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Journal
Respiration physiology, 89(3)

ISSN
0034-5687

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Publication Date
1992-09-01

DOI
10.1016/0034-5687(92)90090-j

Respiration Physiology, 89 (1992) 319–327

RESP 01935

Effect of increased metabolic rate on oxygen isotopic fractionation

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(Accepted 21 April 1992)

Abstract. $^{16}O^{16}O$ is preferentially used over $^{18}O^{16}O$ (a stable isotope of oxygen comprising about 0.2% of atmospheric O_2) as oxygen is consumed during respiration in humans (Epstein and Zeiri, 1988, *Proc. Natl. Acad. Sci. USA* 85: 1727–1731). To test the hypothesis that oxygen isotopic fractionation is related to the metabolic rate, 8 healthy adults performed 5 min of constant work rate cycle ergometer exercise below and above their anaerobic threshold. Moreover, 3 subjects performed an incremental exercise to the limit of tolerance. Oxygen uptake ($\dot{V}_{O_2}$) was measured breath by breath. Samples of the exhaled breath for oxygen isotope measurement were obtained at rest and at various times during exercise and recovery. Oxygen isotopic fractionation was determined by isotope ratio mass spectrometry and calculated as the ratio of the degree of fractionation to the oxygen consumed in the breath sample (Z value). For the constant work rate protocol, both low and high intensity exercise resulted in a significant decrease in Z compared to the rest values ($P<0.01$). However, for the high intensity exercise the reduction in fractionation was greater compared to the low intensity protocol ($P<0.05$). For the incremental test, there was a significant negative correlation between oxygen isotopic fractionation and $\dot{V}_{O_2}$ expressed as percent of the maximal oxygen uptake ($r = -0.91$, $P<0.0001$). These data suggest that during exercise low-fractionating processes become more important as limiting steps for O_2 transport.

Exercise, O_2 fractionation; Isotope, O_2 fractionation, exercise; Mammals, humans

^{18}O is a naturally occurring stable isotope of oxygen. ^{18}O enrichment ($^{18}O/^{16}O$) is greater in the atmosphere than in ocean water, and is thought to be caused by the preferential consumption of $^{16}O^{16}O$ during respiration by different organisms (Dole and Jenks, 1944). In humans, Epstein and Zeiri (Epstein and Zeiri, 1988) found a linear relationship between the $^{18}O^{16}O/^{16}O^{16}O$ ratio and the fraction of oxygen consumed during a single breath hold. These findings were corroborated more recently by Schuster and Pflug (1988). Preliminary observations of Epstein and Zeiri (1988) suggested that factors such as physical exercise could alter the pattern of oxygen isotope fractionation. The purpose of the present study was to examine the effect of precisely controlled

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changes in metabolic rate (induced by exercise) on oxygen isotopic fractionation.

Oxygen isotopic fractionation may be useful in understanding the overall process of oxygen transport. The pathways of oxygen transport to tissues include a number of processes that are likely to cause oxygen isotopic fractionation: namely, diffusion of O_2 through N_2 and CO_2; diffusion of O_2 through membranes (alveolar and red cell); and chemical reactions (*e.g.*, oxidative reactions of the respiratory chain, binding of oxygen to hemoglobin) (Epstein and Zeiri, 1988; Schuster and Pflug, 1988; Pflug and Schuster, 1988). In contrast, other steps in oxygen transport (*e.g.*, bulk flow in the blood, convection) are less likely to result in differences between oxygen isotopes (Schuster and Pflug, 1988). The overall fractionation is determined in large part by two distinct factors: first, the fractionation that occurs at each transport step; and second, by the relative contribution of each step to the rate of oxygen transport. As noted by Epstein and Zeiri (1988), the overall fractionation will be determined by the fractionation associated with the rate-limiting step in the oxygen transport process.

Methods

Ten healthy adults (9 males and 1 female, aged 31–41 years, mean age 34 ± 5 years) comprised the study population. All were volunteers, had no chronic diseases, and did not smoke or use medications. The study was approved by the Human Subjects Committee of Harbor-UCLA Medical Center and informed consent was obtained from each subject.

Protocol

(1) Each subject performed a ramp-type progressive exercise test on a electromagnetically braked cycle ergometer to determine the maximal oxygen uptake ($\dot{V}_{O_2max}$) and the anaerobic threshold (AT) (Whipp *et al.*, 1981).

(2) Eight subjects performed constant work rate ergometry for 5 min corresponding to 80% AT and 40% of the difference between the $\dot{V}_{O_2max}$ and the AT. The protocol included 3 min of rest, followed by 3 min of unloaded pedalling (approximately 7–12 Watts) prior to exercise, and 7 min of rest following exercise.

(3) Three subjects performed an incremental exercise test to the limit of tolerance with work rate increasing every 2 min. The test slope was 20–25 Watts/2 min. The test was preceded by 3 min of rest and 3 min of unloaded pedalling.

Breath samples

Samples of the exhaled breath were taken during each part of the constant work rate test and at each work rate during the incremental test (Figs. 1, 2 and 4). The subjects inhaled atmospheric air, then they held their breath for 5 sec and exhaled some air into a balloon. The latter part of the exhaled air was selected for analyses to maximize the fraction of the O_2 used in the respiration process.

Breath-by-breath measurement of gas exchange

Ventilation and gas exchange were measured breath-by-breath. The subjects breathed through a mouthpiece connected to a turbine flowmeter and a low resistance 2-way valve for continuous measurement of inspired and expired volume. The apparatus dead space was 90 ml. CO_2 and O_2 concentrations were measured by a mass spectrometer that sampled continuously from the mouthpiece at 1 ml/sec. Ventilation (BTPS), $\dot{V}_{O_2}$ (STPD), CO_2 output (STPD), end tidal pressure for O_2 and for CO_2 were computed on-line, breath-by-breath, as previously described (Beaver *et al.*, 1981). Heart rate was measured by a standard lead 1 ECG using three electrodes placed on the chest. The data from each test were stored on digital tape for further analysis.

Analysis of stable isotopes

This technique has been described in detail previously (Epstein and Zeiri, 1988). Briefly, for analysis of ^{18}O, samples of exhaled breath were obtained in plastic syringes of approximately 60 ml volume. The respired CO_2 and H_2O are extracted by cycling a 20–30 ml aliquot of the exhaled gas for about 15 min through a liquid nitrogen-cooled trap. This process isolates the condensable CO_2 and H_2O from the non-condensable gas in the transpired sample. The CO_2 is released by warming the trap in a dry-ice bath and is transferred into a sample tube for manometric and isotopic measurements. The H_2O was pumped away. The exhaled gas O_2 is converted to CO_2 by cycling the remaining aliquot of alveolar gas over a carbon rod that is heated to red heat by passing current through it. Upon formation, the CO_2 was frozen out in liquid nitrogen-cooled traps and isolated for manometric and oxygen-isotope analyses. A modified Nier spectrometer was used to determine the ^{18}O. The enrichment of oxygen with ^{18}O is quantified relative to a standard (standard mean ocean water – SMOW). The units of enrichment in parts per thousand (‰), δ, is given as:

$$\delta^{18}O(‰) = [Rsample \cdot (RSMOW)^{-1} - 1] \cdot 1000$$

where R is equal to the ratio $^{18}O/^{16}O$. The precision of the measurement is $\pm 0.1‰$.

Data analysis

^{18}O enrichment of the breath samples collected in the various experiments described above was compared to that consistently found in atmospheric oxygen ($\delta^{18}O = 23.5‰$). The change in $\delta^{18}O$ was related to the fraction of oxygen used (X).

The latter was calculated as:

$$X = (F_{O_2\ room\ air} - F_{O_2\ sample}) \cdot (F_{O_2\ room\ air})^{-1}$$

where $F_{O_2 room\ air}$ was determined to be 0.21. The Z-value was defined as:

$$Z(‰) = (\delta^{18}O_{sample} - 23.5) \cdot X^{-1}$$

Normalization

The $\dot{V}_{O_2max}$ and the AT were quite variable indicating different degrees of fitness and of functional capability. Thus, to facilitate comparisons, the $\dot{V}_{O_2}$ during exercise of different intensity was scaled as percent $\dot{V}_{O_2max}$.

Statistical analysis

Analysis of variance (repeated measures) and subsequent modified *t*-test (Duncan) were used to determine if there were statistically significant changes in oxygen isotopic fractionation (Z values) during each constant work rate exercise test. Paired *t*-test was used to compare pre-exercise Z values between the low and the high intensity exercise test. Linear and logarithmic regression was used to determine the correlation coefficient between Z values and the ratio of $\dot{V}_{O_2}$ to $\dot{V}_{O_2max}$ during the incremental exercise.

Results

Maximal exercise test

The mean $\dot{V}_{O_2max}$ and AT were 43.6 ± 12.4 ml/min/kg and 22.3 ± 8.2 ml/min/kg respectively.

Constant work rate exercise (Figs. 1 and 2)

The mean Z value was the same under resting conditions for both the high and low intensity exercise protocols. Similarly, there were no differences in the resting $\dot{V}_{O_2}$ between the two protocols. Unloaded pedalling resulted in a significant increase in $\dot{V}_{O_2}$ by 90% ($P<0.01$) but no change in the Z value. Z decreased from 8.9 ± 1.2‰ at rest

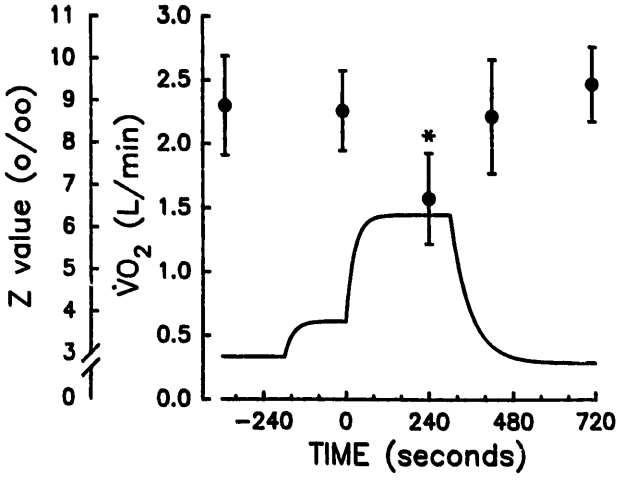

Fig. 1. Group mean $\dot{V}_{O_2}$ and Z values for the low-intensity constant work rate exercise protocol. The Z values (samples for oxygen isotopic fractionation) are represented by closed circles and are expressed as mean ± 1SD. There was a significant decrease in fractionation during exercise (*$P<0.01$).

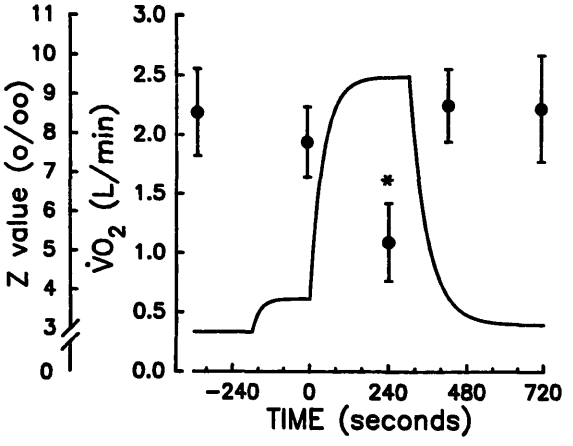

Fig. 2. Group mean $\dot{V}_{O_2}$ and Z values for the high-intensity constant work rate exercise. The Z values (samples for oxygen isotopic fractionation) are represented by closed circles and are expressed as mean ± 1SD. There was a significant decrease in fractionation during exercise (*$P<0.01$).

to 6.7 ± 1.1‰ ($P<0.01$) at the fourth minute of low-intensity exercise while $\dot{V}_{O_2}$ increased from 10 ± 2%max to 42 ± 5%max ($P<0.01$). Similarly, during the high-intensity protocol, Z decreased from 8.7 ± 1.0‰ at rest to 5.2 ± 1.0‰ at the fourth minute of exercise ($P<0.01$) while $\dot{V}_{O_2}$ increased from 10 ± 3%max to 72 ± 8%max ($P<0.01$). By the second minute following exercise, the Z value had returned to pre-exercise levels and no further change was observed at 7 min.

The effect of metabolic rate on fractionation was evaluated by comparing the difference between the resting and exercise Z values (ΔZ) for the two exercise protocols (Fig. 3). High-intensity exercise resulted in a significantly greater reduction in fractionation (mean ΔZ 3.5 ± 1.2‰) than did low-intensity exercise (mean ΔZ 2.2 ± 1.1‰) ($P<0.05$).

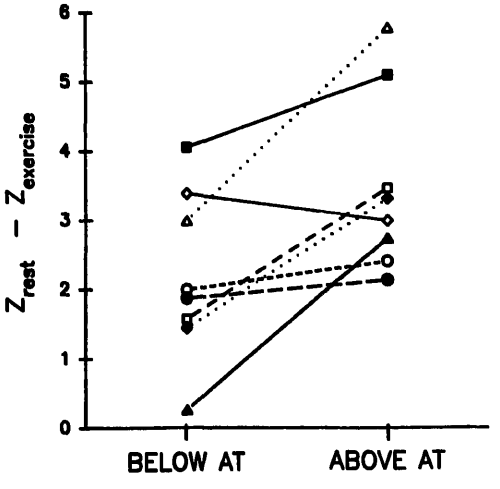

Fig. 3. ΔZ (difference between the resting and the exercise value) for low and high-intensity exercise. High intensity exercise resulted in a greater reduction in Z than did low intensity exercise ($P<0.05$).

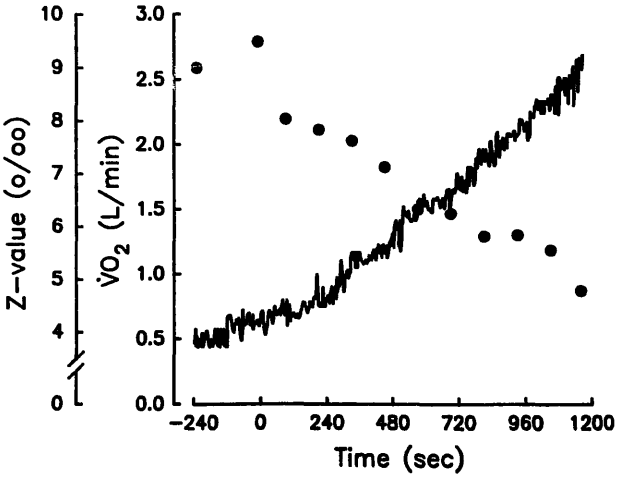

Fig. 4. O_2 uptake and Z during an incremental test in a 34 year old male. The continuous line represents the $\dot{V}_{O_2}$ and the closed circles represent the Z values (samples for oxygen isotopic fractionation). Note that fractionation decreases with increasing $\dot{V}_{O_2}$ throughout the study.

Incremental exercise

An example of $\dot{V}_{O_2}$ and oxygen isotopic fractionation response to incremental exercise is shown in Fig. 4. When the amount of oxygen isotopic fractionation was related to $\dot{V}_{O_2}$ expressed as percent of $\dot{V}_{O_2max}$ for each subject, fractionation decreased as metabolic rate increased throughout the incremental exercise studies (linear regression: $r = -0.86$; $P<0.0001$) (Fig. 5). The correlation coefficient for the logarithmic regression was $r = -0.91$, $P<0.0001$.

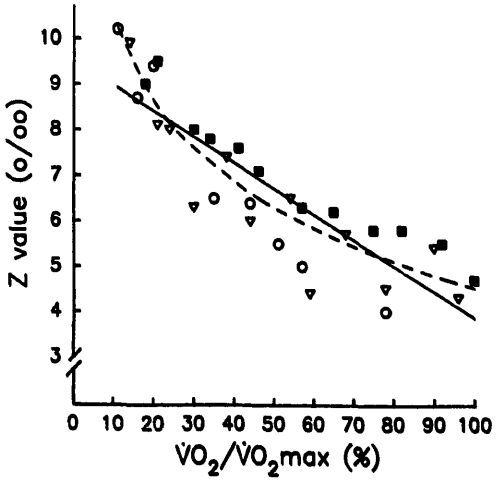

Fig. 5. Fractionation as a function of $\dot{V}_{O_2}/\dot{V}_{O_2max}$ ratio during incremental exercise in three subjects. The continuous line represents the best-fit line for the linear regression ($r = -0.86$, $P<0.0001$). The dashed line represents the curve for the logarithmic regression ($r = -0.91$, $P<0.0001$).

Discussion

There have been few studies of oxygen isotopic fractionation in human beings. Thus it is noteworthy that our Z resting values are similar in magnitude to the previous two studies reported in the literature (Epstein and Zeiri, 1988; Schuster and Pflug, 1988). We found a marked and reproducible reduction in oxygen isotopic fractionation associated with increased metabolic rate induced by exercise. Both constant work rate and progressive exercise test revealed a negative linear relationship between the degree of fractionation and oxygen uptake. Changes in oxygen isotopic fractionation responded rapidly to changes in work rate and $\dot{V}_{O_2}$, and appeared to return to pre-exercise values at about the same rate as well.

It is likely that the oxygen isotopic fractionation results from more than one mechanism. First, as demonstrated by Feldman *et al.* (1959), fractionation can occur during cellular respiration, probably as a result of metalloenzymes. Secondly, transport processes involving gaseous diffusion or diffusion across membranes (*i.e.* alveolar or red cell membrane) are dependent on molecular weight, and, therefore, are associated with fractionation (Epstein and Zeiri, 1988; Schuster and Pflug, 1988). Finally, fractionation also occurs in the chemical combination of oxygen with hemoglobin (Pflug and Schuster, 1988), but whether or not this fractionation is an equilibrium or kinetic phenomenon remains unknown.

It is unlikely that oxygen isotopic fractionation at the cellular level influenced the decrease in alveolar fractionation that we observed during exercise because respiration is associated with *increased* rather than decreased fractionation (Feldman *et al.*, 1959). Moreover, cellular fractionation effects would be manifest in alveolar gas only by preferential back diffusion of ^{18}O from venous blood into the alveoli. Given the small amounts of dissolved oxygen in the blood and the fact that oxygen is predominantly being transported from alveolus to blood, back diffusion could only account for immeasurably small increases in alveolar ^{18}O.

Rather, our findings indicate that as exercise intensity increases there is a significant decrease in oxygen isotopic fractionation. This supports the notion that the rate controlling steps in oxygen transport markedly change during exercise. Controversy still exists concerning the relative roles of diffusion, convection, and chemical combination of oxygen with hemoglobin in determining the overall rate of oxygen transport. Scheid and Piiper (1989), using a relatively simple model, showed that in resting normoxic conditions O_2 exchange is mainly limited by perfusion, but diffusion limitation may become important in certain conditions, such as exercise and hypoxia. Wagner *et al.* (1986) have suggested that for high-intensity exercise only one-third of the total alveolar-arterial O_2 difference is due to ventilation/perfusion mismatch. The remaining two-thirds of alveolar-arterial gradient were speculated to result from alveolar-end-capillary diffusion limitation.

In pure oxygen, isotopic fractionation due to diffusion is directly related to the mass of each oxygen species and is governed by the Rayleigh equation (Epstein and Zeiri, 1988). However, diffusion-related isotopic fractionation is attenuated when oxygen

transport occurs in gas mixtures of oxygen, nitrogen, and carbon dioxide (such as occur in alveolar gas) (Epstein and Zeiri, 1988). The effect of different gas mixtures on diffusion-related oxygen isotopic fractionation is not yet known. Thus, the observations of this study are primarily useful for qualitative assessments of the relative role of diffusion in alveolar gas transport.

The classic Bohr integration assumes that at any point along the pulmonary capillary the rate of diffusion of oxygen is proportional to the P_{O_2} difference between the alveolar gas and the pulmonary capillary blood at that point and the dissolved O_2 in the blood is always in equilibrium with the hemoglobin, in other words that the exchange of O_2 between the red cells and the plasma is instantaneous (Nunn, 1987). Staub *et al.* (1961, 1962), in contrast, showed that the transfer of O_2 from the extracellular fluids into the red cells and into chemical combination with hemoglobin is sufficiently slow to be the limiting factor in the overall rate of O_2 transport. And Coin and Olson (1979) and Yamaguchi *et al.* (1985) found that O_2 uptake by human red cells is much slower than the corresponding rate of O_2 combination with free hemoglobin. It is intriguing that Pflug and Schuster (1988) estimated the fractionation factor for the reaction between oxygen and hemoglobin to be 1.0035 (corresponding to a Z value of 3.5‰). This might suggest that the lower fractionation associated with exercise could reflect the increasingly important role of the chemical reaction between oxygen and hemoglobin in oxygen transport.

The oxygen isotopic fractionation we observed during exercise is virtually the same as that observed previously in anemic subjects (Epstein and Zeiri, 1988). In these patients, the oxygen-hemoglobin interaction is limited by the reduced hemoglobin as well as by increased pulmonary capillary transit. Although specific measurements of cardiac output were not made by Epstein and Zeiri in the previous study, decrease in pulmonary capillary transit time may have occurred in those anemic patients who could increase cardiac output as an adaptation to the anemia. In our study oxygen isotopic fractionation decreases with all work intensities, however for very heavy work rates this may become less evident (note the better fit of the logarithmic model). This might reflect the kinetics of the fall in pulmonary capillary transit time with increasing pulmonary blood flow once the pulmonary capillary blood volume has reached its maximum value (Rowell, 1986). In addition, with increasing work rates other processes with higher fractionating effect, *i.e.* membrane diffusion, might also be involved in O_2 exchange limitation.

In summary, our data show that the relative importance of the processes controlling O_2 uptake changes from rest to exercise. One possible explanation is that oxygen-hemoglobin interaction might become progressively more important as the overall rate determining step in oxygen transport as exercise increases. But this interpretation may be modified as we gain more precise information about the fractionation that occurs with each step of oxygen transport; namely, the as yet undiscovered $\delta^{18}O$ of gaseous diffusion, and the fractionation associated with diffusion across the alveolar and capillary membrane.

Acknowledgements. This work was supported by USPHS HL4053. Stefania Zanconato is a Research Fellow of the American Heart Association, Greater Los Angeles Affiliate. Dan M. Cooper is the recipient of the Career Investigator Award, American Lung Association. Yaacov Armon is a Research Fellow of the Joseph Drown Foundation.

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