Differential changes of lung diffusing capacity and tissue volume in hypergravity

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Rohdin, Malin, and Dag Linnarsson. Differential changes of lung diffusing capacity and tissue volume in hypergravity. J Appl Physiol 93: 931–935, 2002. First published May 10, 2002; 10.1152/japplphysiol.01271.2001.—In normal gravity, lung diffusing capacity (DLCO) and lung tissue volume (LTV, including pulmonary capillary blood volume) change in concert, for example, during shifts between upright and supine. Accordingly, DLCO and LTV might be expected to decrease together in sitting subjects in hypergravity due to peripheral pooling of blood and reduced central blood volume. Nine sitting subjects in a human centrifuge were exposed to one, two, and three times increased gravity (Gz) and rebreathed a gas containing trace amounts of acetylene and carbon monoxide. DLCO was 25.2 ± 2.6, 20.0 ± 2.1, and 16.7 ± 1.7 mL min⁻¹ mbar⁻¹ (means ± SE) at 1, 2, and 3 Gz, respectively (ANOVA P < 0.001). Corresponding values for LTV increased from 541 ± 34 to 677 ± 43, and 756 ± 71 ml (P < 0.001) at 2 and 3 Gz. Results are compatible with sequestration of blood in the dependent part of the pulmonary circulation just as in the systemic counterpart. DLCO, which under normoxic conditions is mainly determined by its membrane component, decreased despite an increased pulmonary capillary blood volume, most likely as a consequence of a less homogenous distribution of alveolar volume with respect to pulmonary capillary blood volume.

IN NORMAL GRAVITY, LUNG DIFFUSING capacity (DLCO), pulmonary capillary blood volume, lung tissue volume (LTV), and central blood volume change in concert during, for example, transitions between supine and upright postures and between rest and exercise (4, 10, 11, 21). This is so, because with increased central blood volume and cardiac output in supine compared with upright or in exercise compared with rest, there is a more homogenous distribution of pulmonary capillary blood volume in relation to the alveolar volume in the human lung (5). By analogy, therefore, the peripheral blood pooling and the decreased cardiac output that occur when sitting humans are exposed to hypergravity might be expected to be accompanied by similar decreases of pulmonary capillary blood volume, DLCO, and LTV. Indeed, arterial desaturation is found in sitting humans in hypergravity (1, 9), pointing to an impairment of the lung as a gas exchanger. However, we reasoned that a simple relationship between DLCO and LTV may not be found if some of the loss of effective circulating blood volume is due to pooling in the dependent parts of the lung circulation and not only in their systemic counterparts. We hypothesized that LTV then would increase despite a falling DLCO, the latter caused by uneven distribution of pulmonary capillary blood. To test this hypothesis, we determined DLCO and LTV during short-lasting hypergravity and we found differential changes of DLCO and LTV in support of the notion of significant sequestration of blood in the pulmonary circulation in hypergravity.

MATERIALS AND METHODS

Subjects. Seven men and two women were studied. Their ages, heights, and body masses ranged from 22 to 32 yr, 169 to 193 cm, and 64 to 90 kg, respectively. They had no history of cardiopulmonary disease, and they were not taking any medication at the time. They were also instructed not to drink coffee or use nicotine-containing products on the day of the experiment. The experimental protocol used in the present study had been approved by the Ethics Committee of Karolinska Institutet.

Equipment and measurements. The experiments were conducted in the human centrifuge at Karolinska Institutet, Stockholm, Sweden. The rotational radius to the center of the centrifuge gondola was 7.2 m, and the roll angle of the gondola was automatically adjusted so that the gondola floor was perpendicular to the resultant of the normal G vector and the centrifugal G vector. Because of the 28° tilt of the backrest, the magnitude of the G vector in the head-to-feet direction (Gh) was in reality (1) 0.88 g, (2) 1.77 g, and (3) 3 g·cos 28° (2.65 g), respectively. The small errors introduced by rounding off to the nearest integer for G have been neglected throughout the text. Multiple slip rings at the center of rotation allowed for audiovisual monitoring, power supply, and transmission of physiological signals between the gondola and a control room. The instrumentation for respiratory measurements included a quadrupole mass spectrometer (AMIS 2000, Innovision, Odense, Denmark) and a manually operated rotary valve assembly with a 4-liter rebreathing bag. The subjects breathed through a mouthpiece and wore a...
During the REB maneuver the DLCO was calculated from the pressure, saturated with water vapor) when appropriate.

20% sulfur hexafluoride, 5% He, 0.63% acetylene (C2H2), 0.3% carbon monoxide (C18O) during rebreathing in 1 subject at 3 times normal gravity. Fa is the instantaneous soluble gas concentration and FFa is the corresponding concentration initially inspired from the rebreathing bag. All data are normalized for concomitant concentrations of an insoluble gas component to adjust for gas mixing and volume shrinkage due to exchange of metabolic gases.

Experimental procedures. The subjects came to the laboratory on two occasions, a first session for familiarization and a few days later for the experiment. The experiments were performed at 1, 2, and 3 Gz, four times at each level and with a randomized order between G levels.

The resting subject sat in the gondola of the centrifuge and breathed air through the mouthpiece. Approximately 1 min after reaching the desired G level, the subject started a rebreathing (REB) maneuver: after an expiration to functional residual capacity (FRC) the subject switched the rotary valve to rebreathe the full bag volume eight times at a rate corresponding to 3 s/breath following a metronome. The gas mixture used for rebreathing contained 35% O2, 5% Ar, 3% sulfur hexafluoride, 5% He, 0.63% acetylene (C2H2), 0.3% carbon monoxide (C18O), and balance of N2. CO with the stable isotope 18O (molecular weight 30) was chosen to permit analysis of CO in the presence of N2 (molecular weight 28). The rebreathing gas volume varied from 1 to 2 liters depending on the stature and the preference of the subject. The subject endeavored to just empty the bag on each inspiration. Repetitions of the REB maneuver were separated by at least 10 min to permit elimination of foreign gases. At least 8 of these 10 min were at normal gravity. Each REB maneuver was ended with a slow (∼0.5 l/s) exhalation to residual volume, where gas tracings were analyzed for phase IV phenomena, indicating poor intrapulmonary gas mixing at the end of the rebreathing.

Data analysis. After intermediate storage in the AMIS 2000 system, off-line data analysis was performed with an Acknowledge 3.2 Biopac digital data handling system (Biopac, Goleta, CA). Off-line computations included algorithms for total dry pressure correction (23) and computation of calibrated values for all dry gas fractional concentrations. Also, concentration readings were corrected for the response latency of the mass spectrometer system, and gas volumes and flows were converted to BTPS (body temperature, ambient pressure, saturated with water vapor) when appropriate. During the REB maneuver the DLCO was calculated from the rate of uptake of C18O and the pulmonary capillary blood flow was calculated as proportional to the uptake of C2H2 (7, 20–22) (Fig. 1). We considered the values of pulmonary capillary blood flow to be equivalent to cardiac output because pure intrapulmonary shunting is negligible in normal subjects also at 3 Gz+ (19). The intercept of the CO regression line with the initially inspired CO level was used to define the instant when the inhaled gas mixture reached the alveoli, i.e., the onset of alveocapillary exchange of the inhaled foreign gases or time zero (21) (Fig. 1). LTV was estimated as described by Sackner et al. (21) from the intercept of the back-extrapolated C2H2 disappearance curve with the ordinate at time zero (Fig. 1). FRC was calculated from the dilution of the insoluble gas Ar in the lung-bag system volume, where all Ar readings were offset by the Ar concentration in atmospheric air. O2 uptake was calculated from the linear slope of end-tidal O2 values during rebreathing and the lung-bag system volume (2).

Statistical techniques. ANOVA (STATISTICA 5.5, Statsoft, Tulsa, OK) with repeated measures design with one dependent factor (Gz+) was used to test for differences between changing G levels with respect to respiratory variables. Planned comparison was used as post hoc test. The two contrasts used were linear and quadratic. Results were considered statistically significant if \( P < 0.05 \), and all tests were two sided. Data are presented as mean and SE, if not otherwise stated. To obtain percent changes, data were normalized to the average 1-Gz+ value of each subject.

RESULTS

Eight subjects completed the experiments, and one subject performed only one-half of the maneuvers because of nausea. No subject reported decreased peripheral vision. Figures 2 and 3 are typical recordings at 3 Gz+, illustrating the fact that no end-expiratory concentration deviations (phase IV phenomena) could be identified in any of the analyzed gases during the slow exhalation to residual volume at the end of the rebreathing. Cardiopulmonary variables are shown in Table 1. Data on LTV and DLCO in percentage of 1 Gz+ control are shown in Fig. 4. All variables changed significantly with increased G level, except for FRC, which remained within 3% of control in both hypergravity conditions. Cardiac output was decreased by
DLCO per unit alveolar volume was 32%. Prisk et al. because FRC was reduced, the concomitant increase oflessness compared with upright ground control, and hypergravity. Accordingly, DlCO was gradually re-
mained during breath holding at total lung capacity
The hypergravity-induced decrements in DLCO (Fig. 1) but with a slightly different method; DLCO was deter-
mined by 12% in sustained weightlessness. Verbanck et al. (25) determined DLCO
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nique; DLCO was increased by 12% in sustained weight-
lessness compared with corresponding measurements in
prone sitting humans in 1-Gz experiments. Rosenhamer (19), who showed that the alveolar-to-
arterial Po2 difference in resting, sitting men was almost doubled from 14 Torr at 1-Gz to 24 Torr at 3-Gz.
Corresponding data from weightlessness have so far not been obtained.

From the above-demonstrated inverse relationship between Gz+ and DlCO it might be expected that, at the same time, the filling of the lung capillaries would be
reduced. This is so, because at normal gravity, DlCO, pulmonary capillary blood volume, central blood volume, and cardiac output change in concert, for exam-
ple, during transitions between upright and supine and between rest and exercise (4, 10, 11, 21). Rosen-
hamer (19) studied sitting humans in 1–3 Gz+ and used central venous injection of indocyanine green and
continuous radial arterial sampling and spectrometric analysis of blood to determine cardiac output, and he

Fig. 2. Typical recording at 3 times normal gravity of the insoluble
gas Ar during the rebreathing maneuver and the following slow
expiration to residual volume at the end of the maneuver. Note that
there is an artifact caused by presence of air in the rotary valve unit,
indicating the transition from the end of the rebreathing maneuver
to the start of the slow expiration into the cabin air. The lack of phase
IV phenomena is indicating a homogenous distribution of Ar
throughout the lung also during hypergravity.

11% at 2 Gz+ and by 16% at 3 Gz+, compared with 1-Gz+ control. DlCO was reduced by 20 and 33% and LTV was increased by 26 and 38% at 2 Gz+ and 3 Gz+, respectively. All subjects had increased LTV values and decreased DlCO values at 3 Gz+ compared with control. O2 uptake was increased by 5% at 2 Gz+ and 16% at 3 Gz+. The arteriovenous O2 difference computed as O2 uptake/cardiac output increased by 19 and 39%, respectively \((P < 0.001)\). All variables, except for FRC, were demonstrated as a linear effect in the planned comparison.

DISCUSSION

The major finding in the present study was the differential responses of lung diffusing capacity and lung tissue volume with increased Gz+ in resting hu-
mans. The findings of decreased cardiac output and maintained FRC with increased Gz+ are in agreement with previous studies by Rosenhamer (19) and Glaister (8), respectively.

Although we are not aware of any previous determina-
tions of DlCO in hypergravity, we expected from previously demonstrated hypergravity-induced im-
pairments of the upright human lung as a gas ex-
changer (1, 8, 9, 19) that DlCO would be reduced in hypergravity. Accordingly, DlCO was gradually re-
duced by up to 33% in the present 3-Gz+ experiments. The hypergravity-induced decrements in DlCO (Fig. 1) can be compared with corresponding measurements in weightlessness. Verbanck et al. (25) determined DlCO in four astronauts using an identical rebreathing tech-
nique; DlCO was increased by 12% in sustained weight-
lessness compared with upright ground control, and because FRC was reduced, the concomitant increase of DlCO per unit alveolar volume was 32%. Prisk et al. (18) obtained similar data in sustained weightlessness but with a slightly different method; DlCO was deter-
mained during breath holding at total lung capacity

Fig. 3. Typical recording of the soluble gas acetylene \((C_2H_2)\) during
the same conditions as in Fig. 3.
found a shortened transfer time for passage of dye through the interposed vascular segment, which included part of the axillary vein, the vena cava, the heart, the lung circulation, a section of the aorta, and the brachial and radial arteries. Rosenhamer cautioned that data could be biased by changes in transfer time in the arterial section. Nevertheless the data could be compatible with a reduced central blood volume at 3 $G_z$ in sitting resting men, which seemed reasonable considering concomitant signs of peripheral venous pooling, such as a 50% reduction in stroke volume. Also, in another study from the same laboratory, Linnarsson and Rosenhamer (15) showed that at 3 $G_z$, sitting resting subjects gradually developed arterial hypotension, which was instantaneously normalized when the subjects started to perform light, dynamic leg exercise, which markedly improved venous return by the action of the muscle pump (6, 13).

The above findings, all in favor of peripheral blood pooling in dependent parts of the body in resting humans at increased $G_z$, appear to be at variance with the present finding of an increased LTV. The most rapidly changing component of LTV is the pulmonary capillary blood volume, and, therefore, the present finding of increased LTV after only minutes of exposure to elevated $G_z$ can only have been caused by an equally increased pulmonary capillary blood volume. This is assuming that there is no rapidly developing lung tissue edema during the short-lasting high-G periods. The absence of time-dependent trends in LTV at normal G between the high-G runs speaks strongly against development of lung tissue edema in the present experiments.

Under normal 1–G, conditions, an increased LTV could be expected to be associated with an overall increase of central blood volume. In the present high-G experiments, however, we propose that there is sequestration of blood in the dependent parts of the lung circulation, just as there is in the dependent part of the systemic circulation. The present data and those of Rosenhamer (19) taken together suggest that during hypergravity there were marked reductions of the blood volume in other segments of the central blood volume than in the pulmonary capillaries outweighing the ~200-ml increase of pulmonary capillary blood volume.

Effects of inhomogeneity. It should be considered whether the present findings of differential responses of $D_{LCO}$ and LTV during hypergravity in sitting men are merely artifacts resulting from increased inhomogeneities of pulmonary ventilation and perfusion. We have, however, several reasons to believe that this is not the case.

The rebreathing method offers several advantages compared with the breath-holding method; during rebreathing, the heterogeneity of alveolar gas composition is diminished by mixing between the alveolar compartments and dead space and between alveolar compartments (17). Accordingly, the lack of phase IV phenomena in the insoluble gas tracings obtained at the prolonged expirations after the rebreathing maneuver (Fig. 3) indicates that the alveolar gas content indeed was well mixed toward the end of the rebreathing to a comparable extent in all experimental conditions of the present study. This is so because sequential emptying and airway closure becomes much more marked with increasing $G_z$ (8, 9, 12) and if there were inhomogeneities in insoluble gas composition between alveolar compartments after rebreathing, this must...
have manifested itself as easily detectable phase IV phenomena.

Also, during the prolonged expiration after rebreathing, the soluble gas tracings showed no clear phase IV phenomena, merely a slope most likely due to continued soluble gas uptake during the ~10 s of the prolonged expiration.

Meyer et al. (16) measured the DLCO determined by rebreathing and drew the conclusion that the distribution of blood flow has little effect on the results, except for very low flows.

For LTV, a modeling study by Burma and Saidel (3) showed that with no or little ventilation-perfusion inhomogeneity, rebreathing measurements tend to overestimate LTV, whereas with increased ventilation-perfusion inhomogeneity LTV tends to be underestimated. To the extent that the relatively simple model of Burma and Saidel is correct, possible errors would be such that we would underestimate the true increase of LTV at hypergravity. Furthermore Petri et al. (17) suggested on the basis of a model analysis that LTV would be relatively insensitive to inhomogeneities of the distributions of pulmonary capillary blood flow and LTV. In contrast, these authors found much larger impacts of inhomogeneities of alveolar volume and ventilation.

Burma and Saidel (3) also predict that the ventilation-perfusion inhomogeneity would lead to an underestimation also of cardiac output as obtained with rebreathing. However, our rebreathing estimates at 1 and 3 Gz+ (6.3 and 5.2 l/min) were remarkably similar to those obtained with dye dilution by Rosenhamer (19) under identical conditions (6.7 and 5.1 l/min). Overall, therefore, the bulk of evidence supports the notion that our observations of an increased LTV reflect a true increase of pulmonary capillary blood volume rather than a distribution artifact.

In summary, we found evidence of blood sequestration in the lungs of sitting, upright humans exposed to hypergravity. From a hemodynamic standpoint, such a sequestration in the lung circulation would be equally disadvantageous for venous return to the heart as sequestration in the systemic circulation.

We acknowledge the dedication of our subjects and the crew at the human centrifuge, Karolinska Institutet, Sweden, in particular the excellent technical support by B. Lindborg.

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