ORIGINAL ARTICLE

Increased resting bronchial tone in normal subjects acclimatised to altitude

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Background: Normal subjects frequently experience troublesome respiratory symptoms when acclimatised to altitude. Bronchial hyperresponsiveness (BHR) and full and partial flow-volume loops were measured before and after ascent to 5000 m altitude to determine if there are changes in resting bronchial tone and BHR that might explain the symptoms.
Methods: BHR to histamine was measured using a turbine spirometer to record partial and full flow-volume curves and expressed as log dose slopes. Twenty one subjects were tested at sea level and after acclimatisation at 5000 m altitude.
Results: No significant change in log dose slope measurements of forced expiratory volume in 1 second occurred after acclimatisation, and the maximal expiratory flow with 30% of forced vital capacity remaining ($MEF_{30\%}$) rose on the full loop and fell on the partial loop. Their ratio (full divided by partial) rose on average by 0.28 (95% confidence limits 0.14 to 0.42) from the mean (SD) sea level value of 0.87 (0.20).
Conclusions: There is no increase in BHR in normal subjects acclimatised to altitude but an increase in resting bronchial tone occurs that could be released by deep inspiration. This may be the result of increased cholinergic tone.

Normal subjects who are exposed to the cold hypoxic environment at high altitude often develop respiratory symptoms,[1] the aetiology of which is unknown. Reports on the effect of both cold exposure and hypoxia on bronchial responsiveness in asthmatic subjects are not consistent. In asthmatic subjects acute isocapnic hypoxia has been found to leave resting airway resistance unchanged[2] but causes an increased bronchial responsiveness to methacholine challenge.[3] However, another study in asthmatic subjects found a reduction in bronchial hyperresponsiveness (BHR) at altitude.[4] We have studied whether exposure to altitude changes resting bronchial tone or BHR in normal subjects by recording partial flow-volume curves[5] to increase the measurement sensitivity to changes in bronchial responsiveness and to assess changes in resting bronchial tone.[6]

METHODS

The studies were approved by the local research ethics committee of St Mary's Hospital, London and informed consent was obtained from all subjects.

Subjects were recruited from a group of over 50 climbers and trekkers on a British expedition to the Kangchenjunga region of Nepal during the post-monsoon season of 1998. Full and partial flow-volume loops were recorded before and after histamine challenge at sea level (barometric pressure 100.7–101.5 kPa) and after trekking to base camp over a period of 14–17 days (altitude 5000 m, barometric pressure 54.6–56.0 kPa). Two subjects with a history of asthma were excluded, as were two subjects who developed unpleasant side effects from the histamine at sea level. Of 38 subjects who started the study, 17 were found post hoc to have taken either salmeterol or nedocromil as part of another study and so were excluded leaving 21 subjects (seven women) for analysis. Measurements at altitude were made inside a tent at temperatures between +9 and +26°C. Ambient temperatures at base camp ranged from –7°C in the tents at night to +10°C outside in the day and up to +26°C in the tents during the day.

Before the expedition BHR was measured in eight subjects (one woman) of mean (SD) age 35.3 (7.4) years (range 23–49) at sea level who were on no treatment. BHR was then measured on consecutive days at a simulated altitude of 5000 m (39.5 kPa) in a hypobaric chamber which, on the first day, was in normoxic hypobaria and on the second day was without supplemental oxygen (acute hypoxic hypobaria).

Spirometric tests and derivation of values

Before histamine challenge three maximal forced expiratory manoeuvres were recorded from each subject using a MicroMedical turbine device (MicroLoop) with specially derived software on a laptop computer. This type of recording device was specifically chosen because ambient air temperature, humidity, and density do not affect its accuracy.[7] At each stage of the histamine challenge subjects performed a partial forced expiratory manoeuvre starting at a point above functional residual capacity (FRC) followed immediately by a full inspiration and then a maximal forced expiratory manoeuvre as one continuous sequence.[5] Subjects were asked to take a small breath in as if they were about to count to 10 out loud and then start the partial forced expiration. This was to help ensure that the start of the partial manoeuvre was between 50% and 80% of total lung capacity (TLC). Blows starting outside this range were rejected. Figure 1 shows partial and maximal manoeuvres (before and after histamine) each as a single continuous sequence.

From the maximal manoeuvre the following indices were derived: forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), peak expiratory flow (PEF), and FEV_1 as a percentage of FVC (FEV_1%). On each manoeuvre the instantaneous flows were recorded at those volumes representing the points where 30% of the largest FVC recorded still had to be expired ($MEF_{30\%}$). The first moment ($\alpha_1$75%) of the maximal blow truncated at 75% of the FVC was determined.[8]

From the partial manoeuvre the instantaneous flow with 30% of the largest FVC still to be expired was recorded ($MEF_{30\%}P$) and the first moment of the partial manoeuvre up

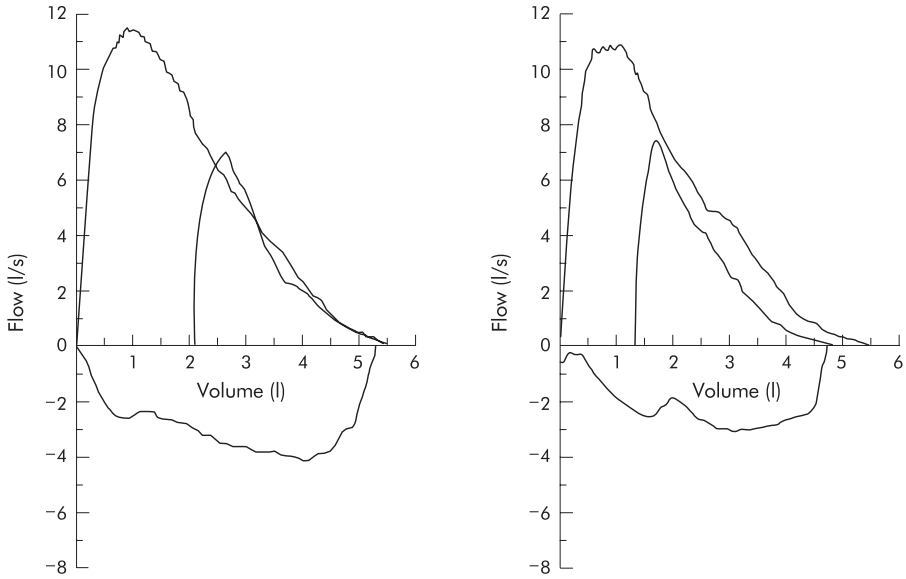

Figure 1 A partial followed by a full flow–volume curve recorded as a single continuous manoeuvre before bronchial challenge (left) and after the highest dose of histamine (right).

to 75% of the volume for the partial manoeuvre was derived ($\alpha_1$75%P). The ratio of the maximal divided by the partial flow (MP ratio) with 30% of the largest FVC to be expired was derived ($MP_{30\%}$). An MP ratio greater than unity indicates an important degree of resting bronchial tone that is removed by full inflation to TLC before the maximal manoeuvre.[6] For those indices with prediction equations, the subjects' baseline values were related to their predicted value using the method of standardised residuals (SR). For FVC, FEV_1, FEV_1%, and PEF the European Coal and Steel Community (ECSC) equations were used[9] to derive FVCSR, for example, and for $\alpha_1$75% previously published equations were used.[10]

Histamine challenge

Subjects undertook a histamine bronchial challenge test using a modified Yan method[11] with histamine being delivered by a Sonix 2000 nebuliser (Clement Clarke International, Harlow, UK). Each subject took three slow vital capacity inhalations from the nebuliser, each over 5 seconds followed by a 5 second breath hold before exhalation. The subject then breathed quietly for 30 seconds before recording a partial/full flow-volume curve. Slow deep inspiration has been shown to have less effect on respiratory resistance than fast inspiration.[12] Because the lower air density at altitude might reduce nebuliser output, we first verified that the output of our nebulisers (0.75 ml fluid/min) was unchanged by experiments at sea level and at a simulated altitude of 5000 m in a hypobaric chamber. Following the first challenge of saline, doubling doses of histamine were administered starting with 0.0475 mg and increasing to a cumulative maximum of 3.04 mg. Higher doses were not delivered to avoid any possible complications when testing in a remote area at altitude. Following each dose, partial and maximal forced manoeuvres were recorded as above. No subjects had a significant fall in FEV_1 after saline alone. Tests were stopped if a 20% fall in FEV_1 occurred (five subjects at altitude, two subjects at sea level).

BHR was expressed as log dose slope[13] which is log to the base 10 of the percentage change in the index from the post saline value divided by the cumulative dose (in mg) of histamine administered plus 1. Thus, for FEV_1:

$$LDSFEV_1 = \log_{10}((\%\ \text{change in } FEV_1)/(\text{total cumulative dose}) + 1)$$

The reason for adding 1 to the calculation is to reduce the number of subjects with missing data because their function index changes a small amount in the opposite direction to that expected (e.g. an increase in FEV_1). If this percentage change divided by the dose administered exceeds 1, a log value still cannot be derived. For a full dose of histamine an increase of more than 1.5% in FEV_1 would still lead to a missing value. Increasing the added value can further diminish the number of subjects with missing data, but at the expense of reducing the spread of data between subjects. We have left a unitary addition. The log transformation is necessary to allow comparison of data between and within subjects.[14] The within person repeatability of this method has been found to be acceptable[15] with a mean within subject variance of 0.02 for two tests of $LDSFEV_1$ on separate days, giving an intraclass correlation coefficient of 0.87 and mean (SD) difference of 0.04 (0.19).

Statistical analysis

Statistical analyses were performed using SPSS for Windows (version 10). Comparisons between data before and after any exposure were by paired *t* test. The Friedman non-parametric equivalent of a two way ANOVA was used to analyse the data from the hypobaric chamber experiments as the variances were unequal for some data. A significance level of less than 5% was taken to reject the null hypothesis, with Bonferroni correction for multiple comparisons where appropriate.

RESULTS

The demographic data and baseline lung function of the 21 subjects are shown in table 1. Table 2 shows the results for the eight subjects who were tested in a hypobaric chamber before the expedition. While PEF increased under both hypobaric conditions together with a shorter mean transit time to 75% of FVC (fall in $\alpha_1$75%), there were no significant changes in other indices, including BHR and the MP ratio, irrespective of whether this was under normoxic or hypoxic conditions.

Table 3 shows the mean (SD) results at sea level, together with the mean change and confidence limits for the effect of altitude on the expedition. There was no significant change in FEV_1 but, as expected, there was a significant increase in PEF and reductions in FVC and $\alpha_1$75%. There was a trend for an increase in $MEF_{30\%}$ on the full loop at altitude and a fall in these flows on the partial loop that is reflected by a significant increase in the MP ratio at altitude (fig 2). For the indices of BHR there were no significant differences between any of the measures at sea level or at altitude. The $MP_{30\%}$ after histamine

Table 1 Demographic data and baseline indices for the 21 subjects studied

	Mean	Median	SD
Age (years)	40.9	41	14.0
Height (m)	1.76	1.76	0.09
FVC (l)	4.95	4.98	0.98
FVCSR	0.87	0.69	1.10
FEV_1 (l)	3.87	3.71	0.77
FEV_1 SR	0.32	0.27	0.93
FEV_1 (%)	78.4	78.7	5.45
FEV_1 (%SR)	–0.28	–0.36	0.60
PEF (l/s)	8.85	8.76	2.08
PEFSR	0.15	0.15	1.26
$\alpha_1$75% (s)	0.31	0.31	0.05
$\alpha_1$75%SR	0.19	0.23	0.75

FVC=forced vital capacity; FEV_1=forced expiratory volume in 1 second; PEF=peak expiratory flow; SR=standardised residuals; $\alpha_1$75%=first moment truncated at 75% of FVC.

Table 2 Mean (SD) values for indices from the eight subjects who underwent simulated altitude in a hypobaric chamber under normoxic and hypoxic conditions.

	Code	Mean (SD)	p value
FVC	1	5.46 (0.95)	0.22
	2	5.26 (0.84)	
	3	5.28 (0.88)	
FEV_1	1	4.25 (0.58)	0.69
	2	4.20 (0.51)	
	3	4.30 (0.55)	
PEF	1	8.74 (1.26)	0.002*
	2	10.48 (1.31)	
	3	10.40 (1.39)	
$\alpha_1$75%	1	0.33 (0.06)	0.002*
	2	0.28 (0.06)	
	3	0.28 (0.06)	
$MEF_{30\%}$	1	2.43 (0.52)	0.04
	2	2.39 (0.61)	
	3	2.61 (0.69)	
$MP_{30\%}$	1	0.97 (0.14)	1.0
	2	1.02 (0.19)	
	3	0.99 (0.18)	
$LDSFEV_1$	1	0.66 (0.40)	0.37
	2	0.51 (0.61)	
	3	0.63 (0.45)	
LDS$\alpha_1$75%	1	0.77 (0.52)	0.88
	2	0.91 (0.34)	
	3	0.72 (0.51)	
$LDSMEF_{30\%}$	1	1.01 (0.32)	0.45
	2	0.85 (0.41)	
	3	1.11 (0.39)	

FVC=forced vital capacity; FEV_1=forced expiratory volume in 1 second; PEF=peak expiratory flow; $\alpha_1$75%=first moment truncated at 75% of FVC; $MEF_{30\%}$=maximum expiratory flow with 30% of FVC still to be expired; $MP_{30\%}$=ratio of $MEF_{30\%}$ on maximum flow loop divided by $MEF_{30\%}$ from partial loop; LDS=log dose slope of index; code 1=sea level; 2=normoxic hypobaria; 3=hypoxic hypobaria. *p<0.05 (Friedman non-parametric test with Bonferroni correction).

challenge increased from 1.22 (0.46) to 1.64 (0.86) (mean difference 0.42, 95% confidence limits –0.05 to 0.88) at altitude, which was a larger increase than before the histamine challenge but the increase was not statistically significant.

DISCUSSION

This is the first study to show an increase in resting bronchial tone that could be reduced by deep inhalation in non-asthmatic subjects after acclimatisation at 5000 m. No evidence of increased BHR was found in these subjects despite this increase in resting tone. The effect of altitude on BHR is not clear. Our finding of no change in BHR at altitude has been

Table 3 Mean (SD) values of indices before histamine challenge, log dose slopes at sea level and mean change at 5000 m with 95% confidence interval (CI) for the difference

	Sea level	Altitude minus sea level	
	Mean (SD)	Mean	95% CI
FVC	4.95 (0.98)	–0.18*	–0.27 to 0.08
FEV_1	3.87 (0.77)	0.02	–0.10 to 0.13
PEF	8.85 (2.08)	1.71***	1.28 to 2.13
FEV_1 %	78.37 (5.45)	2.98**	1.52 to 4.43
$\alpha_1$75%	0.31 (0.05)	–0.05***	–0.06 to –0.04
$MEF_{30\%}$	2.18 (0.77)	0.07	–0.12 to 0.26
$\alpha_1$75%P	0.33 (0.08)	–0.03	–0.07 to 0.01
$MEF_{30\%}$P	2.45 (0.70)	–0.52	–0.92 to –0.12
$MP_{30\%}$	0.87 (0.20)	0.28**	0.14 to 0.42
$LDSFEV_1$	0.68 (0.44)	0.00	–0.32 to 0.33
LDS$\alpha_1$75%	0.80 (0.45)	–0.04	–0.49 to 0.40
$LDSMEF_{30\%}$	0.89 (0.39)	–0.06	–0.37 to 0.26
LDS$\alpha_1$75% P	1.19 (0.38)	0.01	–0.40 to 0.42
$LDSMEF_{30\%}$P	1.31 (0.32)	–0.09	–0.49 to 0.30

For definition of abbreviations see footnote to table 2. The suffix P denotes that this index is taken from the partial loop. ***p<0.0005; **p<0.005; *p<0.05 (paired *t* test with Bonferroni correction for multiple comparisons).

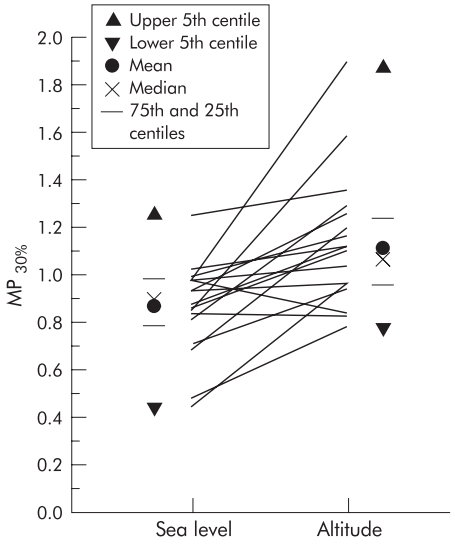

Figure 2 Change in pre histamine $MP_{30\%}$ ratio from sea level to altitude.

noted by others in asthmatic subjects when testing with methacholine,[16] but in the same subjects BHR to adenosine 5′-monophosphate was reduced. Other studies in asthmatic subjects have found a reduction in BHR[4 17] which may be attributed to a reduction in allergen exposure. One study has also used histamine to test BHR at altitude and found an increase in BHR in asthmatic subjects returning to altitude after 2 weeks at sea level.[18] Our subjects were not asthmatic and so we would not expect any effect from possible allergen avoidance.

No other studies have used partial loop analysis at altitude so our methodology should be scrutinised for bias. Our use of a turbine spirometer avoids any effect of temperature or ambient pressure,[7] and the use of ultrasonic nebulisation ensured equivalent delivery at altitude. Histamine is a non-specific agent for provoking bronchoconstriction but has been extensively studied as a bronchial challenge agent and shown to be safe with results being reproducible under controlled conditions. We used log dose slopes that are Normally distributed allowing comparisons to be made to detect a group change in

responsiveness.[14] Since respiratory symptoms such as cough are experienced by up to 42% of subjects at altitude,[19] a group change in BHR should be evident if it was related to this symptom. We have not found convincing evidence for such a change.

PEF was increased by nearly 20% from sea level to altitude in our subjects, as was expected from the reduced air density at altitude. This effect would not alter the output of the nebulisers but it might alter the flow characteristics in airways during tidal breathing when histamine was being inhaled and so influence the deposition of histamine in the airways. Histamine acts on the smooth muscle of the main bronchi and is more effective when delivered here than at the periphery or upper airway.[20] We are not aware that inhaling a lower density mixture with histamine would preferentially deposit in peripheral rather than central airways and so reduce the efficacy of histamine in producing bronchoconstriction. Since five of our subjects developed marked bronchoconstriction in tests at altitude, we believe that deposition to the relevant areas in the lungs was being achieved.

Inhalation of cold air transiently increases bronchial reactivity to other challenge agents such as histamine in non-asthmatic subjects.[21] Cold air appears to have a constrictor effect on the central airways of healthy lungs while inducing a more generalised narrowing of the airways in asthma.[22] Our subjects were retested during the day when acclimatised to altitude and settled inside tents with relatively normal or high ambient temperature, so the acute effect of cold air inhalation was not likely to affect our results.

Our finding of increased bronchial tone should be considered in the light of current concepts concerning airway smooth muscle. When smooth muscle contracts it becomes stiff as a result of the binding of myosin to actin and of temporary bridging with the cytoskeletal matrix. These latter bridges do not turn over very quickly. The rhythmical pattern of tidal breathing and deep sighs that form part of natural breathing make these bridges break down sooner than they otherwise would.[23] At altitude during sleep the normal duty cycle of breathing can become extremely disorganised because of the prevailing hypocapnia.[24] With a slowing and temporary cessation of the tidal breathing pattern during the night, the smooth muscle will become less pliant due to increased levels of cross bridging. This could account for an increase in bronchial tone during the night but would not easily explain the fact that we found resting tone to be increased during the day. However, it is possible that the tendency to tissue oedema that occurs at altitude[25] could influence the delicate balance with regard to airway smooth muscle plasticity.

Acute hypoxia has not been consistently found to affect bronchial reactivity in normal subjects.[26–28] We found no effect in our hypobaric chamber experiment, but accept that the number of subjects undertaking this study limits the power of this conclusion. It is known that low carbon dioxide concentrations lead to a rise in airways resistance in humans.[29] At altitude the arterial carbon dioxide tension is low, and with renal compensation the bicarbonate concentration becomes low, thus restoring pH to normal. Recent work using in vitro animal models has shown that hypocapnia does not affect resting bronchial tone but enhances the effect of carbachol in contracting smooth muscle.[30] This effect is thought to be mediated by intracellular alkalosis affecting L-type calcium channels. These studies were short term experiments with changes occurring in intracellular pH. Studies in rats acclimatised to hypoxia showed an increase in cholinergic tone[31] and this was thought responsible for the lower heart rate found in acclimatised rats. In humans, acute exposure to hypobaric hypoxia for up to 5 days increased sympathetic drive and reduced parasympathetic drive, but after acclimatisation the balance of drive was reversed.[32] Other data have suggested that, after acclimatisation, sympathetic activation in humans is downregulated and vagal drive is thus unmasked[33] leading to the observed lower exercise heart rate. We speculate that acclimatisation may lead to enhanced vagal influence on bronchial tone which could account for the increased resting tone observed.

In conclusion, there is an increase in resting bronchial tone but not in BHR with hypobaric hypocapnic hypoxia at altitude in acclimatised subjects. The hypothesis that parasympathetic action is responsible for the increase in resting tone needs further consideration.

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