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**HIGH INTENSITY INTERMITTENT EXERCISE INCREASES PULMONARY INTERSTITIAL EDEMA AT ALTITUDE BUT NOT AT SIMULATED ALTITUDE**

**Short title: Exercise-induced pulmonary edema at altitude**

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**Conflicts of Interest**:

The above authors have no conflicts of interest to declare.

**Abstract**

**Objective:** Ascent to high altitude leads to a reduction in ambient pressure and a subsequent fall in available oxygen. The resulting hypoxia can lead to elevated pulmonary artery (PA) pressure, capillary stress and an increase in interstitial fluid. This fluid can be assessed on lung ultrasound (LUS) by the presence of B-lines. We undertook a chamber and field study to assess the impact of high intensity exercise in hypoxia on the development of pulmonary interstitial edema in healthy lowlanders.

**Methods**: Thirteen volunteers completed a high-intensity intermittent exercise (HIIE) test at sea level, in acute normobaric hypoxia (12% O2 ~4090 m equivalent altitude) and in hypobaric hypoxia during a field study at 4090m following 6 days progressive acclimatization. Pulmonary interstitial edema was assessed by LUS evaluation of B-lines.

**Results**: Following HIIE no increase in B-lines was seen in normoxia and a small increase in acute normobaric hypoxia (2 ± 2, *P*<0.05). During the field study at 4090m, 12 subjects (92%) demonstrated 7 (± 4) ULC at rest, which increased to 17 (± 5) immediately following the exercise test (*P*<0.001), an increase was evident in all subjects. There was a reciprocal fall in peripheral arterial oxygen saturations (SpO2) following exercise from 88% (± 4) to 80% (± 8) (*P*<0.01). B-lines and SpO2 in all subjects returned to baseline levels within four hours.

**Conclusion**: HIIE led to a rapid increase in pulmonary interstitial edema at altitude after chronic exposure, but not during acute exposure in a chamber at the same simulated altitude.

**Abbreviations**: HIIE = High intensity intermittent exercise, HAPE = High Altitude Pulmonary Edema, LUS = Lung ultrasound, PA = Pulmonary Artery

**Introduction**

Ascent to high altitude leads to a reduction in ambient pressure and a subsequent fall in available oxygen. In the human lung, the resulting hypoxia leads to vasoconstriction and a rise in pulmonary artery pressure.1 This in turn increases microvascular hydrostatic pressure and may lead to the accumulation of a high-permeability-type interstitial fluid (a capillary stress failure) in the presence of normal cardiac function.2 In certain individuals, this can lead to a clinical deterioration, further hypoxemia and the development of High Altitude Pulmonary Edema (HAPE), a potentially life threatening condition for climbers and trekkers in remote locations.1,3

Strenuous exercise, which is often a consequence of the reduction in aerobic power experienced at high altitude, has long been proposed as a potential risk factor for the development of HAPE.4 Even in the absence of HAPE, most climbers and trekkers experience a degree of dyspnea and decreased performance at altitude and it has been hypothesized that this may be due to an increase in interstitial lung fluid.5

Lung Ultrasound (LUS) is a non-invasive technique for the assessment of a range of pulmonary and pleural disease.6,7 Interstitial edema is characterized on LUS by the presence of B-lines also known as Ultrasound Lung Comets.7,8 In hospital practice, B-lines have been correlated with chest x-ray and CT scan assessments of interstitial edema as well as invasive measurements of EVLW using thermodilution.3,9,10

The LUS technique has been used as both a clinical and research investigation at high altitudes and its strength and weakness when used in this environment have been discussed in reviews by Fagenholz *et al* and Wimalasena *et al*.11,12 LUS was first reported as a diagnostic tool for HAPE by Fagenholz *et al*. in a prospective case control study of patients admitted to the Himalayan Rescue Station in the Khumbu valley (5400m). In their observational study, patients with a HAPE had a higher number of lung comets than controls and their presence was negatively correlated with peripheral oxygen saturation.13

The use of LUS to describe *subclinical pulmonary edema* (also described as *clinically-silent HAPE*) was first reported in a study by Pratali *et al.* in 2010.14 The authors performed LUS on 18 subjects during a two-week trek to Everest Base Camp. Fifteen subjects exhibited ULC at 3440m and all subjects showed evidence of increased B-lines at 4790 m. In 2012, they followed up this study using LUS to show that patients suffering from chronic mountain sickness experienced a rapid increase in pulmonary interstitial fluid after exercise at altitude compared to healthy high altitude dwellers.15

LUS has also been used to evaluate exercise-induced pulmonary edema and ventricular function during exercise at sea‐level. Agricola *et al.* reporting that exercise alone can lead to elevation of pulmonary arterial pressure sufficient to induce pulmonary edema.16

Strenuous exercise has long been proposed as a potential risk factor for the development of HAPE with many experienced climbers following the old adage that “slow and steady wins the race”.4 The objective of this study was to evaluate the hypothesis that strenuous exercise in a hypoxic environment will lead to an increase in B-lines in otherwise healthy individuals.

**Methods**

**Subjects**

Thirteen subjects (3 women, 10 men; mean ± 1SD: age 37 ± 10 years; height 179 ± 9 cm; body mass 79 ± 12 kg) volunteered for the study. None of the subjects had been to altitude in the 3 months prior to hypoxic testing. All subjects were non-smokers with no medical history of chronic lung disease. Subjects gave their written informed consent and ethics approval was granted by the University of Chichester Research Ethics Committee (Protocol number 1011\_39).

The study involved a high intensity intermittent exercise test completed at sea level and acute hypoxia in a normobaric chamber (TISS Model 201003-1, TIS Services UK, Medstead, UK) simulating an altitude of 4090 m (O2 12.6%; CO2 0.04%; N2 balance) following a crossover design. Subjects were subsequently tested in hypobaric hypoxia during the 2012 Birmingham Medical Research Expeditionary Society (BMRES) expedition to Bhutan (Jomulhari Base Camp, Jangothan, Bhutan). The acclimatization period comprised a six-day ascent to the test altitude of 4090 m following a gentle ascent profile (~400 m/day). The testing took place in a windproof hut, with the subjects kept warm using sleeping bags and duvet jackets when required.

**High Intensity Intermittent Exercise test (HIEE)**

The HIIE test was completed on a bespoke supine cycle ergometer (Alticycle, BMRES, Birmingham) the details of which have been described in a previous publication.17 Prior to testing, all subjects rested on the Alticycle for 10 minute after which arterial oxygen saturations (SpO2) and heart rate was recorded (Pulse Oximeter model MD300C41, Beijing Choice Electronics, Beijing). Following this LUS was performed and B-line score recorded. For the acute exposure, the subjects entered the chamber approximately 5 minutes before the rest period commenced to allow them to be fitted to the Alticycle, giving a total pre-exercise exposure of 15 minutes in hypoxia. Subjects then commenced a 5-min self-paced warm-up after which they were then asked to complete a best-effort 6000m cycle against a standard resistance. Subjects were instructed not to pace their efforts, but to exercise maximally for as long as possible and then rest and recover before restarting when they felt able. Verbal feedback was given on distance completed only.

**Lung ultrasound**

The LUS was performed using a MicroMaxx® portable ultrasound machine (Sonosite, Bothell, WA, USA) and an 8 MHz Linear array transducer. Subjects were scanned in the supine position using insonnation points on each hemithorax (anterior intercostal rib spaces 2 and 3, in the mid-clavicular line, and lateral intercostal spaces 5 and 6, in the mid-axillary line) giving a total of 8 examination zones.12 In order to reduce bias the LUS were carried out by the same operator for each session who was observed by a minimum of one other trained operator who recorded their scores independently with the mean taken as the score for each site. The mean number of B-lines at each site was recorded and the sum total used in the statistical analysis.

Post-exercise LUS scans were performed immediately following the HIIE test and in the field study, subsequent scans were performed at 1 and 2 hours post-exercise. The subjects whose B-line score had not returned to their baseline after two hours, also had a 4-hour post-exercise scan. Alongside the LUS, subjects were asked to indicate the development of a cough. During the field study, subjects completed a Lake Louise Acute Mountain Sickness questionnaire daily.18

**Statistical Analysis**

Statistical analysis was performed using Statistical Package for Social Sciences version 20.0 (IBM UK Ltd, Portsmouth, UK). The normality of the data was tested with the Shapiro-Wilk test. Paired data were then analyzed using t-tests and multiple comparisons using repeated-measures analysis of variance with sphericity tested with Mauchly’s test, and when necessary Huynh-Feldt Epsilon correction factors were applied. Post hoc comparisons were made using t-tests with a Bonferonni correction applied. Relationships between data were assessed using Pearson product moment correlations. An alpha level of ≤ 0.05 was used for all statistics and all data are presented as mean ± 1 standard deviation (SD).

**Results**

The HIIE test (Table 1) took longer to complete in both hypoxic conditions compared to baseline, with the increase being significant for the acute exposure (*P* < 0.01) but not for the chronic (*P* = 0.08). The HIIE test performance improved following chronic compared to the acute exposure (*P* = 0.018).

No B-lines were evident pre-exercise (baseline) at either sea level or acute exposure to normobaric hypoxia. Following exercise in normoxia at sea level no B-lines were detected, however after acute exposure to hypoxia B-lines were present (range 5; *P* < 0.01) in seven of the subjects (Table 1). During the field study at 4090m, 12 out of the 13 subjects (92%) demonstrated ULC (range 14) at rest (Table 1), which increased (range 20) immediately following strenuous exercise (*P* < 0.001), and were evident in all subjects (Figure 1). B-lines were universally present at one (range 17; *P* = 0.08) and two hours post-exercise (range 22), with values in five subjects remaining above their baseline value. At four hours post-exercise, the B-lines in the five subjects (range 8) had returned to their baseline values (Figure 1). There was no statistical difference in the distribution of B-lines before (anterior vs. posterior) before (4 ± 3 vs. 3 ± 2) or after (9 ± 3 vs. 9 ± 3) exercise.

Baseline SPO2 (Table 1) values were lower during both acute and chronic exposure compared to baseline (*P* < 0.001). Following exercise there was no change in SPO2 at sea level, but a comparable fall in both the acute and chronic exposure (*P* < 0.001), with the latter returning to baseline values at subsequent time points. There was no relationship evident between SPO2 and the number of B-lines. Baseline heart rates (Table 1) were greater than sea level at rest in both the acute and chronic exposure (*P* < 0.01), but no different during exercise. A cough was reported by 11 out of the 13 subjects (84%) following the HIIE test at altitude. Two subjects had Lake Louise scores indicating the presence of mild acute mountain sickness the morning of their test (both 3 and included a headache).

**Discussion**

The principle finding of this study was that HIIE in hypoxia induced an increase in pulmonary interstitial fluid, as measured by the presence of LUS B-lines. This increase was meaningful when subjects were exposed to hypobaric hypoxia over six days, but negligible following acute normobaric hypoxic exposure. In chronic hypoxia B-lines were elevated in all subjects up to 2 hours and in some up to four hours following exercise.

We hypothesize that the HIIE at altitude caused not only increased hypoxemia (by factors such as diffusion limitation and increased oxygen extraction) but also surges in PA pressure as subjects alternated between maximal effort and rest. Subsequent uneven hypoxic pulmonary vasoconstriction led to areas of capillary stress and high permeability type edema.

The increase in interstitial pulmonary edema observed after chronic exposure, but not during acute hypoxic exposure, can be explained by the difference in pulmonary pressures resulting from the length of exposure and exercise. Groves *et al*. reported higher resting PA pressures at altitude, which increased further following 3-5 minutes of strenuous exercise.1 It is therefore likely that our subjects when exposed to hypoxia for longer, as during the field study, had higher resting PA pressure after some acclimatization than with the acute exposure to normobaric hypoxia in the chamber. Given the severity of our exercise test, it is also likely that our subjects had similar increases in cardiac output as those reported by Groves *et al*, and plausible that the PA pressure would have been high enough to cause the interstitial edema to form. Evidently this cannot be proven without direct measurement of PA pressure during HIIE.

The effect of atmospheric pressure on the development of pulmonary interstitial fluid must also be considered in respect of these results. Several authors over the past two decades have investigated the possibility of differing physiological responses between normobaric hypoxia and hypobaric hypoxia but physiologists remain divided over opinions.19,20 The only work of direct relevance to our study is that of Otto *et al*. 2009 who pioneered the use of ultrasound to detect B-lines at high altitude.21 During their pilot study, they exposed two subjects to an acute hypobaric altitude equivalent of 8230m with 100% O2 under positive pressure. Both subjects developed a number of B-lines within 5 minutes despite normal arterial oxygen saturations and the absence of hypoxic symptoms.21 The result suggests that it may be possible to induce B-lines with hypobaria alone but the small study size and severity of the hypobaria used limits its interpretation. Additionally, this study compared the effects of acute exposure to the varying hypoxic conditions with no mention of the effects of chronic hypobaria and did not involve exercise as we did. The consensus statement *working in hypoxic conditions* by Kupper *et al*. in 2011 concludes that the differences between hypobaric and normobaric hypoxia are too small to have any clinical relevance, and thus conditions can be used equivalently.22 In summary, it seems there is evidence that a minor variance in the physiological response to normobaric hypoxia and hypobaric hypoxia exists, however the clinical relevance of such a difference is questionable and thus its influence over the main results of our study is unlikely to be significant. It is more likely that B-lines take between hours to days to evolve and that exercise at high altitudes will exacerbate this, once physiological adaptation has taken place.

Although the presence of B-lines is an ultrasound finding, the majority of our subjects developed a cough following exercise at altitude. This rapidly resolved as the interstitial fluid reduced and peripheral saturation improved. It could therefore be argued that this might represent a mild form of inducible (or sub-clinical) pulmonary edema in otherwise healthy climbers but not enough to fulfill the clinical criteria of HAPE.23 Indeed, one must exercise caution in recommending the use of LUS to define a diagnosis of HAPE. Although patients who were diagnosed by Fagenholz with HAPE did have significantly more B-lines compared to other subjects at the same altitude, the clinical relevance of observing small numbers of B-lines on LUS has yet to be established.13 It is important to recognize that the total numbers of B-lines recorded on our study were small. This was probably due to the choice to use an 8-zone examination rather than the 28-zone examination advocated by Fagenholz *et al*.11 The 8-zone examination has been shown to be equally sensitive and specific at identifying the *presence* of interstitial fluid but the total B-line score will inevitably be smaller. In our subjects, there were no differences in the number of B-lines in each zone scanned.12 This suggests that, in the presence of interstitial lung fluid, the more lung zones scanned, the more B-lines would be found. However, further work is needed to examine the correlation between the number of intercostal spaces scanned, the LUS B-line score and the quantity of edema.

**Limitations**

The multiple B-lines observed in this study were typical of those seen in pulmonary interstitial edema but the differential diagnosis for this sign does include interstitial pneumonia or pneumonitis. However, all subjects were acclimatized at the time of the study and none reported symptoms of productive cough or fever prior to the test. The rapid resolution of the B-lines over a two to four hour period following the test makes this differential very unlikely but the absence of other investigations in the field mean it could not conclusively be excluded. A further limitation was our inability to perform Doppler echocardiography during this study. However, whilst would have been desirable to have an estimate of PA pressure before and after HIIE, it would have been extremely difficult to perform repeated Tricuspid valve Doppler measurements during the multiple short rest periods that characterized this exercise test. Therefore, although echocardiography is a reliable estimate of PA pressure and does correlate albeit weakly with B-lines, the only accurate way to demonstrate our hypothesis would be through direct measurement.24,25

Ideally a blinded independent operator would have confirmed the quantification of B-lines. However, blinded confirmation was not possible due to equipment limitations in the recording of images, therefore steps were taken to reduce the impact of this limitation by using two or more independent observers at the time of the LUS.

**Conclusion**

This study provides the first model for exercise-induced interstitial fluid in otherwise asymptomatic individuals at altitude. Performing severe intermittent strenuous exercise led to a fall in peripheral oxygen saturation and an increase in pulmonary interstitial edema as demonstrated by an increase in B-lines. Interstitial edema may contribute to the dyspnea and reduced performance, often experienced at altitude and may be a precursor to HAPE in susceptible individuals. The reliability of HIIE at inducing LUS B-lines make it a useful model in evaluating pulmonary edema formation at altitude.

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**Table 1. Time to complete high intensity intermittent exercise test, ultrasound B-line count (sum of eight sites), peripheral arterial oxygen saturation and heart rate for each exposure**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | HIIE  test time  (s) | B-line  (count) | | | | | Peripheral arterial oxygen saturation  (%) | | | | | Heart rate  (beats·min-1) | |
|  |  | Baseline | 0 | 1 | 2 | 4  (n = 5) | Baseline | 0 | 1 | 2 | 4  (n = 5) | Baseline | 0 | |
| Sea level  (n = 11) | 656 ± 151 | 0 | 0 | - | - | - | 99 ± 1 | 97 ± 1 | - | - | - | 62 ± 12 | 170 ± 22 | |
| Acute  (n = 13) | 1090 ± 298 | 0 | 2 ± 2\*\* | - | - | - | 90 ± 4 | 83 ± 6\*\*++ | - | - | - | 81 ± 16++ | 155 ± 21++ | |
| Sub-acute  (n = 13) | 884 ± 243 | 7 ± 4 | 17 ± 5\*\* | 12 ± 5 | 9 ± 6 | 5 ± 4 | 88 ± 4 | 80 ± 8\*\*++ | 86 ± 6 | 85 ± 5 | 88 ± 8 | 87 ± 14++ | 159 ± 14++ | |

Notes: HIIE = high intensity intermittent exercise. Numbers (0, 1, 2, 4) indicate the hours post-exercise the measurement was made. The B-line count represents the sum of the mean for two operators at each of the eight sites examined. Sample size equal to the number of participants for that condition unless otherwise indicated. \*\*Greater than baseline, *P* < 0.01; ++ significantly different to sea level, *P* < 0.01.

**![A screenshot of a cell phone

Description automatically generated]()**

**Figure 1. Ultrasound lung comet (ULC) count before and after exercise at 4090m**

Note: \*\* > Baseline *P* < 0.01