Altitude illness is related to low hypoxic chemoresponsne and low oxygenation during sleep

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Abstract

Altitude illness remains a major cause of mortality. Reduced chemosensitivity, irregular breathing leading to central apnoeas/hypopnoeas, and exaggerated pulmonary vasoconstriction may compromise oxygenation. All factors could enhance susceptibility to acute mountain sickness (AMS).

We compared 12 acute mountain sickness susceptible individuals with recurrent and severe symptoms (AMS+) with 12 “AMS non-susceptible” subjects (AMS-) assessing sleep-breathing disorders in simulated altitude as well as chemoresponsive and pulmonary vasoconstrictive responses to hypoxia.

During exposure to simulated altitude, mean blood oxygen saturation during sleep was lower in AMS+ (81.6±2.6 vs. 86.0±2.4% p<0.01), associated with a lower central apnoea-hypopnoea index (18.2±18.1 vs. 33.4±24.8 events/h in AMS+ and AMS- respectively, p=0.038). A lower hypoxic (isocapnic) chemoresponsiveness was observed in AMS+ (0.40±0.49 vs. 0.97±0.46 L·min⁻¹·%⁻¹, p<0.001). This represented the only significant and independent predictive factor for altitude intolerance, despite a higher increase in pulmonary artery systolic pressure in response to hypoxia, a lower lung diffusing capacity and a higher endothelin-1 level at baseline in AMS+ (p<0.05). AMS+ subjects were more hypoxemic whilst exhibiting less respiratory events during sleep owing to lower hypoxic (isocapnic) chemoresponsiveness. Thus, the reduction in peripheral hypoxic chemosensitivity appears as a major causative factor for altitude intolerance.
Glossary:

AMS: Acute mountain sickness
AMS+: acute mountain sickness susceptible subjects
AMS-: acute mountain sickness non-susceptible subjects
HAPE: High altitude pulmonary oedema
HACE: High altitude cerebral oedema
SpO₂: blood oxygen saturation measured by pulse oximeter
PĒTCO₂: End-tidal carbon dioxide pressure
AHI: Apnoea Hypopnoea Index
HVR: hypoxic ventilatory response
ι,HVR₅/₂₀: Isocapnic hypoxic ventilatory response at 5 or 20 minutes
π,HVR₅/₂₀: Poikilocapnic hypoxic ventilatory response at 5 or 20 minutes
HCVR: Hypercapnic ventilatory response
TTE: Transthoracic echocardiography
PASP: Pulmonary artery systolic pressure
PVR: Pulmonary vascular resistance
HPV: Hypoxic pulmonary vasoconstriction
Vₑ: pulmonary ventilation
Vc: capillary volume
Dm: membrane conductance
DL,CO/NO: Diffusion lung capacity for carbon monoxide and nitric oxide
KCO: carbon oxide transfer coefficient
VO₂: Oxygen consumption
NO: nitric oxide
The term high-altitude illness encompasses acute mountain sickness (AMS), high-altitude cerebral oedema (HACE) and high altitude pulmonary oedema (HAPE). Although HAPE represents the major cause of mortality due to high altitude [1], AMS is the most frequently encountered syndrome and is usually considered as an early stage of HAPE and HACE. When subjects rapidly ascend to moderate altitude (2,000-3,000 m), up to 25% will suffer from AMS [2]. When mountaineers ascend to high or very high altitude (>4,500 m) the AMS incidence may exceed 60%[3].

High altitude illness is partly preventable by gradual and stepwise ascent. However, certain individuals are at greater risk to develop high-altitude illness and are more likely to be reproducibly affected during ascents. The ability to predict AMS susceptibility would be highly beneficial, but no predictive test is currently agreed upon by specialists [4].

The mechanisms of high altitude illness remain not fully understood, which precludes any clinical or biological prediction of AMS susceptibility. Characteristics of established AMS and HACE, however, suggest that factors lowering blood oxygenation may play a major role in their development [5, 6]. Thus, a relative hypoventilation induced by low hypoxic chemosensitivity during wakefulness has been suggested as an important feature of AMS susceptibility [7]. Reduced lung diffusing capacity following altitude exposure may also compromise oxygenation [8, 9]. Early fluid retention [10] and increased hypoxic pulmonary vasoconstriction (HPV) in a less adaptable pulmonary circulation (e.g., a smaller pulmonary vascular bed) have also been shown as being important. Both Endothelin-1 levels [11] and exhaled nitric oxide (NO) as well as their changes in response to hypoxic conditions [12] may further affect hypoxic pulmonary vasoconstriction.

Both periodic breathing during sleep [13] and central chemosensitivity (assessed by ventilatory response to carbon dioxide [14]) have not been found as important predictors of AMS susceptibility. Nocturnal oxygenation, however, may play a role in AMS susceptibility. Whether altitude induced central sleep apnoea syndrome by reducing oxygenation, may favour AMS has not been studied. Finally, these factors have usually been investigated separately, making it difficult to determine their individual contribution to high-altitude illness and their ability to predict individual AMS susceptibility.

Our working hypothesis was that AMS susceptibility was associated with low hypoxic chemosensitivity, lower nocturnal oxygenation during sleep and higher pulmonary vasoreactivity when compared with altitude tolerant subjects.
Material and methods

Study population

A total of 24 mountaineers participated in the study. All gave informed consent and the study was approved by the Grenoble Ethics Committee (CPP Sud-Est V). Two distinct groups of subjects were recruited for the study: (a) 12 acute mountain sickness susceptible subjects (AMS+) with recurrent and severe AMS (Lake Louise score $\geq 6$) despite appropriate acclimatization and (b) 12 carefully matched subjects who had always felt well during exposure to equivalent altitudes (AMS-). Subjects were recruited after responding to requests on specialized websites: International Federation of Mountain Guides Association, www.grenoble-montagne.com, French Alpine Club and French Federation of climbing and mountaineering). None of the subjects had previously participated in altitude research. Eighty subjects initially volunteered to participate to the study and a structured phone interview was given to all subjects by two of the investigators (HN and BW), wherein all described their altitude experience. The interviews took place between 2 and 6 months after the last ascent. Tolerance to altitude was assessed by asking specific questions pertaining to clinical manifestations occurring at high altitude. Answers to specific questions and quotations were recorded in respect to the five common symptoms of AMS (headache, fatigue, sleep, vomiting and dizziness). Circumstances of occurrence were checked to exclude obvious acclimatization mistakes. All AMS-sensitive subjects had experienced recurrent and severe AMS (i.e., Lake Louise score $\geq 6$), predominantly at a threshold altitude (between 2500 and 4000 meters high). Lake Louise scores were additionally and retrospectively completed during the inclusion visit (Grenoble: 210m altitude). Diagnosis of HAPE was based on retrospective evaluation of the clinical symptoms (dry cough, hemoptoic sputum, highly disproportionate breathlessness), as well as pulmonary alveolar images on X-ray immediately after descent ($n = 4$). AMS + and AMS- subjects were paired with respect to gender, age, body mass index, physical fitness, and experience of high altitude. All subjects were natives of low altitude and none had gone to altitudes above 2,000 m during the two weeks before the study. All were free of significant cardiac, pulmonary or neurological pathology at the time of the study. All women completed the tests during the first week following menses. Measurements were made at Grenoble University Hospital.

Measurements:
All subjects were asked to avoid caffeine and other respiratory stimulants during all experimental sessions. Subjects were allowed to have regular light meals before all sessions.

Sleep: One full-night polysomnography was performed in normoxia followed by a second one in hypoxia (F_{O2}=14.5%, corresponding to 3,000m), either on two consecutive nights (n=22) or within a few days (<7 days, n=2). Recordings were performed in a hypoxic tent, as previously described by Gilmartin et al. [15]. Subjects were blinded to the condition studied (normoxia or hypoxia). Details of sleep monitoring, and criteria used for scoring apnoeas and hypopnoeas are reported in e-add 1 the online supplement.

Ventilatory responses during wakefulness: Subjects were studied while supine in a quiet room. Conditions of resting eupneic ventilation were carefully controlled: (motor rest without anticipation of exercise, absence of noise, light of moderate intensity, absence of specific mental task and relaxed wakefulness). Subjects breathed through a mouthpiece and wore nose clip. The dead space of the apparatus was minimized and was identical in all conditions [16]. None of the subjects reported any respiratory or general discomfort at any time. All tests were performed on Friday, between 3 and 7 pm.

Hypoxic ventilatory response (HVR) was assessed according to Lake Louise recommendations [17]. In a quiet environment, after habituation to the respiratory valve, subjects breathed spontaneously during hypoxic conditions for 20 minutes. Ventilation (V_{E}), end-tidal partial pressure in CO₂ (P_{ET}CO₂), transcutaneous O₂ saturation (S_{p}O₂) recorded by pulse oximeter were recorded. The hypoxic gas was delivered by the Altitrainer® system (SMTEC, Switzerland) using variable nitrogen fractions whilst CO₂ was adjusted within the inspired air (iso- or poikilocapnic) using a specific device (Isocap®). F_{O2} was set to achieve 80% S_{p}O₂. Acute HVR (i/pHVR_{5-min}) and decline (i/pHVD_{20-min}) were derived in isocapnic and poikilocapnic conditions (i.e., with and without control of P_{ET}CO₂ respectively).

The hypercapnic ventilatory response (HCVR) test protocol was based on the method of Katayama et al [18]. Ventilation and CO₂ fraction were measured with an M’Vmax 229 analyzer (SensorMedics, Yorba-Linda, CA, USA). HCVR sensitivity was determined as the slope of minute ventilation plotted against P_{ET}CO₂ and the P_{ET}CO₂ threshold of the ventilatory response (intercept) was also determined.

Resting baseline V_{E} and P_{ET}CO₂ were analysed during a 2-minute period of relaxed wakefulness at rest, after adaptation to the mouthpiece with steady state ventilation ensured.

Echocardiography: Transthoracic echocardiography (TTE) was performed during normoxia at rest, and after one hour of hypoxic exposure with F_{O2} adjusted in order to achieve 80% S_{p}O₂. Targeting a set value of S_{p}O₂ minimized the effect of inter-individual
differences in arterial oxygenation. The pulmonary artery systolic pressure (PASP) was derived from the maximum velocity of tricuspid regurgitation by Doppler TTE, using modified Bernoulli equation and pulmonary artery diameter estimation (see online supplement) [19]. Pulmonary vascular resistance (PVR) was calculated using Abbas’ equation [20]. Cardiac output was calculated using Huntsman equation [19]. Four subjects were excluded from the analysis due to insufficient Doppler profiles during hypoxic exposure, leaving the sample size for this parameter at n = 20.

Blood analyses: Blood samples were taken in the supine position, upon awakening, after both study nights. Endothelin-1 and Big-endothelin were assessed by ELISA kits. Renin, aldosterone and vasopressin analyses were performed by radio-immunoassays kits (details are available in e-table 5 in online repository).

Pulmonary measurements: Measurements for lung function and lung diffusing capacity for carbon monoxide and nitric oxide \((D_{L,CO} / NO)\) were performed in a BodyBox 5500 (Medi-Soft, Dinant, Belgium). Standardisation of \(D_{L,CO}\) and \(D_{L,NO}\) procedures were those reported by ERS/ATS [21] and Aguilaniu et al. [22], respectively. Diffusing capacity for carbon monoxide per unit of alveolar volume \((K_{CO})\), membrane-diffusing capacity \((D_m)\) and pulmonary capillary blood volume \((V_c)\) [23] were derived.

Protocol

The entire study was conducted in the laboratory. During the initial visit, subjects underwent medical examination (Visit 1). On the second visit, isocapnic and poikilocapnic hypoxic ventilatory response were measured and a maximal incremental exercise test in normoxia \((VO_2_{max})\) was performed. Measurements of pulmonary vascular pressures (using transthoracic echocardiography during normoxia followed by 1 hr-hypoxic exposure), lung function tests, measurement of hypercapnic ventilatory response to CO2 and lung diffusing capacity measurements were done in visit 3. Visits 4 and 5 consisted in the sleep studies during normoxia and hypoxia respectively, followed by blood sample collections. This design avoided any influence of acclimatization to hypoxia.

Statistical analysis:

Statistics were carried out with NCSS software (NCSS, Utah, USA). Results are expressed as the mean ±1.0 SD. For continuous variables, comparisons between ‘AMS+’ and ‘AMS-’ groups were performed using paired Student t-tests or Wilcoxon paired test, depending on the normality of distribution. A value of p<0.05 was considered to be
statistically significant. Univariate conditional logistic regressions were made to determine odds ratio for the occurrence of AMS. Values of each variable were recoded in relation to the median value. Predictive parameters were considered as significant for a probability below 0.05 and if the Odds ratio inferior or superior limits did not include 1.0. To test whether HAPE and HACE and/or AMS were different in ventilatory and circulatory responses, a specific analysis was performed, excluding HAPE subjects and their paired AMS subjects.

Results

Physical characteristics and fitness:

Twelve subjects with typical and recurrent altitude illness, i.e., severe AMS, with an average Lake Louise score of 9.7 and HAPE (n = 4) or HACE (n = 1) and matched AMS non-susceptible subjects are presented in Table 1. None of the subjects were taking medications affecting the control of breathing. One subject was on diuretic therapy for hypertension during altitude exposure and normoxic/hypoxic test sessions.

Table 1: Main characteristics of AMS susceptible vs. AMS non-susceptible subjects.

<table>
<thead>
<tr>
<th></th>
<th>AMS+ subjects</th>
<th>AMS- subjects</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>46 ± 13</td>
<td>47 ± 11</td>
<td>0.31</td>
</tr>
<tr>
<td>Lake Louise Score</td>
<td>9.7 ± 2.0</td>
<td>0.2 ± 0.4</td>
<td>&lt; 0.001 **</td>
</tr>
<tr>
<td>Gender</td>
<td>10 M - 2 F</td>
<td>10 M - 2 F</td>
<td></td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>23.5 ± 2.2</td>
<td>22.3 ± 2.1</td>
<td>0.22</td>
</tr>
<tr>
<td>VO₂ max (ml/kg)</td>
<td>52 ± 15</td>
<td>58 ± 9</td>
<td>0.23</td>
</tr>
</tbody>
</table>

AMS+: acute mountain sickness susceptible subjects, AMS-: acute mountain sickness non-susceptible subjects, BMI: body mass index, LLS: Lake Louise score of AMS symptoms, VO₂max: maximal oxygen consumption. *: p<0.05; **: p<0.01.

Sleep, breathing and blood oxygenation:
Except for one subject presenting with moderate Obstructive Sleep Apnoea with an apnoea/hypopnoea index (AHI) of 27/h, nocturnal ventilation was normal in both groups during normoxia. The mean values in AMS+ and AMS- subjects, respectively, were: AHI = 7 ± 7 vs. 9 ± 9 events/h, p = 0.3, \( S_pO_2 = 94 ± 1 \) vs. 95 ± 1%, p = 0.2. There was no difference in sleep architecture or sleep efficiency between groups. During sleep in hypoxic conditions (Table 2), respiratory events (apnoeas and hypopnoeas) increased with predominantly central events (64% and 74% of total events in AMS+ and AMS- subjects, respectively). AMS+ subjects had a significantly lower AHI than AMS- (18.2±18.0 vs. 33.4±24.8 events/h, p=0.038), whilst exhibiting lower mean \( S_pO_2 \) and minimum \( S_pO_2 \) levels than AMS- (81.6±2.6 vs. 86.0±2.4%, p<0.01 and 73.6±3.0 vs. 78.0±2.6%, p<0.01, respectively). The time spent at low levels of \( S_pO_2 \) was higher in AMS+ subjects than in AMS- subjects (Figure 1). Despite lower AHI, sleep efficiency was also reduced in AMS susceptible subjects compared with AMS- subjects (83.5±9.2% vs. 90.0±6.0%, p=0.02) due to increased wakefulness after sleep onset (44.9±31.1 vs. 70.4±45.1 minutes, p=0.03). Stage I (10.8% vs. 14.8%, p<0.01) and micro-arousals associated with respiratory events (12.9±15.6 vs. 23.8±19.2 events/h) were lower in AMS-susceptible subjects which was consistent with the AHI difference between groups. Also REM sleep was slightly longer in AMS- than in AMS+ subjects (26.6 vs. 21.1% of total sleep time, p<0.01), as shown in Table 2. Details of sleep data are available in E-table 1 in online repository.

Table 2: Sleep characteristics in hypoxia (FiO₂=14.5%) in AMS+ and AMS- subjects.

<table>
<thead>
<tr>
<th></th>
<th>AMS+</th>
<th>AMS-</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sleep Time (min)</td>
<td>354 ± 65</td>
<td>394 ± 25</td>
<td>0.01 *</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>83.5 ± 9.2</td>
<td>90 ± 6</td>
<td>0.02 *</td>
</tr>
<tr>
<td>Stage I (% TST)</td>
<td>10.8 ± 5.9</td>
<td>14.8 ± 7.9</td>
<td>0.003 **</td>
</tr>
</tbody>
</table>
AMS+ subjects exhibited a lower central CO\textsubscript{2} chemosensitivity than AMS- (Figure 2), with a right shift of the response due to a lower slope (2.6±1.5 vs. 4.0±1.8 L·min\textsuperscript{-1}·mmHg\textsuperscript{-1}, p=0.02) and an increased $P_{ET}$CO\textsubscript{2} threshold (48.9±2.8 vs. 45.8±4.0 mmHg, p=0.04). AMS+ subjects also had higher eupneic $P_{ET}$CO\textsubscript{2} (40.2±2.5 mmHg vs. 35.7±2.8 mmHg, p<0.001) than AMS- subjects.

Peripheral O\textsubscript{2} chemosensitivity assessed by the isocapnic hypoxic ventilatory response at 5-minute ($i$HVR\textsubscript{5}) was reduced in AMS+ compared to AMS- subjects (0.40±0.49 vs. 0.97±0.46 l·min\textsuperscript{-1}·%SpO\textsubscript{2}\textsuperscript{-1}, p<0.001), (Figure 3). Hypoxic ventilatory decline in isocapnic hypoxia was not apparent in AMS+ (0.40±0.49 at 5 min vs. 0.13±0.24 l·min\textsuperscript{-1}·%SpO\textsubscript{2}\textsuperscript{-1} at 20 min, p=0.2) compared to AMS- subjects (0.97±0.46 at 5 min vs. 0.40±0.43 l·min\textsuperscript{-1}·%SpO\textsubscript{2}\textsuperscript{-1} at 20 min, p<0.001). Poikilocapnic ventilatory response at 5-minute ($p$HVR\textsubscript{5}: 0.34±0.43 vs. 0.21±0.16 mmHg·%SpO\textsubscript{2}\textsuperscript{-1}, p=0.2), poikilocapnic ventilatory decline ($p$HVR\textsubscript{20}: 0.40±0.43 vs. 0.13±0.24, p=0.2) and $i$HVR\textsubscript{20} (and 0.58±0.45 vs. 0.34±0.52, p=0.1) were not significantly different.

**Pulmonary vasoreactivity:**

Systolic pulmonary artery pressure (PASP), cardiac output and vascular resistances (PVR) are presented in Figure 4. As expected, cardiac output and pulmonary vascular resistance increased after 1h of hypoxic exposure. The mean increase in pulmonary vascular resistance in response to hypoxia ($\Delta$PVR) was not different between groups (+0.31±0.23 vs. +0.33±0.19 Woods in AMS+ and in AMS-, respectively, p>0.05), although AMS+ subjects showed an increase in PASP ($\Delta$) in hypoxia (versus normoxia), which was not observed in AMS- (+6.42±1.85 vs. +0.66±0.19 mmHg, p=0.04), see figure 4. This was essentially attributable to normoxic PASP values in the latter group (AMS– subjects). No significant
difference in pulmonary vasoconstrictive response to hypoxia could be shown between groups in this experimental condition.

Pulmonary diffusion:

None of the subjects displayed abnormal lung function, and both groups had similar ventilatory volumes (FEV₁, FVC). Significant differences in CO/NO diffusion characteristics were observed at rest, in normoxia. Comparatively to AMS- subjects, AMS+ subjects showed lower lung capillary volume (86·2±13·8% vs. 95·0±22·8% of predicted value, p=0·02), a lower CO diffusion (89·8±15·2% vs. 101·8±16·2% of predicted value, p=0·037) and a lower transfer factor: KCO (79·4±9·9% vs. 87·5±8·9% predicted, p=0·02). AMS+ also had a higher DL,CO/NO (5·3±0·2 vs. 5·0±0·2, p=0·03), which suggests the possibility of a vascular restriction (table 3).

Specific analysis of AMS/HACE subjects, excluding HAPE subjects and their paired non-susceptible subjects, did not show any significant difference in delta ΔPASP between hypoxic and normoxic exposure.

### Table 3: CO-NO diffusion characteristics in ‘AMS-susceptible’ and AMS- subjects.

<table>
<thead>
<tr>
<th></th>
<th>AMS+</th>
<th>AMS-</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FVC</strong> (L)</td>
<td>5.3 ± 1.1</td>
<td>5.3 ± 0.6</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>FVC</strong> (% predicted)</td>
<td>119.1 ± 14.6</td>
<td>112.1 ± 12.6</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>FEV₁</strong> (L)</td>
<td>4.0 ± 0.9</td>
<td>4.1 ± 0.8</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>FEV₁ (% predicted)</strong></td>
<td>110.6 ± 15.8</td>
<td>106.3 ± 12.2</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Dₜₗ,CO</strong> (ml/min/mmHg)</td>
<td>32.3 ± 6.9</td>
<td>37.2 ± 6.0</td>
<td>0.03 *</td>
</tr>
</tbody>
</table>
\[
\begin{array}{ccc}
D_{L,\text{CO}} \\ (% \text{ predicted}) & 89.8 \pm 15.2 & 101.8 \pm 16.2 & 0.06 \\
D_{L,\text{CO}}/V_A \\ (\text{ml/min/mmHg/l.}) & 4.0 \pm 0.8 & 4.4 \pm 0.6 & 0.02 \ *
\end{array}
\]

\[
\begin{array}{ccc}
D_{L,\text{CO}}/V_A \\ (% \text{ predicted}) & 79.4 \pm 9.9 & 87.5 \pm 8.9 & 0.02 \ *
\end{array}
\]

\[
\begin{array}{ccc}
D_{L,\text{NO}} \\ (\text{ml/min/mmHg}) & 170.2 \pm 38.2 & 168.4 \pm 43.4 & 0.28 \\
D_{L,\text{NO}} \\ (% \text{ predicted}) & 102.0 \pm 18.8 & 101.0 \pm 21.4 & 0.3
\end{array}
\]

\[
\begin{array}{ccc}
D_{m,\text{CO}} \\ (\text{ml/min/mm Hg}) & 87.3 \pm 19.3 & 86.3 \pm 19.3 & 0.35 \\
D_{m,\text{CO}} \\ (% \text{ predicted}) & 101.8 \pm 18.6 & 100 \pm 18.6 & 0.3 \\
V_c \\ (\text{mL}) & 88.1 \pm 19.6 & 101.6 \pm 30.6 & 0.02 \ *
\end{array}
\]

\[
\begin{array}{ccc}
V_c \\ (% \text{ predicted}) & 86.2 \pm 13.8 & 95.0 \pm 22.8 & 0.02 \ *
\end{array}
\]

\[
\begin{array}{ccc}
D_{L,\text{CO}}/D_{L,\text{NO}} \\ (\text{a.u.}) & 5.3 \pm 0.2 & 4.9 \pm 0.5 & 0.037 \ *
\end{array}
\]

FVC: forced vital capacity, FEV₁: forced expiratory volume in 1 second, \(D_{L,\text{CO}}\): Diffusing capacity for the lungs for carbon monoxide, \(D_{L,\text{NO}}\): Diffusing capacity of the lungs for nitric oxide, \(KCO\) (\(D_{L,\text{CO}}/V_A\)): Transfer coefficient of the lung, \(Dm\): membrane diffusion capacity, \(Vc\): pulmonary capillary blood volume.

**Pulmonary vasoactive biomarkers and fluid retention:**

The plasma endothelin level was markedly increased in AMS+ vs. AMS- subjects, both after the night in normoxia (6·4±1·8 vs. 3·6±1·0 fmol/ml, \(p=0.02\)) and after the night in hypoxia (7·1±1·9 vs. 3·7±1·1 fmol/ml, \(p=0·02\)). AMS+ subjects had a significantly higher increase in Big-endothelin, after the normoxic night than AMS- subjects (1·03±0·29 vs. 0·89±0·14 fmol/mL, \(p=0·04\)). There was no difference in the morning plasma vasopressin level between groups in normoxia (3·8±1·4 vs. 4·5±1·8 pg/mL, \(p=0·12\)) and a lower level in
AMS+ compared to AMS- subjects in hypoxia (3.0±2.4 vs. 4.3±2.5 pg/mL, p=0.04). AMS+ subjects had significantly higher plasmatic bicarbonate ions than AMS- subjects (27.1±1.7 vs. 25.4±2.6 mmol/L, p=0.02) owing to reduced ventilation in hypoxia, which was not found after the normoxic night (25.6±2.5 vs. 25.3±1.7 mmol/L, p=0.2). No difference was found between groups in renin and aldosterone levels after both normoxic and hypoxic nights.

Among factors independently associated with high altitude susceptibility (hypoxic ventilatory response, capillary volume, change in pulmonary arterial pressure with hypoxia, left ventricular ejection fraction, central chemosensitivity), iHVR₅ was the only significant parameter in univariate conditional logistic regression analysis, involved in AMS development. iHVR₅ was a protective factor (low limit=0.016, high limit=0.999, p<0.05). Above the median value of 0.58 l.min⁻¹/%, an odds ratio of 0.125 for the occurrence of AMS was observed (p<0.05). All details of the statistical analysis are available in E-table 4 in online repository.

Discussion

The main result of the present study is that high peripheral hypoxic chemosensitivity appears to be a major protective factor for acute mountain sickness. The presence of more central events with a higher apnoea/hypopnoea index in the altitude tolerant subjects is most likely explained by this higher chemosensitivity. Somewhat unexpectedly, this increase in central respiratory events during sleep was associated with an overall a higher nocturnal oxygen level i.e. higher S₉O₂ levels.

Lastly, hypoxic pulmonary vasoreactivity per se was not involved in AMS physiopathology in the present study. However, there was a reduced capillary bed and subtle differences in diffusion capacity of vascular origin.

Chemical control of breathing

In the present study iHVR₅, the index of peripheral hypoxic chemoresponsiveness without the influence of hypocapnia, represented a highly predictive factor for high-altitude tolerance. Although hypoxic chemosensitivity has always been considered as an important favouring factor, this remains controversial. Methodological issues have been raised [24] As
suggested by Roach et al.[6], studies where ‘$S_aO_2$ after hypoxic exposure’ was the criteria used to predict chemosensitivity and altitude tolerance led to coherent results (i.e., the lower the $S_aO_2$, the higher the severity of altitude intolerance). However, this was less clear when assessing directly the hypoxic ventilatory response (i.e. not only the $S_aO_2$ achieved but also at which level of ventilation). This may be related to hypocapnia[25] but also the time course of the response combining ventilatory stimulation and hypoxic ventilatory decline[7]. The HVR test used in the present study followed the Lake Louise recommendations[17]. Thus we were able to separate the two parts of the response. $iHVR_5$ (i.e. measured after a five minutes hypoxic exposure) corresponds to the stimulating ventilatory phase of the response. Differences in $iHVR_5$ between groups cannot be attributed to baseline conditions of $P_{ETCO_2}$[26], and the targeted isocapnia level, set at 1-2 mmHg above individuals resting normoxic $P_{ETCO_2}$ led to similar level of $P_{ETCO_2}$ between groups during the test. Thus, our study emphasizes the relevance of this index of pure hypoxic chemoresponsivness in predicting altitude tolerance (and AMS) when compared with all the other independent physiological factors tested (ventilatory decline, $\Delta$PASP between hypoxia and normoxia, baseline PVR in hypoxia, endothelin level and central CO$_2$ chemosensitivity). In our study, the risk of developing high-altitude illness was decreased by 80% (corresponding to OR=0.125) when $iHVR_5$ was higher than the median value of 0.58 l.min$^{-1}$.%SpO$_2^{-1}$.

**Sleep apnoea and altitude susceptibility**

Periodic breathing and central events during sleep increase with altitude[27], but whether this higher incidence of central events alters altitude tolerance is still debated. Unlike the hypothesis suggesting that periodic breathing precipitates[13] or is unrelated to[28] AMS, our results suggest that periodic breathing is associated with improved altitude tolerance. We suggest that this is related to an increase in the average nocturnal oxygen saturation.

The association between periodic breathing and improved altitude tolerance is related to the presence of a higher gain of the chemosensitive ventilatory response. It has been evidenced that enhanced ventilatory responses to CO$_2$, to hypoxia or both are critical factors for promoting periodic breathing during heart failure or in hypoxic conditions[29-31]. Thus it is not surprising that the AMS- group having higher chemoresponsiveness exhibited more periodic breathing than the AMS+ group.

More unexpected was the effect of periodic breathing on nocturnal $S_aO_2$. The occurrence of hyperventilation after each reduction in ventilation i.e. apnoeas and
hypopnoeas, which characterises periodic breathing, resulted in an increased overall mean SaO2. A similar relationship between periodic breathing and nocturnal SaO2 was previously reported by Hackett et al. [32]. In this study, Almitrine -an hypoxic ventilatory stimulant- led to a parallel increase in nocturnal SpO2 and periodic breathing during actual altitude exposure [32]. To our best knowledge, our study is the first not only to support this hypothesis but more importantly to correlate with altitude tolerance. Other field studies performed at very high altitude tended to confirm that the higher the AHI, the higher the oxygen saturation achieved [27, 33]. This relationship may be related to hyperventilation-induced improvement in SaO2 associated with enhanced hypoxic chemosensitivity allowing to hyperventilate after central events and thus to reach a higher oxygen saturation.

*Pulmonary circulation, pulmonary diffusion and altitude susceptibility*

The present study does not show any difference in hypoxic pulmonary vasoconstriction between AMS+ and AMS- subjects. A similar increase in pulmonary vascular resistance was induced by hypoxia in both groups. A greater pulmonary artery systolic pressure (∆PASP) in response to hypoxia was only observed in AMS+, which was not associated with greater changes in resistance. A higher cardiac output (NS, trend only) and a high pulmonary artery pressure (PASP) in normoxia found in AMS- subjects are the likely explanations for these findings. Pulmonary artery pressure variations in response to hypoxia remained higher in AMS+ subjects even when subjects with AMS and known pulmonary oedema (HAPE n=4) were excluded from the analysis. Although the pulmonary artery pressure response tended to be lower than previously reported in other studies [34], it is important to note that this is the first study examining altitude intolerance in which differences in response of arterial pulmonary pressure, cardiac output and pulmonary vascular resistance measured by Doppler TTE are investigated at a targeted SpO2 level, instead of iso-altitude or iso-FiO2, as in other studies [34, 35]. This provides a way to measure hypoxic pulmonary vasoreactivity *per se*, i.e. independently from the oxygen desaturation achieved for a given FiO2. Indeed, since desaturation for a given FiO2 tends to be greater in AMS-susceptible subjects (especially in HAPE-susceptible subjects [6]), this may lead to overestimate alterations in pulmonary vascular response during hypoxic exposure. In our study, the FiO2 needed to reach the ‘target’ SpO2 of 80% was higher in AMS+ than AMS-subjects (12.9±0.7% FiO2 vs. 11.8±0.3% FiO2, p<0.05). Overall, our study does not confirm exaggerated hypoxic pulmonary vasoconstriction as a key factor for AMS susceptibility. Nevertheless, subtle differences in the pulmonary circulation at baseline were observed with
reduced diffusing capacity of the lungs for CO diffusion and lower diffusing capacity for carbon monoxide per unit of alveolar volume ($K_{CO}$). Previous functional studies have shown that reduced $D_{L,CO}$ was common in AMS-susceptible subjects during altitude exposure [8]. In our study, diffusion defects of limited amplitude were observed in AMS-susceptible subjects in normoxia at rest. Although clinically unimportant in normoxia, this could lead to lower blood oxygen saturation at altitude. Reduced pulmonary capillary blood volume, measured using CO-NO diffusion, was observed in AMS-susceptible subjects. Combined NO/CO diffusion suggested vascular involvement. Membrane conductance ($D_m$) was similar between groups. The interpretation of these changes and the precise significance in AMS pathophysiology deserve further investigations.

Lastly, after the hypoxic night, plasmatic ET-1 levels were higher in AMS+ than in AMS- subjects. This difference may be linked to an altered vasoconstrictive tone in AMS-susceptible subjects. Unexpectedly, vasopressin level was lower in AMS+ than in AMS-subjects [10] which would tend to compensate for increased diuresis and renal excretion of bicarbonates.

In conclusion, the present study suggests that hyperventilation associated with high hypoxic ventilatory response is a major factor for preventing AMS occurrence. This is also the case during sleep since periodic breathing and central sleep apnoeas result in a better preserved oxygenation probably owing to a more pronounced hyperventilation following the apnoeic events.

$\;HVR_5$ below a 'threshold' of 0·58 L.min$^{-1}$.%$^{-1}$ is a highly predictive factor for AMS occurrence in the present study but should be further tested in a prospective study including non selected subjects.

Conflict of interest statement:
The authors declare that they have no conflict of interest.

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References


CAPTIONS:

Table 1: Physical characteristics, spirometric data and physical fitness of AMS susceptible vs. AMS non-susceptible subjects.
AMS+: acute mountain sickness susceptible subjects, AMS-: acute mountain sickness non-susceptible subjects, BMI: Body Mass Index, LLS: Lake Louise score of AMS symptoms. FVC: forced vital capacity. FEV1: forced expiratory volume in 1 second. VO2 max: maximal oxygen consumption. *: p<0.05; **: p<0.01 (see statistical analysis).

Table 2: Sleep characteristics in hypoxia (FiO2=14.5%) in AMS-susceptible and AMS non-susceptible subjects.
Note the reduction in sleep efficiency and shorter REM sleep in the more hypoxic ‘AMS-susceptible’ patients in comparison with AMS- subjects (p<0.01).

Table 3: CO-NO diffusion characteristics in ‘AMS-susceptible’ and AMS non-susceptible subjects.

Table 4: Sub-analysis with exclusion of HAPE subjects.
Left part of the table (AMS+ vs. AMS-) refers to the entire group with severe AMS and possible signs of HAPE (hemorrhagic sputum, audible chest crackles and/or wheeze, positive chest X-ray after descent, n=4) vs. AMS- subjects. Right part of the panel refers to the same analysis restricted to subjects with severe AMS without edema (AMS without edema vs. paired subjects, n=8).

Fig 1: Nocturnal blood oxygenation difference between AMS-susceptible and AMS non-susceptible subjects in hypoxia.
Case: AMS+ subjects, Control: AMS- subjects. Top panels: Nocturnal SPO2 in a representative AMS-susceptible (left) and tolerant (right) subject. Note the lower oscillations and mean SPO2 in AMS-susceptible. Middle panel: Mean AHI (apnoea left panel, hypopnoea: right panel) in AMS+ (white bars) and AMS- (black bars) subjects. Bottom panel: Mean distribution of nocturnal SPO2 in AMS+ (grey bars, black drawing) and AMS- (white bars, grey drawing) subjects.
Figure 2: Schematic representation of differences in slope and threshold of the ventilatory response to CO₂
HCVR=hypercapnic ventilatory response. P_{ET}CO₂=end tidal carbon dioxide pressure (mmHg).
Figure 3: Mean of ‘isocapnic’ hypoxic ventilatory response in AMS-susceptible (black circles) and AMS- (open circles) subjects. HVR_{5/20}=hypoxic ventilatory response at 5 and/or 20 minutes, respectively.
Figure 4: Pulmonary circulation characteristics in normoxia and difference regarding hypoxia in AMS+ and AMS- subjects. PASP=pulmonary arterial systolic pressure. PVR=pulmonary vascular resistance.