COLD AIR, ASTHMA AND AIRWAYS REACTIVITY

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TO MY BELOVED MOTHER
WHO TAUGHT ME AS A CHILD
THE MOST IMPORTANT LESSON
'THE FEAR OF THE LORD
IS THE BEGINNING OF WISDOM'

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ABSTRACT

A simple and standardised test based on cold air inhalation has been developed to measure the level of bronchial reactivity in patients with asthma. It consisted of stepwise increases in ventilation of dry, subfreezing air at 10, 20, 40 and 60% of predicted indirect maximum breathing capacity (IMBC). For each step the optimal time for inhalation was three minutes and the time between the steps was five minutes. Bronchoconstriction following cold air inhalation reached a maximum when the inhalation was of three minutes duration and the level of ventilation was 60% predicted IMBC. Varying the interval (zero, two and five minutes) between the steps produced no significant differences in the amount of bronchoconstriction produced. Reproducibility following cold air inhalation in individual patients was good when the test was repeated within one week.

Changing the breathing pattern had no effect on airway responsiveness provided that the patient maintained a constant minute ventilation. This implies it is unnecessary to monitor the rate and depth of respiration in order to achieve a given effect, thus making the technique simpler. In addition, a carbon dioxide requirement graph has been constructed at different levels of ventilation. This allows the inspired carbon dioxide concentration to be preset, eliminating the need for continuous monitoring in order to keep the patient isocapnic. This makes the procedure

easier and reduces the cost of the equipment.

The simplified standard method described is easy to use in clinical practice. Bronchial responsiveness to cold air isocapnic hyperventilation was correlated with both exercise and histamine challenges.

Some of the characteristics and mechanisms of cold air-induced asthma were studied. The results indicated that:

1. Cold air inhalation does not produce a cumulative effect.

2. In the majority of patients with asthma, episodes of cold air-induced asthma, were not followed by a refractory period.

3. Evidence has been presented which suggests that respiratory heat loss was the stimulus for the mediator release and bronchoconstriction in both exercise and hyperventilation induced asthma.

4. Stabilization of respiratory heat exchange was achieved within three minutes of moderate hyperventilation with cold air, which could explain why no further bronchoconstriction was observed beyond three minutes.

The method described has been used to detect hyperactivity in

patients who had occupational asthma. These patients had been already removed from their work environment. In such a group of patients it was necessary to have a test which was simple and acceptable for epidemiological studies. The cold air isocapnic hyperventilation test which had been developed fulfilled these requirements.

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ABBREVIATIONS

1 = litre.

kg = kilogram.

cm = centimetre.

ml = millilitre.

mmHg = millimetres of mercury.

h = hour.

min = minute.

sec = second.

% = percentage.

m.p.h. = miles per hour.

BTPS = body temperature and pressure saturated with water vapour.

STPD = standard temperature and pressure of dry gas.

ATPS = ambient temperature and pressure saturated with water vapour.

ATPD = ambient temperature and pressure of dry gas.

BTPS = body temperature and pressure saturated with water vapour.

meq = milliequivalent.

PEFR = peak expiratory flow rate 1/min.

VC = vital capacity 1.

FVC = forced vital capacity 1.

TLC = total lung capacity 1.

FRC = functional residual capacity 1.

RV = residual volume 1.

FEV1 = forced expiratory volume in one second 1.

MMEFR = maximum mid-expiratory flow rate 1/min.

Raw = airways resistance KPa 1-1 s

sGaw = specific airways conductance s-1 KPa-1

VE = minute ventilation 1.

VT = tidal volume ml.

MRC = Medical Research Council.

^{o}C = centigrade.

SRSA = Slow reacting substance of anaphylaxis

TDI = toulene di-isocyanate.

MDI = diphenyl methane di-isocyanate.

NDI = naphthalene di-isocyanate.

HDI = hexamethylene di-isocyanate.

TLV = threshold limit value.

CO2 = carbon dioxide.

IMBC = indirect maximum breathing capacity.

CAIH = cold air isocapnic hyperventilation.

ISH = isocapnic hyperventilation.

EIA = exercise-induced asthma.

PC20 = provocation concentration of histamine in mg/ml inducing 20% fall in in FEV1.

PD10 = the ventilation rate in percentage of predicted IMBC required to reduce FEV1 by 10%.

PD20 = the ventilation rate in percentage of predicted IMBC required to reduce FEV1 by 20%.

PBS = Phosphate buffered saline.

HAP = Histamine acid phosphate.

VA = effective alveolar volume l.

KCO = coefficient of diffusion mmol min-1 KPa-1 l-1.

DLCO = diffusion capacity of carbon monoxide mmol min-1 KPa-1.

HPF = high power field.

RHE = respiratory heat exchange KCal/min.

Ti = inspired temperature ^{o}C.

Te = expired temperature ^{o}C.

WCi = water content of the inspired air mg/l.

WCl = water content of the expired air mg/l.

HV = Latent heat of evaporisation KCal/gm

HC = Heat capacity of the air KCal/l ^{o}C.

KCal = kilocalories.

CIH20 = Water content of the inspired air mg/l.

CEH20 = water content of the expired air mg/l.

g = gram.

PETCO2 = end-tidal CO2 mmHg.

SD = standard deviation.

SEM = standard error of the mean.

r = correlation co-efficient.

n = number of subjects.

p = significance level.

CHAPTER ONE

INTRODUCTION

INTRODUCTION

1.1 DEFINITIONS

Transient or widely variable narrowing of the airways is the functional disorder which defines asthma (Scadding, 1966). This definition underlines the basic functional defect of a variable airways obstruction. In other words, the bronchial tree has hyperreactivity (or increased lability) to a host of stimuli such as allergens, irritants, physical factors (cold air), pharmacological agents (histamine, and methacholine), exercise and emotional factors. Some of these agents are now used for challenge tests in order to investigate the increased airways hyperresponsiveness. Indeed, airway hyperresponsiveness is a central feature of asthma upon which many of the changes of airway calibre depend.

During the last few years, interest has focused on exercise-induced asthma. It has been suggested that the main factor which induces bronchospasm during exercise is hyperventilation with consequent drying and cooling of the airways (Deal et al, 1979a; 1979b). Therefore, breathing cold air has been proposed as a bronchial provocation test. Because it demands high levels of ventilation, hypocapnia has to be prevented by supplementary carbon dioxide. The resultant procedure is known as cold air isocapnic hyperventilation (CAIH).

1.2 HISTORICAL INTRODUCTION

The association between asthma and exercise was first described by Arateus of Cappodocia who lived circa 81-138 A.D. In his early writings on respiratory disorders he stated that "if from running, gymnastic exercises or any other work, the breathing becomes difficult, it is called asthma" (Adams, 1856). Arateus did not comment on the possible reasons why exercise might be responsible for this difficulty of breathing which he called asthma.

It was not until the 17th century that interest was awakened in the association between exercise and asthma. At this time asthma was described as an " obstruction of the bronchi " and it was thought that exercise was capable of acting as a stimulus to change airway calibre by provoking bronchial spasm. This idea was extended in the 18th century by Cullen who reported in his book "First Lines of the Practice of Physic" (1784) that "constriction of the muscular fibres of the bronchiae which not only prevents the dilatation of the bronchiae necessary to a free and full inspiration, but gives also a rigidity which prevents a free and full expiration" and "like many other convulsive and spasmodic affections, is readily excited by a turgescence of the blood or other cause of unusual fullness and distension of the vessels of the lungs". Cullen also made the observation that "asthmatics bear no bodily motion easily"

and recommended riding and especially sailing to be useful exercise for asthmatics. This was in agreement with earlier ideas that exercises which involved the arm were the least agreeable to asthmatics (Floyer, 1717).

The association between exercise, cold and asthmatic attacks was first described by Willis (1679) . He observed that "Whatsoever therefore makes the blood boyl, or raises it into the effervescence as violent motion of the body or mind, excess of extern cold or heat, the drinking of wine, Vinery yea sometimes meer heat of the bed doth cause asthmatical assaults to such as are predisposed".

Although it was generally agreed by Willis (1679), Floyer (1717) and Cullen (1784) that spasm was due to contraction of the bronchi, it was not until the early part of the 19th century that the existence of muscular fibres in the bronchi was demonstrated.

An extensive study was made in the latter half of the 19th Century by Salter (1868). He assessed the the frequency of exercise as a precipitating factor in asthmatic attacks. Of the 223 recorded cases, 54 patients cited exercise as one of the provoking stimuli for their asthmatic attacks.

Despite obvious awareness of the effects of violent exercise in asthmatic patients, physicians of the time generally agreed that some form of exercise was necessary in the treatment of asthma.

Horseback riding and walking were frequently recommended in the medical texts of the period. In "Notes on Asthma" published in 1873, John Thorowgood stated that "Daily exercise in the open air should not be neglected, and several cases testify to the effect of a steady walk in preventing an attack of asthma even when it already threatens". Similarly Salter (1868) commented that he was puzzled by the fact that exercise appeared to help some of his patients. It seems possible that these patients were in fact benefiting from the bronchodilatation which occurs during exercise.

After this time the problem of exercise and the asthmatic appears to have been forgotten until the middle of this century. With the development of the low resistance spirometer, the peak flow meter and the whole body plethysmograph, it became possible to quantify changes in airways resistance. Investigators in the field of asthma research began to use these new techniques to determine the frequency and cause of exercise-induced asthma (EIA).

The importance of hyperventilation in provoking bronchoconstriction was first described by Herxheimer (1946). He was the first investigator to quantify changes in ventilatory volumes following exercise and he reported measurements of changes in vital capacity which occurred after short-term running and cycling exercise. The effect of carbon dioxide inhalation was also investigated by connecting the patients to a rebreathing tube at the end of the exercise. In four patients who were studied in this manner,

Herxheimer noted that no attack of asthma occurred after exercise. These results led to the theory that hypocapnia was reponsible for post-exercise induced bronchospasm which prompted a further investigation into the effect of voluntary hyperventilation. Results in six patients showed that only one failed to wheeze following hyperventilation. Thus it was postulated that excessive overbreathing which had been noted to occur following exercise, produced a fall in alveolar carbon dioxide tension and that this in turn provoked bronchoconstriction.

Following Herxheimer's study a report (Pearson, 1952) suggested that after exercise in asthmatic subjects two opposing factors might occur either independently or consecutively "The one causing improvement and the other an increase in bronchial obstruction". This suggestion was based on the results of measurements made of vital capacity and two-second vital capacity before and after exercise in asthmatic subjects. It appeared that immediately following exercise, some subjects had an increase in two-second vital capacity and some showed no change, but after a period of five minutes there was a reduction in both vital capacity and two-second vital capacity.

Investigations into the forced expiratory volume (FEV1) following exercise in a group of adult subjects some of them with asthma was carried out by Capel and Smart, 1959. It was found that there was a mean increase in FEV1 of 24% of the resting value after exercise

in these subjects while another group of patients either with heart disease or other types of lung disease showed no significant change in FEV1 after exercise. The important observation was made that the combination of exercise and adrenaline inhalation was more effective in increasing the FEV1 in patients with obstructive lung disease than the use of either exercise or adrenaline alone. It was concluded from these and other studies that the increase in inspiratory rate and an increase in lung blood volume due to exercise may be responsible for a fall in airways resistance and thus an increase in the FEV1. This was reasoned on the basis of unequal emptying of alveoli, so that the increased rate of inspiration allowed areas of low resistance in the lung to become overdistended during inspiration. This overdistension would allow the alveoli "to empty into their low resistance airways with less assistance from intrathoracic pressure" and thus there would be less expiratory collapse of the airways.

It was also suggested that increased blood volume in the lungs during exercise would make the lungs more turgid and that this would increase their power of recoil and keep the airways open during a forced expiration.

This last suggestion is an interesting reversal of the idea of the early physicians who considered that it was the increase in blood volume in the lungs during exercise which caused the difficulty of breathing following exercise (Willis 1679; Floyer 1717; Cullen

1784).

In fact the early ideas of there being an increase in blood volume in the lungs in asthmatic subjects had given rise to blood letting as a treatment for asthma.

With the development of the body plethysmography it became possible to measure airways resistance directly and at the same time to determine the functional residual capacity. Using this method of investigation, it was found that airways resistance increased in two asthmatic subjects following brief exercise and that functional residual capacity increased in one subject at a time when airways resistance was high (Butler et al, 1960).

By now, the original observations of Pearson (1952) had been confirmed using different methods for estimation of airways resistance. Asthmatics appeared to be able to respond to exercise in two different ways: by improving their lung function due to a fall in airways resistance and an increase in FEV1 and by increasing the obstruction and having a consequent rise in airways resistance.

The first comprehensive explanation of these two opposing effects of exercise in asthmatic subjects was detailed by Jones, Buston and Wharton in 1962, who suggested that the apparent lack of uniformity was due to the choice of an unsuitable duration and level of

exercise. They studied the effect of brief and prolonged exercise on a group of asthmatic children and showed clearly that exercise of short duration resulted in an increase in FEV1 following exercise, whereas prolonged exercise resulted in a transient fall in FEV1 below the resting level. They also investigated the effect of running exercise in a group of normal children and demonstrated that there was no significant change in the post-exercise FEV1 as compared with the resting value in these subjects. They reported that the increase in FEV1 following short term exercise in the asthmatic subjects, expressed as a percentage of the resting value, was greater in those subjects with the lowest resting values. It was suggested that the reason for the two opposing effects of exercise in the asthmatic subjects was bronchodilatation or bronchoconstriction in bronchi which were already in a state of constriction. This suggestion was supported by the results of a study which demonstrated a diminished or absent response to exercise in asthmatic subjects following administration of isoprenaline sulphate. In addition, it was shown that following exercise there was no bronchodilation or bronchoconstriction of any significance in normal subjects.

The value of exercise as a diagnostic aid and a useful method for the assessment of drugs in asthmatic subjects was emphasized in a further publication (Jones, Wharton and Buston, 1963) which also reviewed several important points including type of exercise used and methods of testing. Firstly, it was commented that the bicycle

ergometer was an unsatisfactory kind of exercise for use in testing asthmatic subjects because it gave variable and unpredictable results. Secondly, it was stressed that frequent interruption of the exercise to make measurements should be avoided because it could diminish the response to exercise.

Peak expiratory flow rate (PEFR) was used for the first time to assess changes in airways resistance during exercise in a large group of adult asthmatic and normal subjects (Irnell and Swartling, 1966). They investigated the effect of progressive bicycle exercise among 101 asthmatic subjects. The results showed that there was an increase in PEFR during exercise and that the highest value was obtained during the highest work load. Following exercise of varying duration, a typical asthmatic attack occurred in fourteen of the subjects. The relatively low proportion who developed bronchoconstriction following exercise, in these asthmatic subjects, was undoubtedly due to the choice of exercise and confirmed the previous suggestion that cycling was an unsatisfactory type of exercise for inducing an attack of asthma (Jones et al, 1963).

At this time other forms of work were being used to study the effects of exercise in normal and asthmatic subjects.

In an investigation of a group of normal and asthmatic adults, airways resistance following stair climbing, was measured in

several different ways. These measurements included FEV1, airways resistance measured in the body plethysmography and distribution of inspired gas using the method of Comroe and Fowler (1951). The results in asthmatic subjects were similar to those reported previously (Butler et al, 1960; Jones et al, 1962 and 1963). However, among the normal subjects although there was no significant change in FEV1 following exercise there was a significant increase in measurements of airways resistance made in the body plethysmograph. This study included the first report on the effect of multiple exercise tests in asthmatic subjects. In two out of four subjects who had repeated exercise tests throughout a day, exercise was shown to have a diminishing effect on the measurement of FEV1. This observation led to the theory that post-exercise bronchoconstriction may be caused by release of an enzyme. It was postulated that repeated exercise resulted in less constriction because stores of the mediating enzyme were depleted throughout the day. In addition to the studies following exercise, the effect of hypercapnic hyperventilation was observed in the asthmatic patients and no fall in FEV1 was demonstrated. This observation did not confirm the previous suggestion that hyperventilation with hypocapnia might be the cause for the observed effects of exercise (McNeill et al, 1966).

The lability index was introduced by Jones (1966), bronchoconstriction (fall in FEV1 from pre-exercise level) was measured following exercise and maximum bronchodilatation (increase

in FEV1) was obtained by administration of isoprenaline sulphate aerosol followed by one minute exercise.

The liability index was defined as the sum of the fall in FEV1 and rise in FEV1 (in litres) below and above resting values, expressed as a percentage of the predicted normal value. It was reported that, with few exceptions, the liability index for normal children was less than 15% while nearly all asthmatic subjects had a liability index greater than 20% (Jones, 1966).

By the late sixties attention had turned towards the measurement of arterial blood gas tension and blood lactate in the hope of elucidating the mechanism of exercise-induced asthma.

Measurements of arterial blood gas tensions and blood lactate were carried out in asthmatics before and after five to eight minutes of running exercise (Seaton et al, 1969). Although significant bronchoconstriction occurred following the exercise there was no significant change in the values of arterial oxygen tension. These workers noted however that a severe metabolic acidosis was present in all subjects at a time when the airways resistance was increased due to exercise, and that this metabolic acidosis was due to an increase in blood lactate. It was found that as the acidosis improved the FEV1 returned towards the normal resting value. Following an injection of bicarbonate and adrenaline there was an immediate reversal of the bronchospasm. In the previous test that

had not reversed with adrenaline alone. From this it was concluded that the metabolic acidosis of exercise was capable of triggering the release of bronchoconstrictor substances such as bradykinin and SRSA (slow reacting substance of anaphylaxis) were suggested.

The observations of Seaton et al (1969) were confirmed in a further publication on the subject of arterial oxygen tension and blood lactate by Fisher et al (1970) and it appeared at the time that lactic acidosis could not be excluded as a possible cause of exercise-induced asthma.

From exercise-induced asthma to cold air isocapnic hyperventilation

In recent years, effects of changes in the temperature and humidity of the inspired air have been studied in subjects with documented exercise-induced asthma. Inhalation of saturated air at room temperature during exercise challenge significantly ameliorates EIA (Weistein et al, 1976; Bar-or et al, 1977; Chen and Horton, 1977; McFadden et al, 1977a) and inhalation of air saturated at body temperature entirely prevents EIA (Chen and Horton, 1977; Deal et al, 1979a; Lee, Assoufi and Kay, 1983). It was shown that cold air could potentiate EIA and that the severity of the airways obstruction, when breathing air of ambient temperture, was inversely proportioned to the water content of the inspired air. Increasing the humidity at ambient tempertures reduced EIA and

inhaling air at body temperature which was fully saturated, completely prevented it from occurring (Deal et al, 1979a). Deal et al (1979a ; 1979b) postulated that the cause for EIA was a function of the heat exchange that occurred in the bronchial mucosa due to the vaporisation of water and that this was necessary for the conditioning of the inspired gas by the airways. They developed quantitative expressions that suggested that respiratory heat exchange was directly proportional to the minute ventilation. They further reasoned that since the level of ventilation was an important determinant to the quantity of heat transferred from the mucosa, it should be possible to reproduce the extent of the airflow obstruction on exercise by performing isocapnic hyperventilation (ISH). To test this hypothesis, they subjected asthmatic patients to isocapnic hyperventilation through a heat exchanger. Hyperventilation with air at body temperature which was fully saturated (heat flux zero) did not result in bronchoconstriction. However, if the water content and temperature of the inspired air was decreased, thereby increasing the thermal burden on the airways bronchoconstriction occurred. They therefore concluded that the major stimulus for EIA is heat loss from the airways which is precipitated by exercise, exercise per se is not essential and serves only as a way to increase ventilation. They have further suggested that the changes produced by isocapnic hyperventilation are a close reflection of EIA (Deal et al, 1980a). Despite these experiments, it is not known how obstruction of airway is induced by cooling. Breathing cold air has been proposed

as a bronchoprovocative test in patients with asthma (Deal et al, 1979a). Because it demands a high level of ventilation hypocapnia has to be prevented by supplement with CO2. The resultant procedure is called cold air isocapnic hyperventilation (CAIH).

1.3 COLD AIR, ASTHMA AND AIRWAY REACTIVITY

It is a well known clinical fact that exposure to weather of an extreme kind may precipitate the onset of symptoms in patients with bronchial asthma. They may complain of cough, chest discomfort, wheezing or dyspnoea during a seasonal change in weather or when they experience an enviromental temperature change.

Exposure to cold air is similar to extreme weather which may aggravate bronchospasm in patients with asthma. Normal man is able to function in an extremely cold environment without experiencing symptoms of respiratory difficulty or suffering injury to his airways. This protective ability may be due to the fact that the normal respiratory tract is highly effective in its ability to heat and humidify inspired air under extreme climatic conditions. Moritz and Weisiger (1945) have shown that air delivered at the lips at -100 ^{o}C is heated to within 1 or 2 ^{o}C of body temperature by the time it reaches the tracheal bifurcation. On the other hand, exposure to cold air may affect the respiratory system severely in patients with bronchial asthma or other chronic respiratory

diseases.

These observations have caused many investigators to study the effect of cold air on airway response in different respiratory diseases. Wells, Walker and Hickler (1960) studied patients with chronic respiratory diseases who had a history of cold intolerance. Measurement of airflow resistance before, during and after inhalation of cold air and revealed a marked rise in airflow resistance in the majority of these patients when they inhaled cold (-30 C) air. A cold air draft delivered across the face of these subjects did not produce significant changes in airflow resistance. This exposure to cold air did not appear to increase the response to cold air inhalation. Normal subjects and patients with chronic respiratory tract diseases who did not give a history of symptoms precipitated by cold were asymptomatic in the cold and were not affected by these stimuli.

The results of Hsieh, Frayser and Ross (1968) support the work of Wells et al (1960). These investigators studied the effect of cold air inhalation on gas flow and distribution in the lungs of normal subjects and patients with chronic obstructive pulmonary diseases. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) maximal expiratory flow rate (MEFR), maximum voluntary ventilation (MVV) and nitrogen elimination after a single breath, were determined during inhalation of air at room temperature and at -40 C in 47 cases of chronic obstructive

pulmonary disease. In normal subjects and patients with no history of respiratory sensitivity to cold air, there was no significant difference in any parameter studied when air was inhaled at room temperature and -40. Patients with a positive history of sensitivity to cold air, however, had a significant reduction in forced vital capacity during inhalation of air at -40 C.

Gullaria et al (1969) studied the effect of cold air inhalation at -30 C for 15 minutes on pulmonary compliance and inspiratory flow resistance. Their results showed that there was significant change in pulmonary compliance but that the inspiratory flow resistance was significantly increased. It was suggested that the direct action of cold air on the upper respiratory tract was responsible for the mild local airways obstruction.

Studies on the effect of cold air by using a large cooled chamber were done by Millar et al (1965) and Ramsey (1977). Millar et al, (1965) investigated the effect of breathing cold air on five normal and ten asthmatic subjects. They used a large refrigeration room with an average temperature of -20 C and a relative humidity of 35%. Measurements of FEV1 were made before, during and after exposure to cold air. There was no significant change in the normal subjects but two asthmatic patients were affected at once and showed a fall in FEV1. Another two patients showed a later fall in FEV1 after returning to normal room temperature. Ramsay (1977) investigated 13 adults with bronchial asthma and a similar

number of controls. They were subjected to a temperature of 2 C for 45 minutes with a controlled relative humidity of 45% in a large, well-ventilated walk-in environmental chamber and then returned to a temperature of 24 C. The asthmatic patients showed a decrease in pulmonary function while no significant changes were noted in the controls. Urinary catecholamines increased in the controls but the asthmatic patients showed no significant increase.

In recent years, breathing cold air has been proposed as a bronchial provocation test in asthma (Deal et al, 1979a). This followed the suggestion that the main factor which induces bronchospasm during exercise is hyperventilation with consequent cooling of the airways

There is little data available on comparison of isocapnic hyperventilation with other standard reactivity tests and reported results are conflicting. Kivity and Souhrada (1981) studied the bronchial responsiveness to methacholine and voluntary isocapnic hyperventilation with room air in five normal subjects and eleven patients. They concluded that methacholine was less sensitive than isocapnic hyperventilation with room air and that there was no correlation between the bronchial responsiveness to the two inhalation challenges. Chatham et al (1982) examined the relationships between responsiveness to methacholine, histamine and respiratory heat loss during exercise in asthmatics, and found that the correlation between the two pharmacological challenges was good

but that the correlation with exercise ventilation and respiratory heat loss was rather poor. In contrast, O'Byrne et al (1982a); Woodrow-Weiss, Rossing and McFadden (1983) reported a good relationship between isocapnic hyperventilation with cold air and methacholine challenge in a group of asthmatics studied. Aquilina, 1983; Heaton et al, 1984 found a good relationship between cold air isocapnic hyperventilation, methocholine and histamine challenges.

1.4 MECHANISMS OF COLD AIR-INDUCED ASTHMA

It was recognised that hyperventilation whether or not alveolar carbon dioxide is allowed to fall, causes airways obstruction whose characteristics and time course are similar to those produced by exercise performed at the same minute ventilation. It was suggested that hyperventilation is the central mechanism in exercise-induced asthma (Zeballos et al, 1978; Deal et al,1979a; Kilham, Tooley and Silverman, 1979; Breslin et al,1980).

Chen and Horton (1977) studied the effects of changing temperature and relative humidity of the inspired gas on EIA. They calculated the respiratory heat loss and they found that the more severe the bronchoconstriction, the greater was the heat loss. Deal et al (1979a) studied the respiratory heat exchange (RHE) in patients with EIA breathing dry air ranging from -13 C to 80C. A very close positive correlation was found between RHE and the fall in FEV1,

MMEF and specific airway conductance as well as with the rise in residual volume. In their study RHE has not been calculated during voluntary isocapnic hyperventilation. However, there is strong evidence that the heat loss also applies to hyperventilation. At two levels of ventilation corresponding to mild and moderate exercise, there was no bronchospastic response when BTPS air was inhaled. At both levels of minute ventilation the fall in FEV1 and airway conductance, and rise in residual volume was greater with subfreezing dry air than with ATPD air and with ATPD than with ATPS air (Deal et al, 1979a). This confirmed that the major stimulus for exercise-induced asthma is evaporation from the mucosa precipitated by the hyperpnoea of exercise but not exercise per se.

Recently, Hahn et al (1984) studied the importance of water loss and heat loss in EIA. They conditioned the inspired air during exercise to the constant water content while changing its temperature and vice versa. They found that airway response to exercise was not significantly different when temperature varied by 25 C, and that the amount of water required to saturate the inspired air remained the same. They concluded that it is the osmotic and not the cooling effects induced by the vaporization of water that is the more important factor determining EIA

Whatever the mechanism of exercise-induced asthma, increased ventilation is an associated feature. A similar obstructive response to exercise could be reproduced by voluntary

hyperventilation if the same ventilation rate and inspired air conditions were used (Deal et al, 1979a).

During hyperventilation, conditioning of the inspired air occurs. This process is a continuous one that begins the moment the air enters the body and involves as much of the tracheobronchial tree as is necessary (McFadden et al, 1982). Heat and water move from the mucosa of the airways to the incoming air as a direct function of the temperature and vapour pressure gradient that exists and inversely with linear velocity of the gas and the geometry of the exchanging surface (Procter and Swift, 1977). A similar gradient exists between the mucosal surface and the deeper tissue of the airways and its blood supply. The evaporation of water and transfer of heat from the mucosal surface stimulates the passage of water and heat transfer from the deeper tissue of the airway to the mucosal surface. The amount of heat gained in a unit time by the inspired air when it leaves the airway passages should be equal to the heat loss by the blood which passes along the wall (Procter and Swift, 1977).

The effect of drying and cooling in hyperventilation lead to airflow obstruction in asthmatic patients and in normal subjects if higher levels of ventilation are used. Drying and cooling of the airway mucosa may produce numerous effects that are not completely understood (McFadden, 1983). For example, the amount and osmolarity of respiratory secretions may increase during airway

cooling (Man et al, 1979). In vitro studies have shown that hyperosmolar solutions can cause the release of chemical mediators from basophils and pulmonary mast cells (Eggelston and Guerrant, 1976). Calcium channel blockers inhibit exercise-induced asthma but do not affect bronchospasm produced by inhaled antigen (Cerrina et al, 1981). This implies that alteration in calcium transport may be important in the response of the respiratory system to airway cooling and drying.

Although recent work has confirmed that the temperature of the airways decreases during exercise or isocapnic hyperventilation, this usually returns to the resting values within five minutes of discontinuing exercise or the isocapnic hyperventilation procedure (Deal et al, 1979a; McFadden et al, 1982). Airway obstruction however, progressively increased over this same period of time and did not resolve for 30 to 60 minutes (Hyanes, Ingram and McFadden, 1976). This indicates that other factors must be sustaining the initiating cold air stimulus.

Recently, it was demonstrated that when the nasal airway is exposed to cold, dry air chemical mediators are released into nasal secretions (Naclerio et al, 1983). These studies support the idea that airway cooling may initiate a sequence of events that may lead to the activation and release of the chemical mediators of immediate hypersensitivity.

The role of the autonomic nervous system in cold air-induced asthma: the precise mechanism by which alpha-adrenergic activity mediates airway activity to cold air and exercise is not known. Reflex vagal bronchonstriction may be important in irritant induced bronchospasm and may partially mediate the response to exercise (Boushey et al, 1980; Griffin et al, 1982) and to cold air (Heaton et al, 1983). The evaluation of the role of the parasympathetic nervous system can be evaluated by administering post ganglionic blocking agents such as atropine. High doses of atropine are known to inhibit exercise-induced asthma in some asthmatics but this agent also causes bronchodilatation, making it difficult to interpret these findings.

Alpha-adrenergic blocking agents also inhibit exercise-induced asthma (Barnes et al, 1981; Bleeker et al, 1983) as well as responses to hyperventilation with cold air, but they do not affect antigen and histamine-induced bronchial reactivity (Walden et al, 1984). In vitro studies have demonstrated increased alpha-receptor activity in tissues that have been cooled (Kunos and Nickerson 1977; Corwin, Cho and Malvin, 1982). During the high levels of ventilation produced with hyperventilation, cooling of the tracheobronchial tree can occur even in peripheral airways (McFadden et al, 1982) where alpha-receptors are known to be present (Kneussl and Richardson, 1978). Therefore alpha adrenergic activity may be increased during airway cooling and this may contribute to the development of bronchoconstriction.

1.5 HEAT EXCHANGE IN THE AIRWAYS

There are two components of heat exchange in the airways : a convective and an evaporative component. The convective component represents the quantity of heat that is necessary to bring the inhaled mass of gas from the temperature of the inspired gas (T1) to that of the expired gas (TE). The evaporative component represents the quantity of heat needed to raise the concentration of water from that of the inspired gas (CIH20) to that of the mixed expired gas (CEH20). In 1966, Houdas and Colin calculated the quantity of heat exchanged per breath (H) by use of equation 1.

$$H = VT\ [a.c.\ (TE-T1) + L\ (CEH20 - CIH20)$$

In this equation VT is the tidal volume, a and c are the volumic mass and the massic heat of the gas respectively, and L is the latent heat of vaporization of water. By multiplying both terms of the above equation by the frequency of breathing, one obtains the flow rate of heat exchanged in the airways (equation 2).

$$H = VE\ [a.c.\ (TE-T1) + L\ (CEH20 - C1H20]$$

By the use of equation 2, Caldwell, Gomez and Fritts (1969) showed that within the range of usual values of T1 and CIH20 the

convective component is about one tenth of the evaporative one. In normal environments, TE is greater than T1 and CEH20 is greater than C1H20 in spite of a slight unsaturation of the mixed expired gas (Ferrus et al, 1980). Therefore, heat is lost by the airways to the environment and the inescapable conclusion follows that breathing in a normal environment causes cooling and drying of the airways. Equations 1 and 2 also predict that cooling and drying of the airways will increase during hyperventilation and during cold and/or dry air breathing. Conversely, the equations also predict that the flow rate of heat will decrease with increasing T1 and CIH20, thus reducing cooling and drying of the airways which will eventually be suppressed when fully saturation air at body temperature is inhaled.

1.6 METHODS FOR MEASURING AIRWAYS RESPONSE

This could be achieved in two ways :

1) Measurement of airways resistance

This provides information on all the airways between the alveoli and the instrument used to measure flow. Thus the results are influenced by events in extrathoracic airways including the glottis. The reciprocal of resistance is called conductance. Airways resistance varies inversely with lung volume so the effect

of lung volume must be taken into consideration in interpreting this measurement. Resistance is usually reported as conductance-volume ratio, known as specific conductance (Mitchell et al, 1967; Payne, Chester and Hsi, 1967; Sobol and Emirgil, 1967). This method is a rapid, easily performed and extremely sensitive index of changes in airways dimensions but it does not distinguish between the response of extrathoracic and intrathoracic airways.

2) Measurements of maximal flow during forced vital capacity mannoeuvre

This can be obtained from flow-volume curves, which reflect the properties of the lung and intrathoracic airways and thus does not share the major disadvantages of conductance measurements. The early part of the maximal expiratory flow volume curve which is effort dependent reflects the rapidity of expiratory muscle contractions, the high elastic recoil pressure at full inflation and the degree of patency of the large central airways. The effort independent portion reflects the resistance from the point of flow limitation towards the alveoli and the recoil pressure of the lung in the mid vital capacity range. Flow is a reflection of the mechanical properties of the lung and intrathoracic airways (Widdicombe and Nadel, 1963; Stein et al, 1966; Sobol and Imirgil, 1967).

1.7 AIMS OF THE PROPOSED STUDY

The aims of the proposed study was to develop a method of estimating airway responsiveness which would be acceptable to working and other populations and therefore applicable to epidemiological studies. The objective assessment of non specific reactivity has involved bronchial challenge tests with various stimuli. Pharmacological, chemical, physical, pharmacological materials (histamine and methacholine) and exercise challenges are most widely used. With pharmacological agents, there might be variability of the response because of inconstant nebulised output, particle size and unpredictable site of drug deposition (Thomson and Pavia, 1974; Pavia et al, 1977; Raffin et al, 1978). In addition, following histamine and methocholine inhalation, side effects such as irritation of the throat, flushing and increased heart rate are often observed (Juniper et al, 1978). Furthermore, when the applications of these tests are considered by industry, there is often reluctance of management or work force to accept tests requiring the administration of a "drug" especially when extremely sensitive workers might have a significant response.

On the other hand, exercise testing to estimate reactivity has some limitations: 1) its use is restricted to those who can perform strenuous physical exercise, 2) the development of bronchoconstriction can alter or mask the effects of cardiac disturbances in situations in which there is a coexistant heart

disease; 3) it is difficult to generate a dose-response curve.

Recently, cold air isocapnic hyperventilation has been introduced as a test of airway reactivity (Deal et al, 1979a). A review of the literature on CAIH suggests that there are four major problems. First, the technique of CAIH has not been standardised. This thesis reports on an attempt to develop a simplified standard test. In essence, it seeks to answer the following questions -

a) What flow rate of cold air is appropriate for use in a standardised CAIH?

b) What duration of exposure is appropriate for use in a standardised CAIH?

c) What is the optimum time between consecutive exposures of cold air to obtain a dose response curve?

d) does the pattern of breathing influence the results?

e) Is it necessary to monitor end-tidal carbon dioxide to keep the subject isocapnic during hyperventilation?

f) how cold should the air be?

g) how repeatable is the test?

h) how does this test compare with other tests of reactivity?

i) what is its specificity (frequency of false positive results) and sensitivity (frequency of potential cases missed by the test)?

Second, little is known about the bronchial provocative characteristics of cold ai isocapnic hyperventilation. An attempt was carried out to study two of the important characteristics of CAIH. These are the cumulative effect and the refractory period The cumulative effect is a feature of some pharmacological challenges like methocholine and carbachol, and of histamine (Orehek and Gayrard 1976, Juniper, 1979, Orehek, 1980). The possibility of a cumulative effect has not previously been investigated for CAIH.

The refractory period is a feature of exercise induced asthma and to a lesser extent of pharmacological substances like histamine (McNeill et al, 1966; James, Faliane and Sly, 1976; Edmund Tooley and Godfrey, 1978; Schoeffel et al, 1980). In CAIH the reports on refractory period were conflicting. Stearn et al (1981), reported that repetition of the exercise test at short intervals produced a diminution in the obstructive response within 30 minutes whereas the degree of obstruction following isocapnic hyperventilation remained constant over the same period of time. Heaton et al (1983); Tal et al (1984) reported no refractory period following CAIH. In contrast Bar-Ysihay, Ben-Dov and Godfrey (1983) reported

a refractory period following cold air isocapnic hyperventilation in seven out of nine of subjects after 30-40 minute intervals. In these studies, a single interval between the challenges was used. Therefore this study has been conducted to determine whether a refractory period exists, following CAIIH using different intervals of time between the challenges.

Third, recent studies have suggested that drying and cooling of the airways precipitated by hyperventilation due to exercise or voluntary hyperventilation is the initiating stimulus for the bronchoconstriction, although the mechanism involved is unknown. The temperature of the airways decreases during exercise or isocapnic hyperventilation but it returns to resting values within five minutes after discontinuation of either exercise or isocapnic hyperventilation (Deal et al, 1979; McFadden et al, 1982). Airways obstruction, however, progressively increases over the same time period and does not resolve for 30-60 minutes (McFadden et al, 1979). Therefore, in this thesis, an investigation was carried out to explore the role of the respiratory heat loss in the airways obstruction in both exercise and isocapnic hyperventilation.

Finally, little is known about the suitability of CAIH in the detection of non-specific reactivity in patients with occupational asthma. In this thesis, CAIH has been used to study non-specific reactivity in patients with asthma due to occupational exposure who had left the factory because of their asthma.

CHAPTER TWO

METHODS AND MATERIALS

METHODS AND MATERIALS

2.1 PATIENT SELECTION

The subjects for this study were volunteers from the staff and patients of the Brompton Hospital. They were able to cooperate with the test procedures and, in the case of patients, were able temporarily to stop from treatment. The subjects were in three groups as follows:

"NORMALS": these had a) no history of cardiopulmonary disease, b) no history of disease which could be expected to affect pulmonary function, c) no evidence of asthma or history of allergic rhinitis (as defined below).

ASTHMATICS: these had a history of episodic symptoms of breathlessness with wheeze and/or chest tightness, and had documented reversibility of 15% or more in FEV1.

ATOPIC ALLERGIC RHINITIS: these were subjects with a history of episodic symptoms of sneezing and/or rhinorrhoea which varied spontaneously or following treatment. Atopy was defined as the presence of positive (> 2 mm) skin prick test reactions to extracts of house dust, mite, cat, or grass pollen extracts.

All subjects were nonsmokers. Subjects were asked to stop any antihistamine preparation 48 hours before testing. Methylxanthine

derivatives, inhaled and oral sympathomimetics and sodium cromoglycate were stopped for 12 hours before all measurements. At the time of the study all subjects had been free of any symptoms of respiratory infection for six weeks. In view circardian variation in lung function and bronchial reactivity, patients were tested at the same time of day if more than one visit was required.

The procedure was explained in detail to all subjects and a consent form was signed. A short history was taken which included individual characteristics, smoking habit, duration and frequency of asthmatic symptoms and precipitating factors. Any other cardiopulmonary illness was also recorded. End-tidal CO2 was then determined, subjects were asked to breathe quietly through a two-way valve (Collins) with a nose clip on. End-tidal CO2 was measured using a capnograph (Godart). A reading was taken when the end-tidal CO2 was constant.

2.2 COLD AIR GENERATION

Subfreezing dry air used for challenging the subject, was generated by passing compressed dry air over a refrigeration coil containing freon (Haak EK 51). The coil was encased in an insulated, sealed cylinder (60 cm long with a 10 cm internal diameter) (Figure 2:1 and 2:2). By this method, the inspiratory air temperature was reduced to between - 15 C to - 20^{o}C (Table 2:1). The subjects were instructed to breathe at a predetermined rate by maintaining the size of a suitable guide balloon (6L bag), placed adjacent to the

Table 2:1

Temperature of the air at the outlet of the exchanger without the subject. Values recorded are (mean ± SD) at different flow rates (10-150 L/min). At each flow rate, the temperature was tested for three occasions.

Flow (L/min)	Temperature of the air (°C) at the outlet of the exchanger without the subject
10	-13·8 (0·52)
20	-13·2 (1·17)
30	-18·1 (3·71)
40	-15·4 (1·63)
50	-18·2 (0·85)
60	-16·5 (0·85)
70	-16·1 (1·28)
80	-13·5 (0·83)
90	-14·9 (1·74)
100	-15·3 (1·30)
110	-13·9 (0·91)
120	-13·8 (0·78)
130	-13·7 (0·70)
140	-13·2 (1·15)
150	-15·4 (1·58)

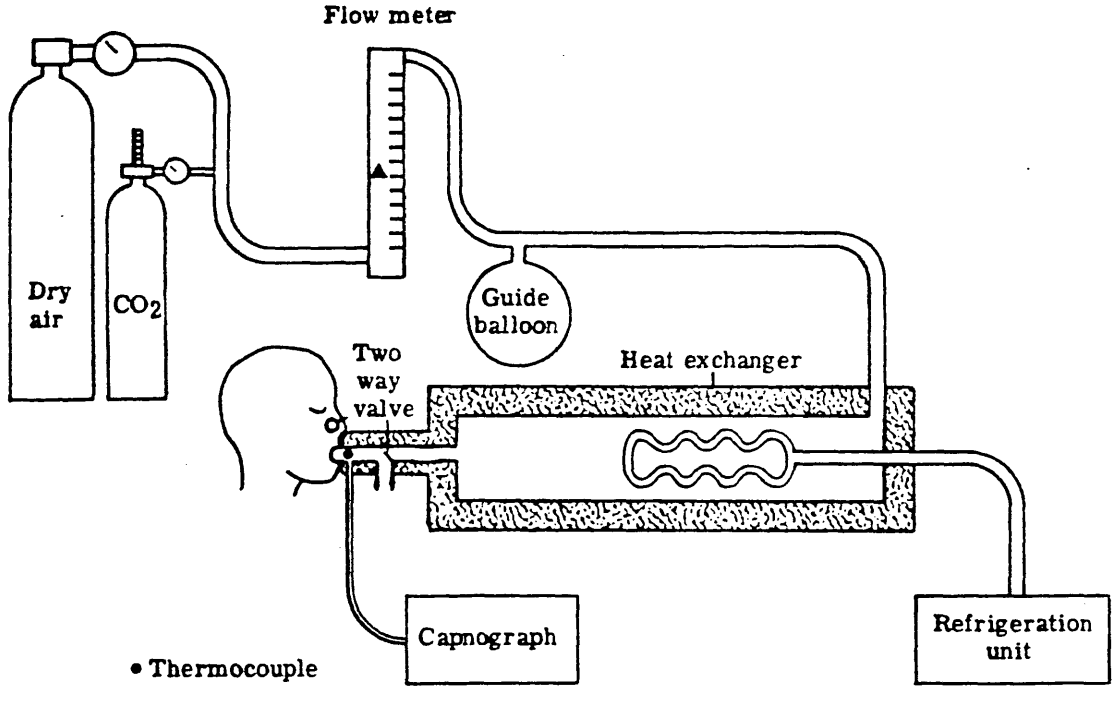


Figure 2:1 Schematic presentation of the apparatus used for delivering a cold air challenge.

Figure 2:2 Photograph of the apparatus used for delivering a cold air challenge.

inspiratory port of the exchanger. The flow of the dry air from the cylinder could be adjusted with a stopcock and measured by a rotameter. End tidal carbon dioxide was maintained at a normal resting level by the addition of a predetermined amount of carbon dioxide at the inspiratory port of the breathing circuit. End tidal carbon dioxide was monitored continuously (Godart capnograph) with the rate of carbon dioxide added being altered as required. A thermocouple situated in the inspiratory port of the valve and located 5 cm upstream from the mouthpiece provided a continuous record of the inspired air temperature.

Following an exposure, spirometry was performed. (The details of the measurements will be described in each of the studies). Exposure continued until the chosen FEV1 fall was achieved (not more than 20%) or the final ventilation was reached. Exposures ceased under the following circumstances: a) evidence of asthma or of any distress while breathing cold air, b) a fall in FEV1 of more than 20% of baseline, c) no reaction after breathing at 80% predicted IMBC. The percentage fall in FEV1 was calculated following each inhalation by:

$$\frac{\text{pre-challenge FEV1 - post-challenge FEV1}}{\text{pre-challenge FEV1}} \times 100$$

The lowest post challenge FEV1 values were taken for analysis following each inhalation.

2.3 RESPIRATORY HEAT EXCHANGE (RHE) MEASUREMENTS:

Subjects were allowed to breathe through an insulated two-way valve (Collins) which was divided into an inspiratory and expiratory part. The temperature of the inspired air was continuously recorded in all experiments by a rapidly responding (0.16 seconds) thermocouple situated in the airstream and located 5 cm from the mouth. The expired air temperature was measured with a second thermocouple which protruded 5 cm into the oral cavity and was shielded so as not to touch any of the mucosal surfaces. Expired gas was directed away from the exchanger into an electronic expiratory monitor, so that the ventilation rate could be recorded continuously.

Respiratory heat exchange (RHE) was computed from the formula of Deal et al, (1979a):

$$RHE = VE\ [HC\ (Ti-Te) + HV\ (WCi - WCe)]$$

RHE = the respiratory heat exchange in KCal/minute.

VE = minute ventilation l/minute (BTPS)

HC = heat capacity of air = (specific heat density) = 0.000304 KCal/l ^{o}C.

Ti = inspired air temperature in ^{o}C

Te = expired air temperature in ^{o}C

HV = latent heat of vapourisation of water = 0.58 KCal/g

WCi = water content of inspired air in mg water/l air.

WCe = water content of expired air in mg water/l air

Since WCe is difficult to measure physically, the assumption was made that WCe was fully saturated at Te. The values for WCe were obtained from standard saturation-temperature tables relationships (Weast, 1978).

2.4 WARM HUMID AIR GENERATION

For the warm, humid air experiments, the inspired air was warmed to approximately 36 ^{o}C and fully saturated. This was achieved by passing the air through an Owen Mumford humidifier (maintained at 55 ^{o}C). Since the inspired air was fully saturated, the water content of the inspired air could be obtained by reference to standard saturation - temperature relationships (Weast, 1978). The temperature of the inspired and expired air and ventilation were measured as described in 2.3.

2.5 HISTAMINE TEST

Airway reactivity to histamine was measured by the method of De Vries (1960) as modified by O'Brien et al (1979). Following satisfactory baseline spirometry, subjects were asked to inhale twofold increasing concentrations of histamine acid phosphate (HAP). Starting with 0.25 mg/ml, 30 second inhalations were given. The aerosol was generated by a Wrights nebuliser driven by oxygen at 6 L/min connected to a rebreathing bag (Figure 2:3). Spirometry was repeated two and five minutes after the end of each inhalation and testing proceeded until a fall in FEV1 of at least 20% of baseline occurred, or up to a maximum HAP concentration of 32 mg/ml. The results were expressed as the provocation concentration of HAP required to produce a 20% drop in FEV1 (PC20) and were calculated from the dose response curve. To obtain the value of the PC10 and PC20, the percentage fall in FEV1 following each inhalation was calculated as follows:

$$\frac{\text{Pre-challenge FEV1 - lowest post-challenge FEV1}}{\text{pre-challenge FEV1}} \times 100$$

The PC20 was obtained from the dose response curve by linear interpolation.

2.6 TREADMILL EXERCISE TESTING

Following satisfactory baseline spirometry, subjects ran on an exercise treadmill (Figure 2:4) - speed 5 kph, slope 10-15%, time 6-8 minutes, seeking to achieve a pulse rate of >170/min at the end of exercise. Running was stopped if asthma developed during the

Figure 2:3 Photograph of equipment used for a histamine challenge.

Figure 2:4 Photograph of the exercise treadmill.

test, or if there was other substantial distress. Lung function was measured at 1, 5, 10, 15, 20 and 30 minutes after the end of the exercise. The percentage fall in FEV1 following the challenge was calculated as follows:

$$\frac{\text{Pre-exercise FEV1 - lowest post-exercise FEV1}}{\text{Pre-exercise FEV1}} \times 100$$

2.7 LUNG FUNCTION MEASUREMENTS FOR BRONCHIAL PROVOCATION TESTS

A dry wedge spirometer (Vitalograph) was used for assessing the lung function changes after bronchial provocation test and the test was performed in the standing position. A noseclip was not used as performance of the test has not been found to be altered by its use (Malik, Moss and Lee, 1972). The procedure was explained and demonstrated. Each subject was asked to take a deep breath and to apply his lips around a disposable mouth piece and to blow as rapidly and as forcibly as possible. The procedure was repeated until three satisfactory curves were obtained and the two highest FEV1 values were within 200 ml. The highest value was taken for the FEV1 and FVC after five minutes and then 10 minutes. Another measurement was performed and again the highest values of FEV1 and FVC were taken. The mean of these three highest readings was considered as a baseline. The gas volume was read directly from the chart at ambient temperature and pressure saturated (ATPS).

2.8 ROUTINE LUNG FUNCTION TESTS

Routine lung function tests were performed for patients with occupational asthma due to isocyanate. The methods used are those in current practice in the department and all subjects underwent the following standard procedures.

1. Maximal flow volume loops

These were obtained using a dry rolling-seal spirometer (Ohio 840) lined to a Prime computer. The volume signal being differentiated to give flow.

2. Body Box Plethysmography

Functional residual capacity (FRC), and hence total lung capacity (TLC) and residual volume (RV) were determined using a constant volume body plethysmograph (Pulmostar SMB, Fenyues and Gut) using the inspiratory technique against the closed shutter rather than the panting procedure (Dubois, Botelho and Comroe, 1956). A similar procedure was used for the determination of inspiratory airways resistance (AWR). Specific (SGAW) airways conductance is calculated from the AWR and FRC.

3. Carbon monoxide transfer factor

Total carbon monoxide transfer (DLCO), accessible gas volume (VA) and transfer coefficient (KCO) were determined using the method described by Oglivie et al (1957), using the transfer test apparatus Model "B" (P.K. Morgan Ltd). The normal values used for

comparison were those of Cotes (1980).

2.9 MEDIATOR ASSAY TECHNIQUES

1. Serum samples

Venous blood was withdrawn through a 19-gauge indwelling needle at a site below the antecubital fossa. No anticoagulants were used; the patency of the catheter was maintained at frequent intervals by small infusions of 0.9% saline. The blood was allowed to clot on glass at 4 C for three hours and the serum was separated, divided into portions, and stored at -20 C until use. Before use in the neutrophil locomotion assay the sera were thawed and heated at 56 C for 30 minutes.

2. Neutrophil chemotactic activity

The preparation of neutrophils for use in a modified Boyden technique was performed as previously described (Lee et al, 1982). Leucocyte-rich plasma, obtained by dextran sedimentation of heparinised blood from normal healthy donors, was applied to a density gradient of 9% Ficoll solution and sodium diatrizoate (density 1:140) in the proportions 2.4 to 1. Following centrifugation at 200 g for 40 minutes at 20 C, the cell-rich pellet was retained, washed in Hank's balanced salt solution and resuspended in Hank's solution containing 0.4% ovalbumin. Eight hundred microlitres of either 20% chromatographic fractions or diluent controls were placed in the lower compartment. Serum was

diluted to 20% with Hank's solution and chromatographic fractions were assayed in phosphate buffered saline (PBS). Following a 90 minute incubation at 37 C, the micropore filters, (Sartorius-membrane filters, 8 um pore size, 34 Gottingen, W. Germany) were removed, fixed and stained as previously described (Turnbull and Kay, 1976), and the number of cells which had migrated through the entire thickness of the filter were counted. The counts obtained with buffer alone (20 to 50 cells per 10 high power fields) were subtracted from those obtained with the test solutions. The results were expressed as the total number of cells in 10 random high power fields (HPF) or as a percent change from baseline, calculated as:

$$\frac{\text{Post-challenge value - pre-challenge value}}{\text{Prechallenge value}} \times 100$$

3. Plasma Histamine

A technique has recently been developed for measuring the concentration of histamine, which has improved the sensitivity and specificity of the radioenzymatic method by the use of rat kidney methyl transferase and the incorporation of a thin layer chromatography step. This method has been further improved by the use of a double isotope technique, originally described for the measurement of plasma catecholamines (Brown and Jenner, 1981) which has permitted the sensitivity to be coupled with a high precision. This assay was performed by Dr. M. Brown at the Royal Postgraduate Medical School and has been described by Brown et al,

(1982) in detail. The sensitivity of the assay was (0.45 nanomoles/L); the intra- and inter-coefficient of variation were 6.9% and 12.8% respectively. The samples were coded and analysed without prior knowledge of the patient from whom the samples had been taken.

4. Basophil Counts

These were performed as described by Hirsch, Rimm and Zastrow (1974). The method consisted of making a thick smear on a glass slide with 2 ml of blood. This was fixed in methanol and stained with toluidine blue. All the basophils on the slide were counted and the smears were performed in duplicate.

2.10 PROVOCATION WITH OCCUPATIONAL AGENTS

This was performed for patients with occupational asthma due to isocyanate. The method used has been described previously (O'Brien et al, 1979; Harries et al, 1979). Inhalation tests were carried out in a 6.0 m closed cubicle. The test period was usually 30 minutes but was curtailed earlier if respiratory symptoms occurred.

Challenge with TDI was performed by painting with a two-part paint system consisting of a varnish (Wilkinson's Marine Varnish) and TDI. Varnish alone was used on the control day. Increasing amounts of TDI were added to the varnish on successive challenge days to a maximum atmospheric concentration of 0.02 ppm or until an

asthmatic reaction was provoked. Testing by soldering or hot tinning of polyurethane coated wire was performed in those patients exposed to TDI in these processes at work.

Testing with MDI was performed by heating Desmodur VL, which primarily contains monomeric diphenylmethane di-isocyanate (MDI) or 1.0 g of crystalline MDI at 110 C. The period of heating was adjusted to produce an atmospheric concentration of <0.001 ppm, which was increased on successive days to a maximum of 0.02 ppm if no asthmatic reaction had occurred.

Testing with HDI was performed by painting with Desmodur N (Bayer) which contains a maximum concentration of free HDI of 0.7% and exposure to NDI was by inhalation of the vapour of 0.3 - 0.5 g of heated NDI crystals for 60 seconds.

A model 700 TDI monitor (Universal Environmental Instruments Ltd) was used to measure the atmospheric concentration of TDI. The concentration of MDI was calculated by multiplying the meter reading by 1.4. Atmospheric levels of HDI and NDI were not measured.

The forced expiratory volume in one second (FEV1) was measured pre-test to ensure a stable baseline and, after the test every five minutes for half an hour and then hourly while awake, for 24 hours. The values for FEV1 post-test were compared with those on a

separate control day without isocyanate exposure.

A positive reaction was taken as a decrease in FEV1 of 15% greater than that on the control day. The pattern of reactions provoked was distinguished by the time of onset. Reactions starting within one hour (usually maximum between 15 and 30 minutes) were regarded as immediate. Reactions starting after one hour (and usually maximum between 4 and 6 hours) were regarded as late. Where both immediate and late asthmatic reactions occurred, the reaction pattern was described as dual.

2.11 STATISTICS

The statistical methods used in this thesis are those in standard practice for the calculation of mean SD, SEM, correlation coefficient and the coefficient of variation (Armitage, 1970)

Data were analysed with the aid of an electronic computer (IBM) and Casio calculator (fx 39).

Student's t-test was used to determine the significance of the difference for paired and unpaired observations, when the observations under study were assumed to be drawn from normally distributed populations (as judged by the Kolmogorov-Smirnoff test). When this assumption could not be made, the Wilcoxon matched pairs signed-ranks test was used.

A value for t was also determined to assess the significance of the correlation coefficient, obtained from the regression analysis. A two tailed test was applied to the value of t to estimate the probability. Differences were considered significant when $p<0.05$.

An analysis of variance was used to determine if there was a significant difference between the values obtained for a measurement made in various groups of subjects. The coefficient of variation was determined in order to assess the reproducibility of a measurement made on several occasions and under the same conditions in the one subject. The coefficient of variation is given by:

$$\frac{\text{S.D.}}{\text{overall mean}} \times 100\%$$

The chi-square test was used to determine whether the observed frequencies differed significantly from the predicted.

2.12 ETHICS

The studies described in this thesis were all performed with the informed consent of each subject and confidentiality was observed at all times. Protocols for each study were approved by the Brompton Hospital Ethical Committee. All challenge procedures were supervised by a physician, exposure was stopped immediately if there was any clinical evidence of asthma or other distress and treatment was given if necessary.

CHAPTER THREE

THE DEVELOPMENT OF COLD AIR TEST FOR AIRWAYS REACTIVITY

THE DEVELOPMENT OF COLD AIR TEST FOR AIRWAYS REACTIVITY

INTRODUCTION

Numerous studies have demonstrated that asthmatic patients are more sensitive to bronchoconstrictor agents than are normal subjects. Consequently, bronchial provocation tests have been proposed as a means of diagnosing asthma (Benson, 1975; Cockcroft et al, 1977a; Boushey et al, 1980). The pharmacological agents histamine and methacholine, and treadmill exercise testing have been used for reactivity estimation in clinical and population based studies of asthma. Treadmill testing is cumbersome and difficult to standardise in unfit adults. Because pharmacological agents can, in sufficient doses, produce airway narrowing even in normal subjects (Fish et al, 1976; Orehek et al, 1977), the discrimination and validity of such reactivity testing is limited particularly in population studies. There may also be variability of response with pharmacological agents because of inconstant nebuliser output, particle size, unpredictable site of drug deposition and dose retention (Thomson and Pavia, 1974; Pavia et al, 1977; Ruffin et al, 1978). In addition, following histamine and methacholine inhalation, side effects such as irritation of the

throat, flushing and increased heart rate are often observed (Juniper et al, 1978). These factors can affect the response to these challenges and render them less specific for determination of the magnitude of airway reactivity.

Recently, cold air isocapnic hyperventilation has been introduced as a bronchoprovocative test in patients with asthma (Deal et al, 1979a; Deal et al, 1980a). However, the technique has not been standardised, In this study an attempt was made to do so. It is aimed to investigate several factors which may determine the responsiveness to cold air isocapnic hyperventilation, as a basis for developing a simple and standardized test. In principle, cold air cools the bronchial tree, both directly and by evapration, which is much the more important component. The distribution and degree of cooling depends upon the dryness, temperature and flow of inspired air, on previous respiratory history and on some physical factors which are easy to imagine but difficult to measure. To develop a standardized cold air test, we need to know the effects of these variables that can be controlled simply; namely minute volume, the duration of, and intervals between, exposures, respiratory flow patterns and inspired air temperature. To do this volunteers with asthma underwent one or more of a series of tests in which these factors were varied. In addition a 'CO_2 requirement graph' was constructed at different levels of ventilation which allows prediction of the rate of addition of CO_2 required for isocapnia during hyperventilation. This avoids the need for

continuous monitoring of end-tidal CO2 with a capnograph.

The efficiency of the test was examined by studying its reproducibility and validity by its comparison with standardised methods of histamine and exercise challenges in normal subjects and in patients with asthma and allergic rhinitis.

CHAPTER THREE

SECTION ONE

THE EFFECT OF INCREASING THE RATE OF VENTILATION

THE EFFECT OF INCREASING THE RATE OF VENTILATION

AIM

The aim of this study was to determine what flow rate of cold air is appropriate for a standardised CAIH test.

METHOD

Six patients were tested on five occasions with subfreezing dry air at different levels of ventilation 10, 20, 40, 60 and 80% of predicted indirect maximum breathing capacity (IMBC), estimated by predicted FEV1 x 35 (Gandevia and Hugh-Jones, 1957). Each level of ventilation was tested on a separate visit. The exposures were randomised and the visits were separated by two to four days. On each of the visits, a satisfactory prechallenge FEV1 was obtained, then the subjects commenced cold air ventilation. The duration of each exposure was for three minutes. Following the inhalations, FEV1 was measured immediately, at two minutes and at five minutes and then every five minutes for up to 20 minutes.

RESULTS

The individual characteristics and baseline lung function of the subjects who participated in this study are shown in Table 3:1:1. The inspired air conditions were kept constant during different challenges (Table 3:1:2). The pre-challenge FEV1 did not differ significantly on each occasion in the group studied. There was a direct but curvilinear relationship between the increase in

Table 3:1:1

Physical characteristics and baseline lung function of the subjects who performed CAIH challenge at different ventilation rates and fixed duration.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred.)
1	M	32	179	68	4·20	101·2
2	F	20	171	60	3·05	86·9
3	M	25	178	72	4·50	103·4
4	M	33	177	70	4·26	105·7
5	M	33	180	75	3·76	91·9
6	M	26	188	70	4·54	98·9
Mean		28·2	178·8	69·2	4·05	98·0
SD		5·34	5·49	5·08	0·564	7·21

Table 3:1:2

Inspired air temperature (^{0}C) during CAIH challenges at different ventilation rates and fixed duration in six asthmatics.

	Level of ventilation (% IMBC)				
Subject	10	20	40	60	80
1	-12·0	-12·0	-19·5	-17·5	-18·0
2	-13·0	-14·5	-14·0	-15·0	-14·0
3	-13·5	-13·0	-18·0	-19·0	-17·5
4	-11·0	-12·0	-19·0	-22·0	-21·0
5	-16·0	-16·0	-15·0	-12·0	-10·0
6	-14·0	-12·0	-11·0	-9·0	-12·0
Mean	-13·3	-13·3	-16·1	-15·8	-15·4
SD	1·72	1·67	3·32	4·75	4·13

Water content was zero as dry air was used.

ventilation rate and fall in FEV1 (Figure 3:1:1). There was no significant difference between the maximum percentage fall in FEV1 obtained following CAIH at a ventilation rate of 60 or 80% predicted IMBC (Table 3:1:3). These were significantly greater than the values obtained at 40% predicated IMBC ($P<0.01$ and $P<0.05$ respectively). The maximum percentage changes in FEV1 following each step are shown in Figure (3:1:2).

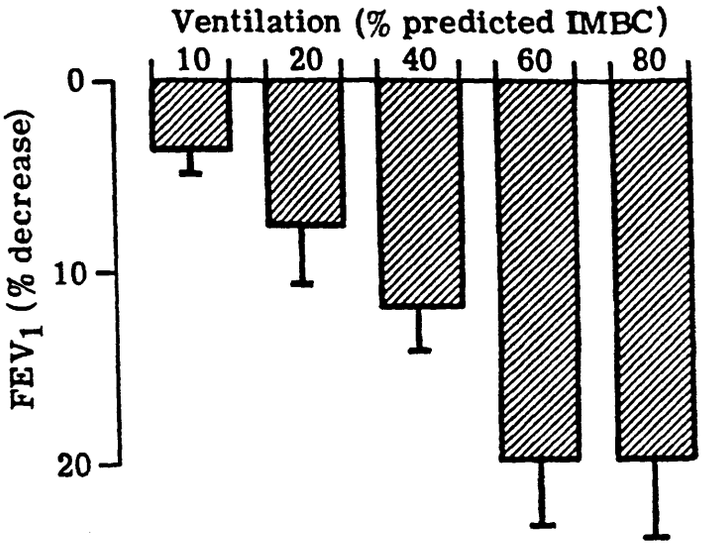
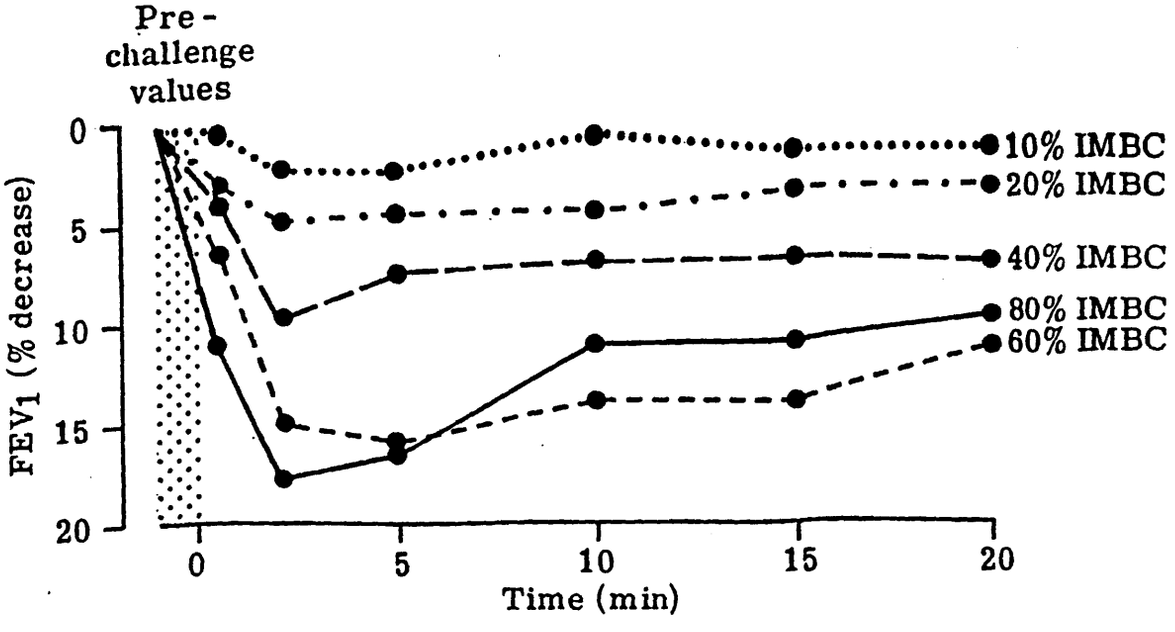


Figure 3:1:1 The effect of increasing the rate of ventilation of CAIH in six asthmatics. The steps 10, 20, 40, 60 and 80% predicted IMBC were administered in random order on separate days. The results shown are mean ± SEM of maximum percentage drop in FEV1 of six asthmatics following each test.

Table 3:1:3

Maximal percentage decrease in FEV_1 following CAIH challenge at different ventilation rates and fixed duration in six asthmatics.

	Level of ventilation (% IMBC)				
Subject	10	20	40	60	80
1	6·7	15·3	12·9	20·3	21·8
2	1·6	1·6	11·2	8·6	11·2
3	-1·7	-0·6	9·8	18·0	16·5
4	3·0	2·9	3·2	9·0	11·3
5	5·3	5·5	16·5	29·3	15·0
6	6·4	19·3	21·0	34·2	42·4
Mean	3·6	7·3	12·4	19·9	19·7
SEM	1·33	3·29	2·47	4·26	4·81



Maximum value for the standard error of each mean :

10% IMBC = 1.9%, 20% IMBC = 3.1%, 40% IMBC = 2.7%,
60% IMBC = 5.3%, 80% IMBC = 4.7%

Figure 3:1:2 Mean changes in FEV1 (percentage of baseline) following CAIH at increasing ventilation rate in six asthmatics. The steps 10, 20, 40, 60 and 80% predicted IMBC were administered for three minutes each. These were randomised and performed on separate days.

CHAPTER THREE

SECTION TWO

THE EFFECT OF INCREASING THE DURATION OF EXPOSURE

THE EFFECT OF INCREASING THE DURATION OF EXPOSURE

AIM

The purpose of this study was to determine what duration of cold air was appropriate for a standardised CAIH test.

METHOD

This study was performed on five asthmatic patients and completed on seven visits. On visit one, preliminary testing was carried out by stepwise increases in ventilation, to determine the ventilation rate required to produce 15-20% fall in FEV1. The prechallenge FEV1 was recorded and then the first challenge commenced at 10% of predicted IMBC for three minutes. The successive challenge periods of three minutes were at 20%, 40%, 60% and 80% of predicted IMBC. Inhalation ceased when there was a 15-20% fall in FEV1 below the prechallenge value, or when a maximum of 80% of predicted IMBC was achieved. The best FEV1 of three determinations was recorded immediately after, at two minutes, and at five minutes following the end of each period. On the subsequent visits the ventilation rate determined on the first visit was given for six different time periods (one, two, three, five, seven, ten minutes) on separate occasions. Following the end of each challenge, FEV1 was measured immediately and at two minutes and at five minutes and then for every five minutes for up to 20 minutes. Each duration of exposure was tested on a separate visit. The visits were separated by 2-4 days and were randomised.

RESULTS

The individual characteristics and baseline lung function for subjects who participated in this study are shown in Table 3:2:1. The inspired air conditions were kept constant during different challenges (Table 3:2:2). There was no significant difference in the pre-challenge FEV1 values in the individual patients studied on different days. The maximum percentage drop in FEV1 was achieved following cold air ventilation for seven minutes but was almost fully developed following three minutes exposure (Figure 3:2:1 and Table 3:2:3). The values obtained following exposure for one or two minutes were significantly smaller ($p < 0.02$, < 0.05 respectively). No significant further fall in FEV1 was observed following exposure beyond three minutes. With increasing duration of exposure, the effect of CAIH started to appear earlier after stopping the exposure and the recovery was slower (Figure 3:2:2).

Table 3:2:1

Physical characteristics and baseline lung function of the subjects who performed CAIH challenge at different durations of exposure and fixed ventilation rate.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	F	24	165	68	3·77	117·4
2	M	55	172	76	1·77	53·6
3	M	49	170	69	2·29	69·4
4	M	43	183	80	3·10	76·7
5	M	27	181	71	4·47	101·8
Mean		39·6	174·2	72·8	3·08	83·8
SD		13·59	7·60	5·07	1·09	25·6

Table 3:2:2

Inspired air temperature (^0C) during CAIH challenge at different durations of exposure in five asthmatics. Level of ventilation used was 40% of their indirect maximum breathing capacity.

	Duration of exposures (min)					
Subject	1	2	3	5	7	10
1	-15·0	-17·0	-14·0	-16·5	-16·0	-12·0
2	-13·5	-15·0	-16·0	-16·5	-17·0	-18·0
3	-14·0	-16·0	-13·5	-16·0	-16·5	-16·0
4	-	-	-15·5	-17·0	-18·0	-15·5
5	-14·5	-16·0	-15·0	-17·0	-16·0	-14·0
Mean	-14·3	-16·0	-14·8	-16·6	-16·7	-15·1
SD	0·65	0·82	1·04	0·42	0·84	2·25

Water content was zero as dry air was used.

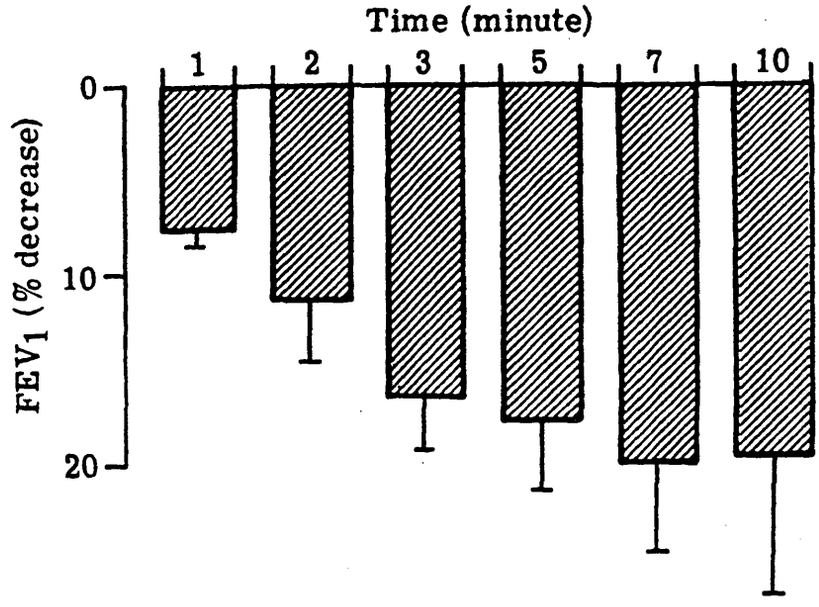
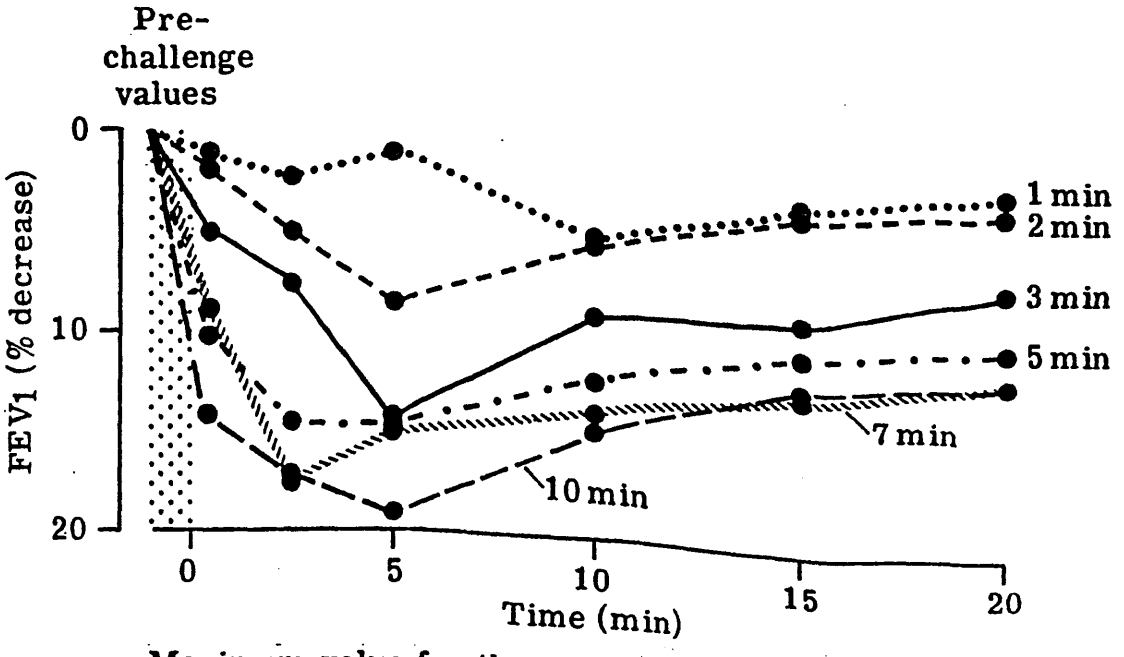


Figure 3:2:1 The influence of increasing duration of exposure of CAIH in five asthmatics. Cold air was administered at a fixed ventilation rate for durations of 1, 2, 3, 5, 7 and 10 minutes in random order on separate days. The results shown are the mean ± SEM of the maximum percentage drop in FEV1 following each test.

Table 3:2:3

Maximal percentage decrease in FEV_1 following CAIH challenge at different durations of exposure and fixed ventilation rate in five asthmatics.

	Duration of exposures (min)					
Subject	1	2	3	5	7	10
1	2·7	3·5	13·8	10·8	14·5	10·4
2	5·8	8·5	16·1	19·1	19·4	16·2
3	14·5	12·5	17·9	14·8	13·4	13·3
4	-	-	13·6	14·6	13·9	12·9
5	7·7	17·4	19·5	29·5	38·5	45·8
Mean	7·7	10·5	16·2	17·8	19·9	19·7
SEM	2·50	2·95	1·15	3·22	4·76	6·58



Maximum value for the standard error of each mean:
1 min = 3.2%, 2 min = 3.7%, 3 min = 4.3%, 5 min = 5.4%, 7 min = 5.8%, 10 min = 8.2%

Figure 3:2:2 Mean changes in FEV1 (percentage of baseline) following CAIH at different durations and at fixed ventilation rate in five asthmatics. The duration of exposures were 1, 2, 3, 5, 7 and 10 minutes. These were randomised and performed on separate days.

CHAPTER THREE

SECTION THREE

THE EFFECT OF INCREASING THE RATE OF VENTILATION GIVEN SERIALLY

THE EFFECT OF INCREASING THE RATE OF VENTILATION GIVEN SERIALLY

AIM

In order to determine the dose response characteristics for CAIH in a safe manner, the effect of consecutive challenges at progressively increasing rates of ventilation has been measured.

METHOD

Five patients were tested with subfreezing dry air at increasing ventilation rates, given serially. Following a satisfactory baseline FEV1 cold air ventilation commenced at 10% of IMBC, for three minutes. FEV1 was recorded at 0.5, 2, 5, 10 and 15 minutes after the inhalation. Following this and irrespective of whether the FEV1 returned to normal or not, the successive challenges were given at 20, 40, 60 and 80% of predicted IMBC separated by 15 minutes and the FEV1 was measured as described for the first challenge.

RESULTS

The individual characteristics and baseline lung function of the subjects in this study are shown in Table 3:3:1. The inspired air conditions were similar for the different challenges performed (Table 3:3:2). Increasing ventilation rates given serially at 15 minute intervals produced an increase in the degree of bronchoconstriction as measured by the fall in FEV1 (Figure 3:3:1;

Table 3:3:1

Physical characteristics and baseline lung function of the subjects who performed stepwise CAIH challenge in serial order (15 minutes between the steps) in five asthmatics.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	F	47	155	63	2·56	109·9
2	F	45	166	76	2·68	100·0
3	M	31	183	75	4·34	101·9
4	M	54	156	54	2·57	79·8
5	F	24	165	68	3·98	102·6
Mean		40·2	165	67·2	3·23	98·8
SD		12·32	11·25	9·09	0·86	11·3

Table 3:3:2

Inspired air temperature (^{0}C) during stepwise CAIH challenges given in serial order (15 minutes between the steps) in five asthmatic patients.

	Level of ventilation (% IMBC)				
Subject	10	20	40	60	80
1	-13·0	-17·0	-19·0	-16·5	-17·5
2	-12·5	-19·0	-18·0	-18·0	-18·0
3	-13·0	-12·0	-14·0	-11·0	-14·0
4	-15·5	-16·5	-17·0	-18·0	-17·0
5	-15·5	-18·0	-17·5	-14·0	-12·0
Mean	-13·9	-16·5	-17·1	-15·5	-15·7
SD	1·47	2·69	1·88	3·0	2·59

Water content was zero as dry air was used.

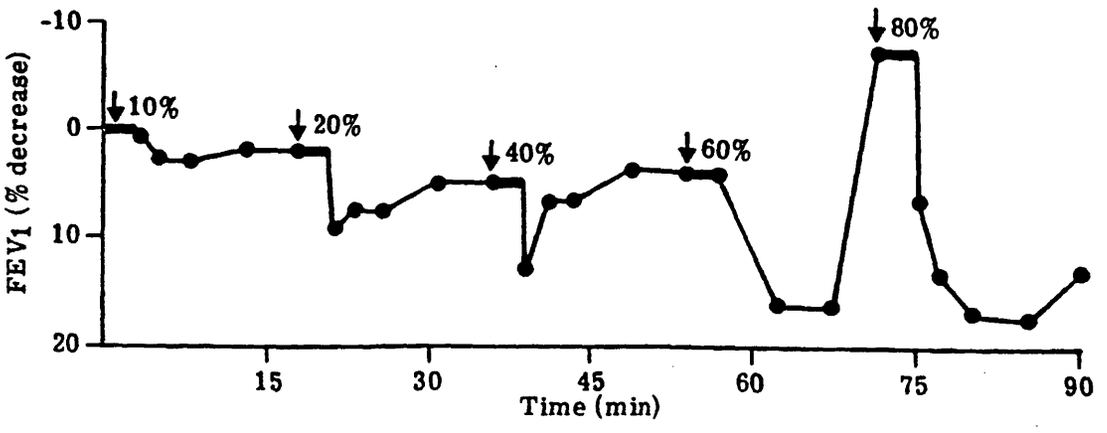


Figure 3:3:1 Changes in FEV1 (percentage of baseline) following inhalation of subfreezing dry air at different ventilation rates (10, 20, 40, 60 and 80% IMBC) performed by a single subject. The inhalation time was three minutes for each step. The interval between the steps was 15 minutes.

3:3:2, Table 3:3:3). The 80% predicted IMBC step produced no more obstructive response than 60%. These observations were similar to those described when the steps were given separately in random fashion. The successive challenges were given irrespective of whether the baseline lung function returned to normal or not. There was a progressive fall in the prechallenge FEV1 following exposure to increasing ventilation rates.

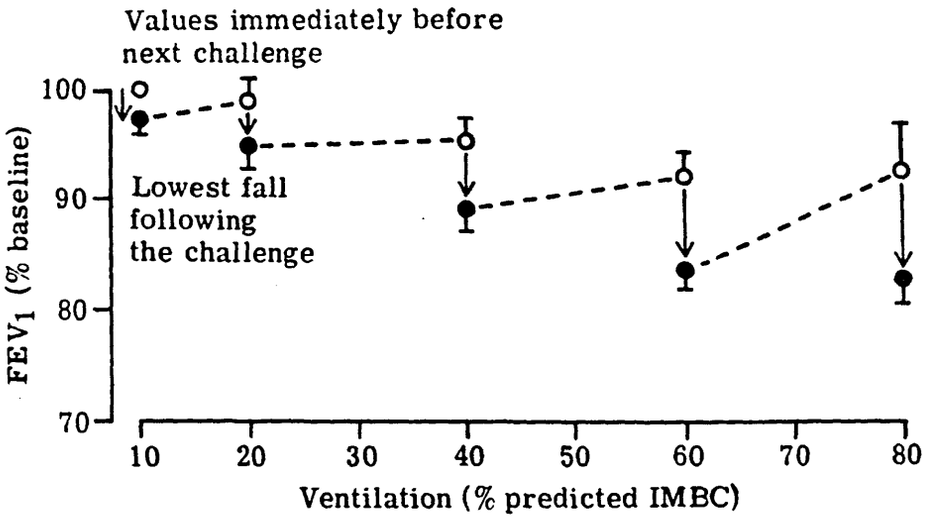


Figure 3:3:2 The effect of increasing the rate of ventilation of CAIH given serially. The steps of 10, 20, 40, 60 and 80% of predicted IMBC were administered in sequence. The interval between the steps was 15 minutes. The results shown are the pre-challenge and lowest post-challenge FEV1 (Mean ± SEM) expressed as percentage of baseline following each step.

Table 3:3:3

Prechallenge (P) and lowest post challenge (M) FEV_1 expressed as a percentage of the baseline following stepwise CAIH challenge given in serial order (15 min between the steps)

	Ventilation steps (% IMBC)									
	10		20		40		60		80	
Subject	P	M	P	M	P	M	P	M	P	M
1	100·0	96·8	98·0	91·4	95·3	87·5	85·7	83·6	107·0	82·4
2	100·0	96·7	97·0	90·3	90·3	84·1	89·4	82·9	89·8	86·4
3	100·0	101·5	102·0	99·6	100·7	93·7	93·7	80·7	81·2	82·8
4	100·0	97·7	103·0	98·8	98·8	90·2	98·8	85·8	93·2	74·6
5	100·0	94·7	94·7	93·0	93·0	91·2	92·2	85·4	91·0	87·7
Mean	100·0	97·5	98·9	94·6	95·6	89· 3	92·0	83·7	92·4	82·8
SEM	0	1·12	1·56	1·92	1·88	1·64	2·18	0·92	4·16	2·28

CHAPTER THREE

SECTION FOUR

THE EFFECT OF VARYING THE TIME INTERVAL BETWEEN EXPOSURES

THE EFFECT OF VARYING THE TIME INTERVAL BETWEEN EXPOSURES

AIM

The aim of this study was to determine interval between consecutive exposures at progressively increasing rates of ventilation.

METHOD

To determine the time interval between one exposure and the next, in a dose response curve, seven patients with asthma were studied and the study was completed in three visits. On each of the visits, the baseline FEV1 was measured and followed by stepwise exposures of 10, 20, 40, 60 and 80% of predicted IMBC. The duration of exposures was three minutes and the intervals between exposures varied with the visit. On one occasion five minutes, on another two minutes and on the other zero minutes. The same steps were used on different testing days for each patient. The order of the intervals was randomised. Following each exposure, FEV1 was recorded immediately and at two minutes and five minutes, when the interval between the steps was five minutes; immediately and at two minutes when the interval between steps was two minutes. When there was zero time between the exposures, measurement of FEV1 was performed during cold air inhalation; the Vitalograph was connected to the circuit by a three-way tap and measurements of FEV1 performed every three minutes during inhalation of cold air.

In order to check whether using a two-way J-valve had an effect on the values of FEV1 recorded during cold air inhalation six subjects were asked to perform both forced expiratory manoeuvres: a) with J-valve b) without J-valve. At least three records were made on each occasion. The highest value was recorded and used for analysis. There was no significant difference between the values recorded with or without the use of the valve (Table 3:4:1).

After the end of the test on each visit, measurements of the forced expiratory manoeurvre were performed at 0.5, two and five minutes and then for every five minutes for up to 20 minutes. The maximum fall in FEV1 following each step was recorded and the maximum percentage drop in FEV1 was calculated.

RESULTS

The individual characteristics and baseline lung function of the subjects tested are shown in Table 3:4:2. The inspiratory air conditions were kept constant during different testing challenges (Table 3:4:3). There was no significant difference in the prechallenge values on different testing days. When stepwise CAIH at increasing ventilation rates were carried out at various intervals between the steps (zero, two and five minutes), no significant differences in % maximum drop in FEV1 were observed or in the recovery from CAIH. (Figures 3:4:1; 3:4:2; 3:4:3; Table 3:4:4).

Table 3:4:1

Comparison between FEV_1 values in litres with and without the use of J-valve.

Subject	Without J-valve	With J-valve
1	5·02	5·17
2	5·00	4·88
3	3·42	3·58
4	3·32	3·26
5	4·00	3·96
6	3·85	3·65
Mean	4·10	4·08
SD	0·75	0·77

Table 3:4:2

Physical characteristics and baseline lung function of seven asthmatics who performed stepwise CAIH at different intervals between the steps.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	M	43	183	80	2·80	69·2
2	M	23	169	80	4·08	100·0
3	M	20	192	74	3·21	76·0
4	M	55	172	76	1·71	51·5
5	M	33	177	70	4·10	101·7
6	M	31	183	88	3·74	85·6
7	M	54	168	78	2·51	79·6
Mean		37·0	177·7	78·0	3·16	80·5
SD		14·1	8·79	5·66	0·89	17·5

Table 3:4:3

Minute ventilation (% IMBC) and inspired air temperature (^{0}C) recorded during stepwise CAIH performed at different intervals between the steps (five, two and zero minute time).

Subject	Minute Ventilation (% IMBC)	Inspired temperature (^{0}C)		
		Five min.	Two min.	Zero time
1	40	-15·0	-14·0	-14·0
2	40	-16·0	-13·0	-13·5
3	40	-16·0	-16·0	-12·5
4	40	-13·0	-16·0	-12·0
5	60	-16·5	-16·0	-14·0
6	40	-13·5	-16·0	-16·0
7	40	-17·0	-13·5	-13·5
Mean		-15·3	-14·9	-13·6
SD		1·52	1·37	1·28

Water content was zero as dry air was used.

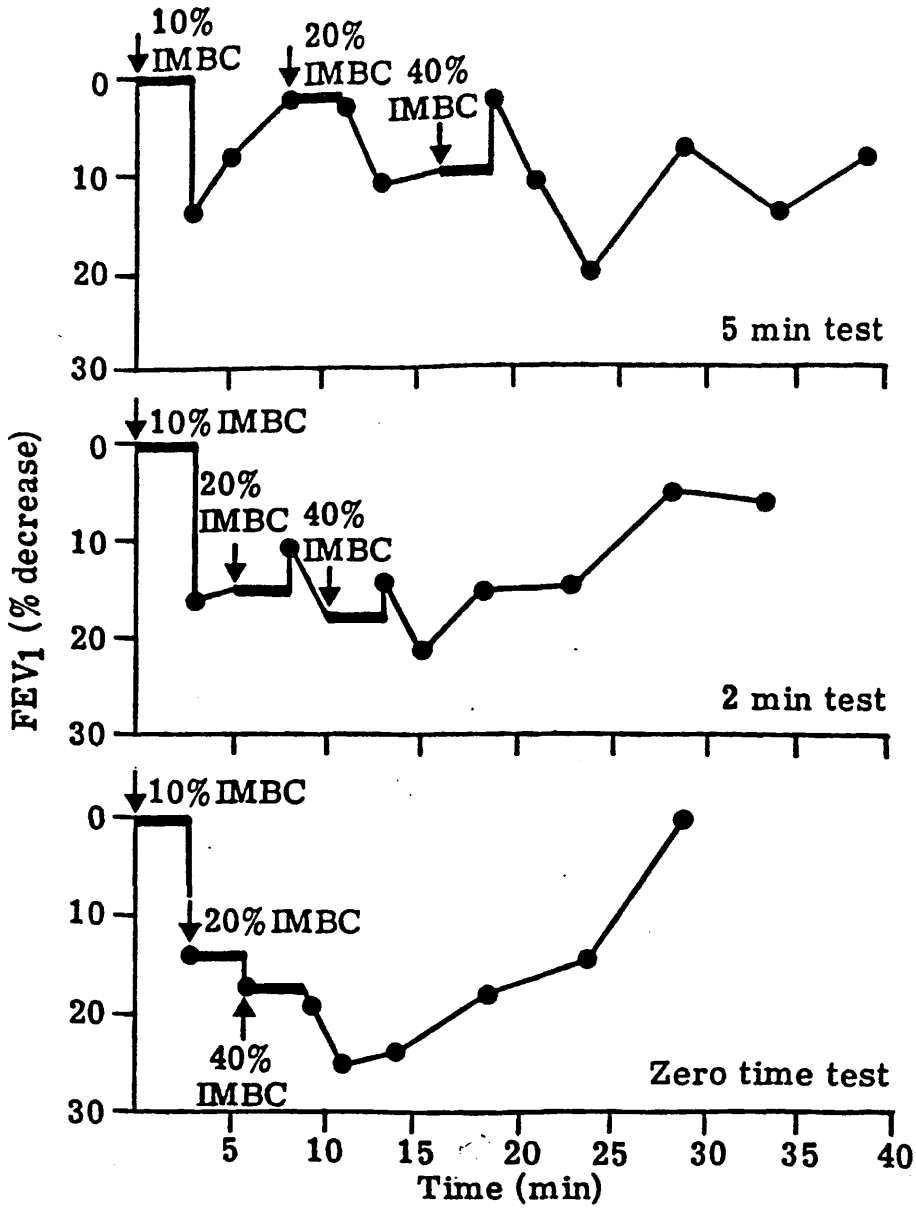


Figure 3:4:1 Changes in FEV1 (percentage of baseline) following CAIH at increasing ventilation rates performed by a single subject. The inhalation time for each step was three minutes. The intervals between steps were a) five minutes; b) two minutes; c) zero time. Inhalation stopped when a 15-20% fall in FEV1 had been achieved.

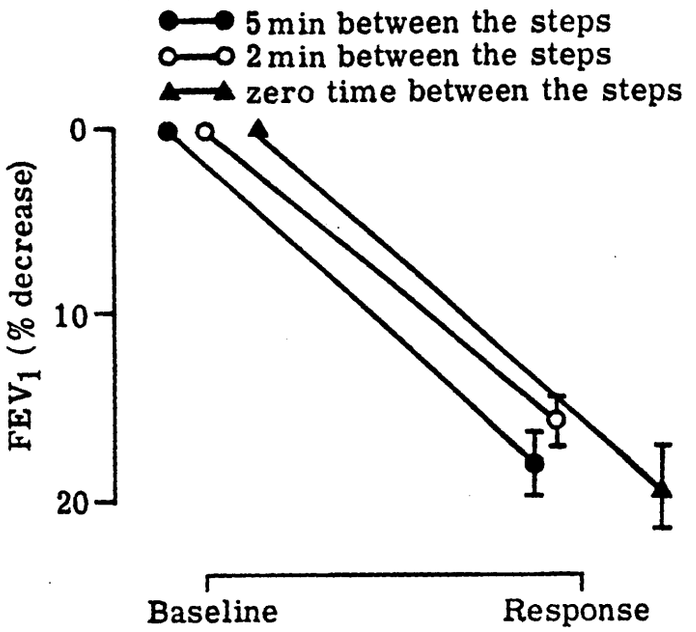


Figure 3:4:2 The effect of varying the time interval between consecutive CAIH exposures at progressively increasing rates of ventilation. The steps of 10, 20 and 40% of predicted IMBC were administered to seven asthmatics on three occasions. The intervals separating the steps varied between occasions (zero, two, or five minutes). The order of the intervals was randomised and was applied on separate days. The results shown are the maximum percentage fall in FEV1 (mean ± SEM) following each test.

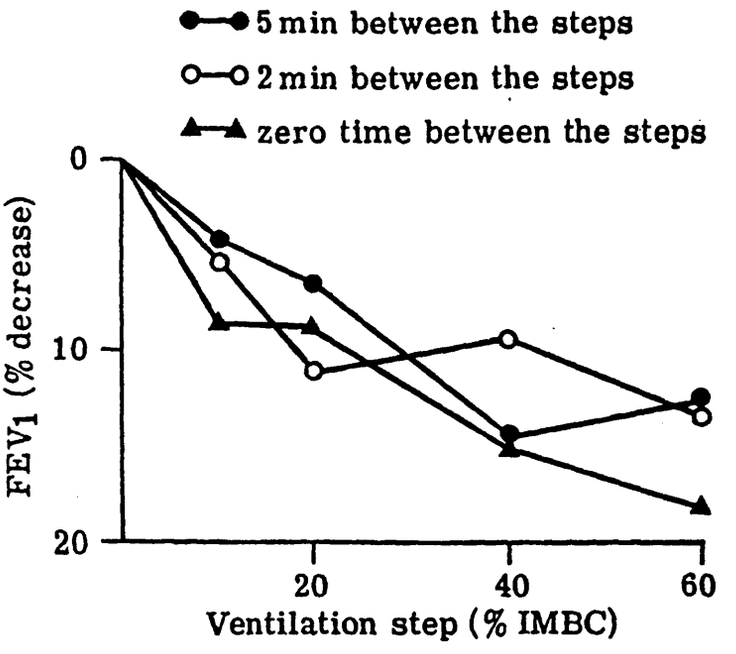


Figure 3:4:3 The dose response curves using stepwise CAIH challenge at increasing ventilation rates varying the time intervals between the steps in a single subject. The duration of each step was three minutes and the intervals between the steps were a) five minutes; b) two minutes; c) zero time. The maximum percentage fall in FEV1 following each step was plotted against the ventilatory rate of the respective challenge period.

Table 3:4:4

Maximum percentage fall in FEV_1 following stepwise CAIH challenge performed at different time intervals between steps (five, two and zero minute time) in seven asthmatics.

Subject	Five min.	Two min.	Zero time
1	21·4	21·1	22·7
2	15·1	13·7	9·7
3	14·6	14·2	20·5
4	21·5	21·1	25·6
5	14·4	14·9	19·8
6	22·5	12·7	13·5
7	15·1	16·7	23·5
Mean	17·8	16·3	19·3
SEM	1·42	1·31	2·16

To determine the dose response curves for individual patients, the maximum percentage fall in FEV1 following each step was plotted against the ventilation rate of respective challenge periods; the ventilation rates producing a 10 and 20% fall in FEV1 were obtained from the dose response curve by linear interpolation (Figure 3:4:3, 3:4:4). The results are expressed as a percentage of subjects predicted IMBC and designated PD10 and PD20 respectively.

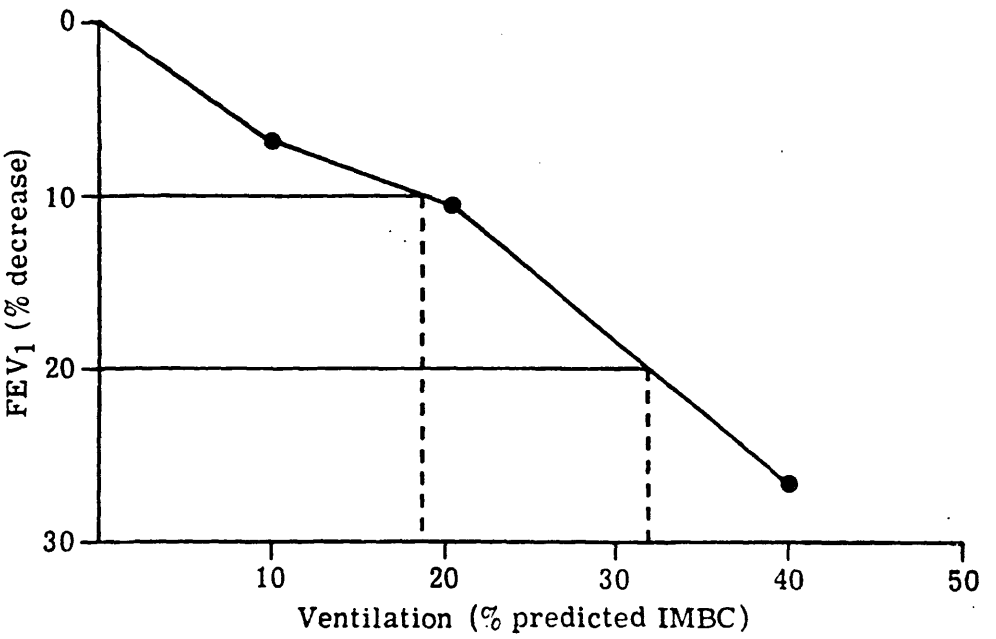


Figure 3:4:4 The dose response curve comparing the level of ventilation (expressed as a percentage of predicted of IMBC) and the decrease in FEV1 (expressed as the percentage decrease from baseline following each ventilatory step) in one subject. Calculation of the ventilation rate causing a 10% decrease (PD10) and 20% decrease (PD20) in FEV1 is by linear interpolation.

CHAPTER THREE

SECTION FIVE

THE REPRODUCIBILITY STUDY

STUDY OF REPRODUCIBILITY

AIM

The purpose of this study is to investigate the repeatability of cold air isocapnic hyperventilation over a short period of time.

METHOD

On each of the four visits, seven subjects underwent a stepwise test as follows: After the establishment of a satisfactory baseline FEV1, challenge with cold air ventilation was started at 10%, followed by successive challenges at 20%, 40% and 60% of predicted IMBC. Inhalation ceased either when there was a 15-20% fall in FEV1 below prechallenge value or when 60% predicted IMBC was achieved. The highest of three records of FEV1 was recorded immediately after and at two and five minute intervals following the end of each period, and then for every five minutes up to 20 minutes following the final step. Subject two was only studied on three occasions.

RESULTS

Individual characteristics and baseline lung function are shown in Table 3:5:1. The inspired air temperature recorded is shown i Table 3:5:2. The maximum percentage fall in FEV1 following sequential challenges on the four occasions is shown in Table 3:5:3. There was no significant difference in the baseline FEV1 on different days. The reproducibility of the responses to challenge

Table 3:5:1

Individual characteristics and baseline lung function of the seven asthmatic subjects who participated in the reproducibility study.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	M	27	181	71	4·48	102·1
2	F	28	179	85	2·58	80·6
3	M	50	174	70	2·93	84·7
4	M	20	177	75	4·45	104·5
5	M	21	178	70	4·52	101·3
6	F	20	170	65	2·66	77·3
7	M	35	175	77	3·48	91·3
Mean		28·7	176·3	73·3	3·59	91·7
SD		10·86	3·64	6·45	0·89	11·13

Table 3:5:2

Minute ventilation (% IMBC) and inspired air temperature (^0C) recorded during repetition of stepwise CAIH challenge on four occasions in seven asthmatics.

Subject	Minute Ventilation	Inspired temperature (^0C)			
	(% IMBC)	Visit 1	Visit 2	Visit 3	Visit 4
1	40	-15·5	-13·0	-14·5	-12·0
2	40	-16·0	-22·5	-11·0	-
3	40	-12·5	-13·0	-16·0	-11·0
4	60	-13·5	-14·0	-13·0	-11·0
5	60	-13·5	-16·0	-11·0	-18·0
6	20	-11·0	-13·0	- 9·0	-15·0
7	20	-10·5	-11·5	-16·0	-14·0
Mean	40·0	-13·2	-14·7	-12·9	-13·5
SD	16·3	2·08	3·70	2·71	2·74

Water content was zero as dry air was used.

Table 3:5:3

Maximum percentage fall in FEV_1 following stepwise CAIH challenge performed on four occasions in seven asthmatics.

Subject	Visits			
	1	2	3	4
1	18·3	16·8	17·0	16·1
2	27·1	39·5	22·3	-
3	14·3	15·0	7·4	14·0
4	18·2	15·9	18·0	6·8
5	15·9	13·4	11·6	15·5
6	25·6	19·1	24·4	22·2
7	20·1	13·5	17·0	20·0
Mean	19·9	19·0	16.8	15·8
SD	4·78	9·24	5·84	5·35

was good, and the coefficient of variation was 17.5% (Figure 3:5:1; Table 3:5:2). In two participants, (Numbers three and four), no reaction was observed on visits three and four respectively, despite the use of similar testing conditions (Table 3:5:3). A response was defined by a fall of 10% or more in FEV1.

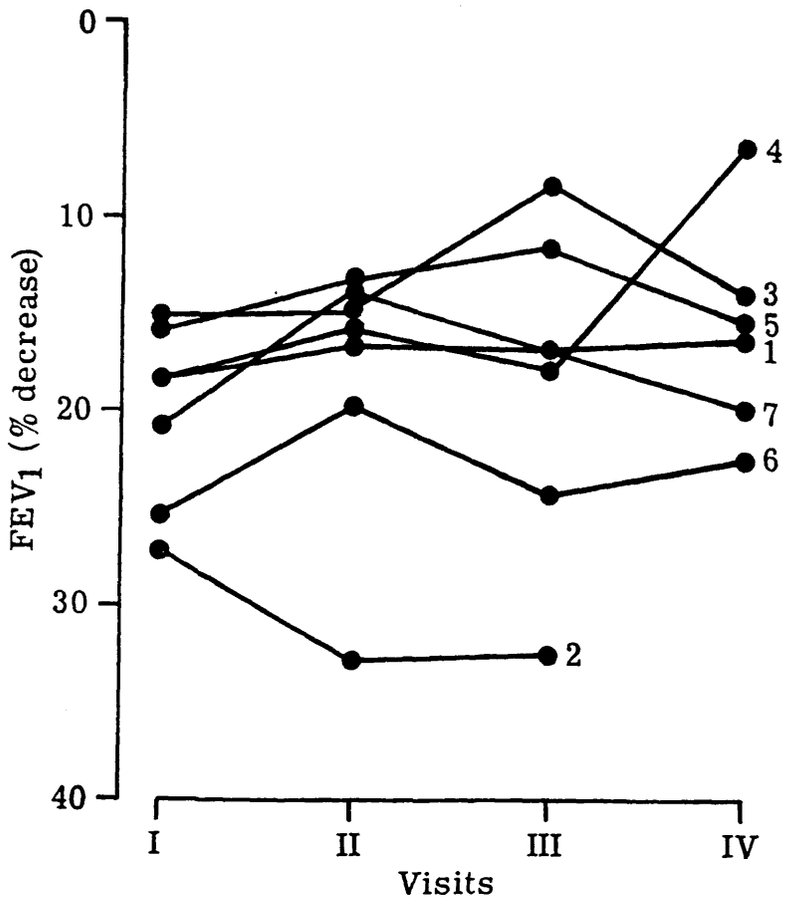


Figure 3:5:1 The reproducibility of CAIH on four occasions in seven asthmatics. Cold air isocapnic hyperventilation was performed at increasing ventilation rates (10, 20, 40 and 60% of predicted IMBC). The duration of each exposure was three minutes and the interval between each step was five minutes. The maximum percentage fall in FEV1 was recorded. The coefficient of variation was 17.5%.

CHAPTER THREE

SECTION SIX

THE EFFECT OF CHANGING THE PATTERN OF BREATHING

THE EFFECT OF CHANGING THE PATTERN OF BREATHING

AIM

The purpose of this study was to determine the influence of breathing pattern on bronchial responsiveness to CAIH.

METHOD

Six patients were tested in this study which was carried out on two visits. On visit one, a stepwise CAIH test at 10, 20, 40 and 60% predicted IMBC was performed to determine the level of ventilation required to cause 15-20% drop in FEV1 as described in Chapter three, section two. The duration of the challenge was three minutes and the interval between challenges was five minutes. On visit two, a single exposure was used at a ventilation rate equal to that required to cause a 15-20% drop in FEV1 as determined on visit one. The patient was asked to keep the balloon full by breathing at a rate of either 30 or 60 breaths/minute. A metronome was used to give the frequency of ventilation to the patient. One hour later, provided the lung function had returned to normal, the same level of ventilation was used, but the patient was instructed to keep the balloon full by breathing at the rate not previously performed. The two challenges were randomised. An electronic respiratory monitor (BOC) placed in the expiratory port of the valve was used to monitor both the respiratory rate and the tidal volume. Following each inhalation of cold air FEV1 was recorded at 0.5, 2 and 5 minutes and then every five minutes for up to 20

minutes.

RESULTS

The individual characteristics and baseline lung function of the subjects tested in the study are shown in Table 3:6:1. There was no significant difference in the mean of the pre-challenge values when using either 30 or 60 breaths/minute. All patients' FEV1 returned to baseline before the next challenges. The inspiratory air conditions were similar in both challenges (Table 3:6:2). The maximum percentage drop (Mean $\pm$ SEM) for the group following challenges at 30 and 60 breaths/min was 13.8 $\pm$ 1.38 and 16.3 $\pm$ 2.49 respectively (Figure 3:6:1; Table 3:6:3). There was no significant difference in the mean of the maximum drop in FEV1 or in the recovery following CAIH at 60 or 30 breaths/minute (Figure 3:6:2).

Table 3:6:1

Physical characteristics and baseline lung function of the subjects tested for the effect of the pattern of breathing cold air on airway responsiveness.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	M	32	181	81	3·70	99·1
2	F	24	148	51	1·60	57·6
3	M	35	175	77	3·32	87·1
4	F	20	170	65	2·74	79·7
5	M	55	172	55	1·72	53·3
6	M	38	185	85	2·32	55·5
Mean		34	171·8	69	2·57	72·1
SD		12·3	13·0	14·1	0·85	19·2

Table 3:6:2

Minute ventilation (% IMBC) and inspired air temperature (^{0}C) recorded during cold air inhalation in six asthmatics at two rates of breathing (30 and 60 breaths/min). The same minute ventilation was used on both occasions.

Subject	Minute Ventilation (% IMBC)	Inspired temperature	
		30 breath/min	60 breath/min
1	40	-14·0	-12·5
2	60	-9·0	-13·0
3	20	-12·0	-11·5
4	20	-11·0	-16·0
5	40	-16·0	-15·5
6	40	-16·5	-19·0
Mean	36·7	-13·1	-14·6
SD	15·1	2·94	2·78

Water content was zero as dry air was used.

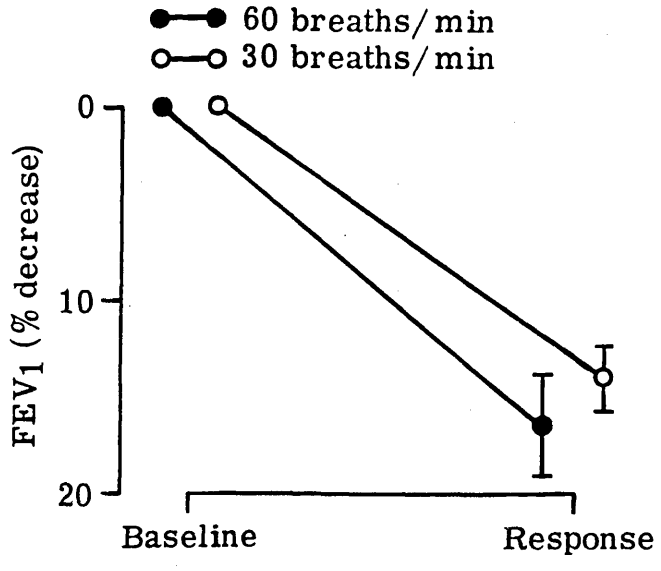


Figure 3:6:1 The effect of changing the breathing pattern of CAIH on airway responsiveness in six asthmatics. CAIH was performed on two occasions in random order using a different rate and depth of breathing (30 and 60 breaths/min). The minute ventilation was the same on each occasion. The results shown are the maximum percentage drop in FEV1 (Mean ± SEM) following each test.

Table 3:6:3

Maximum percentage fall in FEV_1 following inhalation of cold air at different rates (30 and 60 breaths/min) at fixed minute ventilation in six asthmatics.

Subject	30 breaths/min	60 breaths/min
1	8·4	14·6
2	14·6	17·2
3	17·5	10·5
4	11·2	9·2
5	16·3	21·8
6	14·7	24·6
Mean	13·8	16·3
SEM	1·38	2·50

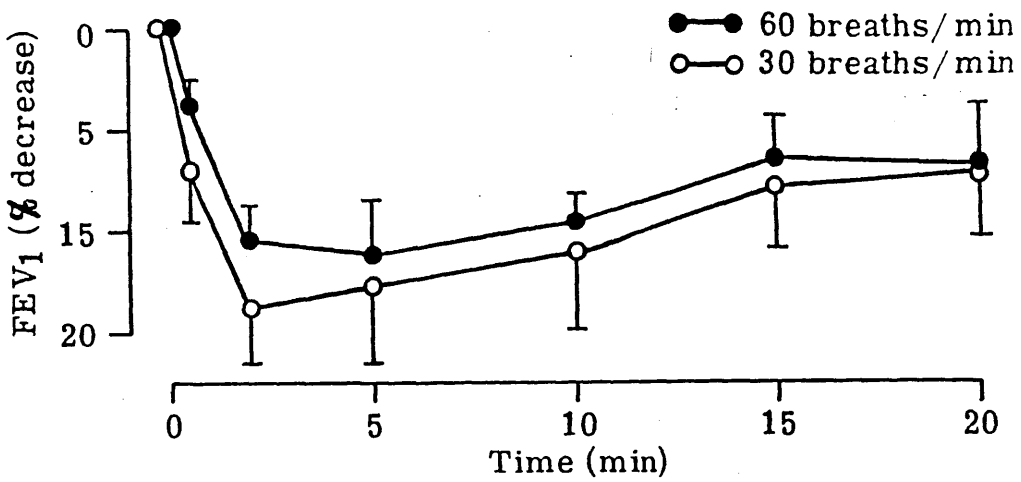


Figure 3:6:2 Changes in FEV1 [percentage of baseline (Mean ± SEM)] following cold air inhalation at different rates (30 and 60 breaths/min) and at fixed minute ventilation.

CHAPTER THREE

SECTION SEVEN

CARBON DIOXIDE REQUIREMENT

CARBON DIOXIDE REQUIREMENT

AIM

The aim of this study was to construct a graph of carbon dioxide requirement and minute ventilation. This would allow prediction of the rate of addition of carbon dioxide required to maintain isocapnia during hyperventilation and would make unnecessary the continuous monitoring of end-tidal carbon dioxide during routine challenge.

METHOD

The apparatus used for this study is shown in Figure 3:7:1. A vacuum cleaner was modified to pump room air instead of using dry air from a cylinder. Subjects were asked to breath quietly through the J-valve (Collins) and end tidal CO2 was measured using a capnograph (Godart). As soon as end tidal CO2 was constant, subjects were asked to breathe at a predetermined ventilation rate by maintaining the size of a suitable balloon (6L) placed adjacent to the inspiratory circuit. Thirty five subjects (28 normal and 7 asthmatics) performed a total of 337 manoeuvres with ventilation rate randomly distributed between 10-120 l/min. Only three or four tests per subject were carried out in a single day. At least one hour was allowed between tests. In each test the end tidal carbon dioxide was monitored continuously and maintained at a normal resting level by the addition of carbon dioxide at the inspiratory point of this breathing circuit. The carbon dioxide reading was

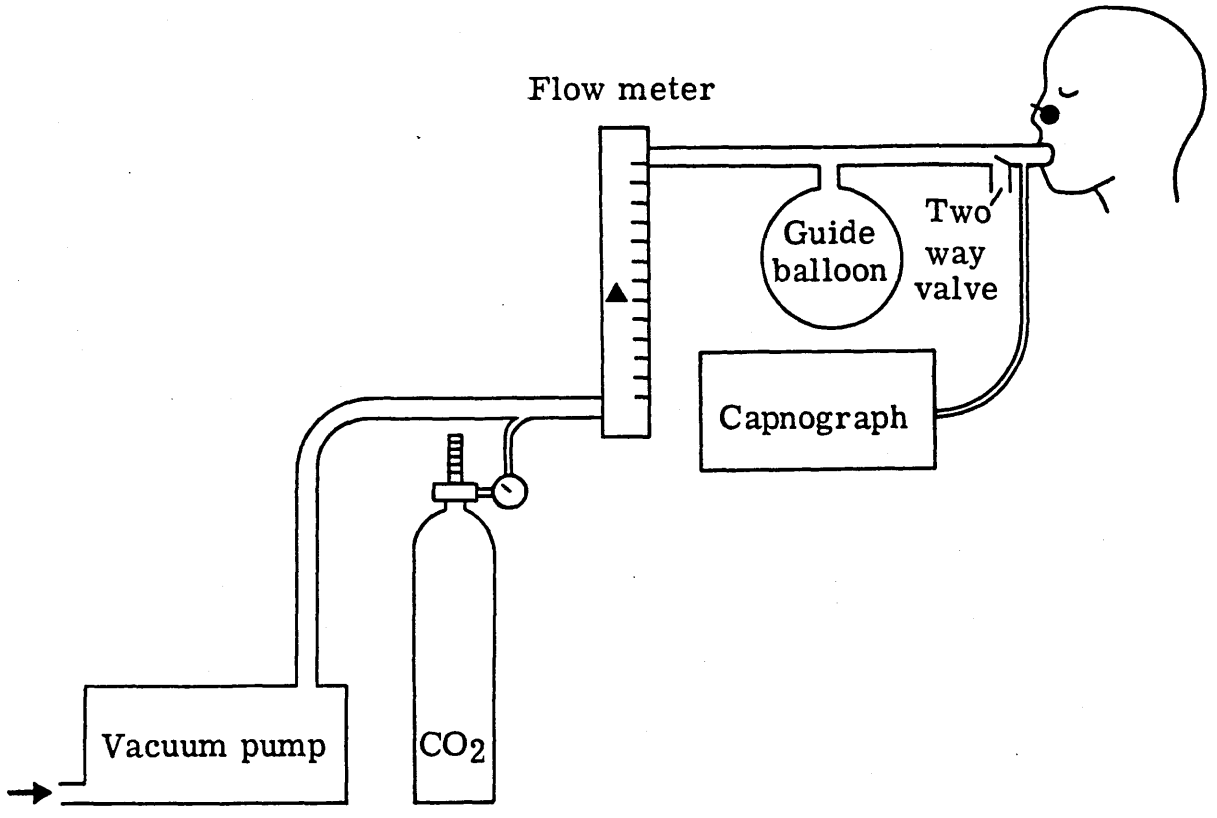


Figure 3:7:1 Schematic representation of the apparatus used to construct the carbon dioxide requirement graph.

taken when the subject achieved a steady state, a graph was constructed from the amount of carbon dioxide added at different levels of ventilation.

RESULTS

The individual characteristics and baseline lung function are shown in Table 3:7:1. All subjects were able to hyperventilate up to 90 L/min. A cumulative total of six, 13 and 19 subjects were unable to cope with hyperventilation at 100, 110 and 120 L/min respectively. There was a direct relationship between the amount of carbon dioxide added at different levels of ventilation. A graph was constructed for the amount of carbon dioxide needed to be added at different levels of ventilation (Table 3:7:2, Figure 3:7:2). There was little individual variation in the amount of carbon dioxide added in L/min at a given ventilation rate.

Table 3:7:1

Anthropometric and spirometric data of subjects tested for carbon dioxide requirement.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% Pred)	FVC (litre)	FVC (% Pred)	FEV_1/FVC %
1	M	35	176	80	3·98	100·0	4·14	83	96
2	M	30	174	81	4·13	102·0	4·57	92	90
3	F	36	165	60	2·91	98·0	3·39	95	85
4	M	30	170	72	3·58	93·0	4·42	96	80
5	F	25	165	64	3·03	94·0	3·51	93	86
6	M	26	187	86	6·11	132·0	7·57	128	80
7	M	30	179	75	4·27	101·0	5·46	104	78
8	M	31	175	76	3·96	99·0	4·57	93	86
9	M	29	172	70	3·77	95·0	4·14	86	91
10	M	20	176	68	4·03	100·0	4·33	89·8	83·6
11	M	38	167	72	2·40	68·4	4·10	98·1	72

cont....

Table 3:7:1 cont....

Anthropometric and spirometric data of subjects tested for carbon dioxide requirement.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% Pred)	FVC (litre)	FVC (% Pred)	FEV_1/FVC %
12	F	50	163	55	2·36	88·0	3·29	100	71
13	M	33	188	82	5·10	113·0	6·04	103	84
14	M	29	182	74	4·75	109·6	5·89	108·1	80
15	F	26	166	59	3·16	97·0	3·84	99	82
16	M	38	164	59	2·84	83·0	3·71	92	76
17	M	21	168	73	4·32	105·0	5·27	108	81
18	M	33	162	55	3·21	92·0	3·60	90	89
19	M	31	168	75	3·50	92·0	3·98	87	87
20	M	35	183	85	4·12	96·0	4·65	85	88
21	F	23	165	65	3·65	113·0	4·27	112	85
22	M	38	181	94	3·37	82·0	3·72	71	90
23	F	32	159	56	3·15	108·0	3·30	98	95

cont.....

Table 3:7:1 cont...
Anthropometric and spirometric data of subjects tested for carbon dioxide requirement.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% Pred)	FVC (litre)	FVC (% Pred)	FEV_1/FVC %
24	F	38	166	55	2·39	79·0	3·19	87	74
25	F	24	150	50	2·55	89·0	2·68	85	95
26	M	26	175	64	4·16	100·0	4·80	94	86
27	M	21	178	70	4·41	98·0	5·39	97	81
28	F	22	160	55	2·98	95·0	3·59	101	83
29	M	28	178	70	5·28	123·0	5·99	112	88
30	F	35	153	50	2·69	99·0	2·89	94	93
31	F	26	160	52	3·58	118·0	4·16	119	86
32	F	24	160	62	3·61	117·0	3·98	113	90
33	M	40	173	60	4·25	113·0	4·89	105	86
34	M	54	160	58	2·50	89·0	2·85	89	87
35	F	26	162	56	3·53	113	3·87	107	91
Mean		30·9	169·4	66·8	3·65	99·8	4·29	97·5	85·0
SD		7·55	9·17	11·15	0·86	12·83	1·03	11·23	6·14

Table 3:7:2

Amount of 100% carbon dioxide in ml/min added to keep the subject isocapnic at different levels of ventilation in 35 subjects.

	Minute ventilation (L/min)											
Subject	10	20	30	40	50	60	70	80	90	100	110	120
1	0	480	950	1200	1850	2350	2800	3270	3850	4150	4920	5700
2	0	480	1100	1150	2100	2500	2950	3350	3800	4150	4400	-
3	0	480	950	1450	1950	2350	3100	3600	3950	4500	-	-
4	0	480	1100	1600	2100	2350	2950	3250	3600	4150	4900	-
5	0	480	1280	1530	1900	2300	3100	3800	4300	4900	-	-
6	0	380	1100	1650	1850	2350	2950	3450	3950	4500	4800	5400
7	0	380	950	1600	2230	2650	3100	3800	4300	4900	5400	-
8	0	380	620	1180	1900	2350	2880	3400	4300	5800	6450	7100
9	0	480	1150	1600	2230	2880	3600	4300	4900	5600	6050	6800
10	200	480	950	1650	2300	2880	3450	3700	3950	4900	5450	6000
11	0	450	950	1400	2450	2880	3450	4300	4900	-	-	-
12	0	380	850	1350	1600	1950	2500	2950	3350	-	-	-

cont...

Table 3:7:2 cont...

Amount of 100% carbon dioxide in ml/min added to keep the subject isocapnic at different levels of ventilation in 35 subjects.

Subject	Minute ventilation (L/min) 10	20	30	40	50	60	70	80	90	100	110	120
13	0	300	650	1120	1900	2230	2650	3600	4500	5130	6050	6700
14	0	600	1350	1650	2100	2880	3450	4200	4700	4500	-	-
15	0	650	1030	1600	1980	2350	3270	3800	4900	5600	-	-
16	0	600	1350	1600	2350	2650	3270	3800	4500	-	-	-
17	0	380	730	1180	1730	2230	2650	3270	3600	3950	4500	5100
18	0	730	1450	2100	2500	3280	3950	4700	5800	6700	7300	-
19	0	400	900	1730	2030	2800	3100	3500	4150	4900	5150	6000
20	0	730	1180	1700	2230	2730	3270	3800	4300	5250	5600	6250
21	0	480	950	1450	1950	2150	2580	3100	3850	4300	4900	5600
22	0	550	1100	1450	1780	2150	2650	3100	3450	4300	4900	5800
23	0	480	950	1350	1700	2150	2650	3050	3850	4830	-	-
24	0	450	930	1630	1980	2500	3050	3800	4400	-	-	-
25	0	650	1100	1750	2000	2350	3100	3800	4300	-	-	-

cont....

Table 3:7:2 cont..

Amount of 100% carbon dioxide in ml/min added to keep the subject isocapnic at different levels of ventilation in 35 subjects.

	Minute ventilation (L/min)											
Subject	10	20	30	40	50	60	70	80	90	100	110	120
26	0	430	750	1280	1700	1950	2500	3270	3600	4900	5380	-
27	0	430	1150	1750	2100	2350	2800	3850	4700	5380	6100	-
28	0	430	950	1450	2000	2350	3030	3450	3800	4150	-	-
29	0	500	850	1600	1950	2350	3100	3600	4330	4900	6100	7100
30	0	625	850	1350	2100	2880	3600	4330	4900	6100	-	-
31	0	400	1100	1600	1950	2350	3100	3670	4300	4900	5800	6480
32	0	650	1200	1650	2230	3100	3950	4630	5350	6100	6600	7500
33	0	730	1280	1900	2350	3100	3800	4700	5600	6480	7150	7500
34	0	450	1280	1600	1950	2500	3030	3450	4150	-	-	-
35	0	480	950	1450	1850	2350	2800	3450	4150	4700	5370	6450
Mean	5·71	498·7	1028·0	1522·9	2024·9	2500·6	3090·9	3688	4295	4987	5603	6343
SD	33·8	111·5	198·6	217·4	217·0	328·4	386·7	463·8	582·3	731·7	803·2	736·1

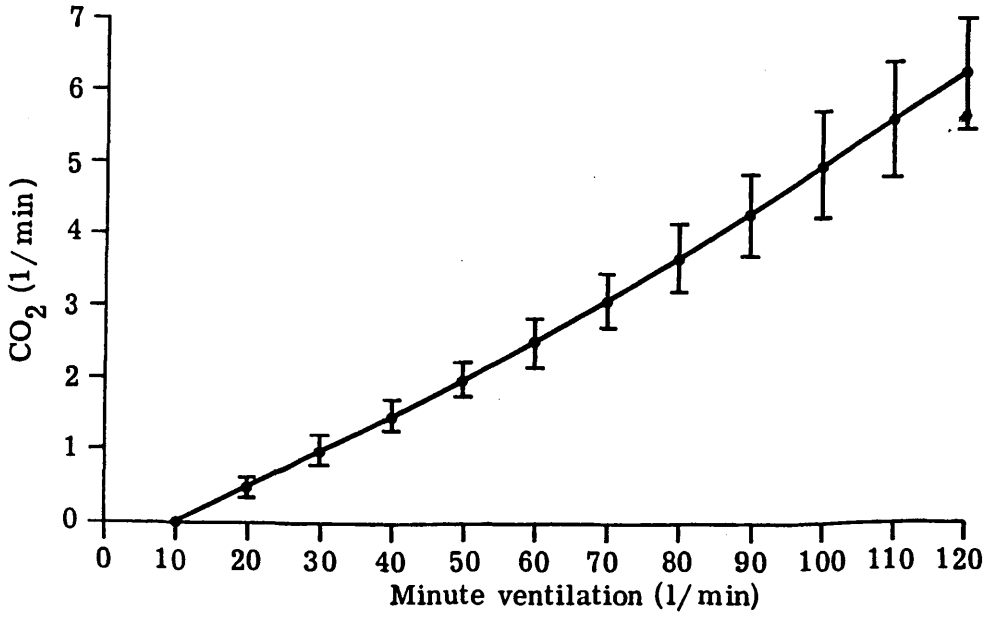


Figure 3:7:2 The carbon dioxide requirement graph showing the amount of 100% carbon dioxide in L/min (Mean ± SD) needed to be added to keep the subject isocapnic at different levels of ventilation.

CHAPTER THREE

SECTION EIGHT

STUDY OF THE DEPENDENCE ON SUBZERO TEMPERATURE

STUDY OF THE DEPENDENCE ON SUBZERO TEMPERATURE

AIM

The purpose of this study was to investigate the influence of temperature on the bronchial response to hyperventilation with dry air.

METHODS

This study was carried out in two parts on 19 patients with asthma. Ten of them were selected randomly for Part A and the remaining 9 for Part B.

PART A

COMPARISON OF A SINGLE EXPOSURE TO SUBFREEDING DRY AIR WITH AN EXPOSURE TO DRY-ROOM TEMPERATURE AIR

This study was carried out on two visits. On the first visit a stepwise test with dry subfreezing air at increasing ventilation rate was performed to determine the level of ventilation required for a 15-20% fall in FEV1 as described previously (Chapter 3, Section 5). The duration of exposure was three minutes and the interval between the exposures was five minutes. On the second visit two challenges were performed, one of them was with dry room temperature air and the other with dry subfreezing air.

1. Isocapnic hyperventilation with dry air: the apparatus used to deliver dry air is shown in Figure 3:8:1. Dry air, at room

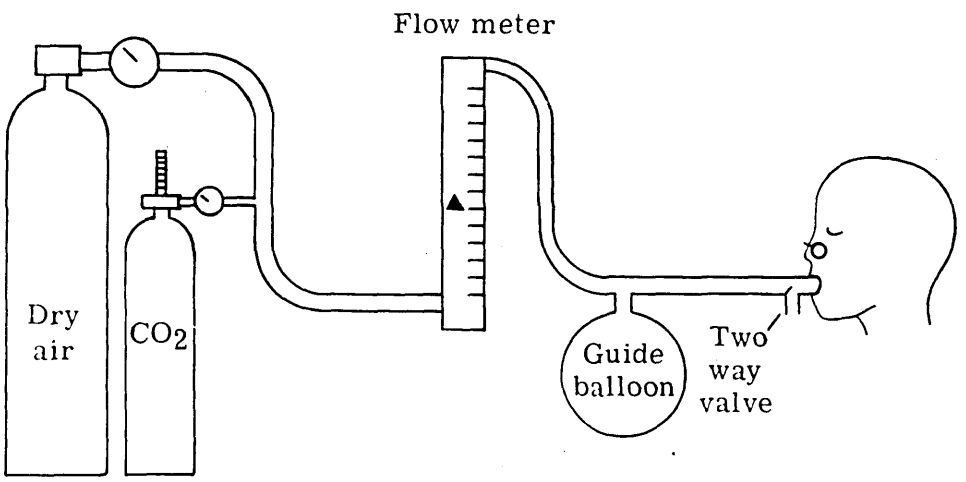


Figure 3:8:1 Schematic representation of the apparatus used for delivering a dry air challenge.

temperature was inhaled from a cylinder whose flow could be adjusted with a stopcock and was measured by a rotameter. A six litre rubber balloon was placed in the respiratory circuit. The desired minute ventilation was maintained by instructing the subject to maintain the size of guide balloon throughout the test. Carbon dioxide was added in sufficient amount and monitored continuously as described before 2.2. For each subject the baseline FEV1 was determined, and then a dry air challenge was performed. The level of ventilation used was that of the final step determined on visit one. The duration of the challenges was three minutes. FEV1 was recorded at 0.5, 2 and 5 minutes and then every 5 minutes for up to 20 minutes and expressed at a percentage of baseline value.

2. Isocapnic hyperventilation with cold air: Subfreezing dry air was generated as previously described (2.2). The same procedure, duration of the challenge and the level of ventilation were used as with the previous test with the dry room temperature air challenge. At least one hour was allowed between the challenges provided lung function had returned to normal.

PART B

COMPARISON OF STEPWISE COLD AIR ISOCAPNIC HYPERVENTILATION WITH DRY AIR ISOCAPNIC HYPERVENTILATION AT ROOM TEMPERATURE

This study was performed on nine asthmatic patients and completed

on two visits. On visit one, a stepwise increasing ventilation test with subfreezing air at 10, 20, 40, 60 and 80% of predicted IMBC was performed, as previously described (3:5) to determine the ventilation rate needed to produce a 15-20% fall in FEV1 fall. The duration of each step was for three minutes and the interval between the steps was five minutes. On visit two, the same procedure was carried out as on visit one but using dry air at room temperature instead of subfreezing air, using the apparatus described in part A. The same ventilation steps were used at both visits. FEV1 was measured at 0.5, 2 and 5 minutes following each step, and for every five minutes up to 20 minutes after the end of the final step.

RESULTS

The individual characteristics and baseline lung function of the subjects participating in these studies are shown in Tables 3:8:1; 3:8:2.

PART A

COMPARISON OF SINGLE EXPOSURE OF SUBFREEZING DRY AIR WITH SINGLE EXPOSURE OF ROOM TEMPERATURE DRY AIR

The inspired air temperature (Mean $\pm$ SD) for dry subfreezing air and dry room temperature air was -13 $\pm$ 2.7, and 20.5 $\pm$ 1.6 C respectively (Table 3:8:3). Maximum % fall in FEV1 achieved (mean $\pm$ SEM) were 17.3 $\pm$ 1.9 and 11 $\pm$ 1.8 respectively (Figure 3:8:2). This challenge was statistically significant ($P<0.02$). Using dry

Table 3:8:1

Individual characteristics and baseline lung function of ten asthmatic patients tested for comparison of single challenge with dry subfreezing and with dry room temperature air.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	F	28	179	85	2·31	64·9
2	M	23	169	80	3·91	97·5
3	M	55	172	76	1·80	55·7
4	M	43	174	85	2·54	69·3
5	M	22	175	75	3·48	87·1
6	F	20	171	60	2·96	84·3
7	M	20	170	48	2·26	66·1
8	M	63	174	75	1·89	62·2
9	M	43	183	80	2·39	59·2
10	F	24	174	68	3·64	105·4
Mean		34·1	174·1	73·2	2·72	75·17
SD		15·77	4·23	11·65	0·74	17·2

Table 3:8:2

Individual characteristics and baseline lung function of nine asthmatics tested for comparison with stepwise challenge of dry subfreezing and with dry room temperature air.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	M	27	161	50	2·61	71·9
2	M	20	192	74	3·52	76·8
3	M	55	172	76	1·81	56·0
4	M	59	169	73	2·66	88·1
5	M	29	171	70	3·32	84·1
6	F	24	148	51	1·50	54·0
7	F	56	164	70	2·31	90·9
8	M	22	175	75	3·29	63·8
9	M	49	170	69	2·09	62·7
Mean		37·9	169·1	67·6	2·57	72·03
SD		16·40	11·77	9·96	0·71	13·78

Table 3:8:3

Minute ventilation (% IMBC) and inspired air temperature (^{0}C) recorded during single challenge of dry subfreezing and dry room temperature air in ten asthmatics.

Subject	Minute Ventilation (% IMBC)	Inspired temperature (^{0}C)	
		Dry sub-freezing air	Dry room temperature air
1	20	-11·0	23·0
2	40	-16·0	19·5
3	40	-14·5	18·0
4	60	-13·5	21·0
5	60	-11·0	22·0
6	60	-16·0	21·5
7	20	-9·0	18·5
8	10	-17·0	20·0
9	40	-13·5	20·5
10	60	-11·0	21·0
Mean	41·0	13·3	20·5
SD	19·1	2·67	1·55

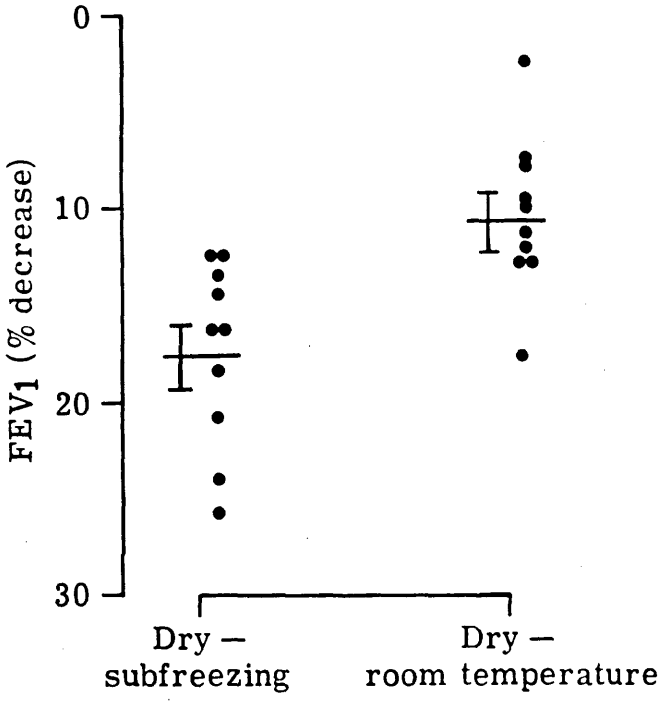


Figure 3:8:2 The influence of temperature on the bronchial response to hyperventilation with dry air. The results shown are maximum percentage drop in FEV1 (Mean ± SEM) following a single exposure to either subfreezing or room temperature dry air in ten asthmatics.

air at room temperature the airflow obstruction was about two thirds of that achieved with dry subfreezing air. The changes in FEV1 following both challenges are shown in Figure (3:8:5). Four patients out of 10 had a fall in FEV1 of less than 10% (a significant response was considered to have occurred when there was a fall of more than 10% in FEV1).

PART B

COMPARISON OF STEPWISE COLD AIR ISOCAPNIC HYPERVENTILATION WITH DRY AIR ISOCAPNIC HYPERVENTILATION AT ROOM TEMPERATURE

The inspired air temperature (Mean $\pm$ SD) for dry subfreezing air experiment was -12.2 $\pm$ 2.3 C and for dry room temperature challenge was 21.5 $\pm$ 2.2 C, (Table 3:8:4). A dose response relationship between % change in FEV1 and the level of ventilation for dry air was determined as described for subfreezing dry air in 5:4. The maximum % fall in FEV1 following both challenges is shown in Figure 3:8:3. Three patients out of nine had a fall in FEV1 of less than 10% of baseline after hyperventilation with dry air at room temperature, while they had more than a 10% fall in their FEV1 with dry subfreezing air. There was no difference in the rate of recovery from both challenges (Figures 3:8:4). There was a good relationship between PD10 dry subfreezing air and PD10 dry room temperature air ($r = 0.95$, $P<0.001$) (Figure 3:8:6). [PD10 was a ventilation rate in percentage predicted IMBC required to reduce the FEV1 by 10%]. However, higher levels of ventilation were needed with dry air at room temperature to achieve a similar

Table 3:8:4

Minute ventilation (% IMBC) and inspired air temperature (^{0}C) recorded during stepwise challenge with dry subfreezing air and dry room temperature air in nine asthmatics.

Subject	Minute Ventilation (% IMBC)	Inspired temperature (^{0}C)	
		Dry sub-freezing air	Dry room temperature air
1	40	-11·0	19·0
2	40	-12·5	20·5
3	40	-16·0	24·5
4	60	-13·0	19·5
5	60	-17·0	20·5
6	40	-15·5	20·0
7	20	-13·0	21·5
8	60	-14·0	23·0
9	40	-16·0	25·0
Mean	44·4	-14·2	21·5
SD	13·3	2·00	2·18

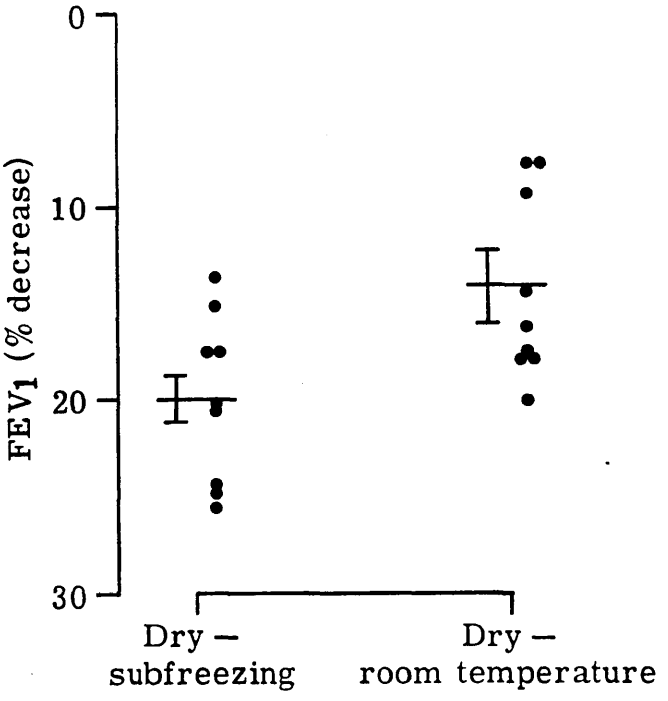


Figure 3:8:3 The influence of temperature on the bronchial response to hyperventilation with dry air. The results shown are the maximum percentage fall in FEV1 (Mean ± SEM) following stepwise tests using either subfreezing or room temperature dry air in nine asthmatics.

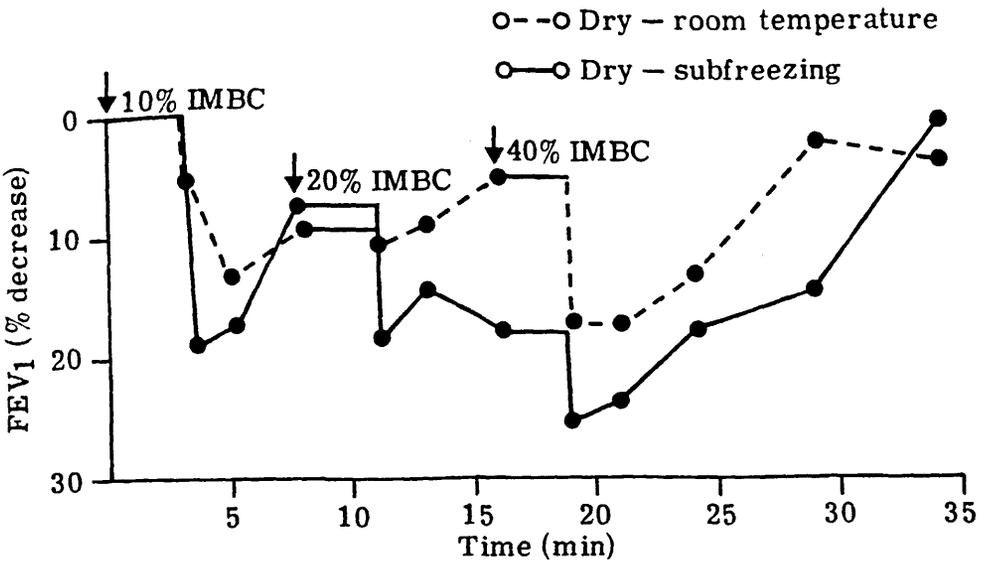


Figure 3:8:4 Changes in FEV1 (percentage of baseline) following inhalation of a) subfreezing dry air and b) dry air at room temperature, performed by a single subject at increasing ventilation rates (10, 20, and 40% predicted IMBC). The duration of each step was three minutes and the interval between the steps was five minutes.

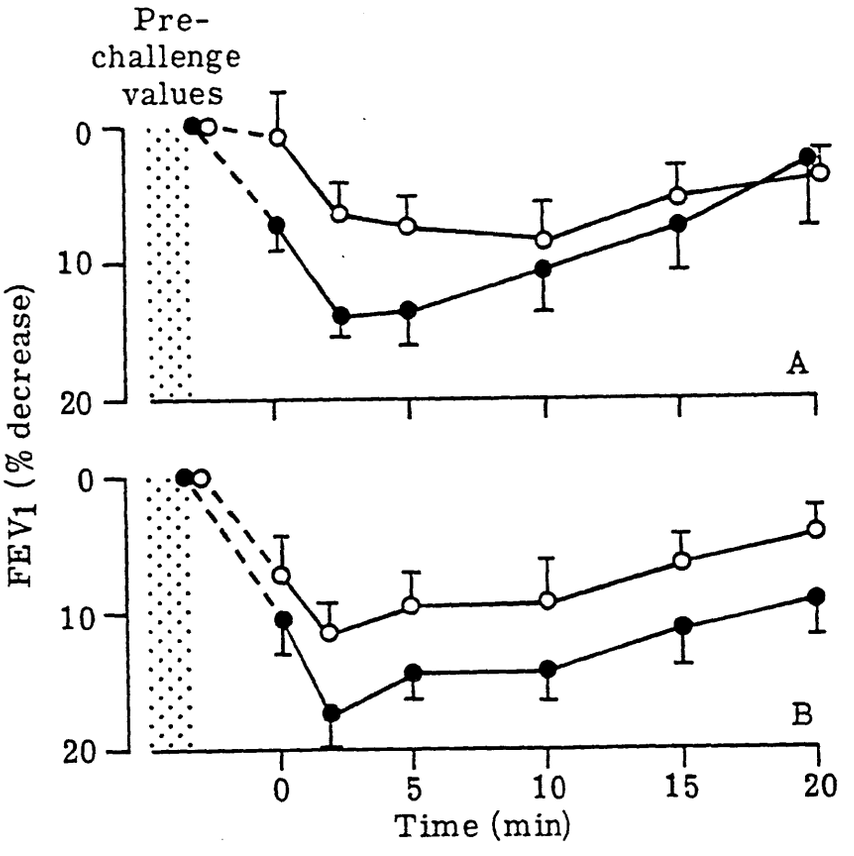


Figure 3:8:5 Changes in FEV1 [percentage of baseline (Mean + SEM)] following isocapnic hyperventilation with either dry subfreezing (●—●) air or dry air at room temperature (○—○) a) Single challenge, b) Stepwise test.

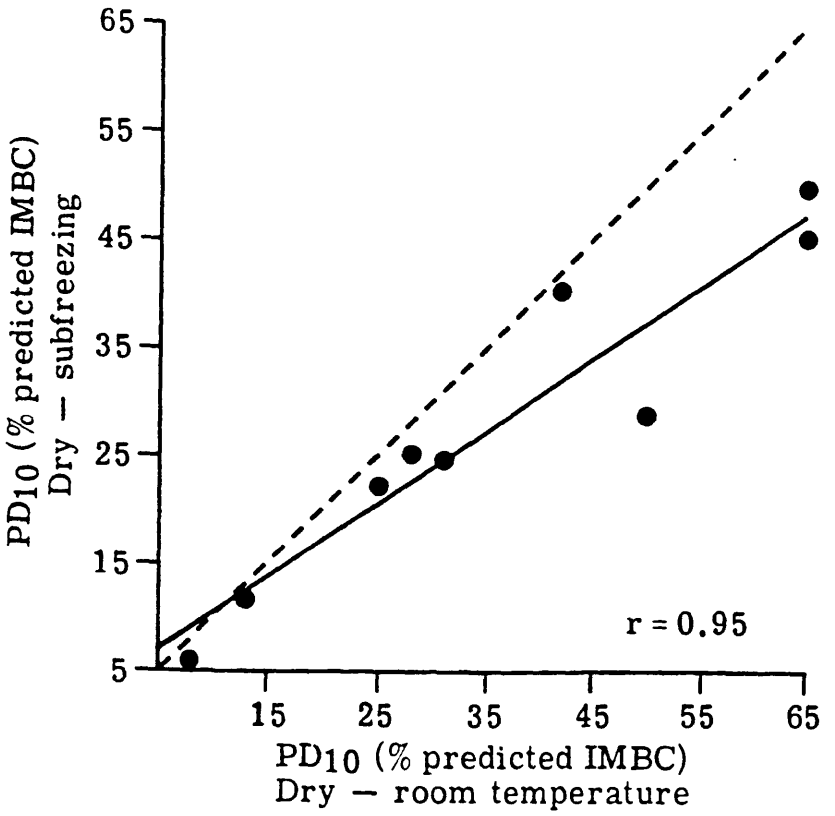


Figure 3.8:6 Comparison of bronchial responsiveness to dry, subfreezing and dry air at room temperature. The responsiveness for each was expressed as PD10 (the ventilatory rate in percentage of predicted IMBC required to reduce the FEV1 by 10%).

obstructive response with that produced by subfreezing air (Figure 3:8:6).

CHAPTER THREE

SECTION NINE

VALIDATION OF THE TECHNIQUE

VALIDATION OF THE TECHNIQUE

AIM

The aim of this study was to compare bronchial responsiveness to standardised CAIH with that of other tests of reactivity (histamine and exercise tests) in normal subjects and patients with asthma and allergic rhinitis.

METHODS

The cold air test was compared with both histamine challenge and exercise testing in 22 asthmatics (only eight of whom performed exercise testing), eight patients with allergic rhinitis and nine normal subjects. The tests were performed on three separate visits within a period of ten days and the visits were randomised.

Cold air test: Cold air was inhaled by the subject at increasing ventilation rates (10, 20, 40 and 60% predicted IMBC). The duration of each exposure was three minutes and the interval between exposures was five minutes). The testing was stopped when more than a 20% drop in FEV1 was obtained, or when 60% predicted IMBC was achieved. The best of three measurements of FEV1 was recorded immediately after two and five minutes following the end of each period. PD10 and PD20 were obtained from the dose response curves and expressed as percentages of each subject's predicted IMBC as previously described (Chapter 3, section 5). These values were also expressed in terms of the absolute level of ventilation

(l/min).

Histamine and exercise tests: were performed as described before (2.5; 2.6).

RESULTS

The individual characteristics and baseline lung function of the subjects who participated in the study validation are shown in Tables 3:9:1, 3:9:2; 3:9:3.

In the asthmatic subjects cold air inhalation caused a decrease in FEV1 of greater than 10% in 21 subjects, and of greater than 20% in 18 of these subjects (Figure 3:9:1) The PD10 and PD20 values were obtained. In the remaining one subject (number 13) (Table 3:9:4), breathing subfreezing air at 60% predicted IMBC in FEV1 of 9.3% and PD10 was measured by extrapolation.

Following histamine inhalation, a 20% decrease in FEV1 was obtained in 18 subjects with asthma (16 of those had PD20 cold air of less than 57.5% of predicted IMBC and in the other two subjects PD10 cold air could only be measured, and the values were 13.8, 40.0 % of predicted IMBC). In the remaining four subjects, inhalation of histamine at 32 mg/ml induced less than 10% drop in FEV1. In the latter four subjects PD10 cold air could be measured and the values obtained were 13.0, 24.3, 47.5 and 61.0 % of predicted IMBC.

Table 3:9:1

Individual characteristics and baseline lung function for 22 patients with asthma tested for the validation study.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	M	55	174	80	1·81	54·5
2	M	27	161	50	2·69	74·1
3	F	20	171	60	3·37	96·0
4	M	59	169	73	2·72	90·1
5	M	27	181	71	4·66	106·2
6	F	56	164	70	2·12	83·5
7	F	28	179	85	2·58	107·1
8	M	43	168	76	2·70	77·8
9	M	32	179	68	4·05	97·6
10	M	65	175	72	1·77	46·9
11	M	20	170	65	2·74	80·0
12	M	61	156	70	1·94	70·0
13	M	50	174	79	3·32	96·0
14	M	43	175	80	2·41	66·9
15	M	43	183	80	2·65	65·6
16	M	32	181	81	3·37	90·3
17	M	43	173	70	3·16	86·3
18	M	43	172	70	3·44	98·0
19	M	43	181	90	2·74	69·5
20	F	31	170	85	4·23	103·4
21	M	35	175	78	3·48	91·3
22	M	34	187	84	3·57	83·0
Mean		40·5	173·6	74·4	2·98	83·4
SD		13·11	7·39	9·16	0·77	16·38

Table 3:9:2

Individual characteristics and baseline lung function of allergic rhinitis patients who participated in the validation study. (Group A subjects with asthma; Group B subjects without asthma).

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
Group A						
1	F	24	158	60	2·85	92·8
2	M	33	168	54	4·21	71·3
3	F	24	165	68	3·21	80·5
4	M	20	172	60	4·26	105·9
Mean		25·3	165·8	60·5	3·63	87·6
SD		5·5	5·91	5·74	0·71	15·0
Group B						
1	M	39	179	110	3·96	65·4
2	M	30	180	65	4·21	108·4
3	M	33	168	54	3·17	120·0
4	M	48	151	87	2·35	117·0
Mean		37·5	169·5	79·0	3·42	102·7
SD		7·94	13·5	24·8	0·84	25·3

Table 3:9:3

Individual characteristics and baseline lung function for normal subjects who participated in the validation study.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	M	32	182	73	4·84	113·9
2	M	54	156	54	2·34	87·3
3	M	26	187	86	6·36	94·6
4	M	33	162	55	3·34	96·5
5	M	30	175	78	4·17	103·7
6	M	34	167	69	4·17	103·7
7	M	22	178	75	4·12	113·8
8	M	27	178	65	3·69	83·3
9	M	31	175	80	4·73	110·3
Mean		32·1	173·3	70·6	4·20	100·8
SD		9·05	9·87	10·94	1·11	11·14

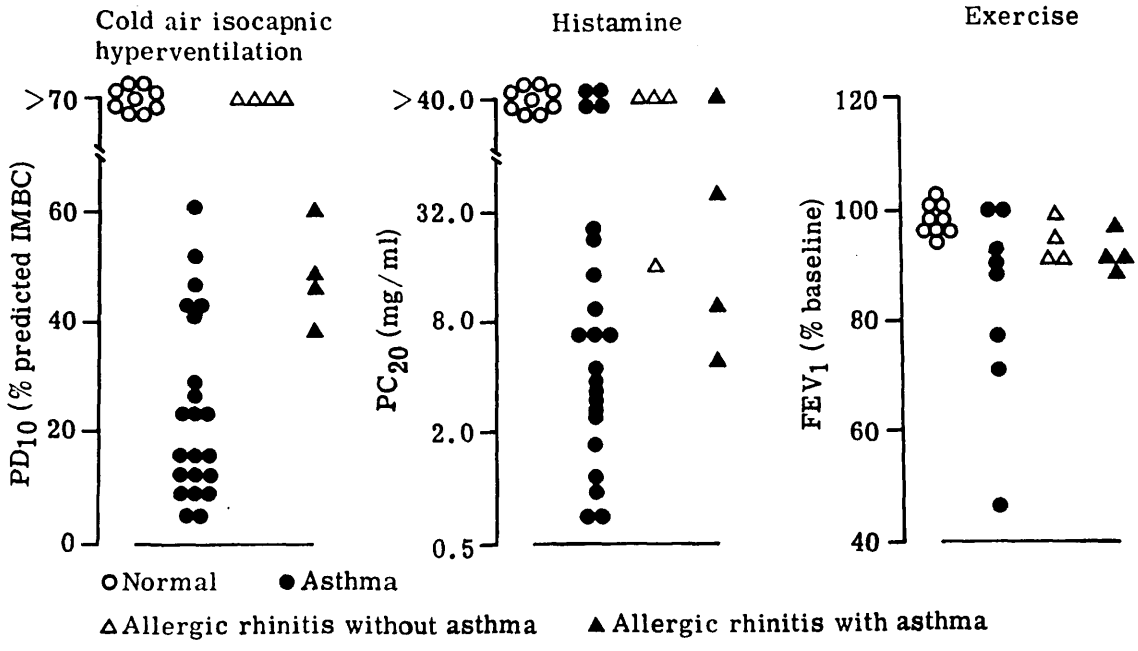


Figure 3:9:1 Bronchial responsiveness to CAIH, histamine and exercise challenges in normal, allergic rhinitis and asthmatics subjects. The results shown are individual values of a) provocative doses of cold air ventilation in percentage of predicted IMBC reducing the FEV1 by 10% (PD10), b) provocative doses of histamine reducing the FEV1 by 20% (PC20), c) the fall in FEV1 in percentage of baseline following exercise.

Table 3:9:4

Variables obtained from different bronchial challenges (a) CAIH, (b) Histamine, (c) Exercise in 22 asthmatics

Subject	Cold air isocapnic hyperventilation						Histamine		Exercise
	Ventilation attained		PD_{10}	PD_{10}	PD_{20}	PD_{20}	Dose of histamine	PC_{20}	FEV_1 max %
	% IMBC	L/min	%IMBC	L/min	%IMBC	L/min	mg/ml		fall
1	40	46·0	5·2	6·0	26·0	29·0	8·0	3·05	29·0
2	60	76·0	22·8	28·2	35·2	44·2	8·0	4·4	-
3	60	77·0	9·6	12·8	26·3	31·5	32·0	0·73	-
4	60	64·0	47·5	52·2	-	-	32·0	-	-
5	40	62·0	28·0	42·8	42·3	61·5	32·0	3·85	7·6
6	40	36·0	13·0	11·0	40·2	18·2	32·0	7·1	-
7	40	34·0	22·1	17·5	32·2	27·5	2·0	1·05	-
8	40	49·0	9·2	11·3	46·5	59·0	16·0	7·6	-
9	40	58·0	24·3	40·7	47·0	90·0	32·0	-	10·3
10	20	26·0	6·0	7·5	18·1	22·8	32·0	9·1	-
11	40	48·0	15·5	20·5	26·0	31·0	4·0	1·65	55·1

cont....

Table 3:9:4 cont...

Variables obtained from different bronchial challenges (a) CAIH, (b) Histamine, (c) Exercise in 22 asthmatics

Subject	Cold air isocapnic hyperventilation						Histamine		Exercise
	Ventilation attained % IMBC	L/min	PD_{10} %IMBC	PD_{10} L/min	PD_{20} %IMBC	PD_{20} L/min	Dose of histamine mg/ml	PC_{20}	FEV_1 max % fall
12	60	58·0	13·8	13·8	-	-	32·0	0·94	-
13	60	73·0	61·0	79·0	-	-	32·0	-	-
14	60	51·0	40·0	51·0	-	-	32·0	22	-
15	60	85·0	43·2	59·5	57·8	81·0	16·0	7·3	0·4
16	40	53·0	23·8	31·2	35·6	46·0	16·0	3·4	11·2
17	20	26·0	10·5	13·0	19·5	24·0	32·0	0·92	-
18	20	25·0	11·3	15·0	17·9	23·2	32·0	2·12	-
19	40	55·0	15·1	21·0	31·8	42·2	32·0	3·35	-
20	60	86·0	44·3	64·0	60·0	85·8	32·0	14·5	-
21	20	26·0	13·0	17·5	19·6	25·8	32·0	-	23·5
22	60	30·0	50·5	75·5	57·0	86·0	32·0	18·2	0·3
Mean	44·5	52·0	24·1	31·4	35·5	46·0	25·0	6·18	17·2
SD	15·0	19·6	16·4	22·9	13·9	24·9	10·97	6·2	18·4

Of the 18 patients in whom PD20 cold air could be measured, 16 had a PC20 of histamine less than 22 mg/ml, and in the other two patients PC20 histamine could not be measured. In the four asthmatics in whom PD10 cold air could only be measured, two had PC20 of 0.94 and 22 mg/ml, and in the other two PC20 could not be measured.

Eight patients with asthma underwent exercise task, five showed greater than 10% fall in FEV1, The remaining three patients showed less than 10% drop. (Figure 3:9:1). In all of the eight subjects PD20 cold air could be measured while the PC20 histamine could be measured for only six of them.

Eight patients with allergic rhinitis participated in this study. four subjects gave a history of occasional wheeze (Group A) and the other four subjects were without wheeze (Group B). Three of the four patients in Group A responded with greater than 10% fall in FEV1 following CAIH (PC20 histamine was measured in only two of these and the values were 10.1 and 5.9; in the other patient PC20 histamine could not be measured). The fourth patient had a fall in FEV1 of 9.5% at a ventilation rate of 60% of predicted IMBC, and PD10 was measured by extrapolation. The maximum fall in FEV1 following exercise in Group A and B were 8.78 $\pm$ 4.56: 7.88 $\pm$ 4.46 (Mean $\pm$ SD) respectively.

All subjects in Group B responded by less than 10% after breathing

cold air at 60% predicted IMBC. PC20 histamine was measured in only one patient in this group, and the value was 15 mg (Figure 3:9:1 and Table 3:9:5).

In normal subjects the maximum drop in FEV1 following CAIH, histamine and exercise challenges (mean ± SEM) in the normal group studied were 3.7 ± 0.72; 2.6 ± 0.73, 1.54 ± 0.74, respectively (Figure 3:9:1; Table 3:9:6). There were no significant difference between these results. The range of the maximum drop in FEV1 achieved in nine normal was 0.6 - 6.6% with a mean and SD of 3.7 ± 2.16. No significant difference in the pre-challenge FEV1 values was observed on the three testing days. An example of bronchial responsiveness to CAIH in two normals and three asthmatics is shown in Figure 3:9:2.

The responsiveness to cold air isocapnic hyperventilation was correlated with responsiveness of histamine challenges. Both PD10 and PD20 for cold air correlated with the fall in FEV1 after exercise ($r = 0.65$, $p < 0.05$, $r = 0.78$, $p < 0.05$) (Figures 3:9:3 and 3:9:4). Similarly, there was a good correlation between PD10 cold air and PC20 histamine ($r = 0.74$; $P<0.001$)(Figure 3:9:5) and PD20 cold air and PC20 histamine ($r = 0.72$, $P<0.002$)(Figure 3:9:6). Expression of PD10 and PD20 in terms of absolute level of ventilation (L/min) showed a similar relationship.

In patients in whom PD20 cold air could be measured, PD10 was

Table 3:9:5

Variables obtained from different bronchial challenge (a) CAIH, (b) Histamine, (c) Exercise in eight allergic rhinitis patients

Subject	Cold air isocapnic hyperventilation						Histamine		Exercise
	Ventilation attained		PD_{10}	PD_{10}	PD_{20}	PD_{20}	Dose of histamine	PC_{20}	FEV_1 max
	% IMBC	L/min	%IMBC	L/min	%IMBC	L/min	mg/ml		fall
Group A									
1	60	65	38·5	40·5	-	-	32	10·1	11·7
2	60	88	48·5	71·5	-	-	16	5·9	12·8
3	60	67	44·5	47·5	-	-	32	-	2·7
4	60	89	61·5	94·5	-	-	32	35·0	7·9
Mean	60	77·3	48·3	63·5	-	-	28·0	17·0	8·78
SD	0	13·02	9·7	24·56	-	-	8·0	15·7	4·56
Group B									
1	60	83	-	-	-	-	32	-	12
2	60	88	-	-	-	-	32	-	1·9
3	60	67	-	-	-	-	32	-	10·4
4	40	32	-	-	-	-	32	15·0	7·2
Mean	55·0	67·5	-	-	-	-	32	-	7·88
SD	10	25·3	-	-	-	-	0	-	4·46

Table 3:9:6

Variables obtained from different bronchial challenges (a) CAIH, (b) Histamine, (c) Exercise in nine normal subjects

Subject	Cold air isocapnic hyperventilation							Histamine			Exercise
	FEV_1 max % fall	Ventilation attained %IMBC	L/min	PD_{10} %IMBC	PD_{10} L/min	PD_{20} %IMBC	PD_{20} L/min	FEV_1 max % fall	Dose of histamine mg/ml	PC_{20}	FEV_1 max % fall
1	2·9	60	119	-	-	-	-	5·3	32	-	0
2	3·8	60	56	-	-	-	-	3·1	32	-	3·6
3	6·6	60	97	-	-	-	-	4·2	32	-	-1·7
4	0·6	60	73	-	-	-	-	4·4	32	-	1·4
5	0·8	60	85	-	-	-	-	2·3	32	-	3·8
6	4·4	60	96	-	-	-	-	-1·5	32	-	3·5
7	5·5	60	93	-	-	-	-	4·0	32	-	4·0
8	6·1	60	90	-	-	-	-	-0·2	32	-	-0·7
9	2·9	60	85	-	-	-	-	2·1	32	-	-
Mean	3·7	60	88·2	-	-	-	-	2·6	32	-	1·54
SD	2·16	0	17·3	-	-	-	-	2·2	0	-	2·22

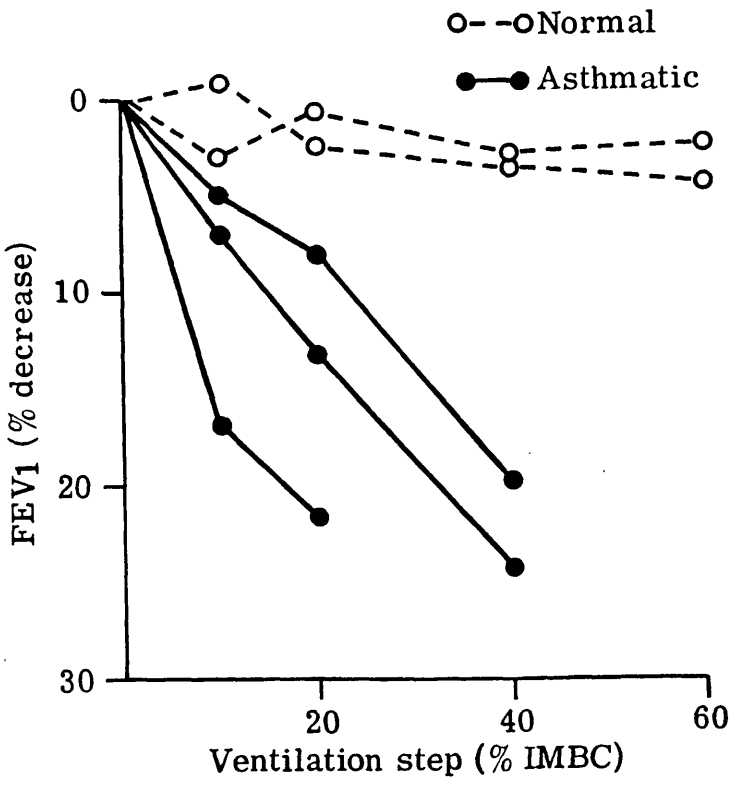


Figure 3:9:2 The dose response curve in three asthmatics and two normal people using CAIH was performed at increasing ventilation rates. Duration of each step was three minutes and the interval between the exposures was five minutes. Maximum percentage fall in FEV1 following each step was plotted against the respective challenge periods.

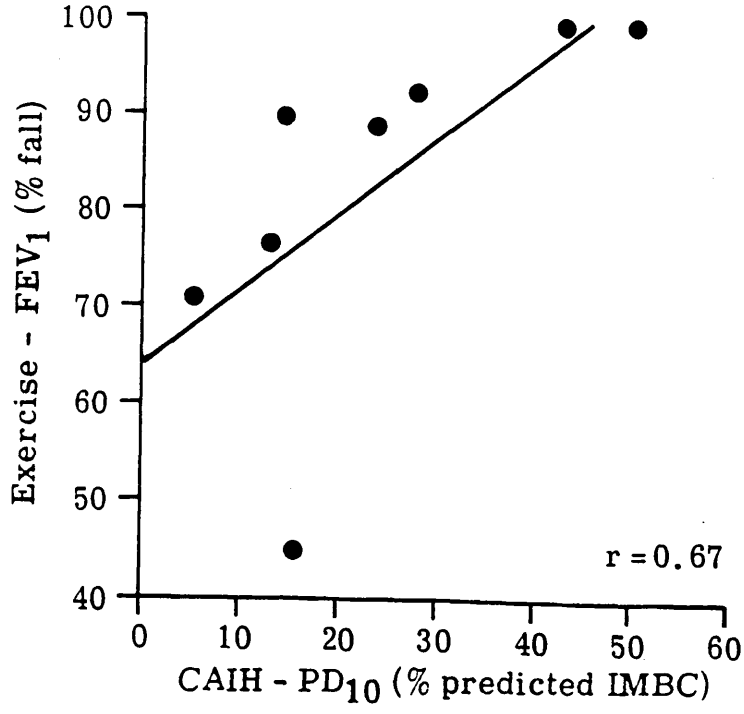


Figure 3:9:3 Comparison of bronchial responsiveness to CAIH expressed as PD10 (provocative dose of cold air ventilation in percentage of predicted IMBC reducing the FEV1 by 20%) and maximum fall in FEV1 following exercise testing.

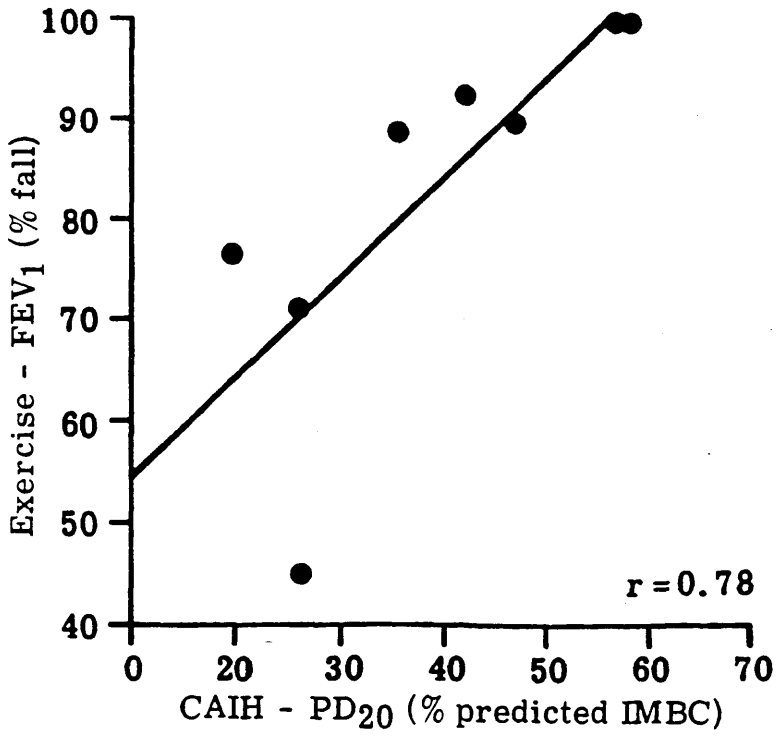


Figure 3:9:4 Comparison of bronchial responsiveness to CAIH expressed as PD20 (provocative dose of cold air ventilation in percentage of predicted IMBC reducing FEV1 by 20%) and the maximum fall in FEV1 following exercise testing.

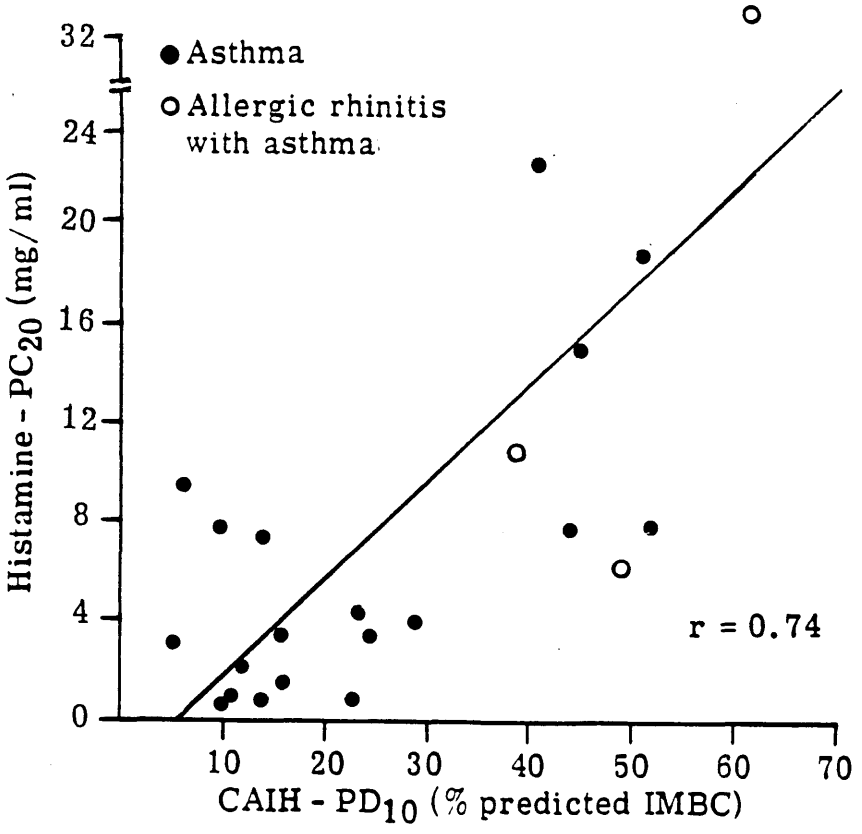


Figure 3:9:5 Comparison of bronchial responsiveness to histamine expressed as PC20, and CAIH expressed as PD10.

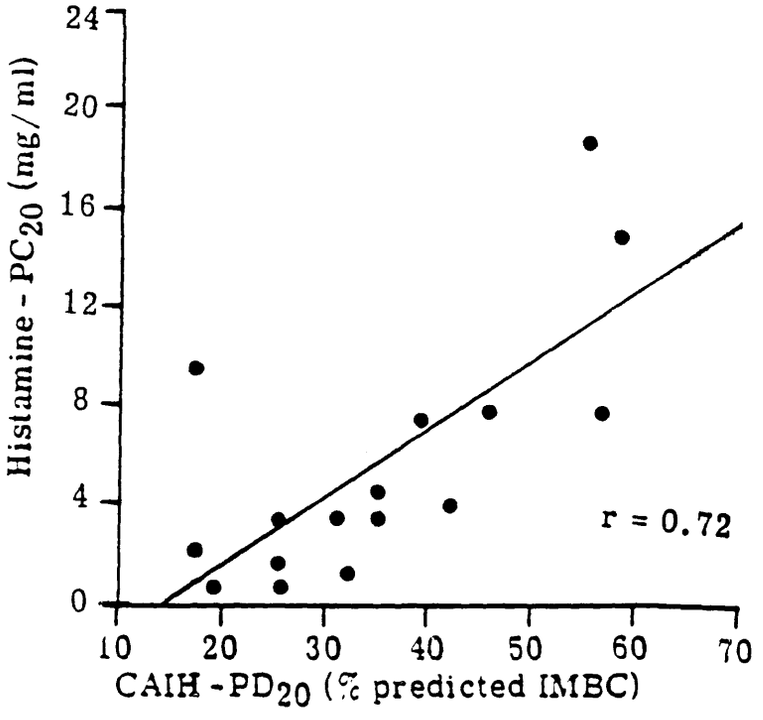


Figure 3:9:6 Comparison of bronchial responsiveness to histamine expressed as PC20 and CAIH expressed as a PD20.

approximatly half of that of PD20 (Figure 3:9:7). These data also show that among these patients demonstrating a 20 % or greater fall in FEV1 with cold air, The PD10 was directly correlated to PD20 ($r = 0.80$; $P<0.001$).

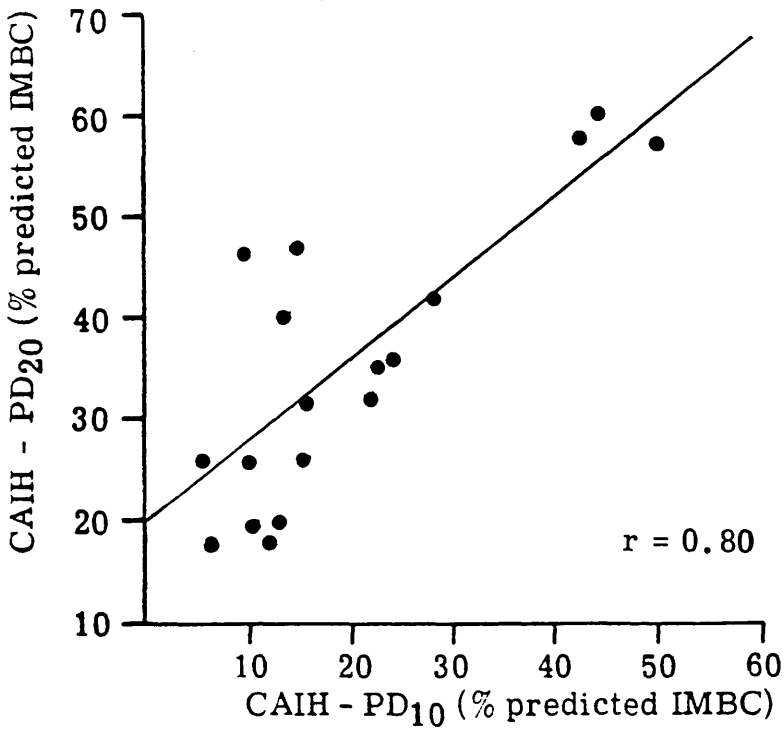


Figure 3:9:7 Correlation between PD10 and PD20 for cold air in those asthma patients demonstrating a 20% or greater fall in FEV1.

CHAPTER THREE

DISCUSSION

DISCUSSION

The factors determining the airway responsiveness to inhaled cold, dry air were investigated as the basis for developing an acceptable, simple and standardised test. First, a carbon dioxide requirement graph was constructed at different levels of ventilation. This allowed the inspired carbon dioxide to be preset, eliminating the need for elaborate equipment and monitoring. Secondly, it was found:

a) it was not necessary to monitor the tidal volume and respiratory rate continuously in order to achieve a given effect,

b) it was better to grade provocation with cold air by varying the level of ventilation than the duration of the exposures,

c) three minutes was sufficient time for test duration,

d) the optimal interval between the steps was five minutes,

e) it was not necessary to challenge patients at a level of ventilation higher than 60% of predicted IMBC,

f) it was sufficient to quantify the stimulus by using the ventilatory rate as a fraction of predicted IMBC. The respiratory heat exchange (RHE) measurement (which needs complex measurements and calculations) is not important in the clinical use of this

test,

g) the reproducibility of bronchoconstriction following cold air inhalation was good (coefficient of variation was 17.5%),

h) the responsiveness to cold air correlated to that with exercise and histamine tests.

i) The detection of normals from asthmatic individuals was better using CAIH test than histamine and exercise tests.

In the last few years, isocapnic hyperventilation with cold air (Deal et al, 1979a and 1979b; Breslin et al, 1980; O'Cain et al, 1980; Deal et al, 1980; O'Bryne et al, 1982b; Aquilina, 1983; Woodrow-Weiss, Rossing, and McFadden 1983; Bar-Yishay, Ben-Dov and Godfrey, 1983; Nagakura et al, 1983; Heaton et al, 1984; Tal et al, 1984) and dry air at room temperature (Strauss at al, 1978; Tweddale, Godden and Grant, 1981; McFadden et al, 1982; Wilson et al, 1983; Denjean et al 1983) have been used as bronchial provocation tests in patient with asthma. Both single and stepwise ventilatory rates have been tried. the latter has the advantages of being safe as it is given in graded doses. In addition, it allowed the generation of dose-response curve. In some cases, respiratory heat loss was determined and used to express the effect of cold air (O'Byrne et al, 1982a and 1982b). Different protocols and techniques have been used for CAIH, making the comparative

analysis of the results difficult. It is a potentially useful measurement of airway reactivity and the need for a simple and standardized test has been emphasized (Menkes, 1980).

The standardised test incorporated a stepwise increase in a ventilation rate (10,20,40 and 60% predicted IMBC). The duration of each step was three minutes duration and the interval between them was five minutes. Using this ventilatory step test, a dose response relationship between the challenging dose (level of ventilation) and subsequent decrease in FEV1 was found. It was better to grade cold air provocation by varying the level of ventilation rather than duration of exposure. Five minutes between the steps was chosen because in most patients, the fall in FEV1 following CAIH occurred within five minutes. In the previous studies of isocapnic hyperventilation, intervals ranging between four to 15 minutes have been used between the ventilatory steps. (O'Byrne et al, 1982b; Gillioz and Orehek 1983; Weiss et al, 1983; Wilson et al, 1984; Heaton et al, 1984). In this study, it was found that using intervals of zero, two and five minutes between the steps produced no significant difference in the test results which implies that CAIH could be performed at any interval between the steps (between zero time and five minutes). But using intervals of less than five minutes an extra step might be needed in order to achieve the required fall in FEV1, because the effect of CAIH may not have manifested itself at intervals of less than five minutes. It is possible therefore that unnecessary

overstimulation in some patients could result if steps are separated by less than five minutes.

In this study it was found that the maximum bronchoconstriction following CAIH was achieved at a ventilation rate of 60% of predicted IMBC, and that moderate isocapnic hyperventilation with cold air beyond three minutes produced no further bronchoconstriction. This implies that it is not necessary to challenge patients at levels of ventilation higher than 60% of predicted IMBC or for a duration of longer than three minutes. In previous studies of iscapnic hyperventilation with cold air or dry ambient temperature air, different durations ranging between two to 12 minutes have been chosen as a challenge time (Deal et al, 1980; O'Byrne et al, 1982a and 1982b; Arnup et al, 1982; Aquilina, 1983; Gillioz and Orehek , 1983; Weiss et al, 1984; Wilson et al, 1984).

The ventilation rate of 60% predicted IMBC seems to be a discriminating level between asthmatic and normal subjects. In the group of normal subjects studied the averate fall in FEV1 was 4% and no subject dropped by more than 10% at 60% predicted IMBC. In contrast, all asthmatic patients with current symptoms had FEV1 falls of greater than 10% (Chapter 3 Section 9). Thus, any fall of more than 10% in FEV1 at a ventilation rate of 60% predicted IMBC should be considered as an indication of increased airway reactivity.

In other inhalation challenges (eg. histamine), the response is usually assessed by the threshold dose (PC20) and the dose-response relationship. Responses to the ventilation step test can be evaluated in a similar way. Orehek et al (1977) have suggested that inhalation dose response curves should be studied in a pharmacological manner, in which distinction is made between sensitivity (the dose at which the reaction is initiated) and reactivity (the slope of the dose response curve beyond this point). The relevance of making this distinction derived from the suggestion that asthmatics can be distinguished from normal individuals by reactivity more than by sensitivity. However, recently it has been suggested that the determination of either the dose-response curves or the log dose-response curves and calculation of the slope provides little information and it was recommended that the bronchial provocation test results should be expressed in term of a threshold dose concentration (Cockcroft and Berscheid, 1983). Moreover, it was reported that the threshold dose of histamine (PC20) is a more useful index than the dose-response slope in characterising the clinical state of asthmatic patients (Beaupre and Malo, 1981).

It is not known what the biological significance is of either the response threshold or the slope. When a strip of muscle is exposed to increasing doses of histamine or cholinergic agents in vitro, the resulting log-dose response curve is sigmoid in shape. It has a threshold, linear slope and a maximum response (the effect

usually assessed in terms of changes in muscle tone or length). A competitive antagonist will produce a parallel shift of the dose response curve to the right (Goldstein, Aronow and Kalman, 1968). The response of the intact bronchial tree is much more complicated. It is usually measured by changes in airways calibre in terms of either airway resistance or airflow. During laminar flow in a straight tube, resistance is inversely proportional to the fourth power of the radius, it follows therefore that a constrictor stimulus will produce a larger increase in resistance in the presence of pre-existing airway narrowing. In addition in normal subjects, the major site of resistance is in the central airway (Hyatt and Wilcox, 1961; Macklem and Mead, 1967), whereas in the disease state, the major changes in calibre occur in the peripheral airways (Hogg, Macklem and Thurlbeck, 1968; Kessler et al, 1973). It is dangerous to extrapolate from the results of a non specific reactivity challenge in vivo, directly to events occurring within the bronchial muscle. Bronchial calibre is influenced not only in a complex way by bronchial muscle tone, but also may be affected by changes in the calibre of pulmonary vessels by the quantity of bronchial gland secretions and the presence of pulmonary oedema, all of which may be altered by administration of the bronchoconstrictor substance. The effect of increasing doses of bronchoconstrictor may be modified by tachyphylaxis, by local neurogenic mechanisms, or by local release of mediators.

Clinically, bronchial responsiveness to some constrictor substances

has been shown to have a clinical relevance. A correlation has been found between the threshold dose of histamine producing a fall of 20% in FEV1 (PC20) and the severity of asthma as judged by symptoms and the quantity of drug therapy required. A correlation was found between the diurnal variation in PEFR and the degree of responsiveness to the bronchodilator drugs (Ryan et al, 1982). However, it was not clear whether this simply reflects a correlation between non specific bronchial reactivity and the severity of asthma, or whether it reflects the dependence of the bronchial response on initial airway calibre.

The choice of the threshold has been generally arbitary. De Vries et al (1960) considered the dose causing a fall in FEV1 of 10% or more as a threshold dose. Orehek and Colleagues (1977) chose a 25% fall in SGAW, read off the dose reponse derived by linear regression. Other investigators have chosen a 20% fall in FEV1 as a threshold dose found by linear interpolation from the dose response curve (Cockroft et al, 1977a; Cropp et al, 1980; Hargreave et al, 1981; Beaupre and Malo, 1981).

Neigens et al (1982) chose a 15% fall in FEV1 derived by square regression of all the data points. Habib, Pare, and Engel (1979) chose that concentration of histamine responsible for a fall in baseline measurements, nearest to the two standard deviations of the mean baseline value. Michoud et al (1981) and Neigens et al (1982) considered twice the coefficient of variation of baseline

FEV1 as a threshold value.

In most of the previous studies of exercise-induced asthma, a fall of 10% in FEV1 or PEFR has been taken as an indication of increased airway reactivity (Deal et al, 1980; Jones, 1966; Anderson et al, 1979; O'Byrne et al, 1982b). With cold air there has been no agreed threshold dose. These present studies suggest that a 10% fall in FEV1 at a ventilation rate of 60% predicted IMBC should be considered as a threshold dose for the following reasons: a) the PD10 could be measured in all current asthmatics but not PD20. b) none of the normal subjects had a fall in FEV1 of 10% or more at 60% predicted IMBC; c) similar information was obtained from either PD10 or PD20 (see Chapter 3, Section 9). d) in subjects responsive to cold air, a lower ventilation rate (approximately half) is needed to achieve a 10% fall in FEV1 than 20%. This makes the test more acceptable and simpler for the patients.

The level of ventilation as a percentage of predicted IMBC was chosen as a measurement of bronchial responsiveness to cold air, rather than the absolute level of ventilation. The inhalations based on percentage IMBC provided doses of cold air proportional to lung size, therefore variations in lung size are adequately accounted for using this method. This allows better comparison in different subjects. Moreover, the results obtained with the method described above have given an indication of the dose required to discriminate asthmatics from normals. The differentiation of

asthmatics from normals is of great value in the studies of hyperreactivity to cold air. This may be difficult to achieve in terms of absolute levels of ventilation, as this could vary in different individuals.

Some workers used a multiple of actual FEV1 to determine the ventilatory rate for thier challenge (Arnup et al, 1983; Heaton et al, 1984; Wiess et al, 1984). Baseline lung function is not the critical factor which determine the bronchial responsiveness to cold air (Assoufi et al, 1985). In addition, patients with asthma have variable baseline lung function (Scadding, 1966). Therefore the use of doses of cold air based on baseline FEV1 would creat problems in reproducibility studies where doses of cold air should be the same in each test trail.

The level of increased airway reactivity to cold air can be arbitrarily divided in term of percentage IMBC into mild (40% < PD10 <60%), moderate (20% < PD10 < 40%), and marked (PD10 < 20%). This test may therefore provide an indication of the severity of asthma, It has been reported that there is a close relationship between the severity of asthma and the level of increased airway reactivity using pharmacological challenges as previously described (Makino, 1966; Cockroft et al, 1977a). The relationship of asthma severity with cold air has not been described, and should be explored using this method.

The expression of cold air responsiveness in terms of RHE in Kcal/min used by other workers (Deal et al, 1979a ; O'Byrne et al, 1982a and 1982b) needs relatively complex measurements and calculations as many factors can affect the results. In addition, it is not known how much of respiratory heat loss is needed in order to distinguish normal subjects from asthmatics.

Some investigators monitored the respiratory rate and tidal volume continuously during isocapnic hyperventilation. Others administered cold air by instructing the patient to breathe at a fixed tidal volume and rate in order to achieve a certain minute ventilations. (O'Bryne et al, 1982a and 1982b). The latter procedure needs respiratory monitoring and careful coaching of the patients. In addition, more than one investigator was required to perform the test. Results from this present study have indicated that changing the pattern of breathing has no effect on airway responsiveness; provided that a constant minute ventilation was maintained by the patient. There has been no report in the literature of the effect of pattern of breathing on airway responsiveness in asthmatics. Therefore this observation documents for the first time this characteristic feature of cold air isocapnic hyperventilation. Patients were asked to hyperventilate at a rate that maintained the size of a guide balloon. In these circumstances, the rate of ventilation is equal to the rate of delivery of air from the cylinder. It is unnecessary therefore to monitor continuously the rate and depth of respiration in order to

achieve a given effect making the technique simpler. Thus, unlike the pharmacological bronchial provocation tests, breathing pattern is not a variable which needs to be controlled when isocapnic hyperventilation is applied as a test for airway reactivity (Strohl et al, 1981).

It is important to keep the patient isocapnic during hyperventilation and this can be achieved by adding sufficient amounts of carbon dioxide to the inspired air. In the laboratory, end tidal CO2 can be continuously monitored to keep the subject at a resting eucapnic level. This procedure requires the availability of a capnograph or similar instrument. It is sometimes difficult to provide continuous monitoring during CAIH procedure, especially in the field. The carbon dioxide requirement (L/min) at different levels of ventilation was calculated and expressed graphically. This allows the inspired CO2 concentration to be preset, eliminating the need for continuous monitoring. It is interesting to note that there was little individual variation in the amount of carbon dioxide added at a fixed ventilation rate in different individuals. The use of a carbon dioxide requirement graph reduces the cost of equipment and makes the technique easier.

In this method, the guide-balloon was placed near the inspiratory port of the exchanger and the subjects were instructed to breathe at a predetermined ventilation rate by maintaining its size half-full. Accurate variable flow could be obtained without the

need for a vaccuum pump. The flow of dry air from the cylinder could be adjusted with stopcock and measured by a rotameter. In other systems of cold air isocapnic hyperventilation, the guide-balloon was located downstream of the subject and it was continuously evaluated by a vaccuum pump (Deal et al, 1979a; O'Byrne et al,1982a; Wodrow Weiss et al, 1983; Aquilina, 1983).

Cold air is more effective in provoking bronchoconstriction than dry air at room temperature. In recent years, isocapnic hyperventilation with ambient air (Kivity and Souhrada, 1981), dry ambient air (Strauss et al, 1978; Tweddale, Godden and Grant, 1981; McFadden et al, 1982; Wilson et al, 1983 ;Denjean et al, 1983) and dry, subfreezing air (Deal et al, 1979a; Breslin et al, 1980; O'Cain et al, 1980; Deal et al 1980a; O'Bryne et al, 1982; Bar-Yishay, Ben-Dov and Godfrey, 1983; Heaton et al, 1983; Nagakura et al, 1983 Tal et al, 1984; Heaton et al, 1984), have been used to elicit bronchoconstriction. In the present study, in the asthmatics, isocapnic hyperventilation with dry air at room temperature induced about two thirds of the airflow obstruction obtained with dry, subfreezing air. Higher ventilation rates were needed in some patients to obtain a similar obstructive response with dry room temperature air to that obtained with dry subfreezing air. Hyperventilation at a higher level may be difficult to achieve in some patients with airways obstruction. In these circumstances cooling of the air may have an additional value in increasing the sensitivity of the test at a lower ventilation rate.

Deal et al, (1979), showed that isocapnic hyperventilation with dry air at ambient temperature caused about 50% and 70% of the airflow obstruction elicited by air at subfreezing temperature when minute ventilation was about 45 and 65 L/min respectively. Therefore, isocapnic hyperventilation was about 45 and 65 L/min respectively.

The efficiency of the CAIH test was investigated by examining the reproducibility of the response. Its responsiveness was also compared to that of other standardised tests of reactivity. Cold air isocapnic hyperventilation was shown to have good reproducibility when it was repeated within a week. Two patients did not react to CAIH on one visit. The unresponsiveness to the test on these visits may be a reflection of altered reactivity of the patient's airways rather than an inadequacy in the CAIH challenge. O'Byrne et al, 1982a; Aquilina 1983; Heaton et al, 1984; found that cold air responsiveness was highly reproducible when it was repeated on two occasions. There is no data available on the reproducibility of isocapnic hyperventilation with cold air on more than two occasions. Tweeddale, Godden and Grant (1981) found a coefficient of variation of 18% in nine subjects who performed a single exposure to isocapnic hyperventilation with dry air repeated on three occasions. Comparing the reproducibility of isocapnic hyperventilation with that of exercise and pharmacological provocational tests, Anderson, Connolly and Godfrey (1971) calculated the coefficient of pairs of treadmill exercise carried out two hours apart and found it to be 25%, while the

variation in bronchial liability reported by Jone (1960) was 20%. The effect of a single dose of non specific bronchoconstrictors are reproducible in asthmatic subjects. De Vries et al (1962) estimated the histamine threshold (the dose of histamine producing a fall in FEV1 or the vital capacity of 10% or more), in 17 asthmatics on five successive days, and found it to be reproducible in 49 of 68 occasions. Woenne et al (1978) reported that the dose producing a 20% fall in baseline FEV1 (PD20) for histamine and methacholine was reproducible in ten asthmatic subjects. However, no statistics were given and there was a threefold difference in PD20 FEV1 in two subjects. Using a paired t-test, Casterline and Evans (1977) showed that in a group of nine asthmatics, the mean of PD20 FEV1 for histamine was not significantly different on different days, despite threefold and tenfold differences in repeated PD20 FEV1 in two of the subjects. In carefully controlled conditions, Juniper and colleagues (1978) found that in a mixed group of normal and asthmatic subjects the provocative dose of histamine and methacholine to produce a 25% fall in FEV1 was highly reproducible (coefficients of determination 0.994 and 0.990 respectively). Whereas Graham, Heim and Constantine (1967) concluded from an earlier small study that the reproducibility of response to carbachol was better in normal subjects than in asthmatics and that results in asthmatics were sometimes dependent on basal bronchial tone.

Cold air isocapnic hyperventilation responsiveness was compared

with that of histamine and exercise challenges in groups of patients with asthma, allergic rhinitis and normal subjects. All asthmatics with current symptoms responded to cold air inhalation. This was in agreement with observations of other workers (Deal et al, 1980a; O'Byrne, 1982a). Histamine and exercise were less able to distinguish asthmatics from normal subjects because there was a considerable overlap in individual responses. In addition, histamine inhalation challenges were more often associated with flushing and headaches which were seen primarily in the normal subjects and patients receiving high doses of this agent. These side effects have been reported by other workers (Hargreave et al, 1981, Orehek and Gayrard, 1976).

The responsiveness to cold air correlated with responsiveness to both exercise and histamine challenges. This finding extends previous work that non specific reacitivity is an important feature of asthmatic patients (O'Bryne et al, 1982b). However, some subjects are more responsive to one stimuli than the other. This suggests that there might be an additional factor besides intrinsic reactivity involved in the development of bronchoconstriction to different challenges. This might be due to the fact that the degree of exercise-induced bronchospasm could not be controlled. In addition, we did not match the level of ventilation and inspired air conditions in both tests, we used subfreezing air for isocapnic hyperventilation and ambient conditions during exercise tests. The response to CAIH was very well controlled by using predetermined

levels of ventilation and more reproducible results were obtained as the temperature and humidity delivered was constant. The variability of response to histamine due to inconstant nebulizer output, particle size and unpredictable drug deposition (Thomson and Pavia 1974; Ruffin et al, 1978), may have influenced the results in some patients. However, some other factors may also be involved.

There is little data available on comparison of isocapnic hyperventilation with other standard reactivity tests and reported results are conflicting. Kivity and Souhrada (1981) studied the bronchial responsiveness to methacholine and voluntary isocapnic hyperventilation with room air in five normal subjects and eleven patients. They concluded that methacholine was less sensitive than isocapnic hyperventilation with room air and that there was no correlation between the bronchial responsiveness to the two inhalation challenges. Chatham et al (1982) examined the relationships between responsiveness to methacholine, histamine and respiratory heat loss in exercise in asthmatics, and found that the correlation between the two pharmacological challenges was good but that the correlation with exercise, ventilation and respiratory heat loss was rather poor. In contrast, O'Byrne et al, 1982b; Woodrow Weiss, Rossing and McFadden (1983), reported a good relationship between isocapnic hyperventilation with cold air and methacholine challenge in the group of asthmatics studied. Aquilina (1983); Heaton et al (1984) found a good relationship

between CAIH and methocholine and histamine challenges. Further studies are needed to explore the relationship between different bronchoprovocation tests.

In patients with allergic rhinitis, CAIH produced a drop of more than 10% in only three subjects who had occasional episodes of wheezing. These observations are similar to those of Deal and his colleagues who found that only subjects with hay fever who had histories of episodes of wheezing developed changes in pulmonary mechanics (Deal et al, 1980) following challenge.

The non-technical factors that might influence the responses were controlled in all the studies, and included the time of day (De Vries et al, 1962); respiratory infection (Empey et al, 1976), degree of initial air flow obstruction (Benson, 1975); smoking and medication (Makino, 1966), allergen exposure (Cockroft et al, 1977b; Chai et al, 1975), or vaccine (Qullette and Reed, 1965). Variation in the influences of these factors may alter bronchial responsiveness.

FEV1 was used in measurement of airway responsiveness to cold air. Changes in airway calibre can be measured by using several techniques (Widdicombe and Nadel, 1963 ; Stein et al, 1966; Mitchell, Watanabe and Renzetti, 1967; Payne et al, 1967). The direct measurement of airway resistance is a good method, but the facilities for such measurements are not widely available and it is

unlikely that significant changes in these more sophisticated indices would not also be reflected in changes in spirometric measurements. Simple, familiar, readily available and sufficiently accurate information is provided by the standard spirometric indices especially FEV1.

There were no unpleasant side effects of using cold air, although some subjects complained of dryness of the mouth. At a higher ventilation rate, the first few breaths induced coughing in some subjects but this quickly abated. No severe reaction was observed. The duration of the CAIH testing was shorter than pharmacological challenges. It was not affected by extraneous factors such as particle size, pattern of breathing, site of deposition in the lung and retained dosage, all of which are known to affect the responsiveness to pharmacological materials (Ruffin et al, 1978; Thomson and Pavia, 1974). Cold air iscocapnic hyperventilation was better controlled than exercise and more reproducible results could be obtained.

The simplified standard method described can be readily employed in the measurement of bronchial reactivity, as a quantatitive test for asthma in the routine pulmonary function laboratory for clinical and research studies. It can be used for the same purposes as other hyper-reactivity tests. For example, it may be used to diagnose the presence of asthma, in the assessment of cases of unexplained dyspnoea or in cases where the diagnosis is uncertain

and spirometric tests fail to show airflow obstruction. It could be used to examine the effect of medication, to provide an index of risk to non specific stimuli in the environment, in the followup of asthma patients over a period of time and to explore the mechanisms of hyperreactivity. As it is portable, it can be used in epidemiological surveys in industry, in the general population and in pre-employment screening in industry. Since cold air is a physiological stimulus there is no ethical problem in using it on apparently healthy individuals. There can be a reluctance of management and employees to accept tests requiring the administration of a 'drug' especially when extremely sensitive workers might have significant responses.

The development of the simplified standard CAIH procedure may provide a new insight into the pathphysiology of airway obstruction, since the hyperreactivity of different individuals could easily be measured and compared quantatively using a natural stimulus. This will advance the understanding of obstructive lung disease and facilitate the development of therapeutic intervention.

CHAPTER FOUR

STUDIES ON THE BRONCHOPROVOCATIVE CHARACTERISTICS OF COLD AIR

STUDIES ON THE BRONCHOPROVOCATIVE CHARACTERESTICS OF COLD AIR

INTRODUCTION

Two important features of cold air inhalation have been studied. These were the cumulative effect and the refractory period. A cumulative effect has been observed with some pharmacological materials, like methacholine and carbachol but not with histamine (Orehek and Gayrard, 1976; Juniper et al, 1978; Orehek, 1980). The refractory period is a characteristic of exercise-induced asthma and to a lesser extent following challenge with pharmacological materials like histamine. Several investigators have demonstrated that, in some patients, when exercise tests are repeated at intervals of less than 2 hours, there is a diminishing bronchoconstrictor response to successive tests. McNeill et al (1966) suggested that repeated exercise challenges at intervals of 45 minutes progressively diminished the post exercise increases in airways resistance in patients with asthma. Jame, Faciane and Sly (1976) confirmed this in a study of ten asthmatics who performed multiple walking tests, at intervals of one hour. Edmund, Tooley and Godfrey (1978) reported a refractory period after exercise-induced asthma in eight asthmatic patients who performed running exercise tests 30 minutes apart. It was suggested by these authors that the reason for this diminished response was a depletion in stores of mediators potentially capable of inducing bronchoconstriction occurring after the initial exercise stimulus.

They also suggested that a time interval might be required to replenish these mediators.

Schoeffel et al (1980) studied the effects of repeated exercise and histamine challenges in a group of asthmatic patients. Twenty nine patients performed three exercise tests, and on a separate day, 16 of these had three histamine inhalation challenge tests. The challenges were separated by 40 minutes. The degree to which a refractory period was induced by repeated exercise challenge was variable. By contrast, the bronchoconstrictor response to repeated challenge with histamine remained relatively constant in most patients. Some of the patients were refractory to the same dose of histamine, after a third challenge. These authors suggested that depletion of pharmacological mediators, rather than a refractory state of the bronchial smooth muscle was one of the possible mechanisms of exercise-induced asthma.

The observation that running causes more bronchospasm than swimming has been extended to show that isocapnic hyperventilation with cold air, will produce bronchospasm, whereas, exercise if performed while inspiring warm, humid air does not (Weinstein et al, 1976; Strauss et al, 1977; Chen and Horton, 1977; McFadden et al, 1982; Lee, Assoufi and Kay, 1983). These observations have led to the hypothesis that exercise-induced asthma is due to respiratory heat exchange across the bronchial mucosa. This hypothesis can now be tested using some features of exercise-induced asthma. One such

feature is the refractory period. Comparing exercise with isocapnic hyperventilation, Stearn et al (1981) found that repetitive exercise tests at short intervals produced a diminution in the obstructive response within a 30 minute interval, whereas the degree of airflow obstruction after isocapnic hyperventilation remained constant over the same period of time. Recovery after exercise was complete after two hours. Further studies have been carried out by Ben-Dov, Bar-Yishay and Godfrey (1982). These authors performed exercise tests with warm humid air. Exercise under these conditions did not alter the FEV1 but rendered 12 out of 15 patients refractory to a second exercise challenge performed breathing cold, dry air, thus suggesting that exercise per se is responsible for the refractory period.

A refractory period following cold air isocapnic hyperventilation has been reported in seven of nine asthmatics studied by Bar-Yishay, Ben-Dov and Godfrey (1983), and in one of the seven patients of Tal et al (1984). However, Heaton et al (1983) were unable to demonstrate a refractory period following CAIH.

In the present study, the cumulative effect and refractory period characteristics of cold air isocapnic hyperventilation are investigated systematically.

CHAPTER FOUR

SECTION ONE

STUDIES ON THE POSSIBLE CUMULATIVE EFFECT OF COLD AIR INHALATION

STUDIES ON POSSIBLE CUMULATIVE EFFECT OF COLD AIR INHALATION

This study was carried out in two parts :

PART 1 : COMPARISON OF STEPWISE TEST WITH SINGLE CHALLENGE

AIM

In order to find whether progressively increasing exposures to CAIH produced a cumulative effect, we have compared the response to stepwise challenges at increasing rates of ventilation with that of a single challenge at the ventilation rate used in the final stage of the stepwise test.

METHOD

This study was carried out on two visits.

On visit one, a stepwise test was performed at an increasing ventilation rate (10, 20, 40, 60 and 80% of predicted IMBC, as described before (Chapter 3, Section 5)). The duration of each challenge was three minutes and the interval between the steps was five minutes. The purpose of this visit was to determine the dose of cold air in terms of ventilation rate capable of inducing a 15-20% fall in FEV1.

On visit two, following a satisfactory baseline value, single

exposure at a ventilation rate equal to the final step of stepwise test was performed. The duration of the challenge was for three minutes. Following each of the challenges, FEV1 was recorded at 0.5, two and five minutes and every five minutes for up to 20 minutes. The study was carried out on 15 patients with asthma. The visits were separated by less than ten days.

RESULTS

The individual characteristics and baseline lung function of the subjects who participated in the study are shown in Table 4:1:1. The inspired air conditions were not different on either testing days (Table 4:1:2). There was a good relationship between the maximum percentage fall in FEV1 following stepwise challenge, performed at increasing ventilation rates with that of single exposure given at the final ventilation rate of the stepwise test ($r = 0.83$ $p < 0.001$) (Figure 4:1:1). There was no significant difference in the mean values obtained on both occasions. There was no difference in the rate of recovery following either challenge (Figure 4:1:2 and 4:1:3).

PART 2: INFLUENCE OF DIFFERING TIME INTERVALS BETWEEN EQUAL EXPOSURES

AIM

To investigate the possibility of a cumulative effort. Further, we have studied the effect of equal exposures to CAIH repeated at

Table 4:1:1

Individual characteristics of subjects tested for comparison of a stepwise test with single challenge of CAIH.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	M	41	183	75	4·00	97·8
2	M	55	172	76	1·81	56·0
3	M	20	192	74	3·70	76·0
4	M	23	169	80	4·03	100·0
5	F	24	173	68	3·52	100·6
6	M	31	183	88	3·74	85·6
7	M	49	170	69	2·14	64·8
8	M	57	176	70	3·00	89·3
9	F	24	165	68	3·82	119·0
10	M	59	169	73	2·72	90·1
11	M	43	174	85	2·78	76·0
12	M	19	170	48	2·25	54·7
13	M	32	179	68	4·04	97·7
14	F	18	170	65	2·74	79·7
15	M	43	173	70	3·15	86·1
Mean		35·9	174·5	71·8	3·16	84·9
SD		14·61	7·05	9·29	0·74	17·6

Table 4:1:2

Minute ventilation (% IMBC) and inspired air temperature (^0C) for the patients who participated in comparison with stepwise test with single exposure of CAIH.

Subject	Minute ventilation (% IMBC)	Inspired temperature (^0C)	
		Stepwise test	Single challenge
1	40	-14·0	-13·5
2	40	-16·5	-12·0
3	40	-11·0	-13·0
4	40	-10·0	-11·0
5	60	-14·5	-9·0
6	40	-15·0	-16·0
7	40	-13·0	-13·9
8	60	-11·0	-15·5
9	60	-11·0	-13·0
10	60	-9·5	-11·0
11	60	-14·8	-11·5
12	20	-13·0	-16·0
13	40	-16·0	-11·0
14	40	-17·5	-13·0
15	20	-9·0	-14·0
Mean	44	-13·1	-12·9
SD	13·52	2·69	2·02

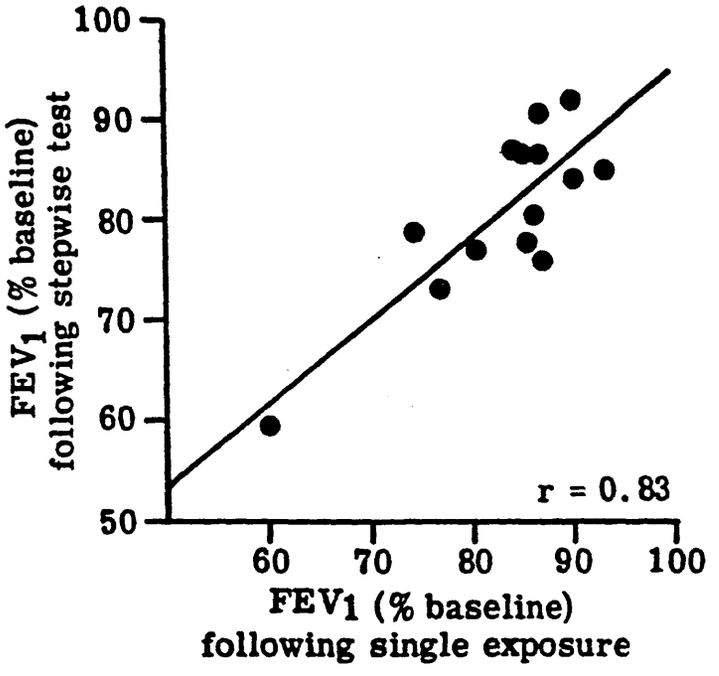


Figure 4:1:1 Correlation between the maximum fall in FEV1 achieved following a stepwise test performed at increasing ventilation rates, with the fall following a single exposure, performed at the final step of the stepwise test.

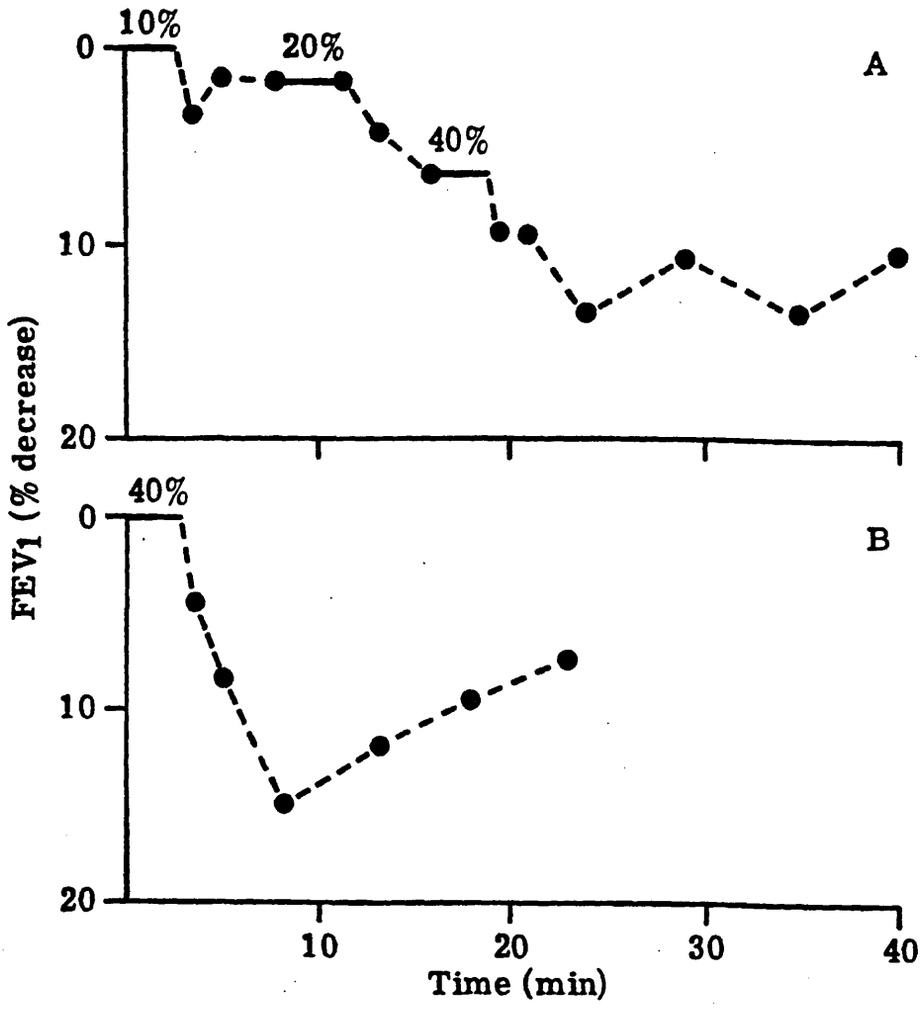


Figure 4:1:2 Changes in FEV1 (percentage of the baseline) following inhalation of subfreezing dry air in a single subject A) a stepwise test at increasing ventilation rates was given serially at 10, 20, 40, 60 and 80% of predicted IMBC. The inhalation time was for three minutes and the interval between exposures was five minutes. Inhalation was stopped when a 15-20% fall in FEV1 had been achieved; B) single exposure at a ventilation rate equal to the final step of the stepwise test.

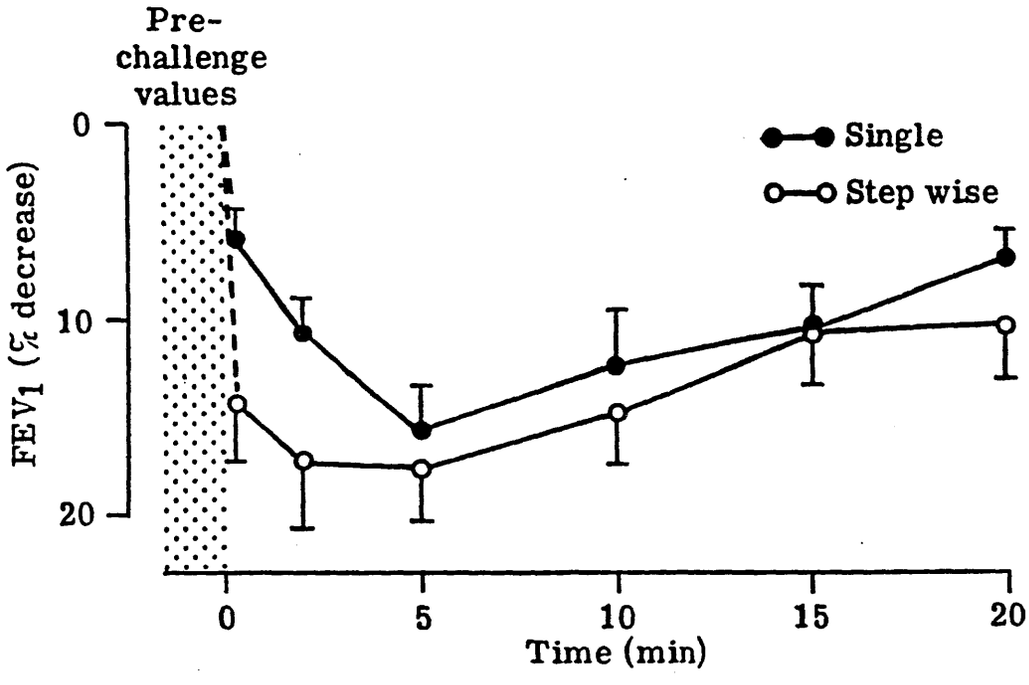


Figure 4:1:3 Changes in FEV1 [percentage of baseline (Mean ± SEM)] following a single challenge and stepwise test with subfreezing dry air in 15 asthmatics.

short intervals.

METHOD

This study was carried out on five asthmatic patients on three visits. On visit one, a stepwise test with increasing ventilation rates (10, 20, 40, 60 and 80% of predicted IMBC to determine the dose of cold air required for a 10-15% drop in FEV1) was performed as described in Chapter 3, Section 5. The duration of the exposures was for three minutes and the intervals between them were five minutes. On visits two and three, following a satisfactory baseline FEV1 value, cold air was inhaled three times at a ventilation rate determined on visit one as the rate producing a 10-15% drop in FEV1. The duration of each exposure was for three minutes and the interval between the exposures was ten minutes and five minutes respectively. The last two visits were randomised. FEV1 was measured at 0.5, 2 and 5 minutes following each challenge and when there was ten minutes between the exposures; measurements of FEV1 were also performed at ten minutes. Following the final challenge at each visit, the FEV1 was recorded at 0.5, 2, 5, 10, 15 and 20 minutes.

RESULTS

The individual characteristics and baseline lung function of the subjects who performed the study are shown in Table 4:1:3. The inspiratory air conditions were similar for each exposure in the three visits (Table 4:1:4). There was no significant difference in

Table 4:1:3

Individual characteristics and baseline lung function for patients tested for possible cumulative effect of CAIH.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	M	33	177	70	4·15	103·2
2	F	35	166	56	2·14	75·2
3	M	55	172	76	1·83	56·7
4	M	43	183	80	3·52	88·4
5	M	41	183	75	3·99	97·6
Mean		41·4	176·2	71·4	3·13	84·2
SD		8·65	7·33	9·32	1·07	18·7

Table 4:1:4

Minute ventilation (% IMBC) and inspired air temperature (^{0}C) in each exposure of the subjects tested for possible cumulative effect

Subject	Minute Ventilation % IMBC	Inspired temperature ^{0}C 10 min between exposures 1st Exp	2nd Exp	3rd Exp	5 min between exposures 1st Exp	2nd Exp	3rd Exp
1	40	-14·5	-13·0	-17·2	-16·5	-14·0	-15·5
2	40	-15·0	-16·5	-14·2	-17·5	-16·2	-14·0
3	40	-12·5	-16·5	-13·6	-14·5	-15·6	-17·0
4	40	-14·5	-17·0	-13·6	-17·5	-16·0	-14·2
5	40	-13·6	-16·5	-15·0	-14·6	-16·6	-13·5
Mean		-14·0	-15·9	-14·7	-16·1	-15·7	-14·8
SD		0·99	1·64	1·50	1·49	1·0	1·42

Water content was zero as dry air was used

the baseline FEV1 on the two visits. The maximum drops in FEV1 following the first exposure on each of the two visits were 16.1 $\pm$ 2.43; and 18.6 $\pm$ 2.93 (Mean $\pm$ SEM). This difference was not significant. The second and third exposure on each visit using ten and five minutes between exposure produced no significant fall in FEV1 (Figure 4:1:4 and Table 4:1:5). The lowest post challenge FEV1 remained constant following each of the challenges and was unaffected by the time interval used. The changes in FEV1 following the three inhalations on both visits, are shown in Figure 4:1:5.

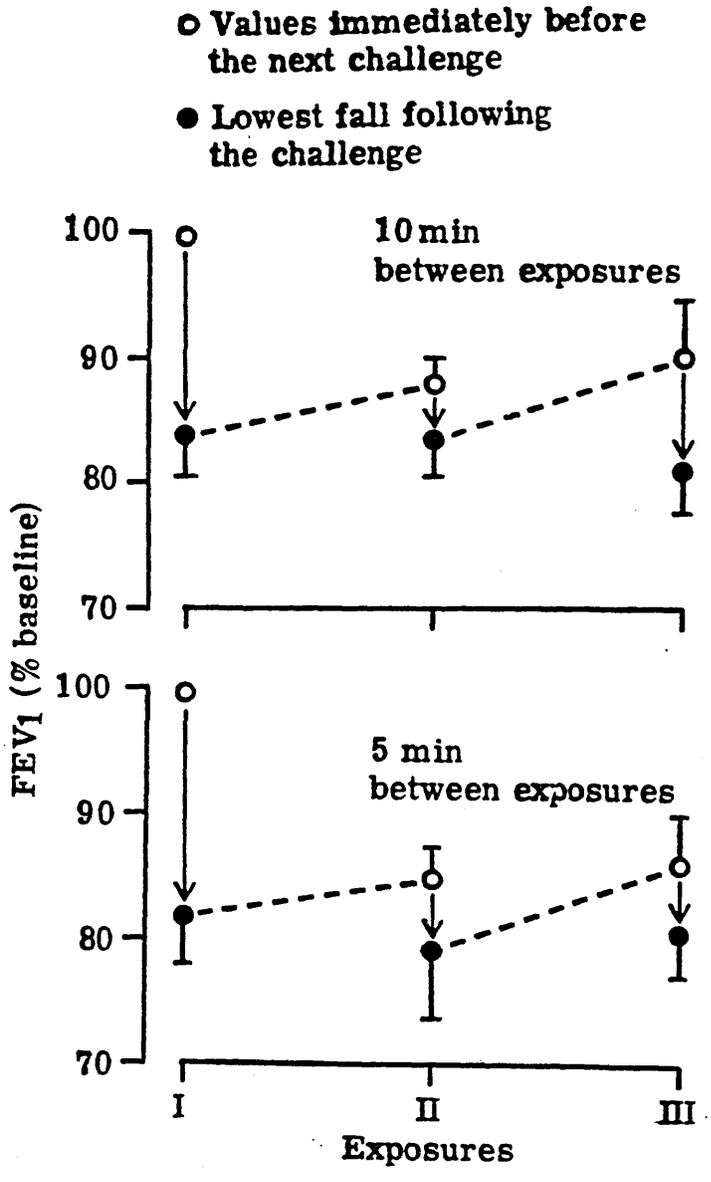


Figure 4:1:4 Maximum percentage fall in FEV1 (Mean ± SEM) following three equal inhalations of cold dry air, in five asthmatics. The periods of exposure was three minutes. Intervals between the exposures were ten minutes and five minutes.

Table 4:1:5

Prechallenge (P) and lowest post challenge (M) fall in FEV_1 expressed as percentage of baseline following three equal exposures of cold air (Ten and five minutes between the exposures)

	Ten min between exposures						Five min between exposures					
	1st exp		2nd exp		3rd exp		1st exp		2nd exp		3rd exp	
Subject	P	M	P	M	P	M	P	M	P	M	P	M
1	100·0	92·0	94·4	91·5	95·9	91·6	100·0	91·3	93·6	92·7	98·8	89·4
2	100·0	77·1	83·1	77·1	77·1	74·2	100·0	81·8	81·8	79·6	79·6	81·8
3	100·0	84·6	84·6	83·0	95·6	85·7	100·0	81·3	81·3	81·9	90·3	85·5
4	100·0	81·5	91·0	84·0	96·3	75·6	100·0	73·0	80·0	69·0	78·8	76·9
5	100·0	83·5	86·2	81·4	84·9	78·1	100·0	79·6	85·0	74·6	82·7	74·8
Mean	100·0	83·7	87·9	83·4	90·0	81·0	100·0	81·4	84·3	79·6	86·0	81·7
SD	0	5·43	4·71	5·24	8·64	7·39	0	6·56	5·49	8·86	8·46	6·01

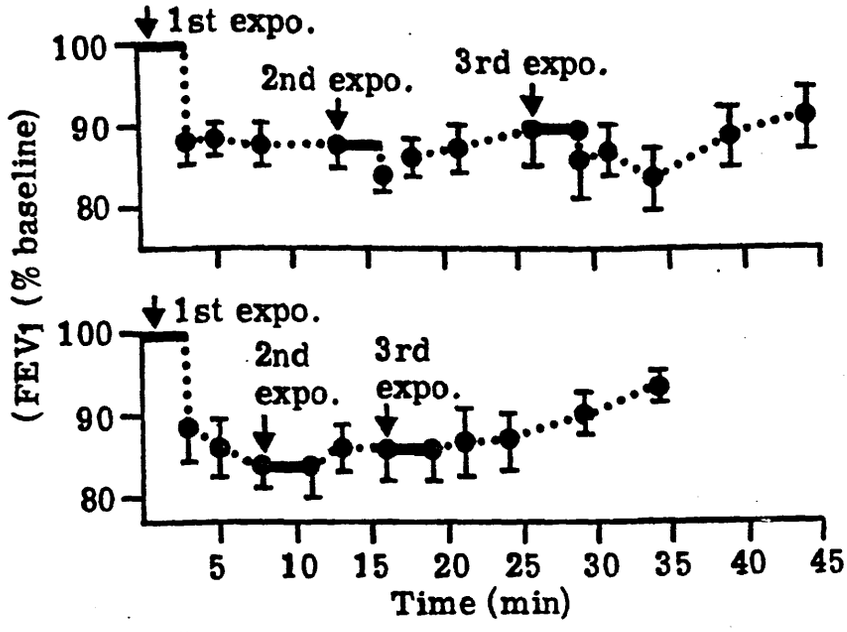


Figure 4:1:5 Changes in FEV1 [percentage of baseline (Mean + SEM)] following three equal inhalations of cold, dry air in five asthmatics. The exposures were of three minutes duration and the interval between the exposures were ten minutes and five minutes.

CHAPTER FOUR

SECTION TWO

STUDIES ON THE POSSIBLE REFRACTORY PERIOD FOLLOWING COLD AIR INHALATION

STUDIES ON THE POSSIBLE REFRACTORY PERIOD FOLLOWING COLD AIR INHALATION

AIM

The aim of this study was to determine whether a refractory period follows asthma induced by CAIH.

METHOD

This study was carried out on two visits. On visit one, a stepwise test with subfreezing air was performed to determine the ventilation rate needed to produce a 15-20% fall in FEV1 as described before (3:4). The duration of exposure was for three minutes and the intervals between the exposures were for five minutes. On visit two, following a satisfactory baseline value, two equal threshold doses were given for three minutes each, at a ventilation rate determined on visit one. Different time intervals between the exposures were used as follows: group 1 - 15 minutes; group 2 - 30 minutes; group 3 - one hour. Each time interval was tested on seven asthmatic patients. Following the first exposure, FEV1 was measured at 0.5, 2, five minutes and then every five minutes for 15 minutes in the first group and for 30 minutes in the second group. In the third group, measurement of FEV1 was continued every ten minutes for 60 minutes. Following the second challenge, FEV1 was measured at 0.5, 2, five minutes and then every five minutes for up to 20 minutes. The maximum values following each challenge were recorded and expressed in terms of predicted

values.

RESULTS

The individual characteristics and baseline lung function of the subjects who performed the refractory period studies are shown in Table 4:2:1. There was no significant difference in the inspired air conditions in the paired challenges in each group (Table 4:2:2). There was no significant difference in the baseline FEV1 values in the different groups studied (Table 4:2:3). The prechallenge values for the second cold air challenge were significantly lower than when the interval between the challenges was 30 minutes ($P < 0.05$), indicating that some subjects were not fully recovered from the first challenge. However, when the interval between the challenges was extended to one hour, all subjects were completely recovered from the first challenge before the second challenge and prechallenge values were not significantly different. In each group, the maximum fall in FEV1 following the second challenge was not significantly different (Figure 4:2:1) from the maximum fall in FEV1 following the first challenge. The changes in FEV1 (% predicted) following the two exposures in each of the three groups are shown in Figure 4:2:2.

Table 4:2:1

Individual characteristics and baseline lung function of patients participating in the refractory period experiment.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
Group 1						
1	M	43	173	70	2·88	78·7
2	M	43	174	85	2·69	73·5
3	M	24	148	51	1·91	54·3
4	F	28	179	85	2·39	67·1
5	M	49	170	69	2·31	70·0
6	F	20	170	65	2·78	80·8
7	F	32	160	69	2·92	84·8
Mean		34·1	167·7	70·6	2·44	72·7
SD		10·98	10·44	11·80	0·46	10·21
Group 2						
1	M	43	173	70	3·26	89·1
2	M	22	175	75	3·08	72·6
3	F	24	174	85	3·77	103·0
4	M	32	179	68	3·95	95·1
5	M	55	172	76	1·75	54·2
6	F	20	170	65	2·15	62·5
7	M	50	174	70	2·75	79·5
Mean		35·1	173·9	72·7	2·96	79·4
SD		14·2	2·79	6·63	0·81	17·63

cont...

Table 4:2:1 cont..

Individual characteristics and baseline lung function of patients participating in the refractory period experiment.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
Group 3						
1	M	55	177	82	3·34	91·7
2	M	20	172	65	4·18	97·9
3	M	55	172	76	1·73	53·6
4	M	43	183	80	2·38	58·9
5	M	43	168	76	2·58	75·6
6	F	20	170	65	2·60	74·6
7	M	21	178	70	4·41	88·9
Mean		36·7	174·3	73·4	3·03	77·3
SD		16·09	5·25	6·88	0·99	16·72

Table 4:2:2

Minute ventilation (% IMBC) and inspired air temperature (^0C) during repetition of CAIH challenge on two occasions using various time intervals between the exposures: group 1, 15 minutes; group 2, 30 minutes; group 3, 60 minutes.

Subject	Minute Ventilation (% IMBC)	Inspired temperature (^0C)	
		1st exposure	2nd exposure
Group 1			
1	20	-11	-15
2	60	-12	-14
3	40	-14	-9
4	40	-16	-14
5	60	-16	-13
6	40	-17·5	-12·5
7	40	-11	-9·5
Mean	42·9	-13·9	12·4
SD	13·8	2·65	2·32
Group 2			
1	20	-12	-15
2	40	-14·5	-14
3	60	-15	-9
4	60	-13	-14
5	40	-14	-13
6	40	-15	-12·5
7	60	-12	-9·5
Mean	45·7	-13·6	-12·4
SD	15·12	1·31	2·32

cont...

Table 4:2:2 cont....

Minute ventilation (% IMBC) and inspired air temperature (^{0}C) during repetition of CAIH challenge on two occasions using various time intervals between the exposures: group 1, 15 minutes; group 2, 30 minutes; group 3, 60 minutes.

Subject	Minute Ventilation (% IMBC)	Inspired temperature (^{0}C)	
		1st exposure	2nd exposure
Group 3			
1	60	-10	-11
2	60	-14	-13·0
3	40	-16	-17·0
4	40	-15	-13·5
5	60	-14	-14
6	40	-15	-16·0
7	60	-16·5	-12
Mean	51·4	-14·4	-13·8
SD	10·7	2·14	2·12

Table 4:2:3

Prechallenge and lowest post challenge FEv_1 as a percent of expected values for paired CAIH test. Various time intervals between the exposures were used:- Group 1, 15 min: Group 2, 30 min: Group 3, 60 min.

	1st exposure		2nd exposure	
Subject	prechallenge	maximum fall	prechallenge	maximum fall
Group 1				
1	78·7	75·4	75·4	63·1
2	73·5	56·3	63·7	58·7
3	54·3	48·2	52·2	47·5
4	67·1	54·2	70·2	55·6
5	70·0	63·0	67·9	64·8
6	80·8	70·9	73·8	64·8
7	84·4	69·4	69·4	66·7
Mean	72·7	62·6	67·5	60·2
SEM	3·83	3·74	2·93	2·57
Group 2				
1	89·1	71·9	85·5	83·6
2	72·6	53·5	59·7	65·8
3	103·0	89·3	103·8	91·8
4	95·1	81·9	90·8	80·7
5	54·2	48·0	52·6	48·9
6	62·5	49·4	55·2	50·0
7	79·5	67·1	71·6	65·6
Mean	79·4	65·9	74·2	69·5
SEM	6·66	6·15	7·44	6·28

cont...

Table 4:2:3 cont...

Prechallenge and lowest post challenge FEv_1 as a percent of expected values for paired CAIH test. Various time intervals between the exposures were used:- Group 1, 15 min: Group 2, 30 min: Group 3, 60 min.

Group 1 Subject	1st exposure		2nd exposure	
	prechallenge	maximum fall	prechallenge	maximum fall
Group 3				
1	100·0	85·6	95·8	83·8
2	97·8	83·1	93·2	83·6
3	53·6	44·9	53·6	45·2
4	58·9	49·5	59·4	49·5
5	75·0	65·4	75·3	61·3
6	74·9	44·6	75·8	57·9
7	98·9	87·2	100·4	89·0
Mean	79·9	65·8	79·1	67·2
SEM	7·35	7·40	6·89	6·79

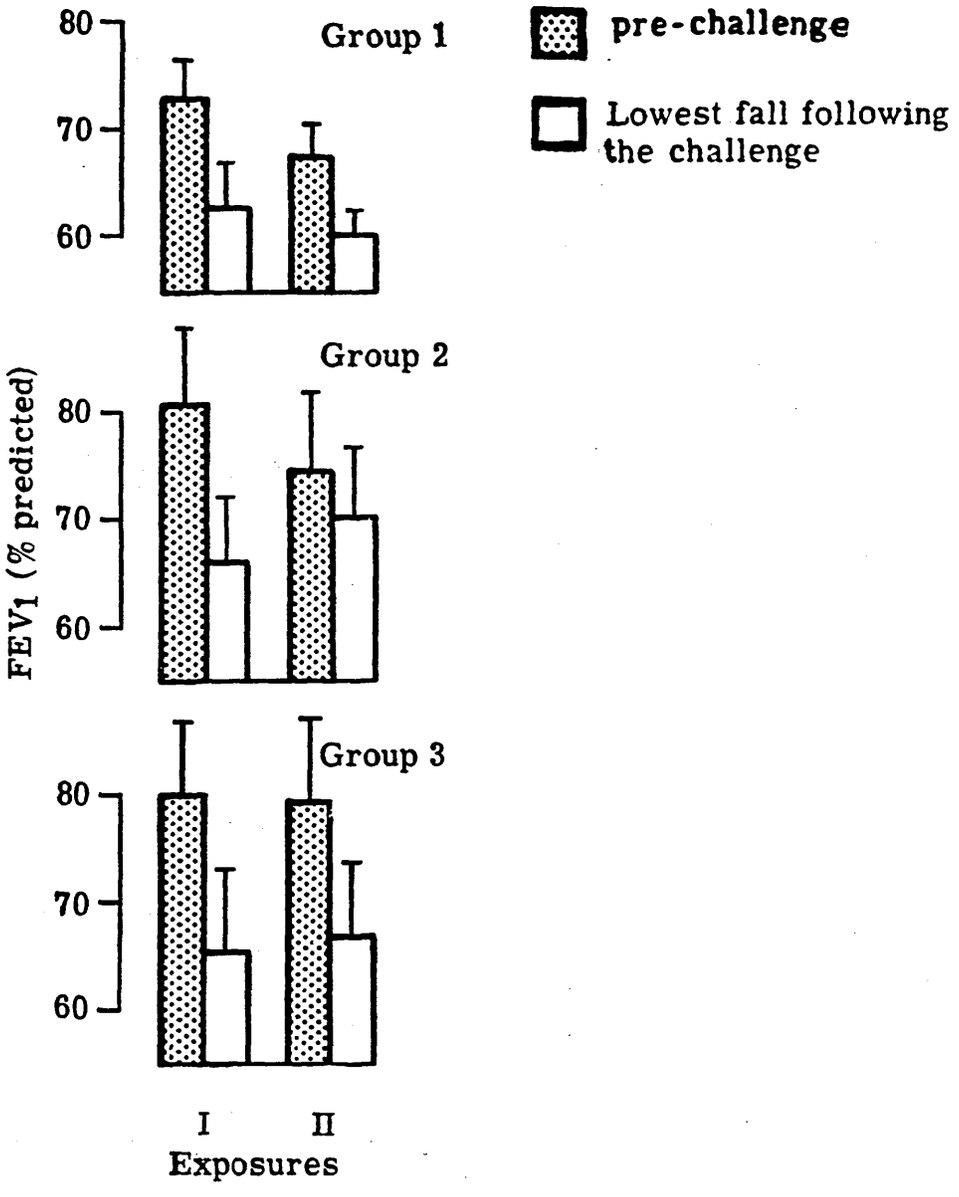
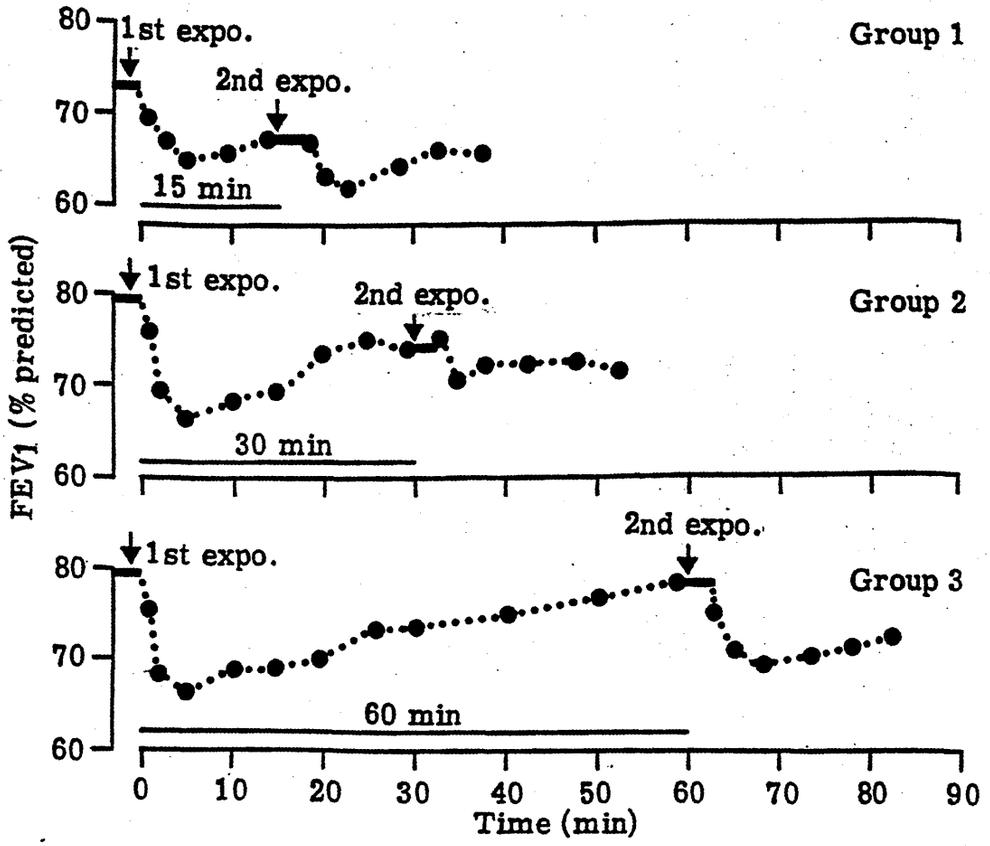


Figure 4:2:1 The pre-challenge FEV1 and maximal fall in FEV1 [percentage of predicted (Mean + SEM)] following two equal inhalations with subfreezing dry air at different intervals between exposures. Group 1: 15 minutes, Group 2: 30 minutes, Group 3: 60 minutes.



Maximum value for the standard error for each mean:

Group 1 = 4.2%, Group 2 = 7.3%, Group 3 = 7.6%

Figure 4:2:2 Mean changes in FEV1 (percentage of predicted) following two equal inhalations of subfreezing dry air at different intervals between the exposures. Group 1: 15 minutes; Group 2: 30 minutes; Group 3: 60 minutes. The duration of each exposure was three minutes.

CHAPTER FOUR

DISCUSSION

DISCUSSION

A cumulative effect has been observed following repeated challenge with increasing doses of pharmacological bronchoconstrictors such as methacholine, carbachol, and histamine Orehek et al, 1977; Juniper et al,1978; Orehek, 1980; Tremblay et al, 1984. The present study which is the first to investigate this aspect of bronchial reactivity using cold air as the provocative stimulus, demonstrates that repeated exposure to CAIH is not associated with a cumulative effect. In this study it was found that the same obstructive response was produced by a stepwise test as that following a single exposure at the ventilation rate used in the final stage of the stepwise test. It is nevertheless important to employ a stepwise test when assessing airways reactivity in order to avoid unnecessary overstimulation in individuals with more hyperreactive airways, and to evaluate the dose-response relationship. It was also found in this study that the lowest post-challenge FEV1 remained constant following exposures repeated after an interval of five or ten minutes. These exposures were made at a ventilation rate (40% predicted IMBC) capable of producing a fall in FEV1 of 10 - 15%, whereas it has been shown previously that a ventilation rate of 60% is required to produce a maximal response (Assoufi et al, 1986). A higher rate of ventilation was avoided in order to make it unlikely that a cumulative effect would be obscured by the prior development of a maximal response. In this study a minimum period of five minutes was left between repeated exposures because in most asthmatics, the

maximum response to CAIH occurs at five minutes and the use of a shorter interval would not have allowed development of the maximal effect following the preceding exposure.

Previous studies investigating the possible presence of a refractory period following CAIH have used intervals between challenges of 30-45 minutes, and provided conflicting results (Deal et al, 1980b; Stearn et al, 1981; Bar-Yishay et al,1983; Heaton et al, 1983; Tal et al, 1984). The results of the present study indicate that in the majority of patients, asthma produced by CAIH was not followed by a refractory period using intevals of 15, 30 or 60 minutes. The smallest interval was 15 minutes because previously it was found that in most subjects the mean FEV1 had not returned to baseline values before this time following challenge. This is in agreement with the observations of Tal et al (1984) who recently reported that mean FEV1 had not returned to within 10% of the baseline values until 11 minutes following CAIH.

Baseline FEV1 before the second challenge was significantly lower than that before the first when the interval between challenges was 15-30 minutes, but the difference was less than 10% and is unlikely to have affected the response to CAIH.

The rapid recovery of lung function is compatible with the reflex triggering of CAIH by a direct neural mechanism; direct stimulation of vagal receptors in the pharynx and large airways can

produce bronchconstriction in the experimental situation (Widdicomb (1975)), and both exercise-induced and hyperventilation induced asthma have been shown to be inhibited by prior administration of ipratropium bromide (Poppius and Solorinne, 1973; Wilson et al, 1982; Wilson, Dixon and Silverman, 1984). Heaton et al, 1984.

It is possible that mediator release plays a role in the development of asthma produced by CAIH in some patients. Histamine release into the systemic circulation has been demonstrated following CAIH (Lee et al, 1983). Two patients in this study demonstrated a refractory period and it has been suggested that this period following CAIH represents the time taken for reaccumulation of depleted mediators (McNeil et al, 1966). A refractory period following CAIH has also been reported in seven of nine asthmatics studied by Bar-Yishay et al (1983) and one of seven patients studied by Tal et al (1984).

It has been proposed that respiratory heat loss or water loss is the initiating stimulus for the development of exercise-induced asthma (Deal et al, 1979a; Anderson et al, 1982a and 1982b), a similar asthmatic response to that seen following exercise can be produced by CAIH (Deal et al, 1979a), and it has been shown that if the minute ventilation of exercise is matched with that of the isocapnic hyperventilation, the same degree of airflow obstruction develops. However, in contrast to CAIH induced asthma, EIA is characteristically followed by a refractory period (Stearn et al,

1981) and is associated with the release of NCF (Lee et al, 1982). This suggest that these two features of exercise-induced asthma cannot be explained by respiratory heat exchange.

There are other features of exercise-induced asthma which cannot be explained by respiratory heat loss, such as bronchodilatation during exercise and the lag period. Furthermore, there are asthmatics who can be rendered refractory to further exercise-induced asthma by exercise when breathing warm humid air which causes no respiratory heat exchange (Bar-Yishay et al, 1983). Considered together, these findings indicate that although respiratory heat loss may initiate EIA, other factors are likely to contribute to the total clinical picture.

CHAPTER FIVE

STUDIES ON MECHANISMS OF COLD AIR-INDUCED ASTHMA

STUDIES ON THE MECHANISMS OF COLD AIR INDUCED ASTHMA

INTRODUCTION

Exercise-induced asthma (EIA) refers to the increase in airflow obstruction which follows physical exertion in many asthmatics. It is not a disease but rather a manifestation of bronchial hyperreactivity. The typical pattern of response is a phase of bronchodilatation during the first few minutes of exercise, followed by a lag period before the development of maximal airflow limitation five to 10 minutes after exercise (Anderson et al, 1975). The airways obstruction usually recovers over the next 60 minutes (Jones, Buston and Wharton, 1962; Anderson et al, 1975). Although this clinical association has been recognised for many years, the pathogenesis of EIA is still not understood.

It is well recognised that swimming is the least likely form of exercise to produce airflow limitation and asthmatics often complain of a deterioration of their asthma in the cold weather. Recent studies have suggested that these clinical observations may be explained by respiratory heat loss or water loss from the airways and that this is the initiating stimulus for EIA (Deal et al, 1979a; Anderson et al, 1981; McFadden, 1983). It was shown that cold air could potentiate EIA and that the severity of the airways obstruction, when breathing air at ambient temperature, was inversely proportional to the water content of the inspired gas. Increasing the humidity at ambient temperatures reduced EIA and

inhaling air at body temperature which was fully saturated, completely prevented it from occurring. Deal et al (1979a; 1979b) postulated that EIA was a consequence of the heat exchange which occurred in the bronchial mucosa due to the water necessary for temperature and humidity conditioning of the inspired air by the airways. They suggested that respiratory heat exchange was directly proportional to the minute ventilation. They further reasoned that since the level of ventilation was an important determinant of the quantity of heat transferred from the mucosa, it should be possible to reproduce the extent of the airflow obstruction on exercise by performing isocapnic hyperventilaterion. To test this hypothesis, they subjected asthmatic patients to isocapnic hyperventilation through a heat exchanger. Hyperventilation with air at body temperature which was fully saturated (heat flux zero) did not result in broncho- constriction. However, if the water content and temperature of the inspired gas was decreased, thereby increasing the thermal burden on the airways, bronchoconstriction occurred. They concluded that the major stimulus for EIA is heat loss from the airways during exercise; the exercise is not essential but serves only as a means to increase ventilation. They have further suggested that the changes induced by isocapnic hyperventilation is a close reflection of EIA (Deal et al, 1980a). Despite these experiments, it is not known how narrowing of the airway is provoked by this cooling. However, there are reasons to believe that airway cooling is unlikely to be the only cause of exercise-induced asthma.

1. The decrease in airway temperature during exercise or hyperventilation returns to resting value within five minutes of discontinuation of exercise when airflow limitation is progressively increasing (McFadden et al, 1982).

2. Late-phase asthmatic reactions after exercise occur when airway cooling consequent upon the hyperpnoea of exertion would have resolved several hours earlier. (Feldman et al, 1983; Lee et al, 1982).

3. Breathing warm, dry air during exercise elicits EIA in the absence of detectable airway cooling (Anderson et al, 1982b).

4. Changing the temperature of the inspired air during exercise without changing the water content produces a reproducible bronchoconstriction (Anderson et al, 1982a).

These observations suggest that the loss of water rather than the loss of heat from the bronchial mucosa can initiate EIA.

Breathing cold air has been proposed as a bronchial provocation to identify patients with asthma (Deal et al, 1979a). Because it demands a high level of ventilation, hypocapnia has to be prevented by supplementation with carbon dioxide. The resultant procedure is called cold air isocapnic hyperventilation (CAIH). In an effort to explore further the role of respiratory heat exchange in airways

obstruction, the following studies were carried out.

CHAPTER FIVE

SECTION ONE

RESPIRATORY HEAT EXCHANGE AND AIRFLOW LIMITATION DURING PROLONGED ISOCAPNIC HYPERVENTILATION WITH COLD AIR

ISOCAPNIC HYPERVENTILATION

INTRODUCTION

Current evidence suggests that airflow limitation produced by exercise or isocapnic hyperventilation of cold air (CAIH) is initated by water and heat loss from the respiratory tract with subsequent drying and cooling of the airways. Strauss et al (1978) and Deal et al (1979) demonstrated a significant correlation between the degree of respiratory heat loss (RHL) and the magnitude of airway obstruction following exercise of CAIH. In these studies, respiratory heat loss was measured during CAIH but airflow limitation was not measured until 5-10 minutes after the end of exposure. In the present study measurements of both FEV1 and RHL during prolonged exposure to cold air were performed and assessment of the changes with time of these variables was made in order to further investigate the relationship between RHL and the development of airflow limitation.

METHODS

STUDY 1

CHANGES IN AIRFLOW LIMITATION DURING PROLONGED EXPOSURE TO CAIH

Five asthmatic patients were studied on two occasions. On visit one, exposures were made at 10, 20, 40 and 60% of predicted IMBC or until a 10% fall in FEV1 occurred. The duration of the challenges

was three minutes and the interval between them five minutes. The ventilation rate producing a 10-15% fall in FEV1 was determined and on visit two, patients underwent exposure to cold air at this ventilation rate for nine minutes. The vitalograph was connected to the circuit by a three way tap and the FEV1 was recorded every three minutes without disconnection from the cold air circuit; measurements of lung function were thus made during hyperventilation with cold air.

STUDY 2

CHANGES IN RESPIRATORY HEAT EXCHANGE

DURING PROLONGED EXPOSURE TO CAIH

Six patients were studied on two occasions. On visit one, exposures were made at 10, 20, 40, 60 and 80% of preducted IMBC or until a 10% fall in FEV1 occurred. The duration of the challenges was three minutes and the interval between them five minutes. The ventilation rate producing a 15-20% fall in FEV1 was determined and on visit two, patients underwent exposure at this ventilation rate for 10 minutes during which time respiratory heat exchange (RHE) was measured every half minute. Five of the patients who participated in this study also underwent CAIH at the same ventilation rate for three minutes and the change in FEV1 was compared with that following exposure for 10 minutes.

RESULTS

STUDY 1

CHANGES IN AIR FLOW LIMITATION DURING PROLONGED EXPOSURE TO CAIH

The individual characteristics of the subjects participating in this study is shown in Table 5:1:1. The inspired air temperature is shown in Table 5:1:2.

The mean maximum fall in FEV1 at three, six and nine minutes after the start of CAIH was 90.1%, 87.9% and 88.1% respectively; there was no significant difference between these values (Figure 5:1:1, Table 5:1:3). The values obtained at five and ten minutes following CAIH were not significantly different from that obtained at three, six and nine minutes after the start of the exposure.

STUDY 2

CHANGES IN RESPIRATORY HEAT EXCHANGE DURING PROLONGED EXPOSURE TO CAIH

The individual characteristics of the subjects participating in the study is shown in Table 5:1:4. The respiratory heat exchange variables were shown in Table 5:1:5. The values of RHE recorded at intervals during exposure are shown in Figure 5:1:2, Table 5:1:6. Respiratory heat exchange was greatest at the beginning of cold air inhalation and values recorded at 0.5 and 1.5 minutes were significantly higher that those obtained at 2.5 and 3.5 minutes of exposure. There was no significant difference in the maximum percentage fall in FEV1 following three minute and ten minute

Table 5:1:1

Individual characteristics and baseline lung function of patients tested for airflow limitation during prolonged CAIH exposure.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	M	23	169	80	3·72	98·1
2	F	24	165	68	3·81	118·7
3	M	33	177	70	4·40	102·0
4	M	43	183	80	2·54	62·9
5	M	41	183	75	1·59	49·2
Mean		32·8	175·4	74·5	3·14	86·18
SD		8·46	8·17	5·55	0·93	25·92

Table 5:1:2

Minute ventilation and inspired air temperatures recorded for the subjects tested for airflow limitation during prolonged exposure to CAIH.

(Three exposure of three minutes each of continuous cold air were administered).

Subject	Minute Ventilation (% IMBC)	Exposures 1st exposure	2nd exposure	3rd exposure
1	40	-12·5	-16·0	-9·5
2	60	-17·0	-13·5	-14·0
3	40	-11·5	-16·5	-15·0
4	40	-15·0	-11·5	-13·5
5	40	-15·0	-13·0	-16·0
Mean	44·0	-14·2	-14·10	-13·6
SD	8·94	2·20	2·10	2·48

Water content was zero as dry air was used

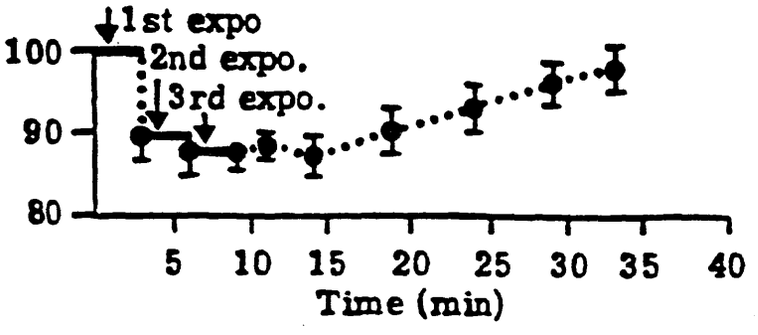


Figure 5:1:1 Changes in FEV1 [percentage of baseline (Mean ± SEM)] following CAIH for nine minutes in five asthmatic patients.

Table 5:1:3

Percentage (P) and lowest post challenge (M) fall in FEV_1 expressed as percentage of the baseline during and following cold air exposure for nine minutes. (Three exposures of three minutes each of continuous cold air exposure were administered). Measurements were made every three minutes without the patient coming off the cold air machine.

	Exposures					
Subject	1st exposure		2nd exposure		3rd exposure	
	P	M	P	M	P	M
1	100·0	98·1	98·1	94·2	94·2	89·4
2	100·0	94·5	94·5	92·6	92·6	92·6
3	100·0	80·1	80·1	86·3	86·3	81·2
4	100·0	88·7	88·7	86·3	86·3	89·4
5	100·0	84·3	89·3	80·3	80·3	87·8
Mean	100·0	90·1	90·1	87·9	87·9	88·1
SEM	0	3·04	3·04	2·49	2·49	1·89

Table 5:1:4

Individual characteristics and baseline lung function of patients tested for the respiratory heat exchange in airway obstruction.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	F	24	165	66	3·77	117·4
2	M	55	172	76	1·77	53·6
3	M	49	170	69	2·29	69·4
4	M	43	183	80	3·10	76·7
5	M	27	181	71	4·47	101·8
6	F	28	179	85	2·58	80·6
Mean		37·7	175	74·5	3·00	83·3
SD		13·0	7·07	7·18	1·00	22·9

Table 5:1:5

Inspired and expired air temperature (^0C) and water content of expired air ($mg/H_2O/L$) (mean $\pm$ SD) during a ten minute challenge with CAIH in six asthmatics.

Time (min)	Inspired temp. (^0C)	Expired temp. (^0C)	Expired water contents $mg/H_2O/L$
0·5	-14·8 (2·93)	25·0 (1·96)	23·2 (2·43)
1·5	-14·5 (3·15)	24·0 (2·65)	21·5 (2·42)
2·5	-13·8 (3·86)	21·9 (2·45)	19·7 (2·67)
3·5	-13·7 (5·22)	21·4 (2·67)	18·9 (2·82)
4·5	-14·8 (4·24)	21·6 (2·85)	19·5 (2·80)
5·5	-14·3 (4·31)	21·3 (2·59)	19·2 (2·68)
6·5	-13·7 (5·36)	21·4 (2·94)	19·1 (3·23)
7·5	-14·3 (3·95)	21·7 (2·70)	19·6 (2·89)
8·5	-14·0 (4·39)	21·9 (2·54)	19·8 (2·18)
9·5	-14·6 (4·12)	21·5 (2·52)	19·2 (2·68)

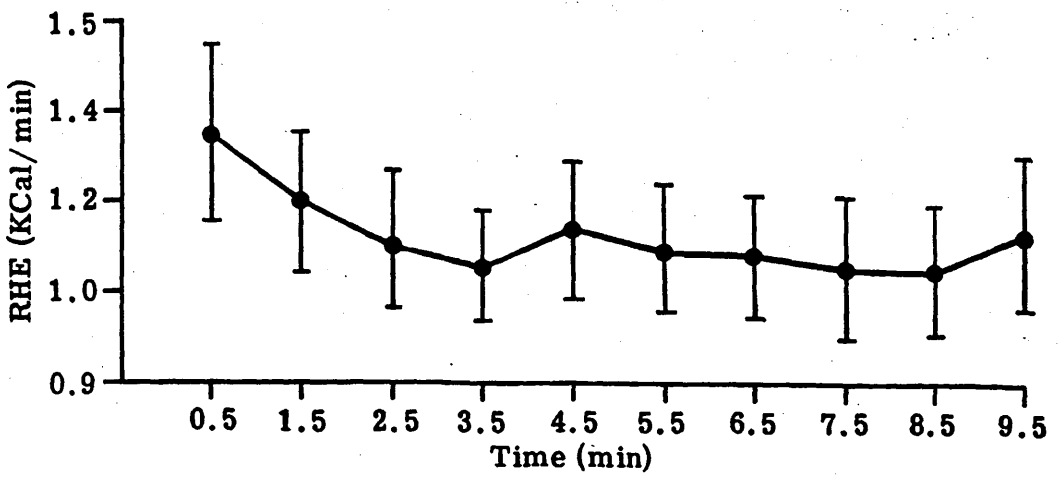


Figure 5:1:2 Changes in the respiratory heat exchange (RHE) in KCal/min (Mean + SEM) during ten minutes inhalation of subfreezing dry air. The level of ventilation used was determined on a separate visit as the dose required to induce a 15-20% fall in FEV1.

Table 5:1:6

Respiratory heat exchange (Kcal/min) during ten minutes challenge with CAIH in six asthmatics (measurement was made every half minute)

	TIME OF EXPOSURE (MIN)									
Subject	0·5	1·5	2·5	3·5	4·5	5·5	6·5	7·5	8·5	9·5
1	1·281	1·203	1·131	1·143	1·112	1·125	1·095	1·095	1·108	1·093
2	1·745	1·226	1·213	1·209	1·424	1·154	1·161	1·219	1·162	1·270
3	0·608	0·580	0·526	0·538	0·545	0·542	0·581	0·477	0·542	0·554
4	1·629	1·649	1·620	1·242	1·529	1·523	1·502	1·590	1·569	1·762
5	1·057	0·972	0·839	0·927	0·906	0·920	0·892	0·920	0·941	0·955
6	1·803	1·594	1·551	1·357	1·376	1·309	1·309	0·979	0·988	1·261
Mean	1·354	1·204	1·147	1·069	1·149	1·096	1·090	1·047	1·052	1·15
SD	0·190	0·163	0·171	0·121	0·152	0·138	0·132	0·150	0·136	0·163

exposures to cold air: 16.2 ± 1.19 and 19.7 ± 6.58 (Mean ± SEM).

DISCUSSION

Isocapnic hyperventilation with cold air appears to induce airflow limitation by water and heat loss from the respiratory tract with subsequent drying and cooling of the airways (Deal et al, 1979a; Anderson et al, 1981). The variables which determine the magnitude of the response are the absolute level of ventilation, and the temperature and water content of the inspired air (Deal et al, 1979a). The higher the minute ventilation, and/or the lower the inspired air temperature or humidity, the greater the heat exchange. Previous work has shown a positive correlation between respiratory heat loss and the magnitude of airways obstruction following exercise or isocapnic hyperventilation (Strauss et al, 1978, Deal et al, 1979a). In their studies, respiratory heat loss was measured during CAIH but airflow limigation was not measured until 5-10 minutes after the end of exposure. Furthermore, measurements made during only the final one minute of a four minute exposure were used to obtain an estimate of RHE. In the present study, we have made measurements of both FEV1 and RHE during prolonged exposure to cold air and assessed the changes with time of these variables in order to further investigate the relationship between RHE and the development of airflow limitation. No further fall in FEV1 occurred after three minutes exposure to cold air and RHE stablized after three minutes. These results are important

when calculating RHE during periods of CAIH, and indicate that this period of exposure is sufficient to measure airway reactivity in asthma.

Conditioning of inspired air is a continuous process that begins immediately air enters the body (McFadden et al, 1981). During hyperventilation with air which has a water vapour saturation of less than 100%, and which is at lower than body temperature, evaporation of water and transfer of heat occur from the mucosal surface of airways to the incoming air as a direct function of the temperature and vapour pressure gradient (Proctor and Swift, 1977), with subsequent cooling (Deal et al, 1979a). The evaporation of water and transfer of heat to the inspired air stimulates transfer to the mucosal surface of water and heat from the deeper tissues supplied by blood. The amount of heat gained per unit time by the inspired air when it leaves the airway passages should be equal to the heat loss from blood which passes along the airway wall (Proctor and Swift, 1977). It is likely that the transfer of water and heat from the mucosal surface to the incoming air, and that from the blood to the mucosal surface reach equilibrium within three minutes, since RHE reached a steady state within this time. The stabilization of FEV1 following three minutes exposure probably reflects the attainment of a steady state of RHE and is compatible with the proposed role of respiratory heat loss in the development of asthma produced by CAIH.

During exercise, the ventilation rate increases to meet metabolic requirements. A large volume of air at ambient conditions is brought into the respiratory tract where it is heated to 37 C and fully saturated with water vapour before it reaches the alveoli (McFadden et al, 1981). The degree of airflow limitation that develops in exercise-induced asthma is directly related to the amount of heat and water transferred (Proctor and Swift, 1977). It is likely that there is a threshold level of ventilation which must be exceeded before a bronchoconstriction response develops and the value of this threshold depends on the temperature and/or water content of the inspired air (Deal et al, 1979a). Exercise produces a gradual increase in ventilation which reaches a steady state within five minutes, whereas in CAIH it is steady from the beginning (Zeballos et al, 1978). Furthermore, additional factors may modify the response to exercise eg. catecholamine release (Barnes et al, 1981). These differences may account for the observation that a greater period of exercise than CAIH is required to produce a maximal asthmatic response; airflow limitation reaches a maximum when the duration of exercise is 6-8 minutes and exercise beyond this time produces no greater response (Anderson et al, 1982a; 1982b).

CHAPTER FIVE

SECTION TWO

THE LINK BETWEEN RESPIRATORY HEAT EXCHANGE, EXERCISE AND MEDIATOR RELEASE

THE ROLE OF RESPIRATORY HEAT EXCHANGE IN BRONCHCONSTRICTION

AIM

The purpose of this study was to explore the role of respiratory heat exchange and mediator release in the mechanism of bronchoconstriction induced by exercise or isocapnic hyperventilation.

Study 1: A similar airflow obstruction and respiratory heat loss were induced using two different tests - exercise and isocapnic hyperventilation

Study 2: A different airflow obstruction and respiratory heat loss were induced using a similar exercise task breathing cold, dry or warm, humid air

METHODS

STUDY 1

Six non-smoking atopic asthmatic patients were subjected to treadmill exercise with two isocapnic hyperventilation procedures. The exercise task was undertaken at a speed of four miles per hour, at a 10-15% incline for six minutes. The exercise apparatus used as previously described (Chapter 2:2). The temperature and humidity were not controlled during the course of these studies. The pulse rate was measured before and immediately after exercise.

The FEV1 values were recorded before exercise and at 2, 5, 10, 15, 30 and 60 minute intervals after the challenge.

Isocapnic hyperventilation with cold air was performed as follows: Cold dry air was generated as previously described (Chapter 2.2). Following baseline lung function measurements of FEV1 and FVC, the subject commenced cold air ventilation at 10% IMBC and continued for three minutes. Following this, FEV1 and FVC were measured immediately, at two minutes and at five minutes. If no reaction was apparant, a further exposure at 20% IMBC was given which was followed by further spirometry. This subsequent exposure was titrated in an identical fashion at 40%, 60% and 80% IMBC until the required fall in FEV1 was obtained. For the purpose of these studies, the percent fall in FEV1 from the baseline following isocapnic hyperventilation was matched to that obtained by treadmill exercise. Exposure ceased if there was no reaction after breathing at 80% IMBC. Three to five days following this initial titration (ie. eight to ten days following the treadmill task) each subject performed another isocapnic hyperventilation procedure at a predetermined IMBC for three minutes and the falls in the FEV1 on this occasion were used for the subsequent analysis. FEV1 was measured following the challenge as described in the exercise challenge. Blood was withdrawn before and after a) the treadmill exercise and b) the second isocapnic hyperventilation procedure. The prechallenge FEV1 did not differ by more than 5% on the exercise and isocapnic hyperventilation days. Neutrophil

chemotactic factor and histamine were measured as previously described Chapter 2:9..

STUDY 2

Cold dry air, and warm humid air was produced as described before (Chapter 2.2 and 2.4). Four patients were subjected to an indentical treadmill exercise task whilst inspiring cold, dry or warm, humid air, on two separate occasions. The test days were randomised and were separated by an interval of less than one week. The prechallenge FEV1 did not differ by greater than 6% between the two days. The subjects inhaled either cold, dry or warm, humid air for at least 15 minutes prior to the treadmill task. During this time the FEV1 measurements were recorded at five minute intervals to ensure that the prechallenge values were consistent. The exercise was then undertaken while the subjects were still inhaling the air under controlled conditions. Following exercise, the patients continued to inspire the controlled air until the end of the experiment. Respiratory heat exchange was measured as previously described Chapter 2, Section 3.

Since the exercise task, performed while breathing cold, dry or warm, humid air, was undertaken by the same asthmatics, they therefore acted as their own controls.

RESULTS

STUDY I

The individual characteristics and baseline lung function of the subjects who participated in the study are shown in Table 5:2:1. There was a significant increase of NCF activity following treadmill exercise but not after isocapnic hyperventilation, despite a virtually identical fall in FEV1 (Table 5:2:2 Figure 5:2:1). The increase in exercise-induced NCF activity accompanied the development of airflow obstruction, although it had returned to baseline by 30 minutes when there was still a decrease in FEV1. The maximum increase in airflow limitation occurred at ten minutes after exercise and at five minutes following isocapnic hyperventilation There was a significant basophilia at five minutes and ten minutes after exercise which was not observed with isocapnic hyerventilation. After exercise, histamine peaked at 10 minutes while after isocapnic hyperventilation it peaked at 30 minutes (Figure 5:2:1).

STUDY 2

The individual characteristics baseline lung function for individuals who participated in the study are shown in Table 5:2:3. The individual percent change in NCF activity and FEV1 in the four asthmatics studied are shown in Figures 5:2:2, 5:2:3. There was an elevation of NCF activity and decrease in FEV1 in all the individuals following treadmill exercise, while inspiring cold/dry air. The respiratory heat exchange values are shown in Table

Table 5:2:1

Individual characteristics and baseline lung function of the subjects tested for comparison of mediators release following CAIH and exercise challenges.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (litre)	FEV_1 (% pred)
1	M	31	183	88	3·74	85·6
2	M	32	179	69	3·95	95·2
3	F	24	174	68	3·53	100·9
4	M	18	192	74	3·71	76·2
5	M	23	169	80	4·03	100·0
6	M	38	185	89	4·05	97·6
Mean		27·7	180·3	78·0	3·84	92·6
SD		7·28	8·19	9·19	0·21	9·73

Table 5:2:2

The pre and maximum post-exercise NCF values and maximum percentage decrease in FEV_1 over a 60 minutes period following challange with exercise and CAIH in six asthmatic patients.

	EXERCISE				ISH			
	NCF Activity				NCF activity			
Patient	pre-	max post-	challenge	FEV_1 (max % decrease)	pre-	max post-	challenge	FEV_1 (max % decrease)
1	150	307	+104·7	36	116	144	+24·1	38
2	153	267	+74·5	12	170	190	+11·8	10
3	139	225	+61·9	14	106	89	-16	15
4	73	142	+94·5	16	99	114	+15	16
5	77	320	+315·6	14	78	74	-5	15
6	96	188	+95·8	16·8	72	67	-6·9	15·7
Mean	114·7	241·5	+124·5	18·1	106·8	113	3·8	18·3
SD	36·91	69·5	94·9	8·91	35·16	47·13	15·40	9·91

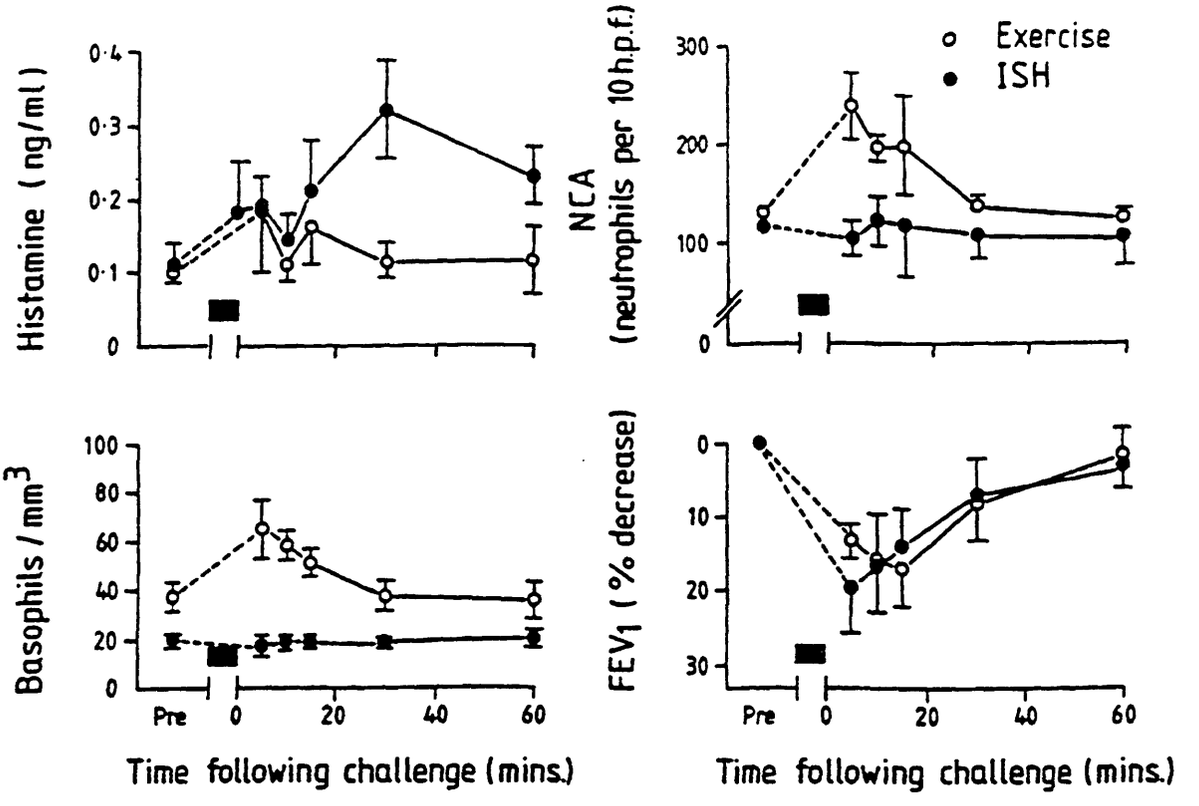


Figure 5:2:1 Plasma histamine, serum NCA, basophil counts, and FEV1 (Mean ± SEM) in five asthmatics who underwent a treadmill exercise (EX) task on day one and isocapnic hyperventilation (ISH) on day seven. The solid bar indicates the period of the challenge.

Table 5:2:3

Individual characteristics and baseline lung function of subjects tested for comparison of exercise challenge with cold, dry or warm, humid air.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)
1	M	38	185	89
2	M	35	175	73
3	M	25	172	68
4	M	32	178	76
Mean		32·5	177·5	76·5
SD		5·57	5·57	8·96

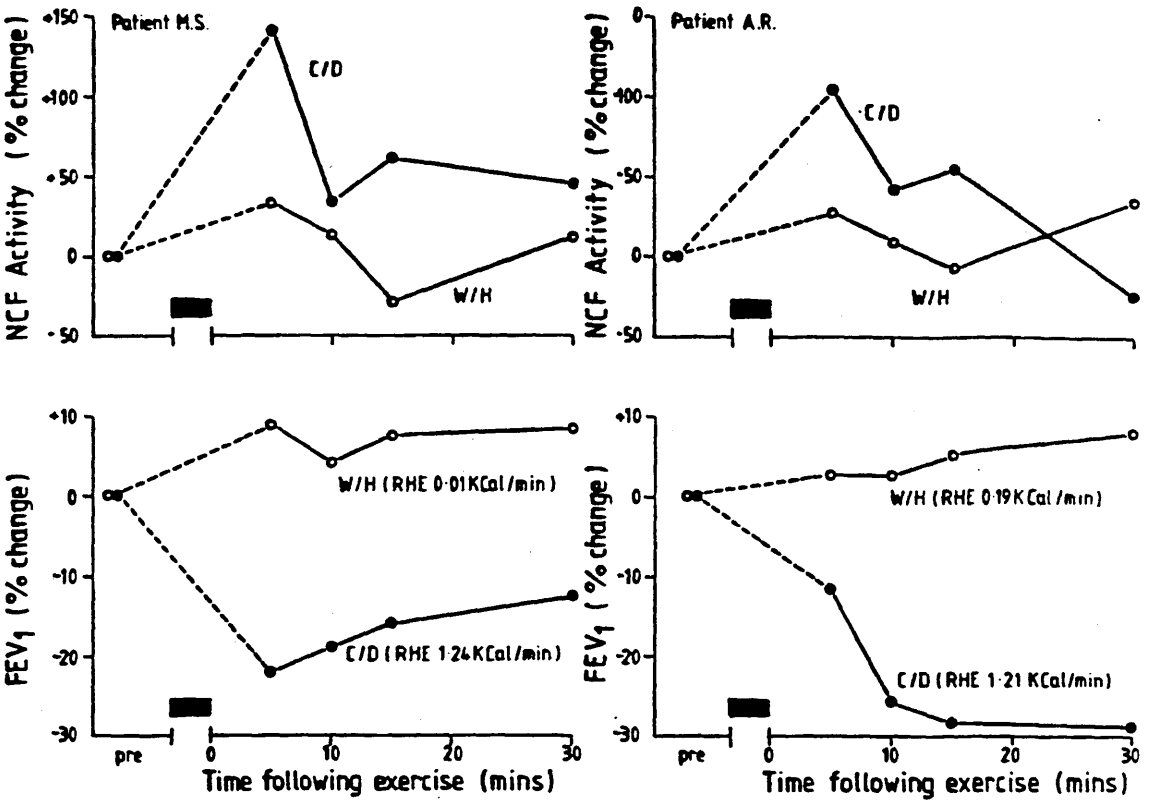


Figure 5:2:2 Effect of breathing cold, dry (C/D) and warm, humid (W/H) during exercise on the time course of release of NCF and percentage change in FEV1. The individual results of two patients (MS and AR) are shown. Respiratory heat exchange (RHE) was expressed as KCal/min, and calculated as documented in the text. The pre-challenge NCF values for patient MS: C/D 100 neutrophils per 10 hpf and W/H 79 neutrophils per 10 hpf; for patient AR: C/D 58 neutrophils per 10 hpf and W/H 116 neutrophils per 10 hpf. The solid bar indicates the period of the exercise test.

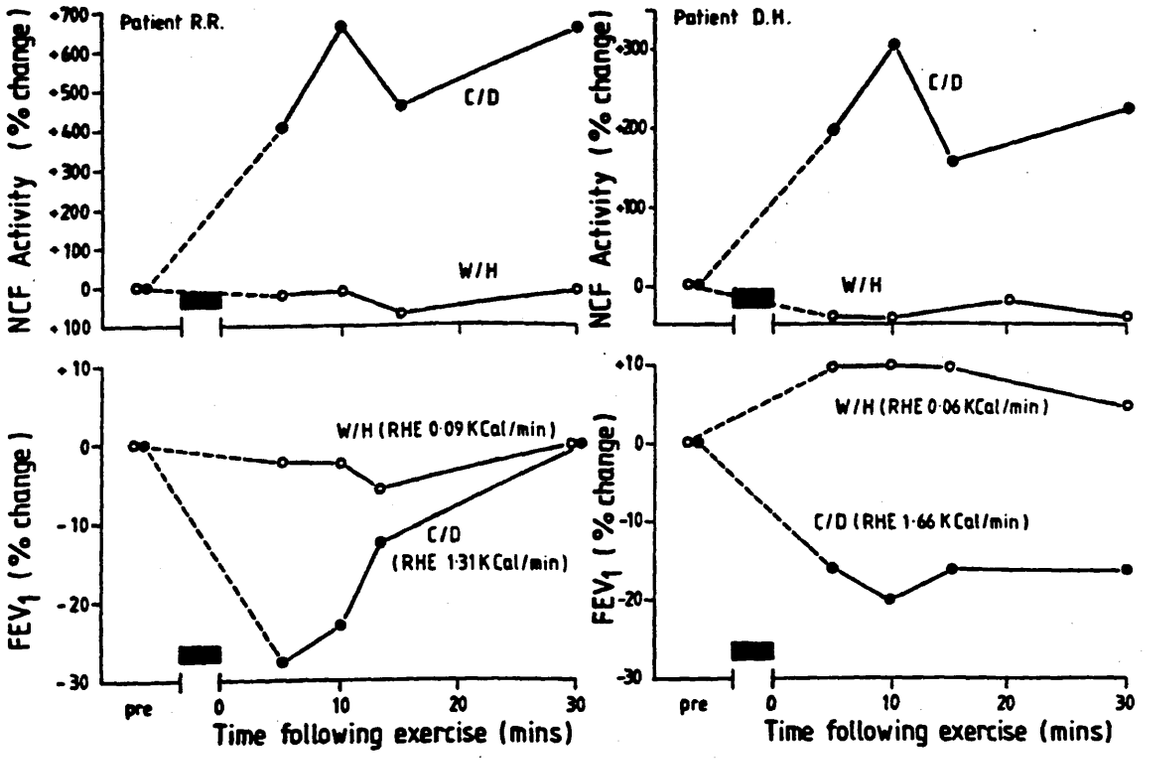


Figure 5:2:3 The effect of breathing cold, dry (C/D) and warm, humid (W/H) during exercise on the time course of release of NCF and percentage change in FEV1. The individual results of two patients (RR and DH) are shown. Respiratory heat exchange (RHE) was expressed as KCal/min, and calculated as documented in the text. The pre-challenge NCF values for patient RR: C/D 20 neutrophils per 10 hpf and W/H 65 neutrophils per hpf; for patient DH: C/D 22 neutrophils per 10 hpf and W/H 34 neutrophils per hpf. The solid bar indicates the period of the exercise task.

5:2:4. In contrast, an identical exercise task while breathing fully conditioned warm/humid air, did not produce appreciable circulatory NCF or reduction in FEV1. There were no significant differences in the increase in the patients pulse rate following cold, dry and warm, humid exercise (cold, dry 78.1 ± 2.6 beat/minutes post 150 ± 3.6 beat/minutes mean ±; warm, humid pre : 76.7 ± 36 beat/minutes, post 143 ± 5.2 beat/minutes).

DISCUSSION

The results of these studies demonstrated that inhalation of warm, humid air during exercise could prevent EIA and mediator release. This suggests that respiratory heat exchange is an important stimulus for both bronchoconstriction and the elaboration of mediators. The observation that histamine and NCF were released in EIA but not following the bronchoconstriction induced by histamine or methocholine (Lee et al, 1982) indicated that the production of mediators was not a consequence of the airflow obstruction alone. The heat loss from the bronchial mucosa alone was clearly insufficient in our patients, to produce detectable circulatory NCF, as isocapnic hyperventilation with cold air was not associated with the appearance of this mediator. Thus, it seems that the appearance of a mediator in the systemic circulation during EIA was related to the combined stimuli of RHE and exercise. In this regard, it is possible that isocapnic hyperventilation released mediators locally in the bronchial mucosa since histamine was detected in the circulation following isocapnic hyperventilation

Table 5:2:4

Respiratory heat exchange in Kcal/min measured during exercise challenge with warm, humid or cold, dry air.

Subject	Warm, Humid	Cold, Dry
1	0·06	1·66
2	0·01	1·24
3	0·19	1·31
4	0·09	1·32
Mean	0·088	1·38
SEM	0·038	0·094

and it peaked at 30 minutes. However, while after exercise histamine peaked at ten minutes. Perhaps after exercise histamine diffuses more rapidly as a consequence of increased cardiac output and possibly NCF is released after isocapnic hyperventilation but its large molecular size prevented it from being transferred to the systemic circulation.

Although recent work has confirmed that the temperature of the airways decreases during exercise or isocapnic hyperventilation, this usually returns to resting values within five minutes of discontinuing exercise or the isocapnic hyperventilation procedure (Deal et al, 1979a; McFadden et al, 1982). Airways obstruction, however, progressively increases over this same period of time and does not resolve for 30 to 60 minutes (Haynes, Ingram and McFadden, 1979). This indicates that other factors must be sustaining the initiating cold air stimulus. It suggests that, in many instances, the release of mediators from mast cell is one of the major factors which sustain the airflow obstruction.

In an attempt to link clinical features with physiological and experimental findings, a hypothesis of the role of RHE in airways obstruction has been shown in Figure 5:2:4. It is suggested that the hyperventilation of exercise leads to RHE (Deal et al, 1979a and 1979b) and that this heat loss can be reproduced by isocapnic hyperventilation (Deal et al, 1980a). RHE causes the release of mast cell mediators which may constrict smooth muscle directly or

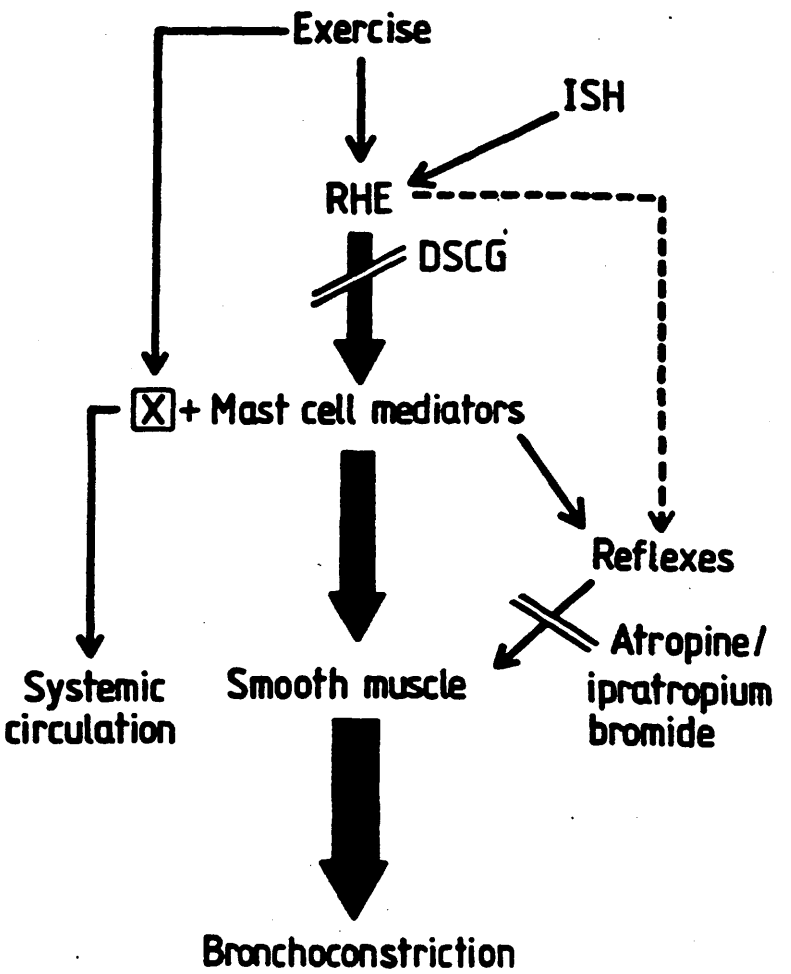


Figure 5:2:4 A hypothesis on the role of respiratory heat exchange in bronchoconstriction. ISH = isocapnic hyperventilation. DSCG = disodium cromoglycate. X = see text for explanation // = inhibition by drugs.

act through reflex mechanisms. The role of mediators in the pathogenesis of EIA may be particularly relevant in asthmatics who have the predominant site of airflow obstruction in the peripheral airways. The EIA in these subjects is inhibited by DSCG but not by anticholinergic agents (McFadden et al, 1977b; Thomson, Patel and Kerr, 1978), and these patients tend to have greater bronchial inflammation and higher plasma histamine concentrations (McFadden, 1981). The increased bronchial inflammation may interfere with the ability of the mucosa to warm and humidify air, thus allowing the cold air stimulus to penetrate further into the intrathoracic airways. There is another group of asthmatics in whom EIA is prevented by the prior administration of either ipratropium bromide or DSCG (McFadden et al, 1977b; Thomson, Patel and Kerr, 1978). This suggests that mediators may cause airways obstruction in these individuals, whose predominant site of airflow limitation is in the large airways, through reflex mechanisms. Finally, there are a small number of asthmatics in whom atropine but not DSCG inhibits EIA (Godfrey and Konig, 1976) suggesting that neurological mechanisms alone may be predominantly involved in the bronchoconstriction. The mechanism whereby RHE initiates this reflex pathway is not understood, but it may involve changes in the pH of bronchial mucosa and its osmotic gradients (Schoeffel, Anderson and Altounyan, 1981). This hypothesis is supported by the observation that hyperventilation induced bronchoconstriction, in subjects whose predominant site of airflow obstruction was in the central airways, was inhibited by the prior administration of

ipratropium bromide. In contrast, the prior administration of ipratropium bromide to asthmatics, whose site of obstruction was in the peripheral airways, did not prevent ISH-induced asthma (Wilson et al, 1984).

It is possible that a high molecular weight mediator is released following isocapnic hyperventilation, but this agent can only be detected in the systemic circulation following exercise. This was labelled as exercise factor as the nature exercise factor as (X). The nature of (X) is unknown, of is unknown, but it may be related to the increased cardiac output of exercise and possible changes in the perfusion of the lungs which do not occur in isocapnic hyperventilation (Matalon et al, 1982).

There is another group of asthmatics in whom exercise-induced asthma is prevented by atropine but not by disodium cromoglycate suggesting that their exercise-induced asthma is mast cell independent and neurological mechanics may be involved in the pathogenesis of airflow obstruction. Hyperventilation with subfreezing air at higher ventilation rates produces a small but significant airflow obstruction in normal subjects (O'Cain et al, 1980). Therefore, it seems likely that the temperature sensitivity of mast cells in normal subjects and asthmatics individuals, differs only in its threshold.

CHAPTER SIX

THE USE OF COLD AIR TEST IN THE FOLLOW-UP OF PATIENTS WITH OCCUPATIONAL ASTHMA

THE USE OF COLD AIR TEST IN THE FOLLOW-UP OF PATIENTS WITH OCCUPATIONAL ASTHMA

INTRODUCTION

Definition

Occupational asthma is a respiratory disorder characterised by variable airways narrowing, casually related to exposure to airborne dusts, gases, vapours or fumes in the working environment (Coutts and Newman-Taylor, 1980).

Airway reactivity and occupational asthma

Occupational asthma from exposure to a variety of organic and inorganic substances has been increasingly recognised in recent years. In many instances the pathogenic mechanism is unknown. Although there is agreement that asthma and airways reactivity are intimately related, the relationship of airway reactivity to occupational lung disease is less clear. It is not known whether the finding of airways hyperreactivity in patients with occupational asthma reflects a genetic predisposition to develop airways obstruction or merely reflects a response to exposure. In either case, if the presence of hyperactive airways indicate an early stage of the disease of prognostic significance, then tests of reactivity are important. For this purpose, pharmacological materials were used to detect hyperactivity in patients with occupational asthma. There have been studies on the aetiological

role of nonspecific bronchial reactivity induced by methacholine or histamine in patients with occupational asthma (Gandevia, 1963; Pain and Symons, 1972; Lam, Wong and Yeung, 1979).

Isocyanate asthma

Asthma due to isocyanates is one example of occupational asthma. The industrial value of isocyanates lies in their reactivity with certain compounds containing hydroxylic groups, such as urethane, or in their ability to form polymerization products. Products made from isocyanate include flexible foam, (mattresses, pillows, seat cushions and packing materials), rigid foam (insulation) surface coating (varnish and paints), adhesives and plastics.

There are four primary isocyanates; toluene diisocyanate (TDI), diphenylmethane diisocyanate (MDI), naphthalene diisocyanate (NDI) and hexamethylene diisocyanate (HDI). Of these, TDI is the most volatile and the most toxic. TDI is well known for its toxicity in low concentration and the threshold limit value (TLV) for workers exposed to this material, set by the American Conference of Governmental Industrial Hygienists is 0.02 ppm (Peter, Murphy and Ferris, 1969).

Clinical response to isocyanate

Two types of clinical response to TDI have been described, the first due to irritation and the second due to "allergy" (Chan-Yeung and Grzybowski, 1976). The irritative effect occurs on exposure to

high concentrations of TDI; burning of the eyes, sore throat, cough and dyspnoea usually appear four to eight hours after the onset of exposure and may persist for three to seven days. The "allergic" response occurs in a minority of individuals after a period of exposure: the onset is insidious, with a nocturnal cough that may progress to severe asthma on exposure to even minute concentrations of isocyanate.

Clinical features of isocyanate asthma

The clinical features of isocyanate-associated illness were summarised by Brugsch and Elkins (1963). Eye, nose and throat irritations are usually the first clinical manifestations. Dry cough with chest pain or tightness often follow. The cough or wheeze is characteristically worse in the evening or at night so the patient or doctor may not recognise its occupational aetiology. Rhonchi or coarse rales are frequently present and in other workers the characteristic pattern of bronchial asthma is the initial manifestation. The TDI workers may frequently present with cough rather than wheeze as the main symptom. Further, the cough may begin during the early hours of the morning, long after the patient has left work. Sometimes the illness remains unrecognised for many years. Nocturnal cough often productive of yellow sputum (containing eosinophils) may be the only symptoms of TDI asthma.

Signs and symptoms of TDI asthma may extend over the weekend and even short holidays adding to the difficulty of the diagnosis. The

time interval between first contact with a material at work and the development of symptoms is variable. In a prospective study of people beginning work in a TDI manufacturing plant, the majority of subjects developed asthma within the first year of employment (Butcher et al, 1977; Peters, Murphy and Farris, 1969). On the other hand, workers may develop asthma after many years of exposure to TDI fumes. Karr et al, (1978) described a case of asthma after six years of exposure in a plant manufacturing TDI.

Diagnosis of isocyanate asthma

The bronchial provocation test is the only method for diagnosing TDI asthma. The test needs to be performed under carefully controlled conditions with continuous monitoring of the levels of TDI inhaled by the subject (see Chapter 2:10).

Asthmatic reaction to isocyanate:

Immediate, non-immediate (late) and dual asthmatic reactions to inhalation of TDI has been described after bronchial provocation testing. The dose response effect in TDI sensitive patients has been studied by Karr et al, (1978) who used increasing concentrations of TDI, namely 0.005, 0.01 and 0.02 ppm to which the subject was exposed for 15 minutes on three separate test days. They found that the inhalation of 0.005 ppm TDI failed to elicit any respiratory reaction whereas higher concentrations produced asthmatic responses. Although no respiratory reaction occurred to the inhalation of 0.005 ppm TDI during the day, PEFR fell

significantly during the evening indicating the occurrence of a non immediate response. However, at the higher concentration of 0.01 ppm, an immediate asthmatic reaction was readily apparent followed by a non-immediate reaction in the evening.

The assessment of the long-term effect of isocyanate asthma

The assessment of the long-term effects in men who were removed from the plants because of respiratory symptoms was studied by Adams (1975). However, the diagnosis was not confirmed by inhalation tests. In addition, it was not stated whether the patients had respiratory symptoms prior to exposure to isocyanate. He studied the respiratory function and symptoms of 46 men who had left a factory because of symptoms. The symptoms were mainly wheezing, varying from mild attacks in the early stages, to fairly severe bronchospasm if the men were allowed to remain in contact with TDI. The acute stage following exposure may last for a week or two, but usually gradually passes off after removal from further contact with TDI. The methods used to assess these men were the MRC questionnaire and lung function (FEV1 and FVC). Men in the affected group have been assessed annually in the same way as the men currently working on the TDI plants. Assessments made six months or more after removal from contact with TDI have been analysed. This group was matched against predicted figures obtained from a control group of 608 subjects living in the same area as the exposed group but not exposed to TDI. It was found that the FEV1 of the affected men was on average 267 ml lower than

predicted and the FVC 269 ml lower. The MRC questionnaire demonstrated that there was a significantly greater number of men suffering from chest symptoms than in the control population. The study demonstrates that sensitization to TDI can lead to long term respiratory symptoms with impaired lung function. The cause of these effects has not as yet been established, but there is general agreement that allergic mechanisms are involved in at least a proportion of cases developing symptoms after exposure (Scheel, Killens and Josephson, 1964).

the aims of the present study were:

1. To assess the cold air isocapnic hyperventilation test in detection of non specific bronchial reactivity in patients with occupational asthma who had been removed from the factory because of their symptoms. There has been no report in the literature exploring the role of bronchial hyperreactivity to cold air in the investigation of occupational asthma due to isocyanates.

2. To determine if removal of the workers from the factory because of asthmatic symptoms lead to the cure of asthma or whether the effects persist leading to permanent changes as assessed by reactivity and lung function studies.

3. To study some of the factors which may influence the outcome of the disease such as smoking or atopic status. The latent period between exposure and the onset of symptoms, the duration of

exposure to dust after symptoms develop and the severity of lung function abnormalities were investigated

METHODS

A total of 56 cases of isocyanate induced asthma were diagnosed at the Brompton Hospital between 1971 and 1979. In each case:

1. Symptoms of asthma had developed after exposure to isocyanates.

2. Inhalation testing with the isocyanate(s) used at work reproducibly provoked an asthmatic reaction.

3. Exposure to isocyanates had been avoided for at least 4 years. At the time of diagnosis, measurements were made of lung function and, in some cases, bronchial reactivity to histamine.

At the time of followup the following were carried out in as many patients as possible. Many patients lived a considerable distance from the hospital and were not therefore able to cooperate in all parts of the study.

a. Questionnaire

At the time of follow-up, patients were asked to answer a modified Medical Research Council respiratory questionnaire. Where possible, those unable to attend the hospital were interviewed by telephone or postal questionnaire. The presence of continuing

symptoms was assessed by asking patients whether they still had episodes of chest tightness or difficulty in breathing. Patients who were able to attend for follow-up also had lung function tests and measurements of bronchial reactivity to inhaled histamine or cold air.

b. Lung Function Tests

Forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) were measured using a regularly calibrated dry wedge spirometer (Vitalograph). Other tests of lung function [total lung capacity (TLc), residual volume (RV), effective alveolar volume (VA), diffussion capacity of carbon monoxide (DLCO), Coefficient of diffusion (KCO)] were made at diagnosis and where possible, after one or more years of avoidance of exposure.

c. Inhalation testing

1. Isocyanates

This was performed as described previously in Chapter 2.10.

2. Histamine

Airway reactivity to histamine was measured by the method of De Vries (1960) as modified by O'Brien et al, (1979). Histamine acid phosphate solution was delivered from a Wright's nebulizer using an oxygen flow rate of eight litres per minute as described before (Chapter 2.6). The aerosol was inhaled via a face mask with a rebreathing bag for 30 seconds and the FEV1 was measured after five

minutes. This was repeated with two-fold increases in concentration until a 20% fall in FEV1 occurred, or the maximum concentration of 32 mg/ml of histamine was reached. A fall in FEV1 of 20% obtained with this method has been found to distinguish asthmatic from normal subjects : bronchial hyperreactivity to histamine was considered to be present if a 20% fall in FEV1 was provoked by 32 mg/ml histamine or less.

3. Isocapnic hyperventilation of cold air

A method developed previously (see Chapter 3) was used. In summary, dry air was fed from a cylinder to the patient through an immersion cooler which lowered the air temperature to -15 degrees centigrade. The rate of delivery of air to the patient was adjusted using a flowmeter. Patients were asked to ventilate at a rate that maintained a guide balloon at constant volume; in these circumstances, the rate of ventilation was equal to the rate of delivery of air from the cylinder. Carbon dioxide was added to the inspiratory circuit according to the ventilatory rate in order to maintain a constant end-tidal CO2.

The best of three measurements of FEV1 was recorded on three occasions at five minute intervals and the mean of these three best values taken as the pre-challenge baseline. Subjects then breathed dry air at -15 degrees centigrade for three minute periods. During the first test period, the ventilation rate was 10% that of indirect maximum breathing capacity (IMBC). Successive test

periods were at 20%, 40% and 60% or until a 20% fall in FEV1 below the pre-test value had occurred. The best of three records of FEV1 was recorded immediately after and at five minutes following the end of each period. With this method of measuring bronchial reactivity to cold air, a fall in FEV1 of 10% or more occurs in asthmatic but not normal subjects. Bronchial hyperactivity to CAIH was considered to be present if a 10% fall in FEV1 occurred.

d. Skin prick tests

Skin prick tests were made with a control solution and extracts of common inhalant allergens which included grass, pollen, Aspergillus fumigatus, cat, hair, dog hair, house dust and Dermatophagoides pteronyssinus (Bencards). A positive reaction was defined as a weal diameter of 3 mm or more greater than that provoked by the control solution. Atopy was regarded as present when one or more positive reactions were elicited.

RESULTS

a. Patients

There were 47 males and 9 females and their ages at diagnosis ranged from 23 to 62 years (mean 46.11). 36% (20/56) of patients were atopic at the time of diagnosis. At diagnosis, 32 patients had inhalation testing with histamine. Fifty cases (89%) of the total 56 cases of isocyanate asthma were studied. Twelve of the 50 seen at folow-up currently smoked cigarettes, 15 had never smoked

and 23 were non-smokers. At follow-up 11 patients had inhalation testing with histamine, and 19 were tested with cold air.

b. Work exposure and symptoms

The occupations of patients at the time of diagnosis are shown in Table 6:1. Symptoms were regarded as work related where there was improvement in symptoms during weekends away from work, or while on holiday. Fifty patients (89.3%) had work related respiratory symptoms and four (7.1%) did not. Information was not available in two cases (3.6%). The mean latent interval of exposure before the development of symptoms was 6.48 years (range 0.5 - 30). The mean period of symptomatic exposure was 2.70 years (range 0.5 - 9).

c. Inhalation tests at time of diagnosis:

1. Isocyanates

The maximum atmospheric concentration of TDI during inhalation tests ranged between an estimated 0.001 0.002 ppm (n = 32). A concentration of 0.02 ppm was reached during only two tests. The range of concentrations of MDI was 0.001 - 0.002 and 0.02 ppm (n = 10). A concentration of 0.02 ppm was reached during only one test.

The most frequent sensitizing isocyanates were TDI (45 patient, 64% of positive reaction), followed by MDI (16 patients, 23% of positive reactions). The majority of patients had late (28 patients, 40% of patients) or dual (31 patients, 44% of reactions)

Table 6:1

Occupations of patients at diagnosis: classification by type and exposure

Flexible packaging and/or printing	27
Polyurethane foam production	8
Soldering and coil winding	5
Painting or paint manufacture	5
Synthetic rubber manufacture (NDI)	3
Office workers sensitised by fumes from adjacent factories	3
Other	5
Total	**56**

reactions provoked. An isolated late reaction occurred in only 9% of tests.

2. Histamine

Sixteen of the 32 patients tested with histamine at diagnosis had bronchial hyperreactivity.

d. Outcome after diagnosis

1. Symptoms:

At the time of follow-up, which was four years or more from the time of diagnosis, 41 (82%) of the patients followed up continued to have episodes of shortness of breath or chest tightness or both. In 19 (46%) this occurred more than once per week, and 22 (54%) required treatment at least once per week.

2. Lung fuction tests:

Thirty two patients had measurements of lung function made both at diagnosis and one or more years later. The results indicate an obstructive ventilatory defect (Figures 6:1, 6:2, 6:3). No significant difference was observed in lung function parameters (FEV1, FVC, FEV1/FVC ratio, RV, TLC, VA, DLCO and KCO) at the time of diagnosis when compared with that at the time of follow-up.

3. Bronchial reactivity to cold air:

Measurement of bronchial reactivity to cold air was made in 19

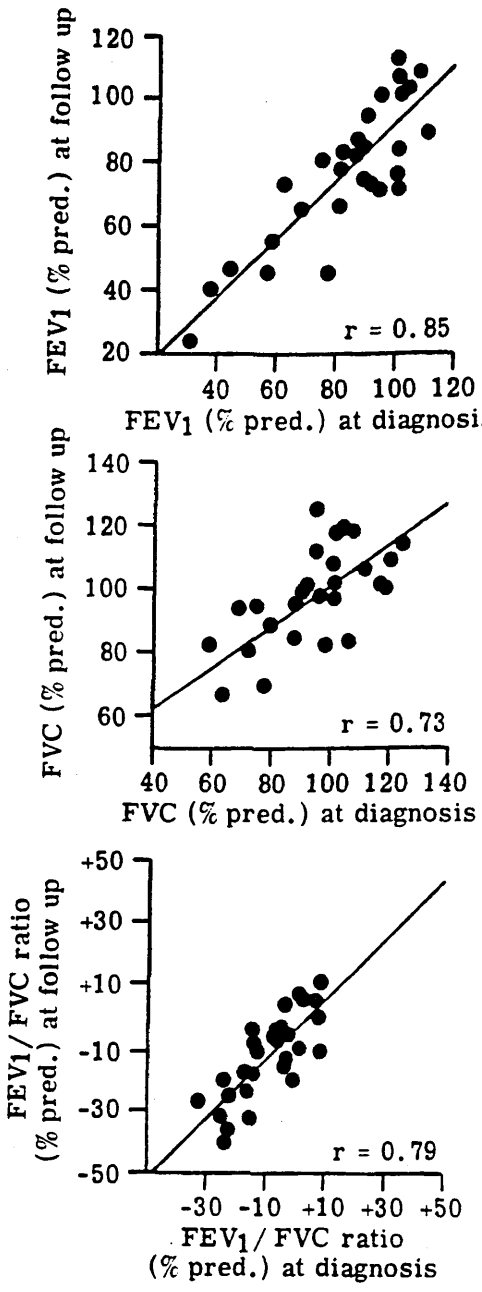


Figure 6:1 Correlation of the spirometric values (FEV1, FVC and FEV1/FVC%) expressed as percentage of predicted values at the time of diagnosis with that at the time of the follow-up.

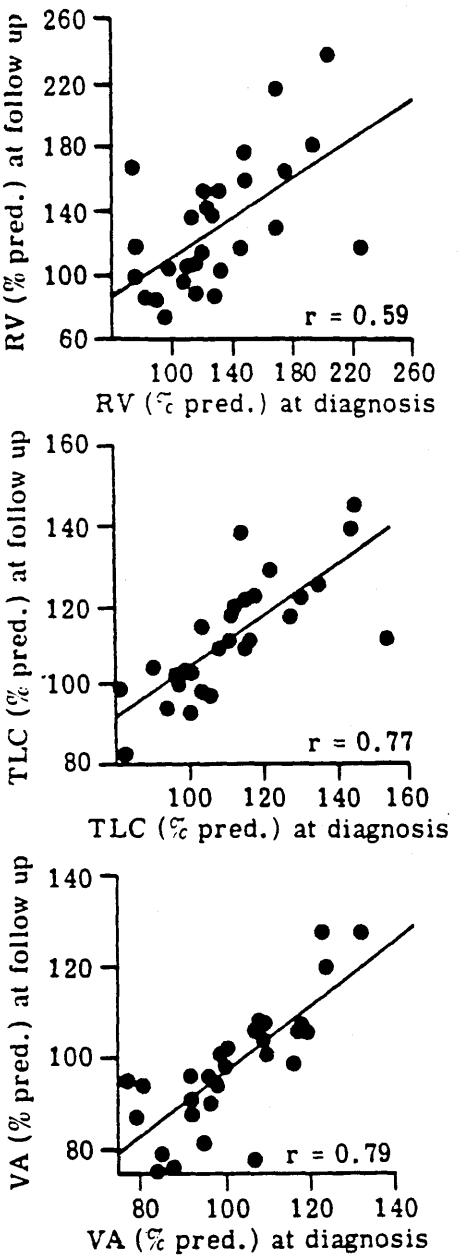


Figure 6:2 Correlation of TLC, RV and VA at the time of diagnosis with that at the time of the follow-up (values were expressed as a percentage of predicted values)

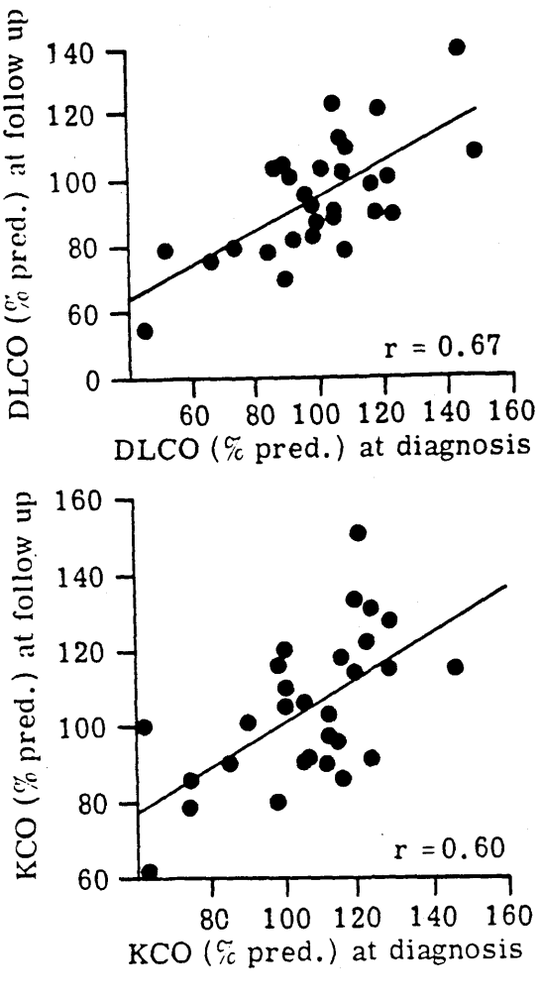


Figure 6:3 Correlation of DLCO and KCO at the time of the diagnosis with that at the time of the follow-up (values were expressed as a percentage of predicted values).

patients at follow-up. The inspired air temperatures , minute ventilation, the maximum percentage fall in FEV1 following CAIH, and the PD10 values for those patients in whom more than 10% fall in FEV1 were obtained with CAIH are shown in Table 6:2. Twelve patients were hyperreactive (these patients had a fall in FEV1 of more than 10%). Of the 19 patients tested with CAIH, eight patients required treatment once per week or more often (seven of these had a 10% or more fall in FEV1 and PD10 could be measured) and 11 patients required treatment less than once per week (only five of these are hyperreactive).

4. Bronchial reactivity to histamine:

Measurement of bronchial reactivity to histamine was made in 11 patients at follow-up: four were hyperreactive.

e. Factors associated with a poor outcome

In order to investigate which factors might be associated with a poor outcome, patients were divided into two groups in relation to frequency of treatment requirement at followup:

Group 1: 22 Patients who required treatment once per week or more often.

Group 2: 27 patients who required treatment less often than once per week.

Table 6:2

Minute ventilation (% IMBC), inspired air temperature (^0C), maximum percentage fall in FEV_1 and PD_{10} cold air for 19 subjects with isocyanate asthma who were tested with CAIH at follow-up.

Subject	Minute Ventilation (% IMBC)	Inspired air temperatue (^0C)	Maximum % fall in FEV_1 following CAIH	PD_{10} (% IMBC) CAIH
1	60	-16·0	3·7	-
* 2	60	-12·5	8·7	-
3	60	-15·3	8·3	-
* 4	60	-16·1	13·6	43·0
* 5	10	-18·2	20·6	7·0
* 6	40	-10·5	19·3	21·0
7	60	-13·6	27·0	49·0
8	60	-14·1	-7·8	-
9	60	-16·6	9·3	-
* 10	20	-17·3	17·3	17·5
11	60	-11·6	10·5	56·0
* 12	60	-17·3	10·0	60·0
13	60	-14·8	4·8	-
14	60	-15·1	6·9	-
* 15	60	-13·8	15·8	41·0
16	60	-11·8	10·0	60·0
17	40	-18·1	18·5	28·0
* 18	20	-16·1	19·8	13·0
19	60	-15·3	14·3	44·0
Mean	51·50	-14·95	12·14	36·63
± SD	16·31	2·22	7·77	18·72

* Patient required treatment $\geq$ once per week. The remaining patients required treatment < once per week.

One patient was known to have continuing symptoms of asthma after more than four years of avoidance of exposure but his treatment requirements were not known.

1. Bronchial reactivity to cold air

It would have been more satisfactory to determine outcome on the basis of bronchial reactivity but only 19 patients attended for measurement of reactivity to cold air at follow-up. Of those whose airway reactivity was tested at follow-up, bronchial hyperreactivity was present in seven of eight patients in group 1, but only five of 11 patients in group 2, providing some validation of the assessment of outcome based on treatment requirement.

2. Bronchial reactivity to histamine

Individuals in group 1 were more likely to have had bronchial hyperreactivity to histamine at the time of diagnosis ($p<0.001$). Similarly, they were more likely to have had an immediate reaction (either isolated or as part of a dual reaction) than an isolated late reaction to inhalation testing with isocyanates at the time of diagnosis (Table 6:3).

3. Lung function tests

FEV1 at diagnosis was significantly ($p<0.02$) lower in the group requiring more treatment at follow-up compared with the group requiring treatment less than once per week. In the first group, FEV1 and FEV1/FVC ratio were significantly ($p<0.05$) lower at the

Table 6:3
Prognostic factors

Details of treatment requirement at follow up were available for 49 patients. In some of these, results of inhalation tests and/or lung function measurements were not available and the number of patients in each analysis is less than 49.

Treatment at follow-up	GROUP I Once per week or more often	GROUP 2 Less than once per week	
Inhalation test reaction at diagnosis			
Histamine			
Increased	10	2	Chi square=9·049
Normal	3	13	$p < 0·01$
Isocyanate			
Immediate or dual	16	12	Chi square=3·58
Late	4	13	$p < 0·10$
FEV_1 (% predicted normal) at diagnosis			
Mean ± SEM	77·1	93·6	$p < 0·02$
	(+5·38)	(+2·90)	(unpaired t-test
	n=21	n=22	

cont..

Table 6:3 cont...
Prognostic factors

Details of treatment requirement at follow up were available for 49 patients. In some of these, results of inhalation tests and/or lung function measurements were not available and the number of patients in each analysis is less than 49.

Treatment at follow-up	GROUP I Once per week or more often	GROUP 2 Less than once per week	
Mean duration of exposure (years ± SEM)			
Before onset of symptoms	6·8 (+1·31) n=22	5·17 (+1·26) n=26	p>0·10
After onset of symptoms	3·0 (+0·76) n=21	2·19 (+0·54) n=26	p>0·10 (t-test)
Atophy at diagnosis			
Atopic	5	12	Chi square=0·461
Non-atopic	12	15	p > 0·10
Cigarette smoking at follow-up			
Current smoker	3	9	Chi square=2·402
Ex-smoker	11	10	p > 0·10
Non-smoker	7	8	
Place of work after diagnosis			
Same factory	13	15	Chi square=0·00172
Left factory	9	12	p > 0·10

time of the follow-up when compared to that at the time of diagnosis (Figure 6:4). In the second group, the FVC was significantly ($p<0.05$) higher at the time of diagnosis, when compared to that at the time of follow-up (Figure 6:4). No significant changes in other lung function were observed.

4. Symptoms, duration of exposure, smoking, and atopic status

The duration of exposure before the onset of symptoms, the duration of continuing exposure after the onset of symptoms, distribution of smoking and atopic status showed no statistically significant differences between the two groups. A subgroup of 12 patients, who developed asthma while exposed to TDI in one printing factory, was analysed separately. These patients probably represent all the cases which developed in this factory. All continued to have symptoms of asthma despite avoidance of further exposure. One patient died during a severe asthmatic episode one year after diagnosis, and the remaining 11 continued to have symptoms of asthma at follow-up. Five (45% of those followed up) had symptoms once per week or more often. Seven (64%) required treatment. Each of six of these patients tested with cold air, demonstrated bronchial hyperreactivity.

DISCUSSION

Parkes (1982) has stated that recovery from asthma due to isocyanates commonly takes several days, occasionally as long as

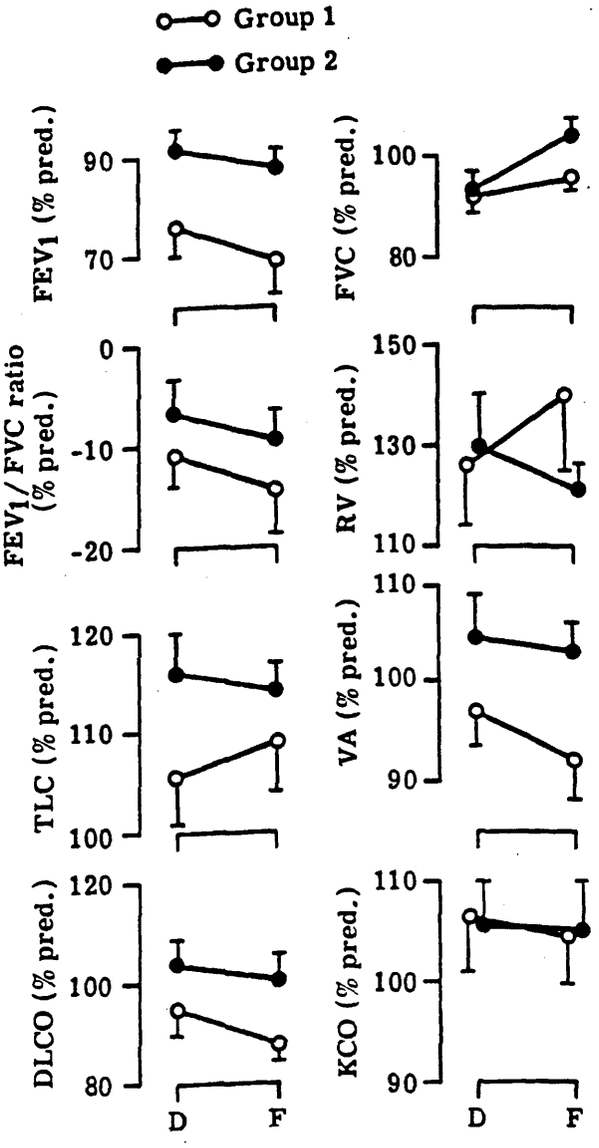


Figure 6:4 Pre-challenge values and changes in different parameters of lung function [FEV1, FVC, FEV1/FVC ratio, TLC, RV, VA, DLCO and KCO. These were expressed as a percentage of predicted values (Mean + SEM)]. Group 1: subjects who required treatment more than once a week, Group 2: subjects who required treatment less than once a week.

ten weeks after stopping exposure. A study by Adams (1975) provided results suggesting that symptoms might continue after cessation of exposure in patients sensitised to TDI, but it was not stated whether the patients had respiratory symptoms prior to exposure to isocyanates, and the diagnosis was not confirmed by inhalation testing. Paggiaro et al (1984) studied 12 patients with TDI asthma proven by inhalation testing and found that two years after cessation of exposure, 67% continued to have dyspnoea and wheezing, it was not stated whether these patients had respiratory symptoms prior to isocyanates and the number of cases was too small to determine the prognostic factors. In this study, 50 patients were followed up out of 56 in whom the diagnosis of asthma induced by isocyanates was proven by inhalation testing at least four years previously. Of these 50 patients, 41 (82%) continued to have symptoms of asthma.

The initial aim of this study was to perform cold air isocapnic hyperventilation test for all patients who had asthma due to isocyanate and had been removed from the factory because of their symptoms, however, it was possible to perform CAIH on 19 patients only; twelve patients were found to be hyper-reactive to cold air (more than 10% fall in FEV1). Bronchial reactivity was present in seven of eight patients in group 1, but only five of 11 patients in group 2. This confirms the validity of assessing the outcome on the basis of treatment requirements. The presence of bronchial hyperreactivity to this challenge and lower values of FEV1 at

diagnosis were each associated with a poor outcome. These results are in agreement with the findings of a study by Chan-Yeung et al, (1982) of patients with occupational asthma due to Western Red Cedar; a poor outcome was associated with a greater degree of bronchial reactivity to histamine, more abnormal results of lung function studies, and a dual (rather than isolated late) reaction to inhalation testing with red cedar extract or plicatic acid at the time of diagnosis. It was also reported that a longer duration of exposure to Western red Cedar was associated with a poor prognosis. The period of exposure to isocyanates was slightly greater in those with a poor outcome, but the difference did not achieve statistical significance. Cautious interpretation of these findings is required since the results depend on patients' memories of distant events, and in many cases, the date of introduction of isocyanates into the working area was uncertain.

There was no difference in outcome between patients who left the factory and those who were relocated. This suggests that it is unlikely that patients who were relocated were still exposed to clinically significant concentrations of isocyanates. The findings do not provide a basis for advising sensitised patients to leave rather than accept relocation within the same factory.

There was a concern that the study patients might not be representative of cases of isocyanate induced asthma. Cases referred to a hospital may be from the more severely affected, and

those with continuing symptoms are more likely to be available for follow-up investigations although this is not supported by the findings with our group. We examined a subgroup of 12 patients in our study population who were all employed in one printing factory and who were probably all those who developed asthma in this factory. The severity if their symptoms influenced neither their inclusion into the study nor their availability for follow-up. All had continuing symptoms when seen at follow-up, and more than half required treatment more than once per week. These results are comparable to those found in the total study population and suggest that it is not unrepresentative of cases of isocyanate asthma generally.

The diagnosis of isocyanate asthma was made only where symptoms had started after the onset of exposure. However, it is possible that some individuals with non-occupational asthma might falsely attribute their symptoms to exposure to isocyanates. This is unlikely to confuse interpretation of the results of this study, since the diagnosis was made only if patients demonstrated an asthmatic reaction to controlled inhalation testing with isocyanates. It has been reported that patients with non-occupational asthma do not have asthmatic reactions following provocation with concentrations of TDI similar to those used in the present study (Chester et al, 1979).

Only 21% of patients were current cigarette smokers at follow-up;

this is likely to be a result of the high prevalence of continuing symptoms of asthma, particularly since only three of the 22 subjects with a poor outcome (group 1) were smokers at followup, compared with nine of the 27 subjects with a better outcome group (group 2). The proportion of atopic paients in the study population was similar to that in the general population which suggests that atopy does not predispose to the development of sensitivity to isocyanates.

The majority of patients developed asthma while working in industries concerned with flexible packaging, printing, polyurethane foam production, soldering, coil winding and painting or paint manufacture. The diagnosis of isocyanate asthma should be considered especially carefully in workers who develop respiratory symptoms while employed in these processes. However, three patients were office workers in buildings adjacent to factories using isocyanates. Similarly, although most patients gave a history of work related symptoms of asthma, nearly 10% did not; the diagnosis is not excluded by the absence of work related symptoms, or a non-industrial occupation.

Both at diagnosis and at follow-up, a significant proportion of patients demonstrated bronchial hyperreactivity. Since different methods of assessing reactivity were used, it is not possible to say whether there was a trend towards a change in bronchial reactivity following removal from exposure. However, measurements

of lung function for the group as a whole, indicated that there was little change between the time of diagnosis and follow-up one or more years later. This is consistent with the high prevalence of continuing symptoms of asthma. Since only a proportion of subjects had abnormal lung function or bronchial reactivity at diagnosis, the demonstration of normal values for these tests does not exclude the diagnosis.

The results of the present study indicate that, patients who develop occupational asthma due to isocyanates are likely to have significant persisting symptoms even after a change of occupation and this should be taken into account in assessing their financial compensation.

This study used cold air isocapnic hyperventilation in the detection of non specific bronchial reactivity in patients with occupational asthma who had been removed from the factory because of their symptoms. Patients found the test very acceptable. Cold air isocapnic hyperventilation is a suitable tool for detecting hyperreactivity in epidemiological surveys.

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