THE LATE ASTMATIC REACTION AFTER EXERCISE CHALLENGE

Nederlands Astmacentum Davos

B. Speelberg

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Contents

CHAPTER 1

INTRODUCTION AND AIMS OF THE STUDY

1-1. General introduction

In 1698 Sir John Floyer discribed for the first time the clinical entity of exercise-induced asthma (EIA).[1] In 1966 Heimlich et al introduced the name "exercise induced asthma".[2] In 1984 Bierman et al confirmed the existence of an early asthmatic reaction (EAR) and late asthmatic reaction (LAR) after exercise challenge.[3] Now, exercise is considered as a common and potent trigger of bronchoconstriction in asthmatic patients [4].

The early fall in PEFR or in FEV1 appears within ten minutes after exercise, reaches a maximum after 20-30 minutes and normally disappears within 1-3 hours.[3] The definition of EAR after exercise is, according to Anderson: the existence of a fall greater than 10% of the pre-exercise value in PEFR (peak expiratory flow rate) or in FEV1 (forced expiratory volume in one second) after exercise [5]. The results in this thesis are reported not according to Anderson's definition of EIA. There is no agreement in the medical literature whether the percentage of a PEFR fall after an exercise challenge should be 10 or 20%. It depends on the spontaneous variability of the bronchial obstruction for the population studied. If a change in PEFR $\geq$ 10% is considered as relevant, spontaneous variability has to be $<$ 10%. The EAR in this thesis is defined as a fall in PEFR of greater than 20% compared to pre-exercise value.

The late reaction can occur after recovery from the early reaction and starts 3-12 hours after the exercise, decreases in severity 12 hours after challenge and has usually disappeared within 24 hours after challenge. The pattern of response is very similar to the pattern observed after allergen provocation. Considering the possibility of a LAR after exercise challenge one should consider the effect of stopping drugs on the course of pulmonary function during the day. The assessment of the course of pulmonary function during a control day seems for this reason particularly important to eliminish the diurnal rhythm.

In this study the PEFR was assessed with the mini-Wright peak-flow meter to measure bronchial obstruction. It is a suitable instrument for recording the PEFR after an exercise challenge. The mini Wright peak flow meter is a reliable, pocket-sized, simple, cheap and robust instrument for following changes in ventilatory function, the measurements appeared to be good reproducible. [6, 7]

The investigations in this thesis firstly dealt with the problem which decrease in PEFR was the most useful in the definition of an EAR and LAR.

1- 2. Controversies about the LAR after exercise challenge

The controversy in the literature about the LAR to exercise is about whether or not late responses to exercise do exist and if so, what is their frequency. The first problem of analyzing this problem is the following: to which value should a fall in PEFR 3-13 hours after exercise be related to? Is this the pre-exercise value, or the value at corresponding clocktime on a control day, or the pre-exercise/predicted PEFR, or the pre-exercise/mean value on the control day, or perhaps another value? Is it to be expected that the LAR after exercise challenge can exist as a clinical entity or is it an aspecific epiphenomenon? The LAR however occurs after allergen inhalation and after metacholine provocation. When the LAR after exercise challenge occurs a consistent protocol for measurements of bronchial obstruction is necessary.

The LAR after allergen provocation is a well known phenomenon which will be discussed briefly in 1-3. It is accompanied by an increased hyperreactivity to methacholine and histamine [8], this is not the case in the LAR after exercise challenge [8, 9, 10]. Thus both LAR after exercise challenge and LAR after allergen exposition must be regarded as distinct specific phenomena.

When methacholine was inhaled in high concentrations to overcome the effect of ipratropiumbromide given before, a prolonged asthmatic response occurs after 5-12 hours which is not directly related to the height of the methacholine dose [11]. This prolonged response has to be considered as an extended EAR. This can be caused by a prolonged high

methacholine level in the blood because methacholine is only slowly hydrolyzed by acetylcholinesterase. [11]. Salbutamol, a beta2-sympathomimetic agent is capable of blocking this reaction. [11]. This is also the case in the LAR after exercise challenge. [3, 12, 13]. Boulet et al. demonstrated that asthmatic adults with a LAR after exercise challenge did not have a LAR after methacholine inhalation one week later. [10]. Thus if one could speak of a LAR after methacholine inhalation, this is distinct from the LAR after exercise challenge. Also in asthmatic children with a LAR after exercise challenge a LAR after acetylcholine exposition does not occur [14]. The arguable LAR after methacholine inhalation is also distinct from the LAR after allergen exposition [11]. The LAR after exercise challenge and methacholine provocation are not comparable as stimulating agents. The LAR after methacholine provocation is not a LAR but an extended EAR and is distinct from the LAR after exercise challenge.

1-3. Mechanisms of the EAR and LAR after allergen inhalation and exercise challenge

The EAR is responsible for bronchoconstriction which causes symptoms of dyspnoe in the patient. Vasodilatation, oedema, mediator release and smooth muscle contraction occur during the EAR after allergen inhalation. [15, 16].This bronchoconstrictive response is mediated by the actions of preformed and newly generated inflammatory mediators released after the IgE-dependent activation of mast cells associated with the bronchial mucosa. [17] Besides mast cells other cells produce and release mediators, such as epithelial cells, alveolar macrophages, basophils, eosinophils and neutrophils. Bronchial hyperreactivity may increase during the EAR after allergen challenge. [18, 19] The mechanisms of that increase in reactivity are not well understood but appear to be related to the airway inflammation after the release of mediators.

The late fall in FEVI or PEFR after provocation with inhalation allergens is a well known phenomenon. The prevalence, mechanisms and treatment are more clarified than the LAR after exercise challenge. It is already well known that the LAR after allergen inhalation can

occur without a preceding EAR. [3, 20, 21]. Mediator release and inflammation are mentioned as factors which cause a LAR. [3]. During the LAR after allergen inhalation a thickening of airway mucosa is present which is the result of plasma exudation and cellular infiltration. [15, 22].

Cells which are playing a role in the LAR after allergen exposition are : mastcells, alveolar macrophages, eosinophils, monocyts, neutrophils and thrombocytes. [8]. When the LAR after allergen exposure gradually disappears, the cellular infiltrate becomes more mononuclear instead of polymorfnuclear.

The cellular events taking place during the LAR after allergen exposure are considered highly relevant to the pathogenesis of asthma. Broncho alveolar lavage (BAL) and studies of the cellular changes during this response have demonstrated the presence of inflammatory cells (e.g. eosinophils and neutrophils), indicating active migration of these cells into the bronchial lumen. Inflammatory cells may be mobilized by chemotactic agents of protein or lipid-nature such as neutrophil chemotactic agent (NCA), serum eosinophilic cationic protein (s-ECP), complement fragments such as C5a, leucotriene B_4 and platelet-activating factor (PAF).

The LAR after allergen inhalation is followed by an increase in bronchial hyperreactivity. [18]

In asthmatics heavy exercise over several minutes may be followed by an increase in airway resistance. Airway cooling, water loss and mediator release are important factors in the mechanisms of the EAR.[23] A neutrophil chemotactic agent (NCA) of high molecular weight can be detected in the bloodstream of 75% of asthmatic subjects during the EAR after exercise challenge. The evidence for the mast cell as the source of NCA is circumstantial, and NCA is also released by mononuclear cells through an IgE-dependent mechanism. However, the release of NCA is paralleled by the release of other mast cell-derived mediators.[24, 25] The intensity of the exercise challenge partly determines the severity of the EAR. Moreover, the EAR after exercise challenge has been observed even after inhalation of water-saturated air of body temperature without heat or water loss [23, 26, 27]. Another mechanism may perhaps play a role in the underlying pathofysiological event. After exercise 40-50% of asthmatic individuals

with an EAR after exercise challenge will be less responsive to an identical exercise task performed within one hour. This is known as the refractory period. [12, 20, 28] Airway inflammation due to the influx of inflammatory cells and the subsequent mediator release is causing narrowing of the airway lumen. [9, 29] Airway inflammation may disrupt epithelial integrity, increase mucosal permeability, and contribute to the smooth muscle hyperresponsiveness. This sequence of events may explain the increased bronchial hyperresponsiveness to histamine or metacholine. [30] Subjects who are hyperreactive to one stimulus also show a greater response to other constrictor stimuli such as exercise. [31]

Controversial data are present about the LAR after exercise challenge. [32, 33]. According to some authors there would be no difference in clinical symptoms, daily FEVI variation, degree of hyperreactivity in patients with an isolated EAR or in patients with an EAR and LAR (dual responders) after exercise challenge. [11, 21, 34]. However, according to others the LAR after exercise challenge would occur more often in patients with a severe EAR (decrease in FEV1 of $\geq$ 40% of predicted value) and an incomplete recovery to baseline during the first 3 hours after exercise. [3]. The severity of the EAR and of the LAR in dual responders are significantly correlated.[34] According to other authors the EAR and LAR are not correlated and it is suggested that the LAR appears without a preceding EAR [35, 36]. In patients with an isolated EAR or isolated LAR, there is no difference in age, sex, atopy, basal FEVI and PC_{20}.[10] The severity of the LAR after exercise challenge is related to the severity and duration of the exercise challenge and is also related to the bronchial hyperreactivity of the patients airways.[37] The same goes for the EAR. The common opinion is that the LAR after exercise challenge is less severe than the EAR. [3] but according to a minority of authors the LAR after exercise challenge in some patients may be more severe than the EAR. [10, 11] However, the LAR after exercise challenge is less frequent and less severe in comparison with the LAR after allergen inhalation. [3]. Both types of LARs are varying in time of occurrence and in duration after exposure to the stimulus. [3].

The LAR after exercise challenge still occurs when the EAR after exercise challenge is inhibited by inhalation of warm humid air, which prevents mediator release. It is found that the

LAR after exercise challenge may occur independently from EAR after exercise challenge [35, 36]. Mediators, released during the EAR would serve as a base for airway obstruction for the LAR. [3]. The late increase in NCA (neutrophil chemotactic agent) after exercise challenge is less in comparison with allergen inhalation. [21]. In addition it is relevant to mention that the LAR after exercise is not IgE dependant in contrary to the LAR after allergen exposure. Neuropeptide release, mediator release from bronchial mucosa resident cells or even the reactive hyperaemia in the bronchial mucosa which follows the vascular constriction due to hyperventilation-induced heat loss can cause delayed bronchoconstriction and increased sensitivity of airway smooth muscle [9]. It is argued that the elevated level of mediators measured in the blood in the EAR and LAR after exercise challenge has no meaning at all [38]. Also, it is well known that inhalation of warm humid air during exercise challenge causes a refractory period for a next exercise challenge, which is a rather stimulus-specific phenomenon [24, 39].

The cellular events taking place during the LAR after exercise challenge are still unknown. [33]

1- 4. Therapy of the EAR and LAR after allergen and exercise challenge

The EAR after allergen challenge can be prevented by the use of bronchospamolytic agents - especially inhaled ß-sympathicomimetics are effective. Also disodium cromoglycate is potent in inhibiting an EAR after allergen challenge[40]. Nedocromil sodium (Tilade^{R}) has been shown to inhibit immediate bronchoconstriction provoked by challenge with allergen.[41] Inhibition of bronchoconstriction exhibited dose dependency up to 4 mg, with nedocromil sodium being up to four times more potent than sodiumcromoglycate.

The LAR after allergen challenge can be prevented by the use of disodium cromoglycate and oral glucocorticosteroids. Theophylline preparations and ß-sympathicomimetics are less

effective. Nedocromil sodium prevents both phases of the dual asthmatic response to bronchial antigen challenge when it is inhaled before provocation. [42]

The EAR after exercise challenge can be prevented by the use of bronchospamolytic agents - especially inhaled ß-sympathicomimetics are effective. Theophylline preparations have also been shown to be (partly) protective.[43] Also disodium cromoglycate is potent in inhibiting an EAR after exercise challenge[40]. Nedocromil sodium (Tilade^{R}) admitted one hour before provocation has been shown to inhibit immediate bronchoconstriction provoked by challenge with exercise.[44] One to four weeks of therapy with inhaled corticosteroids decreases the severity of the EAR after exercise challenge. [45, 46] Oral steroids are also capable of doing so. [47]

Besides glucocorticosteroids, other pharmaceuticals can be used in prevention or therapy of the LAR after exercise challenge. Cromoglycates can prevent the LAR after exercise challenge [3, 10]. Nedocromil sodium might be more effective than disodium cromoglycate in this respect [40, 41, 48, 49, 50]. Anticholinergics might be effective in a subgroup of patients with a LAR after exercise challenge [13, 51]. Theophylline preparations and ß-sympathicomimetics are less effective.

1-5. Clinical practise

It is of very great importance to clinicians to recognize a late fall after an exercise challenge. Patients can visit their physician with pulmonary discomfort which could be related to performed exercise 3-13 hours before the complaints started. A number of nocturnal dyspnoe complaints can also be a late reaction to exercise. The recognition of a late fall is very easily done with a mini-Wright peak-flow meter.

In this thesis the existence and prevalence of a LAR after exercise challenge were investigated in a group of patients hospitalized in the Dutch Asthma Center Davos, Switzerland. In general, this population consists of patients with severe bronchial asthma and chronic obstructive pulmonary disease. All patients used optimal medical treatment for their airway obstruction such as inhaled ß-mimetic agents, inhaled anticholinergic agents, inhaled corticosteroids and nedocromil sodium or disodium cromoglycate. Furthermore, they needed oral bronchodilators such as theophylline preparations, oral ß-mimetic agents, anti-histaminic agents in case of allergy and in a high percentage of patients oral corticosteroids. A standardized exercise challenge was carried out in all patients according to Eggleston to detect exercise-induced asthma. [1,2] [52, 53]

In chapter 2 the prevalence of the EAR and LAR after exercise challenge was studied in patients with reversible airflow limitation. The importance of a control day in the assessment of a LAR after exercise challenge was investigated in relation to other parameters such as the pre-exercise PEFR value.

The normal variability of the PEFR during the course of the day was assessed in chapter 3 and this variability is used to detect late asthmatic responses after exercise challenge. The distinction between real and pseudo late asthmatic responses after exercise challenge was made on stronger criteria to identify late responses. The question was analyzed if it was possible to get more information and a better definition of the LAR after exercise challenge by looking at figures from PEFR recordings instead of looking at percentages peak flow fall.

In chapter 4 the reproducibility of a LAR after exercise challenge was investigated in another group. It was studied if LARs after exercise challenges were still developing when more strict criteria were taken into account such as a PEFR fall on 3 or more time points 3-13

[1]The maximal predicted heart rate was calculated as: R max=209-0.74(age in years)

[2]The predicted maximal workload was calculated for man : $\frac{[0.9(60-0.55\text{age in years})]-(5.8+\text{weight}+151)}{10.5}$

The predicted maximal workload was calculated for woman : $\frac{[0.9(48-0.37\text{age in years})]-(5.8+\text{weight}+151)}{10.5}$

hours after exercise challenge compared to corresponding clock time on a control day. Using these criteria it was studied if the same LAR again was present after a second repeatable exercise challenge. How reproducible was the time at which the LAR occurs?

In chapter 5 a study was performed to start to elucidate the pathophysiological mechanism behind the LAR after exercise challenge. In patients with a reproducible positive LAR after exercise challenge a bronchial provocation test was done on different time points and blood was collected for analysis of inflammatory cells such as neutrophils, eosinophils and basophils. Furthermore, serum histamine and serum-eosinophilic cationic protein were analyzed in order to detect changes in these parameters in comparison with a group of patients with a reproducible negative LAR after exercise challenge.

In chapter 6 the protective effect of nedocromil sodium was investigated on the EAR and LAR after exercise challenge, in a double blind placebo controlled cross-over design study.

In chapter 7 a summary of the investigations in this thesis is given.

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CHAPTER 2

IMMEDIATE AND LATE ASTHMATIC RESPONSES INDUCED BY EXERCISE IN PATIENTS WITH REVERSIBLE AIRFLOW LIMITATION

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Immediate and late asthmatic responses induced by exercise in patients with reversible airflow limitation.

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Abstract.

The existence and prevalence of late asthmatic responses to exercise in patients is uncertain. We investigated whether the late falls of peak expiratory flow rate (PEFR) after exercise challenge were still significant after comparison with the corresponding clocktime PEFR on a control day. We examined 86 patients with reversible airflow limitation, 79 with asthma and 7 with chronic obstructive pulmonary disease (COPD), all under regular treatment with bronchodilators and/or anti-inflammatory agents. Patients were randomized for a control day and an exercise day and their PEFR was recorded hourly. On the exercise day, each patient underwent an 8 minute bicycle ride at 90% of predicted maximal heart-rate. An early and a late asthmatic response to exercise were considered to occur when PEFR decreased by 10% or more on the exercise day compared to the corresponding clocktime PEFR on the control day. Thirtythree patients (38%) had a 10% or greater fall of PEFR at 4 to 13 hours after exercise when PEFR was compared with the corresponding clocktime on a control day. Seven (8%) had an isolated late asthmatic response, and 26 (30%) had a dual asthmatic response. We conclude that true late asthmatic responses develop after exercise in a significant number of patients with well controlled reversible airflow limitation.

Introduction.

Exercise-induced asthma (E.I.A.) was first described in the 17th century by sir John Floyer [1]. At that time this phenomenon was regarded as a distinct clinical entity. Views began to change in 1962, when Jones et al. [2] had established for the first time that exercised-induced asthma could be a normal symptom of asthma. Now, exercise is considered as a common and potent trigger of bronchoconstriction in asthmatic patients [3]. According to Anderson [3], exercise-induced asthma is proved by a 10% or greater fall of peak expiratory flow rate (PEFR) or forced expiratory volume in one second (FEV_1) after exercise when compared to pre-exercise values. Patients with exercise-induced asthma may develop an early and/or a late asthmatic

response [4, 5]. The early fall in PEFR or in FEV_1 develops within ten minutes after exercise, reaches a maximum after 20-30 minutes and normally disappears within 1-3 hours. The late response can occur after recovery from the early response and starts 4-12 hours after the exercise, decreases in severity 12 hours after challenge and has usually disappeared within 24 hours after challenge.

The prevalence of the late asthmatic responses to exercise is uncertain [6, 7]. Rubinstein et al. [8] observed the same delayed asthmatic responses in most of their subjects during an exercise day and during a control day on which the FEV_1 was measured serially but no exercised was performed, demonstrating the lack of specificity of late responses to exercise; however, one of the patients examined had a true late asthmatic response, as shown by a delayed decrease of FEV_1 on the exercise but not on the control day [8]. Unfortunately, in most previous studies, FEV_1 after exercise was compared with FEV_1 before exercise [5, 7, 9, 10] raising the suspicion that observed late asthmatic responses may represent a decrease of pulmonary function related to withdrawal form therapy [8].

In the present study, we examined the bronchoconstrictor response to exercise in a group op patients with reversible airflow limitation, and we calculated the number of late asthmatic responses obtained by comparing the percent fall of PEFR after exercise either with the corresponding clock time PEFR on a control day, or with PEFR before exercise on the exercise day.

Patients and methods.

We examined 115 patients hospitalized in the Dutch Asthma Centre Davos, Switzerland, and included in the study 86 patients with a PEFR greater than 65% of the predicted value [11]. Seventy-nine suffered from asthma, 7 had COPD (table 2-1). All patients with bronchial asthma as defined by the American Thoracic Society, had a documented bronchial hyperresponsiveness to histamine below 8 $mg \cdot ml^{-1}$ as measured according to Cockcroft et al. [12, 13]. Asthmatic

patients showed an FEV_1 reversibility greater than 20% of predicted value after 4 puffs of salbutamol. Also the patients with COPD had a documented bronchial hyperresponsiveness to histamine below 8 $mg \cdot ml^{-1}$. In these cases, FEV_1 reversibility was less than 20% of predicted value after 4 puffs of salbutamol, and the PEFR was greater than 65% of predicted value.

Throughout the study period all patients had to submit to concomitant medication rules: the patients had to stop inhaling bronchodilators 8 hours before the exercise challenge, and during the control day; sodium cromoglycate had to be stopped 24 hours before the test and during the control day; any type of oral bronchodilator had to be stopped at least 48 hours before the start of the exercise challenge and during the control day. The dose of oral and inhaled steroids was kept constant. All patients on steroids were using this treatment for at least 3 months. Patients did not stop smoking during the study.

The control- and exercise days were chosen at random. They were separated by a minimum of 72 hours and a maximum of 6 days in order to minimize the changes in the clinical situation of the patient. Consent was obtained from each of the adult patients and from the parents of the underage children. The protocol was approved by the Ethical Committee of the clinic.

The degree of of airway hyperresponsiveness was measured as the concentration of inhaled histamine which resulted in a 20% decrease of FEV_1, and expressed as $PC_{20}FEV_1$ ($mg \cdot ml^{-1}$) [12].

Table 2-1: Patient characteristics. FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; COPD: chronic obstructive pulmonary disease. PC20: concentration of histamine that gives a 20% decrease in FEV_1.

Characteristics total group with reversible airway obstruction	
Age (yrs)	27 2 ± 14 1
Sex	46 Male, 40 Female
Children/Adults	32/54
Clinical diagnosis	79 Asthma, 7 COPD
Atopic status	71 Atopic, 15 Non-atopic
Smokers	10 Smokers, 76 Non-smokers
Steroids none/oral/inhaled/both	9/7/41/29
Histamine PC 20 (mg/ml)	1.1 ± 1 6
Baseline FEV1 (L, % predicted)	2 85 ± 0 83; 86 7 ± 23.9
FVC (L, % predicted)	4 05 ± 1 00, 101 9 ± 18 6

Characteristics children with reversible airway obstruction	
Age (yrs)	15 2 ± 1 4
Sex	23 Male, 9 Female
Atopic status	31 Atopic, 1 Non-atopic
Smokers	1 Smoker, 31 Non-smokers
Steroids none/oral/inhaled/both	3/3/24/2
Clinical diagnosis	32 Asthma, 0 COPD
Histamine PC 20 (mg/ml)	1 0 ± 1 2
Baseline FEV1 (L, % predicted)	2 67 ± 0 75, 86 8 ± 23 5
FVC (L, % predicted)	3 77 ± 0 89, 101 8 ± 17 2

Characteristics adults with reversible airway obstruction	
Age (yrs)	34 2 ± 13.5
Sex	23 Male, 31 Female
Clinical diagnosis	47 Asthma, 7 COPD
Atopic status	40 Atopic 14 Non-atopic
Smokers	9 Smokers, 45 Non-smokers
Steroids none/oral/inhaled/both	6/4/17/27
Histamine PC 20 (mg/ml)	1.1 ± 1 8
Baseline FEV1 (L, % predicted)	2 95 ± 0 86; 86 6 ± 24 4
FVC (L, % predicted)	4 21 ± 1 03, 101 2 ± 19 5

Exercise challenges were performed on a bicycle ergometer (Erich Jager, Wurzburg, Germany). The workload in Watts was 80% of the predicted maximum workload. The predicted maximum workload was calculated according to Wasserman [14]. Exercise had been performed for 8 minutes during which a heart rate of 90% of the predicted maximum was reached [15, 16]. During the exercise challenge the heart rate was measured by a Siemens Sirecust 341 monitor (Siemens, Germany). The relative humidity of the ambient air was 20-40%, the room temperature was 20-23° Celsius, both on the control and the exercise day and

both were measured with the Hygrotest 6200 (Quarz AG, Zürich, Switzerland). The humidity and room temperature were allowed to oscillate about 10% during control- and exercise day for each patient.

PEFR was measured with the mini-Wright peakflow meter. The best of three measurements was recorded. PEFR was recorded on the control day at t=0 and during the first 13 h after t=0 at hourly intervals; on the exercise day PEFR was recorded at t=0 (pre-exercise PEFR) and 1, 3, 5, 7, 10, 15 and 30 minutes, and then hourly during the next 13 h after the end of the exercise challenge.

The existence of exercise-induced asthma was inferred from the calculation of the formula in table 2-2. The early fall after exercise was calculated with the pre-exercise value as a reference.

The late fall in PEFR after exercise was calculated using four methods.

1. The lowest PEFR 4-13 h after exercise in relation with the pre-exercise PEFR.
2. The lowest PEFR 4-13 h after exercise in relation with the PEFR at the same time on the control day.
3. Because of the broad range of PEFR values in different patients (age, FEV_1), we corrected for the differences in baseline PEFRs among subjects by including the predicted PEFR values in the formula.
4. We calculated the mean PEFR on the control day for each patient and related it to the lowest PEFR 4-13 hours after exercise.

Table 2-2:Formulae used to calculate the early and the late reaction after an exercise challenge.

$$\text{Fall early, \%} = \frac{[\text{PEFR immediately before exercise}] - [\text{Lowest PEFR after exercise 1-30 min.}]}{\text{PEFR immediately before exercise}} \times 100\%$$

$$\text{Fall late \% pre-exercise} = \frac{[\text{PEFR immediately before exercise}] - [\text{Lowest PEFR after exercise 4-13 hrs.}]}{\text{PEFR immediately before exercise}} \times 100\%$$

$$\text{Fall late \% of control day} = \frac{[\text{PEFR on corresponding clocktime on control day}] - [\text{Lowest PEFR after exercise 4-13 hrs.}]}{\text{PEFR on corresponding clocktime}} \times 100\%$$

$$\text{Fall late \% predicted} = \frac{[\text{PEFR immediately before exercise}] - [\text{Lowest PEFR after exercise 4-13 hrs.}]}{\text{PEFR predicted}} \times 100\%$$

$$\text{Fall late \% mean control} = \frac{[\text{Mean PEFR on control day}] - [\text{Lowest PEFR after exercise 4-13 hrs.}]}{\text{Mean PEFR on control day}} \times 100\%$$

A fall in PEFR greater than 10% was considered positive for the existence of the early and/or late bronchoconstrictive response after exercise [3]. We separately registered PEFR falls of 10-20% and falls greater than 20%, because we wondered whether PEFR fall of greater than 10% was sufficient enough to establish a late reaction after exercise.

Results.

The results of this study are presented in table 2-3 and in figures 2-1 and 2-2. The baseline PEFR values on the control and exercise day were not comparable, the baseline PEFR on the exercise day being higher than PEFR on the control day (control day baseline value 333 ± 88 , exercise baseline value 367 ± 65; P<0,01).

Figure 2-1:The percentage of patients with a PEFR fall after exercise. The patients are registered separately, according to whether the pre-exercise PEFR, or the corresponding clocktime on a control day was taken as a reference. Above the dotted line all late respondents with a PEFR fall greater than 20% are registered.

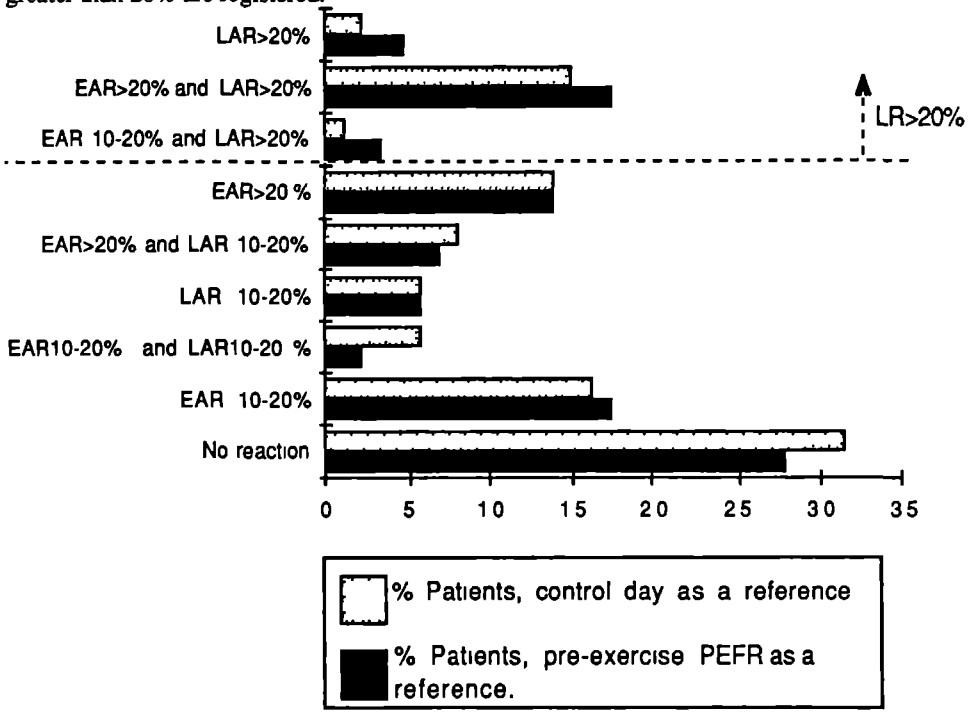


Table 2-3:Number of patients with an early and a late reaction after exercise In one column PEFR fall is compared to pre-exercise PEFR In the other column PEFR fall is compared to the corresponding clock time on a control day. Patients are separately registered as children (< 17 years) and adults (> 17 years).EIA = exercise induced asthma.EAR = early asthmatic response.LAR = late asthmatic response.

Defined subgroups	Maximal fall in PEFR compared to pre-exercise level on same day (exercise)			Maximal fall in PEFR compared to same time level on control day (no exercise)		
	Children	Adults	All	Children	Adults	All
EAR < 10 %, LAR < 10 %	10	14	24	9	18	27
EAR 10-20 %, LAR < 10 %	9	6	15	9	5	14
EAR > 20 %, LAR < 10 %	6	6	12	5	7	12
EAR < 10 %, LAR 10-20 %	1	4	5	1	4	5
EAR 10-20 %, LAR 10-20 %	1	1	2	1	4	5
EAR > 20 %, LAR 10-20 %	4	2	6	3	4	7
EAR < 10 %, LAR > 20 %	0	4	4	1	1	2
EAR 10-20 %, LAR > 20 %	0	3	3	0	1	1
EAR > 20 %, LAR > 20 %	1	14	15	3	10	13
Total	32	54	86	32	54	86

With the pre-exercise PEFR as a reference, 24 patients showed neither an early nor a late response after exercise. A late asthmatic response to exercise with a fall in PEFR of greater than

20% compared to pre-exercise value occurred in 22 (26%) of the 86 patients who completed the exercise challenge. Five patients had an isolated late response with a peak-flow fall of 10-20%. Four patients had an isolated late response with a peak-flow fall greater than 20%. The percent changes of PEFR from baseline on the control and exercise days of 2 patients who developed a late asthmatic response after exercise are illustrated in figure 2-3. In these patients, the maximum decrease of PEFR from pre-exercise PEFR was 33±21% and 36±14% during the early and late asthmatic response, respectively.

Figure 2-2 Number of patients with EIA and late falls in PEFR after an exercise challenge. The fall in PEFR is expressed only for the LAR in 10-20% and >20%.For the calculation of the number of patients with an EAR and LAR see table 2-2.

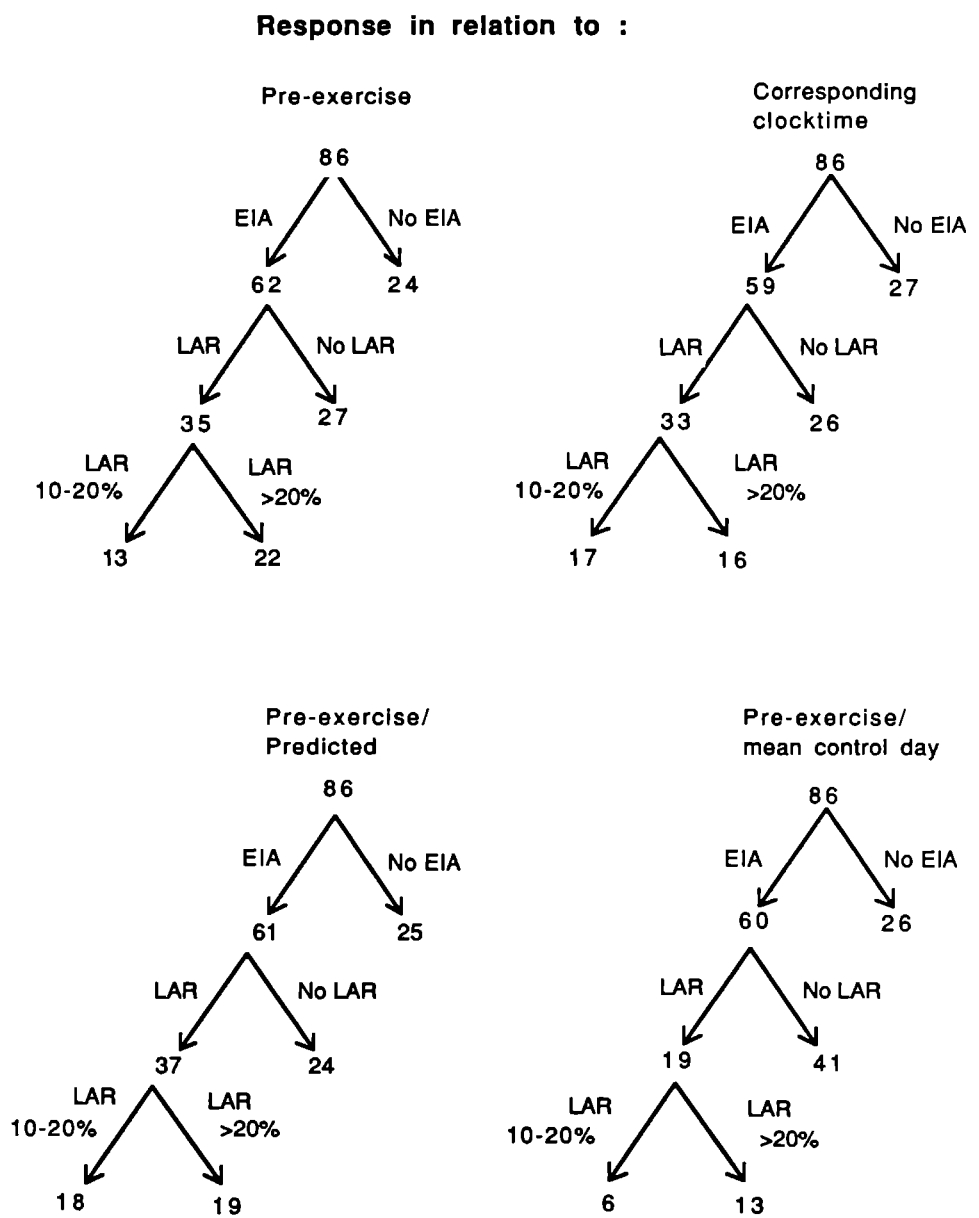
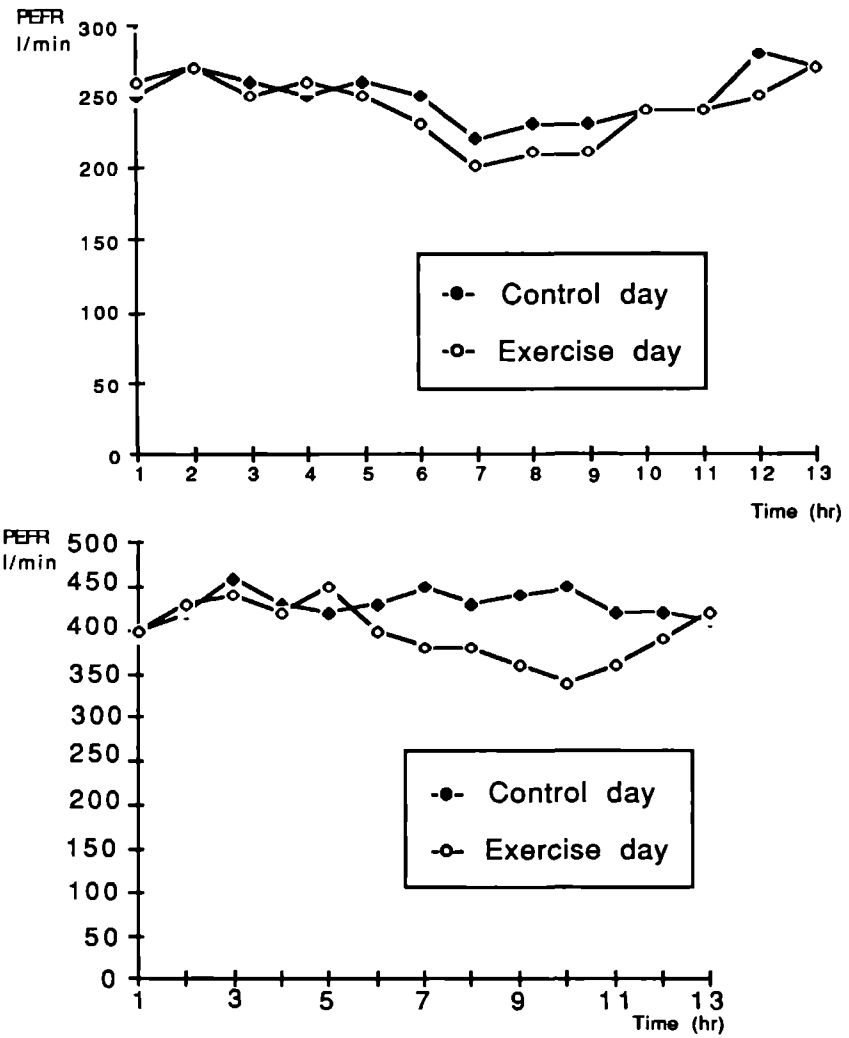


Figure 2-3 PEFR data for 2 patients with a LAR to exercise. The upper curve demonstrates a patient with a late response in relation to the pre-exercise value. PEFR immediately before exercise is 260. The late fall after exercise calculated according to table 2-2 is 23%. The lower curve demonstrates a patient with a late response in relation to corresponding clocktime on a control day. The calculate late fall after exercise according to table 2-2 is 16%.



If PEFR 4-13 h after exercise was compared with the same time on the control day, 27 patients showed no response after exercise. A late asthmatic response to exercise with a fall in PEFR greater than 20% compared to control day occurred in 16 (19%) of the 86 patients who completed the exercise challenge. Five showed an isolated late response with a PEFR fall of 10-

20%. Two patients had an isolated late response with a PEFR fall greater than 20%. Thirteen patients had an early response as well as a late response with a peak flow fall both greater than 20%.

Children developed an isolated early asthmatic response to exercise more frequently than adults, both if PEFR after exercise was compared with PEFR before exercise (children 46.9% adults 22.2%) and with the corresponding clocktime PEFR (children 43.7% adults 22.3%). By contrast adults developed a late asthmatic response to exercise more frequently than children. Twenty-one adults had a fall of PEFR greater than 20% after exercise when PEFR fall was compared to pre-exercise value and 12 when PEFR fall was compared with the corresponding clocktime PEFR on a control day. When the lowest PEFR value 4-13 h after exercise was compared with the corresponding clocktime on the control day, 33 patients (38%) had a fall of PEFR greater than 10% and 16 (19%) greater than 20% on the exercise day.

Despite the use of oral steroids, 3 patients developed a late asthmatic response after exercise when PEFR was compared with the pre-exercise value. Ten patients had a late asthmatic response of greater than 20% using both oral and inhaled bronchodilators. The mean starting time of PEFR greater than 20% was at 6.0 ± 2.1 hours after exercise when PEFR was compared to the control day and 9.0±2.4 hours, when PEFR fall was compared with the corresponding clocktime PEFR on the control day.

All seven patients with COPD developed a late asthmatic response after exercise if the fall of the PEFR was calculated on the pre-exercise PEFR, and 6 of them if the fall of PEFR was calculated on the corresponding clocktime PEFR on a control day.

Discussion.

In our study we compared the post-exercise decrease in PEFR to the corresponding clocktime PEFR on a control day. We found a considerable number of late responses. We demonstrated that the late response can occur without an early response. The late response after exercise can occur as an isolated or as a dual response. A late response was present despite the

use of oral and inhaled steroids. These drugs have been shown to play a protective role in the late asthmatic response after allergen provocation [17]. The mechanisms of exercise-induced late responses seem to be quite different from those responsible for the late responses to allergens, because the late response is much less reproducible and shorter lasting [10].

The prevalence of early, exercise induced asthma (EIA) is as stated in literature [18]. Children are relatively more affected with EIA than adults. For the late response, the contrary is shown. Asthmatic patients older than 17 years are relatively more affected by the late response after exercise than patients younger than 17.

Our results are different from those of Rubinstein et al., who also used a control day in their experiments. Rubinstein et al. demonstrated the lack of specificity of late responses to exercise in most subjects, but found one patient having a true late fall in FEV_1 after exercise challenge. The controversy in the literature about the late asthmatic response to exercise is whether or not late responses to exercise do occur and if so, what is their frequency. The difficulty of analyzing this problem is the following: to which value should a fall in PEFR of for instance >20%, 4-13 hours after exercise be related? Is this the pre-exercise value, or the value at corresponding clocktime on a control day, or the pre-exercise/predicted PEFR, or the pre-exercise/mean value on the control day, or perhaps another value? We do not agree with Rubinstein et al. that the late asthmatic response to exercise is an epiphenomena (figure 2-2) because we demonstrated 16 patients having a LAR >20% to exercise when PEFR fall was compared to corresponding clocktime on a control day. We do agree with Rubinstein et al. that PEFR fall after an exercise challenge should be related to corresponding clocktime on a control day, also without medication. In this way the diurnal variation of airway calibre can be taken into account. Few investigators have used a control day in the examination of the existence of a late fall in PEFR or FEV_1 after an exercise challenge [8-10, 19].

One should consider the effect of stopping drugs on PEFR or FEV_1. For this reason, the control day is particularly important. In studies without a control day the diurnal post-exercise rhythm is not compared with the diurnal rhythm of a day without exercise. We used in our study the PEFR. Other investigators took the FEV_1 [9]. We consider the mini-Wright peak

flow meter a good instrument for recording the PEFR after an exercise challenge. We reported our results according to Anderson's definition of EIA [15, 16]. This definition describes a fall in post-exercise PEFR greater than 10%. There is no agreement in the medical literature whether the percentage of a PEFR fall after an exercise challenge should be 10 or 20%. It depends on the spontaneous variability of the parameters for the population studied. If a change in PEFR > 10% is considered, spontaneous variability has to be < 10%. We considered that a PEFR fall greater than 20% may cause a late asthmatic response.

In contrast to our study Bierman [20] stated that it is important to perform a control day before the exercise day instead of after it, because the preceding late response may have changed the subjects' airway responsiveness and subsequent diurnal variations in PEFR.

In our study we came across a considerable number of patients using oral and inhaled steroids who had a late fall after an exercise challenge. Why these efficient drugs given in adequate pharmacological amounts did not prevent the late fall, is not quite clear to us. It is possible that without steroids, the patients would have had a much more severe late response. There may be two reasons for the occurrence of late responses to exercise in corticosteroid treated patients. First, the dose of corticosteroids may not be sufficient to control symptoms and/or prevent induced inflammatory responses associated with the late responses. Secondly, the late responses to exercise may not be associated with inflammatory responses of the airways. Exercise can induce increased airway responsiveness, with or without late responses as allergen exposure does, but this does not imply that inflammation is involved in the genesis of these phenomena as seen with allergen [10, 21]. Neuropeptide release, mediator release from bronchial mucosa resident cells, or even the reactive hyperaemia in the bronchial mucosa which follows the vascular constriction due to hyperventilation-induced heat loss can cause delayed bronchoconstriction and increased sensitivity of airway smooth muscle [22-24]. Boulet et al. [19] showed an unchanged bronchial reactivity to histamine 24 hours after the exercise.

Lee et al. [25] found no fall in FEV_1 after acetylcholine inhalation, in six persons with documented exercise-induced late response, indicating that an exercise-induced late-phase

response is more than the non-specific sequel of previous bronchoconstriction or a response to drug withdrawal.

It is of very great importance to clinicians to recognize a late fall after an exercise challenge. Patients can visit their physician with pulmonary discomfort which could be related to performed exercise 4-13 hours before the complaints started [26]. A number of nocturnal dyspnea complaints can also be a late response to exercise. The recognition of a late fall is very easily done with a mini-Wright peak flow meter. The early response can be prevented by inhaling ß-sympathicomimetics, or disodium cromoglycate [27, 28].

We should only speak of a late response after an exercise challenge, when the diurnal post-exercise rhythm has been compared with a diurnal rhythm of a day without exercise. This is demonstrated in figure 2-3, in which the upper curve shows a PEFR fall to exercise of 23% and the lower curve of 16%. Although the percentage fall in PEFR after exercise is higher in the upper curve it is nog a late response to exercise because the variation in the PEFR is due to circadian variation in airway calibre. One can never say that a patient has a late asthmatic response to exercise when a PEFR fall is related only to pre-exercise value. To draw a graph, instead of looking at figures, may be more illustrating for demonstrating a late asthmatic response to exercise.

Not all patients who had a late PEFR fall as compared with pre-exercise value, had a late PEFR fall when peak-flow decrease was compared with the corresponding clocktime on a control day. We think a PEFR fall greater than 20% can sufficiently demonstrate a late response after exercise. We demonstrated that the late bronchoconstrictive response after exercise had a prevalence of 38% when PEFR fall greater than 10% was compared with the corresponding clocktime on a control day. The percentage was 19% when PEFR fall of greater than 20% was compared with corresponding clocktime on a control day.

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Résponses asthmatiques immédiates et tardives, induites par l'effort, chez les patients atteints de limitation réversible du débit aérien. B. Speelberg, N.J. van den Berg, C.H.A. Oosthoek, N.P.L.G. Verhoeff, W.T.J. van den Brink.

RÉSUMÉ: L'existence et la prévalence des résponses asthmatiques tardives à l'effort chez les patients sont incertaines. Nous avons investigué si les chutes tardives du débit expiratoire de pointe après une provocation d'effort étaient encore significantives après comparaison avec le débit expiratoire lors d'un jour de contrôle à la même heure. Nous avons examiné 86 patients souffrant d'une diminution réversible des débits gazeux, 79 atteints d'asthme, et 7 de BPCO, tous sous traitement régulier aux bronchodilatateurs et aux agents anti-inflammatoires. Les patients ont été répartis de façon randomisée en un jour de contrôle et un jour d'effort, avec enregistrement horaire du débit expiratoire de pointe. Pendent le jour d'effort, chaque patient a subi une épreuve à la bicyclette pendent 8 minutes à 90% du pouls cardiaque prédit. Une résponse asthmatique précoce et tardive à l'effort a été considérée comme présente si le débit de pointe diminuait de 10% ou davantage le jour de l'effort, par comparaison à la même heure le

jour de contrôle. Trente-trois patients (38%) ont montré une chute du VEMS de 10% ou davantage entre 4 et 13 heures après l'effort, lorsque le débit de pointe était comparé avec le débit correspondant à la même heure le jour contrôle. Sept (8%) n'ont manifesté qu'une résponse tardive isolée, et 26 (30%) ont eu une réaction asthmatique double. Nous concluons que des réactions asthmatiques tardives authentiques se développent après l'effort chez un nombre significantif de patients atteints d'une limitation réversible mais bien controlée des débits aériens.

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CHAPTER 3

REAL AND PSEUDO LATE ASTHMATIC REACTIONS AFTER SUBMAXIMAL EXERCISE CHALLENGE IN PATIENTS WITH BRONCHIAL ASTHMA

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Real and pseudo late asthmatic reactions after submaximal exercise challenge in patients with bronchial asthma.

A new definition for late asthmatic responses after exercise challenge.

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Abstract

The late asthmatic reaction after exercise challenge remains a controversial issue. In this study 21 patients recorded peak expiratory flow rate (PEFR) on two control days without performing exercise. There was no difference between both control days when PEFR at 1 hour was compared to baseline PEFR and when PEFR at 4-13 hours was compared to baseline PEFR. After analyzing variation coefficients of baseline PEFR on a control- and exercise day PEFR was not allowed to differ more than 15.3 % in the same patient, when comparing exercise- and control day for the late fall in PEFR in the study. In 17 out of 81 patients a late asthmatic reaction after exercise challenge was present, when PEFR fall was ≥20% compared to baseline PEFR value. In 8 out of the 17 patients a real late asthmatic reaction to exercise challenge was present with a PEFR fall ≥20% on at least 3 successive time points and who had a PEFR fall ≥20% compared to corresponding clocktime on a control day. The late asthmatic reaction to exercise challenge is characterized not as an non-specific epiphenomenon, but as a fall in PEFR of ≥ 20% both compared to baseline PEFR value and to corresponding clocktime on a control day on at least 3 successive time points. Graphic illustration of airway responses following exercises may facilitate the detection of a late asthmatic responses.

Abbreviations used :

PEFR: Peak Expiratory Flow Rate

EIA: Exercised Induced Asthma

EAR: Early Asthmatic Response

LAR: Late Asthmatic Response

EC: Exercise Challenge

Key words : Exercise - asthma - early asthmatic reaction -late asthmatic reaction -peak expiratory flow rate.

Introduction

In 1980, Bierman et al. reported for the first time a late asthmatic response (LAR) after exercise challenge (EC).[1] The LAR starts 3-12 hrs after EC and may last for several days.[2,3] According to Anderson et al. , a LAR is defined as a fall in peak expiratory flow rate (PEFR) or in forced expiratory volume in 1 second (FEV1) greater than 10 percent of baseline.[4] The prevalence of a LAR after EC has been reported to vary between 2-60 percent.[5-9] Recently, the existence of a LAR after EC has been challenged.[7,10,11] These investigators looked at a LAR after EC as a nonspecific epiphenomenon, to be the result from medication withdrawal before EC, and cyclic changes in pulmonary function and airway hyperresponsiveness. It was stated in a recent review, that cumulative evidence indicates that a LAR after exercise may occur, but that the prevalence of it is uncertain. Furthermore, that decrements in airways calibre may occur simply as the result of withdrawal of medication.[12] In a group of 86 patients with reversible airflow limitation, a LAR after EC, considered to occur when PEFR decreased by 20 percent or more on an exercise day compared to the corresponding clocktime PEFR value on a control day, was demonstrated to have a prevalence of 19 percent. In this study of Speelberg et al. it was suggested that there is no agreement in the medical literature whether the percentage of a PEFR fall after an EC should be 10 or 20 percent, depending on the spontaneous variability of PEFR in the population studied. It was also suggested that drawing graphs, instead of looking at figures, may be more illustrative for demonstrating a LAR after EC.[9]

In the present study we investigated the prevalence and pattern of the fall in PEFR after EC in patients with asthma studied by Speelberg et al. The effects of withdrawal of treatment and EC were separated by monitoring PEFR after medication withdrawal only and after medication withdrawal followed by EC. Spontaneous variability of PEFR in the population is determined and graphs are drawn of those patients demonstrating a late fall in PEFR after EC of more than 20 percent.

Patients and methods

The study consisted of two parts. In study 1, PEFR was measured on two separate study days after withdrawal of medication without exercise. This study was performed to determine spontaneous PEFR variability on two separate days in the population studied. In study 2, PEFR was measured after the same medication withdrawal regimen as in study 1 on a day without exercise (control day) and on a day on which a submaximal EC was performed (exercise day). This study was performed to determine the prevalence of a late fall in PEFR ≥ 20 % after EC in the patients studied and to visualize this late fall in PEFR in graphs, both on control and on exercise day.

In the study, an early asthmatic reaction (EAR) was defined as a fall in PEFR from baseline of at least 20 percent 1-60 min after EC. A LAR was defined as a fall in PEFR from baseline of at least 20 percent 4-13 hours after EC.[9] A true LAR was defined as a fall in PEFR from baseline of at least 20 percent on 3 successive occasions 4-13 hours after EC on the exercise day, without a similar fall in PEFR from baseline at similar time points on the control day. A pseudo LAR was defined as a fall in PEFR from baseline of at least 20 percent 4-13 hours after EC on the exercise day, accompanied by a similar fall in PEFR from baseline at similar time points on a control day.

Study 1

This study was done in 21 asthmatic patients who were treated in the Dutch Asthma Centre Davos and who fulfilled the following inclusion criteria: (a) age 12-65 years; (b) clinical diagnosis of bronchial asthma, according to the criteria of the American Thoracic Society [13]; (c) baseline PEFR (first measurement in the morning on each of both study days) ≥ 65 percent of predicted; (d) PC20 to histamine (the concentration of histamine which resulted in a 20 percent decline in FEVI of baseline was measured) ≤ 8 mg/ml [14]. Patients characteristics are given in table 3-1.

Table 3-1. Characteristics of patients in study 1

Number of patients	21
Age (yrs, mean±SD)	25.8 ±14.1
FVC (% predicted, mean±SD)	98.9 ± 12.4
FEVI (% predicted, mean±SD)	81.0 ± 21.3
Histamine PC20 (mg/ml, mean±SD)	1.5 ± 2.6
Corticosteroids none/oral/inhaled/both (number of patients)	1/5/11/4

Characteristics of patients in study 2

Number of patients	81
Age (yrs, mean±SD)	25.5 ± 11.7
FVC (% predicted, mean±SD)	103.0 ± 18.3
FEVI (% predicted, mean±SD)	89.0 ± 22.9
Histamine PC20 (mg/ml, mean±SD)	1.0 ± 1.7
Corticosteroids none/oral/inhaled/both (number of patients)	9/7/45/26

Oral bronchodilators were stopped 48 hours and disodium cromoglycate 24 hours before the study day and inhaled bronchodilators were stopped ≥ 8 hours before. Oral and/or inhaled corticosteroids were continued.

PEFR was measured with a mini-Wright peak flow meter (Airmed®) ten times in the first 30 minutes in the morning and hourly thereafter for 13 hours. The peak flow meters were cleaned after each measurement to prevent excessive humidity. The best of 3 consecutive PEFR values was recorded.

Study 2

One hundred and ten patients who were treated in the Dutch Asthma Centre Davos and fulfilled the same inclusion criteria as in study 1 were selected. PEFR was first measured on a control day as in study 1 with the exception that in the first 30 minutes in the morning PEFR was measured only once. In 81 patients the PEFR values on the control day and on the morning of the next day were not below 65 percent of predicted value. These patients were exercised. Their characteristics are shown in table 3-1. The patients started EC exactly at the same time as the first PEFR-measurement on the control day. Room temperature was 18-23 °C and relative

humidity of ambient air 20-40 percent. EC consisted of 8 min cycling on a bicycle ergometer (Erich Jäger, Würzburg, Germany). Workload was gradually increased until 80 percent of the predicted maximal heart rate was reached and continued for at least 4 min at this level.[15] During EC, heart rate and ECG were monitored (Sirecust® 341, Siemens. Germany). The subjects wore a nose clip.[16] Prior to EC, baseline PEFR was measured twice with an interval of 5 min. After EC, PEFR was measured at 1, 3, 5, 7, 10, 15, 30 min and 1 hour and hourly for 12 hours as on the control day.

The protocol was approved by the Ethical Committee of the Centre and all patients gave written informed consent.

Statistics

Study 1

The coefficient of variation of the PEFR was calculated over the 13 hour measurement period on both study days in the 21 asthmatic patients.[17] In each patient the 10 PEFR values in the first 30 minutes of both study days were compared by analysis of variance in patients to determine day to day variability in baseline value. From this the within-subject difference in baseline value on the control and exercise days allowed in study 2 was defined. Variability and reproducibility of the PEFR on the entire study days in the 21 patients were analyzed by repeated measurements analysis of variance.[18]

Study 2

The coefficient of variation of the PEFR was calculated over the 13 hour measurement period on the control day in the 81 asthmatic patients who cycled and compared to the PEFR variability in the groups of study 1 and 2. In order to assess whether a late fall in PEFR $\geq$ 20 percent was more frequent on the exercise day than on the control day, Mc Nemar's test for

paired dichotomic observations was used.[18] The magnitude of the early and the late fall in PEFR on the exercise day was compared by a two-tailed paired t-test. In patients in whom no PEFR measurements were missing, the difference between the values of the early and the late fall in PEFR on the exercise and the control day was analyzed by repeated measurements analysis of variance.[18] An early fall was defined here as a >20% fall from baseline PEFR within 1 hour after EC.

Results

Study 1

The mean coefficient of variation in 21 patients of the PEFR on both control days was 4.6 ± 2.9 percent. It was slightly larger on the first day (5.1 ± 3.3 percent) than on the second (4.0 ± 2.6 percent). When these coefficients of variation of the PEFR on the first and second study day were compared by a two-tailed paired t-test, the difference was not statistically significant ($P > 0.15$).

Baseline PEFR variability in the first 30 min of both control days was split up in 3 components, due to different sources of variability. The first coefficient of variation, due to within patient variation of PEFR on one control day, was 3.9 percent. The second coefficient of variation, representing the within patient variation between PEFR on both control days, was 3.7 percent. The third coefficient of variation, describing the variation in mean PEFR levels between patients, was also 3.7 percent. The total within patient coefficient of variation of baseline PEFR values in the first 30 min of both study days was 5.4 percent. Assuming a Gaussian distribution and using 95 percent confidence intervals, we did not allow baseline PEFR on exercise and control day to differ more than 15.3 percent in the same patient when comparing exercise and control day for the late fall in PEFR in study 2.

In 9 out of the 21 patients who completely filled in their PEFR values repeated measurements analysis of variance on hourly recorded PEFR values on both study days revealed no early effect when PEFR at 1 hour was compared to baseline PEFR (P = 0.83) and no late effect when PEFR at 4-13 hours was compared to baseline PEFR (P = 0.32). There was no difference between both study days when PEFR at 1 hour was compared to baseline PEFR (P = 0.12) and when PEFR at 4-13 hours was compared to baseline PEFR (P = 0.88).

Study 2

The coefficient of variation of PEFR on the control day was 4.6 ± 3.1 percent for the entire group of 81 patients who performed EC and 6.7 ± 2.5 percent for 17 patients with a LAR.

On the basis of Mc Nemar's test (table 3-2), the hypothesis that a LAR after EC with a PEFR fall of ≥ 20 percent does not occur more often on the exercise day than on the control day had therefore to be rejected (P= 0.008).

Table 3-2. Comparision of a late fall in PEFR from baseline on the control and exercise day, number of patients.

	Control day	
Exercise day	<20 %	≥20 %
<20 %	64	0
≥20 %	8	9

Table 3-3. Characteristics of patients with a late fall in PEFR < 20 %, a real LAR and a pseudo LAR.

	PEFR fall < 20 %	Real LAR	Pseudo LAR
Number of patients	64	8	9
Age (yrs, mean ± SD)	23.8 ± 12.7	29.9 ± 11.1	33.8 ± 10.9
FVC(% predicted, mean ± SD)	104.2 ± 18.4	107.4 ± 9.2	91.4 ± 20.7
FEVI (% predicted, mean ± SD)	90.3 ± 23.1	87.3 ± 19.1	80.5 ± 24.0
Histamine PC 20 (mg/ml, mean ± SD)	1.1 ± 1.6	1.3 ± 2.1	0.1 ± 0.1
Corticosteroids: none/oral/inhaled/both (number of patients)	6/4/35/19	2/1/3/2	1/2/1/5
EAR ≥ 20 % (number of patients)	18	5	6

The magnitude of the early and the late drop in PEFR on the exercise day in the entire group of 81 patients who performed EC were significantly correlated, although the correlation

coefficient was small ($r = 0.33$; $P < 0.01$). The early PEFR fall (18.9 percent) was significantly greater than the late PEFR fall (9.5 percent) ($P = 0.0001$).

Repeated measurements analysis of variance revealed that the mean level of PEFR after EC was significantly lower on the exercise day than on the control day ($P = 0.004$). Also, the mean level of PEFR 4-13 hours after EC was significantly lower on the exercise day than on the control day ($P = 0.01$).

For the 17 patients in whom PEFR fall was more than 20 percent 4-13 hours after EC, PEFR values on the control and the exercise day were compared at similar time points in graphs taking baseline value at the beginning of the day as 100 percent. There were 2 subgroups: (a) Eight patients in whom late PEFR decreased ≥ 20 percent on the exercise day as compared to the control day on at least 3 successive points of measurement (figure 3-1). These were considered patients with a LAR, probably not caused by medication withdrawal (real LAR). (b) Nine patients in whom late PEFR on the exercise day and the control day showed no consistent difference (figure 3-2). In these subjects, a reason for the fall in PEFR was not clear (pseudo LAR). The characteristics of the patients with a fall in PEFR < 20 percent, a real LAR, and a pseudo LAR are compared in table 3-3.

Figure 3-1.PEFR on the exercise- and control day in 8 patients with a real LAR after EC. Baseline PEFR has been taken as 100% reference value.

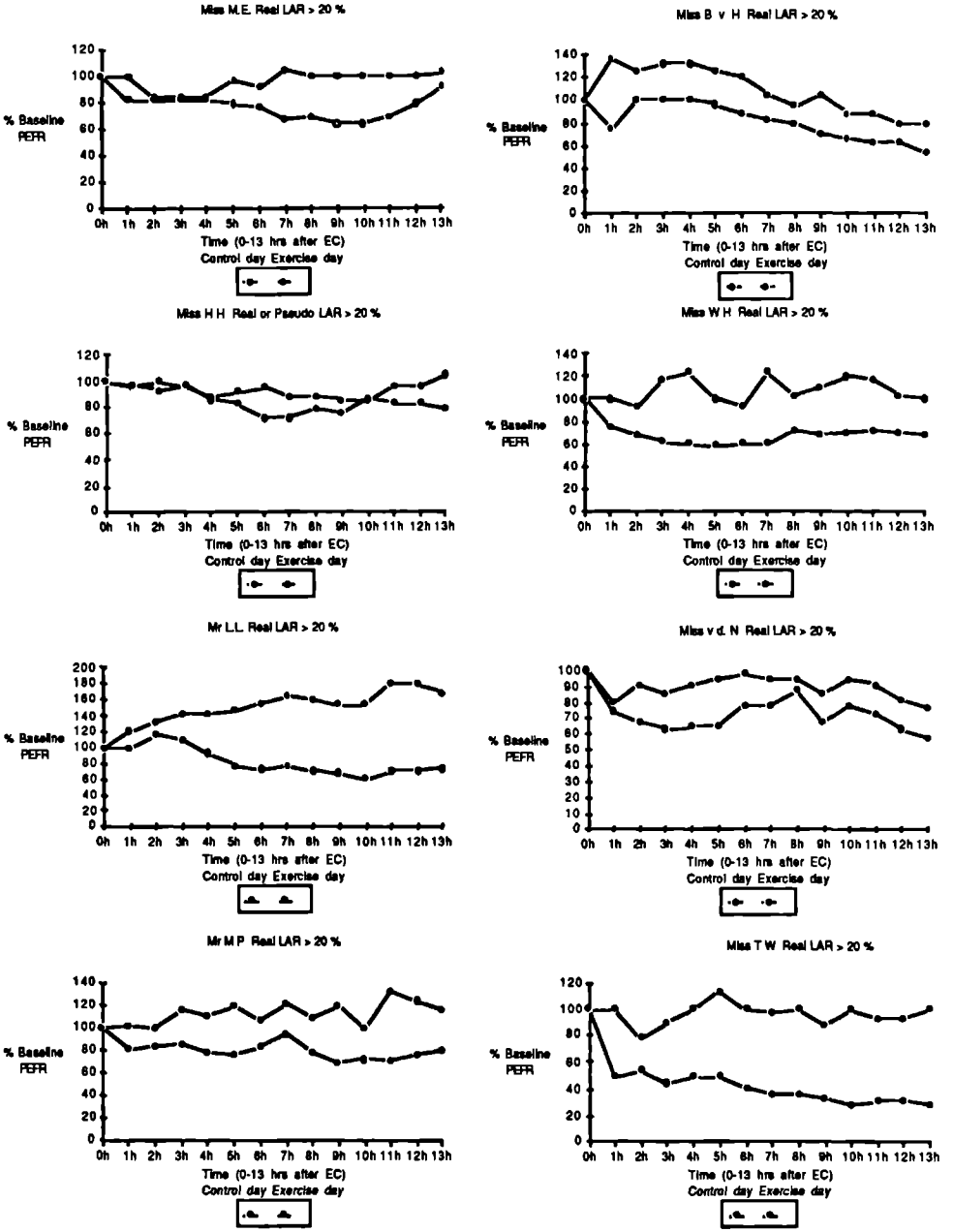
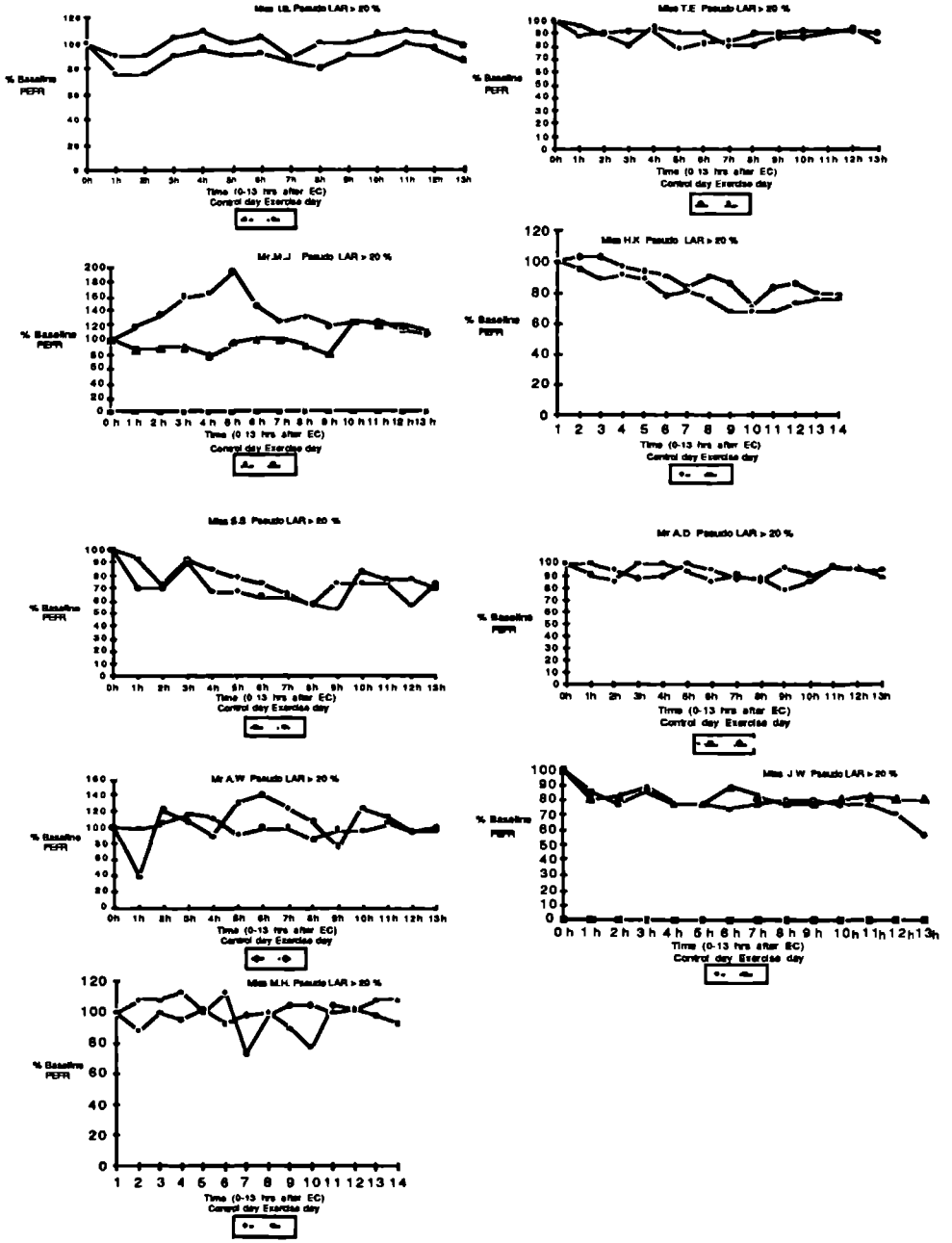


Figure 3-2.PEFR on the exercise- and control day in 9 patients with a pseudo LAR after EC. Baseline PEFR has been taken as 100% reference value.



Discussion

A rather high coefficient of variation for repeated PEFR measurements within 1 control day within 1 asthmatic patient was found in the first 30 min. This may be attributable to the rather high intra-instrumental variability of the Mini Wright Peakflow Meter, which sometimes differs about 15 % from a standardized flow value, especially in the low flow ranges.[19] As there was no statistically significant difference between the coefficients of variation of the PEFR in both control days, the coefficient of variation of the PEFR on control day in the patient group of study 1 was comparable to that in the patient group of study 2.

By taking a limit of a fall in PEFR $\geq$ 20 percent to define a LAR we were sure that this fall could not be explained on the basis of spontaneous variability in PEFR, since a 15.3 % variability is the upper limit of the 95 percent confidence interval. From the Mc Nemar test it can be concluded that a real LAR after exercise did occur in the group of the 81 asthmatic patients who performed an EC. This can also be concluded from the repeated measurements analysis of variance in the asthmatic patients who cycled and had completely recorded PEFR values. Also, the fact that the results were grossly the same when the PEFR value on the same time on the control day was taken instead of the baseline PEFR on the exercise day [9], is an indication that there was a real LAR after EC on the exercise day without an accompanying LAR on the control day.

A real LAR occured in 8 patients, 10 % of the asthmatic population studied. The prevalence may have even been greater since not all patients could be taken off steroids therapy long enough. The pattern of the LAR in these 8 patients showed a variable pattern. PEFR 4-13 hours after EC may fall to return quickly to baseline values, remain down for a long time or to decrease progressively (figure 3-1).

These findings are in accordance with the literature, in which no significant differences were observed in clinical severity of asthma, age, sex, atopic status, and histamine PC20 between patients with a single EAR and patients with both an EAR and LAR.[2,5,20]

An early fall ≥ 20 % occurred more than 2 times as often in patients with a LAR as compared to patients with a late fall in PEFR < 20 %. There was no difference, however, in EAR between the groups with a real and with a pseudo LAR. Two patients with a pseudo LAR and 1 patient with a real LAR did not have an EAR in our study. The more severe the early fall in PEFR, the greater the probability for a severe late fall in PEFR to occur. But a LAR to EC can occur without a preceding EAR. These results are in accordance with the literature in which it was proposed that a more severe EAR and LAR after EC are correlated [20-22], but that a LAR after EC can occur without a preceding EAR.[5,8,9,11]

The early fall in PEFR after EC was found to be significantly greater than the late fall. This is in accordance with the findings in most studies in which a majority contends that the LAR is less prevalent and less severe than the EAR after EC [1,7,11] and in contradiction with a minority that has found that the LAR is more severe.[5]

It could be possible that the LAR after EC results from an inflammatory reaction induced by the combination of airway water loss, airway cooling, hyperventilation and its metabolic consequences and a slow onset of adrenaline release during exercise. Airway inflammation is also an important etiological factor in LAR after allergen inhalation [23,24] and in LAR in occupational asthma.[25-27] In our study, patients continued using steroids. These agents not only alter the late asthmatic response but also the normal variation of airway caliber.[28,29] The observed variability in airway responses may be quite different when using glucocorticosteroids in patients with asthma. In this study is was for clinical reasons not possible to stop GC. The GC did not prevent a LAR to occur in 14 of the 81 asthmatic patients studied, although they used the steroids for at least 3 months before the start of the study. Therefore, either the actual prevalence of the LAR after EC may be greater than found in our study or the role of inflammation may play a less important role in the LAR after EC than in the other LARs discussed.[9] Another possible cause could be the dose of steroids, which was perhaps not high enough.

Our results are in contradiction with the hypothesis that the LAR after EC does not exist or is a non-specific epiphenomena.

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LATE ASTHMATIC RESPONSES AFTER EXERCISE CHALLENGE ARE REPRODUCIBLE.

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Late asthmatic responses after exercise challenge are reproducible.

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Abstract.

In this study the reproducibility of a late asthmatic reaction (LAR) after exercise challenge (EC) has been documented. Eighty three hospitalized asthmatic patients were challenged with exercise. The patients were examined according to a standardized protocol which comprised 8 minutes bicycling at 90% of predicted heart-rate. A LAR after EC was considered to take place when a fall in peak expiratory flow rate (PEFR) ≥20% occurred on 3 or more time points on the exercise day when compared to corresponding clocktime on a control day. According to these criteria 11 patients (13.3%) experienced a LAR. Those patients were rechallenged 21-150 days after the first EC, without changing the therapy regimen of the patients, to study its reproducibility. Eight patients (73%) showed a reproducible LAR after EC based on the criteria for a positive LAR. Although the LAR after EC was reproducible, the time points at which the LAR took place after the second EC differed from those after the first EC. Our results indicate that the LAR after EC occurs in a considerable number of patients with bronchial asthma and is quite reproducible.

Key words :exercise challenge - late asthmatic response - exercise induced asthma - reproducibility.

Introduction

After exercise challenge (EC) asthmatic patients may show an early asthmatic response (EAR) and/or a late asthmatic response (LAR). The EAR is expressed as the fall in peak expiratory flow rate (PEFR) or in forced expiratory volume in 1 second (FEVI) and appears within 10 minutes after EC, reaches a maximum after 20-30 minutes, and disappears normally within 1-3 hours. A LAR after EC may occur after recovery from the EAR and starts usually 3-13 hours after the EC, decreases in severity after 13 hours and usually disappears spontaneously within 24 hours.[1]

Studies describing a LAR after EC have calculated the fall in PEFR compared to the pre-exercise PEFR value. However, it has extensively been documented that it is more appropriate to relate a PEFR fall after EC to the corresponding clocktime PEFR value on a control day instead of to the pre-exercise PEFR value because of diurnal variation.[2,3] Although in this way the LAR after EC can be much better described, we have attempted to further refine its definition in a previous study.[4] Based on this study the LAR was defined as a PEFR fall ≥20% on three consecutive time points, implicating a period of ≥2 hours, 3-13 hours after EC in comparison with the corresponding clocktime PEFR value on a control day without exercise. Although the choice of a PEFR fall ≥ 20% on 3 consecutive time points still is arbitrarily this criteria allowed to discriminate between a LAR and a pseudo-LAR after EC.[4] Furthermore in this study it was stated that graphical analysis of the PEFR changes after EC compared to corresponding clocktime values on a control day are necessary to properly describe the LAR. Moreover, during the LAR clinical symptoms have to be present. Therefore to our opinion this is a careful definition to describe a LAR after EC. Using this definition of a LAR after EC we have investigated whether the LAR after EC was reproducible.

Patients and Methods

Patients.

All patients (n = 83) participating in the study were suffering from bronchial asthma as defined by the American Thoracic Society [5] and the criteria mentioned below. Patients could not be diagnosed as having bronchial asthma and chronic obstructive pulmonary disease (COPD) simultaneously.

Asthmatic patients had paroxysms of dyspnoea, wheezing, and cough. They had fluctuations in the severity of bronchial obstruction characterized by periods of a normal FEVI and by periods of an abnormal FEVI {below 70% of the predicted European Community for Coal and Steel value (ECCS)}, normalization in the severity of airflow obstruction (FEVI

greater than 70% of the predicted ECCS value) following the administration of bronchodilators or corticosteroids.[6] All patients with bronchial asthma had a documented bronchial hyperresponsiveness, measured according to Hargreave et al. and expressed as the concentration of inhaled histamine which results in a 20% decrease of FEV1 (PC20 < 8 mg/ml).[7]

Throughout the study period all patients had to submit to concomitant medication rules. The patients had to stop inhaled bronchodilators 8 hours before the EC and during the control day. Sodium cromoglycate had to be stopped 24 hours before the exercise test and during the control day. Any type of oral bronchodilator had to be stopped at least 48 hours before the start of the EC and during the control day. The dose of oral and inhaled steroids was kept constant. All patients on steroids were using this treatment for at least 3 months. Patients did not stop smoking during the study. Smoking pattern remained the same during the study period. Informed consent was obtained from each of the patients. The characteristics of the patients with a LAR after EC are summarized in table 4-1.

Table 4-1. Patient characteristics of the 11 patients showing a LAR after the first EC. *Atopy is defined as: three or more positive skin tests to common allergens. **Inh.:inhaled.

Nr	Age (years)	Sex	FVC basal (L)	FVC(% pred.)	FEV1 basal (L)	FEV1(% pred.)	PC 20 (mg/ml)	Atopy*	Smoker	Steroids	Diagnose
1	61	Female	3 47	110	2 24	83 9	0 5	+	-	oral	Asthma
2	59	Female	3 39	114 1	2 12	84	0 047	-	-	inh **	Asthma
3	72	Female	2 53	79 9	1 16	67	not done	+	-	oral+inh	Asthma
4	19	Female	3 08	74	2 6	71	< 0 03	+	-	-	Asthma
5	47	Female	3 54	120 9	2 8	111 8	< 0 03	+	+	oral+inh	Asthma
6	49	Female	3 42	115	2 04	80 5	0 26	+	-	inh	Asthma
7	43	Female	4 17	119 9	3 52	117 3	0 17	-	-	oral+inh	Asthma
8	26	Female	4 41	99 5	3 48	89 6	1 6	+	-	oral+inh	Asthma
9	21	Female	4 41	105 32	2 92	80	0 35	+	-	oral	Asthma
10	52	Female	2 19	68	1 52	55 1	< 0 03	+	-	oral+inh	Asthma
11	53	Female	4 31	116	2 64	82 9	0 18	+	-	oral	Asthma

Exercise Challenge (EC)

All ECs were performed on a bicycle ergometer (Erich Jäger, Würzburg, Germany). The maximal workload in Watts was 80% or less of the predicted maximum workload. The predicted maximum workload was calculated according to Eggleston.[8] Exercise was performed

for 8 minutes during which a heart-rate of 90% of the predicted maximum was reached. During the EC the heart-rate was measured by a Siemens Sirecust 341 monitor (Siemens, Germany). The relative humidity of the ambient air was 20-40%, the room temperature was 20-23° Celsius, both on the control and the exercise days and were both measured with the Hygrotest 6200 (Quarz AG, Zürich, Switzerland). The humidity and room temperature were allowed to variate 10% and 2°C respectively during control- and exercise day for each patient. During the 8 min. bicycling the patients wore a noseclip.

PEFR and FEVI measurements.

PEFR was measured with the mini-Wright peakflow meter.[9] All patients participating in the study were well trained in using the mini-Wright peakflow meter. Furthermore, the same peakflow meter was used at each occasion. The best of three measurements was recorded. Before and after each daily recording of peakflow rates, meters were checked and cleaned by the lung function technician. PEFR was recorded on the control day at t=0 (09.00 a.m.) and during the first 13 hrs after t=0 at hourly intervals; on the exercise day PEFR was recorded at t=0 hour (= pre-exercise PEFR) and 1, 3, 5, 7, 10, 15 and 30 minutes afterwards, and also hourly during the next 13 hrs at the end of the EC. In this study the PEFR was assessed with the mini-Wright peak-flow meter to measure bronchial obstruction. It is a suitable instrument for recording the PEFR after an exercise challenge.[10,11]

As reference values the PEFR on corresponding clocktime of the control day was taken. The % PEFR fall for the LAR after EC compared to corresponding clocktime on a control day was calculated as : 100% x (PEFR at corresponding clocktime on control day - lowest PEFR 1-13 hours after EC) / PEFR at corresponding clocktime on control day.

FEVI was measured according the method of the ECCS using a dry spirometer (Schiller, Switzerland) in a group of asthmatic patients with reversible airflow limitation (n=19), in order to study the correlation between FEVI and PEFR measured.[6]

Study-Design.

When patients showed a PEFR fall ≥20% after EC compared to pre-exercise level they were asked to record their PEFR on a control day without exercise, with the same medication restrictions as on the exercise day, 2 days after the first EC. When patients after EC showed a PEFR fall of ≥20% on 3 or more consecutive time points 3-13 hours after EC compared to control day values, a second EC was performed after a minimal interval of 21 days and a maximum interval of 150 days. The second EC was performed with the same medication restriction as the first EC. In between these 2 exercise days the medication was kept unaltered.

Nine at randomly chosen patients who did not show a PEFR fall ≥20% on 3 or more consecutive time points 3-13 hours after EC compared to control day values after the first EC compared to control day were rechallenged for the second time as in the patients with a LAR to EC. (The characteristics of the 9 patients without a LAR after EC are summarized in table 4-2)

The protocol was approved by the ethical committee of the clinic.

Table 4-2. Patient characteristics of the 9 patients without a LAR after EC. *Atopy is defined as : three or more positive skin tests to common allergens. **Inh.:inhaled.

Nr.	Age (years)	Sex	FVC basal (L)	FVC (%pred.)	FEV1 basal (L)	FEV1 (%pred.)	PC 20 (mg/ml)	Atopy*	Smoker	Steroids	Diagnose
1	22	Female	4.5	114	4	116	7.8	+	-	inh	Asthma
2	62	Male	3.15	91.5	2.24	82.3	0.86	-	+	inh	Asthma
3	28	Male	4.71	103	3.4	79.2	0.75	+	-	inh	Asthma
4	65	Male	4.03	111	2.75	71.3	0.7	-	+	inh /oral	Asthma
5	36	Female	3.43	92.4	3.2	101	2.2	+	+	inh /oral	Asthma
6	74	Female	2.46	115	1.24	74.8	1.7	-	-	inh /oral	Asthma
7	74	Male	3.89	103	2.04	73.1	2	+	+	inh /oral	Asthma
8	64	Male	4.2	102	2.36	71.6	3.2	+	+	inh /oral	Asthma
9	35	Female	2.9	90	2.12	76	0.5	+	+	inh	Asthma

Statistical analysis.

Statistical analysis was performed by using Student's t test for paired observations. P values < 0.05 were considered significant. The decrease in PEFR after EC was also calculated from areas under the curve (AUC) [3]for which the trapezoidal method was used.[12] AUC were

[3]AUC describes the surface of the PEFR curve during the day, which is situated below the pre-exercise value line. It is a better method to follow decreases in PEFR in time compared to only one maximal PEFR fall.

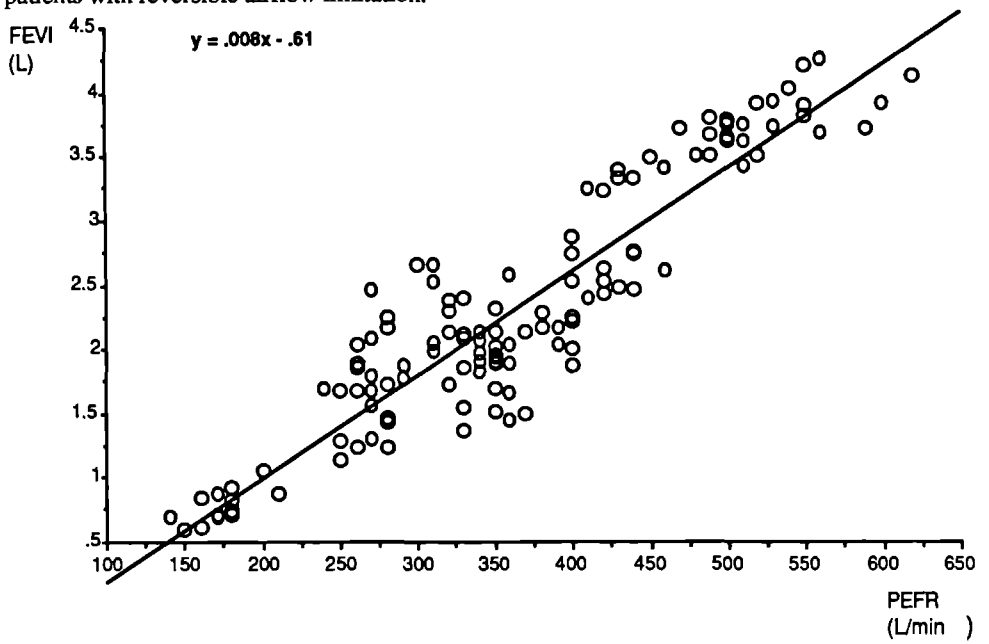
compared by using the analysis of variance. The statistical method from Bland and Altman was used for assessing agreement between the two exercise days.[13] Repeatability was analyzed using repeatability coefficients.[13]

Results

a. Correlation between PEFR and FEVI.

Although extensive literature exists about a good correlation between PEFR measurements and FEVI measurements we have carried out a study to demonstrate this correlation ourselves, and to validate the use of PEFR recordings in the patient population referred to our hospital.[9, 10, 11] Therefore in a group of 19 patients with reversible airflow limitation on various occasions (seven times a day with an interval of 2 hours) PEFR and FEVI were registered (see figure 4-1.) A very close correlation (r=0.926) was observed, which indicated to us that PEFR values could be used to evaluate changes in airflow obstruction after exercise challenge. Analysis according to Bland and Altman showed a good repeatability and agreement. Based on these observations we have chosen for the registration of PEFR values after EC. In particular because most of the tested patients are well trained in using this piece of equipment adequately.

Figure 4-1. Correlation and regression equation between PEFR (L/min) and FEVI (L). Every 2 hours between 09.00 am and 21.00 pm a PEFR and a FEVI were registered in 19 asthmatic patients with reversible airflow limitation.



b. The EAR and LAR after EC in the investigated population.

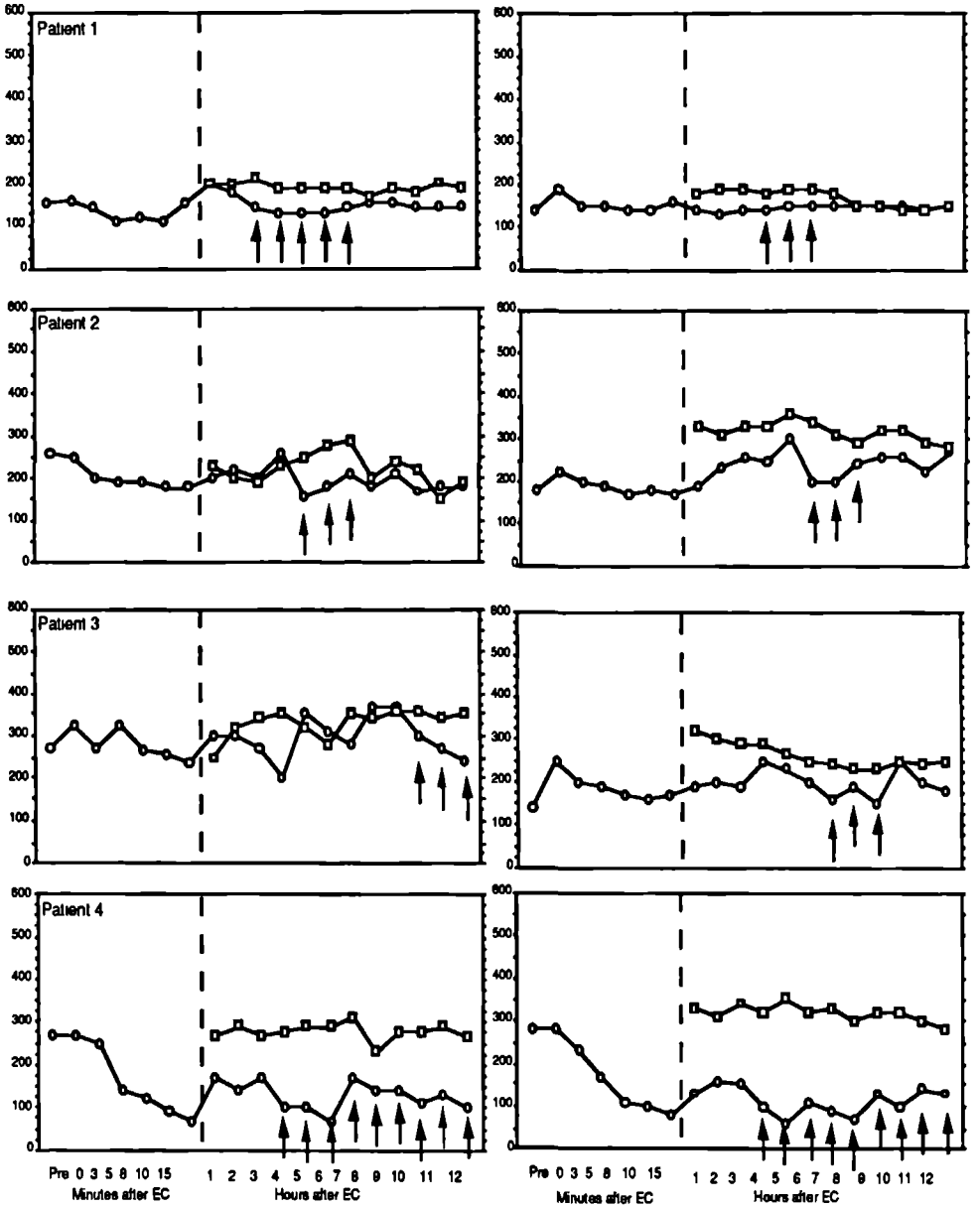
Of the 83 patients examined, 34 (41%) showed an EAR after EC (PEFR fall ≥20% compared to the pre-exercise PEFR value). It appeared that 11 patients had a PEFR fall ≥ 20% on 3 consecutive time points 3-13 hours after EC when compared to the corresponding clocktime on a control day. Of the 11 patients with a LAR after EC 8 patients had a dual response (EAR and LAR), 3 had an isolated LAR after EC. The 11 asthmatic patients with a LAR after EC were rechallenged after a minimal interval of 21 days and a maximal interval of 150 days to test whether the occurrence of the LAR after EC was reproducible.

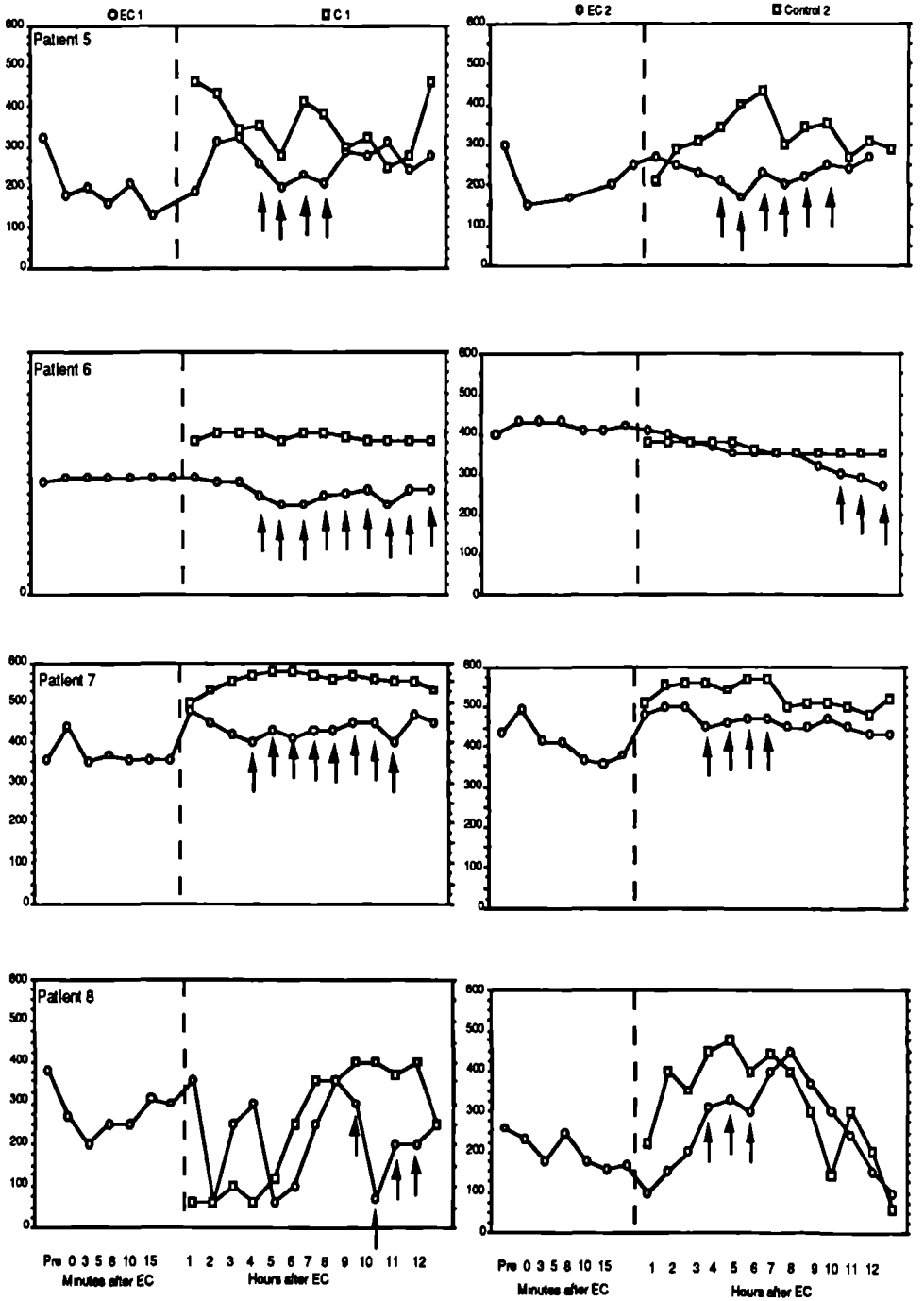
The nine patients who served as controls did not develop a LAR to EC compared to corresponding clocktime on a control day nor did they show an EAR.

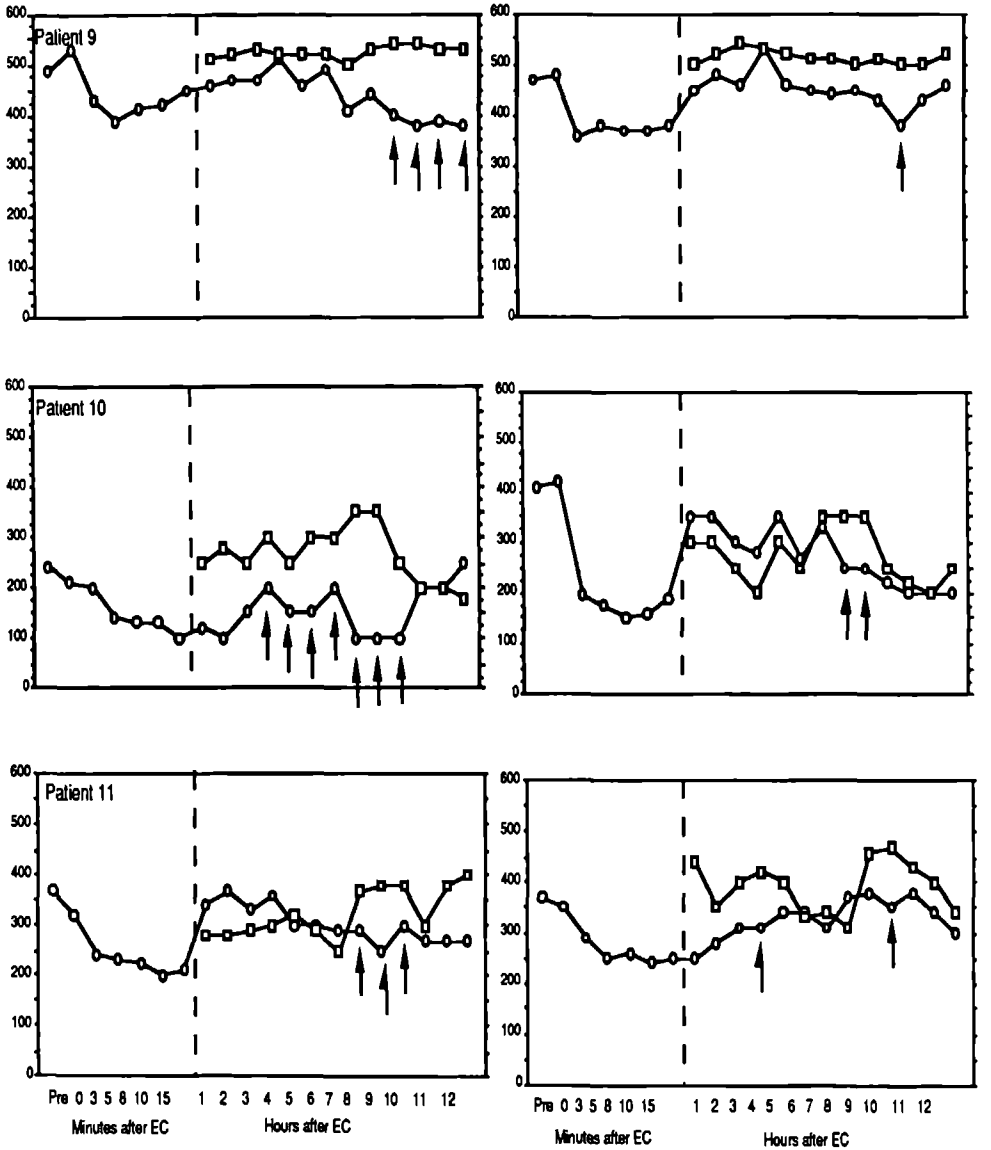
c. Reproducibility of the LAR after EC.

Eight out of the 11 patients who experienced a LAR after EC showed again a PEFR fall ≥20% on at least 3 consecutive time points 3-13 hours after EC compared to corresponding clocktime on a control day when they were rechallenged with exercise. Of those 8 patients who showed a reproducible LAR, 3 patients showed an EAR as well as a LAR after the first EC. In 3 patients the dual asthmatic response proved to be reproducible. Three patients failed to develop a LAR after the second EC. The individual PEFR patterns of the control days as well as the EC days of those 11 patients are presented in figure 4-2. Patients 1, 2 and 4 clearly developed a reproducible LAR after EC. On more than 3 time points in patients 1 and 4 a PEFR fall ≥20% occurred 3-13 hours after EC. Patients 3, 5, 6, 7, and 8 also showed a clearly reproducible LAR, but with a different configuration of the figures. Nevertheless, also in these patients on more than 3 time points 3-13 hours after EC a PEFR fall ≥20% took place. On both control days a stable PEFR pattern was observed in patients 1, 2, 3, 4, 6 and 7. Patients 5 and 8 showed very instable control days. Nevertheless these patients should also be considered as having a reproducible LAR after EC. In patient 9 the change in PEFR gave the impression of the development of a LAR after EC. However after the second EC a LAR seemed to develop only at one time point after EC. Patient 10 had an early occurring reproducible LAR after EC with severe clinical symptoms, which required medical intervention. Patient 11 showed a PEFR fall ≥20% on the first EC day, but not on the second EC day. Therefore patients 9, 10 and 11 should not be considered as having developed a reproducible LAR after EC. Taken together, when a PEFR fall ≥20% on three or more occasions 3-13 hours after EC in comparison with corresponding clocktime on a control day was used as a definition of a positive LAR after EC only 8 patients had a reproducible LAR after EC (patients 1-8).

Figure 4-2. Individual graphs of the 11 patients showing a LAR after EC. Both values of the control days and values of the exercise days are presented. Squares represent control days, open circles represent exercise days. EC1 is the first exercise day, EC2 is the second exercise day. Arrows indicate a PEFR fall ≥20% after EC compared to corresponding clocktime on a control day.




EC 1
C 1
EC 2
Control 2
Patient 5
Patient 6
Patient 7
Patient 8
0
100
200
300
400
500
600
Pre 0 3 5 8 10 15
Minutes after EC
1 2 3 4 5 6 7 8 9 10 11 12
Hours after EC



Based on the combination of PEFR fall ≥20% in comparison with corresponding clocktime on a control day and the occurrence of such a fall on more than three consecutive time points 3-13 hours after EC we were able to discriminate between reproducible and not reproducible LAR's after EC. Table 4-3 summarizes the time points at which the LAR occurred

on the first and second occasion and the maximal % PEFR fall on both occasions, compared to the corresponding clocktime on a control day.

Table 4-3. Comparison of the % PEFR fall for the EAR and LAR and the time point at which the maximal % PEFR fall for the LAR occurred when asthmatic patients with a positive LAR after EC were challenged with exercise on two different occasions.

Patient no.	EAR 1	EAR 2	LAR 1	LAR 2	Days between LAR 1 and 2	LAR 1 after EC (hour)	LAR 2 after EC (hour)
1	27	0	33	31	21	3	2
2	31	6	36	41.1	47	5	6
3	13	0	42.8	34.7	35	4	9
4	74	71	75.8	82.8	28	6	5
5	50	50	44.7	57.5	60	7	5
6	0	0	42.1	34.2	61	5	13
7	3	17	30	20	56	4	4
8	47	38	82.5	31	150	10	4
9	22	23	29	24	15	10	10
10	58	63	71	49	41	8.5	8.5
11	46	35	34	26	62	9	4

From these data we could calculate that the mean maximal % PEFR fall for the LAR after EC on the first- and second exercise day; 48.4±20 % and 41.5±20 % respectively mean ± SD. The pre-exercise PEFR values on both control- and exercise days did not differ significantly. In the 8 patients with a reproducible LAR after EC, the coefficient of variation in PEFR values of the first exercise day was 20.9 ± 12.5 and of the second exercise day 20.7± 13. The correlation coefficient of these variation coefficients was 0.9 ($p<0.001$). The coefficient of variation in PEFR values on the control days was 12.3±7.2 for the 8 patients with a reproducible LAR after EC whereas for the exercise days this coefficient of variation amounted 20.8±12.4 ($p<0.05$). The time points at which the LAR occurred after the first- and second EC did not show a correlation.

In addition in 8 out of the 11 patients with a reproducible LAR after EC, AUC were calculated 3-13 hours after EC in order to study the reproducibility of the LAR after EC. A correlation coefficient of 0.8 ($p<0.001$) was found between areas under the curve for the LAR after EC for the first and second EC. The mean difference for AUC between the first and second EC for the 9 patients with a reproducible LAR was - 6300±44745 (mean ± SD) with 95% confidence interval -43712 and 31113. We expect 95% of differences to be less than two

standard deviations. This is the definition of a repeatability coefficient adopted by the British Standards Institution. In our study all the 8 patients are satisfying this criteria. The sum of the differences squared is $1.433.10^{10}$ so the standard deviation of differences between the 8 pairs of repeated measurements is $1.598 .10^{9}$. The coefficient of repeatability is twice this; or $3.196 .10^{9}$.

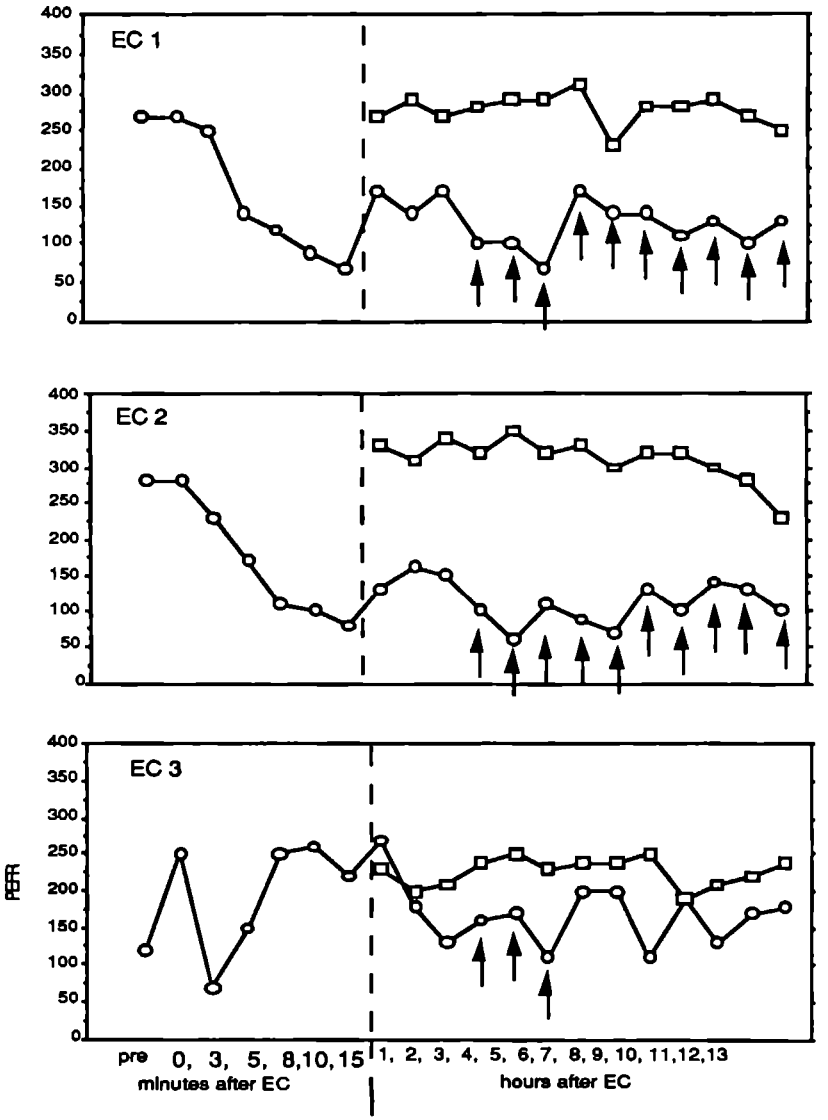
One of the asthmatic patients who experienced a reproducible LAR after EC (patient no.4.) has been challenged with exercise thereafter. In this patient the LAR after EC proved to be reproducible even after 11 months (see figure 4-3.). Also patient no 8 showed a reproducible LAR after 150 days.

The 9 control patients who did not develop a LAR after the first EC also did not develop a LAR after the second EC. They did not show an EAR after the first EC and neither showed this reaction after the second EC. This supports the reliability of the methodology applied.

Discussion

In this study we have been able to demonstrate that the LAR after EC is a reproducible phenomenon when the LAR after EC is defined as: a PEFR fall ≥20% compared to the corresponding clocktime value on a control day on three consecutive time points 3-13 hours after EC. The EC was repeated 21-150 days after the first EC without changing the therapy of the patients in between. In 8 out of 11 asthmatic patients (73%) with a LAR after the first EC, a LAR occurred after the second EC. The LAR after EC was reproducible, although the time points did not correlate. The reproducibility was based on the graphical and statistical analysis of the individual PEFR data on the exercise days compared with the control days. Areas under the curve 3-13 hours after the first and second EC showed a good reproducibility and agreement. In addition the intrinsic variability of exercise and control days was assessed and it appeared that the exercise days differed significantly from both control days. From the individual graphs one may note that baseline airway calibre differs between the first- and second

Figure 4-3. Individual graphs of patient 4. EC 3 is done 11 months after respectively EC 1. Squares are control days, open circles are exercise days. Arrows indicate a PEFR fall ≥20% after EC compared to corresponding clocktime on a control day.



control and exercise days. This likely is due to the fact that the first and second control and exercise day are 21-150 days apart. Moreover, a slight difference in the clinical situation of the patient may be responsible for differences in baseline airway calibre. However, on both

occasions the pre-exercise PEFR values of the control- and exercise days did not differ significantly.

In this study PEFR measurements were used to document the change in airflow limitation, since it was demonstrated that these measurements closely correlate with FEVI measurements. Furthermore, all patients participating in this study were well trained in using the mini-Wright peakflow meter. As described by others this piece of equipment is suitable to register changes in ventilatory function accurately and reproducible when used adequately.[9,10,11]

The question how to define a LAR after EC remains a controversial issue. In earlier papers we have addressed this point extensively.[3,4] The controversy in the literature about the LAR after EC is related to whether or not late responses to exercise really occur and how to properly define them. So far many studies dealing with the EAR and LAR after EC, have compared the PEFR fall after EC in relation to the pre-exercise PEFR value.[1,14-18] Due to diurnal variation later reports have advocated the use of a clocktime comparison on a control day without exercise instead.[2,3] Although the definition is describing the LAR after EC more properly it still does not discriminate between a real and pseudo-LAR after EC. In a previous study we have therefore addressed this problem more closely and we have come to the definition used in this study i.e.: a PEFR fall ≥20% on three or more consecutive time points when compared with the corresponding clocktime value on a control day 3-13 hours after EC. Furthermore the graphs must clearly show a LAR. To our knowledge this definition of a LAR after EC is the most careful one used so far. [4]

Since the therapy regimen for the participating patients was kept unaltered between and before the two challenge occasions this study also argues strongly against the fact that the LAR after EC could be due to medication withdrawal.[2,19] Furthermore, it extends the evidence that the LAR after EC is not a coincidence of a stable control day and an unstable EC day, since both were registered on two different occasions with an in between time period of 21-150 days.

Corticosteroids have been demonstrated to suppress airway responsiveness to histamine and these drugs are effective in suppressing late responses to allergen provocation.[20] It is

noteworthy in this study that LARs after EC do occur, despite the use of oral and/or inhaled corticosteroids. It could be possible that the dose of steroids was not high enough to prevent the LAR after EC, on the other hand it may be possible that the LAR after EC can not be prevented by steroids. In the latter case another pathofysiological mechanism than for the LAR after allergen provocation may be responsible for the LAR after EC.

In the here studied asthmatic patients experiencing a LAR after EC the LAR pointed out to be reproducible in 73 % of the cases. From the presented data it is clear that, when strict criteria are applied for the LAR after EC, it is almost as reproducible as the LAR after antigen challenge.[21,22] Although the LAR after EC in itself was reproducible, the time points at which the maximal PEFR fall occurred were not reproducible (see table 4-3.). One explanation for this discrepancy is that although the therapy regimen for the patients was kept constant throughout the study period a greater part of the participating patients suffered from atopy (see table 4-1.). Since Davos is situated at high altitude (1560 m) the concentration of airborne allergens is low. Therefore patients are less exposed which may lead to lesser complaints of airway obstruction and improvement of PEFR values. This explanation is supported by the finding that the skin reactivity to a panel of common allergens decreased considerably after a stay of 3 months in Davos.[23] Since Davos is surrounded by fields of grass as well as pollinating deciduous trees the skin reactivity towards these allergens did not change. Notwithstanding this decrease in allergen load, the LAR after EC occurred, was reproducible and did not show great seasonal variation. The patients mentioned in this study were tested inside after an overnight sleep and were not allowed to go outdoors.

Taken together, the LAR after EC may be diagnosed on the basis of the PEFR fall measurements compared to control day values on three or more time points 3-13 hours after EC. Based on these criteria the LAR after EC proved to be a reproducible phenomenon in the majority of the tested patients. For this reason we feel the LAR after EC is a reproducible phenomenon.

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CHANGES IN BRONCHIAL HYPERREACTIVITY, INFLAMMATORY CELLS NUMBERS AND DERIVED MEDIATORS IN PERIPHERAL BLOOD BEFORE, DURING AND AFTER THE LATE ASTHMATIC REACTION AFTER EXERCISE CHALLENGE

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Changes in bronchial hyperreactivity, inflammatory cells numbers and derived mediators in peripheral blood before, during and after the late asthmatic reaction after exercise challenge.

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Key words : exercise induced asthma - early asthmatic response -late asthmatic response - exercise challenge - inflammation

Abstract

In order to study whether an inflammatory component could be involved in the late asthmatic reaction (LAR) after exercise challenge (EC), changes in bronchial hyperreactivity to histamine (BHR), inflammatory cell numbers and derived mediators in peripheral blood at fixed time points before, during and after the LAR after EC were analyzed. Nine asthmatic patients with a reproducible positive LAR after EC and three asthmatic patients with a reproducible negative LAR after EC were incorporated in the study. It appeared that BHR to histamine increased 24 and 48 hours after EC, both in most of the patients of the group with a positive LAR and in the group with a negative LAR. No consistent changes were observed in neutrophil, eosinophil and basophil numbers in peripheral blood as well as serum eosinophil cationic protein levels and serum histamine levels in the group with a positive LAR after EC. Therefore from this study it may be concluded that if an inflammatory component is involved in the LAR after EC it is not reflected by the here measured parameters.

Introduction

After exercise challenge (EC) asthmatic patients may show an early asthmatic response (EAR) and/or a late asthmatic response (LAR). The EAR is expressed as the fall in peak expiratory flow rate (PEFR) or in forced expiratory volume in 1 second (FEVI) and appears within 10 minutes after EC, reaches a maximum after 20-30 minutes, and disappears normally within 1-3 hours. A LAR after EC can occur after recovery from the EAR and usually starts 3-12 hours after the EC, decreases in severity after 12 hours and disappears spontaneously within 24 hours. In several studies the existence of the late asthmatic reaction (LAR) after exercise challenge (EC) has been well established, especially when lungfunction changes at the LAR after EC are compared to corresponding clocktime on a control day without exercise [1].

The pathophysiological mechanisms behind the early and late asthmatic reaction after EC have been extensively investigated. Various mechanisms have been postulated: hypoxia [2],

hypercapnia [3], lactic acidosis [4], stimulation of nasopharyngolaryngeal receptors [5], airway cooling during exercise [6-9], airway water loss during exercise [10], condensation of water on airways during exercise [11], a slow onset of adrenaline release and decreased catecholaminergic effectiveness [12]. However, all these hypothesized etiological factors have been criticized [13-17].

In this study we investigated whether an inflammatory component, reflected by changes in bronchial hyperreactivity (BHR) to histamine and changes in inflammatory cell numbers in the circulation and changes in serum eosinophil cationic protein and serum histamine levels would reflect events taking place during a LAR after EC.

Patients and methods

Patients

All patients with bronchial asthma had a documented bronchial hyperresponsiveness, measured according to Hargreave et al. and expressed as the concentration of inhaled histamine which results in a 20% decrease of FEV1 (PC20 < 8 mg/ml) [18]. Asthmatic and COPD patients were classified according to ATS criteria. [19] In COPD patients the FEV1 reversibility after 0,4 mg inhaled salbutamol was less than 20% of the predicted value in contrast to asthmatic patients.

Throughout the study period all patients had to submit to concomitant medication rules. The patients had to stop inhaled bronchodilators 8 hours before EC and during the control day. Sodium cromoglycate had to be stopped 24 hours before the exercise test and during the control day. Any type of oral bronchodilator had to be stopped at least 48 hours before the start of the EC and during the control day. The dose of oral and inhaled steroids was kept constant. All patients on steroids were using this treatment for at least 3 months. Patients did not stop smoking during the study. Their smoking habits remained unaltered during the study period. Informed consent was obtained from each of the patients. The characteristics of the patients

who participated in this study with a reproducible positive and negative LAR after EC are summarized in table 5-1.

Table 5-1. Patient characteristics of the patients with a reproducible positive and negative LAR after EC.

Positive reproducible LAR after EC.

Patient no.	Age (years)	Sex	Diagnose	FEV1(% pred.)	PC 20 (mg/ml)	Atopy	Smoker	Steroids
1	19	Female	Asthma	71	< 0 03	+	-	p o ,inh
2	26	Female	Asthma	90	1 8	+	-	p o ,inh
3	22	Female	Asthma	85	0 35	+	-	inh
4	41	Female	Asthma	71	0 95	-	-	p o ,inh
5	54	Female	COPD	66	0 1	-	+	p o
6	66	Male	COPD	48	0.32	+	+	p o ,inh
7	69	Female	COPD	52 9	< 0 03	-	-	inh
8	55	Female	COPD	52	0 05	+	-	p o
9	63	Female	COPD	68	0 35	+	-	p o ,inh

Negative reproducible LAR after EC.

Patient no.	Age (years)	Sex	Diagnose	FEV1(% pred.)	PC 20 (mg/ml)	Atopy	Smoker	Steroids
10	64	Male	COPD	39	0 08	-	Yes	oral
11	62	Male	Asthma	82 3	0 86	-	Yes	inhalation
12	46	Male	COPD	57 5	0 04	-	Yes	inhalation

Exercise Challenge (EC)

All ECs were performed on a bicycle ergometer (Erich Jäger, Würzburg, Germany). The workload in Watts was 80% of the predicted maximum workload. The predicted maximum workload was calculated according to Eggleston [20]. Exercise was performed for 8 minutes during which a heart-rate of 90% of the predicted maximum was reached. During the EC the heart-rate was measured by a Siemens Sirecust 341 monitor (Siemens, Germany). The relative humidity of the ambient air was 20-40%, the room temperature was 20-23° Celsius, both on the control and the exercise days and were both measured with the Hygrotest 6200 (Quarz AG, Zürich, Switzerland). The humidity and room temperature were allowed to variate 10% and 2°C respectively during the control- and exercise day for each patient. During the 8 min. bicycling the patients wore a noseclip.

Peakflow measurements.

PEFR was measured with the mini-Wright peakflow meter. The same peakflow meter was used at each occasion. The best of three measurements was recorded. Before and after each

daily recording of peakflow rates, meters were checked and cleaned by the lung function technician. PEFR was recorded on the control day at t=0 (09.00 a.m.) and during the first 13 hrs after t=0 at hourly intervals; on the exercise day PEFR was recorded at t=0 hour (= pre-exercise PEFR) and 1, 3, 5, 7, 10, 15 and 30 minutes afterwards, and also hourly during the next 13 hrs at the end of the EC.

As reference values the PEFR on corresponding clocktime of the control day was taken. The % PEFR fall for the LAR after EC compared to corresponding clocktime on a control day was calculated as : 100% x (PEFR at corresponding clocktime on control day - lowest PEFR 1-13 hours after EC) / PEFR at corresponding clocktime on control day. A LAR is defined as a ≥20% fall in PEFR compared to corresponding clocktime on a control day on more than one time point 3-13 hours after EC.

In this study only patients were included who showed a reproducible LAR after EC compared to control day. The reproducibility of the LAR after EC has been extensively documented elsewhere [21].

PC_{20} measurements

Bronchial hyperresponsiveness (BHR) was measured according to Hargreave et al. and expressed as the concentration of inhaled histamine in mg/ml which results in a 20% decrease of FEV1 [18]. PC_{20} was determined before EC and 1, 24 and 48 hours after EC.

Blood cell numbers

At various time points blood samples were collected (see figure 5-1.) in EDTA. These samples were analyzed for eosinophil, neutrophil and basophil numbers by means of an automated cell counter (Technicon A 6000, USA).

Serum-eosinophil-cationic protein (s-ECP) and histamine

At the same time points as blood was collected for blood cell analysis 3-5 ml of blood was collected and allowed to clot at room temperature for 60 minutes. Then serum was collected

and stored in fractions at -20° C until analysis. s-ECP was determined by RIA (Pharmacia Diagnostica AB, Uppsala, Sweden), exactly according to the manufacturer's instructions. Also histamine was determined by RIA (Immunotech, Marseille, France) exactly according to the manufacturers instructions. For the determination of s-ECP and histamine freshly thawed fractions of serum were used. s-ECP values are expressed in µg/l and histamine values are expressed in nmol/L.

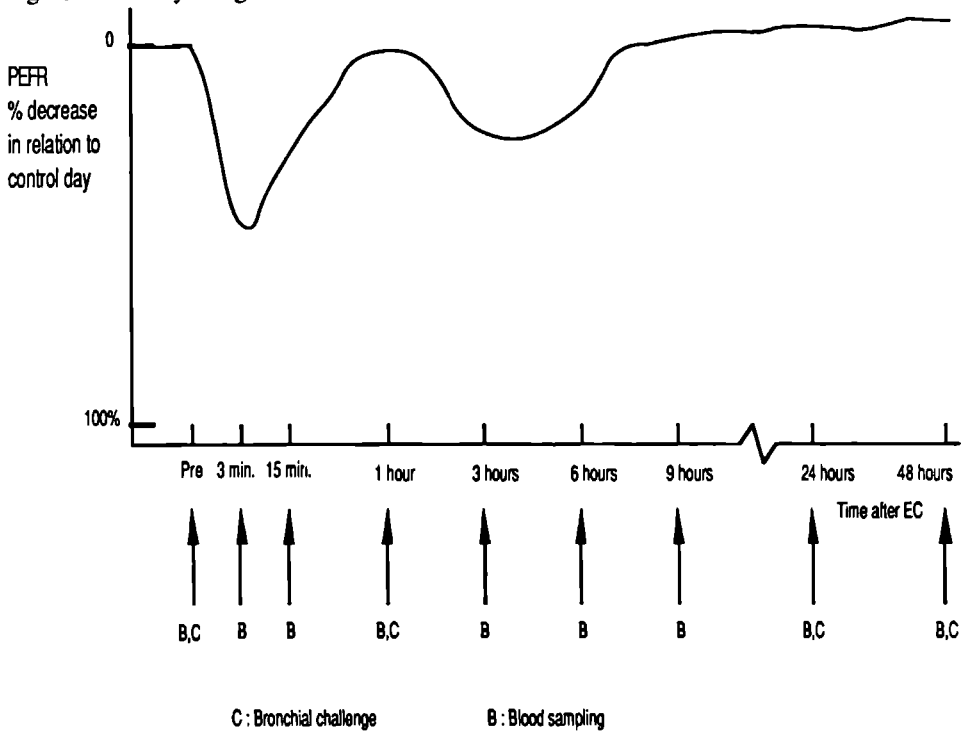
Study-Design.

Only patients who showed a reproducible LAR after EC were enrolled in the study. Nine of those asthmatic patients performed a third EC with the same medication restriction as on the control and exercise days. Before exercise challenge took place an iv catheter was introduced in an antebrachial vein. At various time points before, during and after EC, PC_{20}'s for histamine and blood collection took place. This is outlined in figure 5-1. This study design was also used for the 3 asthmatic patients with a reproducible negative LAR after EC. They underwent the same procedure as the patients with a reproducible positive LAR.

Statistical analysis

PC_{20}'s for histamine, blood cell numbers, s-ECP, and serum histamine were compared at each time point with the pre-exercise value as a reference by means of a paired student's t test. The patient group with a positive reproducible LAR was compared with the one without a LAR for PC_{20}'s for histamine, blood cell numbers, s-ECP, and serum histamine levels using analysis of variance.

Figure 5-1. Study design



Results

In this study 9 patients with a reproducible positive LAR after EC and 3 patients without any reaction (neither EAR or LAR) after EC participated. Four patients (no.1, 2, 3, 4) showed a reproducible positive EAR and LAR after EC. Five patients (no 5, 6, 7, 8, 9) showed an isolated LAR after EC. The other patients (no.10, 11 and 12) showed a reproducible negative EAR and LAR after EC. As described in the materials and methods section of those patients changes in PC_{20} for histamine, changes in neutrophil, eosinophil and basophil counts in peripheral blood and changes in s-ECP and serum histamine were recorded before, during and after EC. The individual data are plotted in table 5-2 and figure 5-2.

Table 5-2. PC_{20} to histamine in mg/ml before, at 1 hour, at 24 and 48 hours after EC in the patient group with a positive and negative LAR after EC.

LAR +	T=0	T=1 HR	T=24 HR	T=48 HR
1	< 0.03	< 0.03	< 0.03	< 0.03
2	0.6	0.6	0.25	6.5
3	0.58	0.67	0.33	0.27
4	4.00	1.83	0.54	*
5	0.038	0.65	0.07	0.037
6	0.45	0.06	0.38	0.32
7	0.5	0.5	0.33	0.25
8	0.4	0.19	0.21	0.11
9	0.35	0.57	*	*
LAR -	T=0	T=1 HR	T=24 HR	T=48 HR
10	0.085	< 0.03	< 0.03	< 0.03
11	5	7	3	5
12	0.42	0.60	0.09	0.05

Changes in BHR before, during and after a LAR after EC.

Only in one out of the 4 patients with a reproducible EAR and LAR after EC a clear decrease in BHR was observed (patient no. 4). In the 5 patients with a reproducible isolated LAR after EC patient no.6, showed a decrease in BHR, whereas patients no. 5 and 9 showed an increase in BHR at 1 hour after EC. In the patient group with a reproducible negative LAR after EC 2 patients (no. 11 and 12) showed a decrease in BHR after 1 hour after EC. At 24 hours after EC an increase in BHR was observed in patients no. 2, 3, 4, 5, 6, 7 and 8 compared with the pre-exercise BHR. However in the patient group with a negative reproducible LAR after EC also an increase in BHR at 24 hours was present in all patients compared to the pre-exercise BHR value. At 48 hours after EC most of the investigated patients showed a further increase in BHR to histamine as compared with the pre-exercise value in BHR and the BHR value at 24 hours. Thus, although most of the patients with a reproducible LAR after EC showed an increase in PC_{20} for histamine at 24 and 48 hours after EC, the control patients showed an identical reaction pattern. (table 5-2)

Changes in neutrophil, eosinophil and basophil cell numbers before, during and after a LAR after EC.

a. Neutrophils

In the patient group showing an EAR and LAR after EC there seems a tendency in the

neutrophil cell number to increase after the EAR (between 15 minutes and one hour). In the patient group showing an isolated LAR after EC there hardly seems a change in neutrophil cell number as is the case in the patient group showing a negative LAR after EC.

b. Eosinophils

In all patient groups there was no clear consistency in the eosinophil cell number to increase or decrease. Also at 24 and/or 48 hours after EC there was no clear increase in the eosinophil cell number in the patients who showed a positive reproducible LAR after EC.

c. Basophils

The basophil counts did not change greatly during and after the LAR in all investigated groups.

Changes in s-ECP and serum histamine levels after a LAR after EC.

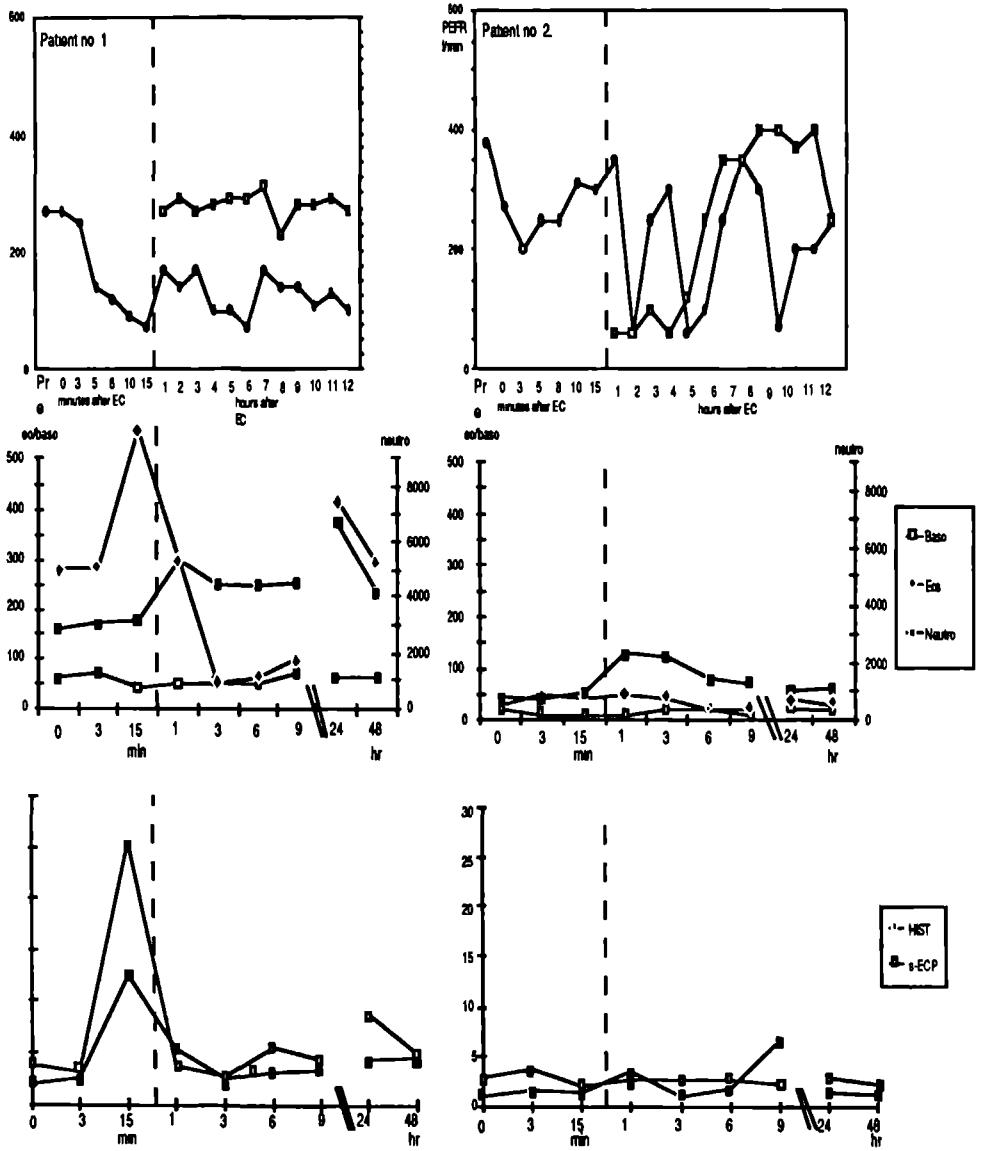
The control group (patient no. 10, 11 and 12) did not show striking changes in s-ECP and serum histamine levels.

In the patient group with a positive reproducible EAR and LAR after EC (patients no. 1, 2, 3, 4) in patient no. 1 a clear increase in s-ECP as well as serum histamine at 15 minutes whereas increase were present at 6 and 24 hours. In patient no.2 a small rise in serum histamine just before the LAR after EC occurred; s-ECP remained unchanged. In this patient the EAR was not reflected by changes in serum histamine or s-ECP. In patient no. 3 a small increase in s-ECP during the EAR after EC and a similar increase in s-ECP at 48 hours after EC was observed; during the LAR no changes occurred. In patient no. 4 serum histamine levels remained totally unchanged during and after EC. The s-ECP levels showed similar findings. Thus in the patient group who showed a reproducible EAR and LAR after EC (patients no. 1, 2, 3 and 4) no clear changes in serum histamine or s-ECP levels in relation to either the EAR or the LAR were observed. In one patient (no. 1) serum histamine and s-ECP rose during the EAR and remained fairly constant thereafter, whereas in another patient (no. 4) there seemed to be a coincidence between changes in these parameters and the occurrence of the LAR.

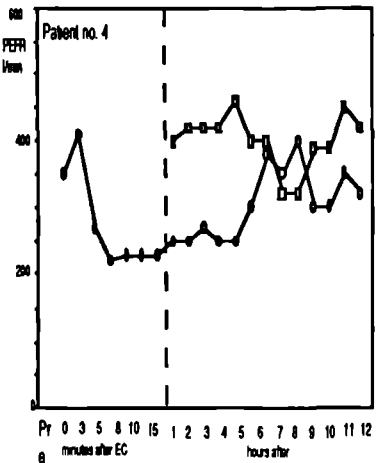
In the patient group who showed an isolated LAR after EC (patients no. 5, 6, 7, 8 and 9) no changes in serum histamine and s-ECP levels were observed in patients no. 5 and 6. In

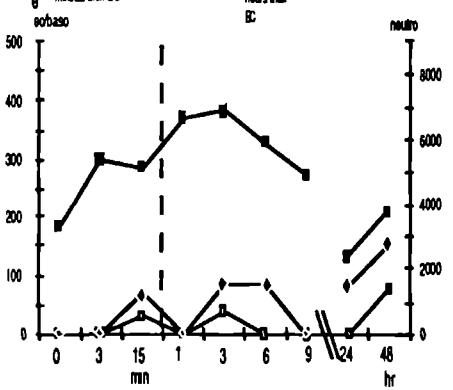
patient no. 7 an inconsistent pattern with respect to s-ECP was observed. In contrast in patient no. 8 the increase in s-ECP and serum histamine levels correlated remarkably well with the occurrence of the LAR after EC. In patient no. 9 an increase in serum histamine levels seemed to parallel the LAR after EC, whereas s-ECP levels did not change.

Figure 5-2. PEFR pattern in l/min after EC (open circles) and on a control day without exercise (open squares). Neutrophil, eosinophil and basophil counts in peripheral blood are expressed x 10^6/L. Histamine levels (black squares) are expressed in nmol/l, s-ECP levels (open squares) are expressed in ug/l




Patient no. 3
Pr 0 3 5 8 10 15 1 2 3 4 5 6 7 8 9 10 11 12
minutes after EC
hours after EC


Patient no. 4
PEFR
l/min
Pr 0 3 5 8 10 15 1 2 3 4 5 6 7 8 9 10 11 12
minutes after EC
hours after EC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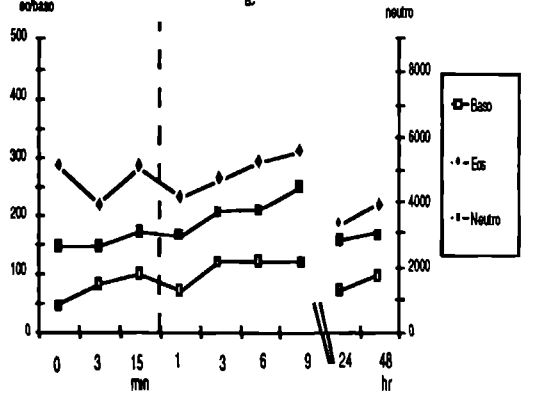
min
hr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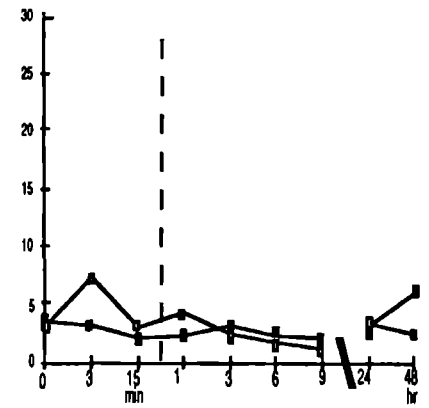
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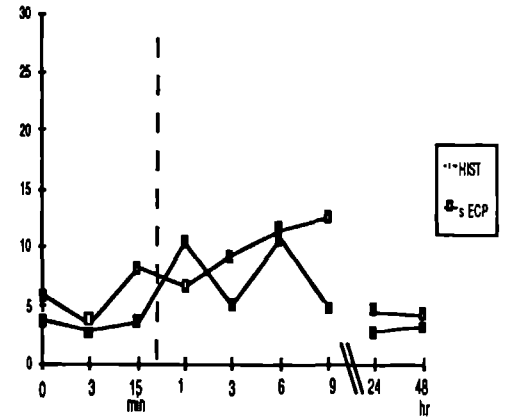
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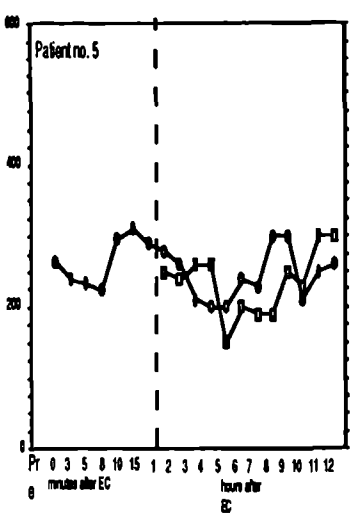
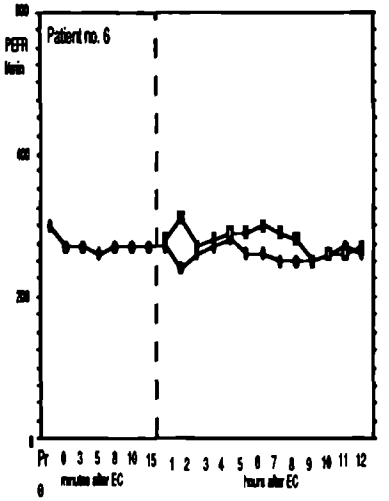
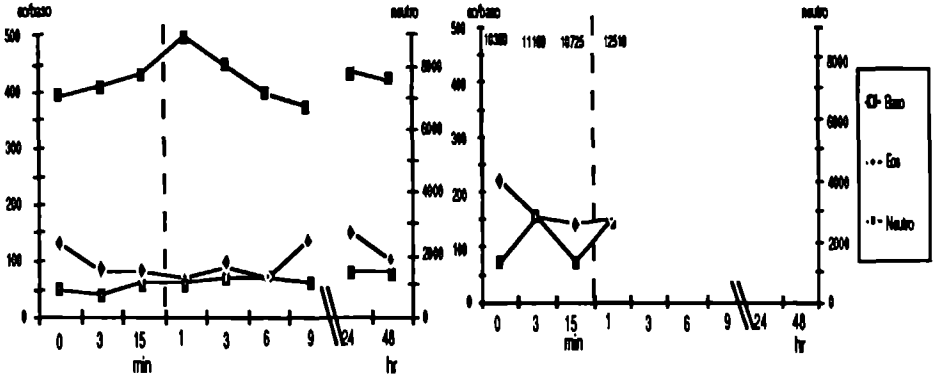

Patient no. 5
minutes after EC
hours after EC


Patient no. 6
PEFR
l/min
minutes after EC
hours after EC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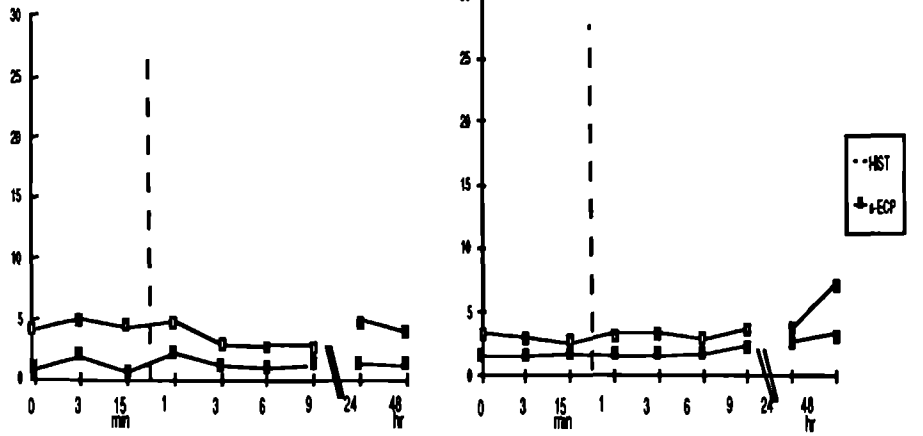
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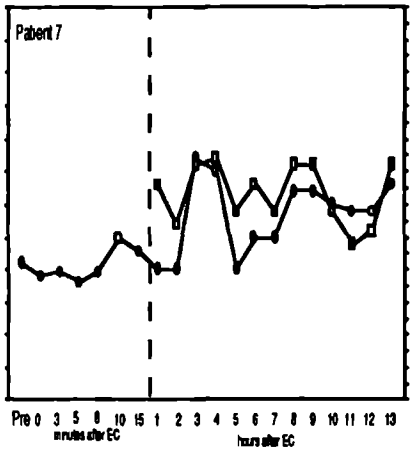
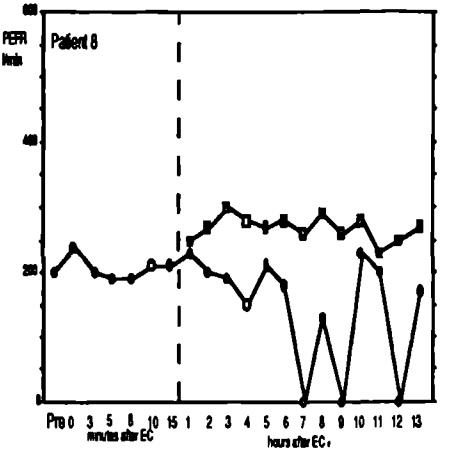
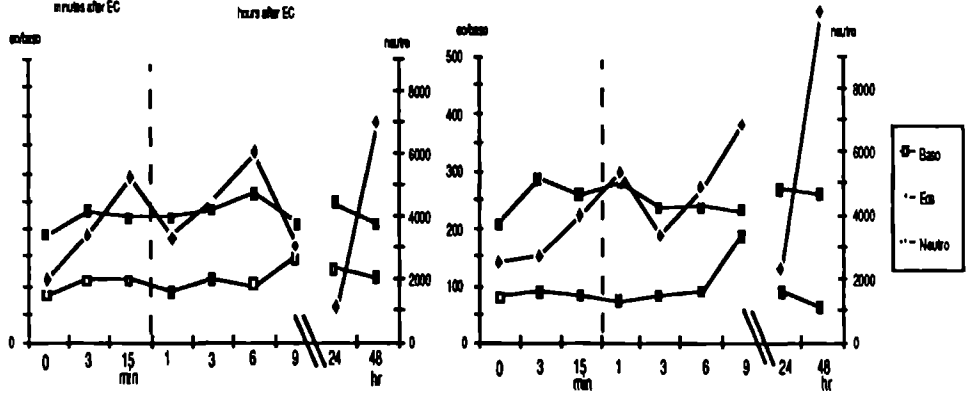

Patient 7
Pre 0 3 5 8 10 15 1 2 3 4 5 6 7 8 9 10 11 12 13
minutes after EC
hours after EC


PEFR
l/min
Patient 8
Pre 0 3 5 8 10 15 1 2 3 4 5 6 7 8 9 10 11 12 13
minutes after EC
hours after EC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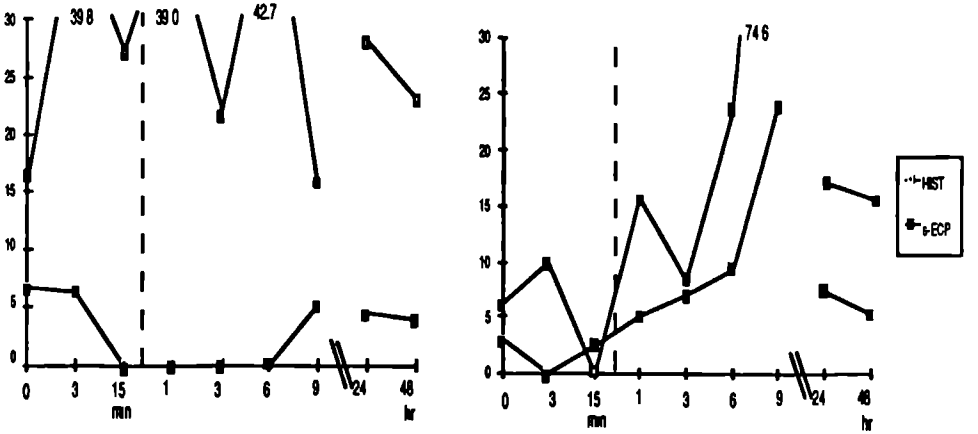
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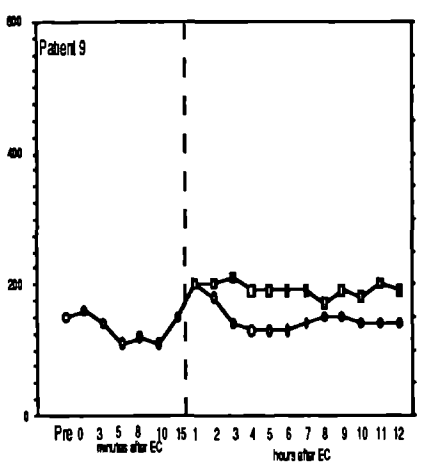
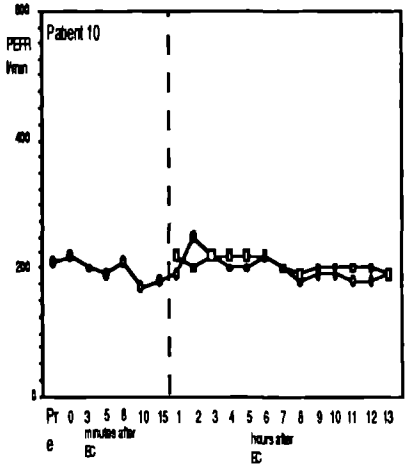
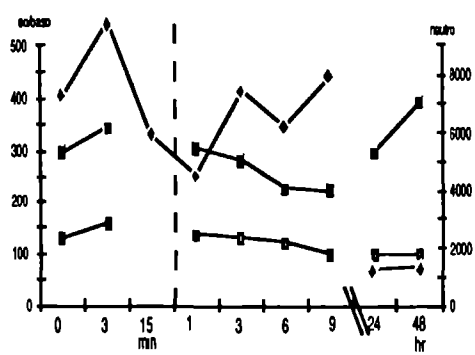

Patient 9
Pre 0 3 5 8 10 15 1 2 3 4 5 6 7 8 9 10 11 12
minutes after EC
hours after EC


Patient 10
PEFR
l/min
Pr e
0 3 5 8 10 15 1 2 3 4 5 6 7 8 9 10 11 12 13
minutes after EC
hours after EC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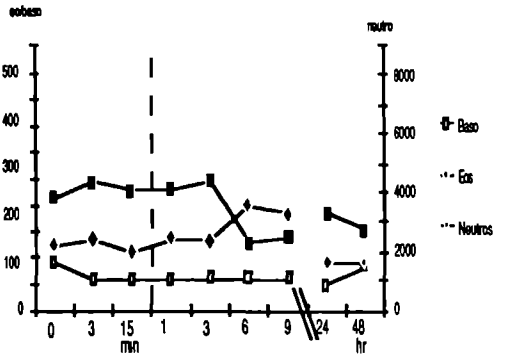
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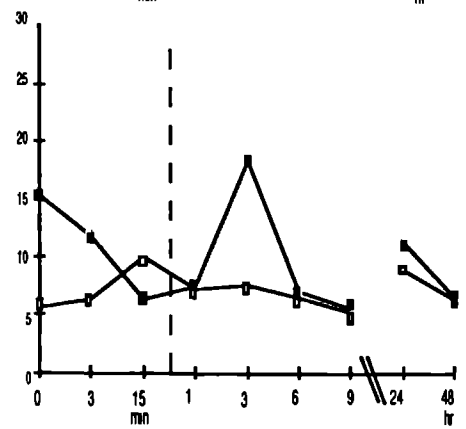
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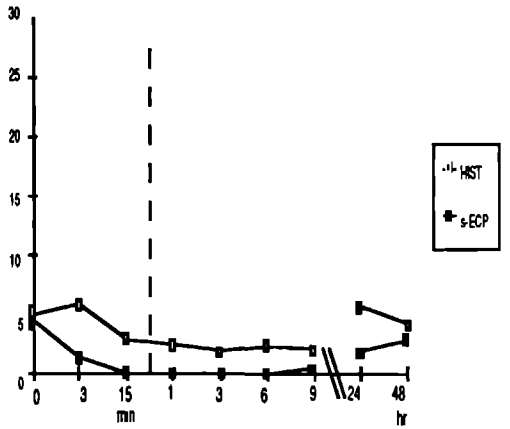
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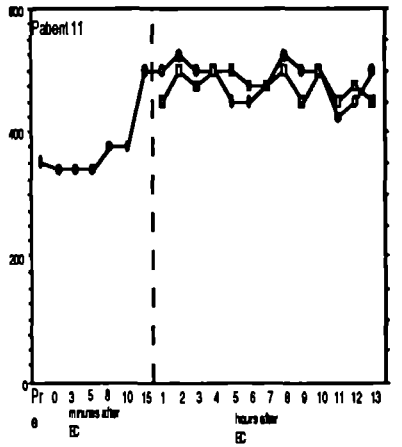
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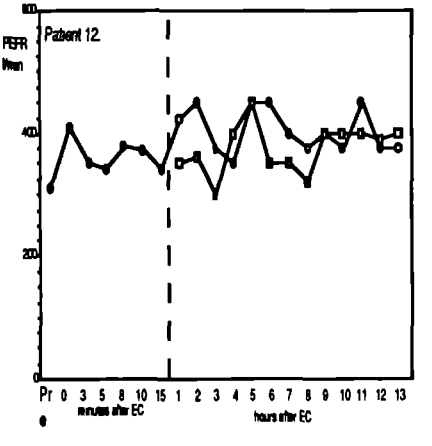
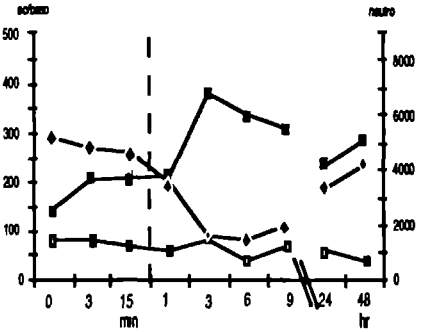
Pr 0 3 5 8 10 15 1 2 3 4 5 6 7 8 9 10 11 12 13
minutes after EC
hours after EC


Patient 12
PEFR
l/min
Pr 0 3 5 8 10 15 1 2 3 4 5 6 7 8 9 10 11 12 13
minutes after EC
hours after EC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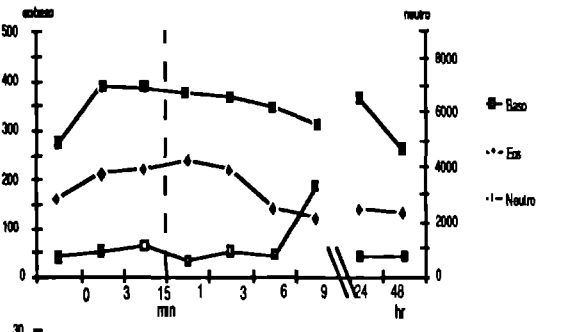
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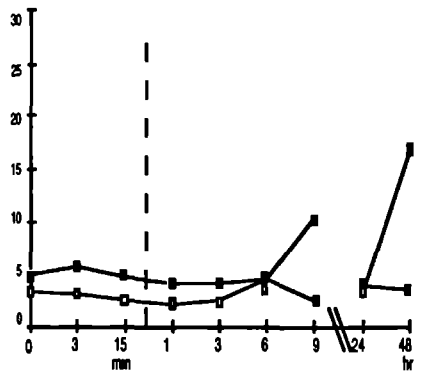
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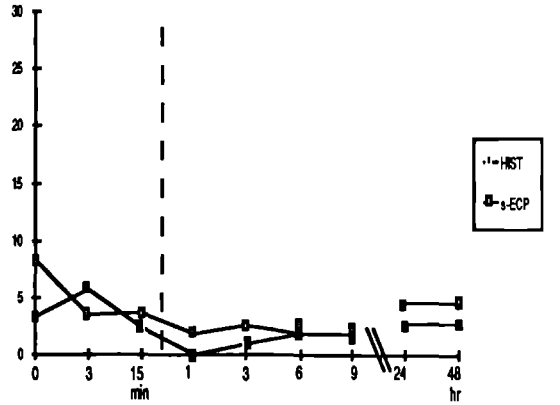
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Since pathogenetic explanations given for the occurrence of the LAR after EC have been unsatisfactory so far, we have tried to find evidence for the involvement of an inflammatory reaction in the LAR after EC. In a previous study the appearance of neutrophil chemotactive activity in the circulation has suggested inflammation to be of some importance for the LAR after EC [22]. Airway inflammation is an important etiological factor in the LAR after allergen inhalation [23] and in the LAR in occupational asthma [24]. The LAR after allergen inhalation may be prevented by the use of glucocorticosteroids (GC) [23,24]. This seems also to be the case in LAR after EC and gives further support for the hypothesis of an inflammatory basis for the LAR after EC [1, 25, 26]. In our study, patients continued using steroids. But the GC did not prevent a LAR to occur in 9 of the 9 asthmatic patients studied, although they used the steroids for at least 3 months before starting the study. Therefore, either the actual prevalence of the LAR after EC may be greater than found in our study or the role of inflammation in the LAR may be of less importance after EC than in the other LARs mentioned above [1].

In this study we tried to gather evidence that inflammation would be involved in the LAR after EC. As parameters reflecting an inflammatory component to be involved bronchial hyperreactivity to histamine, changes in inflammatory cell numbers and changes in s-ECP and serum histamine levels were quantitated before, during and after the LAR after EC.

In accordance with the LAR after allergen seemed the increase in BHR to histamine 24 and 48 hours in the LAR after EC. However, also the control group showed a similar tendency. Therefore these findings do not allow us to draw conclusions. In contrast to the LAR after allergen challenge where 24 hours after challenge an increase in eosinophil counts and an increase in s-ECP may be observed, no such changes were observed after the LAR after EC. Also in the majority of the cases studied the changes in serum histamine levels did not coincidence or precede the occurrence of the LAR after EC. Although in some cases changes in serum histamine and s-ECP levels seemed to correlate with the occurrence of an EAR or LAR after EC. The results as a whole show too little consistence to allow any conclusions. Most

likely changes in the peripheral compartment do not reflect changes in the lung. On the other hand the continuous intake of steroids may have influenced our results. However, for clinical reasons it was not possible to withdraw this treatment from the patients. Therefore further studies, most closely to the lung compartment are necessary to unravel some of the pathogenetic mechanisms behind the LAR after EC.

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CHAPTER 6.

NEDOCROMIL SODIUM INHIBITS THE EARLY AND LATE ASTHMATIC RESPONSE TO EXERCISE: A DOUBLE-BLIND PLACEBO CROSSOVER STUDY

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Nedocromil sodium inhibits the early and late asthmatic response to exercise: a double-blind placebo crossover study.

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Running title: Nedocromil sodium inhibits dual EIA

Abbreviations used:

AUC:area under the curve

COPD:chronic obstructive pulmonary disease

EAR:early asthmatic response

EIA:exercise-induced asthma

FEV1:forced expiratory volume in 1 second

LAR:late asthmatic response

PC20:concentration causing 20% decrease of FEV1

PEFR:peak expiratory flow rate

SD:standard deviation

Abstract

A double-blind crossover study was carried out to determine the effect of nedocromil sodium on the dual asthmatic response to exercise challenge. Nineteen patients with a late response (8 minutes on a bicycle ergometer) to exercise on a screening day were randomly assigned to treatment order with 4 mg nedocromil sodium or a matched placebo aerosol. Exercise challenge was performed on two study days, when two puffs of test medication were inhaled 30 minutes before commencing exercise. Peak flow was measured 10 and 5 minutes before exercise to give the mean pre-exercise baseline, and at 1,3,5,7,10,15,30 and 60 minutes after exercise and each hour thereafter for up to 13 hours post-exercise. Treatment effects on the late (4-13 hour) response were compared primarily from the maximum % fall in peak flow from pre-exercise values. This was reduced significantly by pretreatment with nedocromil sodium compared to placebo (12.4% vs 25.8%; $p<0.01$). The early (1-60 minute) reaction seen in 12 of the patients was also significantly reduced by nedocromil sodium ($p<0.01$). Exercise-induced changes calculated from equivalent diurnal peak flow values showed a smaller late asthmatic response but the protective effect of nedocromil sodium was still evident.

Key words: Exercise-induced asthma, Late asthmatic reaction, Exercise, challenge, Nedocromil sodium

Introduction

Asthmatic patients who react with bronchoconstriction after exercise challenge may show an early asthmatic response (EAR) and/or a late asthmatic response (LAR)[1]. During the EAR, lung function starts to deteriorate within 10 minutes after exercise, shows a maximum fall after 20-30 minutes and generally disappears within 1-3 hours. A LAR can occur, after partial or complete recovery from the EAR, and begins 4-13 hours after exercise, decreasing in severity after 12 hours and normally resolving within 24 hours. The incidence of LARs due to exercise is disputed[2,3] but the development of these reactions has been linked to release of mast cell-derived mediators and possible inflammatory changes in the lung[4]. Recent reports have increased the controversy over the mechanism of exercise-induced bronchoconstriction[5]. Changes in serum levels of neutrophil chemotactic factors after exercise were not found to correlate with the severity of bronchoconstriction[6] and examination of mediators in bronchoalveolar lavage samples after exercise challenge suggested no involvement of airway mast cells in the EAR[7]. In a study by Rubinstein et al[8] five out of six patients who had shown a dual response to exercise challenge suffered a similar 'late' decrease in FEV1 on a no-exercise control day, suggesting the LAR might be caused by diurnal variation of airway calibre in conditions of restricted bronchodilator therapy.

Many of the drugs employed in asthma therapy are effective against exercise-induced asthma[6,9] but in general only the EAR has been studied.

In this study we examined the effect on both EAR and LAR after exercise of a new topical anti-inflammatory asthma treatment, nedocromil sodium (Tilade)[10], which is known to prevent both phases of the dual asthmatic response to bronchial antigen challenge as well as the immediate bronchospasm provoked by exercise challenge[11].

We measured bronchoconstriction primarily by the fall in peak expiratory flow rate (PEFR) from the baseline level recorded prior to each exercise challenge, but a no-exercise control day was also included to investigate the possible influence of diurnal variation on the LAR.

Material and Methods

Study Design

This was a double-blind, randomized, placebo-controlled crossover trial to study the effect of pretreatment with nedocromil sodium on the EAR and LAR following exercise challenge in a group of patients known to develop a LAR. Subjects were selected on an initial screening day from patients with a documented history of asthma or chronic obstructive pulmonary disease (COPD)[12]. PEFR (l/min) was measured, using a mini-Wright peak flow meter and recording the best of 3 measurements, at 10 and 5 minutes before commencing exercise challenge. The mean of these two pre-exercise readings was taken as the baseline PEFR value for that day.

Exercise challenge was carried out on a bicycle ergometer (Erich Jager, Wurtzburg, Germany) with the workload at 80% of the predicted maximum, adjusted for age, sex and height[13].

Exercise was performed for 8 minutes, the workload being reduced if necessary, during which time a heart-rate of 90% of predicted maximum was achieved. Heart-rate was measured by a Siemens Sirecrust 341 monitor (Siemens, Germany). During bicycle exercise each patient wore a nose-clip. Ambient conditions were measured using a Hygrotest 6200 (Quartz AG, Zurich, Switzerland): relative humidity was 20-40% and room temperature 20-23°C. Variations of 10% and 2°C respectively were permitted during any one patient study day. Using the same meter for all tests, PEFR was measured at 1, 3, 5, 7, 10, 15, 30 and 60 minutes after the end of exercise, and again at hourly intervals up to 13 hours after challenge. A positive asthmatic response to exercise was defined as >15% fall in PEFR from the pre-exercise baseline value. The asthmatic response was defined as an EAR at time points from 1-60 minutes after challenge and as a LAR when the >15% fall from pre-exercise PEFR occurred during the period 4-13 hours after exercise challenge.[4] Only patients who developed a LAR (with or without an EAR) were randomized to test treatment.

Medication was restricted on all exercise challenge days: inhaled bronchodilators were not to be used for a period of 8 hours before exercise, nor sodium cromoglycate for a 24-hour period. Use of oral bronchodilators was to be avoided during the preceding 48 hours, and corticosteroid usage was to have been stable for 3 months and be maintained at a constant level throughout the study period.

On test treatment study days the same exercise challenge procedure was carried out. Thirty minutes before starting exercise, test treatment was taken by inhalation of two puffs of medication from an aerosol can that contained either nedocromil sodium (total dose 4 mg) or a matching placebo. An additional PEFR measurement was taken one minute before the test treatment. Treatment order was randomly assigned by coding sheet and all study days were separated by an interval of 4-12 days.

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[4]The 15% level of PEFR fall was choosen to include also 2 patients who had clear clinical symptoms of a LAR after exercise challenge, but showed a PEFR fall of resp. 18.3 and 19.2% compared to baseline value. All of the other patients had a PEFR fall >20%. Furthermore it is noteworthy to mention that this study was the first study we performed in the investigation of the LAR after exercise challenge.

To investigate the influence of diurnal variation on the LAR to exercise challenge, PEFR readings were additionally taken at the same times of day on a separate control day, when all conditions were similar to the screening day except that no exercise challenge was carried out. The LAR expressed as % fall in PEFR from the equivalent 'clocktime' PEFR on this no-challenge control day was also used to examine treatment effects in relation to diurnal variation of PEFR readings, in a secondary assessment made for comparative purposes.

Patients

Out of 86 patients screened, 19 individuals (22%) developed a LAR following exercise challenge and were subsequently randomized to test treatment. Seven of these responders showed an isolated LAR whilst the remaining 12 had a dual reaction. (A further 21/86 patients showed only an EAR.)

The characteristics of the 19 patients who took part in the drug study are summarized in Table 6-1. Nine patients were randomized to the nedocromil sodium/placebo treatment order group and 10 to placebo/nedocromil sodium, the two groups being well-matched.

Seven patients were male and 12 female, with an age range from 17.8 - 62.5 years. Thirteen subjects were classified as bronchial asthmatics and six as COPD patients12, the latter distinguished by FEV1 reversibility <20% predicted after inhalation of 0.4 mg salbutamol. All showed hyperresponsiveness to inhaled histamine (PC20 < 8 mg/ml)14. Five patients were tobacco smokers and continued their usual smoking pattern during the study. After withdrawal of concomitant medication all patients had a PEFR value ≥55% predicted before commencing

exercise challenge. Informed consent to the trial was obtained from all patients, or from the parents of those who were under-age.

Statistical Methods

Parametric statistical methods were applied throughout the analyses. Analysis of variance with patient, order and treatment as factors was used to analyze PEFR differences from baseline at each time point.

The PEFR data were also summarized for each patient as area under the curve (AUC) of the time course and maximum % decreases from baseline, again using analysis of variance. These summary data were regarded as the primary variables, with PEFR changes at individual time points defined as secondary variables. Two-tailed tests were used throughout, with a significance level of 0.05.

Diurnal PEFR changes based on equivalent 'clocktime' control data are presented for comparative reference only since the stated purpose of the study was to compare the effects of nedocromil sodium and placebo on the fall in PEFR after exercise.

Patients who failed to show an EAR to exercise on the screening day were excluded from analyses of the EAR but included in the LAR analysis.

Results

All 19 patients completed the study treatment days as well as the screening day. No unusual symptoms were reported during the study. Each exercise challenge was carried out at the same time of day for each patient except in one case (patient 36) when the two test treatment challenges commenced 40 minutes later than on the screening day. Screening day results are detailed in Table 6-2. All the patients showed a fall from baseline PEFR >18% during the period 4-13 hours after exercise challenge (LAR). Twelve patients had recorded a similar early reduction in PEFR in the period from 1-60 minutes after exercise (EAR) and were therefore included in analysis of the EAR. Seven patients (1, 3, 4, 8, 12, 34, 35) did not show a sufficient fall in PEFR in the first hour after exercise and were excluded from analysis of the EAR.

Five patients (numbers 2, 4, 13, 34, 35) used bronchodilators on the screening day. All showed a (late) PEFR reduction of 20% or more (Table 6-2). Except for patient 34, bronchodilator use occurred only at 9 or 10 hours post-exercise, and was repeated at the identical times on the two test treatment days; the results of these four patients were included in the analysis. Patients 34 and 37 used bronchodilators on the placebo study day but not on the nedocromil sodium day and their data were analyzed only up to the point of medication (5 minutes and 5 hours post-exercise, respectively). Since LAR data from patient 34 were excluded for this reason, 18 patients in total were analyzed for the post-exercise LAR. Using paired t-tests (sample size = 19) pre-exercise baseline PEFR values showed little variation on the three challenge days: (means ± standard deviations of 366.1 ± 68.8; 369.2 ± 73.6 and 371.7 ± 70.6 L/min for screen day, active and placebo treatment days respectively). On both the treatment days, PEFR values recorded just prior to aerosol treatment (at 31 minutes before exercise and 21-26 minutes before the baseline PEFR measurements) were rather lower than

baseline ($p<0.05$): means of 355.0 ± 71.8 and 355.8 ± 67.5 L/min for nedocromil sodium and placebo pre-treatment PEFR values, respectively.

Results of the analyses carried out on absolute differences in PEFR (L/min) from the pre-exercise baseline at each test treatment challenge are summarized in Tables 6-3 and 6-4. PEFR changes on the EIA (no treatment) screen day are included with the treatment group results for reference only. For the EAR (Table 6-3) the maximum % fall in PEFR was significantly less ($p<0.01$) with nedocromil sodium (13.2%) than with placebo (36.6%). The AUC was also significantly better ($p<0.01$) with nedocromil sodium during the EAR. Significant differences ($p<0.05$ - $p<0.01$) in favour of nedocromil sodium continued to occur at individual time points up to 11 hours post-exercise (Table 6-4). AUC for the LAR showed a strong trend in favour of the active treatment, however it should be noted that only the nedocromil sodium treated patients fully recovered their baseline levels of PEFR following resolution of the EAR. The maximum % fall in PEFR during the LAR (Table 5-4) was reduced significantly ($p<0.01$) by pretreatment with nedocromil sodium (12.4%) compared to placebo (25.8%). Individual patient results for maximum fall in PEFR after exercise on the (no- treatment) screen day and following active (nedocromil sodium) and placebo test treatments are presented as % fall from pre-exercise baseline (Table 6-5) and again as % predicted normal PEFR (Table 6-6).

To allow for the influence of diurnal variations in PEFR, measurements taken at the equivalent 'clocktimes' on a separate, no-challenge control day were also used as baseline values for calculation of maximum % fall in PEFR during the LAR. These values are shown (Table 6-7) for comparative purposes but were not used in the statistical comparison of nedocromil sodium and placebo treatment effects on EIA. The group mean maximum % fall in PEFR 4-13 hours after exercise as calculated from the 'clocktime' norm was approximately 12% lesser throughout than the fall from the pre-exercise baseline. Consequently the number of LARs was reduced; however the overall group mean % fall in PEFR on the screenday still reached 22.9%, with 10/18 patients showing an unequivocal LAR ≥19.0% fall in PEFR). This diurnally-adjusted

LAR was considerably reduced by placebo treatment (mean 11.6% fall in PEFR) and ablated by nedocromil sodium (mean increase in PEFR of 1.6%).

Discussion

This study set out to investigate the preventive effect of nedocromil sodium on exercise-induced bronchoconstriction in patients showing a late phase reaction (LAR) 4-13 hours after exercise challenge. The very existence of dual reactions to exercise in patients with airflow limitation remains in itself a subject of continuing debate and the prevalence of LARs after exercise challenge has been variously reported as 2%-60%[1,15,16].

It was therefore important to make sure the response we were measuring was real and not artifactual. The generally accepted method of assessing the effects of bronchial challenge on lung function is to compare serial measurements after challenge against the baseline level measured prior to challenge. In the case of exercise-induced lung function changes, the response may be influenced by many different factors and the mechanism of airway obstruction has yet to be elucidated[17-19]. Current evidence suggests that diurnal factors may have a strong influence on the post-exercise asthmatic response and several lines of evidence are in favour of comparing lung function after exercise with the corresponding 'clocktime' values on a control day[8,15]. Our results support this view since both incidence and severity of LAR after exercise challenge were reduced when the diurnal rhythm was taken into account in this way.

At the same time, this study further substantiates the evidence for the existence of both an EAR and a LAR after exercise challenge in patients with airflow limitation. From a group of 86 asthmatic patients who underwent exercise challenge, 33 (38%) developed an EAR, measured

as fall from pre-exercise PEFR. Using the same pre-exercise baseline, a total of 19/86 patients (22%) developed a LAR. Only 12/86 of these patients (14%) had a dual response, whilst 7/86 (8%) had an isolated LAR. Isolated EAR thus occurred in 21/86 (24%) of the patients. These LAR incidence figures based on the 'standard' fall in PEFR from pre-exercise baseline may have been increased because we looked for LAR in all the patients rather than examining a subgroup known to develop an EAR to exercise: this could be important as 7 out of 19 LAR patients had no preceding EAR. The incidence of LAR was reduced by half when taken as the fall in PEFR from the corresponding 'clocktime' value on a no-exercise control day.

Using this more valid criterion as a baseline measure only 10 of the original 86 patients (11.6%) showed a LAR in response to exercise. Even on this basis, however, the incidence of exercise-induced LAR was considerable and cannot be swept aside. It is possible that our patients were not a truly representative population since they suffered from severe asthma and COPD, such that they had to continue their daily corticosteroid therapy during the study. Nevertheless, some were able to develop a LAR after exercise challenge, which could perhaps indicate that the steroid dose was insufficient. It was interesting to find that all the (6) COPD patients challenged had a LAR after exercise: at present we have no adequate explanation for this.

In view of the fact that the patients maintained their corticosteroid therapy throughout the trial, we were interested to find that nedocromil sodium not only effectively blocked the EAR and the LAR after exercise challenge but that the effect against the LAR was stronger when this was measured from the diurnal equivalent rather than the pre-exercise PEFR. A placebo effect was evident, this too being stronger on the LAR, which introduced the possibility of psychological influences on this response, in addition to doubts about its reproducibility. We have partly answered this question by showing the LAR following exercise challenge to be a highly reproducible phenomenon in repeat tests performed 2-13 weeks after the first challenge (data not yet published). The strongly protective effect of nedocromil sodium against the LAR resulting from exercise challenge appears to tie-in with the efficacy of this compound in

preventing the dual asthmatic response to bronchial allergen challenge[20] and suggests the involvement of an inflammatory component in the exercise-induced LAR also.

It is well-accepted, however, that the pathogenesis of EIA is multifactorial. One major component is considered to be increased water loss from the airway lining fluid, creating a hyperosmolar environment in the bronchial mucosa[21] which could be a stimulus for mediator release from resident cells such as mast cells[4].

The inhibitory activity of nedocromil sodium on mucosal mast cells and other resident cells of the airways would again fit in with this explanation[22,23], which has been countered, however, by the suggestion that exercise increases bronchial obstruction in asthmatics through congestion of the microvasculature[19]. This mechanism also could be moderated by nedocromil sodium, which is known to affect microvascular leakage and neurogenic inflammation in the airways[24].

Whilst both the pathogenesis of EIA and the mechanism of action of nedocromil sodium remain subjects for investigation, our present study confirmed that a proportion of patients with severe asthma and COPD do develop a LAR after exercise challenge and that both this and the immediate EAR are effectively inhibited by pretreatment with a single dose (4 mg) of nedocromil sodium.

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Table 6-1. Patient Characteristics at Admission

Variable	Mean Value/Frequency (n=19)
Age (years)	37.5 ± 13.6
Sex	7 males / 12 females
Diagnosis	13 asthma / 6 COPD
Histamine PC20 (mg/ml)	1.4 ± 0.3
Baseline FEV1: (L)	2.47 ± 0.77
(% predicted)	78.1 ± 24.3
FVC: (L)	3.86 ± 0.93
(% predicted)	99.3 ± 16.7
Atopic status	14 atopic / 5 non-atopic
Smokers	5 yes / 14 no
Current therapy:	
Antihistamine	7
Inhaled corticosteroid	13
Oral corticosteroid	9
Theophyllines/Xanthines	12
Inhaled bronchodilators	13
Oral bronchodilators	2
Sodium cromoglycate	3

Table 6-2. Maximum % Fall in PEFR from Pre-Exercise Baseline on the Initial Screening Day for Patients Showing a LAR

Pat.No.	Order Group	Baseline PEFR (L/min)	EAR (max % fall)	LAR (max % fall)
1	2	375	14.7	33.3
2+	1	425	52.9	20.0
3	1	375	6.7	33.3
4+	2	290	3.5	37.9
5	1	285	40.4	43.9
6	2	390	71.8	28.2
7	2	255	37.3	49.0
8	1	480	10.4	20.8
9	2	495	49.5	19.2
11	1	410	43.9	41.5
12	2	300	13.3	30.0
13+	2	400	55.0	72.5
15	1	425	41.2	27.1
34+	1	320	6.3	37.5
35+	2	400	0.0	21.3
36	2	285	36.8	47.4
37	1	295	39.0	52.5
38	2	355	18.3	18.3
39	1	395	72.2	41.8

Order group: 1 = nedocromil sodium/placebo

2 = placebo/nedocromil sodium

+ No values have been excluded following bronchodilator use by these patients

Table 6-3: Analysis of Absolute Decreases in PEFR (L/min) from Pre-Exercise Baseline Mean (sample size) at Each Time Point Post-Exercise Challenge: - EAR (1-60 minutes)

Treatment	Pre-Exercise Baseline Mean	Absolute Decrease from Pre-Exercise Baseline Mean at Each Time Point (Minutes) Post-Exercise Challenge								Mean Maximum % Fall from Pre-Exercise Baseline	AUC
		1	3	5	7	10	15	30	60		
None (Screen)	368.8 (12)	49.2 (12)	80.8 (12)	110.4 (12)	114.6 (12)	122.1 (12)	126.3 (12)	143.8 (12)	115.4 (12)	47.4 (12)	7434.6 (12)
Nedocromil sodium	370.4 (12)	-11.3 (12)	25.8 (12)	24.6 (12)	25.4 (12)	36.7 (12)	23.3 (12)	14.2 (12)	5.4 (12)	13.2 (12)	933.1 (12)
Placebo	382.3 (12)	26.9 (12)	60.2 (12)	84.0 (12)	95.6 (12)	112.7 (12)	106.9 (12)	131.5 (12)	90.6 (12)	36.6 (12)	6391.0 (12)
Significance of Treatment Comparison	NS	NS	NS+	*	*	*	*	**	**	**	**

Note: results of the screen day challenge are shown for reference only

NS $p>0.05$; NS+ $0.05<p<0.10$; * $p<0.05$; ** $p<0.01$

Table 6-4: Analysis of Absolute Decreases in PEFR (L/min) from Pre-Exercise Baseline Mean (sample size) at Each Time Point Post-Exercise Challenge: - LAR (4-13 hours)

Treatment	Pre-Exercise Baseline Mean	Absolute Decrease from Pre-Exercise Baseline Mean at Each Time Point (Hours) Post-Exercise Challenge										Mean Maximum % Fall from Pre-Exercise Baseline	AUC
		4	5	6	7	8	9	10	11	12	13		
None (Screen)	369.2 (18)	55.0 (18)	69.7 (18)	67.1 (17)	66.5 (17)	80.6 (17)	99.7 (17)	73.5 (17)	69.4 (17)	74.7 (17)	84.1 (17)	35.3 (18)	687.2 (16)
Nedocromil sodium	370.3 (18)	-4.4 (18)	-0.6 (18)	8.5 (17)	7.4 (17)	7.1 (17)	22.4 (17)	24.4 (17)	19.1 (17)	11.8 (17)	23.4 (16)	12.4 (18)	116.4 (16)
Placebo	374.3 (18)	43.8 (18)	52.4 (18)	61.5 (17)	53.8 (17)	52.9 (17)	52.1 (17)	50.3 (17)	59.1 (17)	52.1 (17)	52.8 (16)	25.8 (18)	484.7 (16)
Significance of Treatment Comparison	NS	**	NS+	*	NS	*	NS	NS	*	NS+	NS	**	NS+

Note: results of the screen day challenge are shown for reference only
NS $p>0.05$; NS+ $0.05<p<0.10$; * $p<0.05$; ** $p<0.01$

Table 6-5: Maximum Percentage Fall in PEFR from Pre-Exercise Baseline

Pat No.	Maximum % Fall in PEFR from Pre-Exercise Baseline					
	EAR (1-60 minutes)			LAR (4-13 hours) .		
	Screen	Active	Placebo	Screen	Active	Placebo
1	-	-	-	33.3	15.0	14.3
2	54.0	26.7	54.3	21.8	8.9	23.9
3	-	-	-	33.3	12.7	21.6
4	-	-	-	37.9	-1.4	-11.1
5	40.4	5.7	40.4	43.9	5.7	40.4
6	71.8	5.1	81.6	28.2	-2.6	34.7
7	37.3	16.7	16.0	49.0	41.7	20.0
8	-	-	-	20.8	6.8	10.1
9	49.5	26.9	47.9	19.2	14.0	10.4
11	43.9	4.2	3.8	41.5	1.4	62.0
12	-	-	-	30.0	29.8	22.2
13	55.0	15.2	60.6	72.5	30.4	54.5
15	41.2	22.2	41.9	27.1	22.2	32.6
34	-	-	-	-	-	-
35	-	-	-	21.3	7.8	15.0
36	36.8	0.0	29.0	47.4	20.0	39.5
37	39.0	1.8	7.0	39.0	-7.1	14.7
38	18.3	2.7	0.6	18.3	-16.0	14.8
39	72.2	30.7	56.2	41.8	34.7	44.5
Mean	46.6	13.2	36.6	34.8	12.4	25.8
±SD	±15.2	±11.2	±25.6	±13.7	±15.3	±17.9
Sample size	12	12	12	18	18	18

Table 6-6: EAR and LAR PEFR Values Related to % Predicted Normal Values

Pat. No.	Predicted PEFR	Pre-Exercise Baseline PEFR as % Pred			EAR lowest PEFR as % Pred			LAR lowest PEFR as % Pred		
		Screen	Placebo	Active	Screen	Placebo	Active	Screen	Placebo	Active
1	430	87.2	81.4	93.0	74.4	81.4	97.7	58.1	69.8	79.1
2	439	99.1	104.8	102.5	45.6	47.8	75.2	77.4	79.7	93.4
3	418	89.7	88.5	84.9	83.7	78.9	78.9	59.8	69.4	74.2
4	440	65.9	71.6	80.7	63.6	68.2	47.7	40.9	79.5	81.8
5	391	72 9	72 9	67 8	43.5	43.5	63.9	40.9	43.5	63.9
6	589	66 2	83 2	66 2	18.7	15.3	62.8	47.5	54.3	67.9
7	437	58.4	57.2	54.9	36.6	48.1	45.8	29.7	45.8	32.0
8	429	111.9	103.7	102.6	100.2	90.9	93.2	88.6	93.2	95.6
9	427	115.9	112.4	108.9	58.5	58.5	79.6	93.7	100.7	93.7
11	378	108.5	104.5	93.9	60.8	100.5	89.9	63.5	39.7	92.6
12	403	74.4	67.0	70.7	64.5	62.0	59.6	52.1	52.1	49.6
13	463	86.4	71.3	99.4	38.9	28.1	84.2	23.8	32.4	69.1
15	625	68.0	68.8	57.6	40.0	40.0	44.8	49.6	46.4	44.8
34	477	67.1	68.1	73.4	62.9	71.3	67.1	- -	52.4	
35	519	77.1	77.1	74.2	77.1	79.0	77.1	60.7	65.5	68.4
36	362	78.7	105.0	82.9	49.7	74.6	82.9	41.4	63.5	66.3
37	365	80.8	88.4	76.7	49.3	82.2	75.3	38.4	95.9	54.8
38	497	71.4	85.0	75.5	58.4	84.5	73.4	58.4	72.4	87.5
39	530	74.5	64.6	95.3	20.8	28.3	66.0	43.4	35.8	62.3
Mean	453	82	83	82	55	62	72	54	63	70
±SD	±72	±17	±17	±16	±21	±24	±15	±19	±21	±18

Table 6-7. Maximum % Fall in PEFR (LAR) from Pre-Exercise Baseline and a Comparison of the Maximum % Fall in Relation to the Control PEFR Value at the Corresponding 'Clocktime'

Pat.	LAR % Fall from Pre-Exercise Baseline			LAR % Fall from No-Exercise 'Clocktime' Baseline		
No.	Screen	Placebo	Active	Screen	Placebo	Active
1	33.3	14.3	15.0	34.2	21.1	10.5
2	30.0	23.9	8.9	19.0	-20.7	2.4
3	33.3	21.6	12.7	10.7	-1.8	-24.0
4	37.9	-11.1	-1.4	45.5	0.0	-9.1
5	43.9	40.4	5.7	20.0	15.0	-8.7
6	28.2	34.7	-2.6	24.3	-3.2	2.4
7	49.0	20.0	41.7	0.0	0.0	-7.7
8	20.8	10.1	6.8	11.6	9.1	0.0
9	19.2	10.4	14.0	2.4	4.4	10.1
11	41.5	62.0	1.4	20.0	50.0	-11.1
12	30.0	22.2	29.8	12.5	6.0	25.9
13	72.5	54.5	30.4	72.5	62.5	14.7
15	27.1	32.6	22.2	16.2	19.4	24.3
34	-	-	-	-	-	-
35	21.3	15.0	7.8	7.4	0.0	-18.3
36	47.4	39.5	20.0	16.7	-15.0	-33.3
37	39.0	14.7	-7.1	40.0	8.3	0.0
38	18.3	14.8	-16.0	22.7	-2.9	-17.6
39	41.8	44.5	34.7	36.1	56.8	10.8
Mean	35.3	25.8	12.4	22.9	11.6	-1.6
±SD	±13.4	±17.9	±15.3	±17.6	±23.2	±16.2

CHAPTER 7

SUMMARY AND CONCLUSIONS

7-1. SUMMARY

7-2. CONCLUSIONS

Chapter 1 presented a general introduction and an outline of the objectives of the studies presented in this thesis. The late asthmatic reaction (LAR) after exercise challenge remains a controversial issue. A number of studies in the past have put some evidence for the occurrence of a LAR after exercise challenge. This response after exercise challenge is usually present 3-13 hours after exercise challenge and the decline in peak expiratory flow rate (PEFR) is easily monitored by a mini-Wright peak flow meter. The objectives of the various studies was first to examine the existence and prevalence of the LAR after exercise challenge, and if so to define this reaction properly; second to examine the reproducibility of this reaction and third to look at the protective effect of nedocromil sodium on the EAR and LAR after exercise challenge. Fourth the pathofysiological mechanisms behind the LAR after EC were investigated.

Chapter 2 dealed with the prevalence of the LAR and early asthmatic reaction (EAR) after exercise challenge. There is a discussion going on about the existence of a LAR after exercise challenge. The controversy in the literature is whether the LAR after exercise challenge does occur, and if so what is the frequency of it and to which value should the PEFR after exercise challenge be related to. In this study the post-exercise decrease in PEFR was compared with the corresponding clocktime PEFR on a control day. Patients were randomized for a control day and an exercise day and PEFR was recorded at t=0 (pre-exercise PEFR) and 1, 3, 5, 7, 10, 15 and 30 minutes, and then hourly during the 13 h after the end of the exercise challenge. The control day was monitored in the same way as the exercise day with the only difference that no exercise test took place.

A considerable number of late responses was found: out of 86 patients with reversible airflow limitation 19% had a LAR after exercise challenge when a PEFR fall $\geq$ 20% compared to corresponding clocktime on a control day was used as a definition. Of the examined patients 38% had a LAR after exercise challenge when a PEFR fall $\geq$ 10% compared to corresponding clocktime on a control day was used as a definition. The late response after exercise challenge

can occur as an isolated or as a dual reaction. The dual reaction (LAR + EAR) took place in 13 out of the 86 patients studied. Despite the use of oral and inhaled steroids a LAR after exercise challenge could occur. Concluded is that one should only speak of a LAR after exercise challenge when the diurnal post-exercise PEFR rhythm had been compared with a day without exercise.

In chapter 3 the distinction between true and pseudo LARs after exercise challenge was made, based on fluctuations of the PEFR on control days in relation to days with exercise challenge. The normal variability of the PEFR was identified in another group of asthmatics who did not undergo exercise challenge. Twenty-one patients recorded PEFR on two control days without performing exercise. There was no difference between both control days when PEFR at one hour was compared to baseline PEFR and when PEFR at 3-13 hours was compared to baseline PEFR. After analyzing variation coefficients of baseline PEFR on a control- and exercise day, PEFR was not allowed to differ more than 15.3% in the same patient, when comparing exercise- and control day for the late fall in PEFR in this study. In 17 out of 81 patients with reversible airflow limitation a late asthmatic reaction after exercise challenge was present, when PEFR fall was ≥20% compared to baseline PEFR value. In 8 out of the 17 patients a real late asthmatic reaction to exercise challenge was present on at least 3 successive time points and a PEFR fall ≥20% in comparison with corresponding clocktime on a control day.

Based on the above mentioned observations it was deduced that a LAR after exercise challenge is best described by a 20% or greater fall in PEFR on 3 successive time points in comparison with corresponding clocktime on a control day. Isolated declines in PEFR on three not successive time points or other falls in PEFR not related to corresponding clocktime on a control day were termed pseudo LARs.

This study confirms the presence of late asthmatic responses after exercise challenge. The majority of the patients tested were receiving either or both inhaled and systemic glucocorticosteroids. These agents have been known to inhibit not only late asthmatic responses after antigen but they may also alter the variability of airway calibre. This underscored the

presence of a LAR after exercise challenge. Graphic illustration of airway responses following exercises could facilitate the detection of late asthmatic responses.

In chapter 4 the reproducibility of the LAR after exercise challenge was studied. The examined asthmatic patients performed an exercise test and they recorded thereafter their PEFR as well as on a control day. When a LAR after exercise challenge was present, which was defined as a PEFR fall ≥ 20% on three or more time points 3-13 hours after exercise challenge compared to corresponding clocktime on a control day, they performed a second exercise challenge and control day. We investigated whether this reaction was reproducible when patients were rechallenged 21-150 days after the first exercise challenge. Eighty three hospitalized patients with reversible airflow limitation were challenged with exercise. The patients were examined according to a standardized protocol which comprised eight minutes bicycling at 90% of predicted heart-rate.

Eleven patients (13.3%) had a PEFR fall greater than 20% 3-13 hours after exercise. Those patients who showed a late asthmatic response after exercise challenge were rechallenged in order to study the reproducibility of this reaction. Eight of those patients (73%) showed a reproducible late bronchoconstrictive reaction after exercise challenge when compared with a new control day. Areas under the curve 3-13 hours after the first and second exercise challenge in addition showed a good reproducibility. The presented study clearly supported existing evidence for the occurrence of a LAR after exercise challenge and its reproducibility when very strict criteria as mentioned above were applied. Using these criteria it could even be shown that the LAR after exercise challenge did occur after a period of 11 months. Since the therapy regimen for the patients was kept unaltered between the two challenge occasions it denied the fact that the LAR after exercise challenge could be due to medication withdrawal. Furthermore, it extended the evidence that the LAR after exercise challenge was not a coincidence of a stable control day and an unstable exercise challenge day, since both were registered on two different occasions with an in between time period of 21-150 days. In this selected group of asthmatic patients the LAR after exercise challenge pointed out to be reproducible in 73% of the cases.

From the presented data it was clear that, when strict criteria were applied for the LAR after exercise challenge, it was almost as reproducible as the LAR after antigen challenge. Although the LAR after exercise challenge in itself was reproducible, the time points at which the maximal PEFR fall occurred were not reproducible

In chapter 5 an investigation was done in order to try to clarify the pathophysiological mechanisms behind the LAR after exercise challenge. A reproducible LAR after exercise challenge was defined as a PEFR fall ≥20% on three or more time points 3-13 hours after exercise challenge compared to corresponding clocktime on a control day after the first and second exercise challenge. (as is outlined in chapter 4.) In 9 patients with a reproducible LAR after exercise challenge a bronchial provocation test with histamine was done before exercise challenge, after one hour, 24, and 48 hours after exercise challenge. Before exercise challenge an iv catheter was placed in an antebrachial vene for the investigation of blood eosinophiles, neutrophiles, basophiles as well as serum eosinophil cationic protein levels and serum histamine levels. Blood was collected before exercise challenge, 3 and 15 minutes after exercise challenge and 3, 6, 9, 24, 48, hours after exercise challenge. Three patients with a reproducible negative LAR after exercise challenge underwent the same investigation.

It appeared that the histamine threshold did not differ significantly in the patients with a LAR after exercise challenge; neither there was a significant difference when the positive LAR group was compared with the group without a LAR after exercise challenge. Also the peripheral blood cells measured after exercise challenge showed no significant difference in each of the patients with a LAR after exercise challenge. This was also the case for serum eosinophil cationic protein levels and serum histamine levels. When the positive LAR group was compared with the negative LAR group there was no difference which could elucidate the mechanism behind the LAR after exercise challenge. It was therefore concluded from this study that if an inflammatory component was involved in the LAR after exercise challenge it was not reflected by the here measured parameters. It was concluded that more research is necessary to clarify the mechanism behind the LAR after exercise challenge. Perhaps the methods used in this study

were not sensitive enough to detect the role of inflammation and bronchial hyperreactivity in patients with a LAR after exercise challenge.

In chapter 6 the protective effect of nedocromil sodium on the LAR after exercise challenge was discussed. In a double-blind placebo-controlled crossover design study the effect of nedocromil sodium on the EAR and LAR after exercise challenge with particular emphasis on the LAR, was evaluated. A positive asthmatic reaction was defined as a PEFR fall $\geq$ 15%.

After exercise challenge out of a group of 86 patients with reversible airflow limitation, 33 patients experienced an EAR whereas 19 patients experienced a LAR 3-13 hours after challenge. These numbers were based on comparison of the PEFR fall with the pre-exercise value. In case the PEFR fall was compared with the corresponding clocktime value on a control day without exercise challenge, only 10 out of the 19 patients experienced a LAR. The 19 patients who showed a positive LAR after exercise challenge compared to pre-exercise PEFR value were rechallenged twice after pretreatment with either placebo or 4 mg nedocromil sodium in a randomized order. It could be shown that nedocromil sodium has a significant protective effect in comparison with placebo on both the EAR and LAR after exercise challenge. Although the mode of action of nedocromil sodium remains unclear, the study showed that patients with reversible airflow limitation experiencing EAR and LAR after exercise benefit from nedocromil sodium therapy.

7-2. Conclusions

1.In patients with bronchial asthma and COPD a late asthmatic reaction (LAR) after exercise challenge occurs. The best method to detect a LAR after exercise challenge is when the maximal post-exercise fall in peak expiratory flow rate (PEFR) is compared with corresponding clocktime PEFR value on a day without exercise.

2.The LAR after exercise challenge is best described by a 20% or greater reduction in PEFR on three successive time points as compared to to corresponding clocktime on a control day. Isolated or other declines in PEFR are termed pseudo LARs.

3.The reproducibility of the LAR after exercise challenge is 73 %.

4.Histamine thresholds do not differ significantly in patients with a LAR after exercise challenge during the course of the LAR. Also peripheral inflammatory blood cells measured after exercise challenge showed no significant difference in each of the patients with a LAR after exercise challenge as well as serum eosinophil cationic protein levels and serum histamine levels.

5.Nedocromil sodium demonstrates a significant protective effect on both the EAR and LAR after exercise challenge.

Hoofdstuk 1 bevatte een algemene inleiding over de late astmatische reactie (LAR) en het doel van de studies die in dit proefschrift werden behandeld. De LAR na inspanningsprovocatie is een controversieel gegeven, hoewel enige studies in het verleden bewijzen hebben geleverd voor het bestaan van deze reaktie. De LAR na inspanning is gewoonlijk aanwezig 3-13 uur na inspanning. De vermindering in peak-flow is eenvoudig te volgen door middel van een mini-Wright peak-flow-meter. Het doel van de verschillende studies, die in dit proefschrift zijn vermeld, was om eerst het bestaan van de LAR na inspanning aan te tonen en de prevalentie ervan vast te stellen. Verder werd aandacht besteed aan de moeilijkheid om de LAR na inspanning te definieren. Ten tweede werd de reproduceerbaarheid van de LAR na inspanning onderzocht. Ten derde werd het beschermende effect van nedocromil natrium op de EAR en LAR na inspannings-provokatie vastgesteld en ten vierde werd op mogelijke pathofysiologische mechanismen van de LAR na inspannings-provokatie ingegaan.

Hoofdstuk 2 behandelde de frequentie van voorkomen van de LAR en de vroege astmatische reactie (EAR) na inspanning. Uit literatuur onderzoek blijkt dat er twijfel bestaat aan het bestaan van de LAR na inspanning. Als deze bestaat is het onduidelijk wat de frequentie hiervan is en op welke referentiewaarden de peak-flow daling na inspanning gericht moet worden. In deze studie werd de vermindering in peak-flow na inspanning vergeleken met een peak-flow meting op hetzelfde tijdstip tijdens een controledag. Patiënten werden willekeurig verdeeld om op een controledag en een inspanningsdag de peak-flow bij te houden. De peak-flow werd gemeten op tijdstip t=0 (uitgangspeak-flow) en op 1, 3, 5, 7, 10, 15 en 30 minuten en vervolgens elk uur gedurende de daarop volgende 13 uur na inspanning. Op de controledag werd de peak-flow bijgehouden op dezelfde manier alleen gedurende elk uur tot 13 uur na 09.00 uur, met het verschil dat er geen inspanning plaats vond.

Van de 86 patiënten met CARA had 19% een LAR na inspanning, als de peak-flow daling gedefinieerd werd als een ≥20% daling ten opzichte van de overeenkomstige meting op

hetzelfde tijdstip tijdens een controledag. Achtendertig procent van de onderzochte patiënten had een LAR na inspanning als de peak-flow-daling gedefinieerd werd als ≥10% daling vergeleken met de overeenkomstige meting op hetzelfde tijdstip tijdens een controledag. De LAR na inspanning kan optreden als een geïsoleerde of een gecombineerde reactie. De gecombineerde reactie (LAR + EAR) was bij 13 van de 86 onderzochte patiënten aanwezig. Ondanks het gebruik van orale- en inhalatiesteroïden bleek zich een LAR na inspanning te ontwikkelen. De conclusie van deze studie was, dat men alleen van een LAR na inspanning kon spreken als de peak-flow op de inspanningsdag werd gerelateerd aan die op een controledag waarop geen inspanning werd verricht.

In hoofdstuk 3 werd er onderscheid gemaakt tussen echte en pseudo LAR's na inspanning. Dit onderscheid was gebaseerd op fluctuaties van de peak-flow op controledagen vergeleken met fluctuaties van de peak-flow op dagen met inspanning. De normale variatie van de peak-flow werd vastgesteld in een andere groep astmapatiënten die geen inspanning verrichten. Eenentwintig patiënten hielden peak-flow-metingen bij op twee dagen zonder inspanning te verrichten. Er werd geen verschil gevonden tussen beide controledagen als de peak-flow na één uur vergeleken werd met de eerste peak-flow meting op die dag (uitgangs-peak-flow meting) en als de peak-flow na 3-13 uur vergeleken werd met de eerste peak-flow meting. Na analyse van variatiecoëfficiënten van uitgangs-peak-flow-waarden van patienten die hun peak-flow-waarde bijhielden op een controle- en inspanningsdag, werd besloten, na statistische analyse, de peak-flow niet méér te laten verschillen dan 15,3% bij dezelfde patiënt, als inspannings- en controledag vergeleken werden met de daling in peak-flow in deze studie.

Bij 17 van de 81 patiënten met reversibele luchtwegvernauwing was een LAR na inspanning aanwezig indien het criterium aangehouden werd van een peak-flow-daling ≥20% vergeleken werd met de uitgangs-peak-flow-waarden. Bij 8 van de 17 patiënten was een "echte" LAR na inspanning aanwezig met een peak-flow-daling ≥20% op tenminste drie opeenvolgende tijdstippen. Bovendien was er een peak-flow-daling ≥20% vergeleken met de peak-flow-waarden op de corresponderende tijdstippen van de controledag. Gebaseerd op bovenstaande

observaties werd geconcludeerd dat een LAR na inspanning beter beschreven kan worden door een daling in peak-flow van 20% of meer op drie opeenvolgende tijdstippen, zowel vergeleken met de uitgangs-peak-flow als met de peak-flow-waarde van het corresponderende tijdstip op een controledag. Andere dalingen in peak-flow-sterkte, al of niet geïsoleerd, werden in deze studie pseudo LAR's genoemd.

Deze studie bevestigde het bestaan van een LAR na inspanning. Het merendeel van de patiënten die onderzocht werden, gebruikte inhalatie- en/of systemische glucocorticosteroïden. Deze medicamenten hebben de eigenschap dat zij de late reactie na antigeen kunnen remmen, echter ook de variabiliteit van het lumen van de luchtweg kunnen verminderen. Dit had invloed op het aantal LAR's dat gevonden werd. Luchtwegreacties na inspanning konden ten aanzien van de opsporing van een LAR na inspanning gemakkelijker gezien worden op curven van peak-flow-waarden.

In hoofdstuk 4 werd de reproduceerbaarheid van de LAR na inspanning bestudeerd. De onderzochte patiënten met asthma bronchiale hielden door middel van een mini-Wright peak-flow-meter, de peak-flow bij op een controledag en een dag na een inspanningtest bij. Een peak-flow-waarde-daling van ≥20% op drie achtereenvolgende tijdstippen 3-13 uur na inspanning op de inspanningsdag als deze vergeleken werden met peak-flow-waarden op dezelfde tijdstippen van een controledag werd gedefinieerd als een positieve LAR na inspanning. Als een LAR na inspanning aanwezig was, werden zij onderworpen aan een tweede inspannings- en controledag. Onderzocht werd of de daling in peak-flow reproduceerbaar was, als de patiënten 21-150 dagen na de eerste test voor de tweede maal een inspanningstest ondergingen. Drieentachtig CARA-patiënten die opgenomen waren in het Nederlands Asthmacentrum Davos werden onderworpen aan een inspanningstest. Zij werden onderworpen aan een gestandaardiseerd protocol, hetgeen inhield: acht minuten fietsen bij 90% van de voorspelde maximale hartfrequentie.

Elf patiënten (13.3%) hadden 3-13 uur na inspanning een peak-flow-daling van meer dan 20%. Deze patiënten (die een late reactie na inspanning hadden) werden voor een tweede

maal onderworpen aan een inspanningstest om de reproduceerbaarheid van deze reactie na te gaan. Acht (73%) van deze patiënten vertoonden een reproduceerbare LAR na inspanning vergeleken met een nieuwe controledag. Oppervlakten onder de peak-flow-curve 3-13 uur na de eerste en tweede inspanningstest lieten een goede reproduceerbaarheid zien.

Deze studie gaf duidelijk een bewijs voor de aanwezigheid van een LAR na inspanning, die bovendien reproduceerbaar was als strikte criteria werden toegepast. Van deze criteria gebruik makende, kon aangetoond worden dat de LAR zelfs optreedt na een periode van elf maanden. Aangezien de therapie voor de patiënten ongewijzigd bleef gedurende de twee inspannings- en controledagen, moet het uitgesloten worden geacht dat de LAR na inspanning het gevolg kon zijn van medicatie-onthouding. De LAR na inspanning kon geen toevalligheid zijn van een stabiele controledag en een instabiele inspanningsdag, aangezien beide inspannings-en controledagen geregistreerd werden op twee verschillende dagen met een tijdsinterval van 21-150 dagen. In deze geselecteerde groep van CARA-patiënten bewees de LAR na inspanning reproduceerbaar te zijn in 73% van de gevallen. De LAR na inspanning bleek even reproduceerbaar te zijn als de LAR na antigeen provocatie, als stricte criteria werden gehanteerd. Het tijdstip van optreden van de maximale peak-flow-daling na inspanning bleek echter niet reproduceerbaar (Alhoewel de LAR na inspanning reproduceerbaar was).

In hoofdstuk 5 werd een gedeelte van de mogelijke pathofysiologische mechanismen van de LAR na inspanning onderzocht. Bij 9 patiënten met een reproduceerbare LAR na inspanning zoals beschreven in hoofdstuk 4 (een peak-flow daling $\geq$20% 3-13 uur na provokatie op 2 inspannings dagen vergeleken met de peak-flow-waarden op het corresponderende tijdstip van de 2 controledagen) werd een bronchusprovocatietest gedaan met histamine voor inspanning, 1 uur, 24 en 48 uur na inspanning. Voor dat inspanning plaatsvond, werd een intraveneuze catheter in een antebrachiale vene geplaatst zowel voor onderzoek van bloed op eosinofielen, neutrofielen, basofielen, als voor serum eosinofiel cationic protein (s-ECP) en serum histamine-spiegels. Bloed werd verzameld voor inspanning, 3 en 15 minuten na inspanning en 3, 6, 9, 24

en 48 uur na inspanning. Een controlegroep van drie patiënten met een reproduceerbare negatieve LAR na inspanning onderging dezelfde onderzoeken.

Het bleek dat de histaminedrempel niet significant verandert was na inspanning en het beloop niet anders was dan bij de patiënten met een negatieve LAR na inspanning. Daarnaast bleek uit de perifere inflammatoire cellen dat er geen significant verschil bestond in de patiënten met een positieve en negatieve LAR na inspanning evenals voor serum eosinofiel cationic protein en serum histamine-spiegels. Als ook de totale positieve LAR-groep vergeleken werd met de negatieve LAR-groep ten aanzien van bovengenoemde parameters, kon het mechanisme van de LAR na inspanning niet opgehelderd worden.

Derhalve kon uit deze studie geconcludeerd worden dat als een inflammatoire component betrokken was bij de LAR na inspanning, dit niet weerspiegeld werd door de in deze studie onderzochte parameters. Verder werd geconcludeerd dat nader onderzoek nodig is om het mechanisme van de LAR na inspanning op te helderen. Mogelijkerwijs waren de methoden die in deze studie gebruikt zijn, niet sensitief genoeg om de rol van inflammatie in bronchiale hyperreactiviteit bij patiënten met een LAR na inspanning op te sporen.

In hoofdstuk 6 werd het beschermende effect van nedocromil natrium op de LAR na inspanning onderzocht. In een "double-blind placebo crossover" studie werd het effect van nedocromil natrium op de vroege en late reactie na inspanning bestudeerd, met speciale belangstelling ten aanzien van de LAR. Een positieve reactie na inspanning werd gedefinieerd als een peak-flow-daling ≥15% vergeleken met de uitgangs-peak-flow waarden.

In een groep van 86 patiënten vertoonden 33 patiënten een EAR, terwijl 19 patiënten een LAR na inspanning hadden. Deze getallen werden gebaseerd op vergelijking van de peak-flow daling na inspanning met de uitgangs-peak-flow meting. Als de peak-flow-daling vergeleken werd met de peak-flow-waarde op het corresponderende tijdstip van een controledag, hadden 10 in plaats van 19 patiënten een LAR na inspanning. De 19 patiënten die een positieve LAR na inspanning hadden vergeleken met de uitgangs-peak-flow-waarden, werden onderworpen aan

een tweede en derde inspanningstest na "at random" voorbehandeling met óf een placebo óf 4 mg nedocromil natrium.

Het bleek dat nedocromil natrium een significant beschermend effect had, zowel op de EAR als op de LAR na inspanning. Alhoewel de wijze van werking van nedocromil natrium onduidelijk blijft, bleek uit deze studie dat patiënten met CARA die een EAR en/of LAR na inspanning hadden, baat kunnen hebben bij nedocromil natrium therapie.

Conclusies:

1. Bij patiënten met astma bronchiale en COPD bestaat een LAR na inspanning. De "beste" methode om een late reactie na inspanning te onderzoeken is als de maximale daling in peak-flow vergeleken wordt met de peak-flow-waarde op het corresponderende tijdstip van een controledag waarop geen inspanning plaatsvindt.

2. Een LAR na inspanning wordt "het best" beschreven door een ≥ 20% daling in peak-flow op drie aaneensluitende tijdstippen vergeleken met de uitgangs-peak-flow-waarden én vergeleken met de peak-flow-waarde op hetzelfde tijdstip van een controledag. Andere dalingen in peak-flow op de inspanningsdag worden pseudo LAR's genoemd.

3. De reproduceerbaarheid van de LAR na inspanning is 73 %.

4. Histaminedrempels verschillen niet significant bij patiënten met een LAR na inspanning gedurende het beloop van de late reactie. Ook de perifeer-inflammatoire cellen, gemeten in het bloed van patiënten, laten geen verschil zien bij patiënten met een LAR na inspanning. Dit geldt ook voor serum eosinofiel cationic protein en serum histamine.

5. Nedocromil natrium laat een significant beschermend effect zien op zowel de vroege- als late reactie na inspanning.

Woorden van dank

In de eerste plaats gaat mijn dank uit naar alle patienten die meegewerkt hebben aan de onderzoeken, die in dit proefschrift vermeld staan. Het nauwgezet bijhouden van peak flow lijsten na inspanning en op controle dagen was geen geringe opgave.

Prof.dr.CLA van Herwaarden hoogleraar longziekten Universitair longcentrum Dekkerswald; mijn promotor wil ik danken voor zijn inzet en kritische begeleiding vanaf het begin van de studie naar de late reaktie na inspanning. Beste Cees voor je deskundige begeleiding ben ik je zeer erkentelijk.

De heer J.Kruit, algemeen direkteur van het Nederlands Astmacentrum te Davos (NAD) wil ik danken voor zijn inzet bij het zoeken naar wegen om wetenschappelijk onderzoek te bedrijven in een categoraal ziekenhuis. De Stichting Nederlands Asthmacentrum Davos, de Vereniging Nederland-Davos wil ik o.a. danken voor de financiele steun die tot dit proefschrift hebben geleid.

Collegae specialisten wil ik danken voor de mogelijkheid om onderzoek te doen naast de patientenzorg binnen het NAD.

Artsen hebben in het kader van een onderzoeksstage een bijdrage geleverd aan het onderzoek, waarvan ik in het bijzonder wil noemen: NJ van den Berg, CLA Oosthoek, NPLG Verhoef en E Panis. Hiernaast hebben zij niet geaarzeld mee te denken over bewerking, statistische analyse etc.

Voor het begrip van al het personeel van het NAD om onderzoek te verrichten bij patienten die voor hun hardnekkige soms moeilijk behandelbare longaandoening van Nederland naar Davos werden verwezen ben ik zeer erkentelijk.

Deskundige begeleiding vanaf het eerste moment was aanwezig in de persoon van dr.PJ Sterk, longfysioloog van het Academisch Ziekenhuis Leiden. Later, na de oprichting van het Swiss Institute of Allergy and Asthma Research, Davos, Switzerland, werd dr.PLB Bruynzeel een van de mensen waar ik bij het schrijven van artikelen en het opzetten van klinisch wetenschappelijk onderzoek veel van geleerd heb. Beste Piet nog dank voor de vrijwel dagelijkse contacten die wij onderhielden. Dr.JGR de Monchy, allergoloog Academisch Ziekenhuis Gronongen heeft van afstand en soms van nabij zeer deskundige adviezen gegeven betreffende de late reaktie na inspanning hetgeen verwantschap vertoonde met zijn thesis de late reaktie na allergeen inhalatie.

Allen die maar enigszins te maken hebben gehad bij de tot stand koming van dit proefschrift zoals het medisch secretariaat van het NAD, het laboratorium etc. etc.wil ik dank zeggen voor hun medewerking, het zijn er te veel om op te noemen.

Mijn ouders wil ik dank zeggen voor de kans die zij me gaven te gaan studeren.

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CURRICULUM VITAE

De auteur van dit proefschrift werd op 4 oktober 1954 geboren te Deventer. Hij bezocht de Alexander Hegius Scholengemeenschap te Deventer. In 1972 behaalde hij het diploma HBS-B en startte hij met de studie Geneeskunde aan de Rijksuniversiteit Utrecht. In 1979 werd het arts-examen behaald. Hierna vervulde hij gedurende 16 maanden de militaire dienstplicht bij de Koninklijke Luchtmacht op de Vliegbasis Twente. In september 1980 begon hij de opleiding tot internist in het Diaconessenhuis te Arnhem (opleider dr.C.van Gastel). Na deze 2 jarige opleiding werd de vervolg opleiding gevolgd in het Academisch Ziekenhuis Utrecht (opleiders :prof.dr.A.Struyvenberg en prof.dr.J.van der Sluys Veer). Op 1 september 1985 vond inschrijving als internist in het specialistenregister plaats. Hierna was hij werkzaam op de afdeling klinische oncologie van de Dr.Daniel den Hoed Kliniek te Rotterdam (hoofd dr.G.Stoter). Sinds 1 mei 1986 is hij als staflid en wrnd hoofd medische dienst verbonden aan het Nederlands Astmacentrum in Davos, Zwitserland.

Stellingen behorende bij het proefschrift
DE LATE ASTMATISCHE REAKTIE NA INSPANNING.

B.Speelberg

1. De late astmatische reactie na inspanning is geen epifenomeen, doch een reaktie waarvan de ernst en de gevolgen voor de patient niet onderschat moeten worden.

   Dit proefschrift

2. De beste manier om het bestaan van een late astmatische reaktie na inspanning aan te tonen, is het beloop van de peak flow op een dag met inspanningsprovocatie te vergelijken met een controledag.

   Dit proefschrift

3. Nedocromil natrium is een effectief middel bij de behandeling van een late reaktie na inspanning.

4. Multidisciplinaire behandeling in het hooggebergte is een zinvolle aanvulling in de behandeling van CARA patienten, waarvan de behandeling in Nederland niet het gewenste effect heeft.

5. Rugby lokt bij een aantal mensen bekend met inspanningsastma geen aanval uit; blijkbaar veroorzaakt dus de aggressieve sport een extra catecholamine-uitscheiding die beschermend werkt.

   Anderson K, 1985

6. Astma-aanvallen geprovoceerd door sexuele activiteit ("sexercise induced asthma ") zijn niet zeldzaam en worden niet door de fysieke activiteit veroorzaakt, maar door angst, opwinding, emoties en hyperventilatie.

   Picado S, 1987

7. Een dalend haemoglobine gehalte in het bloed met fragmentocyten in de bloeduitstrijk bij patienten die met chemotherapie behandeld worden voor een gemetastaseerd carcinoom, moet de arts doen denken aan een micro-angiopathische haemolythische aneamie.

   Speelberg B, e.a. NTvG 1986; 130: 2186-88.

8. In abetalipoproteinemie is er een posttranslatie defect in de synthese van apoproteine B-100.

Dullaart RPF, Speelberg B, e.a. J Clin Invest 1986: 78: 1397-1404

9. Plasmacytomen kunnen bij een extramedullaire lokalisatie aanleiding geven tot obstruktie icterus.

Speelberg B, e.a.Neth J Med 1985; 28: 291-294

10. De stapeling van triacylglycerol in patienten met vetlever wordt niet veroorzaakt door een toegenomen aktiviteit van diacylglycerolacyltransferase, maar door een relatief tekort aan phospholipiden.

Speelberg B, e.a. Eur J Clin Invest 1985; 15: A8

11. Slapen is geen geringe kunst :men moet er een hele dag voor wakker blijven.

Friedrich Nietzsche

12. Een geleerde is een persoon die alles weet van wat anderen niet weten, maar niets weet van wat anderen weten.

Albert Einstein

13. De enige methode om gezond te blijven is te eten wat je niet wil, te drinken wat je niet lekker vindt en te doen waar je geen zin in hebt.

Mark Twain