Effects of hypergravity on the distributions of lung ventilation and perfusion in sitting humans assessed with a simple two-step maneuver

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Rohdin, Malin, Patrik Sundblad, and Dag Linnarsson. Effects of hypergravity on the distributions of lung ventilation and perfusion in sitting humans assessed with a simple two-step maneuver. J Appl Physiol 96: 1470–1477, 2004. First published December 12, 2003; 10.1152/japplphysiol.00627.2003.—Increased gravity impairs pulmonary distributions of ventilation and perfusion. We sought to develop a method for rapid, simultaneous, and noninvasive assessments of ventilation and perfusion distributions during a short-duration hypergravity exposure. Nine sitting subjects were exposed to one, two, and three times normal gravity (1, 2, and 3 G) in the head-to-feet direction and performed a rebreathing and a single-breath washout maneuver with a gas mixture containing C2H2, O2, and Ar. Expirograms were analyzed for cardiogenic oscillations (COS) and for phase IV amplitude to analyze inhomogeneities in ventilation (Ar) and perfusion [CO2-to-Ar ratio (CO2/Ar)] distribution, respectively. COS were normalized to analyze inhomogeneities in ventilation (Ar) and for phase IV amplitude to analyze inhomogeneities in ventilation (Ar) and perfusion [CO2-to-Ar ratio (CO2/Ar)] distribution, respectively. COS were normalized for changes in stroke volume. COS for Ar increased from 1-G control to 128 ± 6% (mean ± SE) at 2 G (P = 0.02 for 1 vs. 2 G) and 165 ± 13% at 3 G (P = 0.002 for 2 vs. 3 G). Corresponding values for CO2/Ar were 135 ± 12% (P = 0.04) and 146 ± 13%. Phase IV amplitude for Ar increased to 193 ± 39% (P = 0.008) at 2 G and 229 ± 51% at 3 G compared with 1 G. Corresponding values for CO2/Ar were 188 ± 29% (P = 0.02) and 219 ± 18%. We conclude that not only large-scale ventilation and perfusion inhomogeneities, as reflected by phase IV amplitude, but also smaller-scale inhomogeneities, as reflected by the ratio of COS to stroke volume, increase with hypergravity. Except for small-scale ventilation distribution, most of the impairments observed at 3 G had been attained at 2 G. For some of the parameters and gravity levels, previous comparable data support the present simplified method.

Cardiogenic oscillations; closing volume; single-breath washout; stroke volume

For the first experimental application of the novel technique, we studied sitting subjects, because for sitting normal humans, a relatively large amount of previously reported data could be used for comparison. Generally, previous studies have used separate methods and separate experimental sessions for assessments of the distributions of ventilation and perfusion in the lungs. We now present data obtained with a method where such assessments could be made rapidly and simultaneously. Being noninvasive, it permitted multiple repetitions at several gravity levels. We used a combined rebreathing and single-breath washout (SBW) maneuver to study sitting healthy humans in up to three times normal gravity (3 G).

We hypothesized that indexes of large-scale or apex-to-base ventilation and perfusion distributions would not necessarily show a proportional deterioration with increasing gravity, because lung tissue can be subject to only a finite degree of deformation and because there is a finite amount of blood in the pulmonary vasculature that can be redistributed. We further wanted to confirm in sustained hypergravity that not only large-scale, but also small-scale, distributions (on an acinar level) of ventilation and perfusion would become less homogeneous with increasing gravitational force. This is because, with hypergravity and increasing hydrostatic pressure gradient per unit vertical distance, alveolar expansion and perfusion will be expected to differ also within relatively small regions.

MATERIALS AND METHODS

Subjects

Seven men and two women were studied. Their ages, heights, and body masses were as follows: 22–32 yr, 169–193 cm, and 64–90 kg. They had no history of cardiopulmonary disease and were not taking medication at the time of the study. They were also instructed not to drink coffee or use nicotine-containing products on the day of the experiment.

Ethics

The subjects received written information about the procedure, and informed consent was obtained. 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 and were performed in conjunction with another study (35). The rotational radius to the center of the centrifuge gondola was 7.2 m, and the roll angle of the gondola automatically adjusted so that

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the gondola floor was perpendicular to the resultant of the normal- and the centrifugal-gravity vectors. Because of the 28° tilt of the back rest, the magnitude of the gravity vector in the head-to-feet direction \( (G_{r-e}) \) was, in reality, 1 G * \( \cos 28° \) (0.88 g), 2 G * \( \cos 28° \) (1.77 g), and 3 G * \( \cos 28° \) (2.65 g). The small errors introduced by rounding off to the nearest integer for gravity have been neglected in the results reported here. 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 (model 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 nose clip. Between the mouthpiece and the rotary valve, there was a unidirectional turbine-impeller flowmeter (KL Engineering, Northridge, CA) for measurements of expired flow and an inlet for gas sampling through a 10-m-long capillary tube to the mass spectrometer located at the center of the centrifuge. The volume of the instrumental dead space was 150 ml. Electrocardiogram was monitored from chest electrodes and a clinical monitoring system (type AS2, Datex, Helsinki, Finland). An accelerometer was positioned at heart level behind the subject switched the rotary valve and exhaled completely to residual volume (RV) at a constant flow rate of 0.5 l/s, aided by visual feedback from an airway pressure signal displayed on an liquid crystal display screen. Gas traces during the expiration to RV were analyzed for an airway pressure signal displayed on an liquid crystal display screen. Gas traces during the expiration to RV were analyzed for elimination of foreign gases. At least 8 of these 10 min were at normal gravity.

**Data Acquisition**

After intermediate storage in the AMIS 2000 system, offline data analysis was performed with an Acknowledge 3.2 digital data-handling system (Biopac, Goleta, CA). Offline computations included algorithms for total dry pressure correction (41) 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 when appropriate. The pulmonary capillary blood flow data obtained during the preceding rebreathing maneuver have been reported elsewhere (35) and were used to calculate stroke volume (SV) in the present study. Heart rate (HR) during the 15 to 5 s preceding the rebreathing maneuver was determined from the electrocardiogram, and SV was obtained as pulmonary capillary blood flow \( \div \) HR, with the assumption that the pulmonary capillary blood flow during the SBW maneuver was the same as that during the immediately preceding rebreathing maneuver. Flow was integrated to obtain expired volume.

**Analysis of Expirograms**

Three kinds of expirograms were generated. 1) Expired Ar concentration was plotted as a function of expired volume (Fig. 1). The Ar concentration at the end of the rebreathing maneuver and before the end of the SBW maneuver was the same as that during the immediately preceding rebreathing maneuver. Flow was integrated to obtain expired volume.

**Experimental Procedures**

The subjects came to the laboratory on two occasions: a first session for familiarization and another session, a few days later, for the experiment. The experiments were performed at 1, 2, and 3 G, four times at each level and with a randomized order between gravity levels. The resting subject sat in the gondola of the centrifuge and breathed air through the mouthpiece at the center of the centrifuge. The volume of the instrumental dead space was 150 ml. Electrocardiogram was monitored from chest electrodes and a clinical monitoring system (type AS2, Datex, Helsinki, Finland). An accelerometer was positioned at heart level behind the subject instructed to report whether their peripheral vision was decreased as an early sign of cerebral hypoperfusion.

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onset of phase IV, although not by an iterative process as described by Guy et al. (17). CV was calculated from this point to the end of the expiration and was also expressed as a percentage of VC. Phase IV amplitude was calculated as the vertical distance between the extrapolated phase III slope and the maximal concentration deviation at the end of phase IV. Of the four repetitions at each gravity level, we used the data from the two experiments where the expired VC was larger or, if these two VC values differed by >0.5 liter, only the data from the experiment with the larger VC. We selected the two largest consecutive COS during phase III with Ar, CO2, or CO2/Ar concentrations plotted against volume. We used the R-R interval to find the local maxima and minima, referenced to the line already fitted to phase III. Inasmuch as COS is affected by the size of the SV (20), we normalized COS for changes in SV.

Rationale for the Present Analysis

The Ar value in the expirogram at any time represents the dilution of the resident gas during the preceding inspiration in the lung units that empty at a specific point in time. This is so, because after the rebreathing maneuver and before the VC inspiration, there will be minimal, if any, residual differences in Ar between lung units (43). CO2 (35) and, thereby, CO2/Ar will have an equally homogeneous distribution within the lung. The calculation of CO2/Ar is analogous to the ratio of soluble to insoluble marker gas when lung blood flow and diffusion capacity are calculated from gas traces obtained during the rebreathing maneuver (40); normalization of soluble gas against insoluble gas compensates for the effect of ventilatory distribution, rendering the soluble gas trace specific for the exchange of gas between the gas and the capillary blood in the lung. CO2/Ar would be unity if no CO2 was added from perfusion during the ~20-s-long VC inspiration-expiration maneuver. Thus, to the extent that this ratio is higher than unity, it reflects the extent to which CO2 has been added to lung units by perfusion. The expirogram is considered to represent the sequential emptying of a series of lung units. These units are not necessarily anatomically confined units but represent groups of alveoli, the gas from which reaches the mouth at a certain point in time. Each expired Ar value represents a specific alveolar dilution (7, 22): 

\[ V_b/\Delta V_o + V_k = Ar/100, \]

where \( V_b \) is the RV of the unit before inspiration, initially having an Ar equilibrium concentration of 100%, and \( \Delta V_o \) is the amount of 0% Ar gas received by a unit U. For a typical lung with an RV of 1.5 liters and a VC of 5 liters, one would obtain an overall dilution factor of (6.5 liters – dead space)/1.5, i.e., \(~4\), corresponding to an Ar value of \(~25\).

Each unit has a certain circulatory CO2 delivery, which is assumed to be constant over the time of the maneuvers as a result of assumed constancy of cardiac output and mixed venous CO2 (25). Apart from the circulatory delivery of CO2, the amount of CO2 added to the air space of each unit is determined by the CO2 partial pressure difference between the mixed venous blood and the alveolar space of the unit. Two cases should be considered.

Case A. CO2 transfer into the alveolar space is limited by perfusion; i.e., the ventilatory dilution is so large that there is not sufficient CO2 influx through perfusion to fill the alveolar space to 100% within the 20-s duration of the VC inspiration-expiration maneuver. This is probably so in the present case when the ventilation-perfusion ratio for the lung is considered as a whole during the short-duration non-steady-state period of the VC inspiration-expiration maneuver; during the inspiratory phase, this ratio will be \(~\sim2\), and for the maneuver as a whole it will be \(~\sim2.5\).

Case B. Well-perfused units receive an excess of CO2 from perfusion during the VC maneuver and will then restore the initial CO2 before the expiration is terminated. The finding of expirograms with a time-variable CO2 level of \(<100\%\) would speak against that possibility. A conservative calculation of the lung perfusion that would be required to replenish the CO2 level in the lungs as a whole within the first 5 s of expiration yields a value of \(>30\) liters of blood per minute for the whole lung, which is clearly much in excess of the perfusion that could occur in any lung unit under the present conditions of rest in the sitting position. In summary, therefore, we estimate that it is justified to consider the present CO2/Ar expirograms to reflect perfusion heterogeneity.

The purpose of the present study was to assess inhomogeneities of ventilation and perfusion from variations in the Ar and CO2/Ar expirograms. For a time-varying concentration of a gas to occur in an expirogram, two conditions must be met at the same time: 1) the gas concentration must differ between lung units, and 2) lung units with different gas concentrations must empty sequentially. In this and previous studies in which noninvasive assessments of ventilation (17) and perfusion distributions (33) were performed in the lung, cardiorespiratory oscillations and phase IV amplitude have been used to assess the degree to which gas concentration differences occurred within the lung.

Statistical Techniques

ANOVA (STATISTICA 6.0, Statsoft, Tulsa, OK) with repeated-measures design with one dependent factor (gravity in the head-to-feet direction) was used to test for differences between gravity levels with respect to respiratory variables. Tukey’s honestly significant difference multiple comparisons test was used as post hoc test. Results were considered statistically significant if \( P < 0.05 \), and all tests were two sided. Values are means \pm SE, unless otherwise stated. To obtain percent changes, data were normalized to the average 1-G value of each subject.

RESULTS

Eight subjects completed the experiments, and one subject performed only half the number of maneuvers at each gravity level because of nausea. No subject reported decreased peripheral vision. No end-expiratory concentration deviations (phase IV amplitude) could be identified in any of the analyzed gases during the slow exhalation to RV at the end of the rebreathing maneuver. Typical individual recordings of expirograms from an SBW maneuver are shown in Figs. 1 and 2. VC, HR, SV, and parameters extracted from expirograms are shown in Table 1 and Fig. 3. VC decreased 8% at 2 G and 14% at 3 G compared with 1-G control. HR increased 13 \pm 2 and 22 \pm 3 beats/min at 2 and 3 G, respectively. Corresponding reductions of SV were 20.3 \pm 2.0 and 29.8 \pm 3.8 ml, respectively.

Several parameters extracted from Ar expirograms (Ar parameters) changed significantly with increased gravity level, except COS for Ar, phase III slope for Ar, and CV for Ar. However, COS for Ar normalized for changes in SV showed a

Fig. 2. Ratio of CO2 to Ar concentration under conditions described in Fig. 1 legend.
VITAL CAPACITY

Hyperventilation in hypergravity had modest effects on the magnitude of arterial oscillations. Phase III amplitudes increased significantly with gravity, whereas phase IV amplitudes decreased. The difference increased significantly from 2 to 3 G. Values for CO2/SV and CO2/CV increased significantly with gravity, whereas values for Ar/SV and Ar/CV did not. These results indicate that oscillations may be partially dependent on gravity, but they are not strongly dependent on the respiratory rate.

We also analyzed the parameters obtained for each individual from Ar expirograms. For Ar and CO2, CO2/SV increased significantly with gravity, but the increase from 2 to 3 G was much more modest than for Ar and CO2, if not absent (Table 1). Also for CO2/Ar, the most obvious change in phase IV amplitude was an approximate doubling of the amplitude from 1 to 2 G, with little or no additional change at 3 G.

We analyzed whether parameters obtained for each individual from Ar expirograms were correlated with those from CO2 expirograms for the same gravity condition. The results are shown in Table 2. In summary, neither CO2 for Ar and CO2 for CO2 amplitudes nor phase III slopes were correlated in any of the conditions, but at 2 and 3 G there were strong correlations for CV and phase IV amplitude between Ar and CO2.

DISCUSSION

The distinctive feature of this study was the rapid, simultaneous, and noninvasive assessments of ventilation and perfusion in situ, in the head-to-feet direction.

The HR and SV changes were generally in agreement with previous data from Rosenhammer (38) with progressive HR increase in hyperventilation in hypergravity. The main finding was that indexes of large- and small-scale distributions of ventilation and perfusion showed deteriorations with increasing gravity in the head-to-feet direction, although the deterioration was more extensive for large-scale differences, where most of the changes that were observed at 3 G were attained already at 2 G. We further found differential responses between ventilation and perfusion; small-scale ventilation distribution indexes showed a gradually increasing inhomogeneity, whereas corresponding perfusion distribution indexes showed increasing inhomogeneity from 1 to 2 G but not from 2 to 3 G.

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increase and SV reduction with increasing gravity. The basic mechanism behind the reduced SV is a decrease in effective circulating blood volume in the systemic (38) and pulmonary circulations (35) due to sequestration of blood in dependent parts of these circulations. The tachycardia is of baroreflex origin as a result of reduced arterial blood pressure at the level of the carotid baroreceptors (22), and this tachycardia will further contribute to the SV reduction in response to hypergravity (38).

**Cardiogenic Oscillations**

*General.* Heart-synchronous variations of gas concentration during a slow expiration are known as cardiogenic oscillations and were first demonstrated for CO₂ during tidal breathing and for N₂ during SBW maneuvers when rapidly responding gas analyzers for these gases became available (6, 12). It has been proposed that COS result from the direct mechanical action of the heart on the surrounding lung (13, 18) and/or time-dependent variations in thoracic blood content during the cardiac cycle (2). The now generally accepted concept that the overall cardiac volume does not change much during the cardiac cycle (9, 19) speaks against cardiac volume changes as the principal mechanism. Whatever the mechanism for the mechanical coupling between the heart and the lungs, the beating heart directly or indirectly provokes lung units with different gas concentrations to empty in an alternating manner, producing rhythmical variations of the relative contributions to the expire from different populations of alveoli.

The amplitude of COS is considered to reflect the regional differences in gas concentration and the extent to which the beating heart affects the sequential lung emptying, so to interpret COS data, one has to consider both of these factors. Because cardiac movements and pulsations in intrathoracic vessels are related to the size of the SV, we have normalized COS amplitudes for SV in the present analysis. There is no clear definition of the size and location of regions that contribute to COS by alternating their emptying rate. However, experimental data obtained in humans by Arieli (1) point to a relatively peripheral location of the mixing point where concentration oscillations arise as a result of alternating contributions from lung units with different gas concentrations. On the other hand, Engel et al. (11) argued that gas concentration variations of such peripheral origin would be obscured by diffusion and asynchronous contributions at more central bifurcations. However, the experimental data and model analysis by Arieli indicate that asynchronous contributions at more central sites cause COS to become biphasic but not to be eliminated. These regions are not necessarily equivalent to the vertical regions (apex-to-base) that cause phase IV phenomena due to airway closure but, rather, originate from regions in the periphery on a scale larger than the acinus (32) but smaller than gross apical/basal regions (1). We therefore use the terms small-scale inhomogeneities in the distribution of ventilation and perfusion for phase III slope and COS to distinguish from large-scale (apex-to-base) inhomogeneities, which are reflected by phase IV phenomena, such as CV and phase IV amplitude.

Ar. Michels and West (26) studied COS for Ar and N₂ during an SBW maneuver with an inhaled bolus of Ar at RV followed by O₂. Experiments were done during parabolic flight, and the subjects performed the inspiration and expiration at 1 G, 1.8 G, or 0 G. Oscillation amplitudes were highest at 1.8 G and lowest at 0 G, and the authors suggested that this reflected larger Ar and N₂ differences between lung units with increasing gravity, as a result of less homogeneous ventilation distribution.

This is in contrast to the report of Jones et al. (20). They performed SBW tests with a bolus of N₂ delivered at RV and found lower COS amplitudes during exposure to high gravity than to normogravity in subjects sitting in a human centrifuge. The difference between those two studies that can account for the different results in COS might be the brief duration (10–15 s) of the hypergravity exposure before the measurement during parabolic flights and more of a steady-state situation in the human centrifuge. The longer period of hypergravity in the centrifuge will more effectively reduce the central blood volume and, therefore, also the SV. Therefore, it is interesting to note the COS response in the study of Jones et al., in which the subjects performed the inspiration part of an SBW maneuver at 4 G, and then the centrifuge was stopped, and the subjects

**Table 2.** Correlation coefficient \( r \) between pulmonary function parameters extracted from Ar vs. CO₂ expirograms

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 G</th>
<th>2 G</th>
<th>3 G</th>
</tr>
</thead>
<tbody>
<tr>
<td>COS</td>
<td>0.29</td>
<td>0.22</td>
<td>0.30</td>
</tr>
<tr>
<td>Phase III slope</td>
<td>0.24</td>
<td>0.21</td>
<td>0.06</td>
</tr>
<tr>
<td>CV</td>
<td>0.29</td>
<td>0.88</td>
<td>0.87</td>
</tr>
<tr>
<td>Phase IV amplitude</td>
<td>0.33</td>
<td>0.70</td>
<td>0.70</td>
</tr>
</tbody>
</table>
performed the expiration at normal gravity. The result was larger COS than when the whole maneuver was performed at 4 G, and Jones et al. attributed this result to the sudden increase in venous return and SV on return to 1 G. This finding lends further support to the present use of SV as a normalizing parameter in the interpretation of COS.

In the present study, there was an ~2-min period of hypergravity before the expirogram was recorded: 4–6 s of gradual gravity onset, 60 s of rest at hypergravity, 30 s of rebreathing maneuver, and 20 s of SBW maneuver. During this time, there is likely a more significant reduction of SV in sitting resting humans than during 10–15 s of hypergravity (1.8 G) in parabolic flight. The present constancy of COS for Ar across gravity conditions (Table 1) is therefore likely to be the result of two opposing influences on their amplitude: increased gravity, which is expected to produce larger interunit differences of dilution and Ar concentrations, but, at the same time, decrease SV, thereby decreasing the influence of the heart on the lung. After having taken this into consideration by normalizing the COS for changes in SV, we found signs of gradual deterioration of the homogeneity of small-scale ventilation distribution with increasing hypergravity (Table 1, Fig. 3A).

CO₂. The COS shown in the present CO₂ recordings were 4–6% of the preinspiratory equilibrium level. COS are likely the result of CO₂ concentration differences that originate from varying the dilution of CO₂ between units during inspiration and varying the circulatory CO₂ input to the gas spaces of the same units. In the theoretical case, that is the units with the greatest dilution that have the least perfusion, the dilution and perfusion mechanisms would be in phase and act in the same direction and result in amplification of CO₂ differences between units and, thereby, of COS. On the other hand, should the most well-ventilated units be those with the largest perfusion, COS for CO₂ would be attenuated.

We analyzed the possible correlation between pulmonary function parameters extracted from Ar and CO₂ expirograms (Table 2). The correlation coefficient was 0.30, which indicates that COS for these two gases did not correlate significantly. From this lack of correlation, we conclude that COS for Ar and COS for CO₂ reflect fundamentally different phenomena to such an extent that CO₂/Ar would provide new information not directly provided in the Ar or the CO₂ expirogram.

CO₂/Ar. Michels and West (26) studied COS for CO₂ and O₂ during a maneuver including hyperventilation, breath holding, and slow expiration. We considered such a maneuver unsuitable for use in a sustained high-gravity environment because of the risk that the accompanying hypopcapnia would result in cerebral vasoconstriction and, thereby, increase the risk for a gravity-induced loss of consciousness. Instead, we used a different technique but with analog elements. The hyperventilation period in the study of Michels and West aimed at equilibrating the CO₂ content in the lungs and, therefore, served the same purpose as the rebreathing maneuver in the present study. It also lowered the CO₂ before a period of CO₂ accumulation in the lung and, thereby, served the same purpose as the VC inhalation of CO₂-free air in the present study. The breath hold during ~15 s was to allow CO₂ to accumulate in proportion to the blood flow in each lung unit; in the present study, CO₂ transfer to the alveoli took place during the 20-s VC exhalation-inhalation-exhalation sequence. In the present study, the normalization of CO₂ with respect to Ar compensated for differences in dilution during inspiration. The experiments of Michels and West were done during parabolic flight, and the subjects performed the breath hold and expiration at 1 G, 1.8 G, and 0 G. COS were largest at 1.8 G and smallest at 0 G, and the authors concluded that this was a result of less homogeneous perfusion distribution with increasing gravity.

In the present study, COS for CO₂/Ar normalized for changes in SV showed a significant increase in amplitude from 1 to 2 G, with little or no further increase at 3 G compared with 1-G control (Table 1). These results indicate that the impairment of small-scale pulmonary perfusion homogeneity did not become more severe from 2 to 3 G. This is in contrast to COS for Ar, which showed a gradual change over 1–3 G. This difference in behavior between ventilation and perfusion suggests that, on a regional level, ventilation distribution is a relatively simple function of gravity-induced lung deformation.

**Phase III Slope**

During a VC expiration after an inhalation of an insoluble marker gas, the slope of phase III of the alveolar part of the expirogram represents the sequential emptying of lung units with different ventilations per unit lung volume and asymmetric gas transport at the acinar level (30). By studying these phenomena in microgravity, Guy et al. (17) found that the phase III slope was reduced by only 22% in sustained microgravity compared with 1 G in the standing position and concluded that the genesis of phase III slope was primarily nongravitational. The phase III slope thus appears to depend more on the emptying patterns of small regions with widely varying volume-to-ventilation ratios than on gravity-dependent sequences of emptying (4). The major part of the phase III slope arises from a complex interaction between diffusive and convective transport in the peripheral parts of the lung or diffusion-convective-dependent inhomogeneity (32), which is the major contributing mechanism to inhomogeneity during normal breathing in healthy subjects (5, 10). Indirect effects of the continuing gas exchange of soluble gases in the lung contribute to ~10% of the phase III slope of an insoluble gas (3). Gustafsson et al. (16) showed that phase III slopes for He and SF₆ increased gradually in hypergravity and were 142% and 163%, respectively, at 3 G compared with normogravity. Our data are in accordance with these results. There was a trend for an increasing phase III slope for Ar with a value at 3 G that was 154% of that at 1 G (Table 1).

Because the CO₂/Ar trace is compensated for ventilatory transport phenomena, it reflects the effects of exchange of CO₂ between the blood and the alveoli. We found a trend for the phase III slope for CO₂/Ar to become more negative with increasing gravity, showing a trend for the lung perfusion to have a steeper gradient between lung units that empty early and those that empty late during expiration. However, the lower total pulmonary blood flow in hypergravity (35, 38) may also have contributed to the more-negative slope by reducing the direct effect of continuing gas exchange, which in itself would tend to add a positive component to the slope.

**Phase IV Phenomena: CV and Phase IV Amplitude**

Experiments in normal gravity have shown that airway closure is largely gravity dependent: flow becomes sequential at the end of the expiration as a result of progressive closure of
airs in dependent lung units (8, 44). The reason is the nearly linear vertical gradient in pleural pressure, such that the pleural pressure exceeds airway pressure in dependent lung regions when approaching RV, thus favoring airway closure (21, 27, 42). Phase IV amplitude reflects the vertical gas concentration differences between lung units emptying before and after airway closure and, therefore, increases with increasing gravitational force (15, 20). However, the persistence of phase IV phenomena and airway closure in microgravity demonstrates that inhomogeneity of ventilation to some extent persists in microgravity (17, 33). This also demonstrates that it is merely the topographical sequence of lung emptying that is determined by gravity and not airway closure as such. The authors speculate that the presence of airway closure in the absence of gravity is, rather, caused by differences in mechanical properties between different lung units at low lung volumes, causing patchy, rather than regional, airway closure (17). For phase IV amplitude, it has been suggested that there is a 68% (34) to 75% (17) contribution of gravity at 1 G.

In the present study, phase IV amplitude and CV/VC increased in hypergravity for Ar and CO2/Ar (Table 1). The present Ar results could be compared with those of Jones et al. (20) obtained with a bolus technique. The present trend for an increase of CV (Table 1) is similar to data from the three subjects of Jones et al. Phase IV amplitude was not reported exactly as in the present study, but comparisons of the N2 content between the final and the penultimate expired liter of gas suggest a leveling off at >2 G as in the present study. Our results (CV/VC and phase IV amplitude) show that large-scale ventilation and perfusion distributions become more inhomogeneous in hypergravity, which is in agreement with previous studies using a xenon technique (15). Analysis of the correlation between the parameters (CV and phase IV amplitude) extracted from Ar and CO2 expirograms shows that the large-scale differences changed in the same manner with increasing gravity level (Table 2). This is not surprising, because the resident CO2 and Ar are equally influenced by air dilution and expiratory sequencing during the VC-SBW maneuver. Also the vascular engorgement of dependent lung regions will reduce airway dimensions and, thereby, promote airway closure. There were no significant further deteriorations at >2 G for large-scale distributions of ventilation and perfusion. These results suggest that there is a finite degree by which the lung tissue can be deformed by gravity and, possibly, that changes of intrapulmonary gas distribution beyond 2 G, if any, are mainly secondary to redistribution of the intrapulmonary blood volume (35).

The lack of change of phase IV amplitude for CO2/Ar between 2 and 3 G is in apparent contrast to previous findings that total lung blood flow and lung tissue volume decrease and increase, respectively, in a gradual fashion between 1 and 3 G (35). It must be borne in mind, however, that the increasing lung tissue volume reflects increasing sequestration of blood in dependent lung units. Together with a falling VC, this suggests that lung units with high perfusion to an increasing extent do not contribute to the expirate. Thus the lack of change of indexes of large-scale perfusion inhomogeneities between 2 and 3 G may be an effect of two counteracting mechanisms: one that causes an overall increased gradient of perfusion within the lung and another that decreases the population of lung units that contribute to the expirate. Using radioactive xenon, Glaister (14) scanned the lung in sitting subjects exposed to 1–3 G to measure apicobasal distribution of pulmonary blood flow. There was a gradual increase in the inhomogeneity of pulmonary perfusion distribution, in contrast to our results. However, there are several differences between the present study and Glaister’s study that render a comparison more difficult: most importantly, the subjects in Glaister’s study were wearing inflated antigravity suits, which leads to pulmonary vessel engorgement, basal lung compression, and an increase in gas trapping.

**Relation Between Phase III and Phase IV Phenomena**

In the present study, there was a marked difference in the effect of hypergravity on phase III (COS/SV and phase III slope) and phase IV phenomena (phase IV amplitude and CV/VC). There was a trend for a gravitational effect on phase III slope, and COS/SV was significantly changed with gravity. However, in relative terms, gravity-induced changes were much larger for phase IV phenomena.

Prisk et al. (33) assessed pulmonary perfusion distribution from CO2 expirograms before and during spaceflight and showed that, in microgravity, the reduction in phase IV amplitude was much greater than the change in COS compared with 1-G control. They ascribed this difference to the fact that microgravity eliminates the apicobasal differences in perfusion, in contrast to the more small-scale mechanisms of inhomogeneity, e.g., differences in lung compliance within small regions of the lung that are not affected by gravity (33). At the same time, there were also small-scale ventilation and perfusion inhomogeneities that were affected by gravity, probably represented by small gradients in the dependent-to-nondependent direction within a given lung region. There seems to be a relation between the size of the area of interest and the gravitational influence: from the phase III slope, reflecting inhomogeneities mainly at the acinar level, which are not significantly affected by gravity, through COS at a level larger than the acinus, which are affected by gravity, and further to phase IV phenomena, reflecting apicobasal differences, which are severely affected by gravity.

Finally, we conclude that the validity of the present method is supported by a comparison with results from similar studies to the extent that such data exist. For the insoluble perfusion distribution marker Ar, this is true up to 2 G for COS (26) and up to 3 G for phase III slope (16) and phase IV amplitude (20). For the soluble perfusion distribution marker CO2/Ar, this is true for COS and phase IV amplitude up to 2 G (26).

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