Respiratory Physiology & Neurobiology xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Importance of mitochondrial P_{O_2} in maximal O_2 transport and utilization: A theoretical analysis

I. Cano[a,*], M. Mickael[b], D. Gomez-Cabrero[b], J. Tegnér[b], J. Roca[a], P.D. Wagner[c]

[a] Hospital Clinic, IDIBAPS, CIBERES, Universitat de Barcelona, Barcelona, Catalunya, Spain
[b] Unit of Computational Medicine, Center for Molecular Medicine, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden
[c] School of Medicine University of California, San Diego, San Diego, CA 92093-0623A, United States

ARTICLE INFO

Article history:
Accepted 29 August 2013

Keywords:
Bioenergetics
Mitochondrial respiration
Mitochondrial P_{O_2}
Oxygen transport
$\dot{V}_{O_2}$max

ABSTRACT

In previous calculations of how the O_2 transport system limits $\dot{V}_{O_2}$max, it was reasonably assumed that mitochondrial P_{O_2} (Pm_{O_2}) could be neglected (set to zero). However, in reality, Pm_{O_2} must exceed zero and the red cell to mitochondrion diffusion gradient may therefore be reduced, impairing diffusive transport of O_2 and $\dot{V}_{O_2}$max. Accordingly, we investigated the influence of Pm_{O_2} on these calculations by coupling previously used equations for O_2 transport to one for mitochondrial respiration relating mitochondrial $\dot{V}_{O_2}$ to P_{O_2}. This hyperbolic function, characterized by its P_{50} and $\dot{V}_{MAX}$, allowed Pm_{O_2} to become a model output (rather than set to zero as previously). Simulations using data from exercising normal subjects showed that at $\dot{V}_{O_2}$max, Pm_{O_2} was usually <1 mm Hg, and that the effects on $\dot{V}_{O_2}$max were minimal. However, when O_2 transport capacity exceeded mitochondrial $\dot{V}_{MAX}$, or if P_{50} were elevated, Pm_{O_2} often reached double digit values, thereby reducing the diffusion gradient and significantly decreasing $\dot{V}_{O_2}$max.

1. Introduction

At rest or during exercise, production of ATP requires both physical O_2 transport from the environment to the mitochondria and subsequent chemical utilization of O_2 by oxidative phosphorylation. Oxygen transport has been well described (Dejours and Kayser, 1966; Gnaiger et al., 1998; Weibel et al., 1981) based on the O_2 transport pathway, consisting of the lungs/chest wall, the heart, vascular tree and blood, and the tissues. These structures conduct O_2 as an in-series system in which the main sequential transport steps are ventilation, alveolar-capillary diffusion, circulatory transport, and tissue capillary to mitochondrial diffusion. At each step, the mass of O_2 must be conserved, and this allows a set of simple equations to be defined (Wagner, 1993, 1996b) that quantifies how the transport process at each step integrates with those of the other steps to determine how much O_2 is delivered to the mitochondria per minute (Wagner, 1996a). In this construct, it is shown that each of the four steps contributes to limitation to $\dot{V}_{O_2}$max and that the quantitative effects of changes at each step are similar.

Systems physiological investigations (Wagner, 1993, 1996b) targeting the understanding of the limits to maximal $\dot{V}_{O_2}$, have previously been performed on the basis of an important simplifying approximation. This has been that the downstream mitochondrial P_{O_2} (Pm_{O_2}) is so small in comparison to tissue capillary P_{O_2} that it can be ignored and therefore set to zero, thus making the analyses of O_2 transport much more tractable. However, because O_2 is one of the molecules that drive oxidative phosphorylation according to the law of mass action, this approximation cannot be physiologically correct, or otherwise $\dot{V}_{O_2}$ would itself be zero.

Given that Pm_{O_2} must exceed zero, the P_{O_2} difference between red cells and mitochondria must be less than when Pm_{O_2} is assumed to be zero, and thus the diffusive movement of O_2 between them must also be reduced. Therefore, if Pm_{O_2} is now considered as greater than zero, there is an additional resistance, from the process of mitochondrial respiration, to O_2 movement through the entire pathway of O_2 transport and utilization. We therefore hypothesize that this additional resistance must reduce maximal $\dot{V}_{O_2}$ below that which would be expected if this resistance were ignored. Clearly, the degree to which $\dot{V}_{O_2}$max would be reduced will depend on how the high mitochondrial P_{O_2} rises above zero. This in turn will depend broadly on the capacity for O_2 transport (how many O_2 molecules can be delivered to the mitochondria per minute) compared to the capacity for metabolism (how many O_2 molecules can be consumed by the mitochondria per minute).

The importance of including consideration of oxidative phosphorylation goes beyond asking how much does mitochondrial respiration contribute to the overall impedance to $\dot{V}_{O_2}$. Because the value of Pm_{O_2} is dependent on the mitochondrial respiration curve/O_2 transport interaction, hypoxia-induced biological

* Corresponding author at: IDIBAPS, C/Villarroel 170, 08036 Barcelona, Spain. Tel.: Tel.: +34 666186748.
E-mail address: iscano@clinic.ub.es (I. Cano).

1569-9048/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.resp.2013.08.020

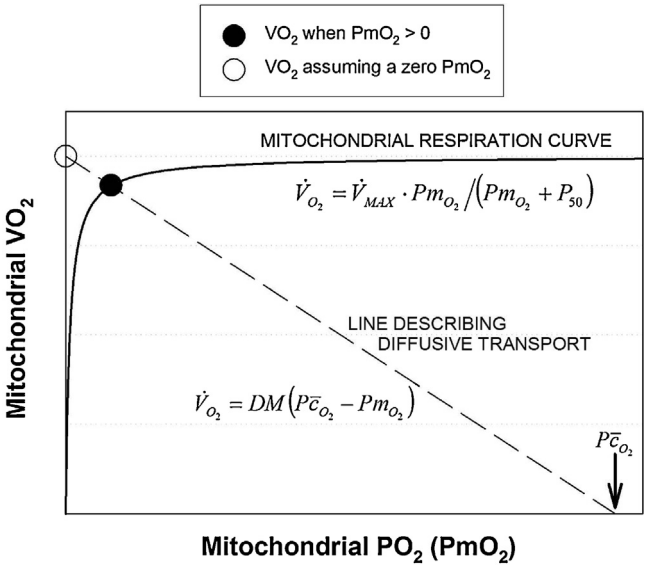

Fig. 1. Graphical analysis of diffusive transport of O_2 from muscle capillary to the mitochondria (dashed line) and subsequent utilization of O_2 through oxidative phosphorylation (solid line). See text for details.

changes may be affected by this interaction. Thus, the significance of the present study is in the degree to which $\dot{V}_{O_2}$max is reduced by the resistance imparted by oxidative phosphorylation and the consequent effect on mitochondrial P_{O_2}, which in turn may affect processes such as generation of reactive oxygen species and hypoxia-induced gene expression.

The purpose of the present paper is therefore to expand the prior theoretical analysis of the integrated O_2 transport pathway (Wagner, 1993, 1996a) by analyzing the consequences for O_2 transport of allowing mitochondrial P_{O_2} to be greater than zero. This requires integration of the previously described O_2 transport equations with an equation for mitochondrial respiration, followed by the application of mass conservation principles to solve this new equation system. The same data that were used in (Wagner, 1993, 1996a) are used here.

2. Material and methods

2.1. Principles

Oxidative phosphorylation ensues via the following Eq. (1) that embodies the law of mass action:

$$3ADP + 3Pi + NADH + H^+ + 1/2O_2 \rightarrow 3ATP + NAD + + H_2O \quad (1)$$

In this equation, Pm_{O_2} corresponds to O_2. Clearly, this mass action equation can only move from left to right and produce ATP if Pm_{O_2} is greater than zero.

To illustrate this effect, a graphical depiction of mitochondrial respiration is presented in Fig. 1. Here, the solid line is the relationship between velocity of the reaction (i.e., mitochondrial $\dot{V}_{O_2}$), and Pm_{O_2}, similar to what has been found experimentally (Gnaiger et al., 1998; Scandurra and Gnaiger, 2010; Wilson et al., 1977). It shows how $\dot{V}_{O_2}$ is a positive but non-linear function of mitochondrial P_{O_2}, and indicates that at low Pm_{O_2}, $\dot{V}_{O_2}$ is very sensitive to (and thus limited by) P_{O_2}, while at higher Pm_{O_2}, $\dot{V}_{O_2}$ becomes independent of P_{O_2}, and is limited by factors other than O_2.

The hyperbolic curve through the origin displayed in Fig. 1 represents mitochondrial respiration. It is of note that despite mitochondrial respiration kinetics is not really a Michaelis–Menten type (Johnson and Goody, 2011; Michaelis and Menten, 1913), experimental data (Gnaiger et al., 1998; Scandurra and Gnaiger, 2010) are well fitted by such a curve. As a hyperbola, it can be represented by Eq. (2):

$$\dot{V}_{O_2} = \frac{\dot{V}_{MAX} \cdot Pm_{O_2}}{Pm_{O_2} + P_{50}} \quad (2)$$

where $\dot{V}_{O_2}$ is mitochondrial $\dot{V}_{O_2}$ (the ordinate in Fig. 1); $\dot{V}_{MAX}$ is the asymptote of the curve, and represents the maximal rate of use of O_2 when O_2 is in excess; Pm_{O_2} is mitochondrial P_{O_2} (the abscissa in Fig. 1) and P_{50} is the P_{O_2} at 50% of $\dot{V}_{MAX}$. Thus, the mitochondrial respiration curve is defined by two parameters: $\dot{V}_{MAX}$ and P_{50}.

Also shown in Fig. 1 is a straight (dashed) line of negative slope. It represents the Fick law of diffusion and depicts diffusive O_2 transport between the tissue capillary and the mitochondria as a function of mitochondrial P_{O_2} for a given tissue O_2 diffusional conductance (DM) and a given tissue mean capillary P_{O_2} ($P\bar{c}_{O_2}$), both at maximal exercise. We previously utilized this representation as a tool for interpreting intracellular oxygenation data obtained using magnetic resonance spectroscopy (Richardson et al., 1999). The equation is as follows:

$$\dot{V}_{O_2} = DM \cdot (P\bar{c}_{O_2} - Pm_{O_2}) \quad (3)$$

As the figure indicates, as Pm_{O_2} is increased, $\dot{V}_{O_2}$ in Eq. (3) must fall because the P_{O_2} difference between mean capillary and mitochondrial P_{O_2} is reduced. Thus, Fig. 1 shows how $\dot{V}_{O_2}$ *increases* with mitochondrial P_{O_2} according to oxidative phosphorylation, but *decreases* with mitochondrial P_{O_2} according to the laws of diffusion.

The key concept in Fig. 1 is that in a steady state of O_2 consumption, $\dot{V}_{O_2}$ given by both Eqs. (2) and (3) must be the same at the same mitochondrial P_{O_2} (i.e., the law of mass conservation applies). This can occur only at the single point of intersection between the two relationships, as indicated by the solid circle placed there. If, as previously approximated (Wagner, 1996b), mitochondrial P_{O_2} were truly zero, $\dot{V}_{O_2}$ would be higher, as indicated by the open circle at the left end of the dashed straight line in Fig. 1. For a given O_2 transport system defined by the conductances for O_2 allowed by ventilation, alveolar-capillary diffusion, circulation, and capillary to mitochondrial diffusion, the values of mitochondrial $\dot{V}_{MAX}$ and P_{50} (Eq. (2)) will thereby influence maximal rate of O_2 utilization, $\dot{V}_{O_2}$max. In the remainder of this paper, it will be important to distinguish between $\dot{V}_{MAX}$ (the asymptote to the mitochondrial respiration curve) and $\dot{V}_{O_2}$max (actual maximal rate of O_2 utilization, solid circle in Fig. 1) to avoid confusion. In general, $\dot{V}_{MAX}$ can exceed $\dot{V}_{O_2}$max, but $\dot{V}_{O_2}$max cannot exceed $\dot{V}_{MAX}$.

2.2. Modeling the O_2 transport/utilization system

The present study augments our prior approach (Wagner, 1993, 1996b) by adding Eq. (2) to the equation system used previously. Fig. 2 recapitulates the O_2 transport pathway, and the associated four mass conservation equations governing O_2 transport at each step. It adds Eq. (2), describing O_2 utilization as a function of Pm_{O_2}. The important point is that in this way, the system has expanded from four equations with four unknowns into a system of five equations and five unknowns.

Briefly, using specified input values for O_2 transport step parameters (i.e., values of inspired O_2 fraction (FI_{O_2}), ventilation ($\dot{V}I$, inspired; $\dot{V}A$, expired), lung diffusing capacity (DL), cardiac output ($\dot{Q}$), [Hb], acid base status, tissue (muscle) diffusing capacity (DM), and mitochondrial respiration curve parameters ($\dot{V}_{MAX}$ and P_{50})), five mass conservation equations are written for O_2 (see Fig. 2). They describe (a) ventilatory transport; (b) alveolar–capillary diffusion; (c) circulatory transport; (d) muscle

Please cite this article in press as: Cano, I., et al., Importance of mitochondrial P_{O_2} in maximal O_2 transport and utilization: A theoretical analysis. Respir. Physiol. Neurobiol. (2013), http://dx.doi.org/10.1016/j.resp.2013.08.020

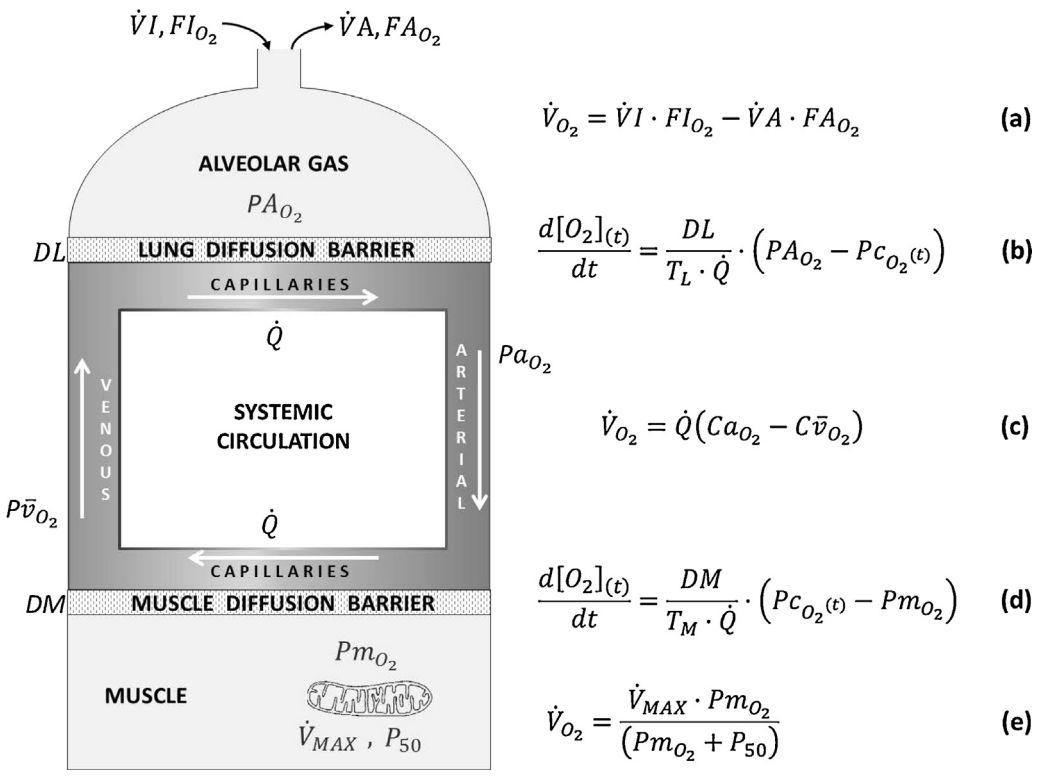

Fig. 2. Schematic representation of the oxygen transport and utilization system considered in this study and the five associated mass conservation equations governing O_2 transport (Eqs. (a)–(d)) and utilization (Eq. (e)).

capillary–mitochondrial diffusion; and (e) mitochondrial respiration. There are five unknowns in these equations: Alveolar P_{O_2} (PA_{O_2}), arterial P_{O_2} (Pa_{O_2}), venous P_{O_2} ($P\bar{v}_{O_2}$), mitochondrial P_{O_2} (Pm_{O_2}) and $\dot{V}_{O_2}$ itself. In Fig. 2, Eqs. (b) and (d) are differential equations describing the process of diffusion across the lung blood: gas barrier and across the tissue capillary wall, respectively. They specifically describe the time rate of change of O_2 concentration, $[O_2]$, along the respective capillary as a function of the diffusing capacity, blood flow, red cell capillary transit time (T_L (lungs); T_M (tissues)) and the instantaneous difference between upstream and downstream P_{O_2} values (alveolar and pulmonary capillary in (b); capillary and mitochondrial in (d)). The two equations are each expressions of the Fick law of diffusion.

The additional inputs of mitochondrial $\dot{V}_{MAX}$ and P_{50}, and the additional coding for the fifth equation were added to the prior model, and the same (numerical) method of solution employed before (Wagner, 1996b) was used to find the solutions for any set of input variables, defined as the unique values of the five unknowns listed above that simultaneously satisfy all five equations for the given input data defining O_2 transport and utilization.

2.3. Input data for simulations

The input data defining the O_2 pathway parameters used in this analysis were essentially identical to those used previously (Wagner, 1996b), and come from Operation Everest II (Sutton et al., 1988). They reflect maximal exercise by normal subjects at sea level, at a chamber "altitude" of 4573 m (approximately 15,000 ft.) and at the chamber altitude of the Everest summit, 8848 m (approximately 29,000 ft.). They are reproduced in Table 1. It is clear that data do not exist for the two new key variables: mitochondrial $\dot{V}_{MAX}$ and P_{50}. Therefore, for each of the three data sets we computed solutions to the equation system over a systematic range of five mitochondrial $\dot{V}_{MAX}$ (1000, 2000, 3000, 4000, and 5000 ml/min) and four mitochondrial P_{50} values (0.1, 0.3, 0.5 and 1.0 mm Hg), resulting in 20 combinations of the two, and thus 20 mitochondrial respiration curves. The values of $\dot{V}_{MAX}$ were chosen to encompass the range of $\dot{V}_{O_2}$ max from the very sedentary to the elite athlete. Values of P_{50} on the other hand were based on physiological studies inscribing the mitochondrial respiration curve from samples of normal muscle (Gnaiger et al., 1998; Scandurra and Gnaiger, 2010).

A typical example from one of these papers is reproduced with permission in Fig. 3, where the hyperbolic character of the curve and its P_{50} can both be seen by the fitted curve. In this and other similar published cases (Gnaiger et al., 1998; Scandurra and Gnaiger, 2010), P_{50} is close to 0.3 mm Hg. This accounts for our choice of P_{50} values – from a third of this typical value to about threefold greater. However it should be stressed that the modeling can be based on

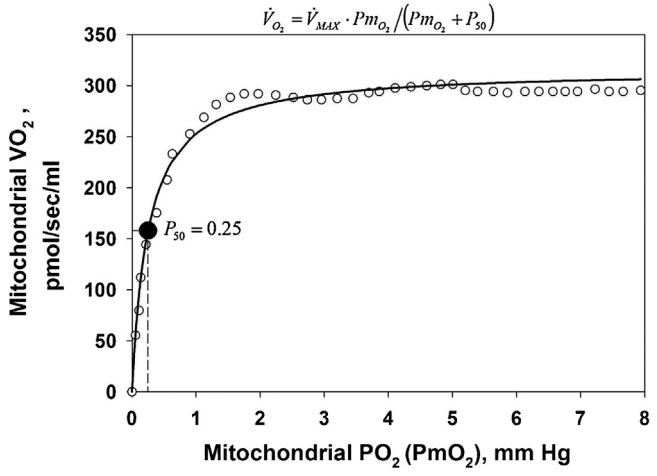

Fig. 3. Graphical depiction of the hyperbolic equation for oxidative phosphorylation fitted to the data of Scandurra and Gnaiger (2010), p16.

Table 1

Parameter	Normal subjects at...		
	Sea level	15,000 ft.	Everest summit
Barometric pressure (PB), Torr	760	464	253
Fractional inspired oxygen (FI_{O_2})	0.2093	0.2093	0.2093
Alveolar ventilation ($\dot{V}$), BTPS, ($L\,min^{-1}$)	112	125	165
Blood flow ($\dot{Q}$), ($L\,min^{-1}$)	23	21	16
Hemoglobin concentration ([*Hb*]) ($g\,dl^{-1}$)	14.5	15.5	18.0
Body temperature (*T*), (°C)	38	38	37
O_2 dissociation curve P_{50} (Torr)	26.8	26.8	26.8
Total lung O_2 diffusing capacity (DL) ($ml/min\,Torr^{-1}$)	51	80	100
Total muscle O_2 diffusing capacity (DM) ($ml/min\,Torr^{-1}$)	102	88	62
Maximum O_2 uptake ($\dot{V}_{O_2}max$) ($L\,min^{-1}$)	3.82	2.81	1.46

any combination of P_{50} and $\dot{V}_{MAX}$, and need not be limited to the choice of specific parameters appearing here.

2.4. Analysis

Across the matrix of $\dot{V}_{MAX}$ and P_{50} values, we posed two questions: First we asked how much would Pm_{O_2} have to rise above zero to satisfy mass conservation and drive mitochondrial respiration for the given set of physiological O_2 transport variables, $\dot{V}_{MAX}$ and P_{50} – and as a result, how much would that cause $\dot{V}_{O_2}$ to be reduced (compared to assuming $Pm_{O_2} = 0$) as per Fig. 1 (comparing the open and closed circles). This question allows a quantitative description of the theoretical consequences for $\dot{V}_{O_2}$max of any combination of mitochondrial $\dot{V}_{MAX}$ and P_{50}. While this is a very useful question to answer, in reality $\dot{V}_{O_2}$max is a directly measured variable. Therefore, asking how much would it be reduced by any pair of $\dot{V}_{MAX}$ and P_{50} values is hypothetical. On the other hand, muscle diffusing capacity, DM, is a variable calculated on the assumption that mitochondrial P_{O_2} can be neglected and set to zero – the very approximation that the present study is addressing.

Thus, another way to interrogate the model system can be proposed, leading to a second question: It recognizes that the muscle O_2 diffusion step was previously modeled, and muscle diffusing capacity estimated, on the basis of $Pm_{O_2} = 0$. However, if Pm_{O_2} is greater than zero, the capillary to mitochondrial O_2 diffusion gradient would be reduced, and this would necessitate, by the Fick law of diffusion, a higher value of DM to accomplish a given, measured $\dot{V}_{O_2}$max (compared to the value calculated assuming $Pm_{O_2} = 0$).

Therefore, for each of the combinations of $\dot{V}_{MAX}$ and P_{50}, we asked how much would muscle diffusing capacity have to increase to maintain $\dot{V}_{O_2}$ constant at the measured value as a result of Pm_{O_2} being greater than zero.

3. Results

3.1. Effects of mitochondrial respiration on Pm_{O_2} and maximal $\dot{V}_{O_2}$

Fig. 4 shows how the different combinations of mitochondrial $\dot{V}_{MAX}$ and P_{50} affect $\dot{V}_{O_2}$max. The upper panel covers the mitochondrial P_{O_2} (Pm_{O_2}) range from 0 to 20 mm Hg; the lower panel shows the same data, but expands the abscissa to better reflect the lower Pm_{O_2} range between 0 and 5 mm Hg. In both panels, each solid curved line emanating from the origin represents one of the 20 mitochondrial respiration curves (as in Fig. 3) for a particular $\dot{V}_{MAX}$ and P_{50} combination. Solid circles reflect sea level conditions; solid squares represent moderate altitude and solid triangles are for the equivalent of the Everest summit. It turns out that at each altitude, an approximately straight line can be drawn through the resulting $\dot{V}_{O_2}max/Pm_{O_2}$ solution points for each mitochondrial respiration curve. These are the dashed lines in the figure.

The values of $\dot{V}_{O_2}$max at each altitude at the point where Pm_{O_2} equals zero (open symbols at zero Pm_{O_2}) are the same as those described in (Wagner, 1996b) where Pm_{O_2} was taken to be zero. The figure shows how relaxing that approximation affects $\dot{V}_{O_2}$max for each combination of mitochondrial $\dot{V}_{MAX}$ and P_{50}.

At sea level (solid circles), results show that allowing for a non-zero Pm_{O_2} has a small but significant impact on $\dot{V}_{O_2}$max. For example, $\dot{V}_{O_2}$max at Pm_{O_2} = 0mmHg (open circle) would be 3827 ml/min, but if $\dot{V}_{MAX}$ were 4000 ml/min and P_{50} 1.0 mm Hg, $\dot{V}_{O_2}$max would be significantly less, by 9%, and would be 3477 ml/min. Moreover, this would require a mitochondrial P_{O_2} of 6.7 mm Hg to drive oxidative phosphorylation, as the figure shows.

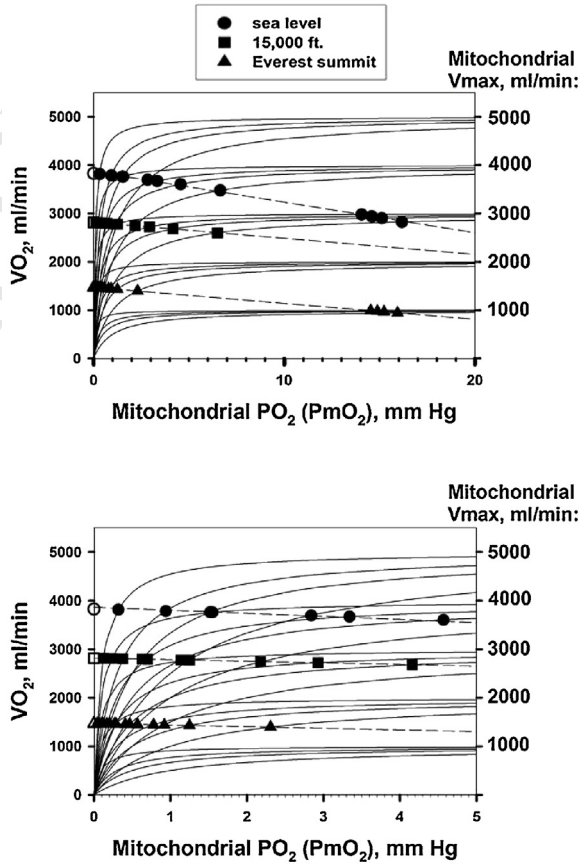

Fig. 4. Effects of considering mitochondrial respiration on maximal $\dot{V}_{O_2}$ and mitochondrial P_{O_2}. For each $\dot{V}_{MAX}$ value, the four hyperbolic curves represent P_{50} values of 0.1, 0.3, 0.5 and 1.0 mm Hg, left to right. See text for details.

In general, for the fixed set of O_2 transport parameters used (see Table 1), the lower the $\dot{V}_{MAX}$ and the higher the P_{50}, the greater is the reduction in $\dot{V}_{O_2}$, and the higher is the Pm_{O_2} required to drive ATP generation. The range of possible values of mitochondrial P_{O_2} is considerable, from a fraction of a mm Hg to more than 10 mm Hg, depending on $\dot{V}_{MAX}$ and P_{50}.

The same outcome is seen at each altitude, but with $\dot{V}_{O_2}$ lower at any Pm_{O_2} as PI_{O_2} is reduced. The reduction in $\dot{V}_{O_2}$ per unit change in Pm_{O_2} is somewhat less at altitude than at sea level, but if examined as a percent of $\dot{V}_{O_2}$ at $Pm_{O_2} = 0$ at each altitude, the effects of allowing for mitochondrial respiration on maximal $\dot{V}_{O_2}$ are relatively similar across altitudes.

In summary, the higher the mitochondrial $\dot{V}_{MAX}$ and the lower the P_{50}, the more O_2 can be metabolized for a given upstream (heart, lungs, blood, muscle) transport system. Mitochondrial P_{O_2} at $\dot{V}_{O_2}$max can be neglected when considering O_2 transport only when mitochondrial P_{50} is low and mitochondrial $\dot{V}_{MAX}$ is high. When $\dot{V}_{MAX}$ is low and/or P_{50} is high, the mitochondrial P_{O_2} required to drive oxidative phosphorylation may reach double digit values, and the impact on $\dot{V}_{O_2}$max can be considerable.

3.2. Maintenance of maximal $\dot{V}_{O_2}$ in the face of non-zero Pm_{O_2}

The preceding subsection showed how $\dot{V}_{O_2}$max would have to decrease as a function of mitochondrial $\dot{V}_{MAX}$ and P_{50} with constant values for all O_2 transport conductances. In this subsection we investigate how much higher the muscle O_2 diffusing capacity would have to be to maintain $\dot{V}_{O_2}$max constant over the same range of $\dot{V}_{MAX}$ and P_{50} values as Pm_{O_2} increases above zero.

The results are shown in Fig. 5, which displays the simulation outcomes across the entire matrix of $\dot{V}_{MAX}$ and P_{50} values, using $\dot{V}_{MAX}$ on the abscissa and isopleths for each P_{50}. Results are shown for each altitude as indicated by the different symbols. The top panel shows mitochondrial P_{O_2} for every combination of $\dot{V}_{MAX}$ and P_{50} examined, and the bottom panel the corresponding values of muscle diffusing capacity (DM), that would have to exist to maintain $\dot{V}_{O_2}$max at measured levels (indicated at each altitude by the vertical dashed lines). Comparing panels shows that when Pm_{O_2} is high (thus reducing the P_{O_2} gradient between capillaries and mitochondria), DM must also be high to maintain diffusive O_2 transport.

Also, when mitochondrial $\dot{V}_{MAX}$ substantially exceeds measured $\dot{V}_{O_2}$max (at each altitude), Pm_{O_2} remains low, and therefore DM does not need to be substantially increased to maintain O_2 flux. However, the closer mitochondrial $\dot{V}_{MAX}$ is to measured $\dot{V}_{O_2}$max, the higher Pm_{O_2} must be (see Fig. 4), and therefore, DM is also required to be elevated to maintain O_2 transport. When $\dot{V}_{MAX}$ and actual $\dot{V}_{O_2}$max are very close, the required DM may be as much as four times the value needed when Pm_{O_2} is (close to) zero, and the associated Pm_{O_2} would reach double digit values.

4. Discussion

4.1. Summary of major findings

This study shows that including mitochondrial respiration in analyzing O_2 transport and utilization generally poses a very small additional resistance to the system (over that of the transport pathway alone), only slightly reducing $\dot{V}_{O_2}$max below that computed ignoring this contribution (Fig. 4). The associated mitochondrial P_{O_2} is also usually low (<1 mm Hg). If however mitochondrial $\dot{V}_{MAX}$ is low in relation to O_2 transport capacity, or if mitochondrial P_{50} is high, $\dot{V}_{O_2}$max may be considerably reduced. Mitochondrial P_{O_2} would then increase more, and may reach double digit values.

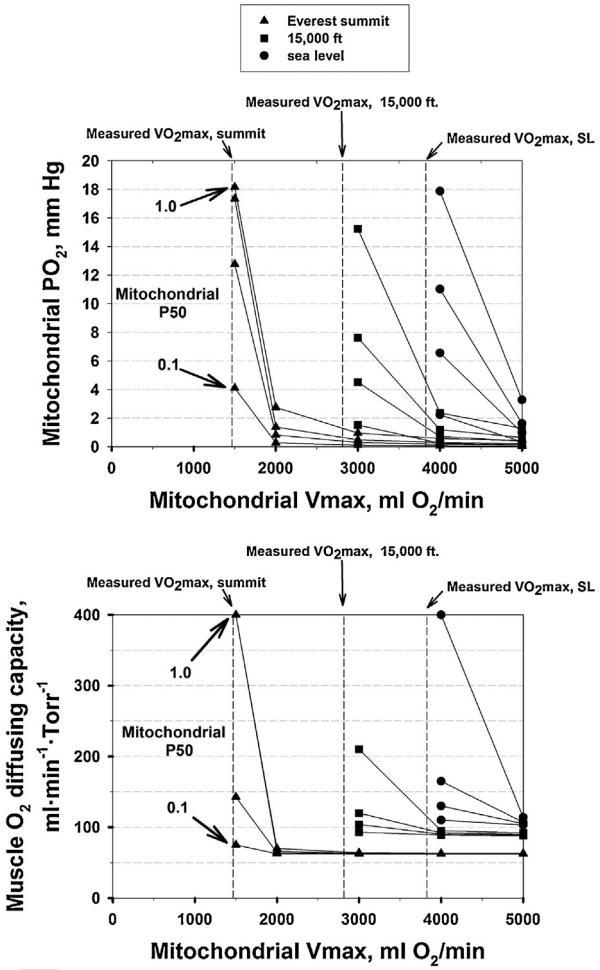

Fig. 5. Mitochondrial P_{O_2} (upper panel) and muscle O_2 diffusing capacity (lower panel) required to maintain $\dot{V}_{O_2}$ constant at the measured value across the domain of $\dot{V}_{MAX}$ and P_{50} values at each altitude studied (see text for details).

In order to maintain $\dot{V}_{O_2}$max when mitochondrial P_{O_2} is high, muscle diffusing capacity (DM) would need to be higher than when Pm_{O_2} is assumed to be zero. Under most conditions, the necessary increase in DM would be minimal, being significant only when Pm_{O_2} is considerably elevated (Fig. 5).

4.2. Unifying principles

The main principle demonstrated in the present study is that the final step in the O_2 pathway – mitochondrial respiration–may contribute a non-negligible resistance to O_2 movement through the system from the air to its conversion to CO_2, resulting in a lower $\dot{V}_{O_2}$max compared to a system where metabolism imposed no resistance to overall O_2 flow. The higher the mitochondrial P_{O_2} required to drive oxidative phosphorylation, the greater would be the relative resistance and thus the more effect there will be on reduction in $\dot{V}_{O_2}$max. When mitochondrial P_{50} is about 0.30 mm Hg as reported by Gnaiger (Gnaiger et al., 1998; Scandurra and Gnaiger, 2010) the effects are generally minor.

The simulations at the three inspired P_{O_2} values shown here demonstrate that it is the relative capacities (rather than individual absolute values) of the physiological transport system and the mitochondrial respiratory chain that effectively determine both the mitochondrial P_{O_2} and the associated effect on $\dot{V}_{O_2}$max, and that both variables, but especially mitochondrial P_{O_2}, may vary over a wide range depending on mitochondrial respiratory function.

Please cite this article in press as: Cano, I., et al., Importance of mitochondrial P_{O_2} in maximal O_2 transport and utilization: A theoretical analysis. Respir. Physiol. Neurobiol. (2013), http://dx.doi.org/10.1016/j.resp.2013.08.020

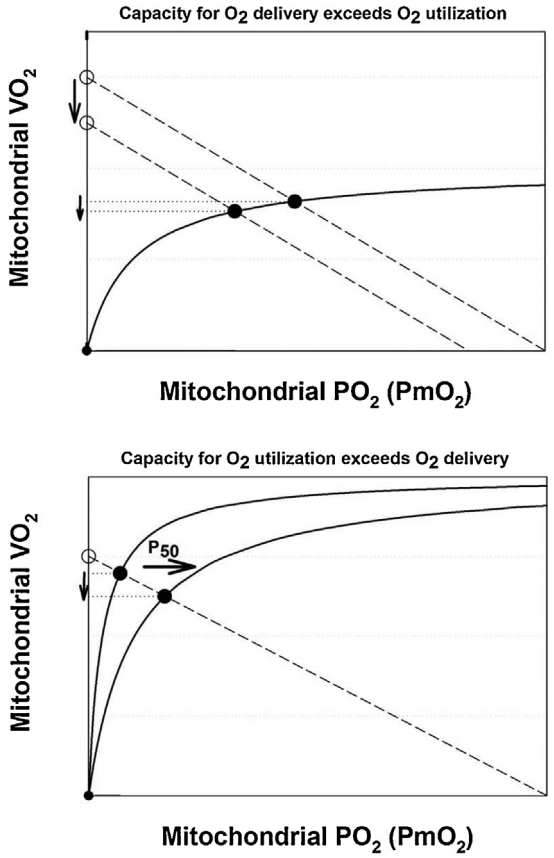

Fig. 6. Graphical depiction of the concept that even when the capacity for O_2 delivery exceeds O_2 utilization (upper panel) a change in O_2 delivery will change actual $\dot{V}_{O_2}$. Conversely, when the capacity for O_2 utilization exceeds O_2 delivery (lower panel) a change in O_2 utilization (increase in P_{50} in this example) will change actual $\dot{V}_{O_2}$. Open circles: maximal O_2 delivery to mitochondria if Pm_{O_2} was zero. Closed circles: actual $\dot{V}_{O_2}$. Solid and dashed lines: as in Fig. 1.

An additional important principle is shown in Fig. 6: even when O_2 transport capacity (i.e., potential for O_2 delivery) is considerably greater than mitochondrial respiratory capacity (i.e., potential for O_2 utilization), as illustrated in concept in the top panel, a change in the former will change overall $\dot{V}_{O_2}$. The converse is also true – that when mitochondrial respiratory capacity exceeds O_2 transport capacity, (lower panel), a change in the former will have an effect on $\dot{V}_{O_2}$. It is thus not correct to think that when one component is greater than the other, only the lesser of the two determines overall $\dot{V}_{O_2}$max. This conclusion is much the same as described for individual components of the physiological transport pathway of the lungs and chest wall, the heart, blood and circulation, and the muscles, where we previously showed (Wagner, 1996a,b) that all components affect $\dot{V}_{O_2}$max, not just the step with the least transport capacity.

4.3. *Effects of mitochondrial respiration kinetics on both $\dot{V}_{O_2}$max and Pm_{O_2} may be small or large*

For the examples shown – fit normal subjects – the effects of considering mitochondrial respiration are generally less on $\dot{V}_{O_2}$ than on the associated Pm_{O_2} (Fig. 4). Examining the sea level results for the example of $\dot{V}_{MAX}$ = 4000 ml/min and P_{50} increasing from 0.1 to 1.0 mm Hg, $\dot{V}_{O_2}$max would fall by 9% while Pm_{O_2} would increase by an order of magnitude, from less than 1 mm Hg to more than 6 mm Hg. Just how much variation there is in mitochondrial P_{50} in the normal population is unknown, let alone whether this may change systematically with training, or in chronic diseases such as chronic obstructive pulmonary disease (COPD) or chronic heart failure. The calculations presented herein however point out that the quantitative nature of the mitochondrial respiration curve may be a critical determinant of the values of mitochondrial P_{O_2} and $\dot{V}_{O_2}$max, over and above any influence of upstream O_2 transport.

Even if the effects on $\dot{V}_{O_2}$max are numerically small, they would likely be important in the competitive endurance athlete where very small differences may separate success from failure. But possibly even more significant might be the potentially large variation in mitochondrial P_{O_2} depending on P_{50} and $\dot{V}_{MAX}$ due to known hypoxia-induced biological effects (Semenza, 2011). Thus, hypoxia-induced gene expression or reactive O_2 species generation may vary according to mitochondrial P_{O_2}.

4.4. *Potential for estimating mitochondrial P_{50} based on the current modeling approach*

The analysis presented here suggests a possible method for estimating the characteristics of the mitochondrial respiration curve in vivo. Currently, mitochondrial $\dot{V}_{MAX}$ and P_{50} are measured in vitro in respirometers where mitochondria are exposed to different levels of O_2 and $\dot{V}_{O_2}$ measured (as in Fig. 3), (Gnaiger et al., 1998; Scandurra and Gnaiger, 2010; Wilson et al., 1977). To obtain this information in humans would therefore necessitate a muscle biopsy, and even if that were done, the result would be subject to the usual sampling constraints as for any other measure of muscle structure or function determined from a single biopsy.

The fitting of a hyperbolic function to paired measured values of $\dot{V}_{O_2}$max and mitochondrial P_{O_2} has the potential for estimating P_{50} and $\dot{V}_{MAX}$ in vivo, and this is illustrated in Fig. 7. The intervention to garner several points on the curve would come from acutely varying FI_{O_2} and measuring $\dot{V}_{O_2}$ and mitochondrial P_{O_2} during maximal exercise at each FI_{O_2}, as indicated by the theoretical example of the two solid circles in the upper panel of Fig. 7. These two points reflect a mitochondrial respiration curve with P_{50} of 0.30 mm Hg and $\dot{V}_{MAX}$ of 4000 ml/min. If such data were to span both the steep and flat parts of the respiration curve, as shown in the figure, identifying the $\dot{V}_{MAX}$ and P_{50} of a hyperbola that resulted in a least squares best fit to the data points would be possible, as shown in the lower panel of Fig. 7. Here, over a range of trial values of both $\dot{V}_{MAX}$ and P_{50}, the root mean square (RMS) residual $\dot{V}_{O_2}$ between the data and the hyperbola corresponding to each trial combination of P_{50} (and the $\dot{V}_{MAX}$ providing the lowest RMS for that P_{50}) is shown. In this error-free theoretical case, one could quite accurately estimate $\dot{V}_{MAX}$ (4000 ml/min) and P_{50} (0.3 mm Hg) from the values at the nadir of the relationship in the figure. However, if measured data happened to lie on only the flat or only on the steep parts of the curve, ability to estimate $\dot{V}_{MAX}$ and/or P_{50} would be considerably reduced.

While whole body or large muscle mass $\dot{V}_{O_2}$ can be measured relatively easily, the experimental challenge would be to measure mitochondrial P_{O_2} (during exercise) (Mik, 2013). The closest approach to date in intact subjects has used MRS-based determination of myoglobin O_2 saturation (Jue et al., 1994; Richardson et al., 1995), where the signal comes from a relatively large muscle region. This approach gives intracellular P_{O_2} estimates of 3–4 mm Hg during exercise (Richardson et al., 1995), but this is the P_{O_2} associated with myoglobin, inferred from the finding of about 50% myoglobin saturation during peak exercise combined with accepted values of myoglobin P_{50} of about 3 mm Hg (Rossi-Fanelli and Antonini, 1958). This P_{O_2} is an order of magnitude greater than that projected at the mitochondria based on the preceding discussion. In the end, a method would have to be developed for direct measurement of mitochondrial P_{O_2}. Whether a candidate signaling atom

Please cite this article in press as: Cano, I., et al., Importance of mitochondrial P_{O_2} in maximal O_2 transport and utilization: A theoretical analysis. Respir. Physiol. Neurobiol. (2013), http://dx.doi.org/10.1016/j.resp.2013.08.020

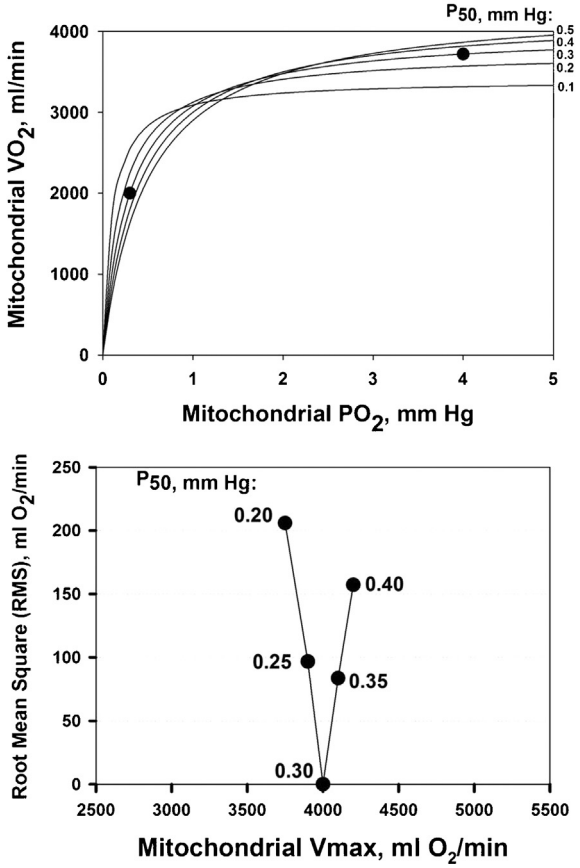

Fig. 7. Estimation of mitochondrial P_{50}. Upper panel: least squares best fit (solid lines) to data (solid circles) for five trial P_{50} values. Lower panel: closeness of fit to data reflected by the Root Mean Square error, showing that a P_{50} of 0.30 mm Hg provides the best estimate.

or molecule can be found for an MRS-based approach is currently unknown.

4.5. Limitations of the analysis

As in previous work (Wagner, 1993, 1996b), the entire analysis is applicable only to steady state conditions (meaning, that O_2 partial pressures are constant in time as is $\dot{V}_{O_2}$ itself). Therefore, the analysis cannot be used to study transient changes in metabolic rate. Another limitation is not taking into account ventilation–perfusion mismatch in the lung and/or metabolism–perfusion mismatch in the muscle as contributors to impaired oxygen transport. However, using methods to quantify both of these phenomena, this limitation could be removed. A final limitation is that non-muscle blood flow during maximum exercise is neglected.

5. Conclusions

Considering the hindrance to overall O_2 flux caused by mitochondrial respiration using an established model of O_2 transport to the mitochondria revealed that in normal subjects exercising maximally, the step of oxidative phosphorylation, with its requirement for a mitochondrial $P_{O_2} > 0$, likely plays only a small role in total O_2 flux resistance. However, we identified conditions in which mitochondrial P_{O_2} can rise to double digit values. This occurs particularly when the mitochondrial respiration curve has either a low $\dot{V}_{MAX}$ (relative to O_2 transport), or a high P_{50}, and under such conditions, mitochondrial function may significantly impair O_2 flux and cell function may be affected, for example, in reactive oxygen species generation and/or oxygen-sensitive gene expression.

Acknowledgements

We would like to acknowledge the contributions made by the Synergy-COPD consortium and NIH P01 HL091830.

References

Dejours, P., Kayser, C., 1966. Respiration. Oxford University Press.
Gnaiger, E., Lassnig, B., Kuznetsov, A., Rieger, G., Margreiter, R., 1998. Mitochondrial oxygen affinity, respiratory flux control and excess capacity of cytochrome *c* oxidase. J. Exp. Biol. 201, 1129–1139.
Johnson, K.A., Goody, R.S., 2011. The original Michaelis constant: translation of the 1913 Michaelis–Menten paper. Biochemistry 50, 8264–8269.
Jue, T., Kreutzer, U., Chung, Y., 1994. ^{1}H NMR approach to observe tissue oxygenation with the signals of myoglobin. Adv. Exp. Med. Biol. 361, 111–118.
Michaelis, L., Menten, M.L., 1913. Die kinetik der invertinwirkung. Biochem. Z. 49, 352.
Mik, E.G., 2013. Measuring mitochondrial oxygen tension: from basic principles to application in humans. Anesth. Analg..
Richardson, R.S., Leigh, J.S., Wagner, P.D., Noyszewski, E.A., 1999. Cellular PO_2 as a determinant of maximal mitochondrial O_2 consumption in trained human skeletal muscle. J. Appl. Physiol. 87, 325–331.
Richardson, R.S., Noyszewski, E.A., Kendrick, K.F., Leigh, J.S., Wagner, P.D., 1995. Myoglobin O_2 desaturation during exercise. Evidence of limited O_2 transport. J. Clin. Invest. 96, 1916–1926.
Rossi-Fanelli, A., Antonini, E., 1958. Studies on the oxygen and carbon monoxide equilibria of human myoglobin. Arch. Biochem. Biophys. 77, 478–492.
Scandurra, F.M., Gnaiger, E., 2010. Cell respiration under hypoxia: facts and artefacts in mitochondrial oxygen kinetics. Adv. Exp. Med. Biol. 662, 7–25.
Semenza, G.L., 2011. Oxygen sensing, homeostasis, and disease. N. Engl. J. Med. 365, 537–547.
Sutton, J.R., Reeves, J.T., Wagner, P.D., Groves, B.M., Cymerman, A., Malconian, M.K., Rock, P.B., Young, P.M., Walter, S.D., Houston, C.S., 1988. Operation Everest II: oxygen transport during exercise at extreme simulated altitude. J. Appl. Physiol. 64, 1309–1321.
Wagner, P.D., 1993. Algebraic analysis of the determinants of V_{O_2} max. Respir. Physiol. 93, 221–237.
Wagner, P.D., 1996a. Determinants of maximal oxygen transport and utilization. Annu. Rev. Physiol. 58, 21–50.
Wagner, P.D., 1996b. A theoretical analysis of factors determining V_{O_2} max at sea level and altitude. Respir. Physiol. 106, 329–343.
Weibel, E.R., Taylor, C.R., Gehr, P., Hoppeler, H., Mathieu, O., Maloiy, G.M., 1981. Design of the mammalian respiratory system: IX. Functional and structural limits for oxygen flow. Respir. Physiol. 44, 151–164.
Wilson, D.F., Erecinska, M., Drown, C., Silver, I.A., 1977. Effect of oxygen tension on cellular energetics. Am. J. Physiol. Cell Physiol. 233, C135–C140.

Please cite this article in press as: Cano, I., et al., Importance of mitochondrial P_{O_2} in maximal O_2 transport and utilization: A theoretical analysis. Respir. Physiol. Neurobiol. (2013), http://dx.doi.org/10.1016/j.resp.2013.08.020