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Oxygen Isotope Fractionation Within Human Mitochondria

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Summary

In a human study we performed isotopic analysis in order to evaluate whether data on oxygen (O_2) isotope fractionation at cytochrome oxidase can be applied to the entire O_2 utilisation within respiration. For this purpose we measured the ratio of the two stable oxygen isotopes $^{16}O_2$ and $^{16}O^{18}O$ within expiratory gas mixtures obtained from subjects who had breathed in O_2-enriched air. With increasing O_2 partial pressures of inspired gas the ratio $^{16}O^{18}O/^{16}O_2$ converged at the corresponding ratio reported for cytochrome oxidase. Thus, although this enzyme merely represents a component of the respiratory chain, its isotopic fractionation reflects the whole body fractionation process of O_2 utilisation within mitochondria. Our finding may indicate that cytochrome oxidase activity dominates cellular O_2 metabolism.

Key Words: cytochrome oxidase, oxygen isotope fractionation, rebreathing method

Introduction

In addition to the past interest shown by others in the chemical (12) and thermodynamic aspects (13) of isotopic exchange reactions, Lane and Dole (10) have studied the fractionation of the two stable oxygen (O_2) isotopes $^{16}O_2$ and $^{16}O^{18}O$ (99.6 % and 0.4 % of atmospheric oxygen) by respiratory processes of aerobic organisms. The authors have put the

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fractionation properties of global respiration and photosynthesis (3) together to form an O_2 isotope cycle of nature. In an attempt to make such effects usable in medicine, we have introduced isotopic analyses for assessing human respiration (7, 8). In order to separate the basic respiratory processes we have used the corresponding fractionation factors. Whereas convective O_2 transport (i.e. ventilation and blood flow) occurs without fractionation, $^{16}O_2$ diffuses 3 % more rapidly than the heavier isotope (4, 6, 9). Owing to the absence of any further data, we applied the fractionation properties of cytochrome oxidase as obtained from an in-vitro study to O_2 utilisation (5). However, it was suspected that the enzyme fractionation data represent the metabolic pathway of human respiration. In the following we present the determination of the fractionation as caused by the entire O_2 metabolism of human mitochondria.

Methods

Experimental protocol. Four healthy men (mean age 38 yr., range 33-49) volunteered for the study after informed consent. All subjects were familiar with the theoretical and practical aspects and were aware that the experiments would inter alia include exposure of ~ 90 % O_2 for several hours. None had any history of serious cardiopulmonary illness and in each case a routine lung function examination had shown no abnormality. Volunteers were seated in a comfortable chair and breathed through a mouthpiece on a 5 liter spirometer that was equipped with a soda-lime containing carbon dioxide absorber. The nose was occluded with a noseclip. The experiments were performed at various levels of hyperoxia in random order and on different days. In each case the spirometer was prepared by adding O_2 and nitrogen so that the target level of O_2 content was reached. During the rebreathing procedure the respective target levels were maintained by addition of O_2 that compensated for the subjects O_2 consumption. One single O_2 source of known isotopic composition was used for preparation of the spirometer, maintenance of spirometric O_2 content and isotopic analysis, respectively. At regular periods we determined the PO_2 values within the spirometer (inspiratory partial

pressure) as well as the overall fractionation factor of respiration by applying mass spectrometry. O_2 consumption of the subjects was gauged by reading the flow rate of O_2 supply to the spirometer. On average the rebreathing experiments lasted for 3-4 hours. During the last hour of rebreathing the α_O values did not change significantly. The data obtained during this plateau phase were hence considered as representing steady-state conditions.

Mass spectrometry. We used a respiratory mass spectrometer (model M3, Varian MAT, Bremen, Germany) that has been especially adapted to measure $^{16}O^{18}O$-$^{16}O_2$-ratios within expired gas mixtures (7, 11). In the present study, the $^{16}O^{18}O$-$^{16}O_2$-ratio was determined within the gas mixture of the spirometer at regular intervals, and was regarded as representing the corresponding ratio of both isotopic species within human respiration. This assumption is only valid for steady-state conditions. The measurement of α_O was performed by simultaneously detecting the ions $^{16}O_2^+$ and $^{16}O^{18}O^+$ at the mass-to-charge ratios 32 and 34, and by repeatedly comparing the content of $^{16}O_2^+$ and $^{16}O^{18}O^+$ ions of the spirometer gas with a reference gas that was equally composed of nitrogen and oxygen. We used one single O_2 source of known isotopic composition to manage the O_2 supply to both the spirometer as well as the reference gas.

Data analysis. We measured the $^{16}O^{18}O$-$^{16}O_2$-ratio within expiratory gas mixtures in order to determine the overall fractionation factor α_O which is the ratio of the relative rates in the consumption of the two isotopes. The O_2 pathways which contribute to α_O are ventilation, pulmonary diffusion, blood flow, tissue diffusion and mitochondrial utilisation. For steady-state transport it is commonly assumed that these pathways are serially connected (1, 2), allowing us to employ Ohm's law. Seen altogether, the component O_2 pathways contribute to α_O to the same extent they play a role in the entire resistance to O_2 transport and utilisation (7). Where F_C, F_D and F_U represent the fractional resistances due to convection (i.e. ventilation and blood flow), diffusion and mitochondrial utilisation, we obtain

$$\alpha_O = 1.0 \cdot F_C + 1.03 \cdot F_D + \alpha_U \cdot F_U \qquad (1)$$

where α_U stands for mitochondrial isotope fractionation. Thus, as α_O increases towards 1.03, the more respiration is operated by diffusion, but α_O is reduced towards 1.0, the more the contribution of convection increases. Since we intended to determine α_U, we had to choose an experimental set-up that led to $F_U \rightarrow 1$. This particular condition was induced by hyperoxic ventilation, i.e. breathing O_2-enriched air. With increasing O_2 partial pressure (PO_2) of inspired gas additional O_2 which is dissolved within blood increasingly contributes to tissue O_2 supply. Because O_2 utilisation within mitochondria is independent of O_2 supply, tissue PO_2 increases with inspiratory PO_2. Since F_U is defined as F_U = (tissue PO_2/inspiratory PO_2) and $1 = (F_C + F_D + F_U)$ holds, F_U converges at unity. According to equation (1) we then obtain $\alpha_O \rightarrow \alpha_U$.

Results

In table 1 we present the mean values of inspiratory O_2 partial pressure and O_2 consumption as well as the α_O mean values for each participant as obtained during the respective plateau phase of the normoxia and hyperoxia experiments. Each subject was exposed to five different levels of inspiratory O_2 partial pressure ranging between 141-624 mmHg. Within this range α_O was increased from 1.00749 to 1.01133. Regression analysis yielded

$$\alpha_O = 1.014 - 0.083 / (\textit{inspiratory } PO_2)^{0.514} \qquad (2)$$

as best fit to data ($P < 0.000001$). The SD value for the intercept was 0.0002 (as obtained from linear transformation).

Discussion and Conclusions

Thus, our experiments provide the first evidence that, while enhancing inspiratory PO_2, the overall fractionation factor of human respiration converges at a fractionation factor (1.014 ± 0.0002) which is not different from the corresponding mean ± SD value (1.0133 ± 0.002) for cytochrome oxidase (5). In our subjects O_2 consumption was normal and independent of

Table 1. *Results of hyperoxic rebreathing experiments in 4 male subjects*

Subject	Inspiratory PO_2 [mmHg]	O_2 consumption [ml/min]	overall fractionation factor of respiration
A	169	267	1.00814
B	146	318	1.00813
C	141	273	1.00763
D	146	277	1.00749
A	241	243	1.00916
B	218	344	1.00887
C	238	287	1.00925
D	241	285	1.00955
A	370	250	1.00980
B	348	287	1.01010
C	364	255	1.01014
D	364	272	1.01031
A	457	250	1.01070
B	484	349	1.01088
C	480	270	1.01062
D	508	263	1.01091
A	590	205	1.01083
B	606	325	1.01133
C	624	307	1.01112
D	622	302	1.01111

inspiratory PO_2, justifying our assumption that a rise in O_2 content of inspired gas would lead to increasing tissue PO_2 values.

We concluded that the enzyme fractionation data about cytochrome oxidase indeed represent the whole body O_2 utilisation within human respiration. According to our in vivo measurements, the fractionation factor of O_2 utilisation seems to be rather independent of the particular in vitro conditions of the enzyme study (5), although the enzyme data were obtained under different conditions of O_2 supply and substrate supply than in our human study. Furthermore, our findings indicate that either cytochrome oxidase predominates cellular O_2 metabolism or that other O_2 activating enzymes such as catalases, peroxidases and the cytochrome P-450 oxygenases preferentially consume $^{16}O_2$ in the same way.

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