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Effect of altitude on urinary leukotriene (LT) E_4 excretion and airway responsiveness to histamine in children with atopic asthma

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ABSTRACT: Asthmatic subjects who are resident at altitude may experience a deterioration in lung function following a stay at sea level. To determine whether measurement of urinary leukotriene E_4 (LTE_4) reflects changes in asthma severity and airway responsiveness, 14 allergic asthmatic subjects resident at altitude (1560 m, Davos, Switzerland) were studied.

Subjects were randomly divided into two groups. Measurements of baseline forced expiratory volume in one second (FEV_1), the concentration of histamine producing a 20% decrease in FEV_1, ($PC_{20}FEV_1$), serum total immunoglobulin E (IgE), eosinophil count, and urinary LTE_4 concentration were determined prior to and following a 2 week stay in The Netherlands (sea level) in eight subjects (4 males and 4 females, aged 14±0.5 yrs) (mean±SEM) and over a similar time period in six subjects (4 males and 2 females, aged 15±0.3 yrs) resident in Davos, Switzerland.

There was no significant difference in total IgE and eosinophil count, and no significant correlation between urinary LTE_4 and $PC_{20}FEV_1$ histamine, FEV_1, total IgE, and eosinophil count. In subjects returning to Davos from The Netherlands there was a significant increase in urinary LTE_4 from a baseline value of 16.9 pg·mg^{-1} creatinine (GM, range 0.3–101.7 pg·mg^{-1} creatinine) to 52.3 pg·mg^{-1} creatinine (GM, range 8.8–301.6 pg·mg^{-1} creatinine), a significant decrease in $PC_{20}FEV_1$ from 1.7 mg·ml^{-1} (GM, range 0.3–16.4 mg·ml^{-1}) to 0.9 mg·ml^{-1} (GM, range 0.1–>32 mg·ml^{-1}), and a significant fall in FEV_1 from 3.0±0.3 to 2.8±0.3 *l* (mean±SEM). There was *no* significant change in urinary LTE_4, FEV_1 or $PC_{20}FEV_1$ histamine during a similar period of time in subjects resident in Davos.

Thus, following a visit to sea level, children with atopic asthma who are usually resident at altitude exhibit a fall in FEV_1 and an increase in airway responsiveness to histamine, which is associated with a threefold increase in urinary LTE_4 excretion.

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The cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) are derived from arachidonic acid by the action of 5-lipoxygenase, which generates 5-hydroperoxyeicosatetraenoic acid and then leukotriene A_4 (LTA_4) [1–3]. LTA_4 is metabolized by the addition of glutathione to form LTC_4. LTC_4 may be converted by γ-glutamyltranspeptidase to generate LTD_4, which is converted by a dipeptidase to yield LTE_4 [4, 5]. *In vitro* the cysteinyl leukotrienes, LTC_4, LTD_4 and LTE_4, contract smooth muscle and enhance microvascular permeability [6–8]. In humans they are potent bronchoconstrictor agents when inhaled, and increase nonspecific bronchial hyperresponsiveness [9–12].

In man, there is rapid metabolism of LTC_4 to LTD_4 and then to LTE_4. LTE_4 may be further metabolized to oxidation products, which are excreted into bile and urine [13–16]. Combined reversed-phase high performance liquid chromatography (RP-HPLC) and radioimmunoassay (RIA) enables urinary LTE_4 to be measured [17], and the values have been used as an estimate of the production of cysteinyl leukotrienes *in vivo*. An increase in LTE_4 excretion occurs during an acute exacerbation of asthma, after antigen challenge in allergic asthmatic subjects [18–22], and following aspirin-induced asthma [23]. Antigen-induced bronchoconstriction is attenuated by prior treatment with leukotriene receptor antagonist [24, 25], supporting a role for the cysteinyl leukotrienes in acute allergic bronchoconstriction. The improvement in basal lung function after ingestion of an oral active LTD_4 receptor antagonist [26], and improvement of pulmonary lung function following long-term administration of leukotriene receptor antagonists [27], supports a

role for the cysteinyl leukotrienes in influencing basal bronchial tone.

Residence at altitude may be beneficial for subjects with atopic asthma, possibly due to the low concentration of house dust mite antigen [28, 29]. A deterioration in lung function may be observed on return to sea level, and this has been attributed to the increased exposure to allergen(s) and pollutants [28–31]. This study involved asthmatic children who were resident at The Netherlands Asthma Centre at altitude, since it had been observed that a return to The Netherlands may be accompanied by a deterioration in lung function.

To assess whether the deterioration in lung function in atopic asthmatic subjects residing at altitude after transient exposure to low altitude is accompanied by elevations in urinary LTE_4, we have measured urinary LTE_4 in eight asthmatic subjects normally resident at altitude who returned to sea level for a short visit and six asthmatic subjects resident at altitude over a similar period of time.

Methods

Subjects

Fourteen asthmatic subjects were studied (table 1). Asthma was defined by a history of episodic wheezing and a >20% reversibility of resting FEV_1 following 400 μg inhaled albuterol. Atopic asthmatic subjects demonstrated a >3 mm wheal as compared to the diluent control in response to skin-prick tests to at least two common aeroallergens: grass pollen, tree pollen, cat dander, dog hair, *Dermatophagoides pteronyssinus* and *D. farinae*. All subjects were positive to house dust mite extracts. Subjects had not taken antihistamines in the month prior to the study, and no subject had experienced an upper respiratory tract infection in the preceding month, or during the study. The study protocol was approved by The Netherlands Asthma Centre Hospital Ethics Committee, and written informed consent was provided by the parent of each subject studied.

Table 1. – Subject characteristics

Subject No.	Age yrs	Sex	Atopy	FEV_1 % pred	TX
Asthmatic subjects returning to The Netherlands					
1	15	M	+	93	A
2	15	M	+	87	AB
3	15	F	+	92	ABD
4	13	F	+	115	AB
5	15	M	+	106	AB
6	12	F	+	109	AB
7	12	M	+	83	AC
8	15	F	+	100	ABC
Mean	14		98.1		
SEM	0.5		3.9		
Asthmatic subjects remaining in Davos					
9	16	M	+	115	AB
10	14	F	+	121	AB
11	16	M	+	92	AB
12	14	M	+	91	AB
13	15	M	+	107	AB
14	15	F	+	53	AB
Mean	15		96.5		
SEM	0.3		9.9		

M: male; F: female; FEV_1: forced expiratory volume in one second; TX: treatment; A: inhaled albuterol; B: inhaled corticosteroid; C: cromoglycate; D: nedocromil. Mean is the arithmetic mean.

Study protocol

The Netherlands Asthma Centre in Davos is a clinic at moderate altitude (1,560 m). Asthmatic children may stay for up to 9 months and during this time return to The Netherlands for a short visit. In a prospective, randomized fashion, mild asthmatic subjects who had been resident in The Netherlands Asthma Centre, Davos, Switzerland (1,560 m) for at least one month, were selected to participate in the study following a full clinical history, examination and skin-prick tests to common inhaled aeroallergens. Subjects were randomly divided into two groups. During the same month, one group remained in Davos, whilst the second group returned to The Netherlands (360 m) for 14 days and then returned to Davos. All subjects attended the laboratory on two separate study days at the same time of day. In asthmatic subjects who remained in Davos, the study days were separated by 3 weeks, whereas in the group of asthmatic subjects who returned to The Netherlands, the study days were performed within 48 h of leaving for The Netherlands and within 48 h of returning to Davos. Medication was withheld for 8 h prior to each study day. On each study day, blood was withdrawn for total IgE measurement and eosinophil count. A urine sample was collected for LTE_4 measurement, and then, within one hour, a histamine inhalation challenge was performed. Two subjects refused blood sampling on the second study day. One further subject selected in addition to the 14 participating in the study was excluded from the study, since medication was altered between the 2 study days when the subject returned to The Netherlands and maintenance therapy with steroids was changed. There was no change in medication between the two study days in the other asthmatic subjects studied.

Histamine inhalation challenge was performed using the Asthma Provocation System (APS) dosimeter (Jaeger, Wuzzburg, Germany), which delivers compressed air at a pressure of 1.6 bar (22.8 psi) for a duration of 0.6 s from the start of each breath. Under these conditions, the nebulizer delivers droplets with a mass median aerodynamic diameter of 1.9 μm. The output of the nebulizer is 9.3 $\mu l \cdot breath^{-1}$. Measurements of FEV_1 were made using a Jaeger Masterlab Spirometer. Three measurements of FEV_1 were made at each time-point and the mean value was recorded. Provided baseline FEV_1 was greater than 70% predicted for the patient, inhalation challenge proceeded. Each inhalation started at functional

residual capacity and terminated at approximately 70% baseline vital capacity; a 5 s breathhold was maintained at the end of each inhalation. Subjects inhaled control solution (five breaths of normal saline). FEV_1 measurements were made at 1 and at 3 min after each inhalation. If the decrease in FEV_1 was <10% baseline value, subjects underwent inhalation challenge with histamine. Serial twofold increasing concentrations of histamine diphosphate (Leiden University Hospital, Leiden, The Netherlands) were inhaled from a concentration of 0.03 $mg \cdot ml^{-1}$ up to a maximum concentration of 32 $mg \cdot ml^{-1}$. Doubling concentrations of histamine were administered until the FEV_1 had fallen by >20% baseline value. The provocation concentration of histamine producing a 20% fall in FEV_1 ($PC_{20}FEV_1$) was determined from the log concentration histamine response curve by linear interpolation.

Measurement of urinary LTE_4

Urine was collected prior to inhalation of histamine. The volume of urine was recorded and a 50 ml aliquot saved. The free radical scavenger, 4 hydroxy-2,2,6,6-tetramethyl-1-piperidino-oxy free radical (4-hydroxy TEMPO; Aldrich Chemical Co., Milwaukee, WI, USA) was added at a final concentration of 1 mM, and the samples adjusted to pH 9.0 with NaOH to stabilize endogenous leukotriene metabolites. The samples were coded and stored at -70°C until measurements of LTE_4 were performed by RP-HPLC and RIA, as described previously [17].

Statistical analysis

Values for urinary LTE_4 and $PC_{20}FEV_1$ histamine were logarithmically transformed prior to analysis, and the results were expressed as geometric mean (GM). For $PC_{20}FEV_1$ histamine, statistical analysis was only performed on subjects in whom a $PC_{20}FEV_1$ histamine was determined. The Wilcoxon samples test was used to compare FEV_1, urinary LTE_4 and $PC_{20}FEV_1$ histamine between the two groups of subjects on study day one, and the changes in FEV_1, urinary LTE_4, $PC_{20}FEV_1$ histamine, total IgE, and eosinophil count between study day one and two in each group of asthmatic subjects. The relationship between urinary LTE_4 and FEV_1, $PC_{20}FEV_1$ histamine, total IgE and eosinophil count in the subjects was analysed using Pearsons correlation coefficient.

Results

Lung function

The FEV_1 values for individual subjects in the two groups on study day one are shown in figure 1 and table 2. There was no significant difference between the baseline FEV_1 in the two groups of asthmatic subjects studied, being 3.0±0.3 *l* (mean±SEM) and 2.9±0.3 *l* (p=0.9)

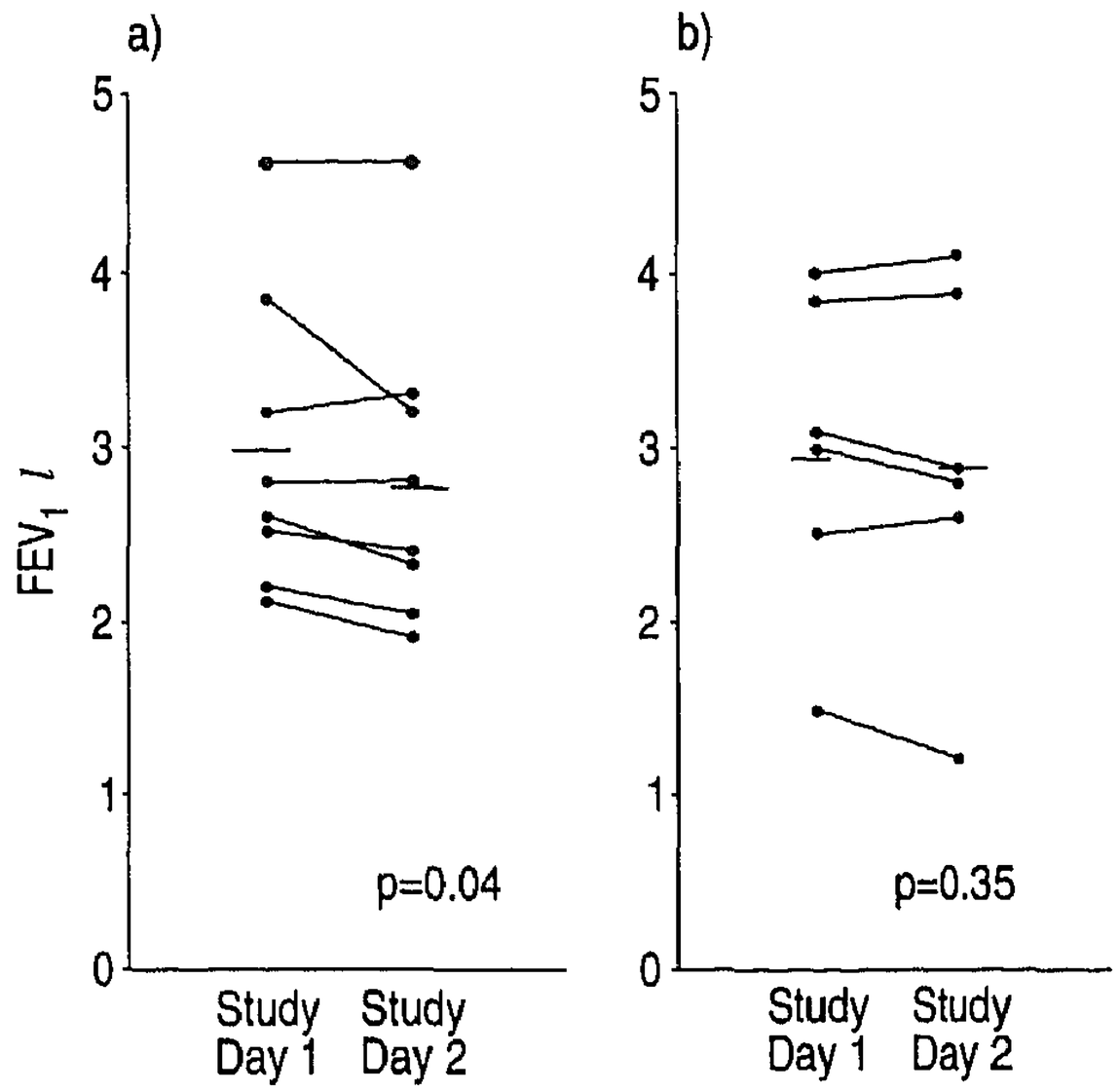

Fig. 1. – The FEV_1 on study day one and two in individual asthmatic subjects: a) returning to The Netherlands; or b) resident in Davos. Bars indicate arithmetic mean. FEV_1: forced expiratory volume in one second.

in subjects returning to The Netherlands and those subjects remaining in Davos, respectively.

In the group of subjects who returned to The Netherlands, there was a significant decrease in FEV_1 on return to Davos from 3.0±0.3 to 2.8±0.3 *l* (mean±SEM) (p=0.04). In subjects who remained in Davos, there was no significant difference in FEV_1 which was 2.9±0.3 and 2.9±0.4 *l* (p=0.35) on study day one and study day two, respectively (fig. 1 and table 2).

Airway response to histamine

The $PC_{20}FEV_1$ histamine in individual subjects on the two study days are shown in figure 2 and table 2. Subject No. 7 (study day 1) and No. 8 (study day 1 and 2) (table 2) did not respond with a 20% fall in FEV_1 after the maximum dose of histamine was administered. Histamine challenge was not performed in subject No. 14 because he had a resting FEV_1 <65% of predicted FEV_1 on study day one and two. There was no significant difference in $PC_{20}FEV_1$ histamine on study day one between the two groups of asthmatic subjects. The $PC_{20}FEV_1$ histamine was 1.7 $mg \cdot ml^{-1}$ (GM, range 0.3–16.4 $mg \cdot ml^{-1}$) (n=5) and 1.5 $mg \cdot ml^{-1}$ (GM, range 0.3–22 $mg \cdot ml^{-1}$) (n=5) (p=0.5) in asthmatic subjects returning to The Netherlands and those subjects remaining in Davos, respectively. There was a significant decrease in $PC_{20}FEV_1$ histamine from 1.7 $mg \cdot ml^{-1}$ (GM, range 0.3–16.4 $mg \cdot ml^{-1}$) to 0.9 $mg \cdot ml^{-1}$ (GM, range 0.1–5.2 $mg \cdot ml^{-1}$) (p=0.04) (n=5) following a 14 day visit to The Netherlands. In subjects who remained in Davos, there was no significant difference in $PC_{20}FEV_1$ histamine, which was 1.5 $mg \cdot ml^{-1}$ (GM, range 0.3–22 $mg \cdot ml^{-1}$) and 1.5 $mg \cdot ml^{-1}$ (GM, range 0.3–32 $mg \cdot ml^{-1}$) (p=0.89) (n=5) on study day one and two, respectively.

Table 2. – The FEV_1, $PC_{20}FEV_1$ histamine and urinary LTE_4 in asthmatic subjects on the two study days

Subject No.	FEV_1 *l*		$PC_{20}FEV_1$ histamine $mg \cdot ml^{-1}$		LTE_4 $pg \cdot mg^{-1}$ creatinine	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Asthmatic subjects returning to The Netherlands						
1	2.6	2.3	16.4	5.2	37.0	130.0
2	3.8	3.2	1.0	0.4	0.3	8.8
3	2.2	2.0	0.3	0.1	40.2	173.6
4	4.6	3.3	1.5	1.3	19.6	14.0
6	2.1	1.9	0.8	0.6	101.7	301.6
7	2.5	2.4	>32.0	13.0	11.3	46.3
8	2.8	2.8	>32.0	>32.0	25.7	134.0
Mean	3.0	2.8	1.7	0.9	16.9	52.3
SEM	0.3	0.3				
Asthmatic subjects remaining in Davos						
9	3.8	3.8	0.3	0.6	10.6	11.7
10	3.0	2.8	1.3	0.7	5.9	5.1
11	3.1	2.8	0.3	0.3	9.4	8.1
12	2.5	2.6	22.0	32.0	14.0	22.0
13	4.0	4.1	2.4	2.0	41.9	25.0
14	1.4	1.2	ND	ND	33.0	13.0
Mean	2.9	2.9	1.5	1.5	14.9	12.0
SEM	0.3	0.4				

LTE_4: leukotriene E_4: $PC_{20}FEV_1$: concentration of histamine producing a 20% decrease in FEV_1; ND: not determined. The mean for FEV_1 is the arithmetic mean. The mean for $PC_{20}FEV_1$ histamine is the geometric mean of subjects with a $PC_{20}FEV_1$ histamine (subjects Nos. 1–6). The mean for LTE_4 concentration is the geometric mean. For further abbreviations see legend to table 1.

Urinary LTE_4 concentration

There was no significant difference between the urinary LTE_4 concentration on study day one between the two groups of asthmatic subjects. The LTE_4 concentration was 16.9 $pg \cdot mg^{-1}$ creatinine (GM, range 0.3–101.7 $pg \cdot mg^{-1}$ creatine) and 14.9 $pg \cdot mg^{-1}$ creatinine (GM, range 5.9–41.9 $pg \cdot mg^{-1}$ creatinine) (p=0.6) in asthmatic subjects returning to The Netherlands and remaining in Davos, respectively.

There was a significant increase in urinary LTE_4 concentration in the group of subjects returning to Davos from The Netherlands, from a baseline value of 16.9 $pg \cdot mg^{-1}$ creatinine (GM, range 0.3–101.7 $pg \cdot mg^{-1}$ creatinine) to 52.3 $pg \cdot mg^{-1}$ creatinine (GM, range 8.8–301.6 $pg \cdot mg^{-1}$ creatinine) (p=0.04) (fig. 3 and table 2). In contrast, there was no significant difference in urinary LTE_4 concentration in subjects resident in Davos, whose urinary LTE_4 concentration on study day 1 and 2 were 14.9 $pg \cdot mg^{-1}$ creatinine (GM, range 5.9–41.9 $pg \cdot mg^{-1}$ creatinine) and 12.0 $pg \cdot mg^{-1}$ creatinine (GM, range 5.1–25 $pg \cdot mg^{-1}$ creatinine) (p=0.24), respectively.

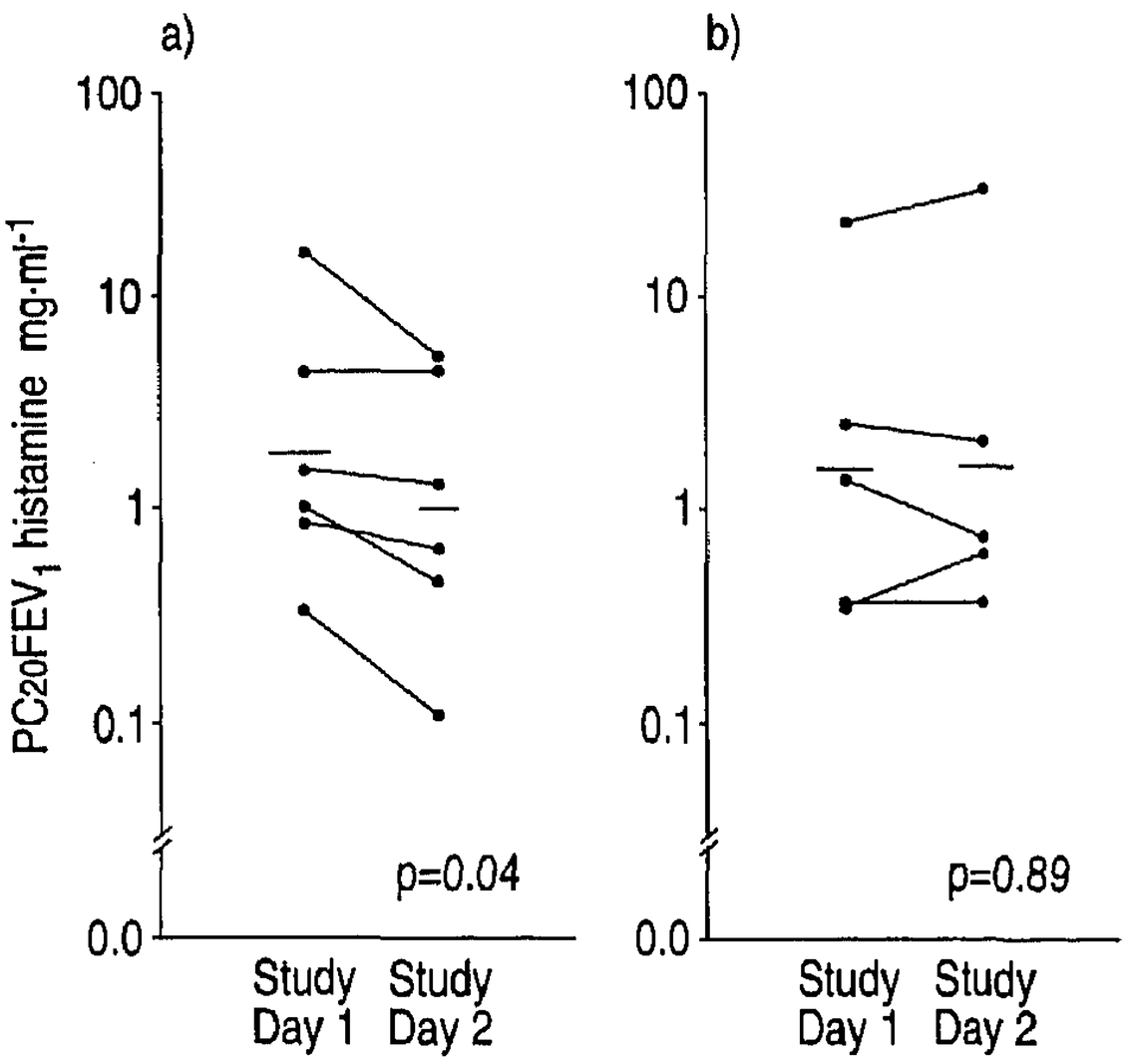

Fig. 2. – The $PC_{20}FEV_1$ histamine on study day 1 and 2 in individual asthmatic subjects: a) returning to The Netherlands; or b) resident in Davos. Bars indicate geometric mean. $PC_{20}FEV_1$: provocative concentration of histamine producing a 20% decrease in forced expiratory volume in one second.

IgE and eosinophil count

The total IgE level and eosinophil count in individual subjects is shown in table 3. There was no significant difference in total IgE levels or eosinophil counts in either group of subjects studied.

There was no significant correlation between urinary LTE_4 and FEV_1 (r=0.25, p=0.19; r=-0.5, p=0.91), urinary LTE_4 and $PC_{20}FEV_1$ histamine (r=0.08, p=0.48; r=<0.01, p=0.97), urinary LTE_4 and eosinophil count (r=0.18, p=0.26; r=<0.01, p=0.87), and urinary LTE_4 and total IgE level (r=0.2, p=0.26; r=0.146, p=0.45) in asthmatic subjects returning to The Netherlands or resident in Davos, respectively.

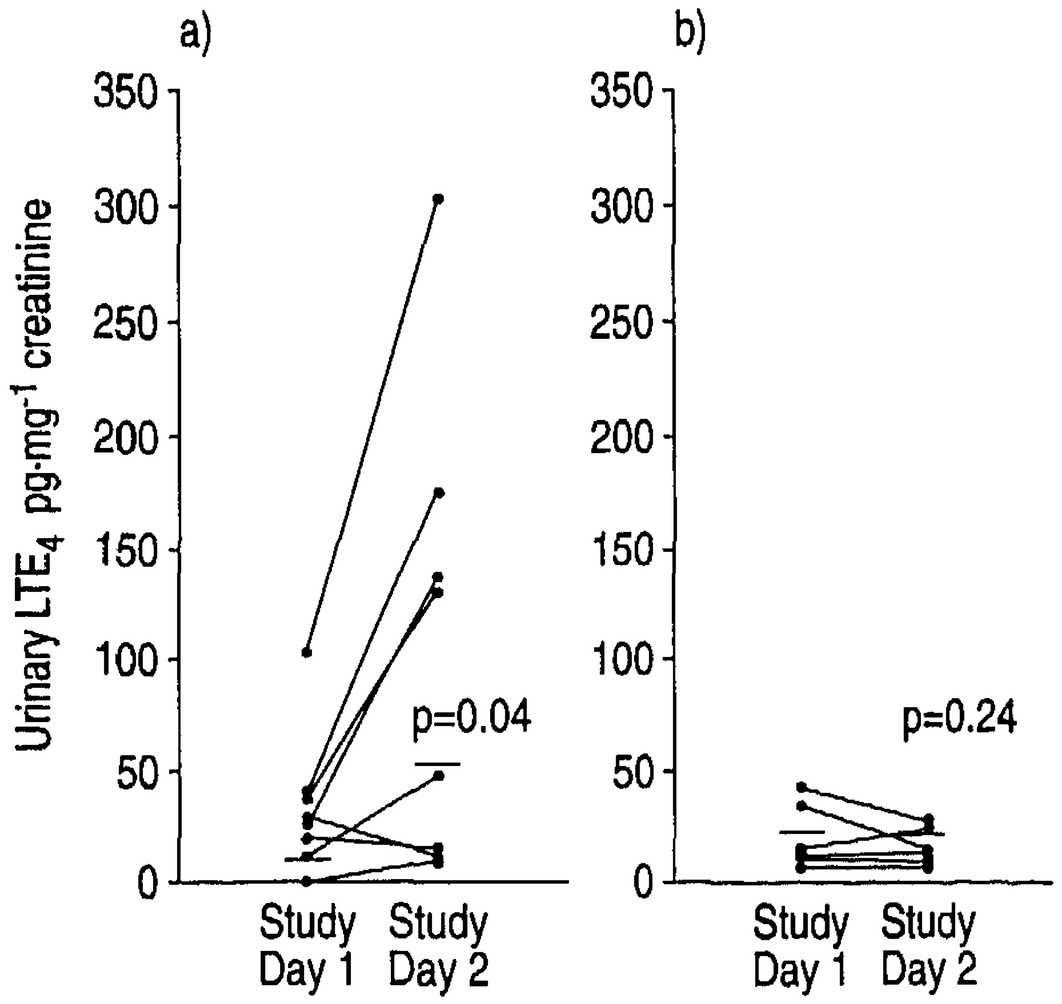

Fig. 3. – The urinary LTE_4 concentration on study day 1 and 2 in individual asthmatic subjects: a) returning to The Netherlands; or b) resident in Davos. Bars indicate geometric mean. LTE_4: leukotriene E_4.

Table 3. – Total IgE and eosinophil counts in subjects studied

Subject No.	Total IgE kU·l^{-1} Day 1	Total IgE kU·l^{-1} Day 2	Eosinophils $\times 10^6 \cdot l^{-1}$ Day 1	Eosinophils $\times 10^6 \cdot l^{-1}$ Day 2
Subjects returning to The Netherlands				
1	572	947	531	866
2	1330	1100	122	559
3	295	339	94	169
4	557	543	3	0
5	3515	4310	288	113
6	233	271	397	297
7	2845	2930	222	125
8	5010	6150	241	497
Mean	1794	2073	237	328
SEM	631	796	60	102
Subjects remaining in Davos				
9	675	570	334	256
10	3960	4005	1012	563
11	814	ND	134	ND
12	1090	858	406	278
13	1142	1077	497	550
14	722	ND	741	ND
Mean	1400	1627	520	411
SEM	517	799	127	837

Mean is the arithetic mean. IgE: immunoglobulin E; Sub: subject; ND: blood sample refused by subject.

Discussion

This study demonstrates that in 6 out of 8 allergic asthmatic children resident at altitude who returned briefly to sea level and then returned to altitude, there was a decline in baseline FEV_1 and an increase in airway responsiveness to histamine. These changes were accompanied by an increase in urinary LTE_4 excretion. The range of LTE_4 concentration on study day one in our subjects was similar to that observed previously in adult asthmatic subjects [20, 32], suggesting that there is no age-related difference in the range of LTE_4 concentration during stable asthma and in the absence of other disease. A raised baseline urinary LTE_4 is suggestive of aspirin-sensitive asthma [23], but there was no clinical history of aspirin sensitivity or other distinguishing clinical features in subject No. 6 who had a baseline urinary LTE_4 higher than the other subjects.

After a 2 week stay in The Netherlands, LTE_4 excretion increased up to approximately threefold, in association with a decrease in baseline FEV_1 and an increase in airway reactivity to histamine. This was not observed in subjects Nos. 4 and 5, who did not demonstrate a change in FEV_1 or $PC_{20}FEV_1$ histamine between the two study days. The cause of this heterogeneity in response is unknown, but may reflect the variation of reactions in asthmatic subjects to environmental factors. Medication was withheld for 8 h prior to FEV_1 measurements, urine collections and histamine challenge and there was no change in medication between the two study days. In healthy subjects, RICHALET *et al.* [33] observed that acute altitude hypoxia is associated with an increase in plasma LTB_4. An increase in leukotriene excretion during acute asthma in adult subjects has been reported by TAYLOR *et al.* [19]. A threefold increase in leukotriene excretion has also been reported following antigen and aspirin challenge in asthmatic subjects [19, 23].

LTE_4 concentration may be used to reflect systemic and pulmonary release of cysteinyl leukotrienes [31]. The increase in leukotriene excretion during a deterioration of lung function reflects an increase in cysteinyl-leukotriene biosynthesis. The cysteinyl leukotrienes may have a central role in the pathogenesis of asthma, as suggested by their recovery from the bronchoalveolar fluid of asthmatic subjects [32], activity as potent lung spasmogens, and ability to increase airway hyperresponsiveness [10–12]. Their presence may explain the decrease in lung function and increase in airway responsiveness to histamine observed in our subjects after a brief visit to The Netherlands. Consistent with prior studies, we were unable to correlate circulating mediator levels of leukotrienes, FEV_1 measurement or airway reactivity to histamine [32]. At altitude, the concentration of house dust mite allergen is reduced [27, 30], and it has been suggested that it is the decreased exposure to house dust mite which accounts for the improvement of lung function in allergic asthmatic subjects after a stay at altitude. All our subjects were sensitive to house dust mite allergen on skin-prick testing, and it is possible that increased allergen exposure to house dust mite after return to sea level resulted in the deterioration of lung function. Other allergens and irritants, such as pollutants and other factors which change with altitude (humidity, temperature, barometric pressure), should also be considered. There was no difference in total IgE level between the two study days in subjects returning to The Netherlands, although changes may not be apparent during this short

period of time. Whilst specific IgE against house dust mite was not determined during the study period in our subjects, dust mite allergen concentration from bedrooms at the Asthma Centre in Davos is low and in the region of 18 $ng \cdot g^{-1}$ dust.

We did not detect a difference in blood eosinophil counts in subjects returning to The Netherlands. This is similar to the study of BONER *et al.* [34], where the influence of allergen avoidance at altitude on serum markers of eosinophil activation in children with allergic asthma was investigated. Whilst there was no increase in peripheral eosinophil count during allergen exposure, there was activation of eosinophils as indicated by the increase of eosinophil cationic protein (ECP) and eosinophil protein X (EPX) serum markers. It is possible that part of the increase in cysteinyl leukotrienes was due to eosinophil activation during exposure to allergen in subjects returning to The Netherlands. The involvement of other cells, such as monocytes or mast cells, as a source for leukotriene synthesis was not determined in this study.

With the exception of subject No. 14, on study day one, all subjects had controlled asthma with FEV_1 measurement >70% of predicted. A decrease in FEV_1 measurement was not observed in all subjects returning to Davos from The Netherlands. Whilst clinical severity could have been additionally assessed using peak flow readings and symptom scores, this was not possible due to poor compliance with completing peak flow charts and symptom questionnaires in the subjects. In this regard, parents were questioned about any changes in medication during the study period. Airway reactivity was assessed using $PC_{20}FEV_1$ histamine, which is used routinely in The Netherlands Asthma Centre. In subjects Nos 7 and 8, airway reactivity to histamine on study day one was >32 $mg \cdot ml^{-1}$. These subjects had a history of asthma and a $PC_{20}FEV_1$ histamine <8 $mg \cdot ml^{-1}$ on admission to the clinic 4 weeks prior to the study. An improvement in lung function and airway reactivity within a week of residence at altitude has previously been observed in altitude clinics. For this reason, the study was conducted after the subjects had been resident for one month at altitude, to optimize stable control of asthma.

In conclusion, utilizing a novel design to provoke a minor deterioration in asthma through a change in the natural environment, we have demonstrated that a decrement in FEV_1 and an increase in airways responsiveness are accompanied by augmented biosynthesis of the cysteinyl leukotrienes. These results add further support to the need to test leukotriene receptor antagonists or biosynthesis inhibitors in the treatment of asthma.

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