Serial changes in nasal potential difference and lung electrical impedance tomography at high altitude

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Although only a minority of those who go to high altitude develop the potentially fatal condition of high-altitude pulmonary edema (HAPE), there is increasing evidence that the majority of people ascending to altitude may develop subclinical pulmonary edema (9). Forced vital capacity (FVC) falls on ascent to high altitude and is thought to be primarily due to subclinical pulmonary edema (32). The control of pulmonary extravascular lung water (EVLW) has traditionally been attributed to the interplay of Starling forces, with the pulmonary capillary pressure attributed the major regulatory role. It is now realized that sodium transport across the respiratory epithelium plays an important role in the removal of alveolar water (34) and that an intact epithelial barrier is necessary for the resolution of alveolar edema (35). Hypoxia reduces epithelial sodium transport in cultured rat alveolar epithelial monolayers (29) and decreases the expression of alveolar epithelial ion transport proteins (8). In addition, treatment of HAPE-susceptible individuals with inhaled β2-agonists, which are known to increase transepithelial sodium transport, decreases pulmonary edema without any effect on pulmonary hemodynamics (44).

Measurement of alveolar ion transport in vivo in human subjects is not feasible; however, measurement of the potential difference (PD) generated by ion transport in the nasal mucosa can be used as a marker of transport in the distal respiratory epithelium (22, 23). By perfusing the nasal mucosa with substances that alter the conductance of specific ion channels, the relative contribution of these channels to the PD may be estimated (21). Amiloride inhibits the amiloride-sensitive epithelial sodium channel (ENaC), whereas a low-concentration chloride solution containing isoprenaline can be used to stimulate chloride secretion, predominantly via the cystic fibrosis transmembrane regulator. Assessing changes in lung water at high altitude has presented problems because of the difficulty in detecting early changes in EVLW. The Sheffield electrical impedance tomography (EIT) system is a portable device that measures the change in electrical resistivity of lung tissue occurring in the presence of EVLW to give a cross-sectional image of the resistive changes in a tissue plane (5).

We therefore measured nasal potential difference (NPD), FVC, EIT changes, and pulmonary circulation pressures by Doppler echocardiography in a group of
normal male volunteers before, during, and after a 2-wk stay at 3,800 m in the Tien Shan mountains of Kyrgyzstan.

METHODS

Subjects.

Twenty male Kyrgyz lowland volunteers (age range 18–35 yr) with no previous exposure to high altitude underwent baseline tests (BL) in Bishkek (altitude 700 m) before being transported by road in 7 h to Kumtor gold mine at an altitude of 3,800 m. High-altitude measurements were repeated on days 1, 2, 5, and 10 for all subjects, on day 14 for half of the subjects, and day 15 for the remaining half (HA01, HA02, HA05, HA10, and HA14/15, respectively). Subjects returned to Bishkek by road, and return to baseline (RBL) measurements were made within 24 h of their departure from high altitude. All subjects gave written, informed consent, and the project was approved by the ethics committee of the Faculty of Medicine of the Free University of Brussels.

Transepithelial NPD. Transepithelial NPD was measured using a 5-French umbilical catheter (Tyco Healthcare, Tul- lamore, Ireland) as the exploring bridge. The reference bridge was a 20-gauge catheter inserted into a forearm vein and perfused with 0.9% NaCl solution. The bridges were marked with an indelible marker pen; these marks were renewed daily so that subsequent recordings were made with the electrodes in an identical position.

FVC. FVC was measured by use of a Micro Medical Micro-Plus Spirometer (Micro Medical, Rochester, Kent, UK). Measurements were made in accordance with the guidelines of the British Thoracic Society (4). The best of three blows were recorded. All volumes are given at body temperature, ambient pressure, and fully saturated with water vapor (BTPS).

Echocardiography. Echocardiography and Doppler estimation of pressures were performed with a Philips SSD-800 echocardiograph (Philips Medical Systems, Bothell, WA). Mean pulmonary arterial pressure (Ppa) was estimated from pulmonary artery acceleration time, and systolic Ppa was estimated from the peak velocity of the tricuspid regurgitant jet, if present, by using the modified Bernoulli formula (36). Left ventricular diastolic function was assessed by comparing the initial peak transmitral velocity of early ventricular filling (E) and the late transmitral velocity of atrial contraction (A) and deriving the E/A ratio (38).

Clinical data. Pulse, blood pressure, temperature, and oxygen saturation (Nellcor N20P pulse oximeter, Nellcor-Puritan Bennet, Warwick, UK) were recorded on the morning of each measurement day. Acute mountain sickness (AMS) was assessed by use of the Lake Louise consensus scoring system (41).

Statistical analysis. Data are presented in text and tables as means ± SE. Normality was assessed by using the Sha- piro-Wilks test. Serial measures were tested for statistical significance by using one-way ANOVA for repeated measures with significant post hoc differences being further analyzed by use of a Student-Newman-Keuls test for parametric data and a Dunnett’s test for nonparametric data. Statistical significance was assumed at P < 0.05.

Because of the repeated nature of the measures, correlation analysis was performed by using the Poon test for the analysis of linear and mildly nonlinear relationships using pooled subject data from all altitudes (40). Analyses were performed by use of SigmaStat 2.0 software (Jandel, San Rafael, CA) and a program written with Microsoft Excel for the Poon test.

RESULTS

NPD. Data were obtained on 18 of the 20 subjects. One subject could not tolerate the procedure because of repeated gagging, and in one subject a stable trace was never obtained. NPD hyperpolarized on ascent to HA01 and remained hyperpolarized at HA02 (both P < 0.05 vs. BL) before returning to BL values on HA05. The PD during infusion with amiloride hyperpolarized on ascent to HA but did not reach statistical signifi-
cance. The PD during perfusion with the low-chloride solution containing isoprenaline hyperpolarized on ascent to HA01 ($P < 0.05$ vs. BL). Neither the amiloride-inhibitable proportion nor the stimulated chloride proportion of the PD changed significantly on ascent to or during stay at altitude compared with baseline (Fig. 1).

**Electrical impedance tomography.** Satisfactory data were obtained on 18 of 20 subjects with data on two subjects being rejected because of consistently poor-quality recordings. The standardized change in impedance between RV and TLC fell on ascent to HA01 ($P < 0.05$) and then returned to BL values on subsequent days (Fig. 2).

**FVC.** Data were obtained on all subjects. FVC fell on ascent to HA01 compared with BL ($P < 0.05$) and remained significantly reduced compared with BL throughout the stay at altitude and at RBL ($P < 0.05$, Table 1).

**Ppa.** Mean Ppa was measurable in 17 subjects at BL and in all subjects at HA and RBL. Systolic Ppa was measurable in 15 subjects at BL and 16 and 18 subjects at HA and RBL, respectively. Both mean and systolic Ppa increased on ascent with maximum values at HA01 and HA02 ($P < 0.01$ vs. BL) and then fell at HA05 although remaining significantly higher than BL throughout the rest of the stay at altitude ($P < 0.05$ vs. BL). Mean Ppa returned to BL values at RBL, although systolic Ppa remained elevated. The E/A ratio fell on ascent to HA and remained reduced throughout the stay at HA and at RBL ($P < 0.05$ vs. BL). These results are summarized in Table 1.

**Oxygen saturation and AMS score.** Data were obtained on all subjects. Oxygen saturation fell on ascent to HA01, remained significantly reduced throughout the stay at altitude compared with BL (all altitudes $P < 0.05$), and then returned to BL values at RBL. Despite a statistically significant increase, AMS score values never exceeded the accepted value of 3 for clinically relevant AMS. These results are summarized in Table 1.

**Relationship between EIT and FVC changes and changes in NPD and Ppa.** There was a positive correlation for FVC and the standardized change in EIT ($R^2 = 0.633$, $P < 0.001$; see Fig. 3) and between the basal nasal potential and both FVC and the standardized change in EIT ($R^2 = 0.418$ and 0.267 respectively, both $P < 0.001$; see Fig. 4). There was no relationship between mean Ppa and FVC or the standardized change in EIT ($R^2 = 0.0046$ and 0.0411, respectively) or systolic Ppa and FVC or the standardized change in EIT ($R^2 = 0.00006$ and 0.0108, respectively).

**DISCUSSION**

This work demonstrates a hyperpolarization in the NPD in normal subjects on ascent to high altitude, a simultaneous fall in FVC, and changes in EIT, which would be consistent with an increase in EVLW. In addition, these findings occurred in the absence of signs of AMS and at the relatively low altitude of 3,800 m.

A fall in FVC on ascent to high altitude has been consistently reported by a number of authors, and our data are in keeping with this (32). The most likely mechanism behind this fall is the presence of subclinical pulmonary edema, although other suggested etiologies include an increase in pulmonary blood volume or reduced respiratory muscle strength. To date there is no evidence to support a sufficient reduction in resting muscle strength at altitude to produce a reduction in FVC.

Despite this circumstantial evidence, the major problem in proving the presence of subclinical pulmo-
nary edema has been the absence of an accurate measure of EVLW that can be used in the mountain environment. Clinical examination and chest radiography are insensitive to small increases in EVLW (31, 47). Magnetic resonance imaging is highly sensitive but of limited practical use at high altitude (10). The thermal dye double-indicator dilution technique remains one of the more accurate methods in intact humans and can determine small changes in EVLW, but it is highly invasive and would be difficult to perform in large numbers of subjects at altitude (42). Transthoracic electrical impedance measurement has been used at altitude (14, 43), but the results have been disappointing and changes in lung volume with ventilation and the lack of spatial resolution further complicate the interpretation of results (11).

The Sheffield Applied Potential Tomograph system was developed to overcome these problems; it provides a portable device that generates a two-dimensional image of the impedance changes occurring during ventilation in a transverse cut through the thorax and allows a region of interest to be delineated for analysis. The parenchyma of the right lower lobe was chosen to be analyzed because in the upright position it has the maximum volume change with ventilation without cardiac interference. There is good correlation between EVLW measured by EIT and the double-indicator dilution technique in ventilated patients with acute respiratory failure (25) and in an oleic acid animal model of the acute respiratory distress syndrome (7).

Possible factors that would confound the EIT results are changes in pulmonary blood volume and lung vol-

![Fig. 3. Relationship between FVC and the normalized change in electrical impedance tomography (EIT) between RV and TLC for pooled data from all altitudes. A statistically significant relationship is present between the change in EIT and FVC. The correlation coefficient and statistical significance are indicated.](image)

![Fig. 4. Relationship between NPD and the fractional change in EIT (A) and between NPD and FVC (B) for pooled data from all altitudes. A statistically significant relationship is present between NPD and both EIT changes and FVC. The correlation coefficient and statistical significance are indicated.](image)
umes. According to the model of Nopp et al. (37), an increase in pulmonary blood volume from 500 to 600 ml (i.e., by 20%) will cause a drop in resistivity of 7% at 50 kHz, whereas a 20% change in extracellular fluid will cause a 40% change in resistivity at 50 kHz. To take account of changes in lung volume, our results were normalized with reference to the FVC at that altitude. However, the raw, nonnormalized results showed a mean reduction in the change in impedance of 22%. For this to be caused by an increase in pulmonary blood volume would require an increase in blood volume of over 300 ml or 60%, and we therefore conclude that it is highly improbable that a change in blood volume explains the change in EIT seen in this study.

Evidence of the effects of acute exposure to hypoxia on RV and TLC suggests that both increase on initial exposure to altitude but return to baseline after around a month at altitude (12). An increase in RV or a fall in TLC could produce a fall in FVC, but as both these volumes increase they would tend to cancel each other out, making it unlikely that changes in lung volume are a sufficient explanation for the observed impedance changes. In addition, the rapidity with which the impedance changes return to normal compared with the much longer time course for the normalization of lung volumes argues against the impedance changes being due to changes in lung volumes. Finally, an increase in lung volume at high altitude might be expected to decrease, rather than increase, the amount of estimated EVLW by augmenting the air content, which would increase resistivity of the lung tissue.

Using EIT, we have demonstrated a marked fall in the normalized change in impedance between RV and TLC on ascent to 3,800 m, which would be consistent with an increase in EVLW. This fall in impedance shows a strong relationship with FVC, with the smallest changes in impedance being associated with the lowest FVCs, suggesting that an increase in EVLW may be responsible for the fall in FVC seen at high altitude. That FVC did not return to baseline levels during the high-altitude sojourn, whereas EIT did, does not exclude the possibility that the fall in FVC is due to increased EVLW but may simply reflect differing sensitivities to the presence of EVLW by EIT and FVC.

Two possible causes for a possible increase in EVLW were addressed by this study. The control of EVLW has conventionally been attributed to the balance between Starling forces causing extravasation of water from the pulmonary capillaries and the ability of the pulmonary lymphatics to clear this water (46). The most important variable that influences the development of clinical pulmonary edema is the pulmonary capillary pressure (Ppc). In an invasive hemodynamic study at 4,559 m in the Swiss Alps, Maggiorini et al. (28) found a cutoff Ppc of 19 Torr for the development of HAPE in a group of HAPE-susceptible subjects. All subjects who developed HAPE had a Ppc > 19 Torr, which corresponded to a mean Ppa > 35 Torr. No HAPE-susceptible subject who developed HAPE had a mean Ppa < 35 Torr.

We chose to use Doppler echocardiography to assess Ppa because it is noninvasive and has been shown to correlate well with the results obtained with right heart catheterization at high altitude (1). None of our subjects’ mean Ppa approached the cutoff point of 35 mmHg observed by Maggiorini (maximum mean Ppa 20.2 ± 0.9 on HA1 of our study) excluding HAPE as a cause of any changes in EVLW in this study. However, if subclinical pulmonary edema is of hydrostatic origin, it is likely to occur at much lower Ppas than those seen in clinical HAPE. Hargreaves et al. (13) demonstrated increased activity in rabbit airway rapidly adapting receptors suggestive of a physiological effect from interstitial edema after an increase in left atrial pressure of as little as 5 mmHg. In keeping with the findings of Boussuges et al. (3), we also found alterations in transmural flow on echo-Doppler with a decrease in the E/A ratio on ascent to high altitude, suggestive of a reduction in left ventricular diastolic compliance. A fall in diastolic compliance would increase the risk of developing pulmonary edema on exercise (45), and one may argue that some of the increase in EVLW seen in this study could be due to repeatedly elevated Ppc during physical activity. Nevertheless, the failure of the Poon analysis to demonstrate any relationship between either mean or systolic Ppa and either the changes in EIT or FVC, while demonstrating a strong relationship between the changes in EIT or FVC and NPD, argues against the subclinical pulmonary edema seen in this study being of predominantly hydrostatic origin. This is also consistent with the work of Sartori et al. (44), in which inhaled β2-agonists, known to increase transepithelial sodium transport, decreased pulmonary edema in HAPE-susceptible individuals without any effect on pulmonary hemodynamics.

During the last decade, it has become apparent that an intact alveolar epithelium is necessary for the resolution of pulmonary edema (35) and that uptake of water across the alveolar-capillary barrier is an active process dependent on transepithelial sodium transport. The sodium current is generated by the basolateral Na-K-ATPase pump, and sodium enters the epithelium through the apical ENaC, the rate-limiting step of this process (34). In patients with acute lung injury and the acute respiratory distress syndrome, reduced alveolar water clearance is associated with increased morbidity and mortality (48), whereas homozygote knockout mice born lacking the α-subunit of ENaC died within 40 h of birth from pulmonary edema (15). A number of factors may influence the sodium current, and thus water uptake across the alveolar epithelium, including β-adrenergic agonists, glucocorticoids, thyroid hormones, insulin, and certain growth factors (34). Studies of cultured respiratory epithelium suggest that hypoxia inhibits both the activity and expression of both ENaC and the Na-K-ATPase pump (29).

It is not possible to measure alveolar epithelial sodium transport in vivo in the human subject, but measurement of the PD generated by ion transport across the respiratory mucosa under the inferior turbinate of
the nose can be used as a surrogate measure for ion transport in the distal respiratory epithelium tract and is a simple and well-tolerated measure to perform (21–23). Measurement of this NPD correlates well with the pathological changes seen in the airways of patients with cystic fibrosis (24). In a study of HAPE-susceptible individuals, Sartori et al. (44) found that their NPD at sea level was 32% less negative than in non-HAPE-susceptible subjects and that, in addition, superperfusion with amiloride produced a significantly smaller depolarization in NPD in the HAPE-susceptible compared with the HAPE-resistant subjects. This work was extended by Mairbäurl et al. (30), who confirmed a less negative NPD in HAPE-susceptible individuals, compared with nonsusceptible controls at low altitude, and stimulated chloride transport with a chloride-free solution containing amiloride and isoprenaline. Although there was no difference between susceptible or resistant subjects at low altitude, ascent to 4,559 m produced a large hyperpolarization in NPD, which was most marked in the nonsusceptible group, in whom it was due to a twofold increase in stimulated chloride secretion whereas sodium absorption was inhibited.

Our results differ from those of Mairbäurl et al. (30) in a number of ways. Although our baseline potentials were only slightly lower, ascent to 3,800 m did not produce such a large hyperpolarization in NPD, nor did we see any reduction in the amiloride-dependent sodium transport or a significant increase in isoprenaline-stimulated chloride transport at altitude. Our study took place at an altitude 750 m lower than that of Mairbäurl et al. and our subjects had almost no AMS. A prevalence of AMS of 53% has been reported at the Margharita hut used by Mairbäurl et al. for the high-altitude part of their study (27). Either the lower altitude with the resultant higher partial pressure of oxygen or the lower incidence of AMS might be responsible for the difference between our results. In addition, the study of Mairbäurl et al. was on white Caucasians, whereas the subjects in the present study were Altai-subtype Mongolians. Ireson et al. (17) have demonstrated a significant difference in NPD between blacks and whites, but little is known about other racial differences in NPD or respiratory epithelial ion transport. It is possible that differences between our results and those of Mairbäurl may in part be explained by racial differences.

The hyperpolarization in NPD on ascent to high altitude could be due either to changes in ion currents or to a change in transepithelial resistance. There is little information available on the changes in transepithelial resistance in hypoxia, and that which is available comes from work in cell monolayers. Mairbäurl et al. (29) demonstrated both small increases and decreases in the transepithelial resistance of rat type II pneumocytes exposed to different levels of hypoxia for between 4 and 24 h. However, in all cases the transepithelial PD fell, equivalent to a depolarization in vivo. From cellular work we can find no evidence to support an increase in transepithelial resistance as a cause for our findings.

Changes in ion currents could be due to either increased cation absorption or increased anion secretion. The exact nature of cation channels in the alveolar epithelium has been debated because of the differing biophysical properties of channels encountered in cultured cells. However, Jain and colleagues (18) demonstrated that the culture conditions under which type II pneumocytes are grown influence their biophysical properties. When ATII cells were grown on glass plates submerged in media, the predominant channel was a 21-pS nonselective cation channel. If they were grown on a permeable support and in the presence of steroids and an air interface, the predominant channel was a low-conductance (6.6 ± 3.4 pS), highly Na+-selective channel, inhibited by submicromolar concentrations of amiloride, and similar in biophysical properties to ENaC. It now seems that the variety of different cation channels seen in alveolar epithelium in part stems from the assembly of different combinations of the α-, β-, and γ-subunits of ENaC with the highly selective 4- to 6-pS channel containing all three of the subunits (33). The absence of any change in the proportion of the NPD inhibited by amiloride on ascent to altitude excludes this being due to increased sodium absorption via an amiloride-sensitive ENaC and suggests that it could be all or partly explained by an increase in sodium absorption via amiloride-insensitive nonspecific cation channels.

If the hyperpolarization is not due to increased cation absorption then it must be due to increased anion secretion. Both chloride and bicarbonate channels have been reported in rat fetal distal lung epithelium (26), although only chloride channels have to date been found in adult rat epithelium (20). Anion secretion would be associated with the secretion of water into the luminal space. This is the normal state in the fetus (39). Chloride transport takes place by a number of apical channels, the major ones being the cystic fibrosis transmembrane regulator (CFTR), the outwardly rectifying chloride channel, and the calcium-activated chloride channel (19). Sympathomimetic agents such as isoprenaline may activate CFTR (16). Such activation in the presence of a favorable chemical gradient will stimulate apical chloride secretion, estimating the transepithelial chloride transport capacity of CFTR. The fact that stimulation of CFTR with isoprenaline and a low-chloride solution showed no statistically significant increase at high altitude in the present study only allows us to conclude that CFTR Cl− transport was already maximally stimulated by the low-chloride isoprenaline solution at sea level and was not further stimulated by the increased levels of sympathetic activity that occur at altitude. This does not exclude an alteration in chloride transport via a channel other than CFTR or exclude bicarbonate secretion.

Although emphasizing that the presence of a correlation does not prove causality, the relationship demonstrated between NPD and both FVC and the change in EIT (Fig. 4) would argue in favor of anion secretion.
over sodium reabsorption as the cause for the hyperpolarization in NPD. The most negative PDs were associated with the lowest FVCs and the lowest changes in EIT between RV and TLC. Both a fall in FVC and a reduced increase in impedance on inspiration would be consistent with an increase in EVLW, a situation more compatible with increased anion secretion than sodium reabsorption.

In conclusion, this work demonstrates a hyperpolarization of the NPD in asymptomatic individuals on ascent to 3,800 m and simultaneous changes in EIT that may be consistent with an increase in EVLW. The changes in NPD suggest either an increase in sodium absorption via an amiloride-resistant cation pathway, either bicarbonate secretion or chloride secretion via a non-CFTR channel, or a combination of both mechanisms. Predominance of anion secretion over sodium reabsorption, if present in the alveoli, would be associated with the secretion of water into the lumen as occurs in the fetal lung (44). Further work is required to confirm the precise nature of these changes.

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