Lung membrane conductance and capillary volume derived from the NO and CO transfer in high-altitude newcomers

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Submitted 7 December 2012; accepted in final form 16 April 2013

Martinot JB, Mulè M, de Bisschop C, Overbeek MJ, Le-Dong N, Naeije R, Guénard H. Lung membrane conductance and capillary volume derived from the NO and CO transfer in high-altitude newcomers. J Appl Physiol 115: 157–166, 2013. First published April 18, 2013; doi:10.1152/japplphysiol.01455.2012.—Acute exposure to high altitude may induce changes in carbon monoxide (CO) membrane conductance (DmCO) and capillary lung volume (Vc). Measurements were performed in 25 lowlanders at sea level (D0), at 4,300 m after a 2- or 3-day exposure (D2,3) without preceding climbing, and 5 days later (D7,8), before and after an exercise test, under a trial with two arterial pulmonary vasodilators or a placebo. The nitric oxide (NO)/CO transfer method was used, assuming both infinite and finite arterial pulmonary vasodilators or a placebo. The nitric oxide (NO) value led to about a twofold increase in DmCO. Compared with sea level, lung diffusion capacity for CO increased by 24% at D2,3 and is returned to

nonnative but long-term residents, previous studies have reported improved CO diffusion (10, 14).

In acute hypoxia or after a short period of acclimatization, pulmonary arteriolar vasoconstriction induces a mild resting increase in pulmonary arterial pressure (Ppa) (34, 35). This is believed to induce pulmonary vascular distension and/or the recruitment of capillaries, despite hypoxic pulmonary arterial vasoconstriction, and is presumed to be unevenly distributed. An increase in capillary blood volume (Vc) follows the increase in Ppa in the compliant pulmonary capillary network of the lung (20). Pulmonary vasodilators, by modifying hemodynamics by reducing Ppa, could support the process of acclimatization and prevent lung interstitial edema during exercise (15). The increase in Vc could also be related to the increase in total lung capacity (TLC) often reported at altitude, which would expand the pulmonary vasculature (32).

Subdivision of DlCO into its components, e.g., the membrane conductance for CO (DmCO) and the capillary conductance (θcO), where θ is the blood conductance of CO (θcO), provide more mechanistic information than DlCO alone (27). The membrane and blood conductances have been previously derived by others from the multistep O2 method, as described in the model of Forster (19). However, compared with this multistep method, the dual-gas nitric oxide (NO)/CO method, applied in this study, allows the derivation of DmCO and Vc in a single step, but requires a value for blood conductance of NO (θNO). In this method, the specific θNO and the PO2-dependent specific θcO are both key parameters (28). While there is some controversy as to whether θNO is infinite or has a finite value, recent reappraisal of the in vivo value of θNO strongly suggests that a finite value has to be given to this coefficient (7). The selection of a finite or infinite value for θNO critically impacts on calculation of membrane component of alveolar-capillary transfer of gases (Dm) and Vc.

Our aim was to study the alterations in DmCO and Vc with the NO/CO method, introducing in the calculations several critical assumptions that will be discussed. Measurements were made at rest at sea level and after acute exposure to HA, in healthy nonacclimatized lowlanders, without preceding climbing effort and without a history of HA pulmonary edema (HAPE). The measurements were repeated after maximal exercise at HA after double-blind randomization for 5 days to placebo, or pulmonary arterial vasodilator (a phosphodiesterase-5 inhibitor and/or a selective endothelin receptor blocking agent). It was presumed that the combination of both drugs would allow more effective control of Ppa. The results using

1This article is the topic of an Invited Editorial by John M. B. Hughes (26a).

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assumptions for an infinite or a finite value of $\theta_{\text{NO}}$ were compared.

**Glossary**

AMS: Acute mountain sickness  
DmCO: Lung diffusing capacity for carbon monoxide  
DmNO: Lung diffusing capacity for nitric oxide  
Dm: Membrane component of alveolar-capillary transfer of gases  
HA: High altitude  
Hb: Hemoglobin concentration  
HR: Heart rate  
LL score: Lake Louise score  
PbO2: Mean pulmonary capillary oxygen pressure  
Ppc: Pulmonary capillary pressure  
Pla: Left atrial pressure  
PO2: Oxygen partial pressure  
Ppa: Pulmonary arterial pressure  
PVR: Pulmonary vascular resistance  
Spo2: Oxygen saturation  
TLC: Total lung capacity  
VA: Alveolar volume  
Vc: Capillary blood volume  
$\theta$: Tissue or blood gas conductance

**MATERIAL AND METHODS**

**Study population.** Healthy nonsmoking subjects with no prior history of HAPE or other significant pulmonary disease were recruited.

**Study design.** Baseline measurements for all parameters were performed at sea level (Brussels, Belgium), within 3 days preceding the departure to Lima, Peru. The subjects were transported from Lima to Cerro de Pasco (altitude 4,380 m) over 2–3 days with an overnight stop in Tarma (altitude 3,000 m). No strenuous exercise was permitted before arrival at Cerro de Pasco. Tests were done at rest at sea level and at HA on day 2 or 3 ($D_{2,3}$). Subjects were then started on double-blind vasodilator therapy, and physiological tests were repeated on day 7 or 8 ($D_{7,8}$) at rest and after an incremental maximal exercise and a subsequent endurance exercise after return of heart rate (HR) to baseline. The incremental exercise was performed on a cycle ergometer (Monark, Ergomedic, 818E). The exercise started at 25 W and increased by step of 20–30 W/min, according to the physical capacity of the subject, until the maximal workload sustainable for at least 30 s was achieved. The following endurance exercise, aimed at inducing some interstitial edema, was performed on an ergometer at 80% of the maximal power. This exercise lasted up to 30 min.

Systemic blood pressure was measured by sphygmomanometry and oxygen saturation ($SpO2$) by pulse oximetry (Onyx 9500, Nomin Medical).

The presence of acute mountain sickness (AMS) was monitored using the Lake Louise consensus scoring system (LL score) (37), with a score of 4 or more suggesting a diagnosis of AMS.

All testing was preceded by a clinical examination with determination of the AMS score and measurements, of blood pressure, $SpO2$, and HR.

**Vasodilator therapy.** After completion of the measurements performed upon arrival in Cerro de Pasco ($D_{2,3}$), the subjects were randomized in a double-blind procedure to one of three groups: group 1, placebo per os ($n = 7$); group 2, sitaxsentan 100 mg/day per os ($n = 8$); or group 3, sitaxsentan 100 mg/day + sildenafil 20 mg 3 times/day per os ($n = 9$) for 5 days (6). Procedures were in place for unblinding if needed. All measurements were repeated at the same time of the day, between 1 and 4 h after the last dose on $D_{7,8}$.

**Measurement of NO/CO transfer.** Spirometry was first performed to obtain pulmonary function. The double NO/CO method has been described previously (15) with the same equipment and software (Hyp’Air compact, Medisoft). The single-breath method was used with a true breath hold of 4 s. The concentrations of CO and NO in the inspired gas were corrected for hemoglobin concentration and PO2; $V_A$, alveolar volume; $PbO_2$, pulmonary capillary O2 pressure.

<table>
<thead>
<tr>
<th>Author (Ref. No.)</th>
<th>Location (Altitude, m)</th>
<th>Previous Climbing Effort to Arrive at HA Newcomers</th>
<th>Single-breath Diffusion Test Postarrival Days at HA</th>
<th>Technique Used for $D_{mCO}, V_c$ Analysis</th>
<th>$D_{mCO}$ Results</th>
<th>Correction For Sea Level Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Bisschop et al. (15)</td>
<td>Khumbu area (5,050 m)</td>
<td>Yes</td>
<td>On day 7</td>
<td>Double NO/CO method</td>
<td>Unchanged</td>
<td>With $PbO_2$</td>
</tr>
<tr>
<td>Agostoni et al. (2)</td>
<td>Everest (5,400 m)</td>
<td>Yes</td>
<td>On day 25</td>
<td>Multiple-step $O_2$ hyperoxic mixture</td>
<td>Increased</td>
<td>With Kanner formulae</td>
</tr>
<tr>
<td>Dehnert et al. (16)</td>
<td>Monte Rosa (4,559 m)</td>
<td>Yes</td>
<td>On day 1/2</td>
<td>Not done</td>
<td>Unchanged</td>
<td>With Kanner formulae</td>
</tr>
<tr>
<td>Cremona et al. (13)</td>
<td>Monte Rosa (4,559 m)</td>
<td>Yes</td>
<td>On day 1</td>
<td>Not done</td>
<td>$D_{mCO}/V_A$ correlated with closing volume</td>
<td>Not done</td>
</tr>
<tr>
<td>Ge et al. (21)</td>
<td>Qinghai, China (4,700 m) Leadville, Colorado (3,100 m)</td>
<td>No</td>
<td>On day 3</td>
<td>Not done</td>
<td>Increased</td>
<td>Not done</td>
</tr>
<tr>
<td>Cerny et al. (10)</td>
<td></td>
<td>Yes</td>
<td>On day 36</td>
<td>Multiple-step $O_2$ hyperoxic mixture</td>
<td>Increased</td>
<td>Not done</td>
</tr>
<tr>
<td>Weiskopf and Severinghaus (48)</td>
<td>White Mountain, California (4,300 m)</td>
<td>Yes</td>
<td>On day 1/2</td>
<td>Multiple-step $O_2$ hyperoxic mixture</td>
<td>Decreased</td>
<td>Not done</td>
</tr>
</tbody>
</table>
~2,000 ppm for CO and 40 ppm for NO. Inspired oxygen and helium fractions were, respectively, 0.19 and 0.12. The alveolar lung volume (VA) was calculated using the dilution technique with helium. The expired fraction of helium was corrected for both the cross-sensitivity of the catharometer for CO2 and O2 and the respiratory exchange ratio.

**Diffusion calculations.** The mean capillary oxygen pressure (PbO2) was calculated from the equation, PNO2 - PbO2 = VCO2/DmCO. The alveolar oxygen partial pressure (PAO2) was measured in the expired sample. Oxygen uptake (VCO2) was calculated from the mass balance of oxygen between inspiration and expiration during the maneuver. The fraction of oxygen in the residual volume preceding the inspiration was assumed to be similar to that found in the midexpired sample. DlCO × 1.23 was used as a surrogate for DlO2. The CO value was calculated according to PbO2 using Forster’s 1987 equation: 1/\( \theta_{CO} \) = 1.3 + 0.0041 PbO2 (19). The linearity of the \( \theta_{CO} \) vs. PbO2 relationship in the low range has been checked in several experiments (45).

**Specific conductance for NO.** \( \theta_{NO} \) has been assumed by several investigators to be either infinite or finite with a value of 4.5 ml NO·ml blood⁻¹·min⁻¹·mmHg⁻¹ calculated from the in vitro value of initial overall rate of NO uptake by red cell (c) of Carlsen and Comroe (8). From experiments in vivo, Borland et al. (7) found a \( \theta_{NO} \) value similar to the in vitro value (9, 28). Both finite and infinite values for specific \( \theta_{NO} \) were used here for the calculations of DmCO and Vc.

The coefficient linking lung diffusing capacity for NO (DlNO) and DmCO was set at 1.97, according to the solubility and molecular weights of both gases; therefore DmCO was equal to DlNO/1.97 when \( \theta_{NO} \) was chosen as infinite in the Roughton and Forster equation (26). Vc values were derived from transfer factor for CO, \( \theta_{CO} \), and DmCO value and corrected (Vc cor) for the standard hemoglobin (Hb) concentration (Hb std) set at 14.6 and 13.4 g/dl for men and women, respectively: Vc cor = Vc × (Hb std/Hb measured).

**Relationships between Vc or DmCO and the ratio of NO and CO conductances for Hb, k.** With \( \theta_{NO} = 4.5 \text{ ml·min}^{-1}·\text{mmHg}^{-1}·\text{ml}^{-1} \), \( k = \theta_{NO}/\theta_{CO} \) would be 7.7 in normoxic condition. It is worth noticing that this ratio is \( \theta_{O2} \) dependent and cannot be considered as a constant.

If \( \theta_{NO} \) is chosen very high, \( k \) would tend to infinity. The relation between the values of Vc or DmCO using one of these hypothesis or the other is developed in the Appendix.

** Corrections for altitude.** The effect of a reduced inspiratory PO2 at altitude was taken into account by calculating the \( \theta_{CO} \) value corresponding to PbO2, assuming that the 1/\( \theta_{CO} \) vs. PO2 relationship remains linear in the hypoxic range. Once Vc and DmCO were calculated in the conditions of altitude, 1/DlCO could be corrected for the effect of hypoxia using the previously calculated values of Vc and DmCO and a normoxic PbO2 value as that measured at sea level: 1/DlCO = 1/DmCO + (1.3 + 0.0041 × PbO2/Nc) (19). All DlCO values reported in the article are corrected for both Hb concentration and altitude.

All gas and volume calibrations were performed before the experiments. A venous sample of blood was drawn for Hb concentration (Hb measured).

**Doppler echocardiographic analysis.** Images were acquired using a Cx50 echocardiographic system (Philips Medical System, Andover, MA) at rest before exercise.

Cardiac output (Q) was estimated from left ventricular outflow tract cross-sectional area and pulsed Doppler velocity-time integral measurements. Systolic Ppa (Ppa0) was estimated from a trans-tricuspid gradient calculated from the maximum velocity (V) of continuous Doppler tricuspid regurgitation, as 4 × V² + 5 mmHg assigned to right atrial pressure. Mean Ppa (Ppam) was calculated as 0.6 × Ppas + 2 (11). Left atrial pressure (Pla) was estimated from the ratio of mitral E flow-velocity wave and tissue Doppler mitral annulus E’ early diastolic velocity, with Pla = 1.9 + 1.24 E/E’. Pulmonary vascular resistance (PVR) was calculated as (Ppam – Pla)/Q. Pulmonary capillary pressure (Ppc) was calculated as 0.4 × (Ppam – Pla) + Ppa (22). This equation is derived from in vitro-determined longitudinal distribution of resistances, which showed that 60% of PVR is normally upstream to the capillaries (20). This distribution has been confirmed in intact normoxic or hypoxic humans, as assessed by the single pulmonary arterial balloon occlusion technique (31).

**Ethics committee.** The study protocol received the agreement of the Ethical Committee of the Erasme Academic Hospital, and a written informed consent was obtained for all the participant subjects.

**Statistical analysis.** Data were analyzed on IBM SPSS 20.0 (Chicago, IL). Our participants were classified into nine subgroups by combination of altitude level and treatments. For each subgroup, homogeneity of variances was confirmed by Levene’s test. Changes in variable values from sea level to HA under three different medical treatments were analyzed by a two-way ANOVA with treatment and altitude as fixed factors. Mean values were compared by using Tukey’s post hoc test, and interaction effect of treatments on each combination with altitude level was assessed by univariate F-test. The effect of exercise on diffusing parameters was analyzed by Wilcoxon signed-rank test. Significance threshold was set at 5%.

**RESULTS**

Twenty-five subjects, 30 ± 12 yr old with body mass index of 22.8 ± 2.3 and height 173 ± 8 cm, were recruited. Vital capacities were normal. One participant developed severe symptoms of AMS during the first night in Cerro de Pasco and could not complete the study. One other subject was excluded for the analysis pre/post because his HR remained up to 120 beats/min, after one-half hour rest postexercise. No other subject had evidence for AMS on clinical scoring (LL) on D2,3 beated/min, after one-half hour rest postexercise. No other subject had evidence for AMS on clinical scoring (LL) on D2,3 beated/min, after one-half hour rest postexercise. No other subject had evidence for AMS on clinical scoring (LL) on D2,3 beated/min, after one-half hour rest postexercise. No other subject had evidence for AMS on clinical scoring (LL) on D2,3 beated/min, after one-half hour rest postexercise. No other subject had evidence for AMS on clinical scoring (LL) on D2,3 beated/min, after one-half hour rest postexercise. No other subject had evidence for AMS on clinical scoring (LL) on D2,3 beated/min, after one-half hour rest postexercise. No other subject had evidence for AMS on clinical scoring (LL) on D2,3 beated/min, after one-half hour rest postexercise. No other subject had evidence for AMS on clinical scoring (LL) on D2,3 beated/min, after one-half hour rest postexercise. No other subject had evidence for AMS on clinical scoring (LL) on D2,3 beated/min, after one-half hour rest postexercise. No other subject had evidence for AMS on clinical scoring (LL) on D2,3 beated/min, after one-half hour rest postexercise. No other subject had evidence for AMS on clinical scoring (LL) on D2,3 beated/min, after one-half hour rest postexercise.
followed the same pattern of changes than Ppa. The indexed PVR were similar for all treatment groups: 5.8 ± 1.0 (placebo), 6.8 ± 0.9 (sitaxsentan), and 5.6 ± 1.4 Wood unit/m² (sitaxsentan + sildenafil) and remained unchanged compared with D2.3. A similar pattern observation was observed for Pla and cardiac index. VA increased significantly from 5.5 ± 1.0 to 6.4 ± 1.2 liters at HA on D2.3 and decreased on D7.8 (5.8 ± 1.0 liters) toward sea level values.

**Diffusion variables.** Sea level and HA values (HA D2.3 and D7.8) of the variables are reported in Table 3 in which all of the subjects data were grouped together, irrespective of drug allocation.

**DmCO.** As no significant effect of the vasodilators on resting preexercise DmCO was observed on D7.8, the data obtained in the three treated groups were combined. The increased DmCO seen at HA on D2.3 has almost reverted to sea level values by D7.8. The effect of the randomized drug treatment allocation started after the D2.3 measurements is illustrated in Fig. 1. The treatments had no effect on DmCO at rest before exercise.

**DlNO/DlCO.** DlNO data for all subjects, irrespective of drug treatment, are plotted against DlCO for the three altitude conditions in Fig. 2. The slope values of the regression lines are estimations of the DlNO-to-DlCO ratios in the group. The ratio DlNO/DlCO decreased significantly from 4.93 to 4.38 between sea level and D2.3, owing to a greater increase in DlCO than DlNO (Fig. 2A). As the minimum value of this ratio is 2 (24), the relative decrease of the ratio was 19% [100 × 0.76/(5.05 − 2)]. After 1 wk at altitude on D7.8 the DlNO-to-DlCO ratio increased significantly coming back to its sea level value. There was no difference between treated groups, thus the changes observed were only due to altitude. The same profile of changes was observed for the ratio of DlNO/VA as DlCO/VA (Fig. 2B). The ratios derived from the slopes of the DlNO vs. DlCO relationships are slightly different from the arithmetic mean of the individual values of these ratios reported in Table 3.

**DmCO.** The effects of altitude and pulmonary vasodilators on resting DmCO at HA (D2.3), and after drug treatment before exercise (D7.8), are reported in Table 3. The importance of the assumption made for θNO in the calculation of DmCO is illustrated by the data in Table 3 and in Fig. 3. Assuming an infinite θNO value, the elevated DmCO seen at HA on D2.3 has almost reverted to sea level value by D7.8. Assuming a finite θNO value, the elevated DmCO seen at HA on D2.3 has not reverted to sea level value by D7.8.
Assuming an infinite value for $\theta_{NO}$, compared with sea level value, $D_{mCO}/VA$ remained stable on D2,3 and D7,8. By contrast, assuming a finite $\theta_{NO}$ value, the $D_{mCO}/VA$ increase observed on D2,3 became significant on D7,8.

$V_c$. The effects of altitude and pulmonary vasodilators on resting $V_c$ at HA (D2,3), and after drug intervention before exercise (D7,8), are reported in Table 3. Whether $\theta_{NO}$ is finite or not, the observed increase in $V_c$ at HA on D2,3 has almost reverted to sea level values by D7,8. The 30% $V_c$ increase, assuming a infinite or a finite $\theta_{NO}$ on D2,3, is reduced to 10% if $V_c$ is divided by VA.

$D_{mCO}/V_c$ results at HA on D2,3 and on D7,8 compared with sea level measurements. The pattern of changes in $D_{mCO}/V_c$ followed that in $DL_{NO}/DL_{CO}$, assuming the infinite $\theta_{NO}$ hypothesis (Table 3). This is in agreement with theory; according to Eq. A3 in the APPENDIX, $DL_{NO}/DL_{CO}$ and $D_{mCO}/V_c$ are linearly related. Both ratios decreased on D2,3 and came back to sea level values on D7,8. Such was not the case with the finite assumption, $D_{mCO}/V_c$ remained stable on D2,3 as both components of the ratio increased similarly. Owing to the decrease in $V_c$ on D7,8 and the constancy of $D_m$ which remained at D2,3 value, the ratio $D_{mCO}/V_c$ increased significantly by $\approx 21\%$ compared with sea level. Drugs had no effect on these changes (Table 4).

Impact of vasodilators on postexercise diffusion measurements on D7,8: The level of exercise was assessed by the maximal power reached (Table 2). Postexercise, $DL_{CO}$ and $V_c$ remained unchanged, irrespective of treatment allocation, with mean changes, respectively, of 0.1 ml·min$^{-1}$·mmHg$^{-1}$ and of 0.5 ml (infinite assumption) or 0.3 ml (finite assumption). Resting pre- and postexercise $D_{mCO}$ values on D7,8 are plotted in Fig. 4, according to the treatment subgroup and the assumptions for $\theta_{NO}$. Exercise induced a slight but significant decrease in $D_{mCO}$ in the placebo group, but not in the treated groups.

Fig. 1. Lung diffusing capacity for carbon monoxide ($DL_{CO}$) changes at rest soon after arrival at high altitude (HA) [day 2 or 3 (D2,3)] and after administration of vasodilator therapy [day 7 or 8 (D7,8)]. The $P$ values are shown for all subjects combined, irrespective of drug treatment. The increases in $DL_{CO}$ seen at HA on D2,3 had almost vanished by D7,8. The data are also analyzed according to the randomized drug treatment allocation, which was started after the D2,3 measurements. There is no discernible impact of drug therapy on D7,8. Open bars, placebo subgroup; hatched bars, sitaxsentan subgroup; dotted bars, sitaxsentan and sildenafil subgroup. NS, nonsignificant.

Fig. 2. A: lung diffusing capacity for nitric oxide ($DL_{NO}$) plotted against $DL_{CO}$ results at rest, at sea level (●, dashed line) soon after arrival at HA (D2,3) (●, dotted line), and after administration of vasodilator therapy at rest before exercise (D7,8) (●, solid line). The data are presented for all subjects, irrespective of drug treatment. All linear regression equations were significant, with a $P$ value < 0.001. Slope values are indicated on the right side. B: the same pattern of changes was observed for the ratios of the NO and CO diffusing capacities to alveolar volume ($VA$).
Table 4. Resting lung diffusion components in healthy participants at high altitude on day 7/8 under trial

<table>
<thead>
<tr>
<th>At High Altitude (Day 7/8)</th>
<th>( \theta_{NO} )</th>
<th>Placebo</th>
<th>Sitaxsentan</th>
<th>Sitaxsentan and Sildenafil</th>
</tr>
</thead>
<tbody>
<tr>
<td>( D_{mCO}, \text{ ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1} )</td>
<td>Infinite</td>
<td>80.3 ± 13.0</td>
<td>81.9 ± 19.5</td>
<td>80.4 ± 17.9</td>
</tr>
<tr>
<td>( Vc, \text{ ml} )</td>
<td>Infinite</td>
<td>92.3 ± 16.7</td>
<td>90.5 ± 21.6</td>
<td>90.1 ± 14.6</td>
</tr>
<tr>
<td>( D_{mCO}/Vc, \text{ ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1} )</td>
<td>Infinite</td>
<td>0.86 ± 0.10</td>
<td>0.91 ± 0.04</td>
<td>0.87 ± 0.08</td>
</tr>
<tr>
<td>( Vc, \text{ ml} )</td>
<td>Finite</td>
<td>183.0 ± 36.2</td>
<td>191.5 ± 45.7</td>
<td>169.3 ± 51.2</td>
</tr>
<tr>
<td>( D_{mCO}/Vc, \text{ ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1} )</td>
<td>Finite</td>
<td>68.7 ± 12.4</td>
<td>67.3 ± 16.1</td>
<td>67.0 ± 10.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. The variables are presented according to the value assigned to the \( \theta_{NO} \). No statistical significant difference was found between groups.
capillary network is considered as a sheet of blood between two layers of membrane, the distension of capillaries would not increase lung surface or $DmCO$ in a meaningful way. Hypoxic vasoconstriction has also been recently documented, in the conditions of a simulated altitude exposure, to recruit pulmonary capillaries (44).

Another factor determining the increase in $Vc$ is the lung capillary compliance. The effect of hypoxia on this compliance is, however, unknown. Capillaries reduce their caliber when endothelial cells are stimulated by various agonists (46), suggesting associated changes in capillary compliance. The present data show that mean capillary pressure increased to a modest degree from a mean of 11 mmHg to 12.5 mmHg at altitude, suggesting a compliance of 11.5 ml/mmHg (20). This value seems much higher than that published by Glenet et al. (24) under normoxia of ~1 ml/mmHg. On D2,3, $Vc$ recovered to the D2,3 value, even though $Ppa_m$ remained higher than at sea level. This is in agreement with a reduction of the vascular compliance following adaptation to hypoxia and/or local active control of the microvessel caliber. The complex biological coupling between hypoxia and endothelial NO production is controlled, at least in part, by local pH. At HA, the respiratory alkalosis can change carbonic anhydrase activity to overproduce NO from nitrite (1). The transcapillary pressure (the mean intracapillary pressure $Ppc$ minus the pericapillary pressure) is largely determined by the elastic fibers present in the alveolar interstitium (49). As $V_A$ increased on D2,3, this could have induced a distension of capillaries. The effect of this stretch on $Vc$ is not well documented, although it is known from morphometric experiments (5) and functional experiments (42) that $Vc$ increases far less than $V_A$ above 60% TLC, leading to a progressive fall in $Vc/V_A$ with the %$V_A$. It seems, therefore, likely that the increase in $Vc$ observed acutely at HA on D2,3 is not, in the main, due to lung distension. It is worth noting that both $Vc$ and $V_A$ were reduced later on D7,8, returning to values seen on D2,3.

Assuming that $Vc$ is a sheet of thickness $K$ and surface $S$, $DmCO$ is proportional to $S$ and inversely related to the total membrane thickness ($\delta$), the ratio $DmCO/Vc$ would be related to $1/\delta$ (24). An increase in $DmCO/Vc$ could, therefore, be explained by either a decrease in $\delta$ or $K$, or both parameters. The $\mu$ includes the thickness of the membrane and the plasma sheet, which varies from one point to another point of the capillaries and with time. The mean thickness depends on the hematocrit. At altitude, the hematocrit increases with time and more rapidly at the onset of exposure (35). For a given $Vc$ value, assuming that no change occurred in the lung surface, an increased hematocrit would reduce the thickness of the plasma sheet. A decrease in $\delta$ value at altitude seems, therefore, likely. $Vc$ remained unchanged on D7,8 at altitude compared with sea level, but this does not mean that the thickness $K$ remained constant if $S$ increased.

The increase in $V_A$ new arrivals at HA has been reported using both plethysmography and inert-gas dilution techniques (12, 25, 39). One hypothesis proposed by Mansell et al. (32) is that pulmonary compliance increases, leading to reduction in elastic recoil, moving the thoracic and lung elastic forces equilibrium point to higher lung volumes. We propose, however, that the more likely explanation for the change in pulmonary compliance is a release of surfactant due to sympathetic stimulation induced by hypoxia and/or sustained hyperventilation, which could mechanically stimulate release of
surfactant by type II pneumocytes. Both factors have been well documented experimentally (3). Compared with sea level, the relative increases in \( V_c \) and \( D_{mCO} \) on \( D_{2.3} \) were similar, suggesting a recruitment of capillaries and lung surface. On \( D_{7.8} \), a discrepancy between the results obtained using infinite or finite assumptions for \( \theta_{NO} \) value was observed. Using the infinite value, \( D_{mCO} \) and \( V_c \) returned to sea level values as \( D_{mCO}/V_A \) did not change. Thus using this assumption for \( \theta_{NO} \) would lead to the conclusion that altitude hypoxia had only a transient effect in the first days of exposure. In contrast, using the finite assumption for \( \theta_{NO} \), \( D_{mCO} \) remained elevated at its previous \( D_{2.3} \) value, and, as \( V_A \) decreased, the ratio \( D_{mCO}/V_A \) increased significantly compared with both sea level and \( D_{2.3} \). This, in turn, could be explained by a redistribution of blood volume in the lung becoming more homogenous and increasing the effective surface of the lung for gas exchange, i.e., the efficient surface for oxygen exchange. A better adaptation of arterial vasoconstriction to local \( P_O2 \) could be the cause of this adaptation, and that could be viewed as part of the acclimatization to preserve oxygen transfer to \( Hb \) (41).

The discrepancy between the results derived from the two assumptions for \( \theta_{NO} \) values is noteworthy as having important impact on the interpretation of \( NO/CO \) transfer. However, the finite hypothesis appears to be more acceptable, as this is based on experimental data and provides \( D_{mCO} \) values better in agreement with morphology (47). Further experiments using the two hypotheses are needed to settle definitely this discrepancy.

The phosphodiesterase-5 inhibitor sildenafil has been shown to decrease pulmonary vascular resistance and improve maximum oxygen consumption at altitude (22); sildenafil also increases arterial oxygenation, suggesting that it may improve exercise capacity in hypoxic conditions related to an improvement of the gas exchange at the alveolar-capillary level. Endothelin receptor antagonism is known to partially reverse the increase in \( P_pA \), under acute hypoxic normobaric conditions and in chronic hypobaric hypoxia (33, 34). We hypothesized that sitaxsentan, a selective endothelin receptor antagonist, combined with sildenafil, would augment this vasodilator effect. No significant effect, however, has been observed, probably because the increase of \( P_pA \) in our subjects remained slight.

The 5-day treatment period required to reach a stable and active blood concentration of sitaxsentan had no effect on the diffusion variables measured at rest before the exercise compared with placebo. The acclimatization, as assessed by diffusion, took place independent of treatment allocation.

Pulmonary vasoconstriction may counteract distension of the capillary bed observed in acute hypoxia. After 5 days of treatment, \( V_c \) returned to sea level value, despite the persistence of a mild pulmonary hypertension. This trend was not altered by the arterial pulmonary vasodilators.

To test the relationship between \( D_{mCO} \), its components, and \( P_pA \), the newcomers were exposed to a maximal exercise followed by an endurance exercise to induce some increase in the filtration pressure and interstitial edema (13, 17). The single-breath CO transfer factor appears to be insensitive to early interstitial edema formation, as only minor changes in \( D_{mCO} \) in subjects were reported in subjects with radiographically evident of HAPE (4). The measurement of \( NO \) transfer should be more sensitive, as the membrane is the main determinant of its transfer; however, data are scarce (15).

The arterial vasodilating agent sitaxsentan alone or combined with sildenafil did indeed prevent the decrease in \( D_{mCO} \) after exercise, independent of the use of a finite or infinite values for \( \theta_{NO} \). This is consistent with a clinically significant effect on exercise-induced pulmonary arterial hypertension (15, 31, 43). The better sensitivity of \( D_{mCO} \), calculated with the \( NO/CO \) method, compared with \( D_{LCO} \) alone to detect interstitial edema is confirmed (Fig. 4).

\( P_pA \) increases at exercise in normoxia. Owing to pulmonary vasoconstriction, higher \( P_pA \) values are expected at altitude with an increased risk of pulmonary edema. Pulmonary vasodilators could reduce this risk by decreasing \( P_pA \) and heterogeneous pulmonary vasoconstriction. That has been suggested in previous experiments after a 15-day period of acclimatization at 5,000-m altitude. A significant effect of a single dose of 50-mg sildenafil was effective in reducing pulmonary arterial hypertension at exercise (18). That is also consistent with a role of \( P_pA \), in inducing a thickening of the alveolar membrane. Our group recently reported that membrane conductance of new arrivals at 5,000-m altitude over 2 wk was significantly reduced, and that sitaxsentan could prevent a further decrease induced by maximal exercise (15). The administration of a specific endothelin receptor antagonist combined with sildenafil allowed a significant decrease in \( P_pA \). This suggests a mechanical stress effect of \( P_pA \) on the pulmonary capillary at HA.

In conclusion, acute exposure to altitude hypoxia induced an increase in \( V_c \) and \( D_{mCO} \) associated with a minimal increase in \( P_pA \) at rest in the first days after arrival. The increase in \( V_c \) resolved within 1 wk. The initial increase in \( CO \) diffusing capacity at acute exposure to HA in newcomers is presumably explained by a recruitment of lung capillaries due to changes in pulmonary hemodynamics and the mechanical effect of the expanded \( V_A \). Afterwards, \( D_{LCO} \) normalized within 1 wk. Changes in \( V_c \) were observed independent of the value assigned to \( \theta_{NO} \). By contrast, \( D_{mCO} \) data derived from the infinite value of \( \theta_{NO} \), which are in better agreement with the data derived from morphometry, −250 ml·min\(^{-1}\)·mmHg\(^{-1} \) (47), could reflect a less heterogeneous distribution of blood capillary flow at the end of the first week stay at HA. Exercise induced a significant decrease in \( D_{mCO} \), which was prevented by pretreatment with pulmonary vasodilators (sitaxsentan + sildenafil).

**APPENDIX**

\( V_c = (1/\theta_{CO})(1 - \alpha/k)(1/D_{mCO} - \alpha/D_{LNO}) \), where \( \alpha = D_{mCO}/D_{LNO} = 1.97 \). \( V_{c7.7} \) and \( V_c \) are the \( V_c \) values for \( k = 7.7 \) and \( k = \infty \), respectively. For \( k = \infty \), \( V_c = (1/\theta_{CO})(1/D_{mCO} - \alpha/D_{LNO}) \).

The \( V_c \) value with \( D_{mCO} \) data derived from the finite value of \( \theta_{NO} \), which are in better agreement with the data derived from morphometry, −250 ml·min\(^{-1}\)·mmHg\(^{-1} \) (47), could reflect a less heterogeneous distribution of blood capillary flow at the end of the first week stay at HA. Exercise induced a significant decrease in \( D_{mCO} \), which was prevented by pretreatment with pulmonary vasodilators (sitaxsentan + sildenafil).

\[ D_{mCO}^{TOT} = D_{mCO}^{TOT} - D_{mCO}^{MIN} \]

\[ D_{mCO}^{MIN} = (1 - \alpha/k)[1 - D_{LNO}/(D_{K} - D_{LNO})] \]

\[ D_{mCO}^{TOT} = D_{mCO}^{TOT} - D_{mCO}^{MIN} \]

\[ D_{mCO}^{TOT} = 0.77/1(D_{LNO} - 0.13/D_{LCO}) \]

\[ D_{mCO}^{TOT} \] value cannot be derived simply from \( D_{mCO}^{TOT} \) value.

\[ D_{mCO}^{TOT}/D_{mCO}^{MIN} \]

\[ D_{mCO}^{TOT}/D_{mCO}^{MIN} = (1 - \alpha/k)[1 - D_{LNO}/(D_{K} - D_{LNO})] \] (Eq. A1).

\[ D_{mCO}^{TOT}/D_{mCO}^{MIN} = 0.74 - 0.13(D_{LNO}/D_{LCO}) \]\n
\[ D_{mCO}^{TOT}/D_{mCO}^{MIN} = 176 \text{ ml·min}^{-1}\text{·mmHg}^{-1} \] and \( D_{LCO} = 37 \text{ ml·min}^{-1}\text{·mmHg}^{-1} \) then \( D_{mCO}^{TOT}/D_{mCO}^{MIN} = 1.95 \).

AUTHOR CONTRIBUTIONS

GRANTS

The authors thank Dr. Philip Silkoff, who revised the article critically for intellectual content. The data management and secretarial assistance of Valerie Cuthbert, Sandrine Lamy, and Liesbeth Orij were greatly appreciated.

ACKNOWLEDGMENTS

This study was supported by the Etna Foundation, Catania, Italy, and by a grant from Pfizer.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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