A COMPARISON OF BRONCHIAL HYPER-REPONSIVENESS AND DIURNAL VARIATION IN PEAK EXPIRATORY FLOW RATE IN CHILDREN WITH ASTHMA.

by

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Thesis submitted for the degree of M.Sc. (Med. Sci.)

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1993

TO
MY DEAR FAMILY

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Abstract

Bronchial hyper-responsiveness and diurnal variability in airways function are thought to be important features of asthma. We have investigated the relationship between bronchial hyper-responsiveness measured by non-specific challenge tests and diurnal variability measured by cosinor analysis in children of varying severities of asthma. Forty asthmatic children and ten normal control children were both tested with a histamine challenge test and standardised treadmill exercise before and after a week long period of home peak flow rate recording. A further 35 normal children (age range 7-14 yrs) underwent only the week long measurement of peak flow at home. For both histamine challenge test and exercise tests there was evidence of a significant difference in reactivity between normals and asthmatic children on inhaled prophylactic medication. However, the range in the asthmatic subjects was wide and overlapped with the normal subjects. The mean diurnal variation in PEF measured as the peak to trough amplitude by cosinor analysis was 13.2% (SD 10.4%; range 0.9% to 45.2%) of the mean PEF in the asthmatic children compared to 6.8% (3.6%; 0.7% - 19.5%) less than has been reported in adult asthmatics. The correlation between the two measurements of bronchial reactivity and the measurements of diurnal variability (Pc_{20}, percent fall in peak flow after exercise and the amplitude of peak flow variability derived from cosinor analysis) was weak (Spearman's Rank correlation: Pc_{20} vs. the cosinor derived peak to trough amplitude $r = -0.483$ ($P<0.02$); per cent fall in peak flow after exercise vs. cosinor peak to trough amplitude $r = 0.481$ ($P<0.02$). This study confirms that both non-specific bronchial responsiveness and diurnal variability are more frequent and more marked in asthmatic children. However, the wide overlap between asthmatics and normal subjects does not suggest that either are particularly well correlated with the presence or severity of asthma.

Author's Declaration

I declare that this thesis embodies the results of my own research, that all the practical work was completed by myself, that it has been written by myself and that it does not include parts of a thesis presented by me for a degree in this or another University. The experimental work for this thesis was completed by June 1988.

Signature _ _____

Date: 10/6/93

Acknowledgements

I am indebted to my supervisor Dr. J.Y. Paton for providing the conditions which made my thesis possible and for his help and guidance. I am grateful to Dr. T. MacDonald my previous supervisor. His friendly help and advice was of great importance throughout the period of my study. I wish him well.

I would like to express my thanks to Professor M. Titterington for his help on statistical problems and to Mr. E. Lindsey who computed the cosinor analysis technique on my findings.

I am also thankful to Professor F. Cockburn and to Dr. W. Hamilton for their help and advice. I wish to thank Miss Caroline King the Lung Function Technician for her help and assistance. I am also thankful to the Consultants of the Royal Hospital for Sick Children, Yorkhill, Glasgow and the rest of the staff who helped me to study their patients or their children.

I wish to thank Miss Lily Plank and Miss Julie Zdanovich for typing this thesis.

Finally, my deeply felt thanks go to my family who made this work possible for their help and understanding.

INTRODUCTION

Historical Overview

Asthma has a long and distinguished medical history. The word asthma is derived from the Greek ασθμα meaning a short drawn breath with noise or panting. Aretaeus the Cappadocian, in the second and third centuries AD (Adams,1858) may have made the first description of an asthmatic attack:

> "If the breathing becomes difficult it is called asthma,...
> but the evil is much worse in sleep."

Maimonides (1135-1204) (quoted in Munter, 1963) who was body physician to Saladin, described sleep in asthma;

> "Sleep in this disease is rather harmful, especially during an attack ..... Those afflicted should therefore sleep as little as possible".

Thomas Willis (1679) outlined a more modern view of asthma when he described "pneumonic" asthma with obstruction of the bronchi with "thick humours" and swelling of their walls and "Convulsive" asthma with cramps of the moving fibres of the bronchi.

Sir John Floyer, himself an asthmatic, in 1698 gave a vivid description of asthma in his book "A Treatise of the Asthma" attributed the symptoms to the compression or constriction of the bronchi.

> "I have assigned the immediate cause of the asthma, to the straitness, compression, or constriction of the bronchi...."

It is interesting to read Floyer describing the muscular fibres of the bronchi as contracted and the contraction responsible for the narrowing of the airways to produce wheezing during expiration. He described "A scheme of the several species of the asthma." In the main these are variety of pulmonary conditions, associated with

dyspnoea, which in modern terminology would not be considered to be asthma. However, the clearest description is of the "Periodical or Flatulent asthma", where Floyer describes his own experiences with what we understand as asthma to-day:

> "My design in this Treatise is to describe the Periodic Asthma, to which I have been long subject and that has given me many opportunities of considering the history of the disease more nicely than it was possible for Physicians who have always an imperfect Account of Chronical Cases from Patients; and to that I must impute their ill success in many Chronical Diseases".

Floyer was also aware of the relationship between exercise and asthma:

"All violent exercise makes the asthmatic to breathe short; because their lungs are frequently oppressed with tubercula; and if the exercise be continued it occasions a fit, by putting the spirits to a great expansion."

Laennec (1827) described the findings on auscultation in his description of asthma. He reported that:

> "The respiration examined by the stethoscope is not at all more perfect than during the severest paroxysms.....sleep acts in this case by diminishing the necessity of respiration".

It is therefore apparent then that these early physicians had a clear appreciation of the episodic nature of asthma and of its relation with exercise. Nowadays it would be recognised that the tendency of bronchi in asthmatics to respond to a variety of stimuli both more readily and with a greater degree of narrowing is a characteristic feature of asthma. In most patients with asthma this phenomenon can be reproduced artificially by bronchial provocation with certain "non-specific" physical or pharmacological

stimuli. It is widely believed that the response to artificial challenge tests reflects the underlying lability of the airways in asthma and that measured levels of bronchial responsiveness reflect the severity of the disease. In the last decade much work has focused on the hypothesis that asthma is an inflammatory disease, effectively returning to Willis' idea of "pneumonic asthma" with an emphasis on the obstruction produced by mucosal oedema.

Bronchial hyper-reactivity

Bronchial reactivity, or responsiveness, is the term used to describe the tendency of the airways to broncho-constrict in response to certain triggers. These triggers may be specific, such as allergens, or non-specific (non-allergic). Non-specific triggers encompass a wide variety of stimuli including responses triggered by chemicals, such as inhaled histamine or methacholine, and physical stimuli like exercise or cold air hyper-ventilation. Bronchial responsiveness to specific (allergic) stimuli is difficult to quantitate because commonly available allergen extracts are not well standardised for either the number or concentration of allergens included. Non-specific responsiveness can be quantitated by inhalation tests with histamine or methacholine, by exercise or by isocapnic hyperventilation of cold air.

Bronchial hyper-reactivity (hyper-responsiveness) is the increased bronchial response to stimuli that can be observed in many asthmatic subjects. Bronchial hyper-reactivity appears to be a consistent feature, important in the pathogenesis of asthma (Boushey. et al 1980, Chatham M. et al 1982).

Bronchial hyper-reactivity in patients with asthma has been known for about seventy years, since Alexander and Paddock (1921) observed that subcutaneous injection of pilocarpine produced asthmatic attack in patients with asthma but not in normal subjects.

Although Dale and Laidlaw (l910) showed that histamine causes contraction of smooth muscle *in vitro*, no study of the effects of histamine on airway smooth muscle *in vivo* was taken until Weiss et al (1929) discovered that intravenous infusion of small amounts of histamine precipitated attacks of broncho-spasm and a decrease in vital capacity. Weiss et al (1932) showed subsequently that in normal subjects, infusion of

histamine caused no change in breathing pattern or vital capacity even when the doses of histamine were large enough to cause severe flushing, nausea, headache and a decrease in arterial blood pressure.

Since these early demonstrations of bronchial hyper-reactivity in response to pilocarpine and histamine, other drugs, including serotonin (Herxheimer 1953; Panzani, 1962; Hajos, 1962), bradykinin (Herxheimer and Stresemann, 1961; Varonier and Panzani, 1961), prostaglandin F2α (Mathe, 1973), and various cholinergic agonists such as methacholine (Curry, 1947; Starr, 1933) and carbachol (Dautrebande and Phillipa, 1941) have also been noted to provoke exaggerated broncho-constriction in patients with asthma. Other stimuli, such as dust (Dubois and Dautrebande, 1958), cold air inhalation (Wells et al, 1960), sulphur dioxide (Nadel et al, 1965), exercise (McNeill et al, 1966) and rapid respiratory manoeuvres (Simonsson et al, 1967) also cause broncho-constriction in susceptible persons. In general, subjects who are hyper-reactive to one stimulus also show a greater response to other constrictor stimuli (De Vries et al, 1964; Laitnen, 1974).

Bronchial reactivity has been shown to be reproducible in any individual patient (Itkin,1967 ; Spector & Farr, 1975). There are, however, variations in the degree of reactivity within any particular subjects. De Vries et al (1962) showed that there is a diurnal variation, with bronchial responsiveness being greatest at night, a time when pulmonary function is often worst. In patients with a tendency to asthma reactivity is greatest during or immediately after an attack of asthma and diminishes as there is clinical improvement (Kerrebijn, 1970; Geubelle et al, 1971). Reproducibility is influenced by recent acute respiratory infections. (Parker et al, 1965; Empey et al, 1976), by recent allergen exposure (Altounyan, 1970), by the overall severity of asthmatic symptoms (Makino, 1966; Muranaka et al, 1974; Cockcroft et al, 1977a),

and by the baseline lung function (Makino, 1966; Benson, 1975; Cockcroft et al, 1977a). For exercise, the response is most reproducible when the interval between tests is less than a week (Silverman & Anderson, 1972).

Mechanisms of Bronchial Hyper-reactivity

There have been a number of hypothesis to explain the mechanism of bronchial hyper-reactivity:

1. Starting airway calibre

It has been suggested that the hyper-reactivity of the airways in disease is due primarily to the fact that the airways are narrower in the control state before bronchial provocation (Benson, 1975). Some studies have shown a correlation between the magnitude of the response to broncho-active substances and the severity of baseline airway obstruction (Parker et al, 1965; Makino, 1966), but it is unclear whether this was due to the severity of the mechanical obstruction or the severity of the underlying disease process. Differences in airway calibre, however, cannot be the sole cause of hyper-reactivity because many subjects with normal airway calibre have developed hyper-reactivity (Empey et al 1976; Holtzman et al 1979). Studies in asthmatic subjects have generally failed to show a relationship between starting airway calibre and non-specific bronchial hyper-reactivity (Ryan G. et al 1982, Rubinfield & Pain 1977). However, these negative studies do not exclude an important role for starting airway narrowing as a contribution to NSBH and theoretical calculations suggest that minor and possibly unmeasurable differences in initial airway sise may have a profound influence on the subsequent response (Moreno et al 1986; Löwhagen and Lindholm 1983).

2. Changes in Airway Smooth Muscle

Hypertrophy and hyperplasia of airways smooth muscle occur in patients with asthma (Huber & Koessler, 1922; Dunhill, 1960; Takizawa & Thurlbeck, 1971) and it is possible that the increased amount of muscle may be capable of developing greater tension and narrowing the airways more than muscle present in smaller amounts. No experimental model of airway smooth muscle hypertrophy exists, but studies of smooth muscle in blood vessels indicate that an increased mass of smooth muscle can increase the thickness of the vessel wall and, by changing blood vessel geometry, can also increase vascular reactivity (Folkow, 1971). Increases in the amount of airway smooth muscle and of airway wall thickness probably contribute to the increased airway responsiveness of severely asthmatic subjects. However, such a mechanism is unlikely to explain the hyperactivity that occurs transiently in normal human subjects during viral infections (Parker et al, 1965; Empey et al, 1976) or after exposure to oxidising pollutants (Golden et al 1978; Holtzman et al, 1979; Orehek et al, 1976) because the mass of smooth muscle is unlikely to change in so short a period of time.

3. Changes in Autonomic Regulation

The autonomic innervation of the airways includes sympathetic, non-adrenergic inhibitory, and parasympathetic nerves and is equipped with membrane receptors for a wide variety of circulating excitatory and inhibitory substances (Boushey et al 1980). Alpha-adrenergic and muscarinic agonists contracts airway smooth muscle, whereas beta-adrenergic agonists and activation of non-adrenergic inhibitory nerves relax it. Bronchial hyper-reactivity could then arise as a consequence of an alteration of the balance between relaxation and constriction.

Parasympathetic Nervous System

The parasympathetic efferent nerve supply to the airways is via the vagal nerves. Preganglionic vagal fibres travel from the central nervous system to parasympathetic ganglia located in the walls of the airways, and post ganglionic fibres from these ganglia extend to airway smooth muscle. The suggestion that parasympathetic nervous pathways may play a role in asthma arose from the fact that the muscarinic antagonist atropine can often relax airway smooth muscle as much as drugs that act directly on the muscle (Cropp, 1975). The hypothesis that exaggeration of parasympathetic reflex activity could be responsible for exaggerated responses in patients with asthma was developed from observations that many of the stimuli that produce broncho-constrictor responses in patients with asthma also stimulate vagal sensory nerve endings (Simonsson et al, 1967). Several studies have confirmed the inhibitory effects of atropine on the exaggerated broncho-constrictor responses to histamine (Holtzman et al, 1980) and sulphur dioxide (Sheppard et al, 1980). However, other studies have reported that muscarinic antagonists such as atropine were either ineffective or only somewhat effective in inhibiting the responses of patients as reviewed in (Boushey et al, 1980; Boulet et al, 1984).

Recently it has become clear that the major effect of histamine is directly on smooth muscle (Sheppard et al, 1984; O'Byrne et al, 1985). Because the study of hyperactivity in patients is complicated by factors such as hypertrophy and hyperplasia of airway smooth muscle, airway obstruction and treatment with drugs, and because certain types of studies are not possible in patients, the study of hyper-reactivity would be advanced if appropriate models, human or animal were available. Unfortunately, studies have been hampered in the past by the lack of an animal model and by the inability to produce hyper-reactivity in healthy subjects. For these reasons it is of interest that viral respiratory infection or exposure to ozone can rapidly produce

reversible hyper-reactivity in otherwise healthy subjects (Empey et al, 1976; Holtzman et al, 1979) and in animals (Laitinen et al, 1976; Lee et al, 1977). The increased broncho-constrictor responses are inhibited by atropine in humans and by vagotomy in animals. These findings together with the findings in asthma patients, imply that parasympathetic pathways are at least partly responsible for the increased responses. It is possible not only that the different types of autonomic systems may cause hyper-reactivity, but also that within each system different parts of the nervous pathway may be altered. For example with the parasympathetic nervous system, it is possible that changes in the sensory nerve endings, the vagal motor pathway including ganglia and post ganglionic nerves, or the muscarinic receptors on the smooth muscle may all be altered in hyperactivity.

Sympathetic Nervous System

The study of sympathetic nervous activity is complicated because there are several different levels at which changes in activity can occur. Thus, not only alpha, and beta adrenergic effects but also α -1, and α -2 and β -1, and β -2 effects have been described, and these effects may vary in different end-organs. In addition, sympathetic nervous activity may affect smooth muscle not only directly, but also through modulation of cholinergic nervous activity (Vermiere and van Houtte 1979). Also, there can be direct effects of neuronally released catecholamines and indirect effects from circulating catecholamines.

One of the most widely held hypotheses to explain airway hyper-responsiveness is that it is caused by decreased responsiveness of β-adrenergic receptors, a hypothesis that rests primarily on evidence from guinea pigs, rats and mice (Szentivanyi, 1968). The importance of decreased β-adrenergic activity in human airways has been explored by studying the effects of β-adrenergic antagonists such as

propranolol as reviewed in (Boushey et al, 1980). In healthy human subjects, propranolol has been reported to cause no broncho-constriction or only mild broncho-constriction. By contrast, patients with asthma may develop severe broncho-constriction after propranolol, but the fact that broncho-constriction can often be reversed by atropine suggests that the broncho-constriction is actually caused by unopposed parasympathetic activity. The small effect of beta-adrenergic blockade on intact parasympathetic innovation, and the sensitivity of asthmatic patients to exogenously administered beta-adrenergic antagonists and agonists all make the hypothesis that beta-blockade causes hyper-reactivity unlikely.

Hyper-reactivity could also be due to increased alpha-adrenergic activity. Although alpha-adrenergic responses are normally weak or absent, pre-treatment with histamine increases broncho-constriction responses to alpha-adrenergic agonist (Kneussl & Richardson, 1978). Limited studies of isolated muscle suggest that alpha-adrenergic activity is increased in some disease states (Henderson et al, 1979). However, additional studies will be required to determine the role of this system in the hyper-reactivity of patients with asthma.

Non-Adrenergic Inhibitory Nervous System

Classically, the autonomic nervous system has been described as consisting of two components, the cholinergic and adrenergic. For sometime, however, the gastrointestinal tract has been known to be innervated by autonomic nerves of another type that, when stimulated, cause smooth muscle relaxation (Crema et al, 1968; Burnstock & Costa, 1973). An absence of this system occurs in Hirschsprung's disease (Frigo et al, 1973), and may be responsible for the characteristic defect in smooth muscle control (Hukuhara et al, 1961). Because the tracheo-bronchial tree arises from the ventral wall of the foregut, it has been suggested that a similar system

exists in the lungs (Burnstock, 1972), and that a loss of its inhibitory action might explain the abnormal response of airway smooth muscle in bronchial hyper-reactivity (Richardson and Bouchard, 1975). Subsequent in vitro studies have demonstrated the presence of this broncho-dilating system in the airways of most mammals, including man (Boushey et al, 1980; Coburn and Tomita, 1973; Richardson, 1976; Davis, 1982) and the intriguing possibility has been suggested that a defective non-adrenergic inhibitory system may be responsible for non specific bronchial hyper-reactivity (Boushey, 1980). In airway tissue from a small number of patients with chronic obstructive pulmonary disease and varying non-specific bronchial reactivity *in vivo*, the magnitude of non-adrenergic smooth muscle relaxation was small in comparison to b-adrenergic relaxation and showed no correlation with *in vivo* responsiveness to methacholine (Matsumoto et al, 1985; Taylor et al, 1985). Maximal non-adrenergic inhibitory system (NAIS) stimulation produced only 10 to 20 percent of maximal theophylline-induced relaxation in human tissue, whereas it accounted for 70 percent of maximal relaxation in the guinea pig airway (Taylor et al,1984).

Taylor et al (1984) have been able to demonstrate the presence of the non-adrenergic inhibitory system in human subjects *in vivo*, and they have also quantified its importance in broncho-dilation and compared its effectiveness in normal and asthmatic subjects (Michoud et al, 1987; Taylor, 1984; Michoud et al, 1988). They mechanically stimulated the larynx during histamine-induced broncho-constriction and showed an abrupt but transient fall in pulmonary resistance that was not mediated through the beta-adrenergic system. Although these results clearly demonstrate the presence of non adrenergic broncho-dilation, there was incomplete relaxation of the airway smooth muscle and no significant difference in the effectiveness of the broncho-dilation between normal and asthmatic subjects (Michoud et al, 1988). At present, a clear picture of the possible role of deficient non-adrenergic inhibitory innervation in the

pathogenesis of non specific bronchial hyper-responsiveness awaits the definitive characterisation of the neuro transmitter and the development of a specific antagonist.

4. Epithelial Damage

Morphologic abnormalities of airway epithelium have been described before in asthma (Glynn and Michaels, 1960; Cunnill, 1975) and it has been suggested that damage to the airway epithelium might play an important role in causing airway hyper-reactivity (Nadel, 1973).

Human and animal studies have shown that viral respiratory tract infections and ozone that cause reversible airway epithelial damage (Hers and Mulder, 1961; Boatman et al, 1974) also result in transient airway hyper-reactivity (Empey et al, 1976; Golden et al, 1978; Holtzman, 1979). Because the various stimuli that cause exaggerated broncho-spasm such as dusts, sulphur dioxide, histamine, all stimulate sensory receptors in the airways with subsequent vagal reflex broncho-constriction in animals (Widdicombe et al, 1962; Nadel et al, 1965; De Kock et al, 1966) it was suggested that damage to the airway epithelium might sensitise these sensory receptors and thus cause exaggerated reflex responses (Nadel 1973). In the airway, the structural change responsible is not known but might be a subtle disturbance in some subcellular component such as the tight junctions between epithelial cells. It has been observed that vagal sensory nerve endings lie beneath the tight junctions of the airway epithelium and damage to the tight junctions could sensitise these receptors and result in exaggerated reflex responses (Widdicombe, 1977).

Increased Bronchial Mucosal Permeability

Epithelial damage might also cause airway hyper-reactivity by increasing airway permeability, thereby allowing higher concentrations of inhaled materials to reach

receptor sites on smooth muscle. For any inhaled substance to affect receptor sites it must cross a layer of respiratory tract secretions, bronchial epithelium and sub-mucosa to reach the receptor sites on smooth muscle. Similarly, agents that cause reflex broncho-constriction through the network of irritant nerve endings must penetrate the bronchial epithelium through tight junctions between epithelial cells (Hogg, 1981). The effectiveness of these barriers may depend on characteristics of the inhaled material such as size, molecular configuration and charge (Diamond, 1978). The amount and concentration of drugs that reaches the smooth muscle will be dependent on the balance between penetration of these barriers and the removal of drug in the bronchial vasculature and lymphatics and by enzymatic degradation. Although animal studies have shown that non-specific bronchial reactivity increases with epithelial damage induced by cigarette smoke (Hulbert et al, 1985) and antigen exposure (Boucher et al, 1979), studies in humans have shown no relationship between airway mucosal hyperpemeability and non-specific bronchial hyper-reactivity (Elwood et al, 1983; O'Byrne et al, 1984).

Methods of Measurement of Bronchial Reactivity

Bronchial reactivity is usually measured by testing the bronchial reaction to increasing doses of an inhaled pharmacologic agent (e.g. histamine or methacholine), or by non-pharmacologic stimuli (e.g. exercise). These three stimuli are commonly used and are becoming increasingly valuable in clinical practice and in research. Other stimuli (e.g. cold air challenge) are useful but are less commonly available.

The generally accepted method of administration of drugs is by inhalation (Curry et al 1946; Kang et al 1976) because it gives an immediate response through direct local action and has a low risk of side effects or severe reactions compared with injections. Most investigators vary the dose of the agonist by increasing concentrations of the test substance while keeping the volume of inhalation constant. A cumulative dose-response relationship is obtained if the drug is administered within the duration of action of the previous dose.

Inhaled histamine acts on the bronchial smooth muscle by direct action and by stimulation of the sensory receptors that cause reflex broncho-constriction (Casterline et al 1976; Widdicombe G. 1977; Woenne et al 1978; Cockcroft et al 1978). Because histamine has a relatively short-lasting action, cumulative responses can be prevented if intervals of three to five minutes are allowed between inhalations (Orehek et al 1976).

Methacholine (Acetylcholine derivative) as a parasympathomimetic agent produces its effect by acting on the muscarinic receptor. It is slowly inactivated by cholinesterase and therefore has a relatively long-lasting effect. Hence cumulative dose response curves will be obtained after serial inhalations with short inter-does intervals.

Solutions of both histamine phosphate and methacholine are relatively stable and can be stored at 4 °C for at least several weeks. Responsiveness to methacholine is similar to responsiveness to histamine when compared under carefully controlled conditions (Juniper et al 1978; Juniper et al 1981; Salome et al 1980). Because they correlate fairly closely either agent can be used for the assessment of bronchial reactivity.

In carrying out inhaled challenge tests it is important to control factors known to influence the measurement of airway reactivity. Technical factors that influence bronchial reactivity include methods of aerosol generation including nebuliser output, and volume and speed of inspiration, preparation and handling of test solutions, pH and temperature (Cockcroft and Berscheid, 1982; Lewis and Tattersfield, 1980; Ruffin et al, 1978; Ryan et al, 1981; Empey, 1976). Methods of measurement and the expression of results can also affect the final results (Fish and Kelly 1979, Orehek et al 1979).

Non-technical factors include medication because drugs administered prior to the test may change reactivity. Drugs (such as broncho-dilators and anti-histamines) should, if possible, be discontinued for at least 12 hours before the test or longer depending on their duration of action. Non-broncho-dilator medications which have no immediate effect on the response (steroids, sodium cromoglycate) can be continued in the same dose (Cockcroft et al 1977(b)). Airway calibre also influences the results of bronchial reactivity. Because of the circadian rhythm in airway calibre, in an experimental study reproducibility will be greatest if the test is performed at the same time of day (Devries et al 1962; Benson 1975). It must also be recognised that respiratory tract infections and allergen exposure will change the bronchial reactivity if it is measured shortly after

such recent exposure (Empey et al 1976; Cockcroft et al 1977 (c)).

Tests to measure bronchial reactivity should be as simple as possible to facilitate routine clinical use and should be standardised to allow results to be compared from different times and from different laboratories.

Various methods has been used to test the level of bronchial reactivity. The most widely used is the method described by Cockcroft (Cockcroft et al 1977(a), Juniper et al 1978). This was modified from the technique of De Vries 1962 who used 30 second inhalations of doubling concentrations of histamine acid phosphate up to 32 mg/ml. The modified method employs two minute inhalations of increasing concentrations up to 8 mg/ml of histamine acid phosphate or methacholine. Aerosols are generated continuously with a Wright nebuliser containing 3 ml of solution at room temperature. Nebuliser output is kept constant at 0.13 to 0.16 ml/min. The presumed degree of bronchial reactivity, based on previous measurements or the severity of disease, may make it possible to start with a concentration higher than 0.03 mg/ml and thereby shorten the test. The doses are given at five minute intervals. Inhalations are discontinued when a significant bronchial constriction is induced. Bronchial response is usually assessed by measuring forced expiratory volume in one second (FEV), before and at 0.5 and 1.5 minutes after each inhalation. The test is stopped when there is either a 20% or greater fall in FEV_1. or when the maximum concentration of histamine used is reached. The level of non-specific bronchial reactivity is calculated as the concentration of inhaled agonist that results in 20% fall in FEV, (PC_{20}) obtained from interpolation from the measured dose response curve.

Exercise is another very commonly used challenge for the assessment of airway responsiveness. Strenuous exercise for 6 minutes induces in many asthmatic children

a short period of broncho-dilation followed at the end of or shortly after the completion of the exercise by a significant degree of broncho-constriction (EIA), which lasts for 15-30 minutes. During an exercise test the child performs at one workload only. In a series of studies varying the duration and severity of running in a group of asthmatic children it was shown that the maximum degree of airway narrowing occurred when the children were exercising between 60 to 85% of their predicted maximum oxygen consumption (Silverman & Anderson 1972; Godfrey 1974). Since direct monitoring of the oxygen consumption is seldom available in laboratories, the metabolic load is usually assessed by heart rate. An increase in the heart rate with exercise to 90% of the predicted maximum for age is comparable to 60-85% of the maximum oxygen consumption.

There are three factors which have been shown to be important in influencing the response to exercise: the nature of the exercise; its severity and duration. Different forms of exercise have been studied in the same asthmatic subjects at the same workload. These studies showed that running induced more broncho-constriction than cycling and swimming and walking were even less effective in causing post exercise fall in PEF (Anderson et al 1971; Fitch & Morton 1971; Silverman & Anderson 1972). Studies have also been carried out in a group of asthmatic children to investigate the effect of altering the severity (by means of increase in gradient) or the duration of exercise (Silverman & Anderson 1972). It was found that maximum airway narrowing occurred when the exercise was performed for a period of 6-8 minutes running up a slope of 10-15% gradient at a constant speed of 5 kph. When the duration of exercise was increased to longer than 8 minutes, less broncho-constriction resulted (Eggleston & Guerrant 1976). This suggests that some subjects can "run through" their asthma if they do not stop exercising. This may arise because the release of broncho-constrictor mediators during exercise is opposed by increased sympathetic drive, and , if the

exercise is prolonged, the constrictor mediators are metabolised before the end of the exercise when sympathetic drive normally falls.

Standardised steady state running gives the most reproducible responses. The reproducibility of (EIA) under laboratory conditions is well defined with a coefficient of variation for the percent fall in PEF or FEV_1 is between 19-25% when an interval of more than two hours and fewer than 28 days elapses between tests (Anderson et al 1975; Anderson and Schoeffel, 1982).The results of multiple running tests in the same asthmatic subject over a period of up to one year have shown that the coefficient of variation was least (21 percent) when the tests were carried out within one week, compared to 53 percent when tests were repeated at monthly or longer intervals and 31 percent when tests were performed within one day (Silverman & Anderson 1972). Standard running was more reproducible than cycling.

Controlling the three factors influencing the exercise will help to standardise the exercise test and make the results more comparable (Silverman & Anderson 1972; Eggleston & Guerrant 1976). As with the histamine challenge, circadian rhythm and drug treatment may influence the result of the exercise test. The potential effect of the pre-exercise level of lung function on the severity of EIA has been the subject of controversy. There appears to be no significant relationship between the value for PEF or FEV_1, before the exercise and the degree of airways narrowing after the exercise (Pierson et al 1969; Silverman 1973; Konig,1974). It has been suggested, however, that the greatest falls in FEV_1, occur in those subjects whose resting values are between 70-80% of the predicted normal (Jones et al 1962). Also it has been argued that the broncho-constrictor response will be greater when the airways have increased tone prior to exercise challenge (Benson, 1975).

The response to exercise can be quantified by various lung function indices, but the simplest and the more reproducible is by lung function measurement of PEF and FEV_1. In individual patients with asthma, the degree of broncho-constriction in response to either exercise or hyperventilation depends on the level of non-specific bronchial responsiveness (Nejens et al, 1981; Weiss et al, 1983; Mellis et al, 1978; Hodgson et al, 1984; O'Byrne et al, 1982). In fact, the responses correlate with non-specific bronchial hyper-reactivity so closely that exercise has been suggested as a test of bronchial reactivity although it does not appear as sensitive as sensitive as histamine or methacholine in spearating normal from asthmatic subjects (Chatham, et al 1982).

A measurement of non-specific bronchial hyper-responsiveness is particularly useful clinically in patients with isolated symptom of chronic cough which can be the sole symptom in some patients with asthma, or in patients who are suspected of having asthma and who complain of breathlessness on or after exertion.

Variability of Peak Flow in Asthma

As noted in the opening historical overview, the early physicians not only recognised that asthma was episodic they also recognised that the symptoms were often most troublesome at night. Thus the ideas of nocturnal asthma and of diurnal variation in airway function as a key feature of asthma have also had a long and distinguished lineage.

In the present century, the aetiology of nocturnal asthma remained unknown until the rhythm in airway calibre was identified in normal subjects (Hetzel and Clark, 1980). In 1951, Israels (1951) demonstrated rhythms in FEV_1 and FVC in asthmatic and bronchitic subjects with an acrophase at 1200. Lewisohn et al (1960) compared an increase in normal subjects of 2.9% in FEV_1 between 0600 and 0800 hours with an increase of 36-60% in patients with airways obstruction. More detailed studies were prompted by the need to exclude the effects of circadian variation in lung function in research work. In normal subjects there is a rise of 0.15 l in FEV_1 in the morning with a subsequent fall of 0.05 l in the afternoon (Guberan et al 1969). $FEV_{0.75}$ falls by 10% in the afternoon (McKerrow et al 1958), and a rhythm in airways resistance shows an acrophase between 0500 and 0800 (McDermott, 1966; De Millas and Ulmer 1971). Studies of subjects in environmentally controlled chambers over six-day periods show a normal rhythm in specific conductance which rises from a mean between 0400 and 1200 hours and mean changes of 0.305 l in functional residual capacity (FRC), 0.17 l in total lung capacity (TLC) and 0.2 l in residual volume (RV) over the 24 hour cycle (Kerr, 1973). Rhythms have also been described in dynamic compliance (Gaultier et al 1977), ventilatory response to CO2 and gas transfer factor (Cinkotai and Thomson, 1966).

Rhythms can be described by their period, amplitude and phase (Figure 1). The period is accepted as 24 hours if the period is entrained to the normal solar day. Amplitude can be most conveniently described as the difference between the highest (acrophase) and the lowest (bathyphase) values in the cycle. Phase is most frequently quoted in degrees with the 24 hours equivalent to 360°.

The normal variability in airway calibre can be most easily studied using a peak flow meter. To establish a picture of this variability single measurements of peak flow rate are inadequate because such random measurements of lung function made in the clinic or at home only reflect the severity of asthma at that moment and gives no indication of the change in severity with time. In order to capture the variability with time multiple measurements of peak flow rate are necessary (Prior and Cochrane, 1980; Connoly and Godfrey; Fallieys et al, 1966). Increasingly, regular monitoring of peak flow has come to be recognised as useful in the diagnosis and assessment of asthma and important in monitoring the response to treatments.(Hetzel, 1981; Muray et al, 1977; Prior and Cochrane, 1980). Children can manage regular peak flows from about 5 years of age.

Hetzel and Clark (1980) pioneered the use of the more sophisticated statistical techniques, of cosinor analysis in analysing rhythms in lung function. Cosinor analysis is a versatile computer technique which enables the phase of the rhythm to be calculated and can detect low amplitude rhythms. It has been widely used in chronobiology. It considers a mathematical model of the circadian rhythm which is best approximated by a sine or cosine wave (Figure 1) with a peak to trough amplitude (a), about a mean value (m), and a period (p), which is usually assumed to be 24 hours. The programme determines the significance, amplitude and phase of the sinusoid which best fits the raw data. Unfortunately, cosinor programmes are not generally

available but regression programmes can be adapted for a sinusoidal regression of PEF upon time. A least squares method is used to estimate the goodness of fit of the raw data to the model. Phase is conventionally expressed as the timing of the acrophase. Amplitude a, is the radius which describes the sinusoid - that is a/2. The peak to trough amplitude, a, has more clinical application and would be identical to the diurnal fall in PEF. The amplitude is most usefully expressed as a percentage of the mean - a/m%.

Measurement of circadian variation in PEF, either as diurnal swings (a/h) or as an amplitude (a/m) is best made in these percentage terms since this allows some comparison between patients with differing degrees of airways obstruction and with normal subjects.

Hetzel and Clark used peak flow monitoring to demonstrate and measure a rhythm in airway calibre over 24 hours. They found that in adults if a normal subject's mean peak expiratory flow rate is calculated from recordings made four times a day for a week, the amplitude (peak to trough is of the order of 8% of the mean value (SD 5%) (Hetzel and Clark, 1980). Using this approach, Hetzel and Clark found a statistically significant rhythm was present in the majority of normal adult subjects (65.6%) who showed a mean amplitude of 8.3% of individual mean PEF (+ SD 5.2%). They also demonstrated a much greater amplitude in asthmatic adults. Asthmatic rhythms were similar in phase to the normals in that the peaks of the cycle occurred at similar times in the afternoon, at 1557 and 1526 respectively. These findings led Hetzel and Clark to propose that a value of > 20% in amplitude might be a useful screening test for the diagnosis of asthma.

From these findings, nocturnal asthma can therefore be viewed as an exaggeration, through increased bronchial lability, of the normal rhythm in airway function. Indeed, this increase in variability of the level of airway obstruction is recognised now as one of the hallmarks of asthma varying both from day to day and in the longer term over weeks months and years.

Hetzel (1981) has pointed out the confusion that has arisen as a consequence of different methods of analysis of the rhythm in PEF . The simplest method merely measures the morning fall in PEF as a percentage of the highest daily reading (a/h%); the diurnal swing or fall in PEF. This is a useful approach clinically since the diurnal fall can be judged accurately on simple inspection of a peak flow chart. If the PEF amplitude is low however, the direct approach is liable to misinterpret biological noises as rhythm.

Variability of Peak Flow in children

Is there any evidence of increased variability in airway function and similar diurnal rhythms in PEF in children?

Johnston, Anderson and Patel (1984) studied variability in PEF in 64 nine to eleven year old asthmatic children and applied the cosinor analysis technique. They found a mean amplitude of 12%.

Sly et al (1985) studied diurnal variation in PEF in 68 asthmatic children and demonstrated that a diurnal variation of PEF exists in asthmatic children. They found a mean PEF amplitude derived from cosinor analysis of 22.6% of the mean value.

However, these two studies using cosinor analysis involved only asthmatic children and no normal children were included.

Usherwood and Barber (1986) studied both asthmatic and control children. They measured daily variation in PEF twice daily (calculated as the difference between the highest and lowest daily values expressed as a percentage of the highest daily value) at home for a week without the use of cosinor analysis. They found a mean daily variation in asthmatic children of 7.1% (± SD 3.4%) while the mean daily variation for control subjects was 4.4% (± SD 2.0%). However, this simpler technique of measuring diurnal variability may underestimate the true amplitude of variability because it only measures variability from the times measured. Cosinor analysis has the advantage of being able to estimate the peak to trough amplitude even if the peak or trough occurs at times different from the time of measurement.

Only one other study by Henderson and Carswell (1989), studied both asthmatic and control children using cosinor analysis. This study found an amplitude of 6.2% in the asthmatic and 4.2% in the control children, much lower than that found in adult asthmatics and lower than those values of Johnson et al quoted above.

There is, therefore, only a limited amount of information available with rather conflicting conclusions about the extent of variability present in normal and asthmatic children. However, there is no information available about the relation between bronchial responsiveness measured by bronchial challenge tests and diurnal variability estimated by cosinor analysis.

AIMS AND HYPOTHESES

Background to research

The present study set out to measure bronchial responsiveness by histamine and exercise challenge tests and compare the responsiveness measured with estimates of diurnal variation in PEF as measured by cosinor analysis in a group of asthmatic children of varying severities and normal controls. We aimed to investigate the relationship between these two quantities and to investigate the relationship with asthma severity.

Hypotheses tested

1. Asthmatic children have a greater degree of bronchial responsiveness as measured by non specific-challenge tests than normal subjects.

2. Children, both asthmatic and normals, have evidence of a diurnal rhythm in PEF and this diurnal variation is greater in asthmatics than in normals and increases in severity with increasing severity of asthma.

3. There is a correlation between bronchial reactivity and diurnal variation in PEF.

Questions posed

1. Do asthmatic children have a greater degree of bronchial responsiveness than normal children? Is responsiveness related to severity of asthma?

2. Is there evidence of a diurnal rhythm in PEF in children?

3. Is the figure 20% or greater variation in peak to trough amplitude as measured by cosinor analysis suggested as a screening test for the diagnosis of asthma in adults (Hetzel and Clark, 1980) applicable to children? Is there evidence that diurnal variation is greater in more severe asthmatics?

4. Is there evidence of a correlation between bronchial reactivity as measured by histamine or exercise challenge test and diurnal variation in lung function as measured by PEF rate?

5. Is there any evidence of a relationship between severity of asthma, bronchial reactivity and diurnal variation in PEF?

METHODS

Plan of Investigation

The basic design of the study was to study two groups of children - a group with asthma and a second group of normal children of similar ages. We planned to perform both a histamine and an exercise challenge tests on two occasions one week apart. Between the two pairs of challenge tests the children were to undertake a period of home peak flow monitoring. For comparative purposes, a larger group of normal children of similar ages were recruited to perform only peak flow monitoring at home for one week.

Subjects

Forty asthmatic children between the age of 7 and 14 years were studied (age 10.4 ± 2.1 yrs, mean ± SD; M = 30, F = 10). All the children with asthma were attending regularly at the respiratory clinic at Yorkhill Hospital, Glasgow and had a history of recurrent, reversible airway obstruction.

The asthmatic children were graded into three groups of severity according to the amount of inhaled medication necessary to control their asthma:

Group I: Mild

Asthma attacks usually infrequent and mild. Long periods without symptoms. Bronchodilators taken as required: 7 children (only 6 underwent challenge testing).

Group II: Moderate

Moderate to severe attacks, often with cough, wheezing and dyspnoea especially at night, particularly during exacerbations. Frequent exacerbations

justifying continuous prophylactic therapy with sodium cromoglycate and bronchodilators: 11 children.

Group III: Severe

Severe perennial asthma with frequent or continuous wheezing with more frequent and severe exacerbations. Not controlled without regular steroids and bronchodilators: 23 underwent challenge testing but only 22 completed peak flow recording.

The number of children in each group roughly reflected the relative proportions of each severity in the population of asthmatic subjects attending the respiratory clinic at the Royal Hospital for Sick Children. Demographic details for the subgroups are shown in Table 1. Age and duration of asthma were similar in the three asthmatic subgroups.

Ten normal children between the age of 7 to 14 yrs (age 10.8 ± 2.1 yrs; 6 M, 4 F) followed the same protocol as the asthmatic children (baseline lung function and challenge tests, peak flow monitoring for a week, second lung function and challenge tests). All were healthy volunteers with no history of atopic or respiratory illness or any family history of asthma. Seven were children of members of the staff; three were attending an endocrinology outpatient clinic at the Royal Hospital for Sick Children.

A further 35 normal children from the same age group of 7 to 14 yeas (25 M, 10 F) underwent only the week long monitoring of peak flow. Again all were normal healthy children with no history of atopy or respiratory illness nor any family history of asthma. Some were selected from children attending the orthopaedic outpatients clinic in Yorkhill Hospital, Glasgow while others were children of some members of staff.

There were a number of exclusion criteria that were used in selecting children to take part in the study:

1. No upper respiratory tract infection in the preceding 4 weeks.
2. No exacerbation of asthma in the previous 4 weeks.
3. No known allergy or undue sensitivity to histamine.
4. FEV_1 greater than 70% of the predicted mean.

Lung Function

All children underwent baseline lung function testing. Peak expiratory flow (PEF) and forced expiratory volume in one second (FEV_1) were measured using a hand-held electronic turbine spirometer (Spirocheck, Morgan). Baseline PEF prior to testing was within 85% of predicted mean values in all but four asthmatic patients (all above 70% of predicted mean). Predicted values for spirometry and PEF were taken from the published data of Godfrey, Kamburoff and Nairn (1970).

For the lung function, all children (asthmatics and controls) were asked to blow as hard as possible into the machine after maximal inspiration. This was repeated three times and the highest of the three values was recorded. The measured value of FEV_1 was multiplied by 1.080 to correct to B.T.P.S. (body temperature and pressure saturated with water). All were standing but nose clips were not used.

In an attempt to minimise changes due to diurnal variation all children, asthmatics and controls, underwent lung function tests and challenge tests at the same time of day. Baseline lung function was checked followed by a histamine challenge test at approximately 10.00 and an exercise test later in the morning at approximately 11.30 on any given morning. The respiratory laboratory was air conditioned and temperature and humidity were kept constant from day to day.

Challenge Tests

General preparation before challenge testing

Asthmatic children undergoing challenge tests were given specific instructions about their inhaled medication before testing. Before attending for challenge testing, they were asked to stop temporarily inhaled sodium cromoglycate therapy for 24 hours; slow release theophyllines, if taken, were withdrawn 48 hours before testing. Subjects were asked not to take any broncho-dilators on the morning of the study. Inhaled steroids, which have no short term bronchodilator effects and no effects on the immediate asthmatic response, were continued. Only 1 child was studied each day.

Histamine challenge testing

Histamine inhalation tests were carried out in all 40 asthmatic children and in 10 controls. All children began by performing baseline lung function. FEV_1 values at the time of the study were above 85% of the predicted normal except in 4 patients where the FEV_1 was greater than 70% predicted. During the challenge tests, FEV_1 and PEF were also measured using the same light weight spirometer (Morgan Spirocheck) four times at the start of the test.

After the initial measurements of baseline lung function, an aerosol of phosphate buffered saline was inhaled as a baseline control. The aerosol was generated continuously by a Wright nebuliser and delivered directly into a mouthpiece. The subjects were asked to breathe normally from the mouthpiece for two minutes using tidal breathing (Figure 2). While breathing from the nebuliser, the children were seated and were wearing nose clips throughout.

Nebuliser output is one of the factors which can influence the results of histamine challenge testing and, therefore, needs to be controlled for. Nebuliser output can vary between nebulisers of different types and even between nebulisers of the same type. For the purposes of this study, nebuliser output was determined as follows: 3 mls of normal saline was placed in each of several nebulisers which were then driven by compressed air at a constant pressure but different flow rates. Flow rates were measured by a calibrated flow meter. Each nebuliser was operated for 2 minutes. The output was determined by measuring the change in weight before and after the period of operation. Two nebulisers with outputs of 0.13 ml/min at flow rate of 8 litres/min were selected and were then used for all studies. To decrease further the possibility of variation, the identical nebuliser was used for both challenge tests in every subject.

Although particle size generated by many nebulisers is known to vary (usually within a range of 1.3 to 3.6 μm aerodynamic mass median diameter) particle size has not been shown to change the broncho-constrictor response (Ryan et al 1981). We did not attempt to evaluate the particle sizes produced by the nebulisers used.

The control aerosol was followed at five minute intervals by inhalations of doubling concentrations of histamine acid phosphate from 0.3 up to 8.0 mg/ml. The broncho-constrictor response was measured by FEV_1 immediately before each inhalation, and at 30 and 90 seconds after. Subsequent inhalations were discontinued when there had been a fall of 20% or more below the lowest post saline value or after the highest concentration had been inhaled.

The percent reduction in FEV_1 was calculated for each histamine concentration from the lowest post saline value to the lowest histamine value at each concentration. Dose response curves were plotted on a log scale by hand drawing the curve through all the

points. The Pc_{20} (Histamine provocation concentration causing a 20% fall in FEV_1) was then calculated from the curve as shown in Figure 3.

Exercise Testing

Standardised exercise testing requires the use of either bicycle ergometer or treadmill. The rate of working on a treadmill depends not only on speed and slope of the treadmill but also on the subject's weight and his pattern of running or walking and is not as precisely controllable as in bicycle ergometry. However, in general, treadmill running machine is more suited to children as it provides a more natural form of exercise. It is also more reproducible in children than bicycle ergometry (Silverman and Anderson 1972). Treadmill exercise was selected for use in these studies.

The treadmill used in this study was a Powerjog M-10 (Cardinal Sports Engineering Limited, Figure 4). This machine is particularly suitable for studying children because it is an appropriate size, runs quietly, is not frightening, and is fitted with a safety bar in case the child stumbles. The treadmill was calibrated by checking the belt speed and slope indicators by direct measurement.

The treadmill speed and slope were adjusted so that the children were exercising at a level of work corresponds to about 2/3 of their maximum oxygen consumption, judged by heart rate response. This level of exercise has been shown by Silverman and Anderson (1972) to induce maximum exercise-induced broncho-constriction in a susceptible person.

Protocol for Treadmill Exercise

Heart rate was monitored continuously throughout the study using a heart rate monitor (Roche Monitor 123-102) to determine the resting heart rate and to monitor the desired level of heart rate during the 6 minute period of exercise. Before starting, the treadmill gradient was increased to 10 per cent. At the start, the treadmill speed was quickly increased to between 5 km/hr and 8 km/hr to a speed sufficient to raise the subjects heart rate to 170 - 180 beats/min.

Lung function measurement during treadmill exercise tests

PEF and FEV, was measured at 2, 4, 6, 8, 12, 16 minutes during and after exercise using the hand-held electronic turbine spirometer (Spirocheck, Morgan).

Measuring the response to exercise

The response to exercise was expressed as a percentage change in Peak Expiratory Flow Rate (PEF) by calculating the following indices:

$$\text{(i) Initial PEF\%} = \frac{\text{Pre-exercise PEF x 100\%}}{\text{Predicted PEF}}$$

$$\text{(ii) \%Fall in PEF} = \frac{\text{Pre-exercise PEF - Lowest PEF x 100\%}}{\text{Pre-exercise PEF}}$$

$$\text{(iii) Lability Index} = \frac{\text{Highest overall PEF - Lowest overall PEF x 100\%}}{\text{Predicted mean PEF at rest}}$$

Diurnal Variation in Peak Expiratory Flow Rate

All children were instructed in the use of mini-Wright peak flow meter (Hetzel et al 1979; Wright 1978). This has been shown to be a reliable instrument for measuring peak flow rate (Perks et al 1979).

Each subject was asked to perform three forced expiratory manoeuvres and record the highest peak flow value obtained on a diary card (Nairn et al 1961).They were asked to perform this manoeuvre four times daily as close as possible to 8.30 a.m, 12.30 p.m., 4.30 p.m. and 8.30 p.m. These times were chosen to coincide with breakfast, lunch, returning home from school and bedtime.

Asthmatic children were maintained on their usual medication and continued with normal activities during the study period. They were asked to record the measurements before taking any medication for a period of one week.

Calculation of diurnal variation

Daily variation in Peak expiratory flow rate was calculated as follows:-

1. For each day the difference between the evening value and morning value and expressed as a percentage of the mean of the two values for that day (e-m/m%) i.e.:

$$\frac{\text{Evening value - Morning value} \times 100\%}{\text{Mean}}$$

2. For each day the difference between the highest and lowest values, whatever the time of day of occurrence, was calculated and expressed as a percentage of the highest daily value (h - l/ h%):

$$\frac{\text{Highest value recorded - lowest valued recorded x 100\%}}{\text{Highest value recorded}}$$

3. For each day, peak to trough amplitude obtained by cosinor analysis was calculated and then expressed as a percentage of the individuals mean PEF over 24 hours period (a/m%). These daily values were then averaged for the 6 days period:

$$\frac{\text{Amplitude (a) x 100\%}}{\text{mean (m)}}$$

Statistics

Data were summarised using standard descriptive measures (mean, median, standard deviation etc).

The reproducibility of the histamine and exercise challenge tests was assessed using an intra-class correlation test.

Differences between the Pc_{20} in the three groups of asthmatics (classified by treatments) and normal subjects were investigated using a one-way analysis of variance with 95% confidence intervals for all pairwise differences between level means calculated using Tukey's method. This approach was used for each data sets from the two challenge tests and also for the peak to trough amplitude data from cosinor analysis to compare the groups. In the case of the Pc_{20} results the data is logarithmic. The analysis was therefore carried out after a logarithmic transformation.

Because the data for the Pc_{20}, the percent fall after exercise and the cosinor amplitude were not known to be normally distributed the relationship between them

was explored using a non-parametric correlation test, the Spearman Rank correlation test.

The cosinor analysis programme developed by Halberg (Halberg et al 1964; Halberg et al 1965) is a computer technique which uses a least squares method to test the goodness of fit of the raw data to a sinusoidal wave form. It considers a mathematical model of the circadian rhythm which is best approximated by a sine and cosine wave, as shown in (Figure 1).

PEF data were analysed by cosinor analysis to determine the statistical significance of 24 hour periodicity in the data, the amplitude of the rhythm as a percentage of the mean PEF value (a/m%), and the phase of the PEF rhythm with respect to 00.00 hours (Figure 1). This was performed using the modified multiple regression technique described by Hetzel and Clark (1980). PEF was analysed against time using the equation:

$$PEFR = Co + a\cos(2\pi t / 24) + b\sin(2\pi t / 24)$$

where Co = constant term or intercept

and t = time the PEF was measured time (in hours from time 00.00).

The coefficients a and b were estimated by multiple regression. Whether the rhythm was statistically significant or not was determined from the F ratio of the co-efficients a and b, accepting a value of $P<0.05$ as significant.

The amplitude and phase were then calculated from the coefficients a and b using the following equations:

$$\text{amplitude} = 2\sqrt{a^2 + b^2}$$

$$\text{phase} = \arctan(\frac{-b}{a})$$

The amplitude in this mathematical model represents half the difference observed between the highest and lowest values in a complete cycle (360° or 24 hours) . The "Peak to Trough" height of the rhythm would therefore be twice this value i.e. amplitude was doubled (a x 2; Figure 1).

The amplitude determined by cosinor analysis was expressed as a percentage of the mean value i.e. ($\frac{a}{m}$ x 100).

Pearson correlation co-efficients were computed to determine the relationship between three different measures of diurnal variation.

Unless otherwise stated, a probability of $P<0.05$ was accepted for statistical significance. Apart from the cosinor analysis, statistical analyses were performed using Minitab v8.

Ethics

The parents and children in the study all agreed to participate in the study. The nature and the reason of the study were fully explained to the parents and the children and informed written consent obtained from the parent. The study protocol was approved by the Ethics Committee of the Royal Hospital for Sick Children, Glasgow.

Limitations

Two limitations of the studied should be acknowledged. Firstly, at a practical level there was no way to assess whether the children recorded their peak flows at the times requested.

Secondly, cosinor analysis assumes that the biological rhythm understudy follows a sinusoidal curve. Carswell and Henderson (1989) have noted that in up to half of children the sinusoidal model did not appear to fit the raw data particularly well. In the present study, there were also some occasions when the data did not fit a sinusoidal model perfectly.

It should also be noted that we did not attempt to confirm the atopic status by skin testing.

RESULTS

Challenge testing

1. Histamine Challenge testing

The reproducibility of the mean provocation concentration of histamine causing a 20% fall in FEV_1 (PC_{20}) was investigated in 32 of the asthmatic subjects by repeating the test one week after the initial test. PC_{20} values were found to be highly reproducible, with an intra-class correlation co-efficient of 0.95.

The results of the histamine challenge tests in normal and asthmatic children are shown in Table 2 and Figure 5. All normal children had PC_{20}s of more than $\geq$8 mg/ml.

The mean baseline FEV_1 (percent predicted) in the asthmatic children was highest in group I (Bronchodilators PRN) and lowest in group III (Beclomethasone and Bronchodilators) (Table 2) as might be expected taking account of the different severities of their asthma.

The Pc_{20} varied in each of the three asthmatic groups was as follows : 2.47mg/ml (0.95 - 8.0) (median, range) in Group I, children with asthma taking only as required bronchodilators; 1.15 (0.115 - 8.0) in Group II, inhaled Sodium Cromoglycate prophylaxis; and 1.40 (0.210 - 8.0) in Group III, children taking inhaled beclomethasone (Figure 5).

Because the data for the Pc_{20} is logarithmic it was not normally distributed. After logarithmic transformation of the PC_{20} in each subject, one-way analysis of variance showed that there was a very significant difference between the 4 levels ($P < 0.0001$). Inspection of the 95% confidence intervals for the difference between the means of

each level calculated using Tukey's method showed evidence of a significant difference between the transformed means for the normal subjects and those on inhaled prophylaxis (Groups 2 and 3) but not those on bronchodilator PRN (Group 1). There was no evidence of differences between the three asthmatic groups.

2. Exercise testing

The reproducibility of the fall in peak flow after exercise was also assessed in 32 of the asthmatic subjects by repeating the test one week after the initial test. The peak fall after exercise was reproducible, with an intra-class correlation coefficient of 0.705. This is lower than with the histamine challenge test. The lower reproducibility of exercise testing has been noted by others (Anderson et al 1975; Anderson and Schoeffel 1982)

Individual and summary results of the exercise tests in both asthmatic and control children are shown in Table 3-7 and Figure 6.

As expected, all the normal control children had a % fall after exercise of less than 10% (Silverman and Anderson (1972); Burretal (1974)) (Figure 6). The lability index was less than 20% in all normal controls, within the normal limit of less than 22% previously reported by Silverman and Anderson, 1972 (Tables 3 & 7).

As expected, the % fall after the exercise in the asthmatic group as a whole (21.4 (15.0)) was significantly different ($P< 0.001$) from the normal control group (6.4 (2.6), (Table 7 and Figure 7) with a higher proportion of the asthmatic children having post-exercise falls greater than 10% (n=32, 80%). The asthmatic group also had a significantly higher lability index (24.9 (14.1) than that of the normal controls (11.0

(4.8); Table 7). The percent fall after exercise and the lability index in all subjects (10 normal controls and 40 asthmatics) were highly correlated (n = 50; r = 0.905). Further analysis therefore concentrated on the percent fall after exercise.

Since the percent fall after exercise and the lability index were highly correlated only the percent fall was investigated further in the 4 subgroups. The results of the exercise tests in the three asthmatic subgroups are best seen in Figure 6. In Group I (Table 4), 4 children (67%) had positive exercise tests while in Group II (Table 5) 10 (91%) were positive and in Group III (Table 6) 18 (78%).

Again the differences between the group means was investigated using one-way analysis of variance with 95% confidence intervals calculated by the Tukey method. The mean % fall after exercise in the 4 subgroups were as follows: controls - 6.47 (2.68); Group I - 11.9 (7.09); Group II - 24.2 (17.73) and Group III - 22.4 (14.67). The 4 levels were significantly different ($P < 0.01$). Inspection of the 95% confidence intervals showed evidence for a difference between the control subjects and those on prophylactic medication (normals vs Group II & III). However, there was no evidence of difference between the asthmatic groups (Group I vs II vs III). Thus the exercise test data is very similar to the results from the histamine challenge tests.

The increased lability in asthmatic children reflected mainly post-exercise broncho-constriction. In contrast, in the normal control group any lability present arose form broncho-dilation during exercise.

As in other studies (Sly 1970; Silverman and Anderson 1972; Konig 1974), the % fall in PEF in this study was found to be independent of the resting PEF. However, there was a highly significant negative correlation between the resting PEF and the duration

of asthma (n = 40; r = - 0.426; P <0.01). The % fall in PEF had no significant correlation with the age of the patient nor was the % fall correlated with the duration of asthma.

The relation between bronchial reactivity measured by histamine challenge test and exercise test is shown in Figure 8. The correlation between the two measures using the Spearman Rank Correlation co-efficient was r = -0.744; P<0.001. Mellis et al (1978) have previously noted a close relation between histamine challenge and exercise challenges in children. Kiviloog (1975) noted a correlation co-efficient in 57 asthmatics adults of r = -0.70, almost identical to the value in the present study.

Diurnal Variation in Peak Flow Testing

Summary results of the indices of diurnal variation in both asthmatic and control children undergoing all three tests are shown in Table 8. The mean diurnal variation in PEF calculated as the difference between the evening and morning daily value was 9.3% (6.8%) in the asthmatic children group and 4.7% (2.7%) in the normal control group (Table 8). However, there was a wide range within both groups and some overlap between groups. Since four measurements of PEF were taken each day, another possible measure of daily variability was the difference between the highest and lowest daily values, whatever the time of measurement. This was calculated for each day. The mean value was 13% (7.0%) in the asthmatic group and 7.7% (3.6%) in the normal control group (Table 8). Again there was a wide range within both groups and some overlap. Finally, the mean diurnal variation in PEF measured as the peak to trough amplitude derived by cosinor analysis in the asthmatic children was 13.2% (10.4%; range 0.9% to 45.2%) of the mean PEF value (Table 8 and Figure 9).

The peak to trough amplitude in the 44 normal subjects (35 who underwent PEF testing alone and 9 who undertook all 3 studies) was 6.8% (3.6%) of mean PEF value. The range is again wide although somewhat narrower than for the asthmatics above, from 0.7% to 19.5%. Figure 10 shows the individual results in the normal subjects who underwent all three tests compared with those who only undertook home PEF monitoring. It can be seen that the mean values were very similar in the two groups.

Twenty four of the 40 (60%) asthmatic children had a significant rhythm ($P < 0.05$) detectable by the cosinor method (Table 9). The mean cosinor derived amplitude for these 24 asthmatic children was 15.9% (11.3%) of their individual mean PEF value. Twenty two of the 44 (50%) normal control children also had a significant ($P < 0.05$)

rhythm and in these the mean cosinor amplitude was 7.7% of their individual mean PEF value (3.6%) (Table 10).

The distribution of the lowest point (bathyphase) of the PEF rhythm in asthmatic children as calculated by cosinor analysis is shown in Figure 11. The lowest point for those 24 asthmatic children with statistically significant rhythm appeared similar to those 16 asthmatic children in whom no significant rhythm was detected with the trough between midnight and 8 a.m. The distribution of the lowest point of the PEF rhythm in normal control children for those 22 children with a significant rhythm also appeared similar to that of the remainder who had no significant rhythm. Again the trough in the majority fell between midnight and 8 a.m. The fact that the pattern of timing of the trough was similar in the majority of both asthmatic and control children is worth noting since it implies that there is no phase difference between the rhythms in the asthmatics and normal controls.

Six of the normal children, but no asthmatics, had their lowest point in the afternoon. This pattern has been noted occasionally by others in both asthmatics and normals, adults and children (Hetzel and Clarke 1980; Johnston et al 1984).

The peak to trough amplitude by cosinor analysis for the 3 asthmatic subgroups were also calculated and are listed in Table 9. The mean amplitude for the group on inhaled bronchodilators when required (prn) was 10.2 (4.7)., for the group on sodium cromoglycate 12.9 (10.4) and for the group on inhaled beclomethasone 14.3 (11.57).

On this occasion, investigating the differences between the peak to trough amplitude calculated by cosinor analysis for the 4 groups using one-way analysis of variance with

95% confidence intervals calculated by the Tukey method showed no evidence of a difference between the 4 levels.

As would be expected, the 3 ways of computing diurnal variations in PEF in the asthmatic group were highly correlated. The correlations between the peak to trough cosinor analysis amplitude (a/m %) and the difference between the evening and morning daily values expressed as (% mean) was r + 0.88, ($p < 0.002$). Also the correlations between the peak to trough amplitude by cosinor analysis (a/m %) and the difference between the highest and lowest daily values as a percentage of the highest was r + 0.90 ($p < 0.002$) (Figure 12). The fact that the correlations are slightly less than perfect may relate to the two techniques other than cosinor analysis being more susceptible to biological noise in those with low amplitude rhythms. Also, the cosinor technique estimates the maximum peak to trough amplitude even if it occurs at a time that is not directly measured.

Relationship between diurnal variation and challenge tests

Finally, the correlation between the two measurements of bronchial reactivity and the measurements of diurnal variability (Pc_{20}, percent fall in peak flow after exercise and the amplitude of peak flow variability derived from cosinor analysis) were compared using Spearman's Rank correlation test in the 48 subjects who had a value for each measurement. The correlation between the two measures of bronchial reactivity was -0.744 ($P<0.0001$). The correlation between Pc_{20} and the cosinor derived variability was -0.483 ($P <0.01$) while between per cent fall in peak flow and cosinor amplitude was 0.481 ($P<0.02$). However, the scatter in the results can be seen to be quite wide (Figure 13).

DISCUSSION

Histamine challenge tests

In the this study, all the normal children had Pc_{20} values greater than or equal to 8mg/ml of histamine.

There was a wide range of Pc_{20} values in the asthmatic children. The median Pc_{20} values in the asthmatic groups classified according to their prescribed treatment were lower than the normal subjects. As noted in the results, the results in the asthmatics children were significantly different from the normal controls only for the groups receiving inhaled prophylactic therapy (cromoglycate or inhaled steroids). For both challenge tests there was no evidence of difference between the three asthmatic groups.

Several other investigators have used treatment requirements as an index of asthma severity e.g. Townley et al (1975) observed a greater responsiveness in asthmatics who had previously required more medication. Similarly, Spector and Farr (1975) studied 200 subjects referred to hospital because of difficulty in controlling their asthma and reported that levels of responsiveness were related to cortico-steroid treatment with the more hyper-responsive patients receiving a higher dose of steroids at the time of discharge from hospital. Cockcroft et al (1977 (a)) retrospectively studied 156 adult asthmatic subjects where minimum medication for control of asthma was applied and found that mean levels of responsiveness increased with increasing treatment requirements. They demonstrated a difference in Pc_{20} between groups of subjects who were asymptomatic and on no treatment, those who required bronchodilators occasionally, those who required them regularly and those who were steroid dependent. However, while the mean Pc_{20} in each asthmatic subgroup was

significantly lower than for the preceding category, there was considerable overlap between the ranges of responsiveness observed in each group.

Avital et al (1991), studied 182 asthmatic children divided into three age groups. Within each group the patients were divided into three clinical groups and classified according to their therapeutic requirements for optimal control of symptoms ranging from bronchodilators as needed to regular inhaled cortico-steroids. They also demonstrated that the mean level of bronchial reactivity to methacholine for the whole group correlated inversely with the severity of bronchial asthma. They reported that in the older children (6 -17 years) the difference between the moderate and severe asthmatics was not significant, perhaps as a result of the effect of steroids in the severe group.

The results in the present studies are similar to the studies above. The most striking finding overall in children with asthma is the wide range of Pc_{20} values and considerable overlap between groups of supposedly differing severities. It is possible that the lack of more significant difference between our three subgroups might relate to the effect of inhaled prophylactic therapy with Beclomethasone dipropionate producing a greater reduction in bronchial hyper-responsiveness (less bronchial responsiveness reflected increase in Pc_{20}) in some patients, as has previously demonstrated (Juniper et al, 1981).

Exercise

Not surprisingly, the asthmatic children in this study showed a higher % fall in peak flow after exercise and higher lability index than the normal control children.

Again, there appeared to be a spectrum of bronchial lability in response to exercise in subjects this study, with the normal subjects displaying almost no bronchial lability on exercise. This has been reported in a number of previous studies (eg Anderson, 1972).

In this study, the fall in PEF after exercise in mild asthmatics appeared to fall between that of normal subjects and of highly labile asthmatics (Figure 6). Such a spectrum has been shown previously in response to exercise tests (Konig, Godfrey and Abrahmov, 1972). The present study confirmed again that a treadmill exercise test and indices of exercise-induced broncho-constriction are helpful in differentiating between asthmatic and normal subjects. From our data, if a patients performs the test properly and has a % fall after exercise greater than 10% or lability index greater than 20% he is unlikely to be normal. The test did not differentiate between the patients who were controlled by sodium cromoglycate and those who received regular inhaled steroids. This finding agrees with Jones (1966) who found the lability index in asthmatics was higher than controls. Our findings agree closely with Konig (1974) in his study of the clinical correlates of exercise induced asthma in 52 asthmatic children who found no difference in percent fall after exercise or lability index between asthmatics in group II compared with group III.

The clinical correlates of exercise induced asthma have attracted considerable interest with some disagreement between the studies. As in other studies (Sly 1970; Silverman and Anderson 1972; Konig 1974), the % fall in PEF was found to be independent of the resting PEF. However, there was a highly significant negative correlation between the resting PEF and the duration of asthma ($n = 40$; $r = - 0.426$; $P < 0.01$). The % fall in PEF had no significant correlation with the age of the patient nor was the % fall correlated with the duration of asthma.

In Sly's study (1970a) in children, there was a significant correlation between the severity of EIA and frequency of wheezing during previous year. However, he used treadmill walking which is a very much less effective inducer of broncho-constriction and only minority of his subjects (47%) developed EIA. Unlike the present study, he showed a significant correlation between % fall and age of the patient. He found no correlation between % fall on exercise and the interval since onset of wheezing or age of onset of wheezing. Similarly, while Silverman (1973) found no correlation existed between the severity of EIA and the number of days of illness in previous year or 3 months, mean PEF recorded twice daily for 1 month before testing, number of positive skin tests, and eosinophil count he did find a significant correlation between % fall after exercise and duration of asthma, age of patient and histamine response. Thus both these studies found a relationship between percentage fall in PEF and age of patient which was not found in this study. Some studies have found results more in line with the present study. For example, Balfour-Lynn et al (1981) found the degree of E.I.A. did not appear to differ in patients requiring steroids, sodium cromoglycate or regular and occasional bronchodilators while Kelemen et al (1987) found no significant correlation between EIA and either age or duration of asthma. The reason for these discrepancies is unclear.

Diurnal variability in peak flow rate

The present study has shown that asthmatic children have greater diurnal variation in peak expiratory rate than normal control children. In our results, we found a significant circadian rhythm in airway calibre was present in 60% of the asthmatic children and in 50% of the normal children. Like Hetzel and Clarke (1980), we found that in the majority of the remaining subjects, in whom rhythm detection did not achieve statistical significance, the computed estimates of the phases of their rhythms showed a very similar distribution to that seen in subjects with significant rhythmicity. In adults, Hetzel and Clarke (1980) suggested that this similarity would not have been expected if the results were an artefact from biological noise alone without any underlying periodicity. They, therefore, suggested that all normal subjects had a circadian rhythm but in the minority the amplitude was too low for rhythm detection. The present data suggests that the same may be true in children.

The mean peak to trough amplitude derived by cosinor analysis in asthmatic children in the present study was 13.2% (10.4) of individual mean PEF value. This value is similar to that reported by Johnston, Anderson and Patel (1984) when they applied cosinor analysis to 64 asthmatic children (9 - 11 years) and found an amplitude of 12%. However, this finding of an amplitude of around 12 -13% in asthmatic children is considerably less than the average amplitude value of 50.9% reported in adult asthmatics by Hetzel and Clark (1980). However, they studied asthmatics shortly after an acute exacerbation. Clearly, the clinical status of their patients may partly explain this particularly high amplitude. The asthmatic children in the present study were clinically stable. They were likely to be of milder severity and were maintained on their normal medication during the study period. In this respect, our population is much closer to that of Johnston et al.

Hetzel and Clark (1980) suggested that in adults an amplitude of 20% might be used as a screening test for the diagnosis of asthma. This equated to their mean amplitude (8.3%) in normal adults plus two standard deviations (SD 5.2%). Like Hetzel and Clarke, the cosinor analysis amplitude in normal children in the present study (6.8% mean (SD 3.6) was lower than in the asthmatics (mean 13.2%). This is just under two standard deviations away from the mean value in the normal subjects. This would suggests that if a cut-off is to be used, a lower one of around 14% might be more appropriate for children.

In this study, high correlations were found between cosinor analysis and other simpler methods of calculating and describing the variability in PEF. In view of the high correlations between these measures and cosinor analysis it seems sensible and appropriate to use the simplest if diurnal variability is to be measured. This would be (evening - morning)/mean%. In this study, the mean + 2SD value was 10.1% providing a convenient number for use in clinical practice. The alternative of (highest - lowest)/highest would give a cut off point of 14.9 (mean + 2SD). These simpler measures avoid the need for complex statistical data handling and provide a simpler and more convenient method of measuring and calculating diurnal variability. They are much more appropriate as an aid to the diagnosis of asthma in clinical practice.

However, we feel it is much more important to note that because of the wide range of amplitudes present in the asthmatic children (range 0.9 - 45.2%) many had values below 14%. The asthmatics in the present study all continued with treatment during the study, had no acute exacerbations and were well-controlled. It is possible that their values without treatment might be higher. Nevertheless the use of a cut-off point to differentiate between those who have asthma and those who do not may be impossible.

The essential problem is that the defining physiological characteristic is a continuous variable that overlaps the variability present in normal children.

Correlation between bronchial responsiveness

How does the diurnal variability in airway function relate to the degree of bronchial responsiveness or the severity of asthma? In this study, there was no evidence of a significant difference between peak to trough amplitude and the severity of asthma as reflected in prescribed therapy. Indeed, inspection of the results (Figure 9) illustrates how wide the spread of amplitudes is and the clear and extensive overlap with normal subjects. In that respect it is interesting to recall that there was no obvious difference in the distribution between the phase as expressed in the bathyphase (trough) between the normals and the asthmatic subjects.

While the correlation between peak to trough amplitude and both Pc_{20} was highly significant, the wide scatter of the data suggests that the relation is at best biologically rather weak (Figure 13). In adults, Bahous et al also found only a modest relationship between diurnal changes in PEF without bronchodilator and Pc_{20} ($r = -0.51$) in 27 asthmatics with mild symptoms of bronchial irritability and low or moderate levels of hyper-responsiveness (Pc_{20} 0.3 - 16 mgs/ml). An even weaker relationship ($r = 0.41$) was observed by Ryan et al (1982) when they examined the relationship between responsiveness and the degree of naturally occurring diurnal variation in PEF in adult subjects with current asthma, in subjects with a past history of asthma and in normal subjects. Thus the relation between diurnal variability and severity of asthma on the one hand and bronchial hyperreactivity on the other is not clear-cut. Both bronchial reactivity and diurnal variation are clearly useful in cross-sectional and longitudinal epidemiological studies but within subjects the relationship with asthma and asthma severity is much less clear.

CONCLUSIONS

Over the last decade many research workers have come to view non-specific bronchial responsiveness as a cardinal feature of asthma. However, it has been pointed out that these conclusion are frequently based on studies, like ours, that compared polarised groups of, often hospital-based, asthmatic subjects with strictly normal subjects. However, as data has accumulated from studies in unselected populations it has become clear that there is indeed considerable overlap in bronchial responsiveness between asthmatics and non-asthmatics (Cockroft et al 1977 (a); Sears et al 1986); Pattemore et al (1990). For example, Pattemore et al (1990) in a large random sample of New Zealand school children found a unimodal distribution of percent change in FEV1 after a cumulative dose of 3.9μmol of histamine, with those in whom asthma had been diagnosed dominating the severe end of the spectrum.

Hyper-responsiveness has been reported in 6-8% of children without asthmatic symptoms (Salome et al, 1987; Sears et al, 1986) but was absent in about a third of those with recurrent wheezing (Lee et al, 1983; Sears et al, 1986). Also hyper-responsiveness has been demonstrated in 31, of normal adults (Cockcroft et al, 1977 (a)) and in 15-22% of subjects who have rhinitis (Cockcroft et al, 1977 (a); Townley et al, 1975). It has also been found in relatives of atopic or asthmatic subjects (Townley et al, 1979). It remains unclear how bronchial responsiveness relates to the severity of asthma, and how and why any relationship between them occurs. It would seem that hyper-responsiveness is a functional abnormality which might reflect underlying pathological processes in the airways rather than relating directly to current levels of airway obstruction.

These findings have led to a view that bronchial responsiveness is not a disease entity resulting from one or two clear-cut aetiologic factors but is likely to be an expression of

heterogeneous factors such as polygenic inheritance modified by environment. Thus, in the present study, while we found a significant difference between normal subjects and those on inhaled prophylactic medication, it is quite possible that these differences represents two ends of a spectrum rather than two distinct populations of subjects.

This study confirms that non-specific bronchial responsiveness is more frequent and often more severe in asthmatic subjects. It also finds that diurnal variability is greater and has a wider range in asthmatics. However, the wide overlap of results between groups of supposedly different severities and with normal subjects does not suggest that it is well correlated either with the presence or severity of asthma.

This is in line with more recent thinking about the relationship between bronchial responsiveness and asthma where bronchial responsiveness has been found to be related to but not identical with asthma (Pattemore et al (1990); Josephs et al (1990)).

A better appreciation of the overlap between bronchial responsiveness, diurnal variability and the presence and severity of asthma will require a much greater understanding of the mechanisms underlying asthma. As noted initially, the clinical appreciation of both bronchial reactivity and diurnal variation in airflow obstruction are now very ancient, dating almost from the dawn of known medicine. If anything the relationships become less clear despite the greater understanding of modern times.

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TABLES

Table 1: Details of asthmatic subjects who underwent challenge tests.

GROUP	n	Sex M:F	Age (yrs) Mean (SD)	Duration of asthma (yrs) (Mean, SD)
I. Bronchodilators PRN	6	5:1	10.4 (2.0)	4.2 (2.9)
II. Sodium Cromoglycate	11	7:4	9.9 (1.7)	5.5 (2.4)
III. Inhaled steroids	23	18:5	10.4 (2.1)	6.2 (2.7)
Total	40	30:10	10.3 (2.0)	5.8 (2.7)

Table 2: Summary of histamine challenge test measurements in normal and asthmatic children.

	Controls	Asthmatics		
		Group 1 Bronchodilator prn	Group II Sodium cromoglycate	Group III Beclomethasone
Number	10	6	11	23
Age (yrs)				
Mean (SD)	10.8 (2.1)	10.4 (2.0)	9.9 (1.7)	10.4 (2.1)
Sex (M:F)	6 : 4	6 : 0	7 : 4	18 : 5
FEV_1 (% predicted)				
Mean (SD)	110 (13.1)	106 (13.8)	98 (11.2)	94 (13.0)
Pc_{20} histamine (mg/ml)				
Median	>8.0	2.47	1.15	1.40

Table 3: Individual results of exercise tests in normal children .

Case No.	Age (yrs)	Resting PEF (%)	% Fall in PEF after exercise	Lability Index
1	13.9	112	10	11.4
2	10.0	92	3.0	5.6
3	12.9	96	4.5	18.5
4	12.11	92.6	6.5	16.1
5	10.7	118	8.5	11.5
6	13.0	122	1.5	1.8
7	8.6	136	7.2	13.0
8	7.0	102	7.0	12.3
9	11.0	98	8.5	12.3
10	9.5	98	8.0	7.8
Mean	10.8	106.6	6.4	11.0
S.D.	2.1	14.7	2.6	4.8

Table 4: Individual results of exercise tests in asthmatic children (Group I - no inhaled prophylaxis).

Case No.	Age (yrs)	Resting PEF (%)	% Fall in PEF after exercise	Lability Index	Duration of Asthma (yrs)
1	7.9	97	4.5	7.8	2
2	9.4	84	15.8	13.3	9
3	10.8	93	11.4	15.2	6
4	14.0	110	21.6	25.0	2
5	10.4	89	15.0	31.0	1.6
6	10.0	98	3.1	12.4	5
Mean	10.4	95.1	11.9	17.4	4.2
SD	2.0	8.9	7.0	8.7	2.9

Table 5: Individual results of exercise tests in asthmatic children (Group II - inhaled prophylaxis with Sodium Cromoglycate).

Case No.	Age (yrs)	Resting PEF (%)	% Fall after exercise	Lability Index	Duration of Asthma (yrs)
1	13.0	79.0	13.9	14.4	6
2	10.0	85.2	37.5	33.8	7
3	8.7	73.0	20.0	28.4	6
4	10.0	85.3	23.9	38.0	8
5	12.2	85.3	10.4	11.0	9
6	7.8	85.2	17.8	17.5	6
7	11.9	88.0	65.3	59.6	7
8	9.5	95.0	43.3	46.6	4
9	8.0	102	15.5	21.2	1
10	8.0	85.0	15.3	12.7	5
11	10.0	86.0	4.1	11.1	2
Mean	9.9	86.2	24.2	26.5	5.5
S.D.	1.7	7.4	17.7	16.0	2.4

Table 6: Individual results of exercise tests in asthmatic children (Group III - inhaled cortico-steroids).

Case No.	Age (yrs)	Resting PEF (%)	% Fall after exercise	Lability Index	Duration of Asthma (yrs)
1	8.0	121.0	16.4	36.6	6.5
2	7.4	120.0	21.8	34.0	4
3	11.0	87.0	49.6	47.7	10
4	11.0	76.0	14.8	14.8	9
5	11.4	90.0	12.0	13.2	4.4
6	9.0	80.0	29.3	41.6	7
7	14.0	86.0	2.2	12.6	13
8	11.0	90.0	52.6	63.1	6
9	9.0	122	21.5	26.0	3
10	7.0	98.0	7.1	27.8	4.5
11	12.5	90.0	40.0	36.4	6
12	9.0	96.0	23.0	25.6	1.5
13	12.0	85.3	49.0	41.9	7
14	14.0	101	31.4	31.8	7
15	14.0	109	3.0	6.9	7
16	12.0	85.0	32.6	30.6	7
17	9.0	101	27.0	27.2	2
18	9.0	87.0	8.5	9.0	8
19	11.6	77.0	15.3	13.4	11
20	8.9	104	9.0	14.4	6
21	10.3	83.0	17.2	14.2	5
22	7.4	106	22.9	24.3	5
23	12.0	97.0	10.6	10.3	9
Mean	10.4	95.2	22.4	26.2	6.4
S.D.	2.1	13.5	14.6	14.2	2.7

Table 7: Summary results of exercise tests in normal subjects and asthmatic subjects as a group.

Group	n	Resting PEFR (%)		Fall in PEF after exercise (%)		Exercise Lability Index (%)	
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
Controls	**10**	106.6	14.7	6.4	2.6	11.0	4.8
Asthmatic	**40**	92.7	12.0	21.4	15.0	25.0	14.1

Table 8: Indices of diurnal variation calculated from the peak flow charts in both normal controls and asthmatic subjects.

Measure of diurnal variation	Control (n = 44)		Asthmatic (n= 40)	
	Mean (SD)	Range	Mean (SD)	Range
1. Difference between evening and morning daily values (% mean).	4.7 (2.7)	0.7 - 13.6	9.3 (6.8)	2.0 - 28.4
2. Difference between highest and lowest daily values (% highest).	7.7 (3.6)	1.9 - 18.7	13.0 (7.0)	4.3 - 31.7
3. Cosinor amplitude (%mean).	6.8 (3.6)	0.7 - 19.5	13.2 (10.4)	0.9 - 45.2

Table 9: Amplitude of PEF rhythm in asthmatic subjects.

Severity of asthma	Subject	Peak to trough Amplitude (%mean PEF)	Significant PEF Rhythm (P<0.05)
Group 1: PRN Bronchodilator			
	1	2.9	-
	2	10.9	+
	3	9.7	+
	4	13.1	-
	5	16.5	+
	6	5.4	+
	7	13.4	-
	Mean (SD)	10.2 (4.7)	
Group 2: Cromoglycate	1	1.9	-
	2	20.2	+
	3	6.0	+
	4	3.7	-
	5	32.6	+
	6	20.9	-
	7	26.5	+
	8	4.2	-
	9	9.3	+
	10	5.5	+
	11	11.8	+
	Mean (SD)	12.9 (10.4)	
Group 3: Beclomethasone			
	1	7.5	-
	2	25.0	+
	3	17.6	+
	4	15.2	-
	5	3.3	-
	6	45.2	+
	7	20.8	-
	8	10.0	-
	9	7.2	+
	10	9.2	+
	11	17.5	+
	12	43.4	+
	13	8.2	+
	14	12.2	+
	15	5.5	-
	16	4.3	+
	17	8.1	+
	18	19.1	-
	19	0.9	-
	20	4.1	-
	21	17.0	+
	22	13.5	+
	Mean (SD)	14.3 (11.57)	
Overall Mean (SD)		13.2 (10.4)	

Table 10: Amplitude of PEF rhythm derived from Cosinor analysis in control subjects.

Subject	Peak to trough Amplitude (%mean PEF)	Significant PEF Rhythm (P<0.05)
1	5.7	+
2	4.8	+
3	2.8	-
4	5.2	+
5	8	-
6	16	-
7	2.9	-
8	5.5	+
9	6.2	+
10	11.7	-
11	4.6	-
12	4.2	-
13	8.3	-
14	10.1	+
15	8.8	+
16	5.7	+
17	7.0	+
18	5.2	-
19	7.1	-
20	6.1	+
21	19.5	+
22	12.6	+
23	5.7	-
24	3.2	-
25	5.1	-
26	6.8	-
27	9.0	+
28	8.0	-
29	12.6	+
30	10.2	-
31	4.9	-
32	5.0	+
33	7.7	+
34	0.7	-
35	5.1	-
36	5.0	-
37	4.4	+
38	11.2	+
39	7.8	+
40	5.7	+
41	4.7	+
42	2.2	-
43	4.5	+
44	2.5	-
Mean (SD)	6.8 (3.6)	

FIGURES

Figure 1: Analysis of circadian variation in PEF.

This can be simply measured as the difference between highest and lowest daily values, expressed as a percentage of highest values (a/h%).

Cosinor analysis allows more detailed analysis, fitting the best sinusoidal waveforem to the raw data. The rhythm is assumed to be sinusoidal with a period of 24 hours. Phase is represented as the timing of the peak (highest daily value, acrophase). The bathyphase is the lowest point and is theoretically 12 hours or 180 degrees from the acrophase. Amplitude is expressed as a percentage of the mean value (a/m%).

a = amplitude; p = period; m = mean; h = highest daily reading;
amplitude % = a/m x 100; diurnal swings = a/h x 100.

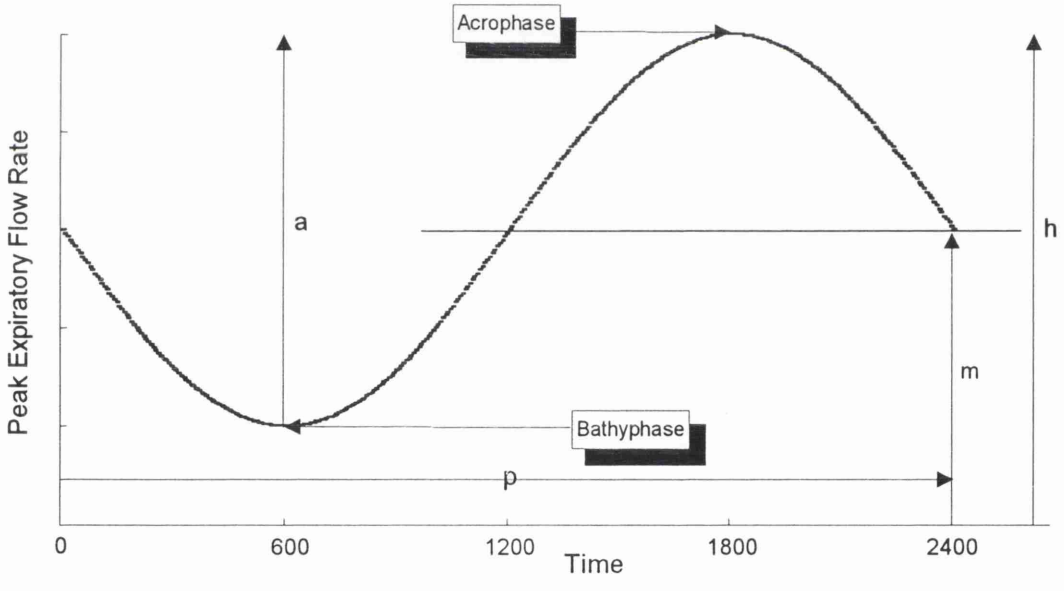

Figure 2: Child undergoing Histamine challenge test.

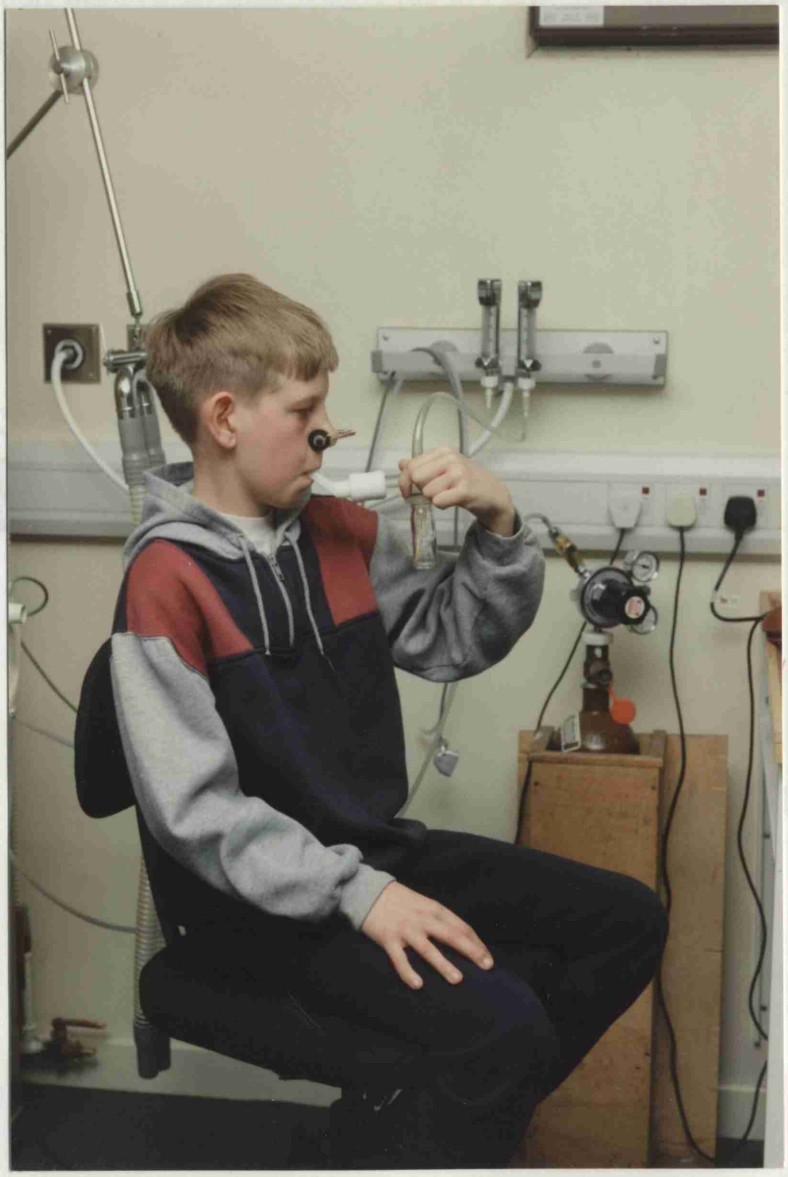

Figure 3: Raw data for a Histamine challenge test in an asthmatic subject.

Results of a Histamine challenge test in an asthmatic subject. Pc_{20} - histamine concentration which provokes a 20% fall in FEV_1 - is estimated by interpolation. In this example the Pc_{20} was estimated to be 2.6mg/ml.

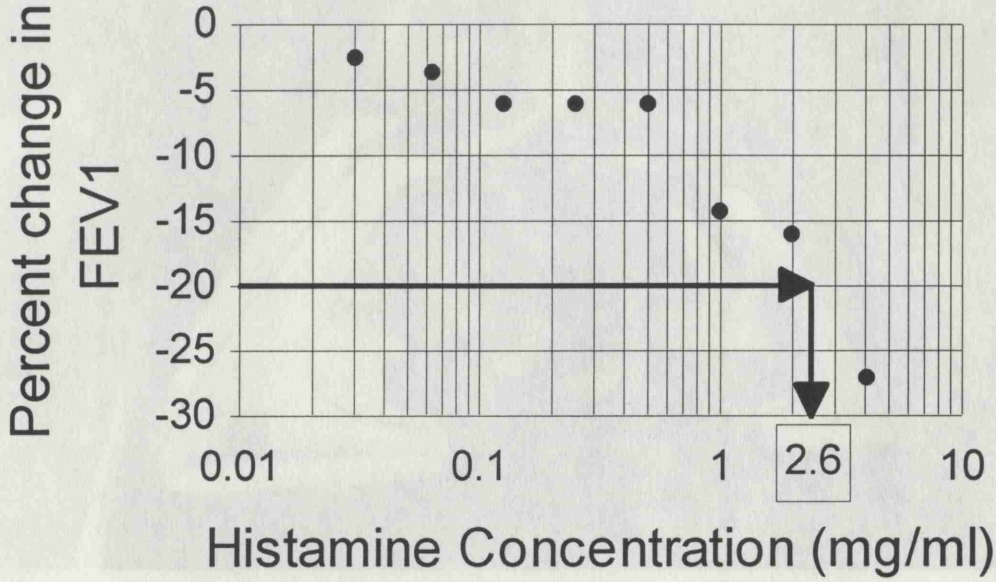

Figure 4: Child undergoing an exercise treadmill test.

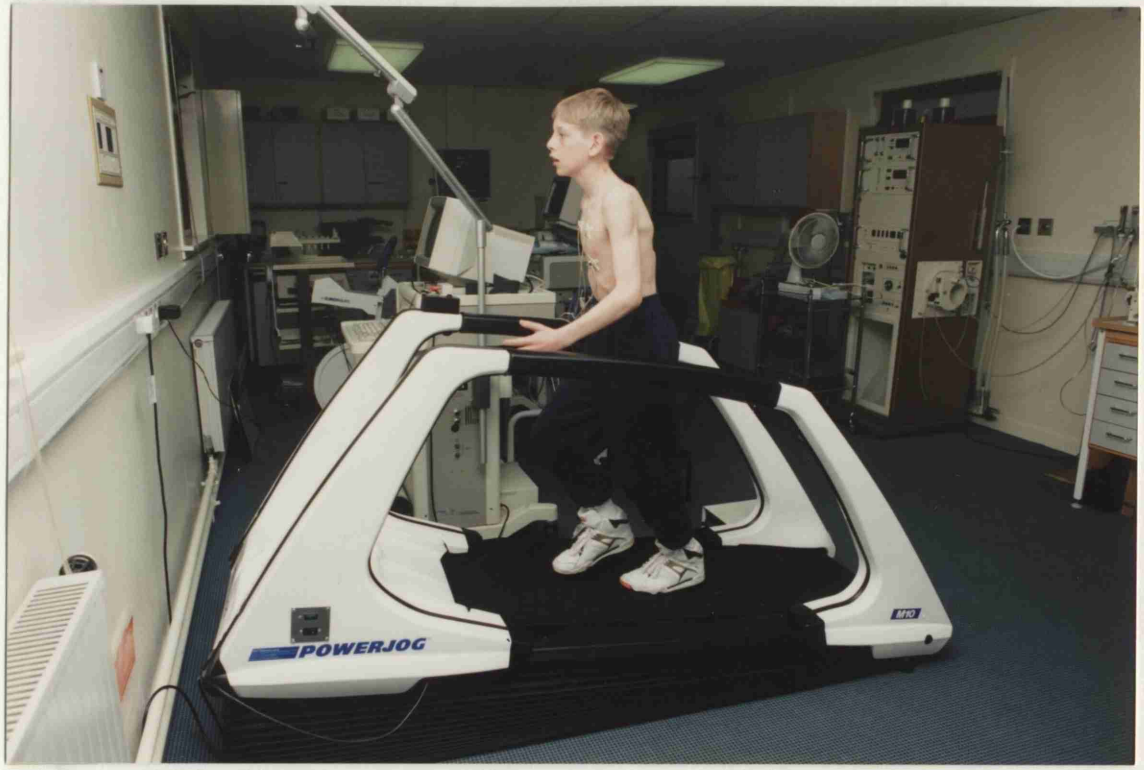

Figure 5: Distribution of Pc_{20} values in control subjects and asthmatics.

The graph shows the Pc_{20} values in the normal subjects and the three asthmatic groups: Group 1- bronchodilators prn; Group 2 - inhaled Sodium cromoglycate regularly and Group 3 - inhaled Beclomethasone Dipropionate regularly. Horizontal lines represent mean values for each group. The normal subjects are those who underwent histamine challenge, exercise tests and PEF recording.

The horizontal bars represent median values of Pc_{20} .

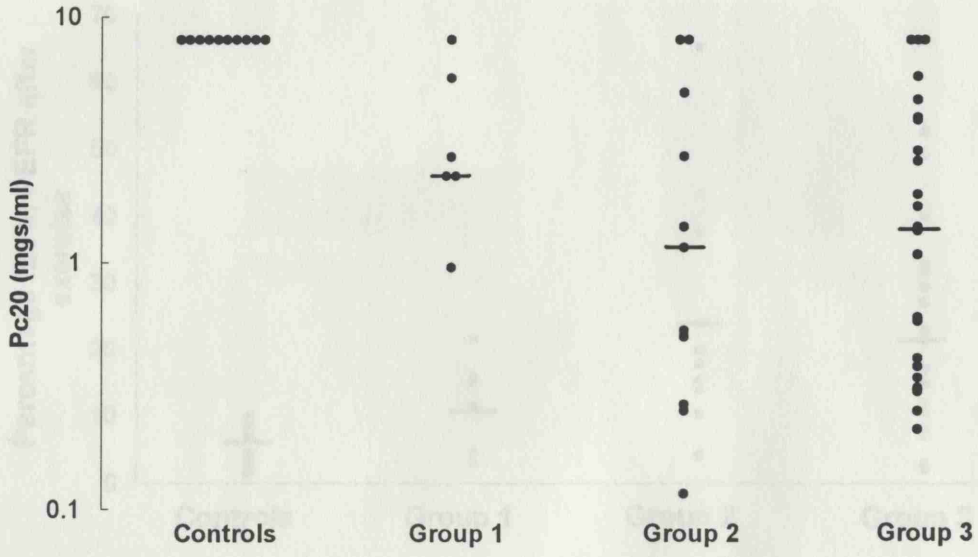

Figure 6: Distribution of percentage fall in PEF after exercise.

The graph shows the percentage fall in PEF in the normal subjects and the three asthmatic groups: Group 1- bronchodilators prn; Group 2 - inhaled Sodium cromoglycate regularly and Group 3 - inhaled Beclomethasone Dipropionate regularly. Horizontal lines represent mean values for each group. The normal subjects are those those who underwent histamine challenge, exercise tests and PEF recording.

Inspection of Tukey confidence intervals from the one-way analysis of variance showed that there were significant differences ($P<0.05$) between the normals and those on inhaled prophylactic therapy (Group 2 and 3) only.

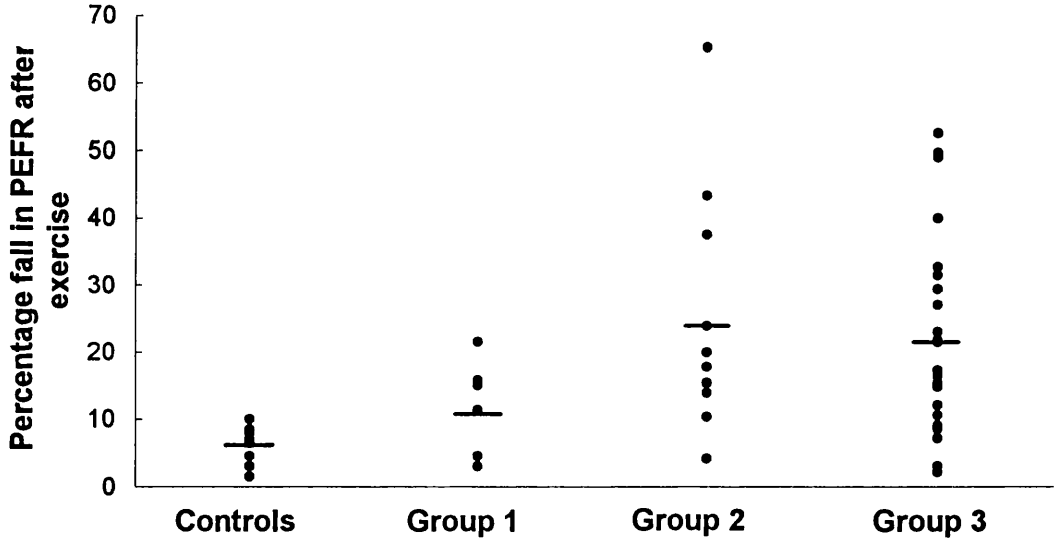

Figure 7: Mean percent fall in PEF after exercise in asthmatics and normal control children.

Error bars represent standard deviation.

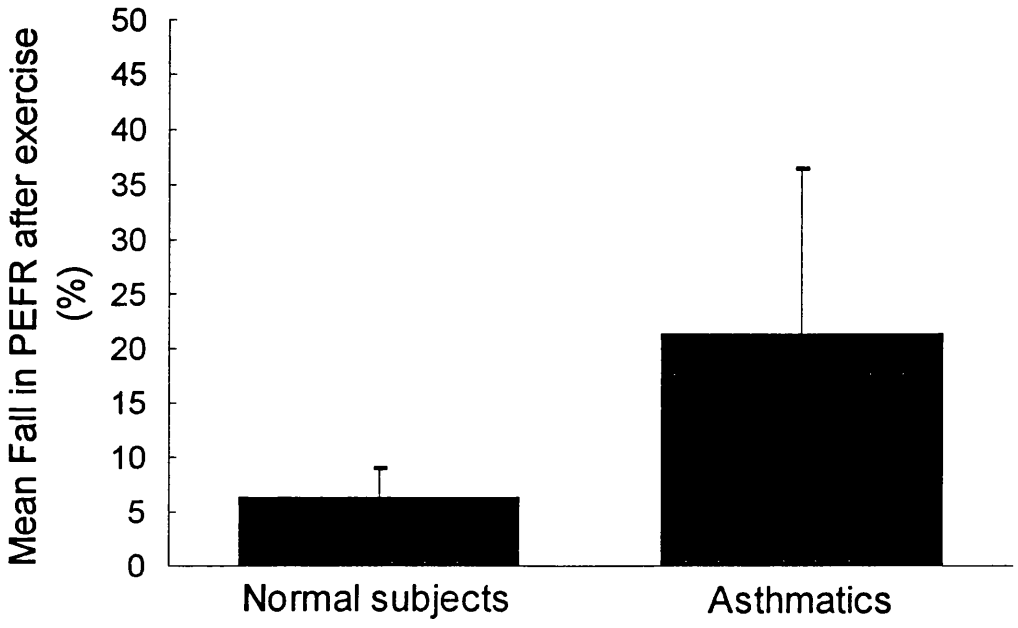

Figure 8: Scatter plot showing relationship between Pc_{20} and percent fall in PEF after exercise.

Pc_{20} values are censored at 8mg/ml as concentrations higher than this were not studied. Because of the logarithmic nature of the Pc_{20} data Spearman's rank correlation test was used. Plot shows data from from asthmatic and control subjects.

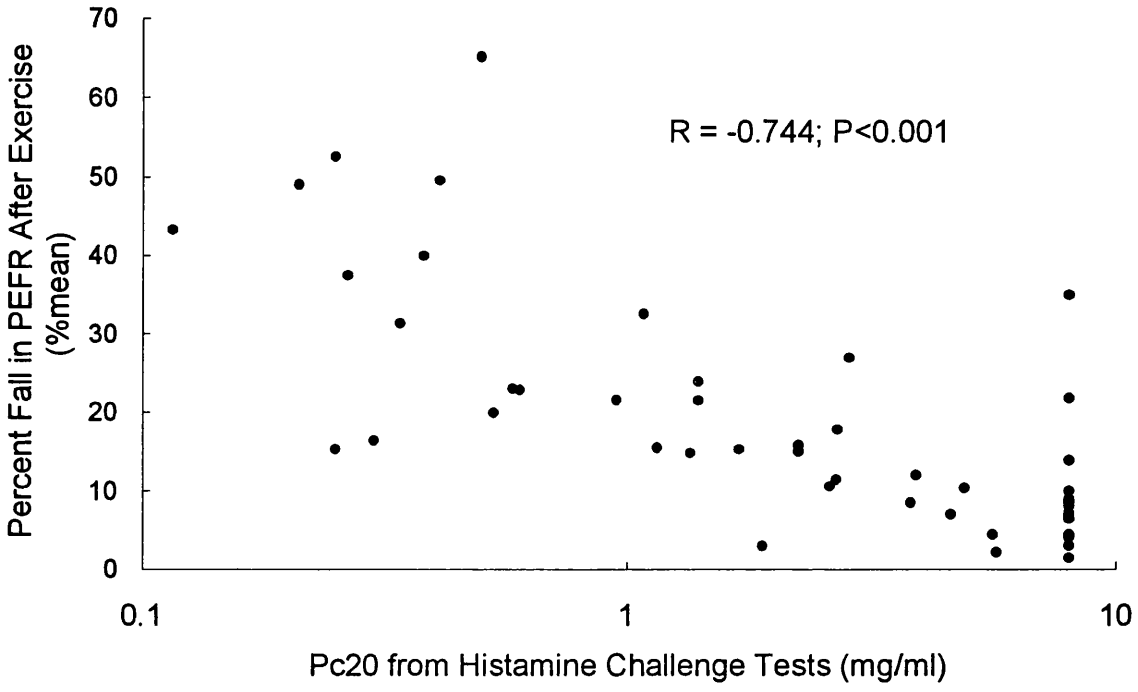

Figure 9: Distribution of amplitude (a/m%) from Cosinor analsysis.

The graph shows the distribution of Cosinor amplitude in PEF in the normal subjects and the three asthmatic groups: Group 1- bronchodilators prn; Group 2 - inhaled Sodium cromoglycate regularly and Group 3 - inhaled Beclomethasone Dipropionate regularly. Horizontal lines represent mean values for each group. The normal subjects only shows those subjects who underwent histamine challenge and exercise tests.

In this case, inspection of Tukey confidence intervals from the one-way analysis of variance showed no significant differences between the normals and the asthmatic groups.

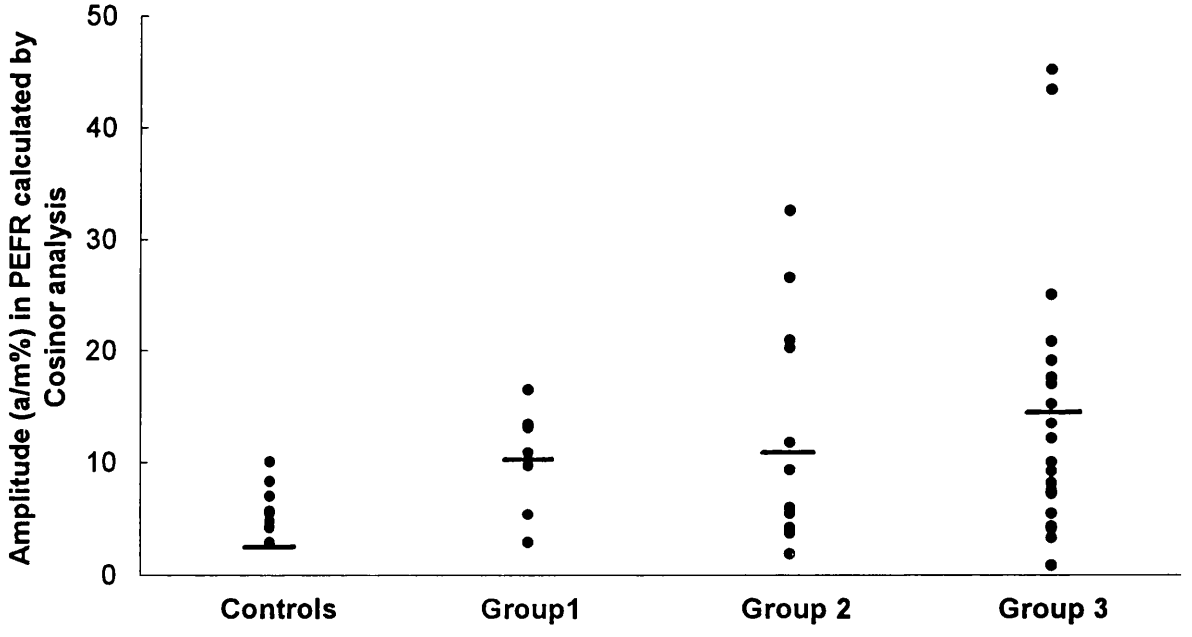

Figure 10: Distribution of amplitude (a/m%) from Cosinor analsysis.

The graph compares the distribution of Cosinor amplitude in PEF in the normal subjects who underwent all three tests (histamine challenge, exercise and PEF recording - n = 9) and those who only took part in PEF measurement (n = 35).

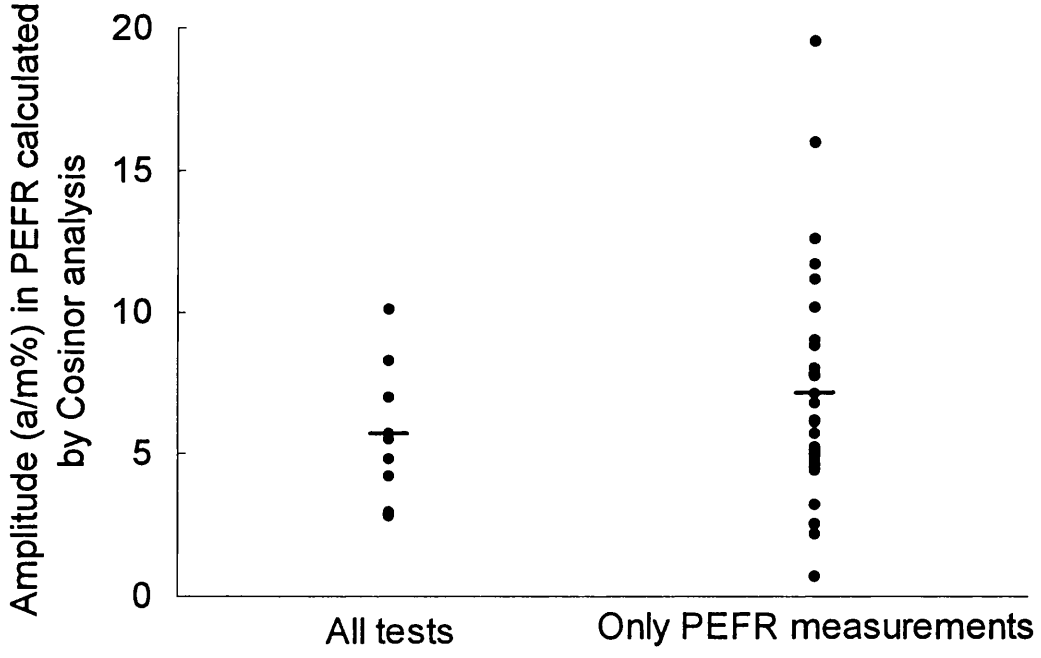

Figure 11: Distribution of lowest point (trough) of the PEF rhythm predicted by Cosinor analysis.

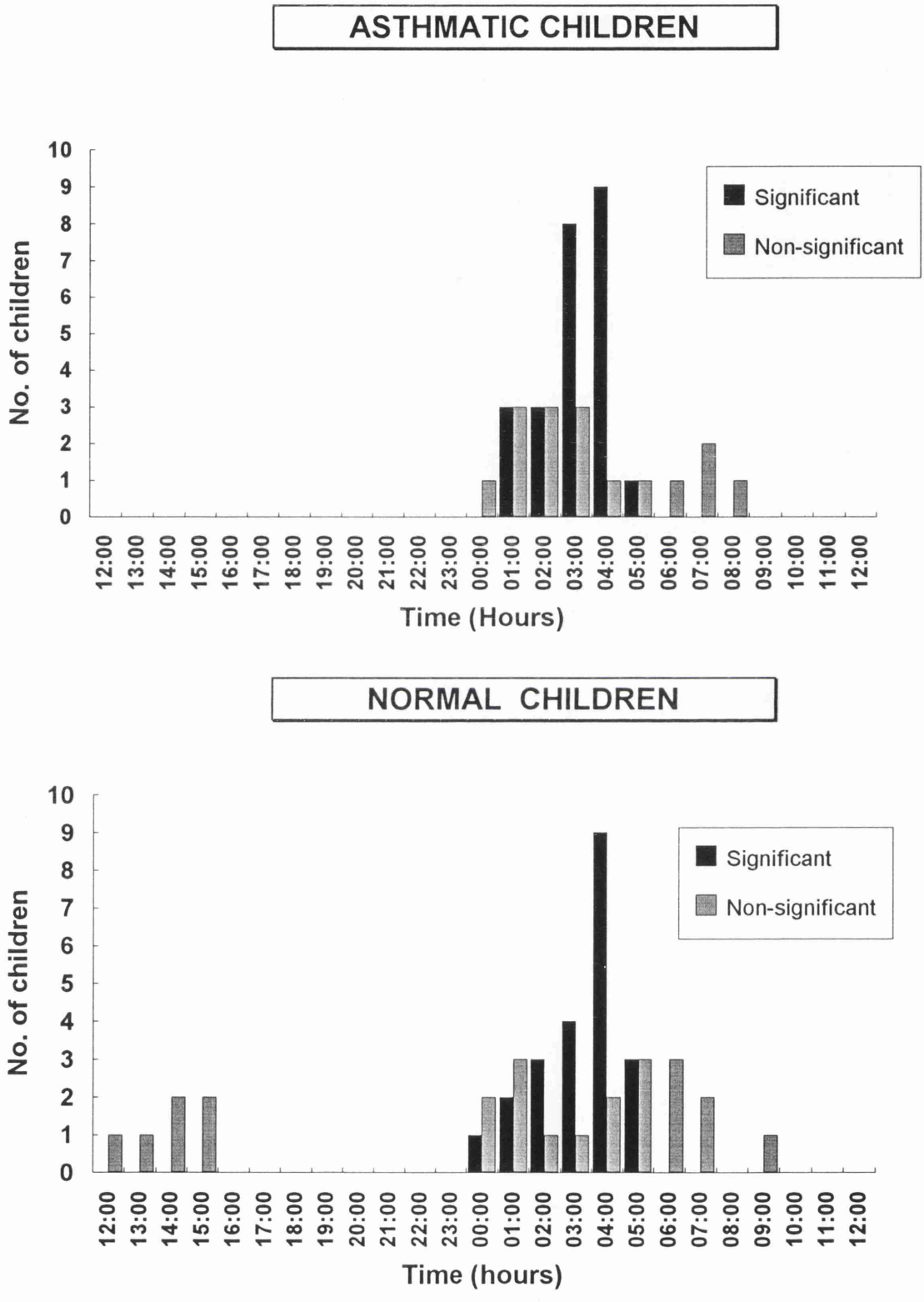

Figure 12: Scatter plots showing the close correlation between 2 different measures of diurnal variation.

The plot shows the individual values of diurnal variation calculated from the same data for each subject using Cosinor analsysis and highest - lowest / highest. The data is from the 48 subjects (39 asthmatics and 9 controls) who took part in all three parts of the study

The Cosinor technique estimates the maximum of the amplitude of the diuranl rhthym even if this occurs at atime that is not directly measured. this may account for some of the the higher values recorded by Cosinor analysis.

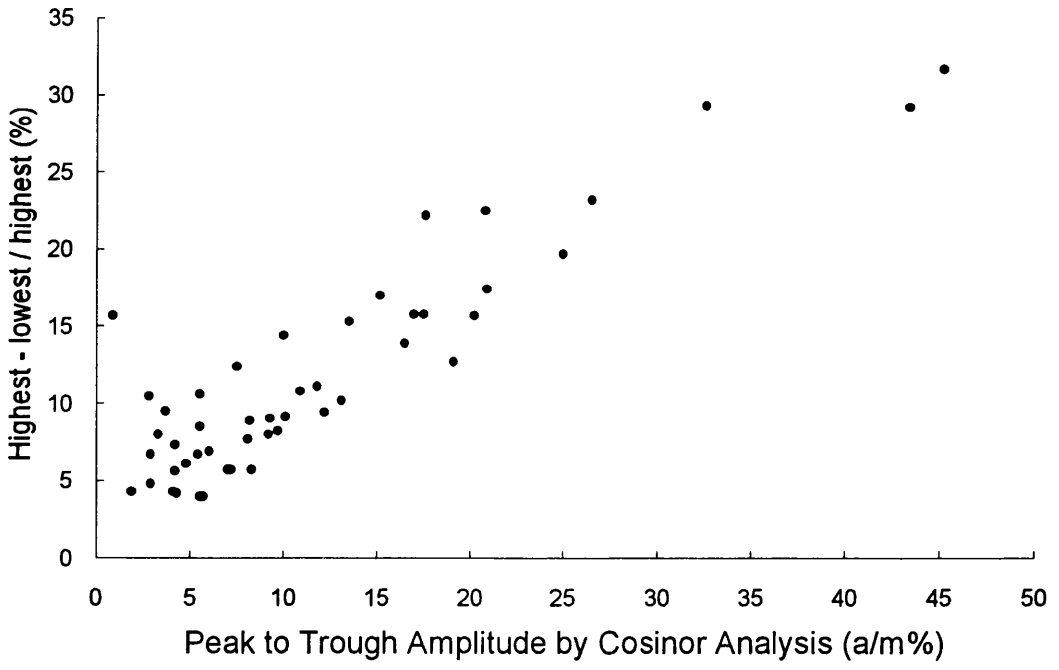

Figure 13: Scatter plots showing relationship between Pc_{20}, Percent fall in PEF and Cosinor peak to trough amplitude.

Pc_{20} values are censored at 8mg/ml as concentrations higher than this were not studied. Because of the logarithmic nature of the Pc_{20} data and the skewed nature of the distribution of values after exercise Spearman's rank correlation test was used for all correlations. Plot shows data from asthmatic and control subjects.