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No renal dysfunction or salt and water retention in acute mountain sickness at 4,559 m among young resting males after passive ascent

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Running Head: Renal function and fluid balance in acute mountain sickness

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Abstract

Purpose: This study examined the role and function of the kidney at high altitude in relation to fluid balance and the development of acute mountain sickness (AMS), avoiding confounders that have contributed to conflicting results in previous studies. Methods: We examined 18 healthy male resting volunteers (18 – 40 years) not acclimatized to high altitude while on a controlled diet for 24 h at Lausanne (altitude: 560 m) followed by a period of 44 hours after reaching the Regina Margherita hut (4,559 m) by helicopter. Results: AMS scores peaked after 20 h at 4,559 m. AMS was defined as functional Lake Louise score ≥ 2. There were no significant differences between 10 subjects with and 8 subjects without AMS for urinary flow, fluid balance and weight change. Sodium excretion rate was lower in those with AMS after 24 h at altitude. Microalbuminuria increased at altitude but not differently between the groups. Creatinine clearance was not affected by altitude or AMS, while clearances of sinistrin and para-aminohippuric acid decreased slightly, somewhat more in those without AMS. Plasma concentrations of epinephrine, norepinephrine, atrial natriuretic factor and vasopressin increased while renin activity, angiotensin and aldosterone decreased at altitude. Circulating hormone concentrations did not differ between those with and without AMS. Conclusions: in healthy resting young men flown by helicopter to 4,559 m renal function is not affected by hypoxia except for minor microalbuminuria, high altitude diuresis does not occur, and AMS is not associated with salt and water retention or renal dysfunction.

(248 words)

Keywords: acute mountain sickness, renal function, fluid balance, hypoxia, high altitude

New & Noteworthy

Kidney function remained essentially unaffected and acute mountain sickness (AMS) was not associated with salt and water retention in healthy young men flown to and resting at the Margherita hut (4,559 m) under strictly controlled conditions maintaining water, salt and food intake at pre-exposure levels. Thus, renal dysfunction and fluid retention are not essential factors contributing to the pathophysiology of AMS.

Introduction

Ascent to altitudes above 2,500 m may cause acute mountain sickness (AMS) (6) with headache as the most frequent symptom, often accompanied by nausea and dizziness. AMS typically occurs with a delay of 8–20 hours after arrival at high altitude and resolves spontaneously after 1–2 days of rest. The risk of developing AMS increases with altitude and rate of ascent, in addition to individual susceptibility. Epidemiological studies predict a prevalence of about 60 % in susceptible, non-acclimatized mountaineers ascending rapidly to 4,559 m (44). Salt and water retention and capillary leakage have been suggested to contribute to the predominantly cerebral pathophysiology of AMS (16, 53), thus ascribing a possible pathogenic role to some dysfunction affecting the kidneys.

Studies, however, on the role and function of the kidney at high altitude, particularly in relation to the development of acute mountain sickness (AMS), have yielded conflicting results. While high altitude induced diuresis (34, 48) possibly linked to suppression of aldosterone synthesis (9, 37) and heightened hypoxic peripheral chemoreceptor activity (49) is considered a normal adaptive response in healthy individuals, sodium and water retention is often found in subjects developing AMS (4, 14, 15, 28). Some studies support the notion that volume retention would be a cause

of AMS (14, 24), whereas other data suggest that retention is rather a consequence of more severe hypoxemia observed in AMS (7) or even that glomerular filtration rate is increased in AMS (35). This lack of consensus in the data regarding renal function at high altitude can most likely be attributed to various uncontrolled confounders such as variability of fluid and salt intake, effects of exercise, systemic hemodynamics, endocrine function and differences in the time course of renal excretory changes(50).

Our study aimed to differ from previous investigations in that renal function was studied continuously, at metabolic steady state, under strictly controlled conditions, with gold standard markers of renal function. The confounding effects of exercise, temperature, and variations in sodium and fluid intake were eliminated by the use of a fixed diet in a controlled indoor environment at normal room temperature, and by investigating the subjects confined to bed rest (except for measurements in standing position, eating, urination or defecation) one day before and following a rapid passive ascent to high altitude. The effect of the circadian rhythm of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) was taken into account by studying the subjects at low and high altitude following an identical daily time schedule.

In this controlled setting, sufficient to cause AMS in roughly 60 % of subjects, we continuously examined renal function and repeatedly measured plasma concentrations of hormones known to regulate fluid and electrolyte homeostasis over 44 h after passive ascent to 4,559 m. Results were compared in those with and without AMS in order to resolve the question whether changes in renal function and fluid balance, if any, are the cause or the consequence of AMS. The study protocol was approved the Ethics Committee of the Centre Hospitalier Universitaire Vaudois, Lausanne, and the study subjects gave written informed consent.

This study was performed 1996 and its main results were presented at two congresses and published as abstracts in 1998 (5, 18). Controversial results of later studies cited above on fluid balance in AMS prompted us to submit a full report of this study, because negative results obtained in a carefully controlled setting are valuable in any field of research and help to build up a more complete data base, which may inform future investigators and limit unnecessary further work along the same lines.

Material and Methods

Run in phase

Healthy male volunteers between 18 and 40 years of age were recruited from hospital staff and medical students. Four subjects were occasional mountaineers, none reported particular susceptibility to AMS or HACE. All but 2 subjects were involved in regular physical activity of at least moderate intensity, on average 3.4 h ± 7.0 per week. During the 5 preceding days and during the whole investigation, the volunteers ingested a standardized controlled diet (2,500-2,700 kcal/day i.e. ~34-38 $kcal.kg^{-1}.day^{-1}$; 150 mmol/day of Na^+ and 100 mmol/day of K^+). They were instructed to eat only and completely the food prepared for them, and to drink a standardized amount of mineral water (2.2 l/day Henniez® mineral water; Ca^{++} 110 mg/l, Mg^{++} 18 mg/l, Na^+ 6 mg/l, K^+ 1.2 mg/l, HCO_3^- 394 mg/l, nitrates 18 mg/l, PO_4^{--} 13 mg/l, Cl^- 10 mg/l). They had to refrain from sport activity, coffee, tea or cola consumption, smoking cigarettes or ingesting any drugs for 5 days before starting the metabolic

preparation until study completion. If necessary, acetaminophen could be administered under supervision by the investigators

Study period 1 (low altitude)

Investigations were performed according to a standard clock time schedule, so as not to be confounded by the physiological circadian rhythms, well known to affect metabolism(21). At low altitude (period 1), the subjects came fasting to the hospital at 7:00, were weighed, placed comfortably in a supine position, their vital signs were measured, indwelling intravenous catheters (Venflon®; 18 gauge) were inserted in a vein of each forearm, one for sinistrin and para-aminohippuric acid (PAH) administration and the other for blood sampling. Thereafter breakfast was served. Around 10:00 and after a 1-hour bed rest, blood samples for biochemical variables and blank sinistrin and PAH levels were drawn. A bolus of sinistrin and PAH was infused over 5 minutes and a constant rate infusion started (syringe pump Perfusor®, Braun, Melsungen Germany) and maintained for 24 hours. The contralateral catheter was kept open by flushing with 0.9% saline after each blood drawing. The amount of 450 ml of blood withdrawn over 3 days was precisely replaced by an equivalent amount of saline and resulted in an excess replacement of about 11 mmol sodium per day in addition to 150 mmol sodium intake per day by standardized food. Hematocrit (figure S1a) shows no progressive effects attributable to repeated blood sampling. AMS scoring (39) was performed iteratively (see below). The meals (diet) were served as usual. Twenty-four hours later, the subjects left the hospital continuing the metabolic diet for at least one day and up to 3 days (according to weather conditions) until being brought to high altitude (study period 2).

Study period 2 (high altitude)

Subjects came to the hospital on day 1 at 6:00 where indwelling intravenous catheters (Venflon®; 18 gauge) were inserted in a vein of each forearm. They had the standard study breakfast served and then were transported by train or car to Sion, where they arrived at the airport around 8:00. They were flown by helicopter to the Regina Margherita hut (alt. 4,559 m). Immediately after arrival, the subjects walked about 20 m to the hut and climbed slowly 4 flights of stairs to the study room where they were placed comfortably on a bed. One hour later (around 10:00), the investigation proceeded as mentioned above (same schedule as period 1) and continued according to the same schedule up to 45 h after arrival at high altitude. At both low and high altitude, the volunteers went walking to the toilets and ate sitting at a table. The average room temperature was 17+2 (6 AM), 19 + 2 (noon) and 21 + 3 °C (8 PM). Corresponding values at low altitude were 25, 26 and 26 °C respectively.

Measurements

During each investigation day, sinistrin and PAH were determined at 0 (pre-dose), 1, 4, 8, 12, 16, 20 and 24 hour; biochemistry variables (urea, creatinine, Na^+, K^+, Cl^-, PO_4^{--}, Ca^{++}, uric acid, albumin, total protein, trace lithium, hematocrit (Ht)) were measured at 0 (pre-dose), 2 , 4 , 8, 12, 16, 20, 24 h, and additionally at 28, 32, 36, 40, 44 h at high altitude. Blood for hormones (ANF, PRA, Ang II, AVP, Aldo) was collected at 0 (pre-dose), 4, 8, 16, 20, 32, 44 h into pre-chilled tubes, immediately centrifuged and quick frozen with liquid nitrogen. The urine collections were made at the following intervals : -24-0; 0-1; 1-4; 4-8; 8-12; 12-16, 16-20, 20-24 h, and 24-28, 28-32, 32-36, 36-40, 40-44 h at high altitude for measuring sinistrin, PAH, lithium, aldosterone, catecholamines, creatinine, urea, Na^+, K^+, Cl^-, PO_4^{--}, Ca^{++}. Blood gases

were measured once at low altitude and repeatedly at high altitude (variable schedule) in capillary blood taken from a hyperemic ear lobe. Body weight was assessed every 4 hours immediately after bladder emptying. Body temperature, blood pressure and heart rate were assessed in both the supine position and after 2 minutes standing at 0, 4, 8, 12, 20, 24, 28, 32, 36, 44 h. AMS was assessed at 2, 5, 9, 21, 25, 29, 33, 45 h by the total (questionnaire plus examination) and functional Lake Louise score(39). Subjects were classified as having AMS when severity of symptoms would have impaired activity (functional LL score ≥ 2) at least once.

Blood pressure measurements were obtained using a semi-automatic Mio-Star Fitness sphygmomanometer (IKS # 50'681) (calibrated at 21°C and certified by Zewa AG, laboratory accredited by the Swiss Calibration Service). Body weight was measured on a digital medical scale (Soehne Digital S) at low altitude and on a mechanical roman scale at high altitude.

Syringe pumps Perfusor® (Braun, Melsungen Germany) were used for perfusing sinistrin and PAH. All devices were calibrated before their use. Pumps and centrifuge at high altitude were powered either directly by the hut generator (daytime), or by an electrical battery (energy valise Oerlikon-Plus) at night.

Laboratory methods

The methods used for measuring Angiotensin II (Ang II ; radioimmunoassay using a monoclonal antibody after solid phase extraction on phenylsilylsilica (29), angiotensin I (Ang I) (radioimmunoassay) (30), plasma renin activity (PRA ; radioimmunological microassay based on trapping of generated angiotensin I with selected high affinity

antibodies) (31), vasopressin (AVP) (radioimmunoassay) (10), atrial natriuretic peptide (ANP ; radioimmunoassay after solid phase extraction on phenylsilylsilica) (32), aldosterone (33) and catecholamines (36), all developed in the Laboratory of Hypertension at CHUV, have been previously published.

Lithium (electrothermal atomic absorption spectrophotometry) (25), PAH, N-methylnicotinamide (HPLC) and sinistrin (high-performance liquid chromatography (HPLC)) (13, 47), were determined in the laboratory of the Division of Clinical Pharmacology; osmolality was measured as the freezing point depression with a Knauer Automatic Osmometer (Berlin, Germany), hematocrit with a dedicated micro-centrifuge, and urinalysis by dry reagents strips (Multistix Bayer) read on a Clinitek 100 (Bayer) apparatus.

Measurements of classical hematology and chemistry variables were made by the Laboratoire d'Hématologie (LCH) and the Laboratoire de Chimie Clinique (LCC) at CHUV using automatized techniques.

Data analysis

GFR and ERPF were determined as the measure of sinistrin (CL_{SIN}) and PAH (CL_{PAH}) clearances respectively. Both renal ($CL_R = U * V/P$) and systemic clearances ($CL_S = R_{in}/C_{SS}$) were calculated (with C_{SS} representing the steady state concentration, and R_{in} the infusion rate). Fractional excretions were calculated as the clearance of substance x divided by the clearance of sinistrin. The filtration fraction was calculated as the ratio of sinistrin over PAH clearances (GFR/ERPF). Fractional Na^+ reabsorption in the proximal and the post-proximal tubule was estimated as 1-

FELi and ((FE_{Li}-FE_{Na})/FE_{Li}), respectively. Absolute proximal reabsorption of Na^+ was estimated as (CL_{SIN}-CL_{Li})* Na_P (with Na_P representing plasma sodium).

The following assumptions were made: first, relative, not absolute, changes are important and the variability of most observed parameters is closer from a log-normal rather than a normal distribution; second, the subjects are studied at steady state (regarding metabolic and sodium balance), therefore no drift or carryover effect are expected; and third, a circadian cycle is present for most variables and time (hour, not day) has to be taken into account.

Statistical analysis

The statistical evaluation of all study variables was performed using univariate ANOVA for repeated measures, the factors being subject (random effect factor), day (low vs high altitude), hour (circadian rhythm), and presence or absence of AMS (fixed effects). We tested the global effect of altitude (day effect period 2 vs period 1), the circadian cycle (hour), the effect of altitude according to hour (interaction day x hour), the global AMS effect (AMS), the AMS effect at high altitude (AMS x day) and the AMS effect at high altitude according to time (AMS x day x hour). The ANOVA was applied on log transformed data (see assumption 1 above). The presence or absence of AMS was defined by a criterion score used in previous studies (4, 7). Under the protection of the overall significance of interactions involving hour, we carried out post-hoc means comparisons between corresponding times using Fisher's least significant difference tests. We performed all statistical evaluations using the general linear module of the Systat software (version 7, SPSS Corporation), while using the Microsoft Excel and Access software (version 7.0) for data management.

No Bonferroni correction- for the significance levels was applied to account for the number of variables and factors tested, considering the exploratory rather than confirmatory use of statistical tests performed on the study results. There were few missing data: none for clinical scores, 1% for vital signs, 3% for clearance and urinary excretion values (due to subjects' difficulties to void or to missing blood samples), 5% for hormone determinations and 7% for biochemistry samples (due to difficulties in blood sampling). The missing values were accounted for during the statistical analyses by adjustment of the degrees of freedom associated to the factors tested by ANOVA, a correction automatically implemented in the SYSTAT software. Considering the essentially exploratory nature of this study, no formal power calculation was performed, and we simply included the maximum number of subjects that we could reasonably include considering the constraints of the investigation due to the assigned time slot and space at high altitude.

Results

Clinical Data

Eighteen male volunteers participated, 8 of whom had no or minimal AMS (AMS- group) and 10 developed AMS that would have affected their physical performance (AMS+ group). The Lake Louise scores of both groups are shown in figure 1 and table 1. There were no significant differences between groups regarding age, height and body mass index, but absolute body weight was slightly lower in AMS+ group (table 2).

Table 3 shows the results of blood gas analysis performed in capillary blood from the hyperemic arterialized ear lobe. There was a significant effect of altitude on all parameters, with lower values for oxygen saturation (SaO_2), partial pressure (PaO_2), carbon dioxide pressure ($PaCO_2$), and base excess (BE) with an increase in pH. The AMS+ group had lower blood oxygenation values vs. the AMS- group, in line with a lesser degree of respiratory alkalosis and higher $PaCO_2$, however not reaching statistical significance. SaO_2, PaO_2 increased and $PaCO_2$ further decreased over time at high altitude, indicating ventilatory acclimatization in both groups (41), while pH remained unchanged and BE decreased over time.

In the AMS+ group, the mean Lake Louise score after 4 hours (figure 1) nearly reached 5, a value indicating clinically relevant AMS at this altitude, and it peaked after 20 h following the first night at high altitude. It recovered somewhat during the second day and rose again above 5 after the second night. The mean Lake Louise score of the AMS- group always remained at or below 3. Supine blood pressure (figure 1) rose equally in both groups by about 10 mmHg at high altitude.

Due to symptoms of AMS, 4 subjects of the AMS+ group could not comply fully with the study protocol. Deviations from the protocol are detailed in the online supplement (table S1). Briefly, during the first 16 h at high altitude, three subjects had a reduced food intake by 12, 20 and 35 %, one subject had a diminished water intake by 300 ml and one had a lower water intake by 400 ml and lost 450 ml through vomiting. In two of these subjects, the deviations increased after 16 h, such that one of them had to

be treated with prednisone and nifedipine for severe AMS and possible early high altitude pulmonary edema after 33 h at 4,559 m. Headache was treated in 3 subjects with acetaminophen.

Fluid balance and weight changes (figure 2) were virtually identical between both groups. Weight changes mirrored the fluctuations of fluid balance with modest increases during the day - most prominent on day 2 at high altitude - and return to baseline overnight.

Renal function

Urinary flow rate (figure 3) and cumulative urine volume (figure S1b) were identical between both groups and not significantly different from values observed at low altitude. The transient increase of urine flow 1 h after arrival at high altitude in both groups is noteworthy. Sodium excretion rate (figure 3) differed between groups at 24 – 36 h at high altitude, but the cumulative sodium excretion (figure S2) remained similar between groups. It was, however, decreased by about 15% at high altitude.

Creatinine clearance (figure S3) was neither affected by altitude nor by AMS. Clearance of sinistrin (figure 4), an exogenously administered compound excreted only by glomerular filtration and thus a better marker of GFR than creatinine, decreased slightly at high altitude. Renal blood flow as assessed by para-amino hippurate (PAH) clearance (figure 4) did also slightly decrease at high altitude, and both interestingly more so in subjects not suffering from AMS. The filtration fraction remained unchanged except for an initial slight decrease at high altitude (figure S4). The fractional excretion of lithium, a marker of proximal tubular sodium handling,

decreased at high altitude (figure S5). Microalbuminuria increased at high altitude, but not differently between those with and without AMS (figure S6 and S7).

Plasma concentrations of hormones relevant to renal function and fluid balance

Epinephrine and norepinephrine concentrations in plasma (figure 5) and their urinary excretion rates (data not shown) were not different between groups at both altitudes, but were overall significantly higher at high altitude. Plasma renin activity (figure 6), angiotensin I and II (figures S8 and S9), and aldosterone (figure 6) were not different between groups at both altitudes, but significantly lower at any time point at high altitude vs. the comparable time of the day at low altitude. Atrial natriuretic factor (ANF) (figure 7) was not different between groups at both altitudes, but was significantly higher at any time point at high altitude vs. the comparable time of the day at low altitude. Vasopressin (figure 7) was not different between groups, but was overall significantly higher at high altitude.

Discussion

Our study demonstrates that renal function remains mostly unaffected by a 2-day acute exposure to 4,559 m and independent from AMS occurrence, in controlled conditions that minimize major confounding factors of previous field studies able to alter renal function independently of hypoxia. These include avoidance of physical exercise, provision of an equivalent steady state diet of food, salt and fluid intake at low altitude and during the altitude exposure, and control of diurnal temperature variation. Furthermore, we demonstrate that the development of AMS with a typical

incidence, time course and hypoxemia for this location is neither preceded nor followed by fluid retention.

Renal function and hormonal responses

Standard measurements of renal function (renal blood flow, glomerular clearances and tubular function) were either unaltered or only minimally affected by hypoxia and not different at any time between those with and without development of AMS. As others have shown, we also found an increase in microalbuminuria, which must be a consequence of hypoxia and not of exercise, since the latter factor was absent in our study (38, 51). Whether this reflects increased glomerular permeability or a reduction in tubular reabsorption remains an open question. Proximal tubular function as assessed by lithium clearance was slightly increased at high altitude. This suggests a contribution of proximal sodium reabsorption to the modest sodium and water retention observed at high altitude. However, a higher lithium clearance might have been expected due to the known reduction of proximal bicarbonate reabsorption in response to the respiratory alkalosis induced by high altitude hypoxia.

The increase of catecholamines and decrease of renin, angiotensin I and II, and aldosterone in plasma are in accordance with the well-established hypoxic sympathetic stimulation (4, 11, 17) and suppression of the renin-aldosterone system (4, 9, 34, 37). The unequivocal increase of plasma ANF and vasopressin suggest that conflicting data of previous investigations can be attributed to confounding factors, which were eliminated in this study. Furthermore, we cannot confirm earlier reports

obtained in less controlled studies indicating higher catecholamines, aldosterone, vasopressin, and higher ANF in plasma of subjects with AMS compared to those without AMS (3, 4, 24, 28). This lack of any significant difference of all hormonal data between the AMS+ and AMS- groups is compatible with the lack of differences of most renal functional data between these groups.

Acute mountain sickness and fluid balance

Defining AMS as a functional Lake Louise score ≥ 2 results in a cut-off value for the "old" Lake Louise score obtained by questionnaire of 5, a value used as reference standard for indicating clinically relevant AMS in a recent meta-analysis (27). The time course and incidence of AMS (figure 1) are compatible with data obtained in other investigations at the same location involving active ascent, unrestricted mobility at 4,559 m and no tight control of food and fluid intake. An epidemiologic study calculated, depending on the degree of susceptibility for AMS, a prevalence of 32 – 60 % for mountaineers ascending in 1-3 days to this altitude (44), and various studies showed similar maximum AMS scores on day 2 at high altitude (4, 7, 19). Furthermore AMS was associated with more pronounced hypoxemia (table 2), which is usually found in subjects with AMS at the Margherita hut (7, 19, 20).

Based on clinical appearance, blood gas analysis and AMS prevalence, which are all typical for AMS in active mountaineers at the Margherita hut, we conclude, that our subjects had the normal incidence and magnitude of AMS despite being physically inactive during ascent and stay at the Margherita hut. Three lines of evidence support our notion further: 1) rigorous bed rest vs ambulatory exposure at normobaric hypoxia simulating ambient PO_2 of and altitude of 4,000 m did not show differences in AMS scores during the first two days (12). 2) the total AMS scores and the scores of each symptom of the Lake Louise score and the AMS-C score (8) were almost

identical between mountaineers after one night at the Margherita hut and healthy volunteers after one night in a normobaric chamber with comparable ambient PO_2 (46). 3) Although there was a small study suggesting that exercise exacerbates AMS (40), three further studies involving more subjects showed that an intensity typical for hiking in the mountains has no effect on prevalence and severity of AMS (26, 42, 45)

Fluid balance and body weight changes (figure 2) did not differ between the groups with and without AMS. Body weight increased similarly in both groups during the day, more so during the second day at high altitude, and it returned to baseline overnight. Urine flow (figure 3) was also identical between both groups. Moreover, it did not increase at high altitude and thus failed to demonstrate “high altitude diuresis”, except at 1 hour after arrival at 4,559 m in both groups. This short-lived peak is most probably explained by stress/excitement and temporary cold exposure during the 30 min helicopter flight and short transfer over about 20 m to the hut. The transiently high plasma epinephrine values (figure 5) are compatible with this hypothesis of heightened sympathetic activity, leading to a short-lived episode of pressure natriuresis.

Sodium excretion rate (figure 3) was slightly lower in the AMS+ group on the second day at high altitude, while it was identical between groups during the first 20 h, when AMS developed and became most prominent. The difference on the second day is probably a consequence of the cumulative effects of four subjects with AMS not being able to fully maintain their controlled water and food intake, two of whom also having losses through vomiting (table S1). Cumulative sodium excretion (figure S2) was identical between groups during the first 20 h at high altitude and tended to be lower in the AMS+ group only on day 2 at 4,559 m. These data demonstrate, that

AMS may reduce fluid and sodium intake and lead to decreased excretion as a consequence of the gastrointestinal symptoms of AMS – an observation that might be relevant for explaining fluid retention in AMS in uncontrolled studies. Compared to the day at low altitude, overall Na^+ excretion was reduced in both groups by about 15 %, which might be explained by hormonal changes with higher catecholamine concentrations contributing to slightly higher systemic blood pressure (figure 1), while decreased plasma aldosterone and increased vasopressin tended to override the effects of slightly increased ANF.

Limitations

Eliminating as many confounders as possible for a well-controlled study on fluid balance and renal function in AMS has the trade-off of deviating from the normal setting of mountaineering or trekking particularly with regard to physical activity in order to isolate the role of one factor. Furthermore, the logistic complexity and the costs limited the number of subjects and careful monitoring of many parameters involving repeated blood sampling. As discussed before, there is enough evidence to conclude that our subjects, despite being inactive, experienced AMS not differently than active mountaineers at this altitude. Since we could not include women in the study because of the uncontrollable influences of the menstrual cycle on fluid balance, our findings apply only to men and particularly to those between 20 and 40 years of age, although AMS susceptibility does not differ appreciably between the sexes and across adult age groups. Considering the number of variables tested, we cannot exclude that some significant differences are produced only by chance, particularly since we did not apply any Bonferroni corrections. Still the number of significant findings (clearly exceeding 5% of all tests), and the consistent picture they

depict leaves little doubt about a reliable pattern of the altitude-induced changes reported in physiological parameters.

On the other hand, one should also consider that, due to the relatively low number of subjects, we cannot exclude a statistical type II error, i.e. that statistically significant differences would appear, e.g. regarding an effect of altitude or AMS on renal parameters, if a much larger number of subjects were examined. With larger numbers, small differences may become significant and one needs to distinguish in these cases between statistical significance and clinical relevance. It should also be noted that several less well controlled studies reporting fluid retention in AMS investigated small groups around 20 subjects as well. Although we replaced the total volume of 450 ml blood sampled over 3 days with isotonic saline, we cannot exclude unknown effects through sampling. In any case, this treatment was identical between groups and should not account for potential differences. We did not see a significant decrease of hemoglobin concentration during the stay at high altitude to which 300 ml of blood sampling during this time might have contributed. Unfortunately, we have no data on hemoglobin over the first two days in the Margherita hut from people that match the activity pattern of our subjects for comparison.

Conclusions

Within the limitations noted above, kidney function remained essentially unaffected during 2 days at 4,559 m in healthy young men adhering to a controlled diet, fixed salt and water intake and with avoidance of exercise after ascent by helicopter. AMS occurred in half of them and was not associated with salt and water retention. These

results support the concept of a predominantly cerebral origin of AMS (53), which may involve activation of the trigemino-vascular system (1, 2, 43) by intermittently increased intracranial pressure (22, 23) with resultant increased permeability of the blood brain barrier (1) or increased vascular pressure due to augmented cerebral blood flow in hypoxia and possibly venous outflow limitation (52). In summary, this study demonstrates that renal dysfunction and fluid retention are not essential factors contributing to the pathophysiology of AMS. Whether salt and water retention or losses will respectively aggravate or ameliorate AMS remain to be examined.

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Legends to Figures

Figure 1:

Upper panel: Lake Louise Score, mean values ± SD. Overall effects: p< 0.0001 for hour, day, and day x hour, p < 0.05 for AMS, p = 0.001 for AMS x day and p < 0.005 for AMS x day x hour. Post-hoc comparisons: *** p <0.001 vs all values at low altitude at corresponding times; ++ p < 0.01 and +++ p < 0.001 between AMS groups at corresponding times.

Lower panel: supine systolic blood pressure (mmHg), mean values ± SD. Overall effects: p < 0.01 for hours, p < 0.025 for day, no significant effects for AMS. Post-hoc comparisons: * p < 0.5 vs values at low altitude at all corresponding times.

Univariate ANOVA for repeated measures and evaluation of global effects of altitude (day), circadian cycle (hour) and (AMS) and interactions in 8 subjects without AMS

694 and 10 subjects with AMS. Post-hoc comparisons between corresponding times
695 using Fisher's least significant difference test when overall significance of interactions
696 involving hour was present.

697

698 **Figure 2:**

699 Upper panel: fluid balance in ml, mean values ± SD. Overall effects: $p < 0.00001$ for
700 hour and for hour x day, no significant effects for AMS. Post-hoc comparisons: ** $p <$
701 0.01, *** $p < 0.001$ vs low altitude at corresponding time.

702 Lower panel: weight change in kg, mean values ± SD. Overall effects: $p < 0.00001$
703 for hours and for hour x day, no significant effects for AMS. Post-hoc comparisons: **
704 $p < 0.01$, *** $p < 0.001$ vs low altitude at corresponding time.

705 Univariate ANOVA for repeated measures and evaluation of global effects of altitude
706 (day), circadian cycle (hour) and (AMS) and interactions in 8 subjects without AMS
707 and 10 subjects with AMS. Post-hoc comparisons between corresponding times
708 using Fisher's least significant difference test when overall significance of interactions
709 involving hour was present.

710

711 **Figure 3:**

712 Upper panel: urinary flow rate (ml/h), mean values ± SD. Overall effects: $p < 0.0001$
713 for hour, $p < 0.00001$ for day x hours, no significant effects day and AMS nor any
714 interactions. Post-hoc comparisons: * $p < 0.05$, *** $p < 0.001$ vs low altitude at
715 corresponding time.

Lower panel: sodium excretion rate (mmol/h), mean values ± SD. Overall effects: $p < 0.00001$ for hour, $p < 0.00001$ for hour x day, $p < 0.025$ for AMS x day x hour. Post-hoc comparisons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs low altitude at corresponding time; + $p < 0.05$, ++ $p < 0.01$ and +++ $p < 0.001$ between groups at corresponding time.

Univariate ANOVA for repeated measures and evaluation of global effects of altitude (day), circadian cycle (hour) and (AMS) and interactions in 8 subjects without AMS and 10 subjects with AMS. Post-hoc comparisons between corresponding times using Fisher's least significant difference test when overall significance of interactions involving hour was present.

Figure 4:

Upper panel: sinistrin clearance per m^2 BSA in ml/min (mean values ± SD). Overall effects: $p < 0001$ for hour, and hour x day, $p < 0.005$ AMS x day. Post-hoc comparisons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs low altitude at corresponding time.

Lower panel: PAH clearance per m^2 BSA in ml/min (mean values ± SD). Overall effects: $p < 0.0001$ for hour and day x hour, $p < 0.005$ for day, no significant effects for AMS. Post-hoc comparisons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs low altitude at corresponding time.

Univariate ANOVA for repeated measures and evaluation of global effects of altitude (day), circadian cycle (hour) and (AMS) and interactions in 8 subjects without AMS and 10 subjects with AMS. Post-hoc comparisons between corresponding times

using Fisher's least significant difference test when overall significance of interactions involving hour was present.

Figure 5:

Upper panel: plasma epinephrine in (mean values ± SD). Overall effects: $p < 0.025$ for hour, $p < 0.0001$ for day, no significant effect of AMS. Post-hoc comparisons: *** $p < 0.001$ for values vs low altitude at all corresponding times.

Lower panel: plasma norepinephrine (mean values ± SD). Overall effects: $p < 0.01$ for hour, $p < 0.0001$ for day, no significant effect of AMS. Post-hoc comparisons: *** $p < 0.001$ for values vs low altitude at all corresponding times.

Univariate ANOVA for repeated measures and evaluation of global effects of altitude (day), circadian cycle (hour) and (AMS) and interactions in 8 subjects without AMS and 10 subjects with AMS. Post-hoc comparisons between corresponding times using Fisher's least significant difference test when overall significance of interactions involving hour was present.

Figure 6:

Upper panel: plasma renin activity (mean values ± SD). Overall effects: $p < 0.001$ for day, $p < 0.005$ for hour x day, no significant effect of AMS. Post-hoc comparisons: *** $p < 0.001$ for values vs low altitude at all corresponding times.

Lower panel: plasma aldosterone (mean values ± SD). Overall effects: $p < 0.0001$ for hour, $p < 0.0001$ for day, $p < 0.0001$ for hour x day, no significant effect of AMS.

762 Post-hoc comparisons: * p < 0.05, ** p < 0.01, *** p < 0.001 vs low altitude at
763 corresponding time.

764 Univariate ANOVA for repeated measures and evaluation of global effects of altitude
765 (day), circadian cycle (hour) and (AMS) and interactions in 8 subjects without AMS
766 and 10 subjects with AMS. Post-hoc comparisons between corresponding times
767 using Fisher's least significant difference test when overall significance of interactions
768 involving hour was present.

769

770 **Figure 7:**

771 Upper panel: plasma atrial natriuretic factor (ANF) (mean values ± SD). Overall
772 effects: p < 0.001 for day, p < 0.0001 for hour x day, no significant effect of AMS.
773 Post-hoc comparisons: * p < 0.05, ** p < 0.01, *** p <0.001 vs low altitude at
774 corresponding time.

775 Lower panel: plasma vasopressin (mean values ± SD). Overall effects: p< 0.01 for
776 hour, p < 0.001 for hour x day, no significant effect of AMS. Post-hoc comparisons:
777 *** p <0.001 for values vs low altitude at all corresponding times.

778 Univariate ANOVA for repeated measures and evaluation of global effects of altitude
779 (day), circadian cycle (hour) and (AMS) and interactions in 8 subjects without AMS
780 and 10 subjects with AMS. Post-hoc comparisons between corresponding times
781 using Fisher's least significant difference test when overall significance of interactions
782 involving hour was present.

783

784

785

Links to Supplemental Material

Figure S1a: https://figshare.com/s/5ed25c088a9b31339198
https://doi.org/10.6084/m9.figshare.13214807
Figure S1b: https://figshare.com/s/8e558238e94c634fef79
https://doi.org/10.6084/m9.figshare.12302723
Figure S2: https://figshare.com/s/6601951f2ce7751ed516
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Figure S5: https://figshare.com/s/67e4d737e381f9d3027f
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Complete Material: https://figshare.com/s/a875b1d13590afbaf184
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Figure 1

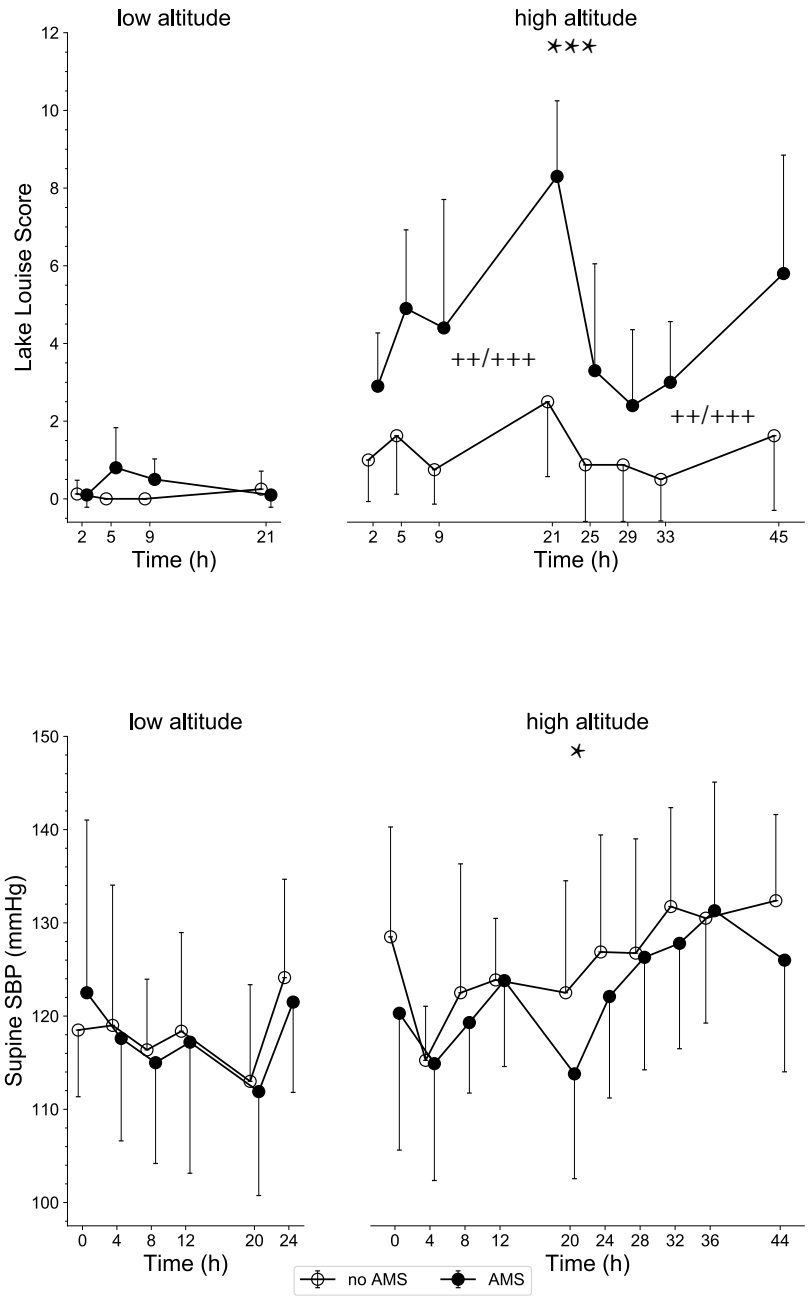
low altitude
high altitude
***
Lake Louise Score
12
10
8
6
4
2
0
++/+++
++/+++
2 5 9 21
Time (h)
2 5 9 21 25 29 33 45
Time (h)
low altitude
high altitude
*
Supine SBP (mmHg)
150
140
130
120
110
100
0 4 8 12 20 24
Time (h)
0 4 8 12 20 24 28 32 36 44
Time (h)
no AMS
AMS

Figure 2

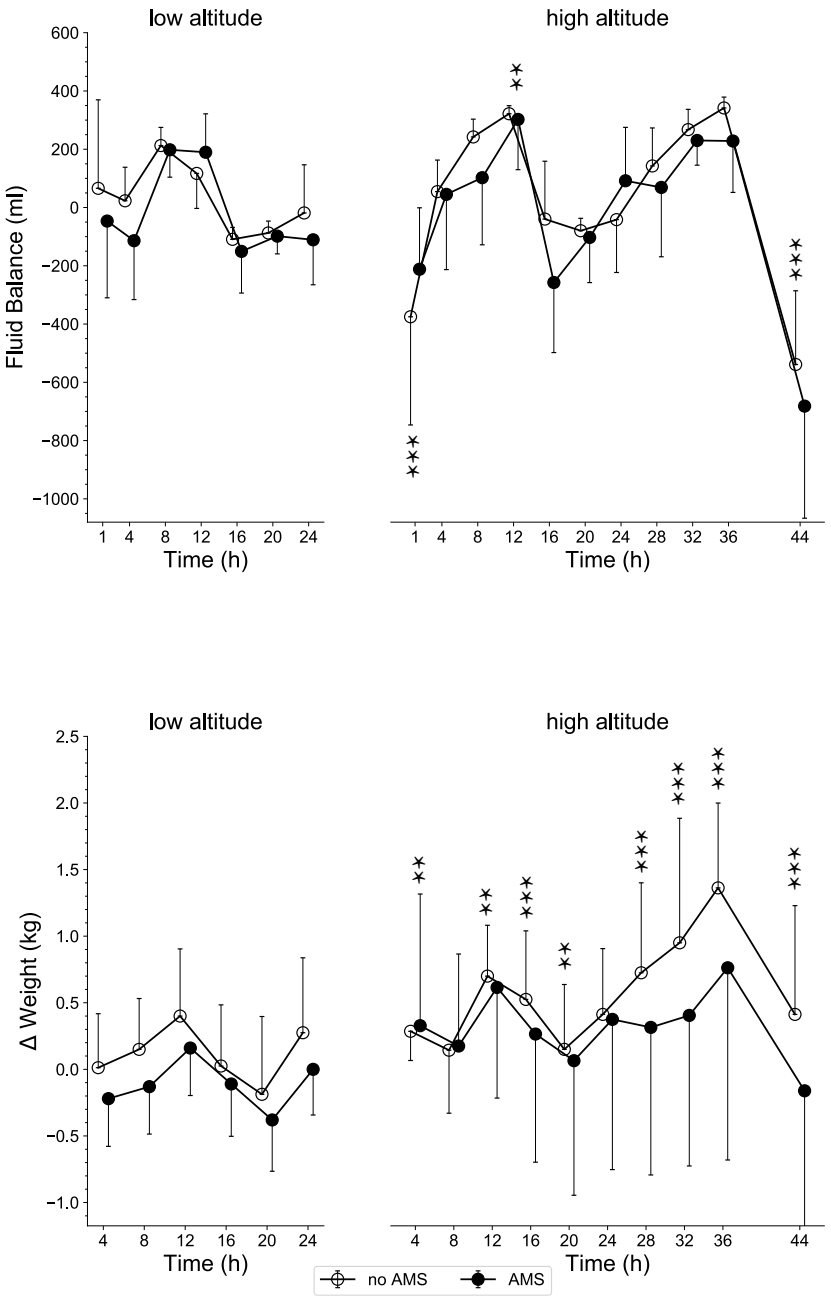
low altitude
high altitude
Fluid Balance (ml)
600
400
200
0
−200
−400
−600
−800
−1000
1 4 8 12 16 20 24
1 4 8 12 16 20 24 28 32 36 44
Time (h)
low altitude
high altitude
Δ Weight (kg)
2.5
2.0
1.5
1.0
0.5
0.0
−0.5
−1.0
4 8 12 16 20 24
4 8 12 16 20 24 28 32 36 44
Time (h)
no AMS
AMS

Figure 3

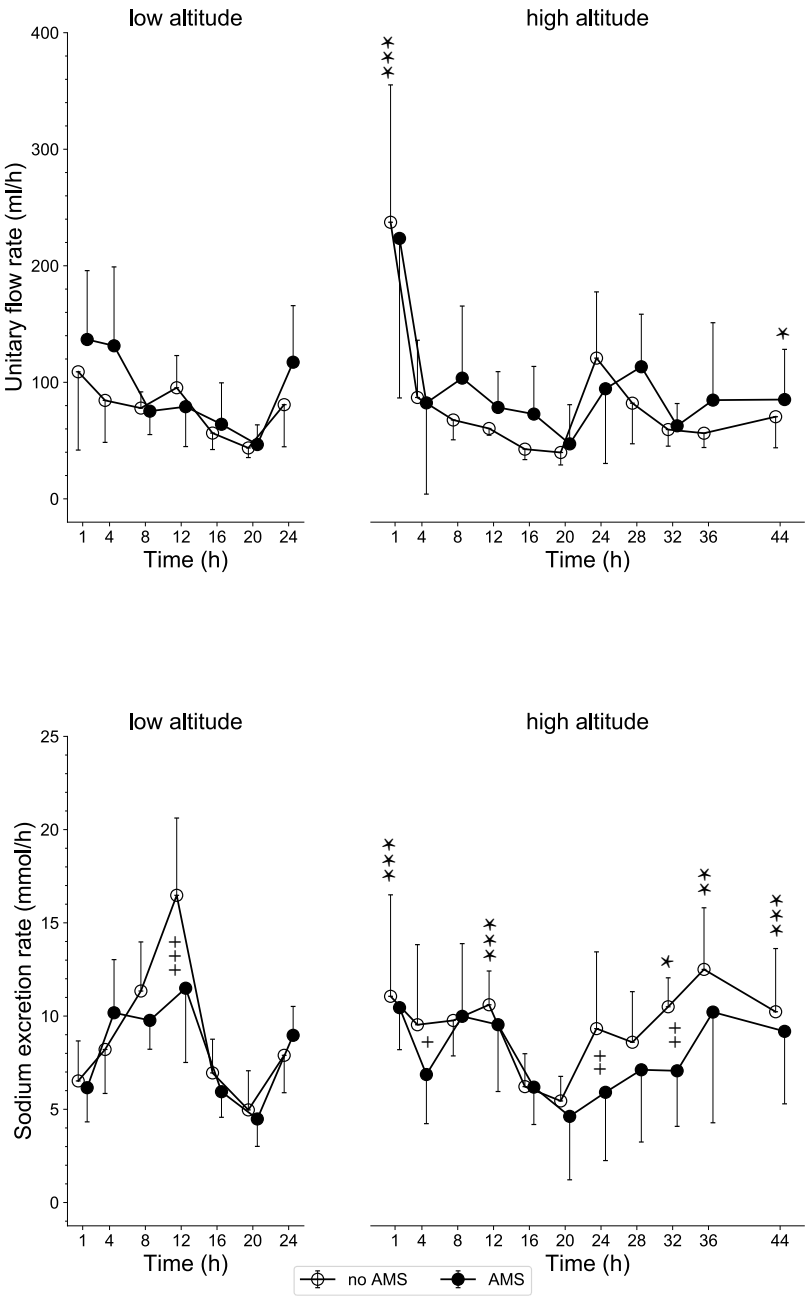
low altitude
high altitude
Unitary flow rate (ml/h)
400
300
200
100
0
1 4 8 12 16 20 24
Time (h)
1 4 8 12 16 20 24 28 32 36 44
Time (h)
low altitude
high altitude
Sodium excretion rate (mmol/h)
25
20
15
10
5
0
1 4 8 12 16 20 24
Time (h)
1 4 8 12 16 20 24 28 32 36 44
Time (h)
no AMS
AMS

Figure 4

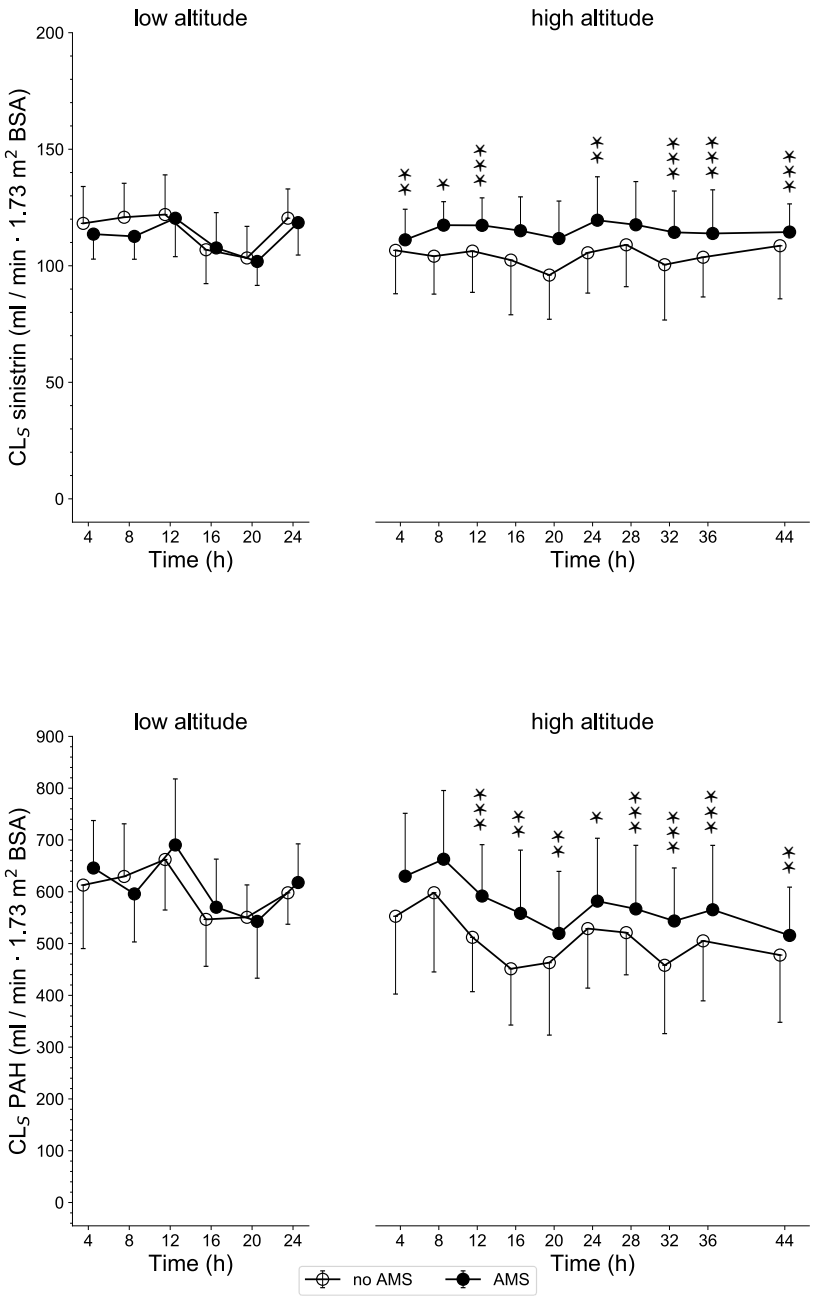
low altitude
high altitude
CLS sinistrin (ml / min · 1.73 m2 BSA)
CLS PAH (ml / min · 1.73 m2 BSA)
Time (h)
no AMS
AMS

Figure 5

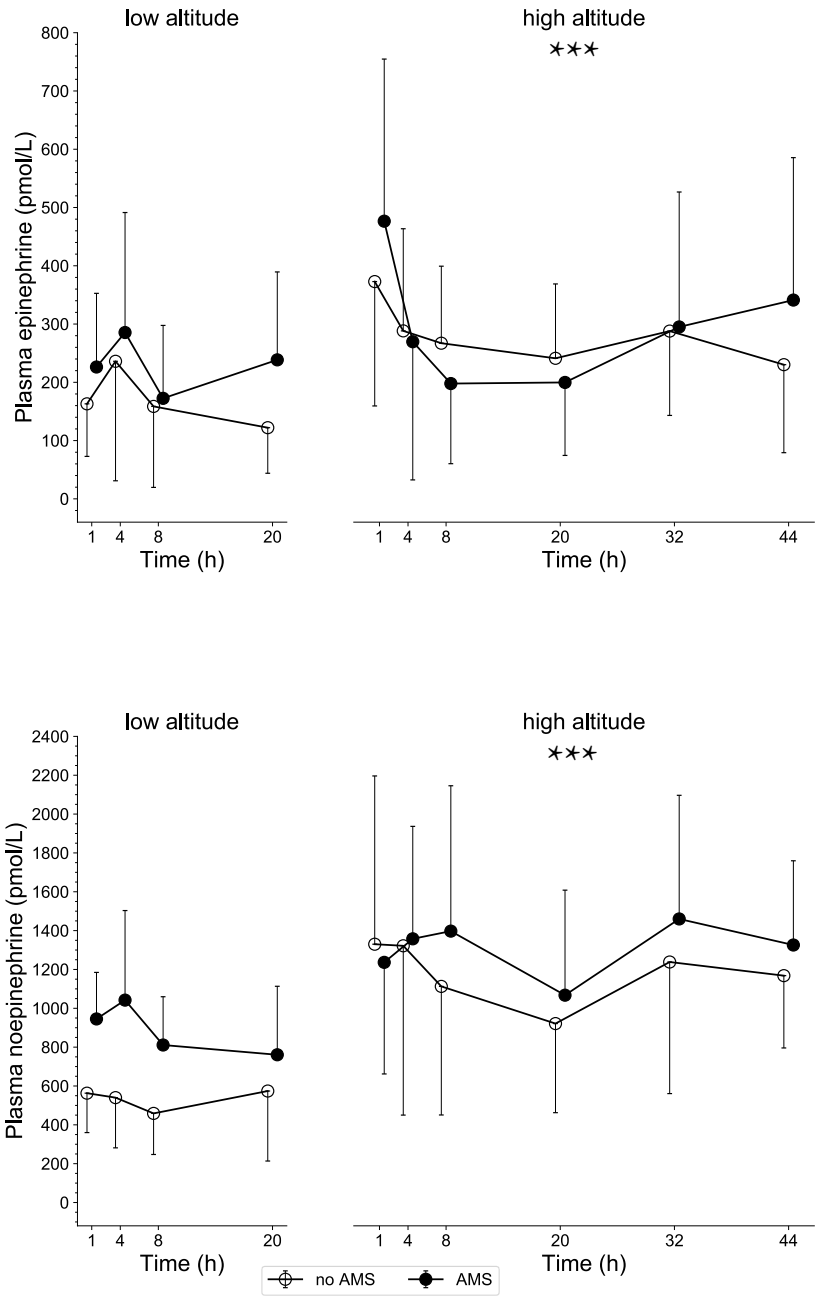
low altitude
high altitude
***
Plasma epinephrine (pmol/L)
800
700
600
500
400
300
200
100
0
1 4 8 20
Time (h)
1 4 8 20 32 44
Time (h)
low altitude
high altitude
***
Plasma noepinephrine (pmol/L)
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1 4 8 20
Time (h)
1 4 8 20 32 44
Time (h)
no AMS
AMS

Figure 6

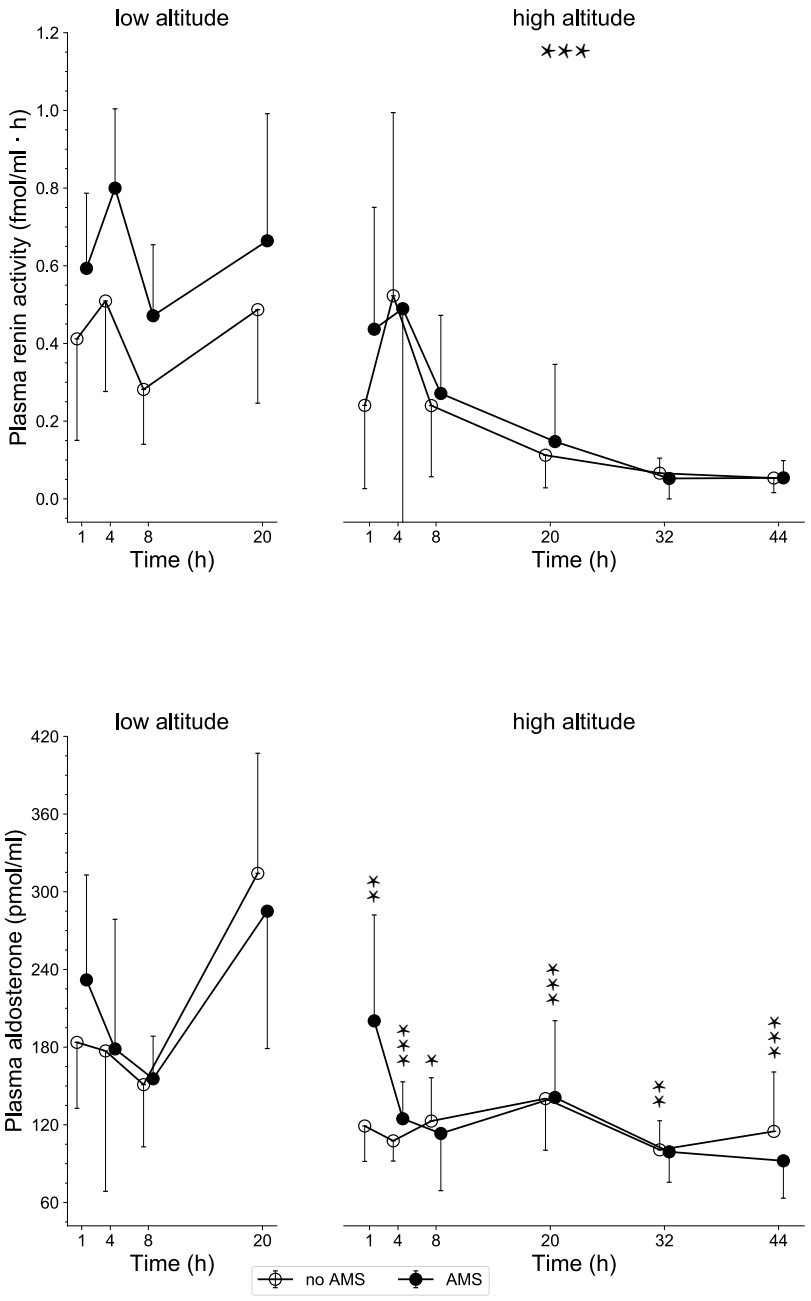
low altitude
high altitude
***
Plasma renin activity (fmol/ml · h)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1
4
8
20
32
44
Time (h)
low altitude
high altitude
Plasma aldosterone (pmol/ml)
420
360
300
240
180
120
60
Time (h)
no AMS
AMS

Figure 7

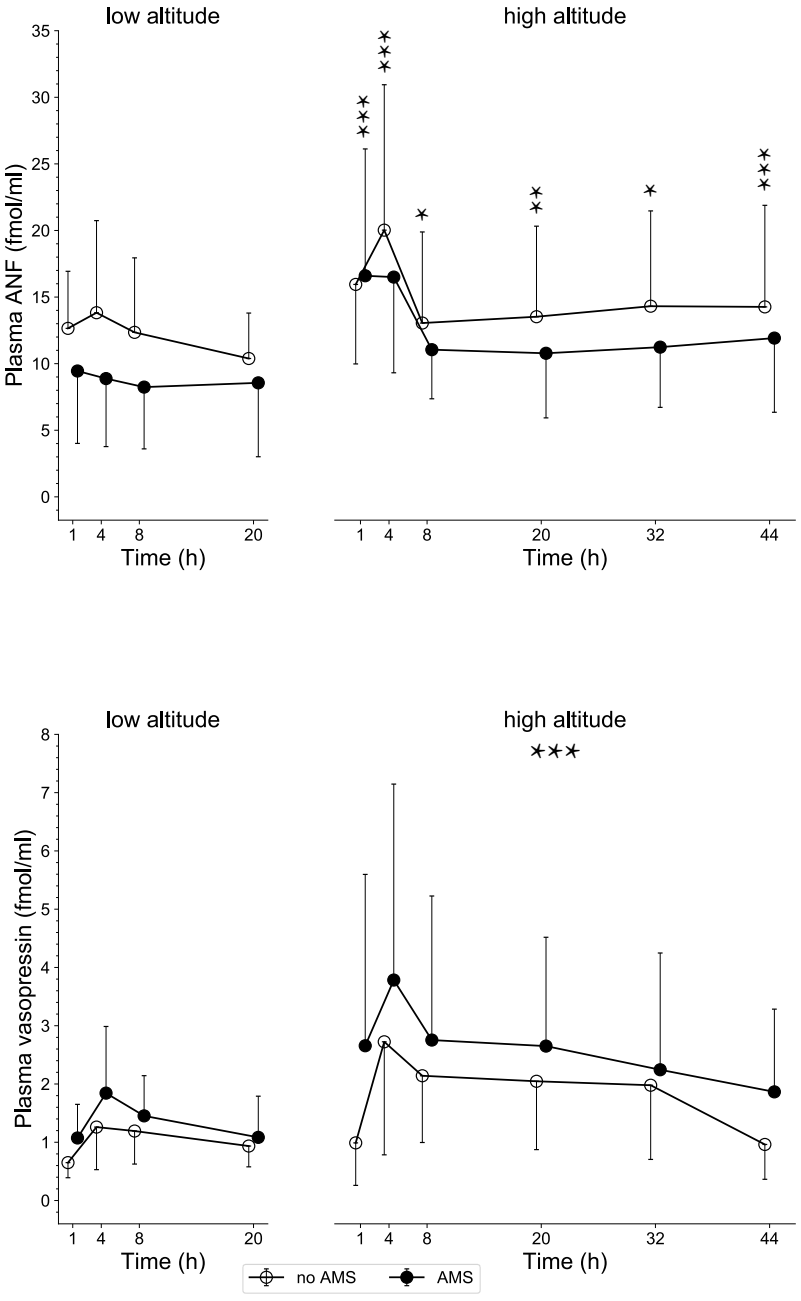
low altitude
high altitude
Plasma ANF (fmol/ml)
Time (h)
low altitude
high altitude
Plasma vasopressin (fmol/ml)
Time (h)
no AMS
AMS

Table 1

	AMS	*Functional Score*	LLS total	LLS Q5	LLS Q4	Headache
2	no	*0*	0	0	0	no
5	no	*0*	2	2	2	no
6	no	*1*	5	5	4	yes
8	no	*1*	3	2	2	yes
12	no	*1*	4	3	3	yes
14	no	*0*	1	1	1	yes
15	no	*1*	4	2	2	no
17	no	*1*	5	5	4	yes
1	yes	*2*	8	6	4	yes
3	yes	*2*	13	9	7	yes
4	yes	*3*	14	9	9	yes
7	yes	*2*	8	6	4	yes
9	yes	*2*	9	7	4	yes
10	yes	*2*	11	7	4	yes
11	yes	*3*	13	9	6	yes
13	yes	*2*	14	9	6	yes
16	yes	*2*	13	10	6	yes
18	yes	*2*	8	5	5	yes

Individual maximal values for functional score, total Lake Louise score (LLS tot), Lake Louise questionnaire old version with 5 questions (LLS Q5) and new version not including sleep (LLS Q4). Subjects #6 and #17 reached maximal values only in one and subject #18 in 3 assessments.

Table 2

	AMS-	AMS+
n	8	10
age (years)	26.0 ± 7.7	29.9 ± 3.6
height (cm)	185,8 ± 6.1	181.4 ± 6.9
weight (kg)	78.5 ± 7.7	70.0 ± 6.7 *
BMI (kg/m^2)	22.7 ± 2.5	21.3 ± 1.7
Hemoglobin[1] (g/l)	157.9 ± 5.8	157.2 ± 8.7
Hematocrit[1] (%)	46.3 ± 1.8	46.6 ± 2.6

Anthropometric data, mean values ± SD, * denotes p=0.023, Student's t-test

[1] Measured at recruitment of subjects

Table 3: Blood Gas Analysis

		560m	-------- 4559 m -------- day 1			---------- day 2 ----------/--- day 3---			p^1	p^2
			1h	5h	9h	22h	33h	45h		
SatO₂ (%)	**AMS-**	**97.1±.3**	**76.0 ±3.0**	**76.3 ±4.1**	**79.1 ±3.6**	**80.0 ±2.6**	**80.1 ±1.9**	**83.0 ±201**	**< .0001**	**< 0.025**
	AMS+	**97.7±1.2**	**74.5 ±6.4**	**74.7 ±5.2**	**76.0 ±3.8**	**74.7 ±7.3**	**76.0 ±5.5**	**79.6 ±5.8**		
pO₂ *(mmHg)*	**AMS-**	**83.7 ±8,3**	**39.5 ±2.5**	**39.2 ±2.9**	**41.9 +3.3**	**44.7 ±2.5**	**42.9 ±1.3**	**46.3 ±2.4**	**< .0001**	**< 0.05**
	AMS+	**79.7 ±5.1**	**38.4 ±5.0**	**37.9 ±3.9**	**38.3 ±3.1**	**40.6 ±4.2**	**40.1 ±3.8**	**42.8 ±4.0**		
pCO₂ *(mmHg)*	**AMS-**	**36.5 ±5.2**	**32.2 ±2.3**	**32.2 ±2.7**	**29.3 ±2.2**	**29.8 ±3.4**	**29.1 ±2.4**	**27.5 ±2.7**	**< .0001**	**ns**
	AMS+	**40.1 ±2.4**	**33.9 ±4.0**	**33.2 ±3.7**	**30.5 ±1.7**	**31.2 ±2.9**	**30.0 ±3.7**	**28.7 ±2.4**		
pH	**AMS-**	**7.44 ±.05**	**7.47 ±.01**	**7.48 ±.03**	**7.49 ±.02**	**7.47 ±.02**	**7.478±.12**	**7.46 ±.07**	**< .0001**	**ns**
	AMS+	**7.43 ±.02**	**7.47 ±.03**	**7.48 ±.02**	**7.49 ±.03**	**7.47 ±.02**	**7.48 ±.02**	**7.49 ±.03**		
BE (mmol/l)	**AMS-**	**-.16 ±167**	**-1.01 ±142**	**0.0 ±2.0**	**-1.56 ±11**	**-2.50 ±1.9**	**-2.50 ±1.5**	**-5.19 ±3.2**	**< .0001**	**= .025**
	AMS+	**1.42 ±1.8**	**.55 ±2.4**	**.56 ±2.7**	**- .41 ±1.7**	**-1.44 ±2.6**	**-1.55 ±2.3**	**-2.14 ±1.8**		

Blood gas analysis in capillary blood obtained from a hyperemic ear lobe, mean values ± SD

p^1 overall value for interaction Day x Hour, significant difference from baseline value at 560 m at all time-points at 4559 m except for BE at 1 and 5 hours

p^2 overall value for comparison AMS+ vs AMS-, no significant interaction for AMS x Day x Hour