Decrease of Asymmetric Dimethylarginine Predicts Acute Mountain Sickness

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Introduction. Each year, 40 million tourists worldwide are at risk of getting acute mountain sickness (AMS), because they travel to altitudes of over 2500 m. As asymmetric dimethylarginine (ADMA) is a nitric oxide synthase (NOS) inhibitor, it should increase pulmonary artery pressure (PAP) and raise the risk of acute mountain sickness and high-altitude pulmonary edema (HAPE). With this in mind, we investigated whether changes in ADMA levels at an altitude of 4000 m can predict an individual’s susceptibility to AMS or HAPE.

Methods. Twelve subjects spent two nights in a hypobaric chamber, the first night without exposure to altitude conditions and the second night at a simulated altitude of 4000 m. At identical time points during both nights (after 2, 5, and 11 hours), we determined ADMA serum levels, PAP by Doppler echocardiography and estimated hypoxia related symptoms by Lake Louise Score (LLS).

Results. Contrary to our initial hypothesis, subjects with a marked increase in ADMA at 4000 m showed PAP levels below the critical threshold for HAPE and were not affected by AMS. By contrast, subjects with a decrease in ADMA suffered from AMS and had PAP levels above 40 mmHg. After 2 hours of hypoxia we found a significant relationship between Δ-PAP (Spearman’s $\rho = 0.30$, $p \leq 0.05$) respectively Δ-ADMA (Spearman’s $\rho = -0.92$, $p \leq 0.05$) and LLS.

Conclusion. After 2 hours of hypoxia, the Δ-ADMA (positive or negative) can predict an LLS of >5 with a sensitivity of 80% and a specificity of 100% and can help assess the risk of an increase in PAP to more than 40 mmHg and thus the risk of HAPE (φ coefficient: 0.69; $p \leq 0.05$).

Worldwide, 40 million tourists are at risk of getting acute mountain sickness (AMS) each year, because they travel to altitudes of higher than 2500 m (AMS-incidence at altitudes of 2500–3000 m: 10–30%). In general, the following conditions are distinguished: AMS, high-altitude cerebral edema (HACE), and high-altitude pulmonary edema (HAPE). An increase in pulmonary artery pressure (PAP), which is subject to individual differences, plays a crucial role in the development of HAPE. The risk of developing HAPE increases massively when PAP exceeds 40 mmHg. The measurement of PAP by Doppler echocardiography usually allows individuals at risk of developing HAPE to be identified, especially in the setting of hypoxia. For methodological reasons, however, Doppler echocardiography can be used only in individuals with (at least minor) tricuspid valve insufficiency. Although this insufficiency is often seen in association with an altitude-induced increase in PAP, high-altitude medical research has revealed the absence of tricuspid reflux in 5–30% of the subjects. In addition, this method requires an experienced examiner and the availability of a suitable (mobile) system. This explains the need for simpler procedures.

Against this background, the measurement of serum levels of asymmetric dimethylarginine (ADMA) may provide a new diagnostic approach. ADMA is a potent inhibitor of nitric oxide synthase (NOS). By increasing

cyclic guanosine monophosphate (cGMP), nitric oxide (NO) causes smooth muscle relaxation and therefore induces rapid vasodilatation. NO plays a key role in oxidative metabolism and modulates the response of the body to hypoxia through hypoxia-inducible factor-1α (HIF-1α). At 4559 m, inhalation of NO led to a marked decrease in PAP and an increase in arterial oxygen saturation especially in subjects susceptible to HAPE. In addition, decreased pulmonary NO production during acute hypoxia was suggested to contribute among other factors to the enhanced hypoxic pulmonary vascular response in HAPE-susceptible subjects and therefore might contribute to exaggerated hypoxic pulmonary vasoconstriction and in turn to pulmonary edema.

As it is an NOS inhibitor, ADMA should cause an increase in PAP and raise the risk of developing altitude sickness and HAPE. By measuring ADMA serum levels during standardized altitude exposure, we were able to assess this approach both from a principal therapeutic perspective as described in the aforementioned studies and from a diagnostic perspective.

This prospective comparative study was conducted to test the hypothesis that there is a relationship between Δ-ADMA in blood and a hypoxia-induced increase in PAP and AMS and that ADMA could be a predictive value for the development of AMS or a PAP > 40 mmHg.

Materials and Methods

The tests were performed in the altitude and climate chamber of the German Air Force Institute of Aviation Medicine in Koenigsbrueck, Germany (134 m). This hypobaric chamber has a capacity of six individuals for an overnight stay. Two tests were performed and 12 subjects could be investigated. Each trial consisted of two overnight stays in the chamber. The subjects were allowed to sleep. For intraindividual comparison, both nights followed the same protocol. Altitude conditions, however, were simulated only during the second night, when the subjects were decompressed over a period of 53 minutes to a pressure equivalent to an altitude of 4000 m. The subjects spent 12 hours in the chamber under these altitude conditions. At all time points, the subjects could have been rapidly recompressed or could have left the chamber through an airlock. An emergency physician with expertise in altitude medicine was continuously present.

The study design had been approved by the ethics committee of the Society of Physicians of the state of Baden-Wuerttemberg, Stuttgart, Germany. All participants had given their written informed consent to take part in the study.

Twelve male subjects (median age: 23 years, range: 18–33 years; median height: 182.5 cm, range: 169–194 cm; median weight: 76 kg, range: 55–100 kg; median body mass index: 22.5 kg/m², range: 19–29 kg/m²) without altitude exposure higher than 1500 m in the last month prior to this study showed a minor tricuspid valve insufficiency found incidentally in the context of this study and were otherwise healthy. Prior to the tests, the subjects received an echocardiogram (ECG). Blood tests (HBG, HCT, RBC, MCH, MCV, PLT) and a 12-channel ECG were performed immediately before the trial. All results were unremarkable.

During the test-period, including the day before, all subjects received the same food that was prepared by the staff restaurant of the Institute of Aviation Medicine. This food was normal German Army diet without any special dietary preparations. During the trials, blood was collected from an indwelling venous cannula (1 3/4 French) in the left forearm without the use of a tourniquet. The blood was centrifuged and the serum was separated and stored at −20°C. The samples were transported on dry ice at a temperature of −40°C and analyzed by enzyme-linked immunosorbent assay (ELISA) (Immundiagnostik, Germany). During the blood collection, the presence or absence of altitude symptoms was documented and rated using the Lake Louise scoring (LLS) system. In accordance with the recommendations by Maggiorini and colleagues, subjects were considered to be affected by altitude sickness in cases where they had shown an LLS of 5 or greater.

PAP measurements were obtained by Doppler echocardiography (vivid i, GE Healthcare) in recumbent position. Color-coded images of tricuspid valve reflux were obtained in the apical four-chamber view and the maximum reflux velocity into the right atrium was measured with continuous wave (CW) Doppler and pressure gradient was calculated by the simplified Bernoulli equation during systole. A measurement session lasted approximately 10 minutes per subject and was conducted four times per night (t1 to t4, respectively t1_4000 to t4_4000). The first measurement (t1/t1_4000) was performed before the subjects entered the chamber (at an altitude of 134 m); the other three measurements were carried out 2, 5, and 11 hours after a simulated altitude of 4000 m had been reached. The subjects were then recompressed. Measurements were always carried out in identical sequence.

Statistical analysis was performed using Spearman’s rank correlation coefficient (Spearman’s ρ) and the χ² test together with Fisher’s exact test. Levels of significance were set at p ≤ 0.05 and p ≤ 0.01.

Results

PAP increased substantially in all subjects during exposure to an altitude of 4000 m. But the most important result is that ADMA was not found to induce this pulmonary hypertension and was therefore not confirmed as a possible trigger of HAPE. Our results support the exact opposite of our original hypothesis. Subjects with a marked increase in ADMA (positive Δ-ADMA) during altitude-induced hypoxia (4000 m)
showed PAP levels below the critical threshold for HAPE (40 mmHg) and were not affected by AMS, whereas subjects with a decrease in ADMA (negative Δ-ADMA) suffered from AMS and had PAP levels above 40 mmHg (Table 1). The higher the increase in PAP, the more severe were the altitude symptoms. As opposed to PAP, Δ-ADMA serum levels were negatively correlated with altitude symptoms. The higher the increase in ADMA at altitude, the milder were the altitude symptoms. The more substantial the decrease in ADMA levels at altitude, more severe were the altitude symptoms.

During the short period of altitude exposure, five subjects (Group 1) were affected by AMS (LLS ≥ 5). They showed a massive increase in PAP > 40 mmHg and, contrary to our hypothesis, a negative Δ-ADMA. However, four subjects had no or only mild AMS (LLS: 0–3) and showed only a minor PAP increase < 40 mmHg, whereas their Δ-ADMA was significantly positive. The three remaining subjects had values in the range of LLS: 3 to 4; PAP levels around 40 mmHg; Δ-ADMA: negative in two subjects and no change in one subject. These results show that the increase in PAP is not caused by an increase in ADMA.

More details are presented in Table 2 showing the absolute values of all participants, but as our study was designed to investigate individual changes at altitude the comparison between the second night (4000 m) and the first night (134 m) is of particular importance. The magnitude of the increase, and the first night (134 m) is of particular importance (Δ-ADMA; Δ-PAP).

These changes are given in Figures 1 and 2 showing Δ-t2, Δ-t3, and Δ-t4, which indicate the differences (t2/t2_4000, t3/t3_4000, and t4/t4_4000). Figure 1 shows Δ-PAP and Figure 2 shows Δ-ADMA levels for Groups 1 and 2. Results for Group 1 (subjects with altitude sickness) are marked in bold and results for Group 2 (subjects without altitude sickness) in italics.

All study participants showed an increase in PAP (Δ > 0) at all time points. The magnitude of the increase, however, varied depending on the group. Group 2 showed a much less noticeable increase in PAP than Group 1 (Figure 1). While Δ-ADMA was negative in Group 1, it was positive in Group 2 (Figure 2).

At t2 (2 h at altitude) we found a significant relationship between Δ-PAP t2 (Spearman’s ρ = 0.30, p ≤ 0.05) and altitude symptoms (LLS). At t3 (5 h at altitude) a significant relationship could be detected between either Δ-PAP t3 (ρ = 0.30, p: n.s.) or Δ-ADMA t3 (ρ = 0.52, p: n.s.) and LLS. At t4 there was a significant relationship between Δ-PAP t4 (ρ = 0.61, p ≤ 0.05) respectively Δ-ADMA t4 (ρ = 0.74, p ≤ 0.01) and LLS.

The analysis of the relationship between Δ-PAP and Δ-ADMA reveals a significant correlation at all time points of measurement (t2: ρ = −0.69, p ≤ 0.05; t3: ρ = −0.79, p ≤ 0.01; t4: ρ = −0.70, p ≤ 0.05). It is interesting to note that this correlation was particularly strong at t1.

These results show that Δ-PAP is positively correlated at t2 and t3 with altitude symptoms expressed by the LLS. In addition, there is an unexpected negative correlation between Δ-PAP and Δ-ADMA. The more pronounced the decrease in ADMA at altitude, the higher is the increase in PAP at the same time point, and vice versa. These findings emphasize the importance of Δ-ADMA and not of the absolute ADMA values. The mean Δ-ADMA (the average increase of ADMA during altitude exposure) of each subject was found to be highly significantly correlated with his altitude symptoms at all time points (mean Δ-ADMA vs LLS t2_4000: ρ = −0.86, p ≤ 0.01; LLS t3_4000: ρ = −0.78, p ≤ 0.01; LLS t4_4000: ρ = −0.76, p ≤ 0.01).

Table 1 Division of the subjects into groups according to the presence (Group 1) or absence (Group 2) of symptoms of altitude sickness as defined by an LLS of ≥ 5 versus 0–3. In Group 1 (subjects with altitude sickness), ADMA decreased and PAP levels exceeded 40 mmHg at an altitude of 4000 m. In Group 2 (subjects without altitude sickness), ADMA increased and PAP levels were lower than 40 mmHg.

<table>
<thead>
<tr>
<th>LLS</th>
<th>n</th>
<th>ADMA ↑</th>
<th>ADMA ↔</th>
<th>ADMA ↓</th>
<th>PAP &lt; 40 mmHg</th>
<th>PAP ≈ 40 mmHg</th>
<th>PAP &gt; 40 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3</td>
<td>n = 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3–4</td>
<td>n = 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 5</td>
<td>n = 5</td>
<td></td>
<td></td>
<td></td>
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</table>

Table 2 Changes of PAP and ADMA at 4000 m of all subjects. Presented are the highest values found during altitude exposure.

<table>
<thead>
<tr>
<th>Changes</th>
<th>Group 1</th>
<th>Indiff. Group</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>at 4000 m</td>
<td>LLS: ≥ 5</td>
<td>LLS: 3–4</td>
<td>LLS: 0–3</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>↑↑↑</td>
<td>↑↑</td>
<td>↑</td>
</tr>
<tr>
<td>46 (+77%)</td>
<td>40 (+74%)</td>
<td>33 (+50%)</td>
<td></td>
</tr>
<tr>
<td>52 (+86%)</td>
<td>43 (+65%)</td>
<td>36 (+38%)</td>
<td></td>
</tr>
<tr>
<td>47 (+81%)</td>
<td>41 (+64%)</td>
<td>33 (+32%)</td>
<td></td>
</tr>
<tr>
<td>53 (+83%)</td>
<td>38 (+37%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65 (+110%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADMA (μmol/l)</td>
<td>↓ ↔</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>0.45 (&lt;44%)</td>
<td>0.30 (&lt;40%)</td>
<td>0.65 (&lt;18%)</td>
<td></td>
</tr>
<tr>
<td>0.45 (&lt;25%)</td>
<td>0.50 (&lt;9%)</td>
<td>0.65 (&lt;63%)</td>
<td></td>
</tr>
<tr>
<td>0.50 (&lt;41%)</td>
<td>0.50 (±0%)</td>
<td>0.80 (&lt;7%)</td>
<td></td>
</tr>
<tr>
<td>0.50 (&lt;38%)</td>
<td></td>
<td>1.10 (&lt;57%)</td>
<td></td>
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<tr>
<td>0.70 (&lt;33%)</td>
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PAP = pulmonary artery pressure during systole; ADMA = asymmetric dimethylarginine; LLS = Lake Louise Score.
Δ-ADMA and AMS

Discussion

Our results did not confirm our hypothesis that an ADMA-induced inhibition of NOS leads to an increase in PAP and to an increased susceptibility to AMS. On the contrary, our tests suggested the exact opposite as described above. The increase in PAP thus may not be caused by an increase in ADMA. Against our assumptions, ADMA was not confirmed as a potential trigger to generate a PAP value of higher than 40 mmHg, which is considered to be the critical threshold for the development of HAPE.

In summary, the reported results show a statistically significant negative correlation of Δ-ADMA and PAP (ρ: −0.74; p ≤ 0.01) and altitude symptoms (ρ: −0.8; p ≤ 0.01). In addition, the measurement of Δ-ADMA under conditions of acute hypoxia (at 4000 m) is also a suitable method for identifying individuals who are likely to develop critical PAP of more than 40 mmHg (ψ: 0.69; p ≤ 0.05) and who are susceptible to AMS (LLS ≥ 5) (ψ: 0.82; p ≤ 0.02). Those at risk can be identified as early as 2 hours after the start of exposure to hypoxia (at an altitude of 4000 m). It is not the absolute ADMA level but rather the change (increase or decrease) in ADMA against the baseline level that plays a key role in this context. In our collective results, the Δ-ADMA value after 2 hours of hypoxia can predict the development of AMS with a sensitivity of 80% and a specificity of 100%. If PAP increases to more than 40 mmHg within 2 hours of exposure, the occurrence of AMS is likely to be expected in 100% of the cases.

In their study on the course of PAP during altitude exposure, Dorrington and colleagues indirectly confirmed that prognostic information can be obtained after such a short period of exposure. Using right-heart catheterization, these authors showed that a maximum PAP level was reached as early as 2 hours after the onset of exposure to hypoxic conditions.

Our results confirmed the findings reported by Song and colleagues. These authors exposed mice to previously lethal conditions—a fraction of oxygen (FO2) of 0.046%—and expected that NOS inhibition (eg, by ADMA) would decrease hypoxic tolerance. Contrary to their original hypothesis, they found that hypoxic tolerance improved greatly and some of the mice that were treated with ADMA even survived. As synthetic NOS inhibitors such as Nω-nitro-l-arginine (l-NNA) also improved hypoxic tolerance, these unexpected results confirmed that it was this NOS-mediated function in the systemic response to...
Table 3 ψ² test results

<table>
<thead>
<tr>
<th>Presence of AMS as defined by</th>
<th>ψ coefficient</th>
<th>Level of significance (Fisher’s exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Decrease in mean Δ-ADMA</td>
<td>LLS ≥ 5</td>
<td>0.690</td>
</tr>
<tr>
<td>(2) Decrease in mean Δ-ADMA</td>
<td>PAP &gt; 40 mmHg</td>
<td>0.690</td>
</tr>
<tr>
<td>(3) PAP &gt; 40 mmHg at t₂</td>
<td>LLS ≥ 5</td>
<td>1.000</td>
</tr>
<tr>
<td>(4) Decrease in ADMA at t₂</td>
<td>LLS ≥ 5</td>
<td>0.816</td>
</tr>
</tbody>
</table>

The mean asymmetric dimethylarginine (ADMA) decrease at 4000 m can predict who will have a Lake Louise Score (LLS) of ≥ 5 (1) and thus will be affected by acute mountain sickness (AMS) or who will develop a pulmonary artery pressure (PAP) of >40 mmHg during a 12-hour exposure to hypoxia (2). If an individual shows a decrease in ADMA serum levels (4) or a PAP of more than 40 mmHg, which is the critical threshold for the development of HAPE (high-altitude pulmonary edema) (3) as early as 2 hours (t₂) after the onset of exposure to altitude conditions at 4000 m, he or she is likely to develop AMS with the stated probability.

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Declaration of Interests

The authors state that they have no conflicts of interest.

References


