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Oxygen and nitrogen isotopic fractionations during human respiration

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Abstract

Both oxygen and nitrogen isotope compositions ($\delta^{18}O$ and $\delta^{15}N$) of exhaled air from 10 individuals were measured. Results show linear relations between isotope variation and the fraction of O_2 used during the respiration process. The isotopic influence of physiological parameters such as smoking habits, age, haemoglobin count, oxygen fixation rate or physical exercise was assessed. Among them, only smoking habits do not have any effect on $\delta^{18}O$. $\delta^{15}N$ differences between inhaled and exhaled air may indicate an active (but minor) role for nitrogen during the human respiration process. Nevertheless, nitrogen fractionation is homogenous among all the individuals, which is coherent with the fact that nitrogen metabolism is controlled by the intestinal bacterial activity. ***To cite this article: D. Widory, C. R. Biologies 327 (2004).***

Résumé

Les fractionnements isotopiques de l'oxygène et de l'azote au cours de la respiration humaine. Les compositions isotopiques en oxygène et azote ($\delta^{18}O$ et $\delta^{15}N$) de l'air expiré par 10 sujets ont été mesurées. Les résultats montrent des relations linéaires entre les fractionnements isotopiques et la fraction d'oxygène utilisée lors de la respiration. L'influence isotopique de paramètres physiologiques tels que le tabagisme, le taux d'hémoglobine, le taux de fixation de l'oxygène ou l'exercice physique est analysée. Seul le tabagisme semble n'avoir aucun effet sur le $\delta^{18}O$. Les différences de $\delta^{15}N$ entre l'air inspiré et expiré pourraient indiquer un rôle actif (mineur) de l'azote durant la respiration. Néanmoins, le fractionnement en azote est homogène pour l'ensemble des sujets, en accord avec le fait que son métabolisme est lié à l'activité bactérienne intestinale. ***Pour citer cet article : D. Widory, C. R. Biologies 327 (2004).***

Version française abrégée

Les compositions isotopiques en O_2 et N_2 de l'air expiré par 10 sujets sains, présentant des caractères

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physiologiques aussi différents que possible, ont été mesurées. Les résultats montrent que le fractionnement des isotopes de O_2, ainsi que de ceux de N_2, est proportionnel à la fraction de O_2 utilisée par la respiration. La respiration consomme préférentiellement ^{16}O, mais il semble qu'une faible fraction de ^{14}N soit aussi utilisée.

L'amplitude du fractionnement (normalisée à la consommation de 100 % de O_2), est représentée par la variable Z pour O_2 et Z' pour N_2. L'influence de certains paramètres physiologiques sur le fractionnement isotopique lors de la respiration a été analysée. Les résultats montrent diverses tendances. Plus le sujet est âgé, moins il fractionne le $\delta^{18}O$ de l'air. Ceci peut s'expliquer par une augmentation du taux de fixation de O_2 avec l'age. Pour un même temps de rétention de l'air inspiré, un sujet ayant un taux de fixation de O_2 plus élevé aura consommé plus de O_2 (et donc de ^{16}O). Son fractionnement sera donc plus faible (cette remarque sera corroborée plus tard dans la discussion par l'étude des relations Z-taux d'hémoglobine). Il est possible de distinguer deux familles de sujets, à l'intérieur desquelles la relation existant entre Z et le taux de fixation de O_2 est conservée. Malheureusement, lors de l'augmentation du taux de fixation de O_2, il n'est pas possible de distinguer le processus de diffusion moléculaire de celui de création d'oxyhémoglobine. Les autres paramètres physiologiques considérés semblent ne pas avoir d'influence sur le fractionnement de O_2. Notamment, les fumeurs (sujets 9 et 10) présentent des valeurs de Z similaires à celles des non-fumeurs (sujets 1, 2 et 7), en contradiction avec les conclusions d'Epstein et Zeiri. L'étude d'un sujet ayant effectué un don du sang confirme qu'il existe une relation positive entre le fractionnement en O_2 et le taux d'hémoglobine ($Z = 1{,}2$ Hb $-3{,}0‰$, où Hb représente le taux d'hémoglobine). Plus la concentration en Hb est élevée, plus le fractionnement isotopique est important. L'extrapolation de cette relation pourrait permettre d'estimer le fractionnement associé à l'étape de diffusion de la respiration. En effet, un sujet ayant un taux d'hémoglobine nul expirerait de l'O_2 n'ayant subi que le processus de diffusion (c'est-à-dire sans formation d'oxyhémoglobine postérieure). L'ordonnée à l'origine de la droite reliant les deux paramètres donne donc une estimation de ce fractionnement associé à la diffusion pulmonaire. Cette valeur est constante, aux alentours de $-3{,}0‰$. Le signe négatif est inattendu, car la théorie prédit que la diffusion enrichit en isotope lourd le résidu de la réaction, représenté ici par l'oxygène expiré. Néanmoins, ceci impliquerait un fractionnement associé à la diffusion homogène pour tous les individus. Cette observation est renforcée par l'étude des isotopes de N_2 (molécule ne subissant que la diffusion lors de la respiration). Le fractionnement isotopique normalisé (Z') semble être similaire pour tous les sujets. Si le fractionnement associé à la diffusion est constant et si le fractionnement normalisé en O_2 varie suivant les individus, cela signifierait que l'étape isotopiquement dominante dans le processus de respiration est la formation d'oxyhémoglobine. L'influence d'un effort physique intense a aussi été testée. Après un effort soutenu de 40 min (squash), Z diminue de 1,7‰, tandis que le taux de fixation de O_2 augmente ($\Delta = +1{,}2\%O_2\,s^{-1}$). Le fractionnement isotopique décroît donc avec l'activité physique. En tenant compte de la relation existant entre Z et Hb, lors d'un exercice physique, les poumons agissent comme si Hb diminuait, relativement.

Comme discuté précédemment, les isotopes de l'azote fractionnent eux aussi légèrement au cours de la respiration (l'azote expiré est enrichi en ^{15}N). Le fractionnement observé est constant ($\approx 1‰$). L'existence d'un fractionnement requiert un rôle actif de N_2 dans la respiration, même si, du fait des faibles valeurs de Z' comparées à celles de Z, celui-ci est mineur.

1. Introduction

The preferential use of the ^{16}O isotope during human respiration was observed by the early works of Dole and Jenks [1], Lane and Dole [2] and Dole [3]. The authors analysed gas samples involved in respiration by plants and by one sample from human individual. Later, Esptein and Zeiri [4] extended this systematic study of O_2 fractionation during respiration by assessing potential effects of certain physiological parameters.

In the present study, exhausted air from 10 persons was analysed for both $\delta^{18}O$ and $\delta^{15}N$ (barely studied so far). In the choice of the individuals' panel, particular physiological characteristics were taken into account: age, weight, height, sex, smoking habits (Table 1).

Table 1
Physiological characteristics of the studied individuals

Individual	Age (years)	Height (cm)	Sex	Weight (kg)	Smoking (years)
1	22	173	M	66	0
2	24	176	M	64	0
3	25	176	F	65	0
4	56	156	M	82	0
5	47	190	M	85	15*
6	59	178	M	75	**
7	52	160	F	59	10*
8	71	167	F	67	21
9	22	179	M	69	9
10	37	173	M	70	25

* Smoked during ... and then stopped.
** Smokes occasionally.

2. Analytical methods

The individuals inhaled air and held their breath for between 10 and 60 seconds, and exhaled it into a Tedlar pack. A fraction of 2.5 ml was extracted and injected into a vacuum line for separation into N_2 and O_2 (under the form of CO_2) for volumetric and isotope analyses. Proportions of O_2 and N_2 in each respired sample were manometrically determined during extraction. The isotope composition measurements were run on a Mat Delta E Finnigan mass spectrometer. Results are given in ‰ relative to the SMOW standard for O_2 and atmosphere for N_2. The reproducibility is 0.1‰ for $\delta^{18}O$ and $\delta^{15}N$.

The fraction of O_2 used during respiration (X) is defined as $X = 1 - V/A$, where V is the concentration of the exhaled O_2 and A the concentration of the initial inhaled O_2. Atmospheric analysis yielded a N_2/O_2 ratio of 3.80 ± 0.03. It comes that $X = 1 - (3.80\,V/B)$, where B is the volume of N_2 in the exhaled air (which is supposed to be equal to the inhaled one). The Z value, the normalised amplitude of the O_2 isotope fractionation (normalised to the consumption of 100% of the O_2), is defined as:

$$Z = \frac{\delta^{18}O_{\text{exhaled air}} - \delta^{18}O_{\text{atmosphere}}}{X}$$

For the determination of the N_2 isotope fractionation (Z'), $\delta^{18}O$ is replaced by $\delta^{15}N$ (Z' is also normalised to the consumption of O_2). The surrounding air prevalent in the laboratory is taken as the standard for N_2 ($\delta^{15}N = 0$‰; [5]), its $\delta^{18}O$ (23.3 ± 0.03‰) is consistent with Kroopnick and Craig's results [6].

3. Gases cycle during respiration

Human respiration is a complex multistep process, in which blood supplies O_2, inhaled through lungs, to the tissues that release CO_2 [7]. Respiration can be modelled by a simple two-step model: (1) O_2 diffuses through the pulmonary membranes with a diffusion constant k_1 and a fractionation factor α_1, followed by oxy-haemoglobin formation with a reaction constant k_2 and a fractionation factor α_2 (followed by other reactions to finally form CO_2). The global fractionation is a combination of the different fractionation factors. The slower the reaction is, the more influence it has on the determination of the final isotope fractionation.

4. Results and discussions

Fig. 1A and B show that human respiration fractionates inhaled-air $\delta^{18}O$ and $\delta^{15}N$, characterised by a heavy-isotope enrichment for both exhaled O_2 and N_2 ($\Delta > 0$).

4.1. Oxygen

The isotopic effect on O_2 is linearly correlated to the fraction of O_2 used and is individual-dependent (Fig. 1A and C). The Z values plot into two distinct populations, the threshold being around $Z = 8.5$‰. The age of the individual seems to be important in the determination of Z: the older the individual is, the lower Z is (Fig. 1D). This has possibly to be linked to the O_2 fixation rate, which increases with the age

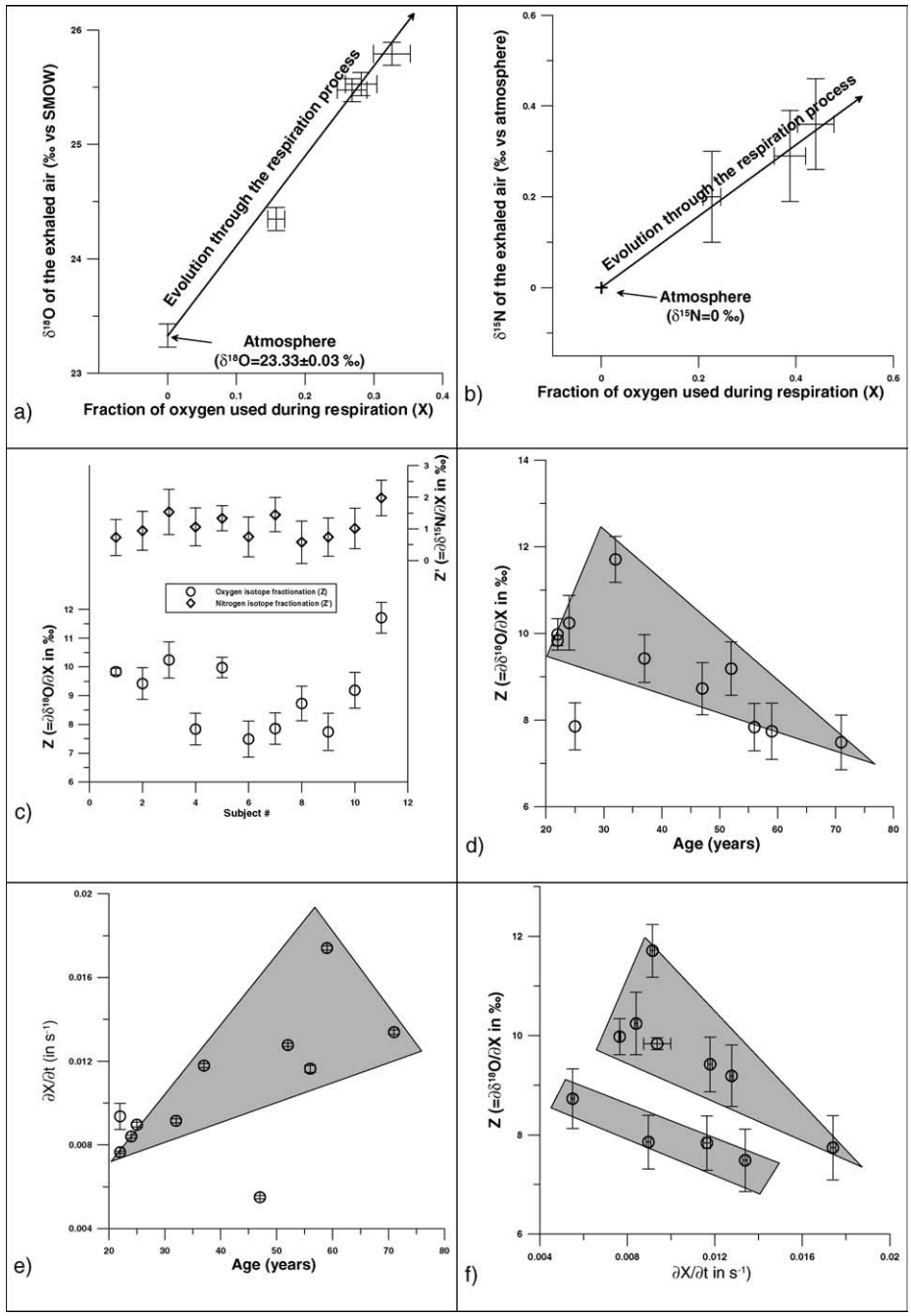

Fig. 1. (**A**) O_2 isotope fractionation during human respiration. (**B**) N_2 isotope fractionation during human respiration. (**C**) Z and Z' values for the studied individuals. (**D**) Relation between Z and the age. (**E**) Relation between the age and the O_2 fixation rate. (**F**) Co-variations of Z and O_2 fixation rate.

of the individual, and on which depends Z (Fig. 1E). For a given time lapse, an individual with a higher X will consume more ^{16}O. This leads to higher values of both the $\delta^{18}O$ of his exhaled air and the fraction of O_2 used, compared to those of a younger individual, and ultimately to a higher Z value (as $\delta^{18}O/X > 1$). The negative linear trend previously observed is conserved within both groups: the higher the O_2 fixation rate is, the lower Z is (Fig. 1F). But, the faster the fixation performs, the faster the total of both diffusion and oxy-haemoglobin formation processes goes. It is thus impossible to determine which step is dominant. Among the other physiological parameters, nothing, and in particular smoking habits, seems to influence the expired $\delta^{18}O$, unlike in a previous study, which indicated that smokers had higher Z values [4]. Smoking lays a tar coat on the pulmonary alveoli, disturbing the O_2 diffusion through the alveolar-capillaries wall. If isotope fractionation differences were observed, it would mean that the diffusion step is prevalent in the determination of the global isotope fractionation. Results show no such discrepancies (Fig. 1C), as heavy smokers (individuals Nos. 9 and 10) have Z values similar to those of non-smokers (individuals Nos. 1, 2, and 7).

Individual 3 had a 400-g total blood donation, yielding two distinct samples: (1) under 'normal' physiological conditions, $Z = 8.5 \pm 0.2‰$, and (2) shortly after the donation, $Z = 7.8 \pm 0.4‰$. After a blood donation, the plasma volume quickly recovers, while restoration of the globular volume is physiologically longer [8]. The observed increase of the O_2 fractionation, from 7.8 to 8.5‰ ($\Delta = +0.7‰$) is in agreement with the positive linear relation between Z and the haemoglobin count (Hb) previously observed: $Z = 1.2\ \mathrm{Hb} - 3.0‰$ [4]. A blood donation of 400 g ($\sim$ 10% of the total blood volume) should have increased the Z value of 0.12‰ ($\Delta Z = 1.2\,\Delta\mathrm{Hb}$), instead of 0.7‰. The time elapsed between both samples (2.5 weeks) may not have been sufficient for the individual's globular volume to totally renew. The extrapolation of the relation existing between Z and Hb could give a better insight of the limiting factor for the O_2 isotope fractionation. If an individual had a nil Hb, he would exhale O_2 that would only undergo the isotope fractionation associated to O_2 diffusion through the pulmonary membranes. The intercept in the Z–Hb relation thus gives an estimation of this fractionation: $\Delta = -3.0‰$. This negative value is unexpected, as diffusion should imply an ^{18}O depletion of the exhaled air. Nevertheless, it still may indicate that isotope fractionation induced by diffusion is constant (assumption later reinforced by results on N_2, which only undergo diffusion). The fact that Z varies among individuals is thus an indication that the oxy-haemoglobin formation is the prevalent step in the determination of the final O_2 isotope fractionation. This conclusion could be reinforced by further in vitro studies, especially focusing on the ^{18}O affinity for hemoglobin.

4.2. Exercise influence

A sample was taken immediately after a 40-min intense effort (squash) and analysed on individual 6. The result yields a Z value of $6.0 \pm 0.4‰$ and a fixation rate of $2.5 \pm 0.4\%O_2\,s^{-1}$. Under normal physiological conditions, his fixation rate is only $1.7\%O_2\,s^{-1}$ for $Z = 7.7 \pm 0.2‰$. Vigorous exercise drastically increases the O_2 fixation rate. During effort, lungs are to take O_2 in the blood up to 20 times more than usual. This increase occurs in two ways: (1) increase of the number of open capillaries – O_2 diffusion between the alveoli gas and the blood increases ($\sim$ 3 times the value in rest conditions) –; (2) increase of the heart rate, with a concomitant increase of the pulmonary blood flow (4 to 6 times), the O_2 fixation rate being also increased [9]. At the same time, Z decreases with the effort [4]. According to the relation with the haemoglobin count, this suggests that, as blood flow increases, lungs behave as if Hb were to decrease, relatively.

4.3. Nitrogen

Like O_2, human respiration induces an isotope fractionation of the exhaled N_2, linear with the consumption of O_2 (Fig. 1B), Z' variation between individuals is really small (average of $1.1 \pm 0.4‰$; Fig. 1C). The existence of a N_2 isotope fractionation during human respiration implies an active role for this molecule. As Z' values are significantly smaller than Z ones, this role is minor (but not nil) compared to O_2. If the O_2 isotope fractionation associated to diffusion is constant ($\sim -3‰$), it can be assumed that it is also true for N_2. This would thus explain why no noticeable differences are observed among the Z' values, as N_2 only

undergoes pulmonary diffusion. Further experiments should consist in ensuring the independence of $\delta^{15}N$ towards Hb, and in performing in vitro analysis of bacterial strains (as nitrogen metabolism is controlled by the intestinal bacterial activity).

5. Conclusions

Our study confirms that humans preferentially use ^{16}O in respiration, and may put to the fore that they also use ^{14}N (in a smaller proportion). All isotope fractionations are linear with the amplitude of O_2 consumption, and vary from one individual to the other (for O_2). Physiological parameters such as exercise, haemoglobin count, O_2 fixation rate and age (which influences the O_2 fixation rate), all affect O_2 fractionation. Isotope effect on N_2 seems to be individual-independent. There is no clear evidence for determining the prevalent step (diffusion or O_2 fixation) in human respiration, even if O_2 isotopes favour the latest. But the study of the (non-)relation between Hb and both O_2 and N_2 should bring more constrains and help discriminating both processes. The existence of a blood flow measurement, displaying significant variations, would be a huge step in the comprehension and investigation of gas fractionations during human respiration.

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