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High-altitude exposure of three weeks duration increases lung diffusing capacity in humans

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Agostoni P, Swenson ER, Bussotti M, Revera M, Meriggi P, Faini A, Lombardi C, Bilo G, Giuliano A, Bonacina D, Modesti PA, Mancia G, Parati G. High-altitude exposure of three weeks duration increases lung diffusing capacity in humans. *J Appl Physiol* 110: 1564–1571, 2011. First published March 24, 2011; doi:10.1152/japplphysiol.01167.2010.—*Background*: high-altitude adaptation leads to progressive increase in arterial Pa_{O_2}. In addition to increased ventilation, better arterial oxygenation may reflect improvements in lung gas exchange. Previous investigations reveal alterations at the alveolar-capillary barrier indicative of decreased resistance to gas exchange with prolonged hypoxia adaptation, but how quickly this occurs is unknown. Carbon monoxide lung diffusing capacity and its major determinants, hemoglobin, alveolar volume, pulmonary capillary blood volume, and alveolar-capillary membrane diffusion, have never been examined with early high-altitude adaptation. *Methods and Results*: lung diffusion was measured in 33 healthy lowlanders at sea level (Milan, Italy) and at Mount Everest South Base Camp (5,400 m) after a 9-day trek and 2-wk residence at 5,400 m. Measurements were adjusted for hemoglobin and inspired oxygen. Subjects with mountain sickness were excluded. After 2 wk at 5,400 m, hemoglobin oxygen saturation increased from 77.2 ± 6.0 to 85.3 ± 3.6%. Compared with sea level, there were increases in hemoglobin, lung diffusing capacity, membrane diffusion, and alveolar volume from 14.2 ± 1.2 to 17.2 ± 1.8 g/dl ($P < 0.01$), from 23.6 ± 4.4 to 25.1 ± 5.3 ml·min^{-1}·mmHg^{-1} ($P < 0.0303$), 63 ± 34 to 102 ± 65 ml·min^{-1}·mmHg^{-1} ($P < 0.01$), and 5.6 ± 1.0 to 6.3 ± 1.1 liters ($P < 0.01$), respectively. Pulmonary capillary blood volume was unchanged. Membrane diffusion normalized for alveolar volume was 10.9 ± 5.2 at sea level rising to 16.0 ± 9.2 ml·min^{-1}·mmHg^{-1}·l^{-1} ($P < 0.01$) at 5,400 m. *Conclusions*: at high altitude, lung diffusing capacity improves with acclimatization due to increases of hemoglobin, alveolar volume, and membrane diffusion. Reduction in alveolar-capillary barrier resistance is possibly mediated by an increase of sympathetic tone and can develop in 3 wk.

hypoxia; respiration; heart failure; ventilation; oxygen

HIGH-ALTITUDE ASCENT leads to arterial oxygen desaturation, which lessens over time with adaptation to the hypoxic environment. The initial drop in arterial oxygenation with ascent results directly from a lower inspired P_{O_2}. During the first few days at altitude, arterial oxyhemoglobin saturation (SpO_2) is decreased (30), not only due to low inspired P_{O_2}, but also to gas exchange impairment at altitude, possibly as a result of increased ventilation perfusion inequality from a subclinical increase in interstitial lung fluid (2, 11, 47). The evidence for this, however, is equivocal (13). With further time and acclimatization at high altitude there is a significant progressive increase in arterial oxygenation occurring over days to weeks from an augmented hypoxic ventilatory response and more hyperventilation (6, 30, 52). In addition to greater ventilation, improved arterial oxygenation may also reflect an increase in pulmonary gas exchange efficiency (30). This has been demonstrated in studies of animals raised under high-altitude conditions (23, 25) and in high-altitude human populations (8, 12, 42, 51). Whether this occurs in lowlanders in the immediate weeks to months after arrival at high altitude is unknown.

One easily measured index of gas exchange efficiency is the carbon monoxide diffusing capacity of the lung (D_{LCO}) with the reciprocal of D_{LCO}, $1/D_{LCO}$, being the resistance to gas transfer across the alveolar-capillary membrane (1). Roughton and Foster (44) proposed the existence of two resistances in series, the alveolar-capillary membrane resistance ($1/D_M$) and the red cell resistance ($1/\theta\ V_C$), with θ as the rate of blood transfer conductance for the gas, in this case CO, measured in vitro for a given P_{O_2} and V_C the amount of blood undergoing gas exchange, the so-called pulmonary capillary blood volume. Thus $1/D_{LCO} = 1/D_M + 1/\theta\ V_C$ with each of the two being responsible for ~50% of $1/D_{LCO}$ (22). Numerous variables may influence D_{LCO}, including hemoglobin and carboxyhemoglobin concentrations, inspired oxygen partial pressure, alveolar-capillary barrier thickness, pulmonary capillary blood volume, gas exchange surface area, and cardiac output (1, 22, 44). Recently attention has been focused on the possible role of active sodium and fluid transport across the alveolar-capillary membrane in modulating D_{LCO} possibly at the level of the alveolar-capillary barrier thickness (3, 19, 36). Several groups have measured D_{LCO} at variable times after arrival at high altitude (13, 27, 47, 52), and, in general, they found that D_{LCO} (when corrected for hemoglobin and inspired oxygen differences) does not increase in the first several days, but begins to rise within several weeks.

The present study was undertaken to analyze pulmonary gas exchange in high altitude-acclimatized healthy lowlanders to specifically explore whether changes in D_{LCO} play a role in

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high-altitude adaptation and, if so, what components of the diffusing capacity contribute to its improvement.

METHODS

Lung diffusion measurements were performed at sea level and at Mount Everest South Base Camp after ~3 wk of high-altitude sojourn within the framework of the HIGHCARE (HIGHaltitude CArdiovascular REsearch) research project. The expedition included a total of 47 subjects. As part of a larger study examining the effects of angiotensin II receptor antagonism at high altitude, the subjects were randomly treated with either telmisartan 80 mg once daily ($n = 24$) or placebo ($n = 23$). Telmisartan/placebo treatment started at least 1 mo before ascending and continued until subjects returned to Milan. Drugs were not allowed for acute mountain sickness (AMS) prevention, but whenever it was deemed clinically necessary, subjects were treated and withdrawn from analysis. Thirty-seven (18 on placebo and 19 on telmisartan) of these 47 subjects were randomly assigned to diffusing capacity evaluation, of which 34 were nonsmokers and 3 were smokers. Professional climbers, athletes, and subjects with recent (<6 mo) exposure to altitude were excluded from this set of experiments. The three smokers abstained from tobacco use 24 h before the D_{LCO} measurements. All subjects underwent standard spirometry in Milan (altitude: 337 ft or 102 m).

High-altitude ascent technique, safety controls, and laboratory setting. Mount Everest South Base Camp is located at 5,400 m in Nepal on the south face of Mt. Everest below the "Ice Fall" Glacier and was reached by the subjects in a 9-day hike. During the sojourn at Mount Everest South Base Camp, subjects were not allowed to perform any further ascent (>300 m), relevant trekking, or strenuous physical activity. Gas exchange evaluation was done 12–14 days after arrival at Mount Everest South Base Camp. The experiments were performed in a heated tent with temperature and barometric pressure always recorded. Sea level evaluation was done in Milan in the month before arriving in Nepal.

During the HIGHCARE Expedition, mandatory medical checks were performed at Namche Bazar (3,400 m) and 1 and 2 days after arrival at Mount Everest South Base Camp. During these sessions several parameters were measured including heart rate, systemic blood pressure, respiratory rate, transcutaneous oxygen saturation (SpO_2, pulse oximeter, Life Scope I, Nihon Kohden, Tokyo, Japan) and pulmonary artery systolic pressure (PASP) by echo guided Doppler ultrasounds (Vivid I, General Electric, Tirat Carmel, Israel). SpO_2 and PASP were also measured before each D_{LCO} measurement. The occurrence and severity of AMS was assessed by means of the Lake Louise Score (43) during each safety check and before D_{LCO} measurements at Mount Everest South Base Camp. Norepinephrine plasma concentrations were measured in Milan by HPLC (reagents: CHROMSYSTEMS Instruments & Chemicals, Munich, Germany; instrumentation: HPLC Cromat, BioRad Laboratories, Hercules, CA) on blood collected at Milan and at Mount Everest South Base Camp where plasma samples were stored in liquid nitrogen.

Acclimatization parameters. Evaluation of high-altitude acclimatization was done at Namche Bazar and at Mount Everest South Base Camp 1–2 days after arrival and then again between *days 12* and *13* before the gas exchange measurements. Acclimatization parameters included Lake Louise Score, SpO_2, hemoglobin, venous total carbon dioxide concentration, heart rate, respiratory rate, systemic blood pressure, and pulmonary artery systolic pressure.

Diffusing capacity measurements. D_{LCO} at low altitude (Milan) and at the end of the 2-wk sojourn at 5,400 m was measured with the single breath-constant expiratory flow technique (exhalation rate = 0.5 l/s; Sensor Medics 2200, Yorba Linda, CA) and calculated according to American Thoracic Society 1995 update and ATS-ERS 2005 guidelines (1, 24, 31, 53). We performed duplicate D_{LCO} measurements. While it was our intention to measure D_{LCO} sequentially in all subjects while ascending to Mount Everest South Base Camp and immediately on arrival (roughly *day 9* at high altitude), we could not obtain these data due to logistical difficulties in the timely delivery of the gases and equipment during the trek. To rigorously compare changes in D_{LCO} with time at altitude, it is necessary to account and correct for differences in inspired O_2 (P_{IO_2}) due to differences in barometric pressure and changes in hemoglobin concentration due to altitude-induced erythropoiesis, both of which lead to an increased D_{LCO} independent of any adaptive changes in lung structure or function. Thus D_{LCO} at Mount Everest South Base Camp corrected for inspired P_{O_2} was calculated as D_{LCO} measured $\times$ [1.0 + 0.0031(P_{IO_2} − 150)] (31). Normative D_{LCO} values were those of Crapo et al. (10) who used the single breath technique. Wilson et al. (53) showed normative values for single breath and intra-breath techniques to be comparable. D_{LCO} was also corrected for hemoglobin concentration using the following formula: D_{LCO} measured $\times$ [(10.15 + hemoglobin)/1.7 $\times$ hemoglobin] (9). Diffusion subcomponents, D_M and V_C, were also measured by applying the Roughton and Forster (44) method to hemoglobin corrected D_{LCO}. For these measurements, subjects inhaled gas mixtures containing 0.3% methane (CH_4), 0.3% CO, with three different oxygen fractions equal to 0.2, 0.4, and 0.6, respectively, balanced with nitrogen (9). Due to differences in P_{IO_2} the 1/θ values used for the different F_{IO_2} were 1.71 (F_{IO_2} 20%), 2.21 (F_{IO_2} 40%), 3.17 (F_{IO_2} 60%) in the Milan laboratory and 1.44 (F_{IO_2} 0.2), 1.68 (F_{IO_2} 0.4), and 1.99 (F_{IO_2} 0.6) at Mount Everest South Base Camp, respectively (44). V_A was measured by CH_4 decay slope during single breath constant expiratory flow measurements (39). At Mount Everest South Base Camp we also assessed D_{LCO} repeatability over a 1-h interval in 16 subjects.

The study was approved by the ethical committee of Istituto Auxologico Italiano, IRCCS, with a registration code number of EudraCT 2008-000540-14. All subjects provided written informed consent.

Statistical analysis. Data are reported as means ± SD. Gas diffusion repeatability was assessed by the Bland and Altman method. Effects of high altitude on gas diffusion were analyzed by paired *t*-test. Effects of telmisartan on D_{LCO} were measured by unpaired *t*-test. Changes during high-altitude exposure were assessed by repeated-measures ANOVA followed by post hoc analysis corrected by Bonferroni method. *P* values <0.05 were considered as significant. All tests were two-sided and were performed using SAS statistical package V9.13 (SAS Institute, Cary, NC).

To account for the effect of heart rate increase, D_{LCO} at altitude was analyzed by repeated-measures ANCOVA including heart rate change as a covariate.

RESULTS

Four subjects developed severe AMS early after reaching Mount Everest South Base Camp, which required medical treatment and urgent temporary descent to a lower altitude. All four subjects returned a few days later and remained on medical treatment. Of these four subjects, one was on telmisartan and three were on placebo. None of the data measured in Milan could identify subjects who developed AMS (Table 1). In these subjects, at the end of the Mount Everest South Base Camp stay, Lake Louise Score, hemoglobin, and SpO_2 were 1.8 ± 2.1, 18.5 ± 1.1 g/dl, 84.0 ± 2.2%, respectively, while D_{LCO} decreased from 24.3 ± 5.6 predicted in Milan to 16.3 ± 4.9 at Mount Everest South Base Camp ($P < 0.05$).

Mean atmospheric pressure and oxygen pressure were 402 and 75 mmHg at Mount Everest South Base Camp during D_{LCO} measurements. Furthermore, we show that that D_{LCO} measurements at high altitude demonstrate excellent reproducibility over a 1-h interval (Fig. 1). Telmisartan treatment did not influence D_{LCO} in Milan or at Mount Everest South Base

Table 1. *Anthropometric data of subjects with and without acute mountain sickness*

	33 Subjects	4 Subjects
	Without acute mountain sickness	With acute mountain sickness
Sex, M/F	22/11	2/2
Age, yr	40.8 ± 10.4	33.8 ± 5.9
Placebo/Telmisartan	15/18	3/1
Actual smokers	2	1
Weight, kg	68.4 ± 12.6	60.0 ± 14.9
High, cm	173 ± 8	176 ± 12
BMI, kg/m^2	22.8 ± 3.2	19.3 ± 3.2
FEV_1, liters	3.5 ± 0.6	3.0 ± 0.8
FVC, liters	4.4 ± 0.7	4.1 ± 1.2
FEV_1/FVC, %	80 ± 7.6	75 ± 8.6
D_LCO, ml·mmHg^{-1}·min^{-1}	23.8 ± 4.9	24.3 ± 5.6

Data are expressed as means ± SD and were obtained in Milan before ascent. M, male; F, female; BMI, body mass index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; D_LCO, diffusing capacity of the lung for carbon monoxide; D_M, membrane diffusion; V_C, pulmonary capillary blood volume.

Camp (Table 2) as expected according to previous studies done in heart failure patients (20). Furthermore, the two groups (placebo = 15 and telmisartan = 18) did not differ with regard to SpO_2, heart rate, respiratory rate, and systemic and pulmonary artery systolic pressure. Most importantly, results in the placebo-treated subjects paralleled those of the entire group and D_LCO, D_M and D_M/V_C all were significantly higher at Mount Everest South Base Camp compared with Milan (Figs. 2–5, Table 2). Therefore, data from the telmisartan and the placebo group were analyzed together (Table 3). In the 33 AMS-free subjects, acclimatization was achieved by all as demonstrated by improvement in the Lake Louise Score, an increase in hemoglobin and SpO_2, and decreases in venous total carbon dioxide (Table 3). Heart rate, respiratory rate, venous total carbon dioxide, plasma norepinephrine, and systolic pulmonary artery pressure are also reported in Table 3.

In the AMS-free subjects after 12 days at Mount Everest South Base Camp, hemoglobin concentration increased from 14.2 ± 1.2 (sea level) to 17.2 ± 1.8 g/dl ($P < 0.001$). Table 4 gives the D_LCO data at Milan and after 12 days at Mount Everest South Base Camp. The measured D_LCO increased from 23.8 to 35.1 ml·min^{-1}·mmHg^{-1}. When corrected for actual hemoglobin concentration, the D_LCO rose to 32.7 ± 6.9 ml·min^{-1}·mmHg^{-1}. Correcting for both hemoglobin and $P_{I_{O_2}}$ differences, the D_LCO nonetheless still rose significantly from 23.6 ± 4.4 to 25.1 ± 5.3 ml·min^{-1}·mmHg^{-1} ($P < 0.033$) at Mount Everest South Base Camp (Fig. 2). D_M, V_C, and V_A are shown in Fig. 3. We found that D_M rose from 63 ± 34 to 102 ± 65 ml·min^{-1}·mmHg^{-1} ($P < 0.01$) while V_C was 67.8 ± 20.0 and 70.8 ± 33.3 ml ($P = 0.88$) in Milan and Mount Everest South Base Camp, respectively. An increase of D_M at altitude was still present after normalization for V_A and V_C. D_M/V_A rose from 10.9 ± 5.2 to 16.0 ± 9.2 ml·min^{-1}·mmHg^{-1}·l^{-1} ($P < 0.01$) and D_M/V_C rose from 0.94 ± 0.5 to 1.8 ± 1.3 ml·min^{-1}·mmHg^{-1}·ml^{-1} ($P < 0.01$).

We also analyzed the correlations between norepinephrine and lung diffusion parameters increases between Milan and Mount Everest South Base Camp. Statistical relevance was observed between norepinephrine increase and D_M/V_A increase ($r = 0.505$, $P = 0.014$). In contrast D_LCO increase at altitude was not significantly related to heart rate changes and the D_LCO increase remained statistically significant also after adjustment for heart rate change ($P = 0.021$).

DISCUSSION

The results of the present research demonstrate that high-altitude acclimatization over several weeks is associated with a relevant increase in SpO_2 and diffusing capacity of the lung. The novel contribution of our study is the demonstration that the increase in diffusing capacity is in large part related to an increase in the membrane diffusing capacity (D_M).

To our knowledge D_LCO and its subcomponents have never been measured in acutely acclimatizing lowlanders. Two previous studies (27, 49) comprising six and five subjects at lower altitudes (<4,600 m) did not find an increase in D_LCO over a period of 10–20 days, although in the study by Vincent et al. (49) D_M did rise and V_C fell. Finally De Bisschop et al. (12) showed in 16 subjects at high altitude (4,000 m) a reduction of D_M and D_M/V_A at the fourth day. Thus, compared with our study, the De Bisschop data were obtained at lower altitude and after a much shorter acclimatization period. Compared with previous studies, we examined a larger set of subjects ($n = 33$, placebo 15, telmisartan 18) at a higher altitude (5,400 m), and we demonstrated that D_LCO and its subdivisions can be reliably measured at high altitude in a field laboratory setting. At altitude the reduction in atmospheric P_{O_2} per se increases D_LCO values, because there is less competition by oxygen for CO-hemoglobin binding. Furthermore, the rise in hemoglobin with adaption will also increase D_LCO due to the greater number of heme binding sites that CO can occupy. Nonetheless, it is possible to account for ambient P_{O_2} differences and hemoglobin changes in the calculation (26, 31) so that the corrected values can be made comparable with those obtained at sea level, and indicative of actual changes at the level of the alveolar-capillary surface occurring at high altitude. The Roughton and Forster (15) method used to calculate D_M and V_C was also adjusted for $P_{I_{O_2}}$ at Mount Everest South Base Camp.

High-altitude acclimatization is a long process involving numerous organs with variable time courses (6, 30, 52). The

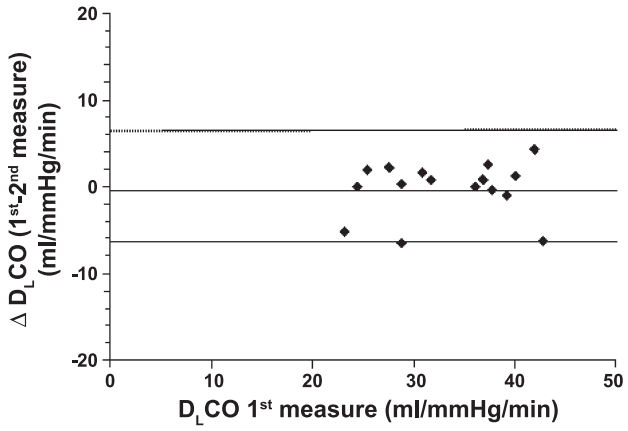

Fig. 1. Bland and Altman plot of the first and second lung diffusion for carbon monoxide (D_LCO) recorded at Mount Everest South Base Camp in 16 subjects.

Table 2. *D_LCO and D_LCO subcomponents measured in subjects without acute mountain sickness receiving telmisartan or placebo (33 subjects)*

		Milan		Mount Everest South Base Camp			DELTA			
	n	mean	SD	mean	SD	Test T	mean	SD	Lower Confidence limit	Upper Confidence limit
D_LCO, ml·mmHg^{-1}·min^{-1}										
Placebo	15	24.7	±3.9	27.3	±5.4	$P < 0.01$	2.6	±3.1	0.9	4.4
Telmisartan	18	22.7	±4.6	23.3	±4.5	NS	0.5	±3.8	−1.4	2.4
Combined	33	23.6	±4.4	25.1	±5.3	$P < 0.03$	1.5	±3.6	0.2	2.7
D_M, ml·mmHg^{-1}·min^{-1}										
Placebo	11	80.3	±39.6	124.2	±75.5	$P < 0.05$	56.9	±75.4	6.3	107.5
Telmisartan	18	51.9	±25.0	84.0	±49.0	$P < 0.01$	32.0	±43.4	10.5	53.6
Combined	29	62.7	±33.7	102.3	±64.7	$P < 0.01$	41.5	±57.6	19.6	63.4
V_C, ml										
Placebo	11	67.2	±17.2	87.0	±26.3	NS	24.0	±36.5	0.7	47.2
Telmisartan	18	68.2	±22.2	59.1	±33.5	NS	−9.1	±43.9	−30.9	12.7
Combined	29	67.8	±20.0	70.8	±33.3	NS	4.1	±41.2	−12.2	20.4
V_A, liter										
Placebo	15	5.7	±0.7	6.5	±1.0	$P < 0.01$	0.8	±0.5	0.5	1.1
Telmisartan	18	5.6	±1.1	6.2	±1.2	$P < 0.01$	0.7	±0.6	0.4	1.0
Combined	33	5.6	±0.9	6.3	±1.1	$P < 0.01$	0.7	±0.5	0.6	0.9
D_LCO/V_A, ml·min^{-1}·mmHg^{-1}·l^{-1}										
Placebo	15	4.4	±0.8	4.2	±0.8	NS	−0.2	±0.4	−0.4	0.1
Telmisartan	18	4.1	±0.5	3.8	±0.5	$P < 0.05$	−0.3	±0.7	−0.7	0.0
Combined	33	4.2	±0.7	3.9	±0.7	$P = 0.01$	−0.3	±0.6	−0.5	−0.1
D_M/V_A, ml·min^{-1}·mmHg^{-1}·l^{-1}										
Placebo	11	13.5	±5.9	19.0	±10.4	$P = 0.05$	8.6	±10.5	2.3	14.9
Telmisartan	18	9.4	±4.1	13.7	±7.7	$P < 0.05$	4.3	±6.9	0.9	7.7
Combined	29	10.9	±5.1	16.0	±9.3	$P < 0.01$	6.1	±8.7	2.9	9.3
D_M/V_C, ml·min^{-1}·mmHg^{-1}·ml^{-1}										
Placebo	11	1.2	±0.6	1.7	±1.2	NS	0.7	±1.2	−0.1	1.5
Telmisartan	18	0.8	±0.3	1.8	±1.4	$P < 0.01$	1.0	±1.4	0.3	1.7
Combined	29	0.9	±0.5	1.8	±1.3	$P < 0.01$	0.9	±1.3	0.2	1.3

Data are expressed as means ± SD. D_M, mMembrane diffusion; V_C, pulmonary capillary blood volume; V_A, alveolar volume.

present experiments were conducted after a 9-day climb and a 12- to 14-day stay at Mount Everest South Base Camp. Therefore it is likely that acclimatization processes may not have been complete. Regardless, we found a considerable degree of acclimatization, as evidenced by resolving symptoms of AMS, increase in arterial hemoglobin saturation, reduction in plasma norepinephrine, and fall in arterial carbon dioxide pressure. The progressive SpO_2 increase during the sojourn at high altitude, although reflective of increasing ventilation as indicated by the fall in arterial carbon dioxide pressure, also suggests that the efficiency of gas exchange improved as well (2). In normal lungs, gas exchange efficiency at sea level is largely dependent on regional ventilation-perfusion (V/Q) matching, but at high altitude and with exercise, diffusion limitation can occur (16). Studies in humans performed with multiple inert gas elimination technique found that V/Q matching does not improve over time at high altitude (38), although the alveolar-arterial P_{O_2} difference (A-a P_{O_2}) is reduced (7). A decrease in the A-a P_{O_2} without a reduction in V/Q mismatching is consistent with less diffusion limitation (7). Histological analysis of dogs and guinea pigs raised at high altitude show a detectable lower thickness of the blood-gas diffusion barrier corresponding physiologically to greater D_{LCO} and D_M and reduced A-a P_{O_2} (23, 25, 41). Our study offers the first, albeit indirect, demonstration of this phenomenon in humans, developing within 2 wk in the course of high-altitude adaptation. The advantage of diminished alveolar capillary diffusion resistance at high altitude will be to promote alveolar-capillary P_{O_2} equilibration and thus greater arterial oxygenation in the face of altitude associated decrement in the driving gradient for lung oxygen uptake, greater hemoglobin concentration, and shorter capillary transit times with higher cardiac output (50). These factors become especially limiting on arterial oxygen-

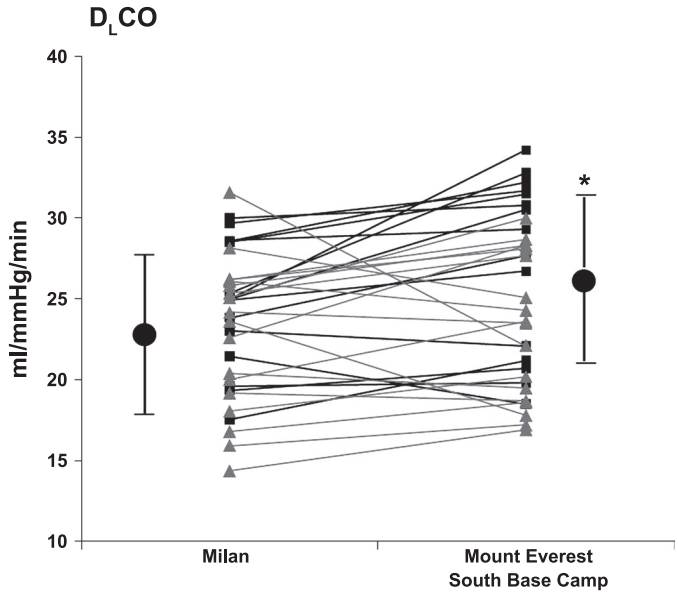

Fig. 2. D_{LCO}, corrected for Hb and $P_{I_{O_2}}$, at Milan and after ~3 wk residence at Mount Everest South Base Camp in 33 subjects who did develop severe acute mountain sickness. ■, Placebo group; shaded triangles, telmisartan group. ●, Mean of the 33 subjects + SD. *$P < 0.01$.

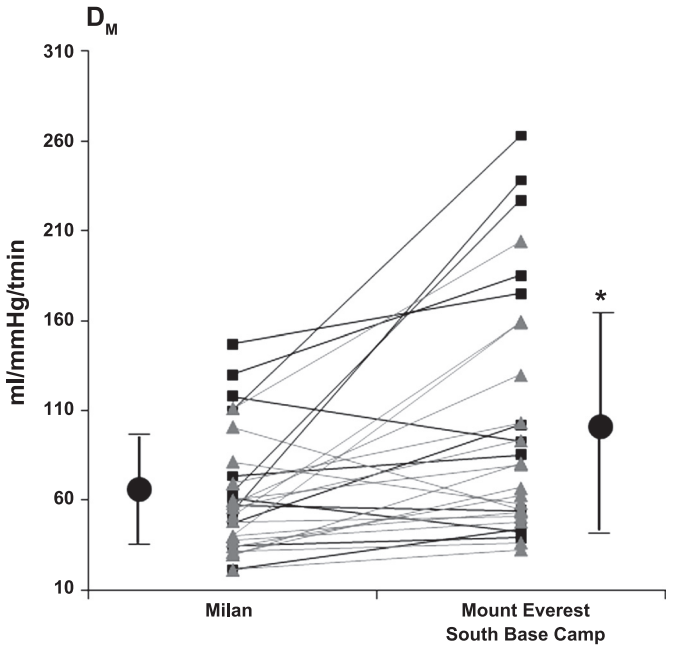

Fig. 3. Membrane diffusion (D_M) at Milan and after ~3 wk residence at Mount Everest South Base Camp in 29 subjects who did not develop severe acute mountain sickness. In 4 subjects, quality of data was considered not sufficient to reliably calculate D_{LCO} subcomponents. ■, Placebo group; shaded triangles, telmisartan group. ●, Mean of the 29 subjects + SD. $P < 0.01$.

ation with exercise. In a theoretical analysis of pulmonary factors determining maximal oxygen consumption (V_{O2max}) at high altitude, Wagner (50) found that only increasing lung diffusing capacity and alveolar ventilation improved V_{O2max}

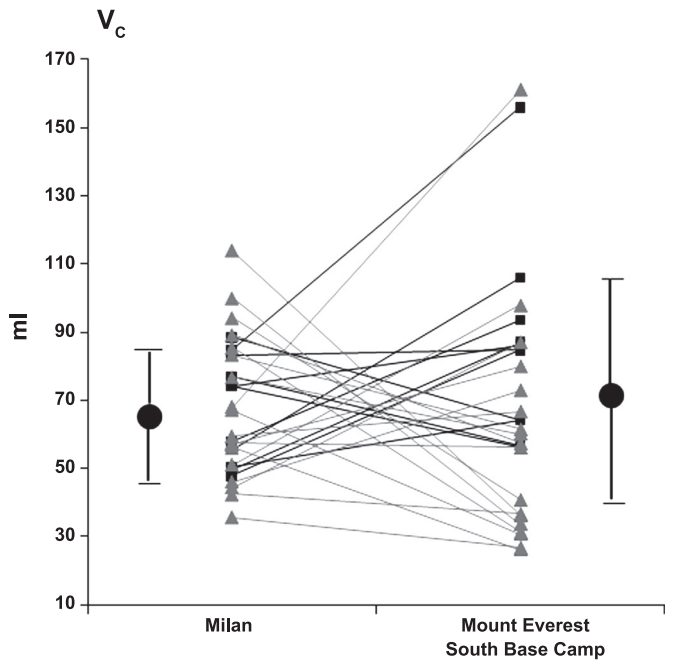

Fig. 4. Pulmonary capillary blood volume (V_C) at Milan and after ~3 wk residence at Mount Everest South Base Camp in 29 subjects who did not develop severe acute mountain sickness. In 4 subjects, quality of data was considered not sufficient to reliably calculate D_{LCO} subcomponents. ■, Placebo group; shaded triangles, telmisartan group. ●, Mean of the 29 subjects + SD. P = NS.

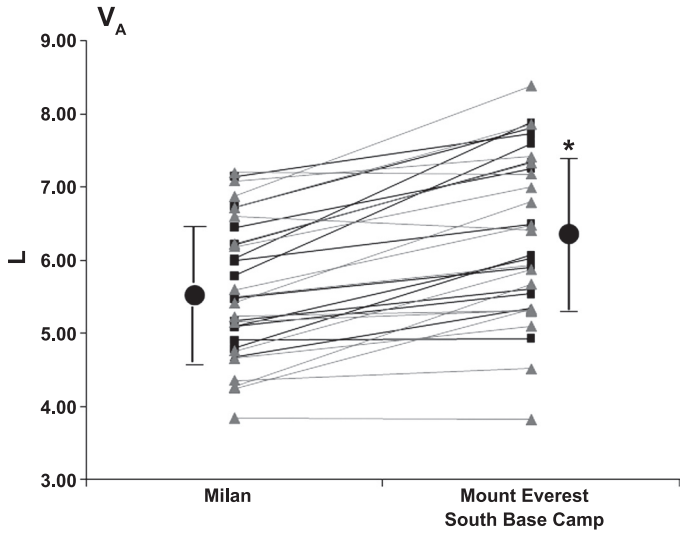

Fig. 5. Alveolar volume (V_A) at Milan and after ~3 wk residence at Mount Everest South Base Camp in 33 subjects who did not develop severe acute mountain sickness. ■, Placebo group; shaded triangles, telmisartan group. ●, Mean of the 33 subjects + SD. *$P < 0.01$.

significantly, in contrast to any increases in cardiac output or hemoglobin.

Several mechanisms may be responsible for the D_{LCO} increase we observed, including increases in hemoglobin, gas exchange surface area, and V_C, in addition to a reduction in the alveolar-capillary barrier thickness. As expected, a relevant increase of hemoglobin was observed after 2 wk at Mount Everest South Base Camp (6). The hemoglobin increase accounts for ~30% of the D_{LCO} (P_{IO_2} adjusted) increase. Mansell et al. (34) showed a relevant increase of total lung capacity at altitude. Similarly we observed a V_A increase of ~12% accounting for some additional D_{LCO} increase. However, V_A and hemoglobin changes were not the sole reason for D_{LCO} improvement. Indeed D_M/V_A and D_M/V_C remained significantly higher at Mount Everest South Base Camp compared with sea level. Although high-altitude ascent is associated with pulmonary artery pressure and cardiac output increases that could play a role in altering D_{LCO} (6, 11, 32, 33) acting mainly to increase V_C, due to vessel recruitment and distension, V_C was unchanged in our subjects.

Our data show that at high altitude D_M, D_M/V_A, and D_M/V_C increase. All these changes imply a reduced resistance to gas transfer across the alveolar-capillary membrane. Functional changes of the alveolar-capillary membrane might have been induced through mechanisms that act at the membrane level (14, 36). One such mechanism is the stimulation of several active Na^+ transport mechanisms on the epithelial layer of the alveolar-capillary barrier (36). These mechanisms are under β_2-receptor control and are activated by sympathetic stimulation (5). Indeed, high-altitude pulmonary edema may be prevented by the use of a β_2-stimulating agent (46). Several observations made in our study suggest that the sympathetic activity during our high-altitude sojourn increased, such as the increased heart rate, systemic blood pressure, and the accompanying increase of norepinephrine plasma concentrations (21, 35). Another benefit to increased β_2-receptor stimulation is a tightening of cell to cell contacts in the vascular endothelium,

Table 3. *High-altitude adaptation*

	Milan	Namche Bazar	Mount Everest South Base Camp	Mount Everest South Base Camp	ANOVA
		Day 1–2	*Day 10–11*	*Day 24–25*	
Lake Lousie Score	—	0.9 ± 0.9†	1.9 ± 1.4†*	0.6 ± 0.8°	<0.01
Hb, g/dl	14.2 ± 1.2	14.4 ± 1.7	16.0 ± 2.0†‡	17.2 ± 1.8†*#	<0.01
O_2sat, %	97.6 ± 0.6	90.5 ± 2.8†	77.2 ± 6.0†*	85.3 ± 3.6†*°	<0.01
HR, beats/min	71 ± 971 ± 9	73 ± 13	82 ± 19§	77 ± 18	<0.03
RR, breaths/min	10.3 ± 1.0	12.0 ± 2.2§	15.4 ± 3.2†*	14.2 ± 2.6†‡	<0.01
DBP, mmHg	73 ± 11	87 ± 9†	85 ± 9†	85 ± 8†	<0.01
SBP, mmHg	116 ± 12	128 ± 18†	126 ± 13†	132 ± 18†	<0.01
PASP, mmHg	22.7 ± 3.5	27.3 ± 4.3§	35.5 ± 5.7†*	35.8 ± 6.1†*	<0.01
HCO_3 venous, mmol/l	—	22.8 ± 1.6	20.1 ± 1.4*	20.4 ± 1.6*	<0.01
NE, pg/ml	332 ± 196	581 ± 177	999 ± 351†°	769 ± 667§	<0.01

Data are expressed as mean ± SD. Hb, hemoglobin; O_2sat, hemoglobin oxygen saturation; HR, heart rate; RR, respiratory rate; DBP, diastolic blood pressure; SBP, systolic blood pressure, HCO_3, bicarbonate; NE, norepinephrine. *$P < 0.01$ vs. Namche Bazar; †$P < 0.01$ vs. Milan; ‡$P < 0.01$ vs. Namche Bazar; §$P < 0.05$ vs. Milan; °$P < 0.01$ vs. Mount Everest South Base Camp *day 10–11*; #$P < 0.05$ vs. Mount Everest South Base Camp *day 10–11*. Days are from the beginning of the ascent.

which would act to reduce capillary permeability and fluid flux into the lung interstitium (4). These results support the suggestion that prolonged sympathetic stimulation may reduce the amount of fluid along the alveolar-capillary membrane. Accordingly, we observed a correlation between norepinephrine and D_M/V_A increase. The possibility that this mechanism is at work and/or can be pharmacologically stimulated in other hypoxic conditions, such as pulmonary edema, is a fascinating hypothesis and under study in the acute respiratory distress syndrome (37).

We acknowledge a few study limitations. First, it is recognized that the number of subjects studied in the present study, particularly in the placebo arm, was small. However, it was not possible to have more subjects due to logistic difficulties and to the relatively high number of subjects who had mountain sickness needing treatment. Second, the experiments were conducted only once after a 9-day high-altitude trek and a subsequent 2-wk stay at high altitude. Consequently, we cannot describe the complete time course of lung diffusion adaptation with residence at high altitude. Some acclimatization was achieved in our subjects; however, it is likely that a more prolonged sojourn at altitude might have produced additional adaptive changes. Moreover, different degrees of adaptation may occur at different altitudes and individuals may adapt at different rates. Therefore, the results of the present study may apply only to our experimental settings. Third, the correction factor we applied for inspired P_{O_2} at Mount Everest South Base Camp has not been validated below an inspired P_{O_2} of 80 mmHg (31) and thus our use of it is an extrapolation to the average $P_{I_{O_2}}$ of 75 mmHg. We do not believe this should introduce a significant over- or underestimation because earlier work of Lilienthal et al. (29) and Roughton and Darling (45) found that the relative affinity difference of CO and O_2 for binding to hemoglobin was not altered over a large range of hemoglobin oxygen desaturation. Fourth, arterial blood gas data were not measured and, indeed, these measurements would have significantly strengthened the SpO_2 data. Unfortunately repeated arterial blood samples were considered unacceptable by our trekkers and by our ethics committee. Fifth, although we made adjustments for hemoglobin and $P_{I_{O_2}}$ differences, other factors at high altitude that might also alter $D_{L_{CO}}$ independent of changes in the function and structure of the lung were not incorporated. High altitude leads to hypocapnia, respiratory alkalemia, and changes in 2–3DPG, which might affect CO uptake by hemoglobin. These factors, however, when reproduced in vitro with the magnitude of changes typically occurring at high altitude, do not introduce any measurable alteration in the reaction kinetics of CO and hemoglobin (28, 40, 48). More importantly, we could not measure carboxyhemoglobin concentrations and thus make appropriate corrections of $D_{L_{CO}}$ for any back partial pressure of capillary CO and the "anemia" effect of carboxyhemoglobinemia (18). Animal studies show a rise in carboxyhemoglobin of 1–2% with several weeks of altitude exposure (17) resulting from upregulation in the lung and other tissues of heme-oxygenase, whose catalytic breakdown of heme yields bilirubin and CO. The very limited data available in humans are less convincing, but even assuming that these changes might have occurred in our subjects, any altitude-mediated rise in hemoglobin-CO would have only led us to underestimate the

Table 4. *Comparison of D_LCO at low and high altitude with adjustments for hemoglobin and inspired PO_2 differences*

	Milan (102m)	Mount Everest South Base Camp (5,400 m)	delta	Lower Confidence limit	Upper Confidence limit
D_LCO measured, ml·mmHg^{-1}·min^{-1}	23.8 ± 5.3	35.1 ± 8.3*	11.3 ± 6.5‡	8.9	13.6
D_LCO adjusted for Hb (ml·mmHg^{-1}·min^{-1}) to a standard Hb concentration of 14.6 g/dl	23.8 ± 4.9	32.7 ± 6.9*	8.3 ± 5.8‡	6.2	10.5
D_LCO adjusted for Hb and P_IO_2 (ml·mmHg^{-1}·min^{-1}) to a standard Hb concentration of 14.6 g/dl and inspired PO_2	23.6 ± 4.4	25.1 ± 5.3†	1.5 ± 3.6§	0.2	2.7

Data are expressed as mean ± SD. *$P < 0.01$ vs. Milan. †$P < 0.03$ vs. Milan. ‡power 99%; §power 36%. D_LCO Measurements were obtained at Mount Everest South Base Camp after a 9-day trek plus 2 wk of sojourn.

increases in D_{LCO} we observed. Finally, it is possible that a direct effect of physical activity on improvement of D_{LCO} exists. We have excluded athletes, professional climbers, or subjects recently exposed to high altitude from the present research and asked all subjects, while at Mount Everest South Base Camp, not to perform any further ascent or relevant trekking. However, a role of physical activity on D_{LCO} changes we observed cannot be excluded.

In conclusion, a prolonged (3 wk) high-altitude exposure leads to an increase in lung gas exchange capacity, as one mechanism of adaptation. Although it is not possible to precisely calculate the role of each of the variables involved in the measurement of D_{LCO} that contributes to its increase at altitude, it is clear from our data that all do have a role. Indeed, hemoglobin concentration and V_A increases as did D_M. We can thus speculate that the increase in D_M observed in native high-altitude populations occurs relatively early in the acclimatization process of lowlanders and contributes to more efficient gas exchange and oxygenation in the hypoxic environment.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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