Hyperoxia and hypergravity are independent risk factors of atelectasis in healthy sitting humans: a pulmonary ultrasound and SPECT/CT study

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Dussault C, Gontier E, Verret C, Soret M, Boussuges A, Hedenstierna G, Montmerle-Borgdorff S. Hyperoxia and hypergravity are independent risk factors of atelectasis in healthy sitting humans: a pulmonary ultrasound and SPECT/CT study. J Appl Physiol 121: 66–77, 2016. First published April 21, 2016; doi:10.1152/japplphysiol.00085.2016.—Aeroatelectasis has developed in aircrew flying routine peacetime flights on the latest generations of high-performance aircraft, when undergoing excessive oxygen supply. To single out the effects of hyperoxia and hypergravity on lung tissue compression, and on ventilation and perfusion, eight subjects were studied before and after 1 h 15 min exposure to +1 to +3.5 Gz in a human centrifuge. They performed the protocol three times, breathing air, 44.5% O2, or 100% O2 and underwent functional and topographical imaging of the whole lung by ultrasound and single-photon emission computed tomography combined with computed tomography (SPECT/CT). Ultrasound lung comets (ULC) and atelectasis both increased after exposure. The number of ULC was <1 pre protocol (i.e., normal lung) and larger post 100% O2 (22 ± 3, mean ± SD) than in all other conditions (P < 0.001). Post 44.5% O2 differed from air (P < 0.05). Seven subjects showed low- to medium-grade atelectasis post 100% O2. There was an effect on grade of gas mixture and hypergravity, with interaction (P < 0.001, respectively): 100% O2, 44.5% O2, and air differed from each other (P < 0.05). SPECT ventilation and perfusion were always normal. Ultrasound concurred with CT in showing normal lung in the upper third and ULC/atelectasis in posterior and inferior areas, not for other localizations. In conclusion, hyperoxia and hypergravity are independent risk factors of reversible atelectasis formation. Ultrasound is a useful screening tool. Together with electrical impedance tomography measurements (reported separately), these findings show that zones with decreased ventilation prone to transient airway closure are present above atelectatic areas.

+Gz accelerations; lung collapse; echography; computerized tomography; excessive oxygen supply

NEW & NOTEWORTHY

This paper reports the first-time use of pulmonary ultrasound and single-photon emission computed tomography combined with computed tomography (SPECT/CT) to study the influence of hyperoxia and sustained moderate hypergravity on lung tissue compression, and on ventilation and perfusion. Together with electrical impedance tomography measurements (reported separately), a functional and topographical imaging of lung function before, during, and after exposure to hyperoxia and hypergravity is obtained: zones with decreased ventilation are present above atelectatic areas and are prone to transient airway closure.

BILATERAL ATELECTASIS of the dependent lung has been found on low-dose computed tomography (CT) performed in fighter pilots presenting with respiratory complaints. These pilots were flying the latest generations of combat aircraft.

Flight atelectasis was reported for the first time in 1958 (34), following the implementation of aircraft capable of producing high sustained inertial forces in the head-to-foot direction (+Gz) (17) combined with the use of anti-G trousers (AGT) and of hypoxia mitigation by constant 100% oxygen supply regardless of flight altitude (3–5, 19, 51). Diagnosis was made from chest X-rays and vital capacity measurements performed before and after flight and from blood gases showing large pulmonary shunting of blood. Following these results, oxygen supply was modified in all aircraft for inspired oxygen fraction (FiO2) to be adapted to altitude, and the complaints stopped.

Combat aircraft today are even more powerful than the previous generations; however, current missions often do not involve exposure to more than +4 Gz. Oxygen is provided by the On-board Oxygen Generating System, a molecular sieve-based oxygen concentrator. In-flight measurements have suggested that the current oxygen supply is excessive (7).

Following these findings, we aimed at singling out the effects of hyperoxia and moderate hypergravity (the expression hypergravity refers to the vectorial sum of gravitational and inertial forces) on breathing pattern and regional lung volumes. Functional imaging of regional lung volumes and regional ventilation distribution was performed during combined exposure to mild sustained hypergravity and hyperoxia in a human-use centrifuge, using electrical impedance tomography (EIT) (7). The protocol mimicked in-flight conditions of fighter aircraft pilots. When breathing air (control), exposure to +3.5 G combined with AGT inflation caused a rapidly reversible decrease in dorsal ventilation at xiphioid level, suggesting air trapping due to airway closure. When breathing 100% O2, the decrease in regional ventilation was both dorsal and ventral, suggesting rapid absorption of trapped gas (7).

Functional and topographical imaging of the whole lung was also performed in that study, on the same subjects and at the same occasion, before and after the protocol, looking for 1) increased density of the subpleural lung parenchyma, i.e., comet tail artifacts [or ultrasound lung comets (ULC)], using pulmonary ultrasound; and 2) atelectasis and ventilation-perfusion defects, using high-resolution single-photon emission computed tomography combined with computed tomography (SPECT/CT). Indeed, in the last decade, several studies have provided evidence that lung ultrasound is useful for the diag-
nosis of pulmonary syndromes (35). Sonographic semiology of lung diseases is based on the recognition of artifacts rather than visualization of real structures (54). CT is considered the best imaging technique currently available for diagnosing atelectasis (15).

Results of functional and topographical imaging of the whole lung are reported in the present paper. We expected that airway closure occurring in hypergravity and hyperoxia would ultimately lead to absorption atelectasis formation in dependent dorsal and ventral lung areas, confirming EIT findings. In addition, we hypothesized that ventilation and perfusion would be decreased in these areas, as described in patients ventilated with hyperoxic air (27).

**MATERIALS AND METHODS**

**Subjects**

Eight healthy male nonsmoker volunteers were included. Their mean ± SD age, height, and mass were 32 ± 3 yr, 1.74 ± 0.06 m, and 74 ± 9 kg, respectively. Procedures for inclusion were the following: volunteers first underwent a thorough medical examination including history, clinical examination focusing on the respiratory and cardiovascular systems, and 12-lead rest ECG to rule out arrhythmias and conduction disorders. Pulmonary function tests checked for normal lung function and were conducted using a portable spirometer Quark B2 (2004; COSMED, Rome, Italy). Interpretation was performed according to the recommendations of the European Respiratory Society and the American Thoracic Society (38).

Volunteers were then tested for tolerance to +Gz accelerations in the human centrifuge.

The protocol (Clinical Trials registration no. NCT01993394) adhered to the principles of the 1964 Declaration of Helsinki and had been approved by the local Research Ethics Committee. Subjects received oral and written information before the start of the experiment and provided written informed consent to participate, in accordance with the current national French ethical guidelines.

**Human-Use Centrifuge**

Experiments were carried out in the 8-m-radius human centrifuge of the Armed Forces Biomedical Research Institute in Brétigny-sur-Orge, France. This centrifuge was built in 1999 (Latécoère, Toulouse, France) and includes a rotating arms equipped with a gondola with two degrees of freedom. The seat (MK 10; Martin-Baker, Higher Denham, UK) in the gondola reclined at 30°. Thus the inertial effect of +1.1, +1.6, and +3.5 Gz exposures in the head-to-foot direction was +0.83, +1.39, and +2.92 Gz, respectively. Accordingly, there was also an inertial force in the anterior-posterior direction of +0.5, +0.8, and +1.75 Gz.

Slip rings at the center of the centrifuge allowed audiovisual monitoring, power supply, and transmission of the physiological signals between the gondola and a control room. Visual fields were checked with a proprietary developed light rack and software.

**Subject Instrumentation**

Subjects sat in the gondola during all tests. They wore flying suits and shoes and were equipped with pneumatic ARZ 825 AGT, combat jacket (both Aérazur, Plaisir, France), Ulmer UA21S oxygen mask (Ulmer Aéronautique, Bobigny, France), and Gallet CGF LA-100 flight helmet (MSA Gallet, Châtillon-sur-Chalaronne, France). ARZ 825 are extended-coverage trousers, which include five connected bladders disposed in an inverted U: two on the calves, two on the thighs, and one on the abdomen. They cover 65% of the total leg and abdominal area. Trousers were fitted snugly on each subject. The upper edge was placed at iliac crest level and the lower edge at ankle level. Inflation started automatically at +2 Gz, at a rate of 70 hPaG, using 100% O2 supplied by a bottle fixed in the gondola. No ready pressure at +1 Gz was applied, although trouser pressure increased slightly after fitting (range 5–8 hPa). The Aérazur combat jacket includes a lifejacket (Mae West) and inextensible pockets. It comes in three sizes, is not adjustable, and weighs 6.4 kg. The UA21S is a demand oxygen mask. No safety pressure or positive pressure breathing was applied. The mask was connected to the helmet by a rack and pinion, fitted snugly, and coupled to a 50-cm-long hose, which was supplied either with air, 44.5% O2, or 100% O2 (L’Air Liquide, Sassenage, France). No pressure was applied.

Measurements performed during hypergravity are detailed elsewhere (7).

**SPECT/CT**

Tracer administration and imaging were performed with the subjects being supine.

**Radiopharmaceuticals.** Regional distribution of ventilation was marked by inhaled radioactive Kr81m provided by a rubidium Rb81 generator (Mallinckrodt, Paris, France). The average activity delivered by this generator was 200 ± 80 MBq. Regional distribution of perfusion was marked by macroaggregates of albumin labeled with radioactive technetium, Tc99m (Pulmocis; CisBio International, Gif-sur-Yvette, France), average activity 180 ± 20 MBq. They inhaled Kr81m during quiet breathing through a box that mixed krypton with ambient air. Pulmocis was injected via an infusion set (Venoflux; Vygon, Ecouen, France). The subjects were estimated to receive a total effective dose of 5 mSv per SPECT/CT. Ventilation and perfusion were acquired simultaneously during inhalation.

**SPECT/CT.** The combination of scintigraphy with CT enables acquisition of both functional and anatomical data. SPECT images were obtained with a double-headed gamma camera integrating an attenuation device, which consisted of flat panel X-ray imaging components (Bright-View-XCT; Philips Medical Systems, Cleveland, OH) equipped with low-energy high-resolution parallel-hole collimators. SPECT scans were performed with 90 projections (45 per head) at 360° (elliptic orbit). Each projection lasted 20 s and included two energy windows centered on each photopic (171–209 keV for Kr81m and 126–154 keV for Tc99m). Two sets of projected images were obtained: one for Kr81m (ventilation) and one for Tc99m (perfusion).

Multislice helical CT scanning was performed using three rotations to cover three 14-cm axial fields of view, for attenuation correction and CT diagnosis. Each rotation lasted 12 s (120 kV, 5 mA). The obtained volume-weighted computed tomography dose index (CTDIL_w) was 1.63 mGy [dose length product (DLP), 70 mGy-cm].

**Macroaggregates as markers of regional perfusion.** With SPECT, imaging of regional blood flow is accomplished by microembolization of radionucleide-labeled particles in the arterial pulmonary circulation. It is based on the principle that the number of particles trapped in a lung volume is proportional to regional blood flow. Macroaggregates of albumin have been shown to faithfully measure regional pulmonary blood flow compared with other measurement methods, in animals and in humans (21, 42). The size of 80% of macroaggregates ranged from 30 to 50 μm.

**Krypton as marker of regional ventilation.** Imaging of regional ventilation is proportional to Kr81m distribution (50).

**Pulmonary Ultrasound**

Thoracic ultrasound was performed with the subject in sitting posture, by an experienced investigator using a Mylab 30CV device (Esaote, Genoa, Italy) with a 2.5–3.5-MHz probe.

Sixty-three separate sites were analyzed. Anterior and lateral chest walls were studied at the anterior, midaxillary, midclavicular, and parasternal levels: 2nd to 5th intercostal and 2nd to 4th left intercostal spaces. At the posterior wall, explorations were paraver-
tebral, 2nd to 10th intercostal spaces, and posterior, axillary line, 2nd to 5th intercostal spaces.

The overall examination duration was ~5 min.

Protocol

The design and setup of the study aimed at reproducing flight conditions, mimicking specifically in-flight conditions of fighter aircraft pilots. Subjects were their own controls. On study day, the subjects first underwent cardiopulmonary auscultation and ECG. They were then equipped and installed in the centrifuge gondola. The protocol was designed from flight recordings and started with breathing air, 44.5% O$_2$ and balance N$_2$, or 100% O$_2$ for 1 h. Following this period, the centrifuge was started and two runs were performed (Fig. 1): 1) a 2-min warm-up run, which reached +4 Gz; and 2) an 8-min sustained hypergravity run composed of three 2-min periods at +1.6 Gz and four 15-s plateaus at +3.5 Gz. Onset rate was +0.5 G/s during warming up and +1 G/s during the second run. The complete sequence, including the resting period and the two centrifuge runs, was performed three times. Each subject was exposed to the three gas mixtures and the order was randomly allocated. Each mixture was breathed throughout the whole protocol. The minimal interval between two sequences was 3 days.

During and also after completion of the centrifuge runs and in order not to remove potential protocol-induced atelectasis, subjects were instructed to rest and breathe as calmly as possible.

Ultrasound examinations were performed before protocol start (control, pre protocol) and ~2 min after centrifuge stop (post protocol).

Control SPECT/CT (pre protocol) was carried out a few days before protocol start, 1 wk at the most. The procedure for post protocol imaging was the following: as soon as possible after completion of the post protocol ultrasound examination, subjects were transported in the sitting posture to the Val-de-Grâce hospital. SPECT/CT was immediately carried out on arrival. The delay from centrifuge stop to SPECT/CT imaging ranged from 1 to 1.5 h. Additional CTs were performed half an hour later in two subjects who presented atelectasis on the post protocol CT. SPECT/CTs were carried out in the supine posture.

When imaging was completed, subjects performed a recruitment maneuver composed of three successive inspirations and expirations to vital capacity, to remove potentially remaining atelectasis. This maneuver was completed by a final medical examination, which included breathing frequency and pulmonary auscultation. The recruitment maneuver was repeated if the subject was not eupneic and did not present normal breath sounds.

Data Analysis

Double-blind interpretation of ultrasound and SPECT/CT data was performed by experienced specialists: the investigator and a cardiologist interpreted ultrasound images, and two senior nuclear physicists interpreted SPECT/CT images.

Ultrasound. Pulmonary ultrasound recordings were interpreted offline. Ultrasound lung comets (ULC, Fig. 2) were counted in each site and sorted according to lung side (left or right), coronal localization (anterior, lateral, posterior), and axial localization (upper, middle, and lower third), per subject and condition [pre or post protocol (pre-post) and gas mixture breathed (air, 44.5% O$_2$, 100% O$_2$)].

SPECT/CT. Iterative processing was used for reconstruction of CT images: 512 × 512 matrix 1-mm-thick CT images were obtained. Both sets of projected SPECT images were corrected for attenuation during reconstruction using ordered subset expectation maximization (OSEM; 3 iterations, 9 subsets) with Butterworth filtering (cutoff 0.53, order 5).

Ventilation and perfusion reconstructed images were 128 × 128 pixels, with pixel size 4.66 mm.

VENTILATION. Ventilation images were screened for visual defects. The following classification was used: no defects, minor, moderate, and major.

PERFUSION. Perfusion images were also screened for visual defects, using the same classification. Perfusion was analyzed by comparing the left and right lungs: total perfusion was set to 100%, and the relative percentages of total perfusion present in the right and left lungs were measured. Each lung was then divided into three segments—upper, middle, and lower—using the System-X software (SystemX France, Levallois-Perret, France).

CT. CT-diagnosed atelectases were sorted by subject, exam date, pre or post protocol (pre-post), gas mixture breathed (air, 44.5% O$_2$, 100% O$_2$), lung side (left or right), coronal localization (anterior, lateral, posterior), axial localization (upper, middle, and lower thirds), and grade (0–5 and diffuse). Grade 0 was defined as normal lung;

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**Fig. 1. Study protocol.** The subject, equipped with AGT, sat resting for 1 h in the centrifuge gondola, then encountered two runs in the human centrifuge (duration of the runs: 10 min). The whole sequence (1 h 15 min) was performed with three gas mixtures: air, 44.5% O$_2$, and 100% O$_2$. Ultrasound examinations were performed in the left lateral semi-reclining posture, before protocol start (control, pre protocol) and about 2 min after centrifuge stop (post protocol). SPECT/CT was performed with the subject being supine. Pre protocol imaging was carried out a few days before protocol start, 1 wk at the most. Post protocol SPECT/CT was carried out ~2 h after centrifuge stop. [Borrowed with permission from Springer (7)].

**Fig. 2. Three typical ultrasound lung comets (ULC, arrows): mobile densifications of the subpleural lung parenchyma, which follow respiratory movements.**
grade 1, minor atelectasis; grade 3, airplane wing (bilateral); grade 5, white lung. Grades 2 and 4 were intermediates between 1 and 3, and 3 and 5, respectively.

Statistical Analysis

The statistical analysis was carried out by a qualified statistician. No analysis was applied to ventilation and perfusion data, as image interpretation was normal on all occasions.

ANOVA. For normally distributed variables, a two-way repeated measures ANOVA with two factors (time/+Gz accelerations and gas mixture) was applied on the ultrasound and SPECT/CT data (STATISTICA 8; Statsoft, Tulsa, OK). Ultrasound data were grouped into 14 zones (7 in the left lung, 7 in the right lung), regardless of intercostal space. $P < 0.05$ was considered significant. When significance was observed, Tukey’s honest significant difference post hoc test was performed for all pairwise comparisons.

ANOVA could not be used to test the effect of localization, because of few observations in several categories.

Logistic regression. Logistic regression enabled us to test the influence of gas mixture (air, 44.5% O$_2$, and 100% O$_2$) and of hypergravity (pre vs. post protocol) on ULC, and on atelectasis formation, separately. The first step is descriptive (regression model): classification regrouping similar observations. The second step is a comparison within classes, looking whether variables of interest can be predictive of classes: univariate analysis followed by multinomial logistic regression is applied to the sorted data.

In the first step, a mixed classification of ULC and atelectasis was performed using the following variables: lung side, coronal and axial localizations, number, and grade. The best regression model included five classes. Pearson $\chi^2$ and Fisher’s exact test were applied to these classes. Significance was accepted at the $P < 0.05$ level.

In the second step, individual variables that could be predictive of classes, independently of gas mixture and pre vs. post conditions, were then tested using univariate analysis: age $> 30$ yr, height $> 1.75$ m, body surface $> 2$ m$^2$, forced vital capacity (FVC) $> 5.5$ liters, forced expiratory volume during 1 s (FEV$_{1.0}$) $> 4.5$ liters, peak expiratory flow (PEF) $> 10$ l/s, FEV$_{1.0}$/FVC $> 0.8$, maximal midexpiratory flow rate between 25 and 75% of FVC (FEF$_{25-75}$) $> 4.5$ l/s, and vital capacity (VC) $> 6$ liters. Significance for inclusion in the multinomial logistic regression analysis was accepted at the $P \leq 0.25$ level.

Multinomial logistic regression was applied on the retained variables, adjusted for gas mixture and pre-post conditions. Significance was accepted at the $P \leq 0.05$ level, and trend was defined as $0.05 \leq P \leq 0.10$.

Finally, multiple logistic regression was applied on the variables presenting least correlation, including gas mixture and pre-post in the model. $\chi^2$ was calculated. Significance was accepted at the $P \leq 0.05$ level, and trend was defined as $0.05 \leq P \leq 0.10$.

RESULTS

As reported previously, subjects tolerated the protocol well but complained of fatigue after the 100% O$_2$ breathing sequence. In three subjects, cough was observed during and for about 2 h after completion of that sequence (7).

Ultrasound

Three typical ULC are presented in Fig. 2. Fig. 3 shows the evolution of the number of ULC before and after the centrifugation protocol.

ANOVA. Results are presented in Fig. 4. Pre protocol, the average (mean $\pm$ SD) number of ULC was $< 1$ (air, 0.223 $\pm$ 0.7; 44.5% O$_2$, 0.321 $\pm$ 0.8; 100% O$_2$, 0.527 $\pm$ 1.15), regardless of location. There was no difference between gas mixtures.

Contrastingly, the number of ULC post protocol was influenced by gas mixture ($P < 0.05$).
The ANOVA revealed that the number of ULC increased in hyperoxia: the condition post 100% O₂ differed from all other pre-post situations (P < 0.001 or less) but for post +44.5% O₂ breathing (trend). The number of ULC was larger in post +44.5% O₂ breathing than pre air breathing.

Multivariate analysis. Table 1 shows the results of the multivariate analysis for ULC. More than 70% of observations showed no or one ULC.

Class 1 included exclusively ULC located in the upper third of the lung, of which 62% were anterior and 56% were located in the right lung. Sixty-eight percent of observations showed normal lung, and 32% showed one ULC. Of class 1 ULC, 28.1% occurred post 100%.

Class 2 included primarily (98%) 0–2 ULC, all located in the lateral lung. They accounted for 39 out of 40 lateral ULC found in the whole set of observations. Eighty-two percent belonged to the right lung, and 59% belonged to the upper third independently of the lung side. Class 2 ULC occurred in all conditions.

Class 3 included primarily (93%) 1 or 2 ULC, location anterior or posterior. Sixty-five percent belonged to the right lung. Eighty-eight percent were located in the middle third of the lung and represented 77% of all middle lung ULC. Thirty-four percent occurred post 100%.

In class 4, the number of ULC was 0, 1, 2, or 4. Noticeably, the most common (92%) ULC number per observation was 1 or 2. All ULC were located in the posterior and inferior lung. Of the ULC of this class, 51.3% appeared post 44.5%.

Class 5 included only and all observations with 3 ULC, of which 60% were located in the right lung, 78% posterior, and 64% in the middle third of the lung. Fifty percent of class 5 ULC occurred post 100% O₂.

Predictive variables and influence of gas mixture on the adjusted predictive model. The following variables were retained: age > 30 yr (P = 0.0078), FEV₁0 > 4.5 l/s (P = 0.039), FEF₂₅–₇₅ ≥ 4.5 l/s (P = 0.034), and VC ≥ 6 liters (P = 0.033).

Hyperoxia (44.5 or 100%) differed globally from air (P = 0.01); 100% O₂ differed from 44.5% for classes 4 and 5 compared with class 1 (P = 0.01).

Odds ratios are presented in Table 2. Briefly, the risk of class 3 ULC decreased with VC ≥ 6 liters and increased when breathing 100% O₂. The risk of class 4 and class 5 ULC increased post protocol compared with pre protocol.

Table 1. Constitution of classes of ULC (according to lung side, coronal and axial localizations, and grade) and comparison of pre/post protocol and gas mixture conditions between classes

<table>
<thead>
<tr>
<th>Variables included in the construction of classes</th>
<th>Class 1</th>
<th>Class 2</th>
<th>Class 3</th>
<th>Class 4</th>
<th>Class 5</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right lung</td>
<td>114 (56)</td>
<td>32 (82)</td>
<td>86 (65)</td>
<td>18 (46)</td>
<td>17 (61)</td>
<td>267 (60.5)</td>
<td>0.008</td>
</tr>
<tr>
<td>Left lung</td>
<td>89 (44)</td>
<td>7 (18)</td>
<td>46 (33)</td>
<td>21 (54)</td>
<td>11 (39)</td>
<td>174 (39.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Coronal localization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>126 (62)</td>
<td>0 (0)</td>
<td>72 (54.5)</td>
<td>0 (0)</td>
<td>5 (18)</td>
<td>203 (46)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lateral</td>
<td>0 (0)</td>
<td>39 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (3.5)</td>
<td>40 (9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Posterior</td>
<td>77 (38)</td>
<td>0 (0)</td>
<td>60 (45.5)</td>
<td>39 (100)</td>
<td>22 (78.5)</td>
<td>198 (45)</td>
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<tr>
<td>Axial localization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper third</td>
<td>203 (100)</td>
<td>23 (59)</td>
<td>16 (12)</td>
<td>0 (0)</td>
<td>4 (14)</td>
<td>246 (56)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Middle third</td>
<td>0 (0)</td>
<td>16 (41)</td>
<td>116 (88)</td>
<td>0 (0)</td>
<td>18 (64)</td>
<td>150 (34)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lower third</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>39 (100)</td>
<td>6 (21.5)</td>
<td>45 (10)</td>
<td>0.003</td>
</tr>
<tr>
<td>Number of ULC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>139 (68.5)</td>
<td>13 (33.5)</td>
<td>0 (0)</td>
<td>1 (2.5)</td>
<td>0 (0)</td>
<td>153 (34.5)</td>
<td>0.003</td>
</tr>
<tr>
<td>1</td>
<td>64 (31.5)</td>
<td>17 (43.5)</td>
<td>59 (45)</td>
<td>22 (56.5)</td>
<td>0 (0)</td>
<td>162 (37)</td>
<td>0.003</td>
</tr>
<tr>
<td>2</td>
<td>0 (0)</td>
<td>8 (21.5)</td>
<td>63 (48.5)</td>
<td>14 (36)</td>
<td>0 (0)</td>
<td>85 (19)</td>
<td>0.003</td>
</tr>
<tr>
<td>3</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>28 (100)</td>
<td>28 (6.5)</td>
<td>0.003</td>
</tr>
<tr>
<td>4</td>
<td>0 (0)</td>
<td>1 (2.5)</td>
<td>8 (6)</td>
<td>2 (5)</td>
<td>0 (0)</td>
<td>11 (2.5)</td>
<td>0.003</td>
</tr>
<tr>
<td>5</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (1.5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (0.5)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are presented as number of observations (percent of the total number of observations for the class). Significance is accepted at P < 0.05.
SPECT/CT

Ventilation and perfusion. No ventilation and perfusion defects were noted pre and post protocol. Mean perfusion repartition was the following: left lung, 46 ± 2% of total perfusion (upper third, 11 ± 1%; middle third, 22 ± 2%; lower third, 13 ± 2%); right lung, 54 ± 2% of total perfusion (upper third, 11 ± 1%; middle third, 27 ± 2%; lower third, 16 ± 2%).

CT ANOVA pre protocol vs. post protocol. Seven subjects out of eight showed low- to medium-grade atelectasis post 100% O2 (posterior and dependent lung areas) (Fig. 5). No grade 5 atelectasis was found.

Multivariate analysis. Table 3 shows the results of the multivariate analysis for atelectasis. Fifty-one percent of observations included atelectasis. Although there was no overall difference between the right and left lungs, atelectasis prevailed in one lung side in classes 3 and 4.

Table 2. Predictive factors of ULC classes and results of analysis adjusted for gas mixture and pre/post (class 1 as reference)

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>Class 2 OR 95% CI</th>
<th>Class 3 OR 95% CI</th>
<th>Class 4 OR 95% CI</th>
<th>Class 5 OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>age ≤ 30 yr</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>age &gt; 30 yr</td>
<td>2.85 [0.85; 9.62]†</td>
<td>3.65 [0.72; 18.58]</td>
<td>0.73 [0.31; 1.70]</td>
<td>0.76 [0.27; 2.15]</td>
</tr>
<tr>
<td>VC &lt; 6 liters</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>VC ≥ 6 liters</td>
<td>4.43 [0.39; 49.8]</td>
<td>0.10 [0.01; 0.93]*</td>
<td>1.64 [0.46; 5.86]</td>
<td>1.3 [0.15; 11.72]</td>
</tr>
<tr>
<td>FVC &lt; 5.5 liters</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>FVC ≥ 5.5 liters</td>
<td>0.31 [0.03; 3.23]</td>
<td>3.76 [0.66; 21.31]</td>
<td>0.48 [0.16; 1.44]</td>
<td>0.16 [0.03; 0.88]*</td>
</tr>
</tbody>
</table>

Effect of gas mixture and pre/post with variables adjusted for age, VC, and FVC (according to the predictive model)

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>Pre OR 95% CI</th>
<th>Post OR 95% CI</th>
<th>Air OR 95% CI</th>
<th>44.5% O2 OR 95% CI</th>
<th>100% O2 OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>1.00</td>
<td>1.20 [0.49; 2.97]</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Post</td>
<td>1.00</td>
<td>2.31 [0.83; 6.44]</td>
<td>1.00</td>
<td>0.49 [0.16; 1.53]</td>
<td>0.88 [0.31; 2.51]</td>
</tr>
<tr>
<td>Air</td>
<td>1.00</td>
<td>2.31 [0.83; 6.44]</td>
<td>1.00</td>
<td>2.04 [0.37; 11.42]</td>
<td>7.34 [1.53; 35.09]*</td>
</tr>
<tr>
<td>44.5% O2</td>
<td>1.00</td>
<td>2.04 [0.37; 11.42]</td>
<td>1.00</td>
<td>0.71 [0.27; 1.84]</td>
<td>0.88 [0.31; 2.51]</td>
</tr>
<tr>
<td>100% O2</td>
<td>1.00</td>
<td>7.34 [1.53; 35.09]*</td>
<td>1.00</td>
<td>1.64 [0.46; 5.86]</td>
<td>2.28 [0.96; 5.46]*</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; VC, vital capacity; FVC, forced vital capacity; pre, pre protocol (control); post, post protocol. *P < 0.05, †trend (0.05 ≤ P ≤ 0.10).

There was an effect on grade of gas mixture and pre-post, with an interaction between these variables (P < 0.001 in all cases). The post hoc analysis showed differences between pre and post (P < 0.001) and between air, 44.5% O2, and 100% O2 (P < 0.001; but for air vs. 44.5% O2, P = 0.045).

Fig. 5. Tomography performed post protocol in two subjects post 100% O2. Top: axial low-dose CT. Bottom: coronal reformatted slice. A: unilateral atelectasis grade 3 (arrows). B: bilateral atelectasis grade 3 (arrows). Pre protocol lung tomography was normal.
Table 3. Constitution of classes of atelectasis (according to lung side, coronal and axial localizations, grade, and number) and comparison of pre/post protocol and gas mixture conditions between classes

<table>
<thead>
<tr>
<th>Lung</th>
<th>Class 1</th>
<th>Class 2</th>
<th>Class 3</th>
<th>Class 4</th>
<th>Class 5</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right lung</td>
<td>38 (53%)</td>
<td>6 (15%)</td>
<td>7 (14%)</td>
<td>4 (29%)</td>
<td>19 (58%)</td>
<td>74 (50%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Left lung</td>
<td>34 (47%)</td>
<td>11 (15%)</td>
<td>4 (36%)</td>
<td>10 (71%)</td>
<td>14 (42%)</td>
<td>73 (50%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Coronal localization</td>
<td>72 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>72 (49%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Anterior</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>3 (27%)</td>
<td>14 (100%)</td>
<td>0 (0%)</td>
<td>17 (12%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lateral</td>
<td>0 (0%)</td>
<td>1 (6%)</td>
<td>3 (27%)</td>
<td>0 (0%)</td>
<td>15 (45%)</td>
<td>19 (13%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Posterior</td>
<td>0 (0%)</td>
<td>16 (94%)</td>
<td>5 (46%)</td>
<td>0 (0%)</td>
<td>18 (55%)</td>
<td>39 (26%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Axial localization</td>
<td>72 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>72 (49%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Upper third</td>
<td>0 (0%)</td>
<td>2 (12%)</td>
<td>11 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>13 (9%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Middle third</td>
<td>0 (0%)</td>
<td>15 (88%)</td>
<td>0 (0%)</td>
<td>14 (100%)</td>
<td>33 (100%)</td>
<td>62 (42%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Lower third</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Grade</td>
<td>0  72 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>72 (49%)</td>
<td>0.001</td>
</tr>
<tr>
<td>1  0 (0%)</td>
<td>13 (76%)</td>
<td>4 (36%)</td>
<td>8 (57%)</td>
<td>4 (12%)</td>
<td>29 (20%)</td>
<td>36 (24%)</td>
<td>0.001</td>
</tr>
<tr>
<td>2  0 (0%)</td>
<td>4 (24%)</td>
<td>6 (55%)</td>
<td>6 (43%)</td>
<td>20 (61%)</td>
<td>36 (24%)</td>
<td>56 (38%)</td>
<td>0.001</td>
</tr>
<tr>
<td>3  0 (0%)</td>
<td>0 (0%)</td>
<td>1 (9%)</td>
<td>0 (0%)</td>
<td>8 (24%)</td>
<td>9 (6%)</td>
<td>17 (12%)</td>
<td>0.001</td>
</tr>
<tr>
<td>4  0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (5%)</td>
<td>1 (1%)</td>
<td>2 (1%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of atelectases</td>
<td>0  72 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>72 (49%)</td>
<td>0.001</td>
</tr>
<tr>
<td>1  0 (0%)</td>
<td>0 (0%)</td>
<td>11 (100%)</td>
<td>14 (100%)</td>
<td>33 (100%)</td>
<td>58 (39%)</td>
<td>82 (54%)</td>
<td>0.001</td>
</tr>
<tr>
<td>≥2 and diffuse</td>
<td>0 (0%)</td>
<td>17 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>17 (12%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are presented as number of observations (percent of the total number of observations for the class). Significance is accepted at \( P < 0.05 \). Grade 0 is defined as normal lung; grade 1, minor atelectasis; grade 3, airplane wing (bilateral); grade 5, white lung. Grades 2 and 4 are intermediates between 1 and 3, and 3 and 5, respectively.

**Class 1** included all normal observations, which were all located in the upper third of the lung. There was no difference between the right and left lungs.

**Class 2** included 23% of atelectasis observations. All atelectasis ≥2 and diffuse belonged to this class. They were mostly grade 1, with some grade 2. In addition, they were all post 100% O2, 88% located in the inferior lung and 94% posterior.

**Class 3** included 15% of atelectasis observations. They were all located in the middle lung, predominantly in the right lung, and represented 85% of overall middle lung atelectasis. Seventy-six percent were post protocol 44.5% and 100% O2. There was only one atelectasis per observation.

**Class 4** included 19% of atelectasis observations. They were all anterior, in the lower lung, and represented 82% of overall anterior atelectasis. There was only one atelectasis per observation, the grade of which was 1 or 2. The left lung prevailed.

**Class 5** included the remaining 44% of atelectasis. Ninety-eight percent were post 44.5% O2 and post 100% O2, corresponding to 45% of all post 44.5% O2 and 63% of all post 100% O2 atelectasis. They were exclusively lateral and posterior, in the lower lung. There was only one atelectasis per observation.

**Predictive variables and influence of gas mixture on the adjusted predictive model.** The following variables were retained: age > 30 yr (trend \( P = 0.051 \)), height > 1.75 (\( P = 0.040 \)), PEF ≥ 10 l/s (\( P = 0.031 \)), FEV\(_{1.0}\) ≥ 4.5 liters (trend \( P = 0.14 \)), FVC ≥ 5.5 liters (trend \( P = 0.057 \)), and VC ≥ 6 liters (\( P = 0.039 \)).

The effect of gas mixture was due entirely to 100% O2 (\( P = 0.065 \)) and not to 44.5% O2 (\( P = 0.14 \)).

After multivariate descending analysis, only age and PEF were retained. Odds ratios are presented in Table 4.

The risk of presenting one middle lung atelectasis (*class 3*) was increased in the following situation: PEF < 10 l/s, post protocol, and in hyperoxia; 44.5% O2 did not differ from 100% O2. The risk of presenting one anterior atelectasis, predominantly in the left lung (*class 4*), was largely increased with PEF < 10 l/s and moderately increased post protocol and with 100% O2.

**Half an hour post protocol vs. post protocol.** Both CTs performed half an hour after the post protocol CT were normal, showing recovery from grade 3 atelectasis diagnosed post protocol in the two subjects.

**Ultrasound vs. SPECT/CT**

The localization and number of ULC could not be compared statistically to those of CT atelectasis, as multiple logistic regression models differed for both data sets. Visually, common localizations were normal upper lung third and ULC/atelectasis in posterior and inferior areas (Fig. 6). Divergent findings were anterior (middle lung ULC and inferior atelec-
DISCUSSION

Summary of the Main Results

A specific feature of the present protocol is that both topographical and functional imaging techniques were used to assess the effects of hyperoxia and sustained moderate hypergravity on lung function. Inhaled Kr81m and intravenous macroaggregates of albumin labeled with Tc99m enabled simultaneous determination of both regional ventilation and regional perfusion.

Our main finding is that hyperoxia and hypergravity are independent risk factors of atelectasis formation in healthy sitting humans. Contrastingly, gas exchange is not impaired to a large extent, as neither ventilation nor perfusion defects were found with SPECT/CT. The effect of hyperoxia is larger than moderate hypergravity. Atelectasis formed with the present protocol is reversible within 2 h of completion of exposure.

![Diagram of lung regions]

Fig. 6. Localization of atelectasis (panels at top) and ULC (panels at bottom) for the five classes defined after multiple logistic regression analysis, seen from the front and back sides of each lung. Spots (atelectasis) and squares (ULC) represent the percent of observations in proportion to their respective class 1 (normal lung). The large class 1 spot is exclusively normal lung without atelectasis. The small class 1 squares show that class 1 included few ULC, one at a time. Zero or one atelectases is considered to be normal lung. For detailed description of classes 1–5 for ULC and atelectasis, see Tables 2 and 4, respectively.

Table 4. Predictive factors of atelectasis classes and results of analysis adjusted for gas mixture and pre/post (class 1 as reference)

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>OR 95% CI</th>
<th>OR 95% CI</th>
<th>OR 95% CI</th>
<th>OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>age &lt; 30 yr</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>age &gt; 30 yr</td>
<td>5.99 [0.80; 44.70]†</td>
<td>0.72 [0.11; 4.64]</td>
<td>2.68 [0.47; 15.34]</td>
<td>0.48 [0.11; 2.18]</td>
</tr>
<tr>
<td>PEF &lt; 10 l/s</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>PEF ≥ 10 l/s</td>
<td>0.66 [0.11; 3.88]</td>
<td>0.22 [0.03; 1.58]</td>
<td>0.03 [0.002; 0.32]</td>
<td>0.58 [0.12; 2.73]</td>
</tr>
</tbody>
</table>

Effect of gas mixture and pre/post with variables adjusted for age and PEF (according to the predictive model)

<table>
<thead>
<tr>
<th>Pre</th>
<th>Post</th>
<th>Air</th>
<th>44.5% O₂</th>
<th>100% O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>NA</td>
<td>42.26 [4.90; 364.21] *</td>
<td>10.18 [1.73; 60.02] *</td>
<td>NA</td>
</tr>
<tr>
<td>1.00</td>
<td>1.00</td>
<td>0.66 [0.11; 3.88]</td>
<td>0.22 [0.03; 1.58]</td>
<td>0.03 [0.002; 0.32]</td>
</tr>
<tr>
<td>1.00</td>
<td>1.00</td>
<td>54.24 [3.15; 932.75] *</td>
<td>10.17 [1.23; 84.11] *</td>
<td>NA</td>
</tr>
</tbody>
</table>

PEF, peak expiratory flow; pre, preprotocol (control); post, postprotocol; NA, not applicable. *P < 0.05, †trend (0.05 < P < 0.10).
Another important finding is that both ULC and atelectasis increased in hyperoxia and hypergravity, showing that ULC detected in these conditions are caused by atelectasis. This validates the use of ultrasound in hyperoxia and hypergravity to detect atelectasis. A downside is that localization cannot be ascertained with this technique.

Other findings are as follows: 1) small-size atelectasis (grades 1 and 2) occur predominantly in the middle and inferior lung zones, anterior and posterior; 2) large-size atelectasis (grades 3 and 4) occur in the middle and inferior lung, predominantly posterior.

**Effect of Hyperoxia**

The rationale behind the use of oxygen during flight is that atmospheric pressure, hence PO2, decreases with altitude. In fighter aircraft, the decrease in pressure is partially but not fully compensated for by cabin pressurization (22). Beyond 12,000 ft of cabin altitude, an external supply of oxygen, i.e., increasing FIO2, is required for hypoxia mitigation.

It is known that oxygen supply in altitude has deleterious effects if it exceeds needs and is combined with exposure to hypergravity (6, 9, 14). We are not aware of studies on the sole effect of hyperoxia in altitude. However, respiratory complaints—cough, dyspnea, chest pain—coming from pilots flying at cabin altitudes ranging from 14,000 to 20,000 ft for 5 h or longer while breathing 100% O2 suggest that atelectases do form in this context.

Literature on hyperoxia-related atelectasis formation in awake healthy humans is scarce. Nunn (40) reports several studies conducted by his group in the 1960s. In one of them, one subject breathed 100% O2 with small tidal volumes starting from residual volume during two sessions of 5 min. Chest radiographs performed 20 min later showed atelectasis in dependent lung zones and in the right middle lobe. Recovery occurred between 4 and 48 h after exposure. The authors concluded that airway closure and absorption atelectasis had occurred.

The physiology of atelectasis formation has been studied extensively in anaesthesia patients. The mechanism is absorption. Either the airway is completely occluded or the inspired ventilation/perfusion ratio falls below a critical level (11). Ventilation/perfusion mismatch and shunt measured by multiple inert gas elimination technique (MIGET) increase (29). Triggering factors are lung volume reduction, airway closure, FIO2, and duration of exposure (13, 31–33, 48). The lower the initial lung volume, the faster the alveolar collapse occurs (27). The higher the oxygen concentration in the airways, hence the lower the nitrogen concentration, the faster the reduction in alveolar volume in poorly ventilated areas. The mean delay to alveolar collapse is 3 h when FIO2 = 0.3 and 8 min when FIO2 = 1 (28, 29, 48, 53).

EIT recordings showed that, when breathing 100% O2, tidal and end-expiratory lung volumes decreased similarly in dorsal and ventral regions, suggesting rapid absorption of trapped gas. Contrastingly, tidal volume increased with FIO2 on average +100 ml when 44.5% O2 was breathed and +200 ml on 100% O2 compared with air breathing (7).

Hedenstierna reports that the physiological vascular shunt, defined as perfusion of regions with ventilation/perfusion ratios <0.005, has a certain dependence on FIO2 during anesthesia, as increasing FIO2 from 0.21 to 0.5 causes a 3–4% increase in shunt (29). A 10% shunt has been measured in elderly patients ventilated with FIO2 = 0.85. Increased shunt measured by MIGET correlates with the formation of atelectasis assessed by CT. The suggested explanation is attenuation of hypoxic pulmonary vasoconstriction with increasing FIO2 (29). Above atelectatic areas, ventilation is poor in relation to perfusion, suggesting intermittent airway closure and trapped gas (27). Contrastingly, Bradley et al. found no change in perfusion in atelectatic areas in anesthetized supine dogs breathing in hyperoxia (8). In their study, the final measurement of perfusion distribution with 15-μm radioactive microspheres was accomplished 15 min after the onset of atelectasis. Bradley et al. suggest that the effects of altered lobar pressures and volumes cancelled each other, because of the distortion which occurs with lobar atelectasis in intact animals (8).

**Effect of Hypergravity**

Existing literature on the effect of hypergravity on ventilation was extensively reported in our first article on the present study (7). Briefly, the work of breathing increases, as does ventilation heterogeneity (24, 39, 46). A pleural pressure gradient is produced and results, during breathing at functional residual capacity (FRC) or higher, in a greater expansion of the apical than the basal alveoli and ventilation to be directed preferentially to the basal lung zones. At lung volumes below FRC, the positive basal pleural pressure leads to increasing airway closures (10, 18, 20, 24). At the most, compressed alveoli can be completely emptied of air. This compression of the dependent parts of the lung, added to a decrease in functional residual capacity, is the most likely mechanism responsible for atelectasis formation in hypergravity (52).

In addition, the heterogeneity of perfusion distribution increases, altering the ventilation/perfusion ratio (25). Yet there is little change in total venous admixture up to 6-min exposure at +3 Gz, even though the physiological dead space to tidal volume ratio is larger than that at +1 Gz and the effective alveolar to total ventilation ratio is smaller (47). Abdominal compliance and contribution to tidal volume decrease gradually from +1 to +3 Gz.

We observed a minor effect of hypergravity on atelectasis formation. This result is coherent with the immediate and reversible decreases in regional dorsal tidal and end-expiratory lung volumes in the upper part of the lower third of the lung recorded by EIT at 3.5 Gz, which speak for transient airway closure at +3.5 Gz and airway reopening at +1.6 Gz (7). Thus, when the duration of exposure to +3.5 Gz is short, 15 s at a time, no atelectases are formed. Long-duration exposure to +1.6 Gz is not a risk factor of atelectasis.

**Combined Effect of Hyperoxia and Hypergravity**

The absence of post protocol perfusion defects on SPECT/CT is in agreement with the study of Bradley et al. on dogs (8). The finding of normal ventilation and perfusion can be interpreted in different ways: 1) Ventilation and perfusion were indeed normal throughout the protocol. 2) Ventilation and/or perfusion were affected by hyperoxia and hypergravity but recovered within 2 h after the end of exposure, i.e., the delay to post protocol SPECT/CT. 3) Ventilation and/or perfusion were affected by hyperoxia and hypergravity to some degree, had not recovered when the post
The protocol SPECT/CT was performed, but could not be detected because of insufficient resolution: SPECT resolution is ~13 mm whereas that of CT is <1 mm (49). In addition, we used 30–50-μm macroaggregates for the determination of regional perfusion. Melsom et al. have reported that the best resolution is obtained with 15-μm microspheres (37). 4) The regional distribution of ventilation and pulmonary blood flow is known to be influenced by posture. Going from sitting to supine causes gravity-induced movements of tissue and blood, i.e., less heterogeneity in ventilation and perfusion along the cranial-caudal axis, and the ventilation to perfusion ratio comes close to 1 (12, 41). Thus small ventilation and perfusion defects may have been present post protocol that were removed on posture change.

Our results show that atelectases caused by exposure to hyperoxia and hypergravity occur mainly at the lung bases, which are poorly ventilated areas in standing and sitting positions, and in the posterior parts of the lung, i.e., following the main gravity vector. This localization is in agreement with descriptions of flight atelectasis on chest radiographs made by other teams: infralobar images of increased tissue density may have been present post protocol that were removed on posture change.

In contrast with SPECT/CT, which images the whole lung, EIT measures ventilation in a 5–8-cm-thick slice centered on the fifth intercostal space, which corresponds roughly to the upper part of the lower third of the lung on CT. While breathing 100% O2, there was no difference in the pattern of ventilation distribution between 1.6 and 3.5 G (7). Considering that atelectasis occurred in not only posterior and inferior lung zones but also anterior, it is likely that the EIT-measured poor ventilation at 3.5 G in anterior and posterior regions of interest reflected ventilation in lung zones located immediately above atelectatic areas.

### Ultrasound vs. SPECT/CT

Pulmonary ultrasound has been validated against chest CT for the diagnosis of alveolar consolidation in intensive care patients (35). In poorly ventilated zones, lung sliding is abolished, and comet tail artifacts originating from water-thickened interlobular septa are often present (2, 30, 36, 43). Pulmonary ultrasound has also shown high sensitivity and specificity for detecting lung collapse (1, 55). In the present study, ULC must have represented lung collapse, because of the following: 1) The protocol lasted 1.5 h, including 1 h at 1 G and 10 min of hypergravity. The delay to pulmonary edema formation in healthy humans at 1 G, in the context of high-altitude pulmonary edema, is at least a few hours, more often 8–24 h, upon acute ascent to a new high-altitude location (57). Weidner and Hoffman have shown in chickens that exposure to 3 G for 120 min does not cause pulmonary edema in most animals studied (56). 2) CT images post protocol showed atelectasis with no signs of edema.

Ultrasound is nonirradiating, easy to carry, and relatively cheap. Unfortunately, it is operator dependent. Another drawback specific to pulmonary ultrasound is its limitation to subpleural imaging. This contrasts with SPECT/CT, not operator dependent, which offers imaging of the whole lung but cannot be repeated endlessly because it is irradiating.

Some common features to ultrasound and SPECT/CT were observed, even though both techniques could not be statistically compared because different logistic regression models described their data sets. Both ultrasound and SPECT/CT detected normal lung in the upper third and ULC/atelectasis in dependent lung zones. In addition, the total number of ULC increased concomitantly with atelectasis formation as FIO2 increased. A downside is that the number of ULC was not representative of the degree of atelectasis in a given subject, as intersubject variability was high for ULC.

Discrepancy between findings in anterior and lateral lung zones may be a consequence of the delay between ultrasound and SPECT/CT imaging post protocol.

### Effect of Individual Protective Gear on Atelectasis Formation

Individual protective gear could have enhanced atelectasis formation by causing chest compression: the upper edge of the AGT was placed above the abdominal ribs and could have compressed the dependent parts of the lung bases when inflated (6). In addition, Eiken et al. have shown that a portion of the pressure applied in inflated full-coverage anti-G trousers in hypergravity is transmitted from the abdominal bladder to the lower thorax (approximately +6 hPa at +3.5 G0) (16). Finally, the heavy Mae West (7 kg) was likely to prevent lung expansion during inspiration.

In the present study, the increase in lower thoracic pressure was likely less than 6 hPa because ARZ 825 trousers have lower coverage and are inflated at lower pressures than full-coverage anti-G trousers (145–158 hPa at +3.5 G0, vs. ~209 hPa). Inflation lasted 1.5–2 min per protocol. As we observed few ULC and atelectases after the air protocol, we conclude that hypergravity did not lead to major airway closures and, following, that G-trouser inflation and the Mae West did not contribute to the development of compression atelectasis to a large extent. This result is in accordance with the findings of Glaister on AGT (18): up to +3.5 G0, at least, inflation to 207 hPa has no effect on tidal volume and hardly any on functional residual capacity (~5% decrease). Concerning the Mae West, a likely explanation is that this garment was adjusted but not tightly fitted on the subject. Conversely, as atelectasis increased in number and grade with increasing FIO2, it cannot be excluded that G-trouser inflation promoted closure and collapse of airways presenting hyperoxia-induced decrease in alveolar volume.

### Symptom Intensity Related to Ultrasound and SPECT/CT Findings

Aeroatelectasis manifests most often after flight, and discomfort persists for a few minutes to several hours (14, 52). Typical symptoms are moderately increased breathing frequency, difficulty in taking a full inspiration, tightening sensation in the midprecordium or soreness at the xiphoid area, and cough (26, 52). Pulmonary auscultation is either normal or shows basilar rales and/or diminished breath sounds (58). Symptom intensity is not always correlated to the degree of atelectasis found on chest radiography: atelectasis of the lung bases has been found in asymptomatic pilots, and conversely...
often no dyspnea arises when the loss in vital capacity is less than 0.5 liters (26).

Thanks to higher resolution of the imaging techniques used in the present protocol, we could see a closer relationship between symptom intensity and the amount in CT atelectasis and ULC. In particular, high-grade atelectasis, which occurred after 100% O2 breathing, manifested by cough and chest pain. The relationship was less obvious for small-grade atelectasis, which occurred after 44.5% O2 breathing.

Conclusions

Hyperoxia and hypergravity are independent risk factors of atelectasis formation in healthy sitting humans, with some degree of interindividual variability. Atelectasis occurs predominantly in the posterior and inferior lung and is reversible within 2 h without sequelae. Further experiments are required to assess whether repeated exposures would lead to less reversible atelectasis and impair lung function.

Atelectases formed in hyperoxia and moderate sustained hypergravity are caused by a combination of absorption and compression. This process is progressive. Zones with decreased ventilation are present above atelectatic areas and are prone to transient airway closure.

Ultrasound is a useful nonionizing radiating screening tool for atelectasis detection, although it cannot reliably assess their localization.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

C.D., E.G., and S.S.M.-B. performed experiments; C.D., E.G., C.V., M.S., and S.S.M.-B. analyzed data; C.D., E.G., and S.S.M.-B. interpreted results of experiments; C.D., E.G., and S.S.M.-B. wrote the manuscript; C.D., E.G., and S.S.M.-B. revised and edited the manuscript; C.D., E.G., C.V., and S.S.M.-B. approved the final version of the manuscript; C.D., E.G., M.S., and S.S.M.-B. conceived and designed research.

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