Eur Respir J, 1995, 8, 742–751
DOI: 10.1183/09031936.95.08050742

European Respiratory Journal
ISSN 0903 - 1936

Changes in mucociliary clearance during and after isocapnic hyperventilation in asthmatic and healthy subjects

E. Daviskas*, S.D. Anderson*, I. Gonda+, H.K. Chan+, P. Cook**, R. Fulton**

Changes in mucociliary clearance during and after isocapnic hyperventilation in asthmatic and healthy subjects. E. Daviskas, S.D. Anderson, I. Gonda, H.K. Chan, P. Cook, R. Fulton. ©ERS Journals Ltd 1995.

ABSTRACT: Hyperpnoea with dry air could lead to a reduction in depth and hyperosmolarity of the periciliary fluid layer (PFL) as a consequence of evaporative water loss. We investigated whether mucociliary clearance (MCC) is likely to be affected by dry air hyperpnoea, which also results in airway narrowing in asthmatics.

MCC was measured by radioaerosol technique, for about 1 h, in 10 asthmatic and 8 healthy subjects on 3 separate days: 1) nasal resting breathing with ambient air; 2) isocapnic hyperventilation (ISH) with dry air; and 3) ISH with warm humid air.

Analysis of the initial and post-intervention lung radioactivity for the whole right lung and for defined regions of interest showed that, compared to ISH with warm humid air and nasal resting breathing, MCC was reduced during and increased post-ISH with dry air in the whole right lung of both groups. The mean reduction in clearance (± 95% confidence interval (95% CI)) was -9.3% (-3.1 to -15.6%) and -3.6% (-2.0 to -9.1%), and the mean increase (±95% CI) was 19.2% (11.8 to 26.6%) and 14.8% (7.1 to 22.5%), compared to warm humid air, in asthmatic and healthy subjects, respectively. However, regional analysis showed that the changes were present in all lung regions of the asthmatics, whilst only in the central region of the healthy subjects. The duration of the increased clearance rates post-ISH was also different in both groups.

The changes in mucociliary clearance during and after isocapnic hyperventilation with dry air were probably related to the water content of the inspired air, causing transient changes in the periciliary fluid layer.
Eur Respir J., 1995, 8, 742–751.

Depts of *Respiratory and **Nuclear Medicine, Royal Prince Alfred Hospital, Sydney, Australia. +Dept of Pharmacy, University of Sydney, Sydney Australia. Currently at Genentech Inc., South San Francisco, CA, USA.

Correspondence: S.D. Anderson
Dept of Respiratory Medicine
PCP Level 9
Royal Prince Alfred Hospital
Missenden Rd
Camperdown NSW 2050
Australia

Keywords: Asthma
dry air
mucociliary clearance

Received: January 19 1994
Accepted December 15 after revision

This study was supported in part by a grant from the Asthma Foundation of New South Wales and the National Health and Medical Research Council of Australia.

Exercise or isocapnic hyperventilation whilst breathing dry air, at high flow rates, results in airway narrowing in most asthmatic subjects [1]. The severity of the airway narrowing is directly related to the water loss from the airways, in bringing the inspired air to alveolar conditions [2]. The amount of water loss depends on the ventilation reached and sustained during hyperpnoea, and the water content of the inspired air. The airway narrowing is inhibited by inspiring air conditioned to the vapour pressure and temperature of the alveoli [2, 3].

Evidence from experimental [4] and theoretical studies [5, 6] has shown that a significant proportion of the water lost during hyperpnoea comes from the intrathoracic airways. The extent to which the intrathoracic airways are involved depends on the ventilation and the inspired conditions [7]. It is thought that some of the water lost during hyperpnoea to condition the inspired air comes from the periciliary fluid layer.

The mechanism whereby the loss of water causes airway narrowing is thought to relate to its thermal and dehydrating effects. Dehydration could cause cooling and a reduction of the periciliary fluid volume, with subsequent increase in the ion concentration leading to hyperosmolarity [8]. Studies in animals support this hypothesis [9]. If the rate of water loss during hyperpnoea exceeds the rate of water return, then the depth of the periciliary fluid layer should be reduced. If the depth of the periciliary fluid layer is decreased by evaporation, the ciliary beat frequency will be impaired in the viscous gel layer, and mucociliary clearance rate will decrease.

Ciliary beat frequency has been found to decrease with a reduction in temperature [10], and dry air inhalation has been found to decrease mucus velocity in dogs [11–13], and increase mucus secretion in cats [14]. Although there is evidence that in humans dry air reduces clearance in the nose [15], there is no evidence that dry air might reduce the clearance in the lower airways, especially at high ventilation rates. The need to humidify the air during mechanical ventilation [16] that bypasses the upper airways, suggests that the evaporative loss of water may not be replaced instantaneously, which has consequences for the mucociliary clearance.

The aim of the present study was to investigate whether changes in mucociliary clearance occur during and

after isocapnic hyperventilation (ISH) with dry air, which would reflect the changes in the periciliary fluid layer. To achieve this, mucociliary clearance during and after isocapnic hyperventilation with dry air was compared with the mucociliary clearance during and after resting nasal breathing and isocapnic hyperventilation with warm humid air.

Material and methods

The study was approved by the Ethics Review Committee of Central Sydney Area Health Service, and informed consent was obtained in writing from all subjects before participation.

Subjects

Ten asthmatic and eight healthy volunteers took part in the study and their characteristics are given in table 1. The asthmatic subjects had stable asthma and a resting forced expiratory volume in one second (FEV_1)≥85% of predicted. No asthmatic subject had a reduction in FEV_1 >40% after challenge with dry air. Most of the asthmatics were using beta-agonists, as needed or regularly, and were taking aerosolized corticosteroids. No asthmatic subject was taking theophylline. All subjects were asked not to take any nonsteroidal anti-inflammatory drugs for at least 48 h prior to each study day.

All asthmatics withheld their medications for at least 8 h. Subjects with a history of smoking, or subjects who had a lower respiratory tract infection in the last 6 weeks prior to each study, were excluded.

All asthmatics and three of the healthy subjects were atopic according to skin-prick tests.

Study design

All subjects had their lung function measured on two occasions, prior to mucociliary clearance studies. All volunteers had their MCC measured on three occasions.

The procedure on each day was as follows: 1) spirometry; 2) radioaerosol inhalation; 3) spirometry (to ascertain that there was no response to the radioaerosol); 4) emission anterior/posterior images (static) to obtain initial dose in the lung; 5) intervention for 6–8 min; and 6) emission anterior/posterior images (dynamic) for 45 min.

The reference state was: nasal resting breathing with ambient air (temperature (T) 23°C and 50% relative humidity (RH)). The intervention was: 1) isocapnic hyperventilation with dry air (T 23°C and 0% RH); and

Table 1. – Anthropometric data, baseline lung function, ventilation rate (% maximum voluntary ventilation (MVV)) duration of isocapnic hyperventilation (ISH), responses to ISH with dry and warm humid (WH) air of asthmatic and healthy subjects and medication taken by the asthmatic subjects

Subj. No.	Age yrs	Sex	Height cm	Baseline FEV_1 % pred	Ventilation L·min^{-1}	Ventilation % MVV	Duration min	% fall FEV_1 Dry ISH	% fall FEV_1 WH ISH	Medication Drug	Medication Steroid dose µg	Medication Time on months
Asthmatic												
1	29	F	168	94	58	51	8	24	4	Nil		
2	20	M	177	96	99	62	8	32	4	Nil		
3	18	M	176	86	58	36	6	17	0	S, BEC	2000	1.5
4	21	M	181	94	95	57	6	39	8	S, BEC	400	96
5	41	M	173	88	73	54	6	10	-	S, BUD	1600	2
6	30	M	180	92	79	51	8	17	-	S		
7	29	M	181	100	99	62	8	19	-	S, BEC	100	48
8	18	F	155	105	45	45	8	39	3	S, BUD	1000	2
9	21	F	171	85	41	34	6	29	5	S, BEC	400	7
10	19	F	156	93	50	49	6	62	1	S, BEC	400	3
Mean	25		172	93	70	50		29				
±SD	8		10	6	23	10		15				
Healthy												
11	20	M	174	88	93	60	8	4	-			
12	20	M	178	120	96	59	8	5	-			
13	23	F	180	89	88	60	8	4	-			
14	23	M	168	108	92	65	8	4	-			
15	35	F	164	122	63	66	8	3	-			
16	46	F	165	107	75	59	8	1	-			
17	30	F	163	96	59	56	8	9	-			
18	20	M	183	94	97	56	8	7	-			
Mean	27		172	103	83	60		5				
±SD	9		8	13	15	4		2				

Subj: subject; FEV_1: forced expiratory volume in one second; % pred: percentage of predicted; S: salbutamol; BUD: budesonide; BEC: beclomethasone.

2) isocapnic hyperventilation with warm humid air (approximately T 29°C and 99% RH). The choice of intervention was random. A 7 day interval existed between the ISH with dry air and the following study.

Measurement of lung function

Spirometry, using a hot wire anemometer (Minato, AS-500, Osaka, Japan), was measured before and after isocapnic hyperventilation challenge with dry and warm humid air on two different occasions. The warm humid air challenge was omitted with the healthy subjects and with the very mild asthmatics, as it could be predicted that there would be no airway response to this challenge. All subjects had normal and reproducible spirometry at rest. This was necessary, so that their response to isocapnic hyperventilation and the deposition of the aerosol would be the same. In all studies, predicted values for lung volumes were taken from GOLDMAN and BECKLAKE [17] for adults.

To avoid possible changes in mucociliary clearance with the forced manoeuvres, lung function after the ISH was not measured on the mucociliary clearance study day. All subjects, except one, experienced the same symptoms following dry air isocapnic hyperventilation on the study day. One subject unpredictably developed severe airway narrowing. This response was gradual, and the camera was stopped after 20 min from the start of the dynamic images. Thus, only the data for the first 20 min could be included in the analysis.

Isocapnic hyperventilation

The technique used was similar to the one described by PHILLIPS *et al.* [18]. Dry compressed air containing 21% oxygen, 4.9% carbon dioxide, and nitrogen to balance was passed *via* a demand resuscitator to a rotameter, and then to a metereological balloon (approximately 30 L capacity) that served as a target. The subject, wearing a noseclip, breathed through a low-resistance two-way valve (Hans-Rudolf No. 2700, Kansas City, Mo, USA). The subject was asked to breathe for 6–8 min at a rate equivalent to 60% of predicted maximum ventilatory ventilation (MVV), taken as $FEV_1 \times 37.5$ [19]. If a subject was unable to hyperventilate on 60% MVV, this was reduced appropriately. However, the ventilation and duration of ISH was kept the same for the two study days within a subject.

When a warm humid ISH was performed, a kettle humidifier was placed between the balloon and the two-way valve. The kettle humidifier was set to warm the air to approximately 29°C and 99% RH, at ventilation around 90 $L \cdot min^{-1}$.

Measurement of mucociliary clearance

Inhalation of radioaerosol. Mucociliary clearance was assessed using a radioaerosol technique. ^{99m}Tc-sulphur colloid (Australian Radioisotopes, ANSTO, Sydney, Australia), approximately 1 GBq, was diluted in 5 ml of isotonic saline. The radioaerosol was generated by an Acorn (Medic-Aid, Peckham, Sussex, UK) nebulizer using oxygen from a cylinder set to 6 $L \cdot min^{-1}$. The droplets had a mass median aerodynamic diameter (MMAD) of 6 µm and a geometric standard deviation (GSD) of 1.7, measured by a seven stage cascade impactor (DCI6, Delron, Columbus, Ohio, USA). The dilution air supplementing the flow to the mouthpiece was humidified to maintain the characteristics of the droplets [20]. The radioaerosol was delivered with a controlled breathing pattern, in order to maximize deposition in the conducting airways. A closed breathing circuit was linked to an IBM computer, that allowed monitoring and control of the breathing pattern using a target volume and target inspiratory and expiratory times [21, 22]. The target volume, set to 450 ml, was displayed on the screen together with the target line oscillating at set rates, aiming at a peak inspiratory flow rate of 60 $l \cdot min^{-1}$. The inspiratory time was set higher than the expiratory time, and the subject tried to breath according to the set tidal volume and flow rates. The patient inhaled the radioaerosol for approximately 2 min. This delivery time was chosen so that the lung counts were about 2,000 $counts \cdot s^{-1}$ over the posterior thorax. Upon termination of the delivery of radioaerosol, the subjects removed the radioactivity from their oropharynx and oesophagus by rinsing and gargling with water and expectorating, and by swallowing some bread and water.

Imaging. Lung images were obtained using a gamma camera (Phillips Diagnost Tomo; Hamburg, Germany) fitted with a low energy, all purpose, collimator and linked to an on-line computer (DEC PDP11, Maynard, MA, USA). The images were collected in a 64×64 matrix.

In order to delineate the lung fields, the subjects had an anterior and posterior transmission image [23] taken using a flood source containing approximately 1.5 GBq of ^{153}Gd in water. An anterior and a posterior emission image (static), 1 min each, were collected for each subject approximately 10 min after the inhalation of the radioaerosol. The purpose of this image was to define the initial deposition of the radioaerosol in terms of distribution and intensity. As soon as the intervention finished, the subject was placed in the supine position and successive anterior and posterior emission images (dynamic) of 20 s each were collected dynamically for 45 min [24]. Care was taken that the collection of the dynamic images started within a maximum of 3 min post-intervention. In order to align the lung fields between the transmission, static and the dynamic studies, images of markers placed on premarked positions, on the subject's body, were also collected at the end of each study. Care was taken that the static and dynamic images and the interventions occurred at the same time point after the mid-inhalation time of the radioaerosol for the three study days.

Image and data analysis

All the images were decay corrected to the mid-inhalation timeof the radioaerosol. A geometric mean image was obtained from the anterior and posterior images for the transmission, static and dynamic emission images [24].

The left lung was not analysed, as there would be interference from the activity in the stomach. The right lung was divided into three regions of interest, central, intermediate and peripheral [21], in order to estimate the initial deposition of the radioaerosol. An estimate of the initial deposition was obtained from the activity of the static image by calculating the penetration index (PI), as follows:

$$PI = \frac{\text{activity in peripheral region}}{\text{activity in central region}}$$

A bi-exponential function was fitted to the curve obtained from the dynamic images, using a computer program that uses a nonlinear least squares method (PCNONLIN, SCI, Software, Lexington, Kentucky, USA). The fitted data were extrapolated to one minute post-intervention, if the start of the dynamic images was delayed up to 3 min. The total activity of the right lung in the static image and in each region for the regional clearance assessment was taken as the initial activity before the intervention and represented 100% retention. The activity measured serially over 45 minutes post-intervention time was converted as counts per minute and expressed as a percentage of the initial activity. The percent of the activity cleared is the difference between the initial activity and the percent of the activity retained. Data from the best-fit were used to calculate the % clearance during and post-intervention as follows:

$$\%\ \text{clearance during intervention} = \frac{\text{initial activity - activity at 1 min post-intervention}}{\text{initial activity}} \times 100$$

$$\%\ \text{clearance post-intervention} = \frac{\text{activity at 1 min post-intervention - activity at 45 min post-intervention}}{\text{activity at 1 min post-intervention}} \times 100$$

Statistical analysis

Two-factor analysis of variance (ANOVA), with repeated measures, was performed to compare the two groups in terms of the effect of intervention on clearance during and post-intervention. Duncan's multiple range test was used to compare the clearance of the three study days. The same analysis was also performed for the clearance rate for every 10 min interval post-intervention. If the two groups were different, then one-factor ANOVA, with repeated measures, was performed for each group separately.

Comparison of the deposition of radioaerosol on the three study days of both groups was made with two-factor ANOVA, repeated measures.

Spearman's correlation analysis was performed between: 1) the change of the % clearance and the percentage change of the PI from the resting values for each region; 2) the % fall in FEV_1 in the asthmatic subjects and the change of % clearance during and post-ISH with dry air; and 3) the change of % clearance during and post-ISH with dry air.

Results

The key finding of this study was that isocapnic hyperventilation (ISH) with dry air changed mucociliary clearance (MCC), compared to nasal resting values, both in the asthmatic and healthy subjects, whilst ISH with warm humid air did not.

Changes of MCC during intervention

Whole right lung. Mucociliary clearance was significantly ($p<0.002$) reduced during ISH with dry air compared to ISH with warm humid air and nasal resting breathing in the whole right lung both of asthmatic and healthy subjects (fig. 1a). This reduction was not statistically different between the two groups ($p>0.1$), although the asthmatic subjects had larger changes. In the asthmatic subjects, the mean reduction in clearance (±95% CI) was -9.3% (-3.1 to -15.6%) compared to ISH with warm humid air, and -9.75% (-2.5 to -17.0%) compared to nasal resting breathing. In the healthy subjects, the mean reduction in clearance (±95% CI) was -3.6% (-2.0 to -9.1%) compared to ISH with warm humid air, and -2.7% (0 to -5.5%) compared to nasal resting breathing.

Regional analysis. During ISH with dry air, the reduction in MCC approached significance ($p=0.0519$) in the central region, and there was no significant difference between asthmatic and healthy subjects ($p>0.1$) (fig. 1b). In the asthmatic subjects, the mean reduction in clearance (±95% CI) was -5.2% (+3 to -13.3%) compared to ISH with warm humid air, and -8.6% (+0.1 to -17.3%) compared to nasal resting breathing. In the healthy subjects, the mean reduction in clearance (±95% CI) was -4.5% (0.8 to -9.8%) compared to ISH with warm humid air, and -0.4% (+3.9 to - 4.8%) compared to nasal resting breathing.

During ISH with dry air, MCC was significantly reduced in the intermediate ($p<0.03$) (fig. 1c) and peripheral region ($p<0.003$) (fig. 1d) of the asthmatic subjects only. The mean reduction in clearance (±95% CI), in the intermediate region of the asthmatic subjects, was -9.5% (-0.3 to -18.6%) compared to ISH with warm humid air, and -11.4% (-3.9 to -18.8%) compared to nasal resting breathing. The mean reduction in clearance (±95% CI), in the peripheral region of the asthmatic subjects, was -22.9% (-12.9 to -32.9%) compared to ISH with warm humid air and -19.3% (-3.9 to -34.8%) compared to nasal resting breathing.

Changes of MCC post-intervention

Whole right lung. Mucociliary clearance was increased significantly ($p<0.0001$) (fig. 1a) post-ISH with dry air in the whole right lung, compared to ISH with warm humid air and nasal resting breathing, of all asthmatic and healthy subjects. Although the asthmatic subjects had a larger increase in MCC following ISH with dry air, compared to the healthy subjects, this increase of

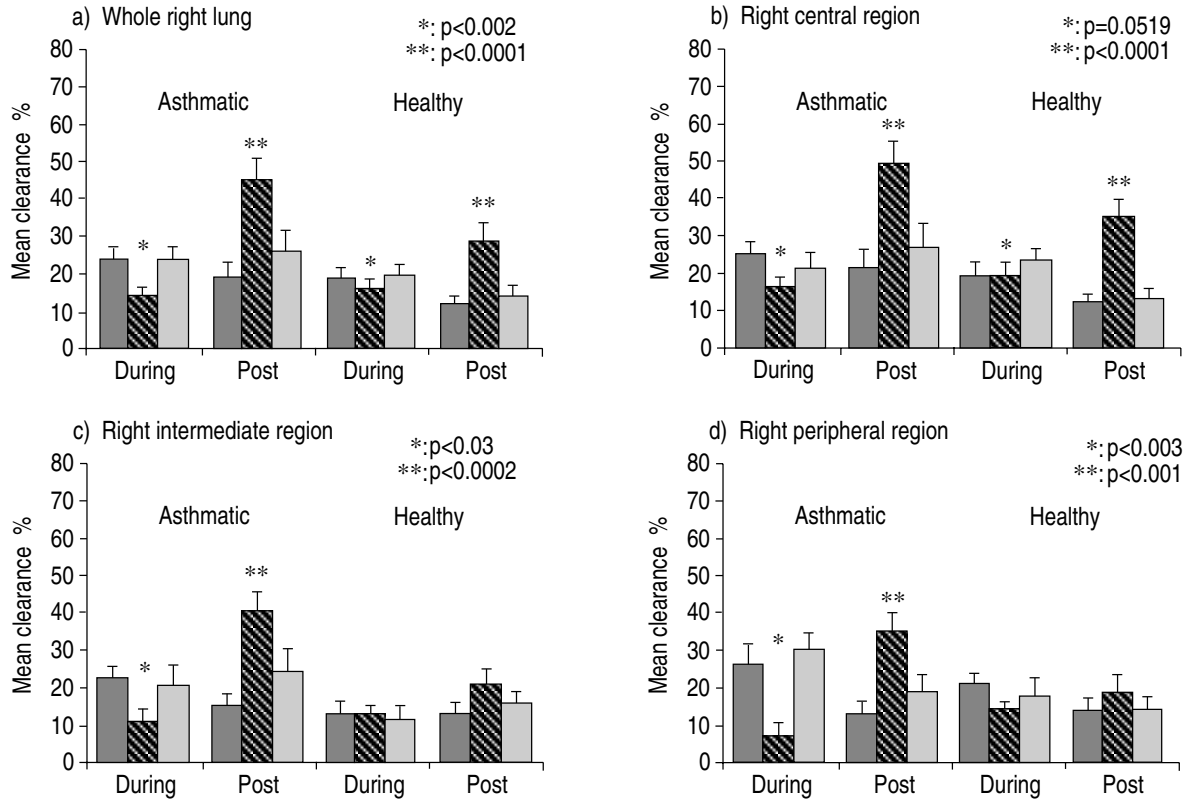

Fig. 1. – Comparison of the mean % clearance (±SEM) of: a) the whole right lung; b) central; c) intermediate; and d) peripheral region during and post-ISH with dry ( ) and warm humid air ( ) and nasal resting breathing ( ) at the same time intervals in the asthmatic and healthy subjects. In the asthmatic subjects, mucociliary clearance decreased during and increased post-ISH with dry air in all regions of the lung, in contrast to the healthy subjects who had these changes only in the whole right lung and central region. There was no significant difference between clearance with nasal resting breathing and ISH with warm humid air.

MCC was not different between groups (p>0.1). In the asthmatic subjects, the mean increase in clearance (±95% CI) was 19.2% (11.8 to 26.6%) compared to ISH with warm humid air, and 26.2% (17.0 to 35.4%) compared to nasal resting breathing. In the healthy subjects, the mean increase in clearance (±95% CI) was 14.8% (7.1 to 22.5%) compared to ISH with warm humid air, and 16.9% (8.3 to 25.5%) compared to nasal resting breathing.

ISH with warm humid air increased MCC post-intervention, compared to the nasal resting values, however this increase was significantly less (p<0.001) than the increase of MCC brought on by ISH with dry air.

Regional analysis. MCC increased significantly (p<0.0001), following ISH with dry air, in the central region both of asthmatic and healthy subjects (fig. 1b), and the increase was not different between groups (p>0.5). For most asthmatic and healthy subjects, the increase was more than twofold in the whole right lung and central region (fig. 1a, b). In the asthmatic subjects, the mean increase in clearance (±95% CI) was 22.5% (13.3 to 31.7%) compared to ISH with warm humid air, and 28.0% (18.6 to 37.4%) compared to nasal resting breathing. In the healthy subjects, the mean increase in clearance (±95% CI) was 22.4% (14.8 to 30.0%) compared to ISH with warm humid air, and 23.0% (12.9 to 33.1%) compared to nasal resting breathing.

The two groups were significantly different (p<0.02) in the responses of the MCC in the intermediate (fig. 1c) and peripheral region (fig. 1d). The asthmatic subjects, in contrast to the healthy, had a highly significant (p<0.001) increase in MCC both in the intermediate and peripheral region following ISH with dry air, compared

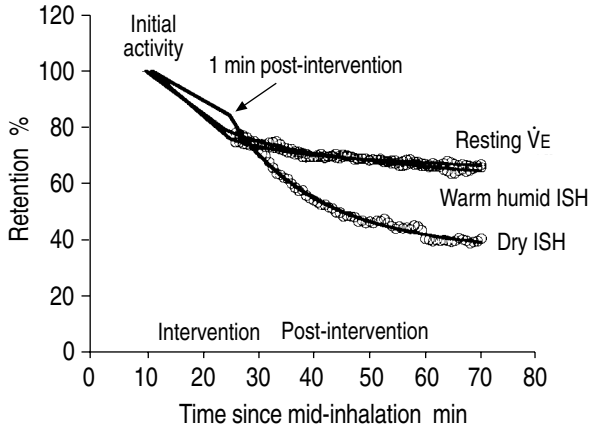

Fig. 2. – Example of the % retention curves of the whole right lung, in an asthmatic subject, on three study days: 1) nasal resting breathing; 2) ISH with dry air and 3) ISH with warm humid air. The % retained activity was plotted *vs* time since mid-inhalation of the radioaerosol. This figure demonstrates that mucociliary clearance (initial activity - % retained activity) decreases during and increases post-ISH with dry air.

to ISH with warm humid and nasal resting breathing. There was no significant difference between MCC with dry and warm humid air, in the healthy subjects, in this region. There was a tendency for MCC to increase following ISH with warm humid air, compared to the nasal resting values, in all regions for both groups. However, this increase never reached statistical significance apart from the whole right lung, and was distinctly different ($p<0.001$) to the increase in MCC that followed ISH with dry air. The mean increase in clearance (±95% CI), in the intermediate region of the asthmatic subjects, was 16.2% (3.3 to 29.0%) compared to ISH with warm humid air, and 25.0% (15.1 to 34.9%) compared to nasal resting breathing. The mean increase in clearance (±95% CI), in the peripheral region of the asthmatic subjects, was 16.1% (5.4 to 26.8%) compared to ISH with warm humid air, and 22.6% (10.4 to 34.8%) compared to nasal resting breathing.

An example of the percentage retention curves for the whole right lung, for an asthmatic subject, at rest and with the two interventions is shown in figure 2.

The clearance rate calculated for every 10 min interval post-ISH with dry air remained significantly increased ($p<0.0001$) for the whole time of measurement (45 min), in the whole lung (fig. 3a) and central region (fig. 3b) of the asthmatic subjects, compared to the clearance rate at rest and ISH with warm humid air. However, in the intermediate (fig. 3c) and peripheral region (fig. 3d) the increased clearance rate lasted 30 and 20 min, respectively.

In the healthy subjects, the clearance rate increased for only 30 min, and mainly in the central region (fig. 4). However, there was no difference ($p>0.5$) in the magnitude of this increase in the clearance rate between asthmatic and healthy subjects for the first 20 mins post-ISH with dry air in the whole right lung (fig. 4a) and central region (fig. 4b).

The resting lung function, the ventilation (%MVV) achieved during ISH with dry and warm humid air, its duration, and the response are presented in table 1 for the asthmatic and healthy subjects. The mean ventilation (±SD) that the asthmatic and healthy subjects achieved during ISH was 70 (±22) and 83 (±15) $L \cdot min^{-1}$, respectively. The mean ventilation expressed as %MVV (±SD)

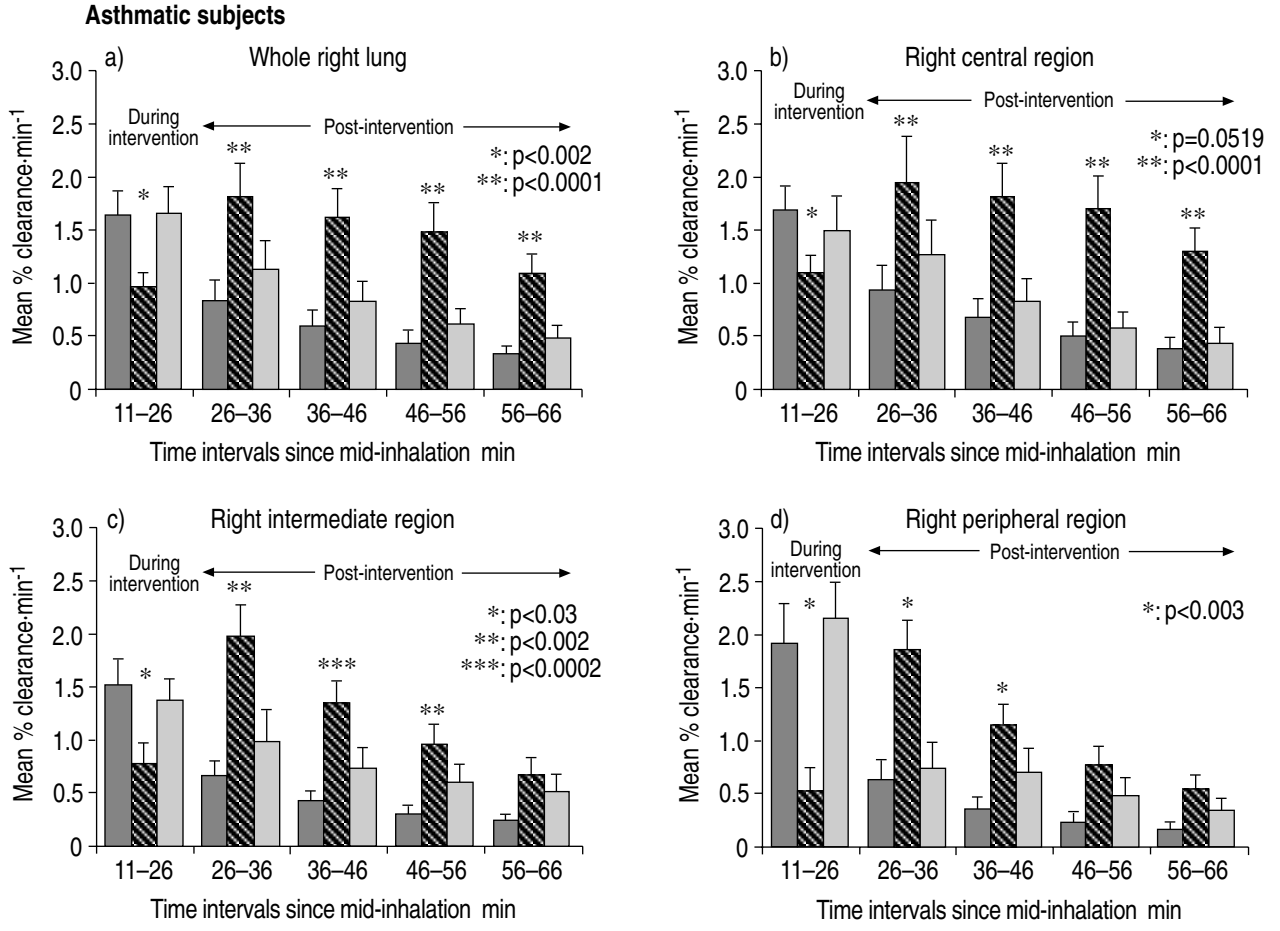

Fig. 3. – Mean % clearance rates (±SEM) of the three study days during intervention and for four consecutive 10 minute intervals post-intervention in: a) the whole right lung; b) central; c) intermediate; and d) peripheral region in the asthmatic subjects. The mean % clearance rates were plotted *vs* the corresponding time intervals based on the time since mid-inhalation of radioaerosol. The study days were: 1) ISH with dry air (▩); 2) ISH with warm humid air (□); and 3) nasal resting breathing over a similar time period to ISH days (■). The clearance rates when the intervention was ISH with dry air were compared to the clearance rates when the intervention was ISH with warm humid air and nasal resting breathing and significant differences are expressed with the p-values. This figure demonstrates that the decrease of the clearance rate during ISH with dry air was followed by a marked increase in all regions of the lung in the asthmatic subjects. The duration of this increase in clearance was less in the intermediate and peripheral region.

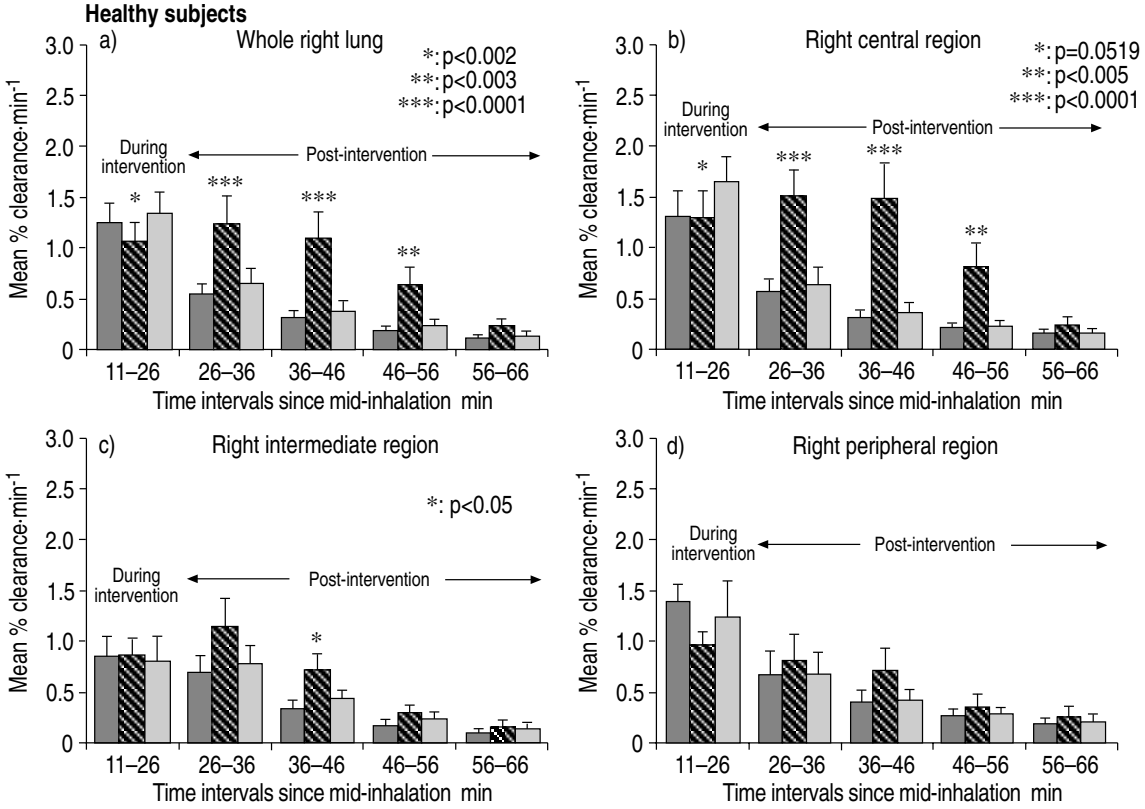

Fig. 4. – Mean % clearance rates (±SEM) of the three study days during intervention and for four 10 minute intervals post-intervention in: a) the whole right lung; b) central; c) intermediate; and d) peripheral region in the healthy subjects. The mean % clearance rates were plotted *vs* the corresponding time intervals based on the time since mid-inhalation of radioaerosol. The study days were: 1) ISH with dry air ( ); 2) ISH with warm humid air ( ) and; 3) nasal resting breathing over a similar time period to ISH days ( ). The clearance rates when the intervention was ISH with dry air were compared to the clearance rates when the intervention was ISH with warm humid air and nasal resting breathing and significant differences are expressed withe the p-values. This figures demonstrates that the decrease of the clearance rate during ISH with dry air was followed by a marked increase in the whole right lung and central region only. By contrast to the asthmatic subjects, this increase in clearance lasted for only half an hour post-ISH with dry air.

Table 2. – Penetration indices (PI) for asthmatic and healthy subjects on the three study days: nasal resting breathing (rest), ISH with dry air and ISH with warm humid (WH) air

Subject No.	Penetration index Rest	Dry	WH
Asthmatics			
1	0.60	0.58	0.66
2	0.34	0.31	0.39
3	0.15	0.15	0.15
4	0.34	0.34	0.32
5	0.12	0.08	0.11
6	0.24	0.27	0.22
7	0.16	0.20	0.23
8	0.18	0.14	0.14
9	0.18	0.19	0.22
10	0.17	0.17	0.13
Mean	0.25	0.24	0.26
±SD	0.15	0.14	0.17
Healthy			
11	0.39	0.42	0.41
12	0.42	0.38	0.40
13	0.31	0.32	0.36
14	0.21	0.22	0.18
15	0.11	0.12	0.13
16	0.37	0.31	0.32
17	0.46	0.42	0.41
18	0.49	0.39	0.46
Mean	0.34	0.32	0.33
±SD	0.13	0.11	0.12

ISH: isocapnic hyperventilation.

was 50 (±10)% and 60 (±4)% for asthmatic and healthy subjects, respectively. Although the actual ventilation was not significantly different ($p>0.15$) between groups, the %MVV was significantly different ($p<0.02$), indicating that the asthmatic subjects, in fact, achieved a lower ventilation for their lung volume.

The individual and mean (±SD) data of the penetration indices are given in table 2. The initial distribution of the radioaerosol, as shown by the PI, was well-matched on all study days in both groups, and it was not different between groups ($p>0.4$). No significant correlation ($p>0.2$) was found between the small percentage changes in the PI and the changes in the clearance of all regions during ISH with dry air.

No significant correlation ($p>0.2$) was found between the changes in the % clearance of asthmatic subjects during and post-ISH with dry air and the percentage fall in FEV_1. Also, no significant correlation ($p>0.2$) was found between the changes in % clearance during and post-ISH with dry air.

Discussion

This study demonstrates that: 1) isocapnic hyperventilation (ISH) with dry air changes mucociliary clearance (MCC), compared to nasal resting values, both in the

asthmatic and healthy subjects, whilst hyperventilation with warm humid air does not; 2) asthmatic subjects have a significant reduction in MCC during and a significant increase in MCC post-ISH with dry air, in the large and small airways, in contrast to the healthy subjects who have similar changes only in the large airways; 3) the increase in the clearance rates in the central region after ISH with dry air is of shorter duration in the healthy subjects in contrast to the asthmatics, in whom the clearance rates remained high for the whole time of measurement; and 4) the decrease in MCC during ISH with dry air always preceded the increase in MCC after ISH. The finding that mucociliary clearance decreases during dry air hyperventilation is consistent with the view that the airways dehydrate and cool during ISH with dry air. The finding that the MCC increases after hyperventilation with dry air is probably related to the low water content of the inspired air, causing a transient hyperosmolarity of the periciliary fluid layer and the release of mediators that stimulate ciliary beat frequency and chloride ion secretion [25].

The differences in MCC between ISH with dry and warm humid air cannot be accounted for by differences in airflow, and are most likely to be due to the differences in the water content of the inspired air resulting in different evaporative losses of water from the intrathoracic airways. ISH with warm humid air involves increased airflow, like ISH with dry air, but no evaporative loss of water from the intrathoracic airways. The conclusion that the low water content of the inspired air is responsible for the changes in MCC rather than the increased airflow is also supported by the observation that there was no difference in MCC between rest and ISH with warm humid air.

It is not clear why there were local differences in the reduction of MCC during ISH with dry air between asthmatic and healthy subjects. As there is no difference in the cooling of the airway walls between asthmatic and healthy individuals [26], the reduction in MCC observed in the asthmatic subjects during ISH in the smaller airways is unlikely to be due to changes in temperature. Based on the measurements of the water content of the expired air, it can be argued that both asthmatic and healthy subjects lose the same amount of water, under the same inspired conditions. Therefore, we should have observed the same changes in MCC in both groups. Perhaps, the hydration of the airways is different in the asthmatics, and air has to travel deeper in the lungs to be humidified. Also, it is possible that there is a delay in the water return in response to the osmotic stimulus in the asthmatic airways due to the pathological changes associated with asthma. Asthmatics have a thicker basement membrane [27, 28] compared with healthy subjects, and this could reduce water diffusion simply by increasing the diffusion path. This could not easily be detected at the mouth, as the expired air rapidly adjusts to the wall conditions of the upper airways on its way to the mouth. However, the data from TABKA *et al*. [29] support a difference in water transport between healthy and asthmatic subjects.

The changes in mucociliary clearance caused by isocapnic hyperventilation in both groups and between groups cannot be explained by the deposition of the aerosol. The aerosol deposition was well-matched in the three studies, and it was similar in both groups ($p>0.4$). Also, the differences in MCC between asthmatic and healthy subjects cannot be attributed to the airway narrowing induced in the asthmatic subjects by the ISH with dry air. There was no correlation between the changes in mucociliary clearance during and after isocapnic hyperventilation in all regions with the percentage fall in FEV_1 in the asthmatic subjects. This is not surprising, as the healthy subjects had significant changes in the mucociliary clearance of the whole lung and central region after ISH with dry air but no airway narrowing. Also, no correlation was found between the changes during and after ISH with dry air in any region of the asthmatic and healthy subjects. The finding that airway narrowing had no relationship with either the decrease during or the increase of MCC after ISH with dry air, is consistent with the work by O'RIORDAN and SMALDONE [30]. These workers found no impairment of MCC in asthmatics after acute bronchoconstriction induced by inhaled methacholine.

WOLFF *et al*. [31] observed similar findings in mucociliary clearance during and after exercise and ISH in healthy subjects. They found a delay in the onset of the observed increase in mucociliary clearance and the maximum increase was after 2 h. In the study by WOLFF *et al.* [31] ISH was performed 30 min after the radioaerosol was inhaled, and in five intervals of 4 min, with interruptions of 2 min, for clearance measurements. In the present study, ISH was performed close to 10 min after the inhalation, and it was of shorter duration (6–8 min) but continuous. WOLFF *et al.* [31] explained the observed increase in mucociliary clearance afterwards as being due to increased lung movement. Hyperventilation could stimulate lung stretch receptors that result in increased parasympathetic activity. If the increase in mucociliary clearance is due purely to lung movement, then the increase in mucociliary clearance post-ISH with dry and warm humid air should have been identical. We showed, however, that MCC post these two interventions was different.

BENNETT *et al*. [32] have shown that rapid airflow, as in cough or rapid inhalations, regardless of its direction increases mucociliary clearance. They concluded that this results from stimulation of mucociliary clearance rather than two-phase gas-liquid flow interaction. They took no account of the fact that these experiments involved repeated inhalations with dry air, 60–90 in 1 h. If it is true that rapid airflow alone stimulates mucociliary clearance, then hyperventilation with warm humid air would have resulted in the same increase in mucociliary clearance as hyperventilation with dry air. Stimulation of irritant receptors by rapid lung deflations has been shown to stimulate airway secretions in dogs [33]. We agree that mucociliary clearance is stimulated *via* some mechanism that stimulates the ciliary beat frequency, either directly or indirectly, however, our results indicate that this cannot be due to increased airflow alone.

Recent work of ISHII and KITAMURA [34] has shown

that ISH with dry air stimulates the release of prostaglandin E_2 (PGE_2) in humans. There is also evidence that hyperosmolarity induces the production of prostaglandins, in particular PGE_2 [35]. PGE_2 is known to increase secretions and the ciliary beat frequency [25]. Prostaglandins can cause a parasympathetic reflex that activates the ciliary stimulatory mechanisms [36–38]. The release of PGE_2 is, therefore, a possible candidate responsible for the observed increase of the mucociliary clearance in all subjects.

Asthmatics probably have additional mediators present in their airways, due to inflammatory cells. Since ISH with dry air induced airway narrowing in the asthmatic subjects, this warrants the presence of the mediators after ISH. This has been shown by the work of PLISS *et al.* [39]. Inflammatory cells, like mast cells, are known to degranulate in the presence of hyperosmolarity [40], which is regarded as the likely mechanism of hyperpnoea-induced asthma [1]. Mediators, for example histamine, can influence mucociliary clearance by increasing ciliary beat frequency or by increasing the secretions and the epithelial water transport towards the lumen [41, 42]. Histamine has been found to cause a prolonged stimulation of mucociliary clearance in asthmatic subjects at relatively lower concentrations than in normal subjects [43]. This could explain the differences in the duration of the increase in MCC in the central region between asthmatic and healthy subjects. The state of the airways of the asthmatics post-ISH with dry air should not be confused with the state of the airways when they are undergoing exacerbations of their asthma which lead to impairment of MCC. Their secretions during exacerbations of asthma have even been found to contain a ciliostatic factor [44], which could contribute to the impairment of MCC.

In summary, ISH with dry air changed MCC compared to ISH with warm humid air and with resting ventilation. MCC was significantly reduced during and increased after ISH with dry air in the large airways both of asthmatic and healthy subjects. In addition, MCC was reduced during and increased after ISH with dry air in the small airways of the asthmatics only. Furthermore, the increase in MCC was persistent in the large airways in the asthmatic subjects. We conclude that the differences in MCC between dry and warm humid ISH were most likely to have resulted from the low inspired water content, causing changes in the periciliary fluid layer. The hypothesis that during ISH with dry air the rate of water loss probably exceeds the rate of water return, resulting in a reduction of the depth of the periciliary fluid layer and subsequent hyperosmolarity of the airway fluid, is consistent with the observations of this study.

Acknowledgements: The authors would like to thank D. Bailey, G. Bautovich and the technical staff of the Department of Nuclear Medicine and J.P. Seale of the Department of Respiratory Medicine for their help. They would also like to thank M. Okazawa of St Pauls Hospital in Vancouver for his incisive question that led to the carrying out of this study.

References

1. Anderson SD. Exercise-induced asthma: *In*: Middleton E, Reed CE, Ellis EF, Franklin Adkinson N, Yunginger JW, Busse WW, eds. Allergy Principles and Practice. 4th edn. St Louis, Mosby, 1993; pp. 1343–1368.
2. Anderson SD, Schoeffel E, Follet R, Perry CP, Daviskas E, Kendall M. Sensitivity to heat and water loss at rest and during exercise in asthmatic patients. *Eur J Respir Dis* 1982; 63: 459–471.
3. Strauss RH, McFadden ER Jr, Ingram RH Jr, Deal EC Jr, Jaeger JJ. Influence of heat and humidity on the airway obstruction induced by exercise in asthma. *J Clin Invest* 1978; 61: 433–440.
4. McFadden ER Jr, Pichurko BM, Bowman HF, *et al.* Thermal mapping of the airways in humans. *J Appl Physiol* 1985; 58(2): 564–570.
5. Hanna LM, Scherer PW. A theoretical model of localized heat and water vapor transport in the human respiratory tract. *J Biomed Eng* 1986; 108: 19–27.
6. Daviskas E, Gonda I, Anderson SD. Mathematical modeling of heat and water transport in human respiratory tract. *J Appl Physiol* 1990; 69 (1): 362–372.
7. Daviskas E, Gonda I, Anderson SD. Local airway heat and water vapour losses. *Respir Physiol* 1991; 84: 115–132.
8. Smith CM, Anderson SD. Hyperosmolarity as the stimulus to asthma induced by hyperventilation? *J Allergy Clin Immunol* 1986; 77: 729–736.
9. Sheppard KL, Rahmoune H. Evaporation-induced changes in airway liquid on an isolated guinea-pig trachea. *J Appl Physiol* 1994; 76(3): 1156–1165.
10. Konietzko N, Nakhsteen JA, Mizera W, Kasparek R, Hesse H. Ciliary beat frequency taken from normal persons and patients with various lung diseases. *Chest* 1981; Suppl. 80 (6): 855–857.
11. Hirsch JA, Tokayer JL, Robinson MJ, Sackner MA. Effects of dry air and subsequent humidification on tracheal mucous velocity in dogs. *J Appl Physiol* 1975; 39(2): 242–246.
12. Puchelle E, Zahm JM, Jacquot J, Pierrot D. Effect of humidity on spinability and transport capacity of canine airway secretions. *Biorheology* 1989; 26: 315–322.
13. Winters SL, Daza AV, Yeates DB. Direct and indirect manipulation of mucosal Na^+ and Cl^- on the mucociliary transport system in the anesthetized dog. *Am Rev Respir Dis* 1993; 147, A1010.
14. Peatfield AC, Richardson PS, Wells UM. The effect of airflow on mucus secretion into the trachea of the cat. *J Physiol* 1986; 380: 429–439.
15. Salah B, Dinh Xuan AT, Fouilladieu JL, Lockhart A, Regnard J. Nasal mucociliary transport in healthy subjects is slower when breathing dry air. *Eur Respir J* 1988; 1: 846–849.
16. Chalon J, Day L, Malebranche J. Effects of dry anaesthetic gases on tracheobronchial ciliated epithelium. *Anaesthesiology* 1972; 37: 338–343.
17. Goldman HI, Becklake MR. Respiratory function tests. *Am Rev Respir Dis* 1959; 79: 457–467.
18. Phillips YY, Jaeger JJ, Laube BL, Rosenthal RR. Eucapnic voluntary hyperventilation of compressed gas mixture. *Am Rev Respir Dis* 1985; 131: 31–35.
19. Smith CM, Anderson SD. A comparison between the airway response to isocapnic hyperventilation and hypertonic saline in subjects with asthma. *Eur Respir J* 1989; 2: 36–43.
20. Phipps PR, Gonda I. Droplets produced by medical

nebulizers: some factors affecting their size and solute concentration. *Chest* 1990; 97: 1327–1332.
21. Phipps PR, Gonda I, Bailey DL, Borham P, Bautovich G, Anderson SD. Comparisons of planar and tomographic gamma-scintigraphy to measure the penetration index of inhaled aerosols. *Am Rev Respir Dis* 1989; 139: 1516–1523.
22. Phipps PR, Gonda I, Anderson SD. Apparatus for the control of breathing patterns during aerosol inhalation. *J Aerosol Med* 1992; 5: 155–170.
23. Bailey DL, Hutton BF, Walker PJ. Improved SPECT using simultaneous emission and transmission tomography. *J Nucl Med* 1987; 28: 844–851.
24. Bailey DL, Fulton RR, Jackson CB, Hutton BF. Dynamic geometric mean studies using a single headed rotating gamma camera. *J Nucl Med* 1989; 30: 1865–1869.
25. Wanner A, Sielczak M, Mella JF, Abraham WM. Ciliary responsiveness in allergic and nonallergic airways. *J Appl Physiol* 1986; 60(6): 1967–1971.
26. Zawadski DK, Lenner KA, McFadden ER Jr. Comparison of intra-airway temperatures in normal and asthmatic subjects after hyperpnoea with hot, cold and ambient air. *Am Rev Respir Dis* 1988; 138: 1553–1558.
27. Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989; March: 520–524.
28. Jeffery RK, Godfrey RW, Adelroth E, Nelson F, Rogers A, Johansson SA. Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma. *Am Rev Respir Dis* 1992; 145: 890–899.
29. Tabka Z, Jebria AB, Guénard H. Effect of breathing dry warm air on respiratory water loss at rest and during exercise. *Respir Physiol* 1987; 67: 115–125.
30. O'Riordan TG, Smaldone GC. Induced bronchoconstriction and mucociliary clearance in asthma. *J Aerosol Med* 1993; 6 (Suppl.): 50.
31. Wolff RK, Dolovich MB, Obminski G, Newhouse MT. Effects of exercise and eucapnic hyperventilation on bronchial clearance in man. *J Appl Physiol: Respirat Environ Exercise Physiol* 1977; 43(1): 46–50.
32. Bennet WD, Foster MW, Chapman WF. Cough-enhanced mucus clearance in the normal lung. *J Appl Physiol* 1990; 69(5): 1670–1675.
33. Yu J, Schultz HD, Goodman J, Coleridge JCG, Coelridge HM, Davis B. Pulmonary rapidly-adapting receptors reflexly increase airway secretion in dogs. *J Appl Physiol* 1989; 67: 682–687.
34. Ishii Y, Kitamura S. Hyperventilation stimulates the release of prostaglandin I_2 and E_2 from lung in humans. Prostaglandins, 1990; 39(6): 685–691.
35. Assouline G, Leibson V, Danon A. Stimulation of prostaglandin output from rat stomach by hypertonic solutions. *Eur J Pharmacol* 1977; 44: 271–273.
36. Wong LB, Miller IF, Yeates DB. Regulation of ciliary beat frequency by autonomic mechanisms *in vitro*. *J Appl Physiol* 1988; 65(4): 1895–1901.
37. Wong LB, Miller IF, Yeates DB. Stimulation of tracheal ciliary beat frequency by capsaicin. *J Appl Physiol* 1990; 68(6): 2574–2580.
38. Wong LB, Miller IF, Yeates DB. Pathways of substance P stimulation of canine tracheal ciliary beat frequency. *J Appl Physiol* 1991; 70(1): 267–273.
39. Pliss LB, Ingenito EP, Ingram RH Jr, Pichurko B. Assessment of bronchoalveolar cell and mediator response to isocapnic hyperpnea in asthma. *Am Rev Respir Dis* 1990; 142; 73–78.
40. Silber G, Proud D, Warner J, *et al*. *In vivo* release of inflammatory mediators by hyperosmolar solutions. *Am Rev Respir Dis* 1988; 137: 606–612.
41. Wanner A, Maurer D, Abraham WM, Szepfalusi Z, Sielczak M. Effects of chemical mediators of anaphylaxis on ciliary function. *J Allergy Clin Immunol* 1983; 72: 663–667.
42. Mussatto DJ, Garrard CS, Lourenco RV. The effect of inhaled histamine on human tracheal mucus velocity and bronchial mucociliary clearance. *Am Rev Respir Dis* 1988; 138: 775–779.
43. Garrard CS, Mussatto DJ, Lourenco RV. Lung mucociliary transport in asymptomatic asthma: effects of inhaled histamine. *J Lab Clin Med* 1989; 113: 190–195.
44. Dulfano MJ, Luk CKA. Sputum and ciliary inhabition in asthma. *Thorax* 1982; 37: 646–651.