weibel 1963].

I have noted several minor improvements to the writing:

-2nd paragraph of Discussion (page 7):

Delete first “the” and replace the second “the” with “specifically”, “especially” or “such as”

“This raises the question about the influence of the other respiratory gas, the carbon dioxide.” → “This raises the question about the influence of other respiratory gases, and specifically, carbon dioxide.”

-6th paragraph of Discussion (page 9):

Replace “Actually” with “Instead”

“Actually, it predicts that small mammals should exhibit a breathing rate at...” → “Instead, it predicts that small mammals should exhibit a breathing rate at...”

-Final sentence:

Bring “more specifically” after “these configurations.”

“Our model could be used to study more specifically these configurations” → “Our model could be used to study these configurations more specifically”

Corrected, thanks a lot!

I would like to add a few points from my side (which you can choose to address or ignore):

- (1) Regarding the last point by reviewer 1 (what branching generations your model predicts), since you do not predict lung geometry directly, but use it to predict breathing volume and frequency, I would like to develop this question even further. Do you think that your results (breathing volume and frequency for basal, field, and maximum metabolic rates) may have a bearing, in reverse, on the ideal lung geometry (considering that in evolution, lung geometry and breathing patterns evolve together)?

Yes, indeed. Actually, it is reasonable to think that both geometry and ventilation parameters did evolve together. We already suggested this idea in the discussion, with the sentence:

“Since morphology itself has probably been selected by evolution in order to minimize the hydrodynamic resistance in a constrained volume [Mauroy et al. 2004, Dubois de la Sablonière et al. 2011], morphology and ventilation patterns are intertwined together in order for the lung to function with a low global energetic cost, i.e. a low hydrodynamic resistance R and a low ventilation cost $P(V_T, f_b)$ that also depends on R .”

but we made it clearer by adding next:

“Actually, this suggests that coevolution of these traits might have occurred in order to keep the cost of breathing as low as possible.”

And, given that animals use their lungs in different “modes” (BMR, FMR or MMR), and that the relative importance of these modes may differ between species, do you think that one should differentiate between species (not just regarding body mass, but also regarding these different lifestyles) when predicting optimal lung geometry?

Again, yes. This is very interesting. Actually, in Weibel et al. 2005, they discussed athletics species versus sedentary species. According to Weibel, the controversies about the exponent of the allometric scaling of metabolism at BMR and VO_{2max} comes from whether we consider subgroups of the species or not. Typical subgroups are the athletic animals or the sedentary ones. Weibel argues that the exponents $3/4$ at rest and $7/8$ at VO_{2max} are valid for the whole group but probably not for some of the subgroups. We suggested this on the last paragraph of page 5 with the sentence:

“Other exponents for metabolic rates, less pertinent for our study, have also been derived for specific subsets of mammals’ species, based for example on the size or on the athletic capacity [49, 50].”

Coming back to lung geometry, indeed, the geometry should depend on the lifestyle of the animals. We already know that the air fluid mechanic (hence the ventilation) plays a role on the geometry of the airways [Mauroy et al. 2003 and 2004], so if the “normal” life of an athletic animal needs a higher ventilation, there should be some specific adaptations of the geometry that, in turn, are reflected in the ventilation parameters.

Moreover, these questions are connected to others about the lung of marine mammals (out of the scope of this paper), for which the environment is drastically different.

- (2) At the end of “Ventilation pattern and energy cost of ventilation”, it could be didactically good to explicitly state your optimality problem as a formula, something like

Maximize $P(V_T, f_b)$ with respect to V_T, f_b subject to $fO_2(V_T, f_b, M) = fO_2^{demand}(M, regime)$

You could continue by saying that the two functions $f_{O_2}(\cdot)$ and $f_{O_2}^{\text{demand}}(\cdot)$ will be described next, that the optimization needs to be carried out numerically, and that the resulting optimal V_T and f_b , again, according to these calculations, show a scaling behavior.

Thanks for the suggestion, we amended this part of the text:

“The mechanical power has to be minimized with the constraint of an oxygen flow to the blood induced by the ventilation $f_{O_2}(V_T, f_b)$ matching the oxygen flow demand \dot{V}_{O_2} of the metabolic regime considered. For a given mammal's mass, this formulates mathematically as searching for

$$\begin{aligned} \text{Min}_{(V_T, f_b) \in \mathcal{H}} \tilde{P}(V_T, f_b) \\ \text{with } \mathcal{H} = \{ (V_T, f_b) \mid f_{O_2}(V_T, f_b) = \dot{V}_{O_2} \} \end{aligned} \quad (3)$$

In the following, we will estimate the oxygen flow $f_{O_2}(V_T, f_b)$ transferred to the blood during the ventilation and the oxygen flow demand \dot{V}_{O_2} according to the metabolic regime. Then, due to the complexity of the model, the optimization of the mechanical power will be carried out numerically. Our model will predict that the optimal tidal volume V_T and breathing rate f_b follow an allometric scaling law.”

(3) To justify Eq. (2), it seems that you argue that the lung tissue is fully elastic. Shouldn't there also be extra energy loss because the tissue is not non perfectly elastic? Or is this a negligible effect?

Indeed, the tissues are viscoelastic. But the viscous part of the tissues is small relatively to the viscous effects due to the air circulation in the airways, hence they are, in general, neglected and the lung is represented with its compliance (elasticity) and its hydrodynamic resistance (= viscous part corresponding to the air circulation in the airways), as we did in this work.

(4) The way your simulation works is not fully clear to me. Do you simulate many rounds of inhaling and exhaling until all variables become periodic, and then determine the oxygen transfer? I'm asking this because I was wondering about your initial conditions for the oxygen partial pressure (and how much of an effect they have on the end result).

Yes, this is exactly what we did. We made this clearer by adding the sentences:

* in the Appendix “initial condition”: “This initial condition allows to speed up the algorithm by giving a non-trivial and physically relevant oxygen distribution at the start of the algorithm. Nevertheless, it is necessary to run the model of oxygen transport for several ventilation cycles to reach oxygen profiles in the airways that are periodic in time.”

* in the Appendix “numerical simulations”: “All the model predictions are based on computations made when the oxygen profile is periodic.”

And how do you run the numerical optimization?

Indeed, this was not detailed. We added the details in the Appendix “numerical method”: “The optimization process is made by inverting numerically the implicit constraint $f_{O_2}(V_T, f_b) = \dot{V}_{O_2}$ with the secant method. The inversion is equivocal and allows to compute numerically the non-linear function $f_b \rightarrow V_T(f_b)$. Then, the optimization is performed on the unidimensional function $f_b \rightarrow P(V_T(f_b), f_b)$ by computing explicitly $dP(V_T(f_b), f_b)/df_b$ from equation (2) and by solving $dP(V_T(f_b), f_b)/df_b = 0$. The derivative $dP(V_T(f_b), f_b)/df_b$ depends on $V_T(f_b)$ and

$dVT(fb)/dfb$. The quantity $dVT(fb)/dfb$ is estimated numerically using the approximation $dVT(fb)/dfb = (VT(fb + m) - VT(fb))/m$ with m a scalar small relatively to fb .”

(5) Your appendix VII is fantastic! Listing the main assumptions behind a model is a great service to the reader! Personally, I would suggest putting this appendix section first in the appendix because it provides a really good overview of the model.

Thanks a lot, we moved it at the beginning of the appendix, as suggested.