EXERCISE-INDUCED BRONCHOCONSTRICTION

clinical studies in childhood asthma

INSPANNINGSGEÏNDUCEERDE BRONCHUSOBSTRUCTIE

klinische studies bij kinderen met astma

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Aan mijn moeder en vader†

aan Marcel, Thomas en Wouter

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chapter 1 chapter

General introduction

1.1 Asthma and childhood

At present, asthma is regarded as a chronic inflammatory disorder of the airways. In susceptible individuals, asthma causes symptoms, that are usually associated with variable, but often reversible airflow obstruction[1]. Asthma is the most common lung disease in childhood, with a symptom-based asthma prevalence of approximately 11% in The Netherlands[2]. Worldwide, asthma prevalence appears to be on the increase[3]. Although mortality rates are low, asthma causes significant morbidity and school absenteism in children[4].

The clinical expression of asthma varies from patient to patient and from time to time within each patient. Anamnestic features suggestive of a diagnosis of asthma are intermittent episodes of wheezing, chest tightness and shortness of breath, as well as recurrent or persistent cough. Symptoms may worsen at night or in the early morning, and are precipitated by viral infections, allergen exposure, exercise, chemical irritants, tobacco smoke and strong emotional expressions[5-7].

Diagnosis may be especially difficult in infancy. Wheezing in infancy as a reflection of early-onset asthma appears to be associated with increased sensitization to allergens and a deterioration of lung function in the first 6 years of life[8]. Risk factors for development of asthma in older children and adolescents also include atopic sensitization as well as the occurence of bronchial hyperresponsiveness (BHR)[9,10]. The BHR in children decreases with age, but the tendency to retain BHR is closely related to markers of atopy, such as serum IgE levels and positive skin prick tests[11]. These epidemiological data underline the close association between atopy, BHR and asthma. Supportive evidence for a genetic basis of this association has now been provided by the identification of a gene cluster on chromosome 5, linked to both elevated IgE levels and BHR[12].

1.2 Pathophysiology of asthma

Prominent infiltration of the airway wall with inflammatory cells, such as mast cells, eosinophils and lymphocytes, together with hyperplasia and hypertrophy of the muscular layer and extensive damage to the epithelium was identified in lung tissue of patients who had died from status asthmaticus[13]. *In vivo* fiberoptic bronchoscopy studies in adults asthmatics have subsequently shown that these inflammatory changes in the airways are also present in mild, stable asthmatics[14-16]. The chronicity of the inflammatory responses is reflected by the thickening of the lamina reticularis beneath the basement membrane[14]. Consequently, the consensus has emerged that airway inflammation is a consistent feature of asthma[1].

Mast cells are recognized as key cells of type I hypersensitivity reactions[17]. Experimental

allergen challenge leads to the production and release of mast cell derived mediators such as histamine, tryptase, prostanoids and leukotrienes, which are involved in the early asthmatic reaction[18]. In addition, mast cells are capable of generating an array of cytokines, among which IL-4 and IL-13, involved in switching the B-lymphocyte to IgE production, as well as IL-5, and granulocyte-macrophage colony stimulating factor (GM-CSF), known to promote eosinophil priming and activation[17]. Thus, mast cells may be important for initiating allergen-induced airway inflammation[19].

Activated T-lymphocytes seem to regulate the chronic inflammatory response in atopic asthma through the production of cytokines pertinent to allergy[20]. The T-helper cell population can be divided into two subsets: the Th1 cells, that predominantly produce IL-2 and interferon-gamma, and are associated with delayed hypersensitivity reactions. The second subset consists of the Th2 cells, and is predominant in allergic asthma[21,22]. Th2 cells synthesize various cytokines, including Il-3, IL-4, IL-5, and IL-10, and GMCSF. These cytokines enhance allergic responses by activating mast cells and eosinophils, as well as prolonging the survival of the latter granulocyte[23].

The eosinophil leucocyte appears to be the predominant effector cell in asthma, and is seen to infiltrate the full thickness of the airway wall[24,25]. Eosinophils are capable of producing a wide range of vaso- and bronchoactive mediators, including cysteinyl-leukotrienes, PAF, prostanoids and neuropeptides, as well as cytotoxic proteins, such as eosinophil cationic protein, eosinophil-protein X and eosinophil peroxidase[26]. These latter products may cause epithelial injury and denudation resulting in an increased permeability to irritant stimuli for sensory nerves, while repeated denudation-restitution processes may lead to thickening of the reticular basement membrane[27]. In asthmatic adults, an association between the inflammatory cellular infiltrate, especially eosinophils, and the degree of bronchial hyperresponsiveness has been observed[28,29]. Likewise, a correlation between airway responsiveness and cellular activation was documented in children with asthma[30].

1.3 Bronchial hyperresponsiveness

Bronchial hyperresponsiveness (BHR) refers to the exaggerated response to bronchoconstrictor stimuli of physical, chemical or pharmacological origin[31]. Bronchial responsiveness is expressed as the provocative dose or concentration of an agent which induces a predefined fall in air flow, commonly a 20% decrease in forced expiratory volume in 1 second (FEV_1)[32]. In a random population sample of children, bronchial responsiveness to histamine was shown to

have a continuous, unimodal, log-normal distribution, with asthmatic subjects at the more responsive end of the distribution[33]. The degree of BHR correlates with respiratory symptoms. However, there is a wide overlap between symptomatic and asymptomatic individuals, reducing the predictive value of a positive test for the presence of asthma in the general population[34,35].

The various bronchoconstrictor stimuli used to assess the degree of BHR act either directly through stimulations of the bronchial smooth muscle in the airway wall, or indirectly through infiltrative or resident pulmonary cells or neural pathways[36]. For example, methacholine has a direct bronchoconstricting effect on the airway smooth muscle[32], while cysteinyl-leukotrienes mediate their bronchoconstrictor effects via stimulation of various cell types, and possibly through the secondary release of neuropeptides[37]. Therefore, it can be postulated that differences in responsiveness to directly and indirectly acting bronchoconstricor stimuli may reflect differences in the underlying pathophysiology of asthma[29,38] and may be helpful in the evaluation of drug treatment for asthma.

Exercise, especially in childhood, is a common trigger of acute, usually short-lived asthma attacks, referred to as exercise-induced bronchoconstriction (EIB)[39]. The current knowledge on its mechanism suggests that inflammatory mediators are released in response to airway cooling and/or drying due to the hyperventilation of exercise[40]. The main goal of this thesis is to broaden our understanding of the pathophysiologic mechanisms underlying the manifestations of EIB and its significance as an expression of BHR. In addition, treatment strategies for the prevention of EIB in childhood asthma are evaluated.

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chapter 2 chapter

Exercise-induced bronchoconstriction and childhood asthma

2.1 Introduction

Currently, asthma is viewed as a chronic inflammatory disease of the airways, characterized by variable airways obstruction over time[1]. Fluctuations in airway patency can occur either spontaneously or in response to bronchoconstrictor stimuli[2]. The degree of response to such stimuli is a measure for the severity of the underlying bronchial hyperresponsiveness, currently thought to be a hallmark of asthma[3]. Exercise is a very common, physiologic trigger of acute, usually short-lived asthma attacks in daily life. And although three hunderd years have passsed since Sir Floyer in 1698, observed that "All violent exercise makes the asthmatic to breathe short"[4], it has only been for the past thirty years that substantial progress has been made regarding the understanding of asthma and its relationship with exercise[5].

In this chapter, the current knowledge on EIB with respect to clinical characteristics in asthma, bronchial hyperresponsiveness, pathophysiologic mechanisms, diagnosis and drug treatment will be reviewed, with special emphasis on its occurence in childhood.

2.2 Clinical characteristics

2.2.1 Symptomatology

In the daily life of many asthmatic children, exercise is a common cause of short-lasting asthma-attacks, usually referred to as "exercise-induced asthma" (EIA) or "exercise-induced bronchoconstriction" (EIB)[5,6]. It refers to the airway narrowing that occurs minutes after the onset of vigorous exercise. A history of cough, wheezing, or shortness of breath during or after moderately severe exercise, is suggestive of EIB. In addition, chest pain in otherwise healthy children is often overlooked as its symptom[7]. Likewise, endurance problems with exercise are more readily but wrongly associated with poor physical fitness than with EIB[8]. Finally, it is not unusual that EIB goes unnoticed by the patient itself[9].

In the typical response, the bronchoconstriction after exercise reaches its maximum within the first 10 minutes after exercise, and is most marked at 5 minutes post-exercise[6,10,11]. Generally, the recovery of EIB occurs spontaneously within 30 minutes, but may occasionally last more than 60 minutes in individual asthmatic children[10,12]. It has been suggested in the earlier studies on EIB that children reach their maximum level of airway obstruction sooner, and subsequently recover more quickly to baseline levels than adults[6].

2.2.2 Exercise refractoriness

When exercise is repeated at intervals of two hours or less, there is a diminishing bronchoconstrictor response to successive exercise, known as refractoriness[13]. The time period during

which this is present, is called the refractory period. Allthough the refractory period can extend up to 4 hours after exercise, it is strongest during the first hour post-exercise[13,14,15]. Refractoriness to subsequent exercise can be induced in many asthmatic children suffering from EIB, allthough in some it will not occur[15,16]. The extent of refractoriness is variable, and not dependent on the degree of bronchoconstriction in the first exercise[17]. In fact, refractoriness to repeated exercise can occur without prior bronchoconstriction in the initial exercise[16,18], suggesting that exercise itself is an important factor for its occurence.

2.2.3 Late asthmatic response

The potential development of a late asthmatic response (LAR) to exercise in asthmatic children and adults is a subject of considerable controversy. Several studies have been published describing the occurence of a LAR three or four, or up to ten hours after exercise[10,19-23]. In these studies, a late asthmatic response was prevalent in 10% to 89% of the population studied[10,19-23]. However, other investigators have not succeeded in documenting such a late response post-exercise[24-27]. When evaluating the results of these studies, the design of many can be critisized for absence of appropriate control days[28], on which to study the normal variation in lung function. Contributing to the confusion concerning the existence of a LAR, is the lack of agreement on its definition[19-27]. Thus far, the controversy around the LAR has not been solved[29].

2.3 Exercise-induced bronchoconstriction and bronchial hyperresponsiveness

2.3.1 Definition and epidemiology

The bronchial response to exercise in the general population is represented by a unimodal normal distribution, with the asthmatic subjects at the more responsive end of the distribution[30-33]. By definition, the response to exercise testing is considered abnormal, when the decline in lung function from the pre-exercise value, expressed as the percentage fall in forced expiratory volume in 1 second (FEV_1) or peak expiratory flow rate (PEFR) (%fall), exceeds twice the standard deviation of the mean %fall obtained in normal children[6,11,34]. Applying this definiton, a %fall in FEV_1 of 10% or more is indicative of EIB (figure 1). The response to PEFR is more variable, the upper limit of normal varying from 10%[6] to 12.5% [35] to 17.5%[11] fall. Hence, in epidemiological studies, a fall in PEFR exceeding 15% is considered to be abnormal[36].

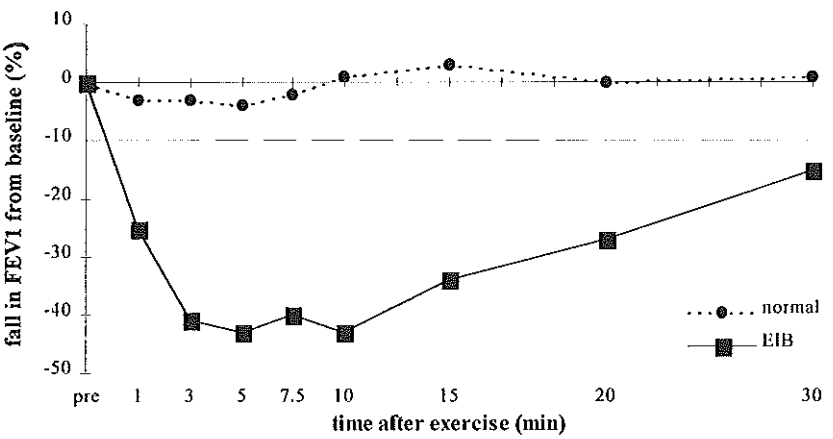

Figure 1: example of the bronchoconstrictor response after exercise (pre = baseline pre-exercise)

The prevalence of EIB in a random sample of children and adults has been reported to vary between 4% to 16%[31,32,35,37-39], with the prevalence of EIB in the general population being lowest in Africa[35,37]. As was previously observed for BHR to pharmacological stimuli[30,40], an age-dependent decrease in BHR to exercise has been described in non-asthmatic subjects[30,31].

A positive response to exercise testing is significantly associated with history of respiratory symptoms[41], the frequency of wheeze attacks [32], atopy [42], as well as to symptomatic asthma [43]. However, EIB is not limited to children diagnosed with asthma, an abnormal response also occurring more frequently in atopic non-asthmatics (approximately 14%[44] to 23%[42]), as well as in non-asthmatic relatives of asthmatic children[45]. In addition, an increased occurence of an excessive bronchoconstrictor response to exercise can be observed competitive ice rink skaters[46], possibly as a consequence of performing exercise in extreme conditions of temperature and humidity[47].

Allthough in one study the occurence of EIB in asymptomatic children and adolescents was shown to be a risk factor for the subsequent development of asthma[33], this could not be confirmed in another study[43]. Hence, despite the strong association between EIB, atopy and asthma, EIB is not equivalent with asthma. Therefore, exercise-induced bronchoconstriction is a more appropriate term in describing the phenomenon than the often used term exercise-induced asthma.

2.3.2 Relationship of EIB with bronchial hyperresponsiveness in clinical asthma

Several studies have looked at the prevalence of EIB in children diagnosed with asthma. When defining EIB as at least 10% fall in FEV_1 after a standardized exercise test, the prevalence of documented EIB averages around 74%[34,48,49] to nearly 90%[11] in untreated asthmatic children. The severity of EIB is generally related to the severity of clinical asthma[50]. Considerable overlap has been shown to exist between the response to exercise and other non-specific bronchoconstrictor stimuli, such as histamine[32,48,51,52] and methacholine[53,54]. The relationship between the response to exercise challenge and the response to histamine challenge is generally good, with a correlation coefficient of approx. 0.78 in the asthmatic population[52]. The relationship with methacholine challenge seems to be less strong[53,54]. This implicates that some children are more responsive to histamine than to exercise[52], or vice versa[32]. In addition, it was shown that exercise is better than methacholine in discriminating asthma from chronic lung diseases in children[49]. These data support the view that although exercise (a physiologic stimulus), and histamine or methacholine (a pharmacological stimulus), both reflect the severity of the underlying bronchial hyperresponsiveness in asthma, they measure different components of the airways dysfunction[55].

The precise mechanism by which airway inflammation results in bronchial hyperresponsiveness is not known, yet the two are closely related[1,56]. Therefore, factors inducing (transient) changes in airway inflammation most likely will influence the degree of EIB. It has been shown that allergen exposure aggrevates the severity of EIB, in laboratory challenges[57,58] as well as in natural circumstances[59], while allergen avoidance leads to a decrease in the degree of EIB in asthmatic children[60]. Viral infections are known to exacerbate asthma in children[61], suggesting a worsening of EIB during an upper respiratory tract infection. Thus far, studies investigating the effect of experimentally administered viral infections on EIB in asthmatic adults, have been unable to confirm this[62]. Lastly, regular treatment with inhaled steroids, drugs that exert their inhibiting effects on many aspects of airway inflammation[63], attenuate the bronchoconstrictor response in asthmatic children[64] and adults[65].

2.4 Pathophysiology of EIB

2.4.1 The initiating stimulus

One of the earliest observations on the pathophysiology of EIB was made when it was shown that the bronchoconstriction post-exercise was dependent upon the type of exercise[66], its intensity and duration[14]. As it is known that one of the physiological effects of exercise is, to

increase the minute ventilation[67], it was postulated and subsequently shown that the severity of EIB was related to the level of ventilation reached during exercise[68,69]. In addition, it was observed that the bronchoconstrictor response to exercise could be modified by varying the temperature and the humidity of the inspired air[70], the largest bronchoconstrictor response induced while breathing dry air during exercise, whereas warm, fully saturated air completely inhibited EIB[71].

During normal breathing, heat and water are transferred from the mucosa of the upper airways, including the nose, to the incoming air to warm and humidify inspired air to alveolar conditions[72]. During the high ventilatory drive of exercise, this mechanism is not sufficient, and consequently, the lower respiratory mucosa compensates to complete the conditioning process. This results in both evaporative and conductive cooling of the airways. Based on measurements of ventilation, temperature, and humidity of the inspired air, the total heat flux from the airways could be quantified during an exercise task[70], the ensuing bronchoconstriction proportional to the respiratory heat exchanged. Similar observations were made with hyperventilation-induced bronchoconstriction[73], leading to the conclusion that the severity of EIB was determined by the hyperpnoea of exercise, as well as the temperature and the humidity of the inspired air during exercise[73]. Thus it was proposed that the prime stimulus for EIB was airway cooling secondary to respiratory heat loss during hyperpnoea of exercise, and not exercise itself[74]. Airway cooling was later confirmed by direct temperature recordings from the pharynx up to the subsegmental bronchi[75].

The total heat loss during exercise however, is caused by a combination of heat loss due to differences in the in- and expiratory air temperature as well as heat loss due to the evaporation of water. And since some of the observations on EIB were difficult to explain by heat loss alone, for example the breathing of hot dry air also inducing bronchoconstriction[71], it was postulated that respiratory water loss during hyperpnoea of exercise was more important than heat loss in EIB. This was supported by studies showing that the severity of EIB was not altered with increasing temperature when the water content was kept constant[76]. Further evidence was put forward when experiments, using gasses with similar water contents but different volume heat capacities, showed that the bronchoconstriction post-exercise correlated closely with evaporative water loss, but poorly with the temperature gradient[77]. Lastly, significant EIB was found to be provoked, without significant heat loss or airway cooling, but mainly determined by amount of water loss[78]. The mechanisms by which water loss from the bronchial mucosa induces bronchoconstriction was not known, but increasing evidence suggested that it might be due to hypertonicity of the airway lining fluid[79].

The debate on the initiating stimulus remained inconclusive, and yet an alternative hypothesis on the reaction sequence was forwarded. Since airway cooling occurred both in normal and asthmatic subjects, the effects of breathing air at different temperatures immediately after exercise were studied, to investigate whether post-exertional thermal events might explain the difference in response between asthmatics and normals[80]. Immediately post-exercise, the airways of asthmatic adults rewarm twice as rapidly as those of normal subjects[81]. Attenuation of the bronchoconstrictor response occured when rewarming was slowed down by breathing cold air[80]. It was then postulated that the thermal gradient during and after exercise and the rate of airway rewarming provided the initiating stimulus for EIB[82].

To date, it is agreed upon that the airway microvasulature has the potential for contributing to the pathophysiology of EIB. However, discussion remains to whether the increased blood flow during airway rewarming may be the consequence of mediator release in response to osmolarity changes rather than the initiating stimulus itself[83]. Evidence in favor of this view comes from studies showing that EIB can occur in the absence of a thermal gradient[70]. EIB can already occur during exercise, that is before rewarming occurs[84]. Lastly, breathing of dry air increases airway blood flow in animals[85]. Although one should be cautious when extrapolating data from animal studies to man, if asthmatic subjects would respond to breathing of dry air in similar fashion as other mammals, reactive hyperaemia is unlikely to occur after exercise[85]. In contrast, an increased blood flow following exercise in asthmatics could well be explained as a consequence of mediator release due to changes in osmolarity induced by the hyperpnoea of exercise, with mediators either directly relaxing precapillary sphincters of blood vessels, or indirectly causing leakage of the microvasculature through one or more of the sensory neuropeptides[83]. Consistent with the hypertonicity-mediator-release-hypothesis is that hyperosmolar stimuli induce pulmonary mast cell degranulation *in vitro*[86], and that hypertonic solutions induce bronchoconstriction in asthmatic subjects[87,88]. However, conclusive evidence of the hypertonicity theory based on direct measurement of osmolarity during hyperpnoea of exercise is still lacking.

2.4.2 Mediator release in EIB

Many investigators have tried to elucidate the relative contribution of different mediators to the severity of EIB, either by direct measurements of their concentrations in biological fluids, or indirectly by determining the effect of specific mediator antagonists or synthesis inhibitors.

Histamine. Most histamine is stored preformed in cytoplasmic granules of mast cells and basophils[89]. Its bronchoconstrictor effect is mediated through H_1-receptors [90]. Exercise-induced release of histamine has been observed in asthmatic children[91], however, measurements were not compared to a control group of normal children. In adults, histamine in serum post-exercise was increased for both normal and asthmatic subjects, with the difference between groups not significant[92]. Direct measurements of the level of histamine pre- and post exercise in broncho-alveolar lavage fluid did not succeed in implicating histamine in the reaction sequence[93,94]. In contrast, pretreatment with the highly potent and selective H_1-receptor antagonist, terfenadine, almost completely blocked the response to exercise in asthmatic children[95], while in asthmatic adults[96,97] the largest inhibition occurred during the first 5 minutes of the bronchoconstrictor response.

Cysteinyl leukotrienes (LTC_4, LTD_4, and LTE_4). Cysteinyl leukotrienes (Cys-LT) are metabolites of arachidonic acid, and represent a heterogeneous group of biologically active mediators[98]. They are preferentially generated by mast cells, eosinophils and basophils[89]. In addition to being potent spasmogens of airway smooth muscle, they also induce increased micro vascular permeability and mucus hypersecretion[99]. In asthmatic children, but not adults[100], a significant rise in urinary excretion of Cys-LT after exercise was found compared to normal children[101], it being most evident in those children with the most severe EIB[102]. In adults, direct measurements of increased levels of Cys-LT in BALfluid post-exercise could not be observed[93]. Yet, following pre-treatment with potent leukotriene receptor antagonists[103-106] and synthesis inhibitors[107], involvement of Cys-LT in the reaction sequence was demonstrated. Studies investigating the involvement of Cys-LT in EIB in pediatric asthma are currently under way, with preliminary reports confirming their role in EIB in childhood asthma [108,109].

Prostaglandins. Prostaglandins are metabolites of arachidonic acid via the cyclo-oxygenase pathway[110]. Prostaglandin D_2, its principal metabolite $PGF_2\alpha$, and thromboxane (TX) A_2 are potent bronchoconstrictors, while PGE_2 is a major bronchodilator prostanoid, able to antagonize $PGF_2\alpha$-induced bronchoconstriction. Again, direct measurements of prostaglandins before and after exercise did not show differences in the level of prostanoids in BALfluid in adult asthmatics[93]. Neither did pretreatment with a potent thromboxane receptor antagonist attenuate EIB[111]. Evidence for involvement of prostanoids comes from studies showing inhibition of EIB following pretreatment with the cyclo-oxygenase inhibitor flurbiprofen[97]. Indomethacin, another less potent cyclo-oxygenase inhibitor, did not inhibit EIB in asthmatic adults[112] and adolescents[18] when given orally. However, pre-treatment with inhaled indomethacin did show attenuation of the bronchoconstrictor response in asthmatic children[113]. Since exercise refracto-

riness was significantly reduced after treatment with indomethacin, inhibitory or bronchodilating prostaglandins, such as PGE_2, and PGI_2 , are currently thought to be involved in its mechanism[18,114].

2.4.3 Neural mechanisms in EIB

The role of an increase in vagal discharge has been investigated by determining the effect of anticholinergic drugs such as atropine or ipratropiumbromide in protecting against EIB. Ipratropiumbromide attenuates the bronchoconstrictor response to exercise in asthmatic children, however, its protective effect is weak and inconsistent[115,116]. This suggests that the importance of the vagal reflex in EIB may vary in different patients, and also in time.

Recently, it has been suggested from studies using animals models of EIB[85], that neurogenic mechanisms are involved in its mechanism. These neural mechanisms particularly refer to nonadrenerge, noncholinerge pathways, which can either be excitatory, or inhibitory[117]. There is growing evidence that after hyperventilation with dry air, the release of tachykinins may contribute to the subsequent airway obstruction in guinea pigs[118,119], possibly through the release of cysteinyl leukotrienes[120]. However, it has been suggested that in humans, neuropeptides are released secondary to leukotrienes[121]. Thus far, in mild to moderate asthmatic adults, pre-treatment with thiorphan, an inhibitor of the neuropeptide degrading enzym neutral endopeptidase (NEP), significantly attenuated the bronchoconstrictor response to exercise, specifically during the recovery period[122]. However, NEP-inhibition with thiorphan could equally well have resulted in a prolonged action of the bronchorelaxing peptide atrial natriuretic peptide (ANP), known to protect against histamine-induced bronchoconstriction[123]. Further studies are needed to elucidate the role of neuropeptide release in asthma and EIB.

2.4.4 Cellular involvement

The mast cell has always been regarded as an important effector cell for EIB[124]. Because a difference in mast cell mediators post-exercise as compared to pre-exercise values could not be measured in bronchoalveolar lavage fluid, it was thought that EIB was not associated with mast cell activation[93,94]. However, after using mediator antagonists or synthesis inhibitor to unequivocally implicate histamine[95-97], cysteinyl leukotrienes[103-109] and prostaglandins[18,97,113] in EIB, the mast cell, being an important source for these mediators[110], is currently thought to be an important effector cell[3]. Further supportive evidence for its role in EIB is forwarded by the inhibitory effect of heparin on EIB[125,126] most likely through an inhibitory modulation of mast

cell activation[127]. Secondly, a greater percentage of degranulated mast cells in bronchial biopsies is observed after exercise- as compared to methacholine-induced bronchoconstriction[128].

On the other hand, eosinophils are nowadays put at the forefront of the effector leucocytes in chronic asthma[129,130]. Numerous studies have shown a relationship between bronchial eosinophil activation, airway inflammation and bronchial hyperresponsiveness in adult[131,132] and pediatric asthma[133,134]. Indeed, eosinophils are potent generators of leukotrienes[98] . In adults asthmatics, a good relationship was found between the level of serum cosinophil cationic protein (sECP) and the severity of EIB[135]. However, to date, no studies adressing the relationship between sECP and EIB have been adressed in childhood asthma.

Other pulmonary cells. It is known that the number of basophils increases after exercise, and thereby may account for some of the histamine released post-exercise[92,136]. In addition, bronchial epithelial cells may play an important role in asthma, not only through their role of airway protectors against noxious agents, but also as cells that can synthesize and release a wide array of mediators, including prostanoids and leukotrienes, either spontaneously or after stimulation[137]. Given their close contact with the airway microvasculature, they may potentially be influential in EIB[138].

Conclusion. Studies aimed at directly measuring mediators in biological fluids, have not unequivocally implicated their release in EIB. This might be related to methodological issues, such as the timing of measurements after challenge[93,94], (in)sensitivity of assay techniques[136] , or the relatively small contribution of locally released mediators to the total body content[100]. Notwithstanding, the available evidence provided from studies using mediator antagonists, supports a strong case for mediator release as the mechanism of EIB[139].

2.5 Diagnosis of EIB

EIB should be suspected in any child who presents with wheezing, cough, chest tightness or dyspnoea during or shortly after exercise[6]. Epidemiological studies have shown that these symptoms, as well as sleep disturbance due to wheeze, are risk factors for the prevalence of bronchial responsiveness to exercise[39,41]. In addition, chest pain[7] or exercise intolerance[8] in otherwise healthy children or adolescents can be symptoms of EIB. Due to the inherent variability of asthma with intermittent exacerbations and remissions, the percentage of patients reporting exercise-induced symptoms is expected to be higher than the percentage of patients in which EIB can be documented at a specific time point using exercise testing[39,41,140,141].

When evaluating EIB, changes in spirometry after exercise are used to assess the degree of bronchusobstruction[14,36]. As mentioned previously, various factors, such as humidity and temperature of the air inspired during exercising[142], exercise intensity, duration of the test, and time since last exercise period will influence the bronchoconstrictor response to exercise[14]. Therefore, it is a prerequisite that the method of exercise testing is standardized, leading to adequately reproducible results[36]. A duration of the test of 6 to 8 minutes at a workload at 60% to 85% of the predicted maximum oxygen consumption[14] or at 90% of predicted maximum heart rate[143] has been recommended earlier. As the level of hyperventilation reached during exercise is an important determinant of the bronchoconstrictor response, it is nowadays recommended to measure ventilation, and to select a subject's workload between 40% to 60% of the predicted maximum voluntary ventilation during the last 4 minutes of the test[36]. In addition, it is strongly recommended that air inspired during exercise has a water content less than 10 mg per litre (equivalent to relative humidity less than 50% between 20-25°C). The nose must be clipped during running to ensure mouth breathing. Repeated exercise tests should be separated by at least 2, but preferably 4 hours to avoid influence of the refractory period[13].

Lung function measurements (FEV_1 , PEFR) are performed before exercise, and should be at least within 80% of the subjects' usual value, and preferably better than 75% of their predicted value. Post-exercise, measurements of lung function are repeated at regular intervals up to 30 minutes after exercise, or until lung function has recovered to within 10% of pre-exercise value. The severity of EIB is usually reflected in the %fall index (%fall), which is calculated by subtracting the lowest value of FEV_1 or PEFR recorded after exercise and expressing it as a percentage of the value recorded immediately before exercise. In formula:
%fall = [(FEV_1 pre-exercise - lowest FEV_1 post-exercise) / FEV_1 pre-exercise] * 100%.
In addition, calculating the area under the time-response curve post-exercise may provide valuable information on the recovery phase of EIB[97,103,122].

Reproducibility of the response to exercise testing has been shown to vary with the time interval between tests, the coefficient of variation (CV = [standard deviation/mean] * 100%) varying from 12 to 35% with repeated challenges at different time intervals[14,143,144]. However, the CV will vary with the mean value of EIB in a study group, making comparisons between studies difficult to interpret[143]. Nowadays, it is recommended to use the intra-class correlation coefficient as an index of repeatability[145]. Despite the inherent variability in EIB[146] , exercise testing has proven very useful for evaluating drug treatment in asthma.

2.6 Treatment of EIB

One of the aims of asthma treatment, as summarized in the latest guidelines, is the participation of children in play and sports without limitations[147,148]. This can be achieved through non-pharmacological interventions, as well as the prescibing of drug therapy shown to provide adequate protection against EIB[149].

2.6.1 Non-pharmacological intervention

Since warm, humified air blocks EIB, exercising in a warm, humidified environment might be beneficial in inhibiting its occurence[17]. Local hyperthermia by breating hot and fully humidified air 30 minutes prior to exercise has been shown to attenuate EIB[150]. Breathing through the nose, or wearing a scarf over the nose and mouth in cold weather are effective ways of increasing the temperature and humidity of the inspired air.

Effectively treating nasal blockage is capable of attenuating EIB[151]. This is in accordance with earlier observations on the beneficial effect of nose breathing as compared to oral breating in reducing EIB[152]. However, it is not easy for many subjects to keep up nose breathing while performing strenuous exercise.

In some asthmatic patients, inducing refractoriness to submaximal exercise by a preceding bout of repetitive exercise of short duration might be helpful to prevent exercise-induced complaints[153]. The ability to induce refractoriness to subsequent exercise is maintained despite an initial exercise being performed in warm, humidified air[18]. It has been suggested that patients not protected by cromolyn sodium will not be rendered refractory[154]. Unfortunately, no formal studies have been performed on what clinical or spirometric parameters accurately predict which patients are likely to benefit from refractoriness. Empirical induction for all patients with EIB is therefore recommended.

The role of physical training in the prevention of EIB in asthmatic children has been subject to investigation. Normal cardiovascular fitness does not prevent the occurence of EIB[155]. Some studies have described amelioration of EIB after a training programme[156,157], whilst others could not observe such an effect[158,159]. These inconsistencies may be related to methodological issues. Physical training improves cardiovascular fitness as assessed by the maximal oxygen uptake, and the workload performed[160]. Thus, when assessing the effect of training on the severity of EIB, the workload during the exercise test post-training should not be similar to the workload during the test before training, lest the apparent beneficial effect be the consequence of a significantly lower ventilation rate post-training. However, regardless of the effects of training on EIB, it is clear that psychological benefits are gained by increasing physical fitness,

since children become more self-confident and may better participate in group activities[159].

Lastly, allergen avoidance has been shown to improve the severity of EIB[60]. Therefore, interventions aimed at reducing or removing relevant allergen exposure, such as bed covers or removal of pets, should be strongly supported when treating EIB in paediatric asthma[147].

2.6.2 Pharmacological intervention

The actions of drugs known to protect against the occurence of EIB can be divided into two categories: quick-relief actions, working acutely to prevent or reverse exercise-induced airway narrowing, and long-term preventive actions, preventing symptoms and attacks by treating the underlying bronchial hyperresponsiveness[148]. The institution of prophylactic treatment is warranted if symptoms occur more than once a week, but less than once a day[147,148].

In an earlier study , it was proposed to assess the protective effect of a drug against EIB by either of three methods[149]:

1. By determining the statistical significance of the difference in protective effect between an active drug and its placebo using appropriate statistical tests.
2. By determining the percentage of subjects who experience a fall in lung function post-exercise within the normal range for either placebo or active drug (= complete protection).
3. By determining the protection index of a drug, i.e. the ability to reduce the severity of EIB as compared to placebo (= clinical protection). In formula:
   $\%protection = ([EIB_{placebo} - EIB_{active\ drug}] / EIB_{placebo}) * 100\%$.

As a test for statistical significance does not provide an estimate of the effect size that is clinically relevant[161], the protection index has now become most accepted for assessing the preventive effects of drugs. Based on previous studies on the repreducibility of EIB in asthmatic subjects[14,143,144], a protection index of at least 50% on the maximal %fall was found to be clinically relevant[15]. Although the %fall does not take into account the effect of a drug on the recovery phase of EIB, the protection index for assessing drug effects on %fall will be used in this thesis to facilitate comparison between studies.

Beta-2-agonists. Used as quick-relief medications, inhaled short-acting β_2-agonists are highly effective in preventing execise-induced bronchoconstriction when given shortly before exercise[162-166]. The protection index (PI) of a dose of 200 mcg, usually prescribed in these studies, varied from 50% to 80%. Protection may be increased using higher doses, as dose-response effects for protection against EIB have been shown in adults[167]. The duration of

protection with short-acting β_2-agonists is usually less than two hours[163,164,168]. In contrast, single doses of long-acting β_2-agonists offer prolonged protection, varying from 8 [164,169] up to 12 hours after dosing[168,170,171]. The protection index at these time points ranges from 30% up to 80%.

However, chronic dosing with both short- and long-acting β_2-agonists in adults asthmatics resulted in a decreased protective effect against EIB[172,173]. Although no such studies investigating the effect of chronic dosing on the protection against EIB, have been performed in asthmatic children, a decreased protection against methacholine-induced bronchoconstriction with regular salmeterol has been described in this age group[174]. Therefore, one should be cautious when prescribing regular mono-therapy with β_2-agonists in children as treatment for EIB.

Anticholinergics. These drugs are mainly used for their quick-relief actions. The few studies that have been performed on the effectiveness of anticholinergic drugs in preventing EIB in children, have shown only weak protective effects[115,116,175-177]. The protection index on the maximal %fall varies from 25% to 50% at the most. The protection is not dependent upon the dose, higher doses being no more protective than lower doses[116]. Notwithstanding these results for asthmatic children in general, individual subjects have been shown to derive substantial benefit from inhalation of ipratropiumbromide before exercising[175], underlining the heterogeneity of EIB in childhood[115].

Cromolyn sodium. Over the years, cromolyn has been extensively studied with respect to its protective effect on EIB in paediatric and adolescent asthma[154,162,163,165,176-183]. The protective effect of a single dose of cromolyn sodium in these studies, given less than 20 minutes before exercise, varies from 26% [163] to nearly 80% [183]. Some of this variability in the inhibiting effect on EIB is explained by the dose used, with the higher dose range (20 mg or above) giving at least 50% protection[165,177,182,183], while the protection of a lower dose (less than 20 mg) usually does not exceed 50%[163,180,181]. In general, the duration of protection of a single dose lasts less than 2 hours[163,165,181].

Surprisingly, while many studies have looked at the quick relief actions of cromolyn sodium, only few studies have investigated the long term preventive properties of cromolyn sodium on EIB in asthma[65,184]. Regular use did not reduce the bronchial responsiveness to exercise in children[184] or adults[65].

Corticosteroids. Inhaled corticosteroids are currently the most effective long-term preventive medications[148], most likely through their inhibitory effects on many aspects of the airway inflammation[63]. Single doses of inhaled corticosteroids are not helpful in preventing EIB[185] And although the first reports of the protective effects on EIB during short term treatment were

not very encouraging[186], later studies documented reductions in EIB of at least 50% within 3 to 8 weeks after starting drug therapy[64,187,188]. In moderate to severe asthmatic children, a dose response effect for the degree of protection was observed[188]. In part of the asthmatic population, EIB can still occur despite the use of maintenance treatment with EIB[64]. To date, it is not known whether increasing the dose of inhaled steroids might be beneficial in these patiens.

Anti-leukotriene therapy. Cysteinyl leukotriene receptor antagonists or synthesis inhibitors are a new class of drugs, targeted at inhibiting specific mediators in asthma[98,99]. Although they have shown promising results in pre-clinical studies, their place in anti-asthma therapy is not yet clear[189,190]. Single doses of Cys-LT_1 receptor antagonists as well as synthesis inhibitors have shown to provide good protection against EIB in adult asthmatics[103-107] as soon as 20 minutes after dosing[103]. The protection index on the maximal %fall in these studies varied from 40% to 63%[103,104,106,107], with the protection afforded up to 8 hours after dosing[106] . Time to recovery from EIB was significantly reduced, the protection index for the area under the time-response curve ranging from 60%[104] to 86% [107] . Preliminary reports on pre-treatment with single doses of anti-leukotriene therapy against EIB in paediatric asthma seem to suggest similar efficay[108,109], the protection extending up to 24 hours after dosing[109].

To date, one study has investigated the long-term preventive effects on EIB after chronic dosing during one week. It was found that the degree of protection was sustained for the higher doses, but not the low dose[107]. However, further studies are needed to confirm or refute these findings. Likewise, the role of antileukotriene therapy as regular monotherapy or as adjuvans to maintenance treatment with inhaled steroids needs to be investigated.

2.7 Conclusions

Exercise-induced bronchoconstriction in childhood asthma, as assessed by > 10% fall in FEV1 from baseline after standardized exercise testing, is an exaggeration of the bronchial response to exercise in normal children. EIB is related to the clinical severity of asthma, as measured by the severity of the underlying bronchial hyperresponsiveness. The postulated pathophysiology of EIB fits in well with the concept of asthma being an inflammatory disorder of the airways. The cardinal issue at hand is, whether EIB is merely a reflection of that airway inflammation, or whether exercise itself is capable of maintaining or contributing to the underlying airway disease in asthma. Obviously, the answer to that question will determine the significance of EIB in the management of childhood asthma.

2.8 References

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chapter 3 chapter

Aims of the studies

In this thesis, we aimed to clarify some of the questions pertaining to the symptom of exercise-induced bronchoconstriction in childhood asthma. The primary objective of the studies was to gain more insight in the pathofysiologic mechanisms underlying EIB in childhood asthma. The second, equally important objective was to evaluate treatment strategies in the prevention of EIB. To that end, we raised the following questions:

- ✶ Is the bronchoconstrictor response to exercise testing for diagnosis of EIB using a standardized protocol adequately reproducible to allow the set-up of research studies in a limited number of subjects with sufficient power? Hence, we determined the index of repeatability of two repeated exercise challenges on two separate days, using a standardized treadmill exercise protocol, and applied the data to construct power curves that allowed estimations on sample size requirements for studies of EIB in children (*Chapter 4*).
- ★ Is the clinical expression of EIB the same throughout the years of childhood? Based partly on clinical observation, and partly on comparison with literature in adults, we hypothesized that the recovery from exercise-induced bronchoconstriction is prolonged with increasing age. This would reflect the underlying mechanism to change with rising age. To verify this hypothesis, we measured the rate of recovery of EIB in two different age groups of children, and compared this to their rate of recovery from histamine-induced bronchoconstriction (*Chapter 5*).
- ✶ Does exercise challenge result in a late asthmatic reaction (LAR) in all or part of the asthmatic children with EIB? The occurrence of a LAR could have important consequences for understanding the pathophysiologic mechanisms of EIB, as the late asthmatic reaction after allergen challenge is shown to be associated with influx of inflammatory cells and the development of bronchial hyperresponsiveness. To answer this question, we repeatedly measured lung function up to eight hours after exercise challenge, and compared this to lung function measurements on a control day without exercise, as well as to lung function measured on a control day after histamine challenge inducing a matched level of bronchoconstriction to that observed after exercise (*Chapter 6*).
- ★ Does seasonal allergen exposure result in increased bronchial responsiveness to exercise and to methacholine, and are these effects mediated through changes in the eosinophil-induced arway inflammation? Secondly, are inhaled steroids beneficial in modifying these effects? We postulated that the effects of the pollen season on airway inflammation and bronchial responsiveness would be dependent upon the cumulative pollen counts, as well as the

patients' sensitization to grass pollen. We assumed the serum level of eosinophil cationic protein (sECP) to reflect the allergen-induced eosinophilic inflammation. To test this hypothesis, we concomitantly measured bronchial responsiveness to exercise and methacholine, and sECP before and during the grass pollen season in non-steroid and steroid treated grass pollen allergic asthmatic children and their controls, and related these to the severity of the allergen exposure (*Chapter 7*).

* Is the inhibiting effect of inhaled steroids against exercise-induced bronchoconstriction time- and dose-dependent during long-term treatment? Does a similar time- and dose-dependency exist for the improvement in methacholine-induced bronchoconstriction during treatment with inhaled steroids? We postulated that the protection afforded by inhaled steroids against directly (methacholine) and indirectly (exercise) acting bronchoconstrictor stimuli would be mediated through different mechanisms. Therefore, we repeatedly measured the severity of EIB, and bronchial responsiveness to methacholine during 24 weeks of treatment using two dose levels of fluticasone propionate in a placebo-controlled, parallel group study (*Chapter 8*).
* Do leukotrienes play a role in the early asthmatic reaction of EIB in childhood asthma? Cysteinyl leukotrienes were hypothesized to be predominantly active in prolonging the bronchoconstriction reaction, as based on studies descibed in *chapter 5*. To test this hypothesis, the efficacy of zafirlukast, a cysteinyl leukotriene receptor antagonist, in inhibiting EIB in asthmatic adolescents was evaluated, using a randomised, double-blind, placebo-controlled cross-over study design (*Chapter 9*).

chapter 4 chapter

Sample size estimation in studies monitoring exercise-induced bronchoconstriction in asthmatic children

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4.1 Summary

The repeatability of the response to standardized treadmill exercise testing using dry air and monitoring of heart rate in asthmatic children suffering from exercise-induced bronchoconstriction (EIB) has not been well established. Therefore, twentyseven asthmatic children with documente EIB performed standardized exercise testing twice within a period of three weeks. The tests were performed on a treadmill while breathing dry air. During both tests heart rate had to reach 90% of the predicted maximum. Response to exercise was expressed as %fall in FEV_1 from baseline (%fall) and as area under the curve (AUC) of the time-response curve. The repeatability was assessed bycalculating the intra-class correlation coëfficiënts (ICC) for %fall and AUC (logtransformed), which were 0.57 and 0.67, respectively. From these data power curves were constructed allowing sample size estimations for studies monitoring EIB in children, indicating that if a drug is expected to reduce EIB by 50%, as few as 12 patients in total suffice to demonstrate this effect (90% power), using a parallel design study. Thus, standardized exercise testing for EIB using dry air and monitoring of heart rate is adequately repeatable for use in research and clinical practice in children with asthma.

4.2 Introduction

At present, exercise-induced bronchoconstriction (EIB) is regarded as an expression of airway hyperresponsiveness to non-sensitizing bronchoconstrictor stimuli, which is a common characteristic of current symptomatic asthma[1]. One of the goals of asthma treatment is to enable the patients to participate in activities and exercise without limitations[2]. Exercise testing for determining the severity of EIB can therefore be a helpful tool in asthma diagnosis and management, and in research studies investigating drug therapy, provided the method of exercise testing is standardized, and reproducible. It is recommended to use inspired dry air and to adjust the work intensity of the subject achieving 40-50% of predicted maximal voluntary ventilation[3] or, alternatively, a heart rate ≥ 90% of maximum predicted during the last minutes of the test[4]. Allthough data are available on the reproducibility of the response to standardized exercise testing[4-7], data for dry air exercise testing in children are lacking. Therefore, we investigated the repeatability of EIB induced by standardized treadmill exercise testing with breathing of dry air in asthmatic children with documented EIB, and used the data for sample size estimations for studies of EIB.

4.3 Materials and methods

4.3.1. Patients

Twentyseven asthmatic children (12 male, 11 female; age 6-14), with a current history of EIB, were recruited from the clinic of the Juliana Childrens' Hospital, The Hague, and 't Lange Land Hospital in Zoetermeer. Pre-exercise, forced expiratory volume in 1 second (FEV_1) was above 70% of predicted for all children, while post-exercise testing, all showed a $\geq$15% fall in FEV_1 compared to baseline. Four children used continuous treatment with inhaled corticosteroids, but not in the week before, nor during the study period. All children used short-acting inhaled bronchodilators on demand only, which were withheld for 8 hours before exercise testing.

4.3.2 Study design

After the screening exercise, the children attended twice at approximately the same time of day on separate days (interval range: 6 - 24 days). At both visits, a standardized treadmill exercise challenge was performed. The two tests were considered to be acceptable for analysis if the child had reached the target heart rate in both tests, regardless of the speed of the treadmill used, and if the duration of the two tests did not differ by more than 30 seconds.

4.3.3 Exercise challenge

Before exercise, baseline FEV_1 was measured in triplicate, with the largest FEV_1 used for analysis. Exercise testing was performed by running on a treadmill[4] (LE 2000, Jaeger, Germany or Tunturi J880, Finland), while breathing dry air[3] (relative humidity <10%) during running. Dry air was obtained by pressurized medical air, collected in a Douglas bag (contents 150 litre), and inhaled by the child through a face mask (Hans-Rudolph) with an in-and expiratory port. Heart rate was monitored by a radiographic device (Polar Sport Tester). The incline of the treadmill was set at 5 to 10%, depending on the physical condition of the child. During the first minute of the test, the children walked at slow speed to familiarize themselves again with the procedure. Subsequently, the speed of the treadmill was increased during the first three minutes of the test to induce a heart rate $\geq$ 90% of the predicted maximum (approx. 210-age[4]) by the third minute of the test. Thereafter, the children ran for another three minutes, unless dyspnoea made further running impossible. FEV_1 was measured in duplicate at 1, 3, 5, 7.5, 10, 15, 20 and 30 minutes after running, with the largest FEV_1 at each time point retained for analysis.

4.4 Statistical analysis

The severity of EIB was expressed as maximal percent decrease in FEV_1 post-exercise as compared to baseline FEV_1 (%fall), and as area under the time-response curve (AUC) between 0 and 30 min post-exercise. The values of the test days for heart rate during the last minute of the test, %fall and AUC, respectively, were compared using Students' t-test (paired samples), with p-values <0.05 (two-sided) considered as statistically significant. Analysis of the repeatability was performed according to published guidelines[8] by calculating the intra-class correlation coëfficiënt (=between subject variance/within+between subject variance).

Curves for estimation of sample size were constructed using within-subject variability measurements and published power functions (one-sided) for predicting sample size[9]. For comparison of active versus placebo treatment, the change over time in the outcome variable was chosen as the main efficacy parameter, the treatment-induced change over time being a more sensitive indicator of drug effect than the absolute values of the outcome variable at each time point of measurement. Assuming absence of period or carry-over effects, the approximate number of subjects required in a cross-over design study was subsequently calculated using the formula:

N (number of subjects) = $[(Sdd * \{Z_\alpha - Z_\beta\}) / D]^2$.

with: Sdd = standard deviation of the change in outcome variable;

Z_α = standard normal deviate corresponding to a right-hand tail area of α;

Z_β = standard normal deviate corresponding to a left-hand tail area of β;

D = expected mean change in EIB produced by treatment (i.e. 40%, 50% etc)

For a parallel group design study, comparing the change over time of the outcome variable for the two groups, the number of subjects was calculated according to:

N (number of subjects) = $2* [(Sdd * \{Z_\alpha - Z_\beta\}) / D]^2$ in each group.

With estimated sample sizes $\leq$ 30, Z-values should be replaced by Student's t values for the same α and β, but based on n-1 degrees of freedom [with n=estimated sample size].

4.5 Results

All children completed the study according to the protocol. Between children, the duration of the exercise tests varied from 3.5 to 6 minutes. Heart rate during the last minute of the test did not differ significantly between the test days (mean (SD)): 188 (9) min^{-1}, and 189 (8) min^{-1}, respectively, p=0.44). Mean (SD) %fall for the first test was 32.1 (10.5)%, and for the repeat

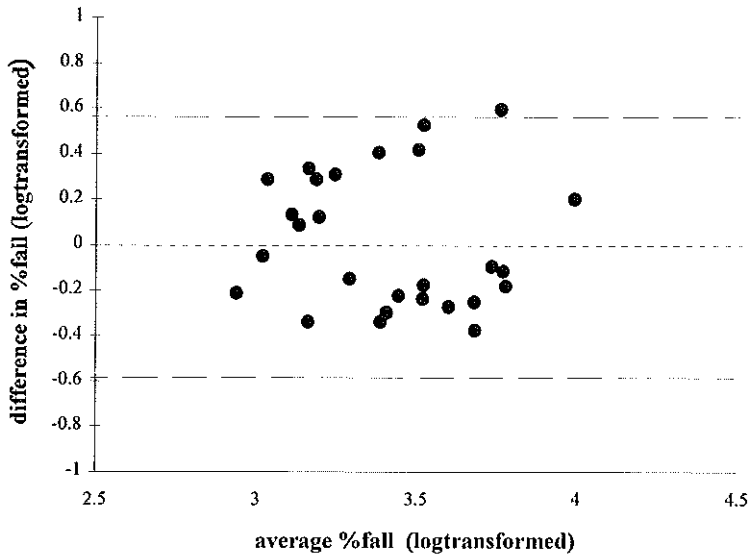

Figure 1a: repeated measures of %fall in FEV1 (logtransformed), the difference in repeated measures plotted against their mean, and shown with line of no difference (dotted line) and 95% confidence interval (dashed line).

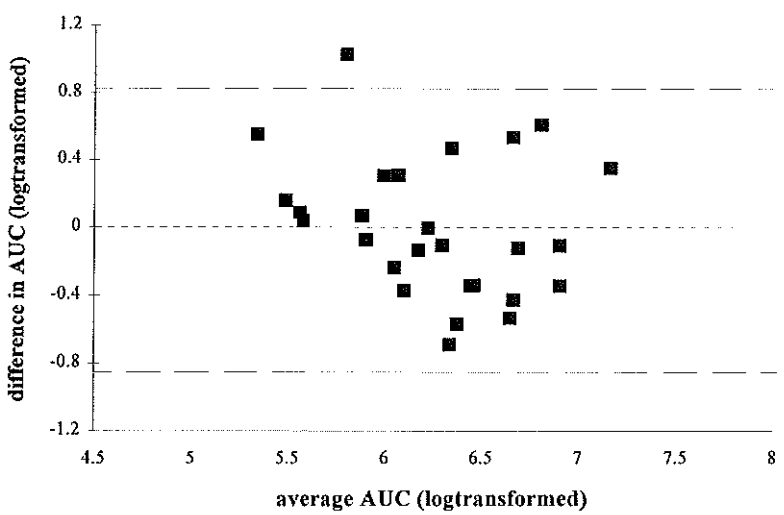

Figure 1b: repeated measures of AUC (logtransformed), the difference in repeated measures plotted against their mean, and shown with line of no difference (dotted line) and 95% confidence interval (dashed line)

test it was 31.7 (10.3)%. Mean AUC was 582 (307) %min for test 1, and 596 (297) %min for test 2, respectively. The severity of EIB did not differ significantly between the two tests (%fall:p=0.87; AUC:p=0.80). Because the differences in %fall and AUC between the two tests were proportional to their mean, both parameters were log-transformed, thereby achieving normality of the differences (figure 1a, 1b).

The ICC (based on these log-transformed data) for %fall was 0.57 and 0.67 for AUC. Curves for estimation of sample sizes at given power levels were constructed for parallel group studies (figure 2: %fall in FEV_1) and for cross-over studies (not shown). The standard deviation of the difference in EIB for the two tests (=Sdd) used in the formulae to calculate sample size, was 0.296 for the log-transformed data of %fall, and 0.416 for the log-transformed data of AUC. In table 1, estimated sample sizes needed at different power levels to discriminate statistically significant reductions in EIB of 40% and 50% respectively, are presented, the required number of subjects dependent on the choice of design (cross-over or parallel) and outcome variable (%fall or AUC). For example, if a drug-induced reduction of 50% is considered to be clinically effective, only 5 (%fall) to 6 (AUC) patients are needed to achieve statistical significance (α=0.05, two-sided, β=0.90, one-sided) using a cross-over design, while a parallel group design requires a total of 12 (%fall) to 20 (AUC) patients only.

Table 1: Sample size estimations (n) at given power levels required in cross-over and parallel group studies to discriminate the expected protective effect of a drug and placebo against EIB with statistical significance (α=0.05, two-sided).

		CROSS-OVER		PARALLEL§	
PI (%)	power (%)	%fall	AUC	%fall	AUC
40	80	5	8	16	26
	90	6	10	20	32
	95	7	11	24	40
50	80	4	6	12	16
	90	4	6	12	20
	95	5	8	16	24

PI= protection index= $\{(EIB_{post\text{-}placebo} - EIB_{post\text{-}drug})/ EIB_{post\text{-}placebo}\}*100\%$;

§ : n for drug and placebo group together, hence ½n per treatment arm.

4.6 Discussion

This study has shown that in asthmatic children the response to standardized exercise testing is adequately repeatable using inspiration of dry air and monitoring of heart rate. Sample sizes needed to detect significant differences in EIB are relatively small, supporting the feasibility of research studies monitoring EIB in childhood asthma.

Two methodological points need to be adressed when comparing our results to those in the literature[4-7]. Firstly, the repeatability is influenced by the degree of EIB of the selected patients. This is illustrated by an epidemiological study of Haby et al[7], in which the reproducibility of the %fall to a standardized free range running exercise test was assessed by the calculation of the single determination 95% range. Taking into account all children in that study, a 95% range of ±12 % was calculated, meaning that there is a 95% chance of the true value for a subject to be found within the range of 12% fall in FEV_1 around the single measurement value. However, when re-analysing the published data in children with at least 20% fall in FEV_1 post-exercise (comparable to our study population), we have estimated the single determination 95% range to be ±18.5%[7].

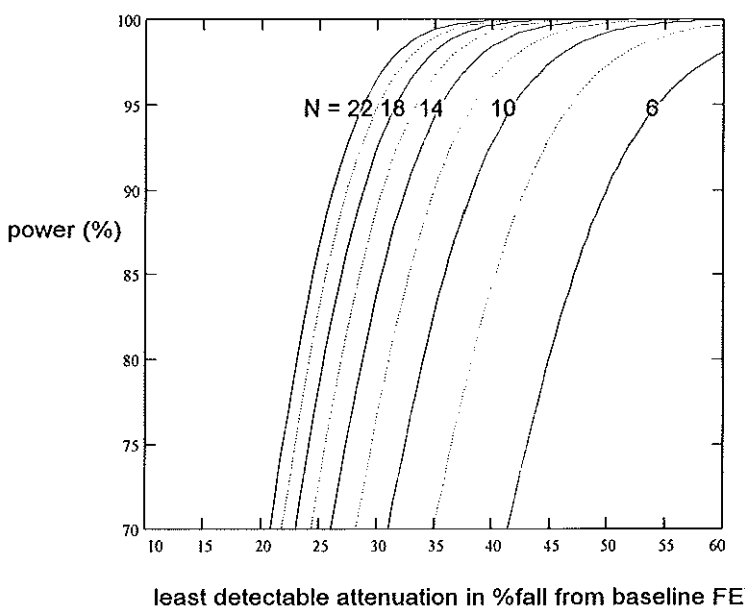

Figure 2: Curves, based on log-transformed data, for estimation of sample size needed in a parallel group study to show variable attenuations in EIB at certain power levels for %fall in FEV1, with N = required subjects per treatment arm

Secondly, in the studies published, different indexes of repeatability are used, such as coëfficient of variation (CV = standard deviation divided by mean)[4,5], the intra-class correlation coëfficiënt[6], or the 95% CI of a single measurement[7] . The CV is only to be used when the standard deviation is proportional to the mean[10], otherwise the CV will vary with the mean value of EIB, as was elegantly shown for %fall by Eggleston et al[4]. In our study, we choose to use ICC as an index of repeatability, as has recently been advised, because in all circumstances the ICC relates the size of the error variation to the size of the variation of interest[10]. However, a low ICC does not necessarily implicate a larger sample size, as the sample size estimation is dependent on the standard deviation of the difference between two tests, and not the ICC itself[6].

What are the implications of these data? The short-term repeatability of the response to standardized dry air exercise testing is adequate enough to allow drug evaluation for EIB in limited numbers of children, with the required study sample sizes influenced by the choice of design (cross-over or parallel) and outcome variable (%fall or AUC). Thus, standardized dry air exercise testing can be an important tool in management of childhood asthma. Whether repeatability can be improved by measuring ventilation instead of heart rate during testing, remains to be investigated.

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chapter 5 chapter

Prolonged recovery from exercise-induced asthma with increasing age in childhood

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5.1 Summary

It has been suggested that children recover more quickly from bronchoconstriction after exercise than adults. Based on clinical observation we hypothesized that recovery rate from exercise-induced asthma (EIA) in childhood decreases with age. In 14 children aged 7 to 12 year, with a history of EIA we measured spontaneous recovery from bronchoconstriction induced by two different stimuli: exercise and histamine. The children attended the laboratory on three visits. After a screening exercise test on the first visit, standardized bronchoprovocation tests with either exercise or histamine were performed on the following visits in random order. The degree of bronchoconstriction induced by histamine was matched for that observed after exercise. During recovery forced expiratory volume in 1 second (FEV_1) was measured repeatedly up to 2 hours postchallenge. The recovery rate (%increase in FEV_1/min) was calculated from the linear slope of the time-response curve. Differences in recovery rate between the two stimuli were analysed by paired t-test, while age related differences were analysed using multiple regression analysis. For the group as a whole, recovery rate was not different between the two stimuli (mean±SD: 1.22±0.91 for exercise and 1.46±0.65 for histamine, p=0.31). However, the recovery rate for exercise-induced bronchoconctriction decreased significantly with age (r=-0.74, p=0.003), in contrast to the recovery rate for histamine (r=-0.15, p=0.60). Consequently, in the oldest age group (11-12 yr, n=5) recovery rate for exercise was significantly lower as compared to the younger age group (7-10 year, n=9): 0.54±0.17 and 1.60±0.93, respectively, p=0.009, and also as compared to recovery rate for histamine: 0.54±0.17 and 1.33±0.54, respectively, p=0.03. In the younger age group the recovery rates for exercise and histamine were not different: 1.60±0.93 and 1.54±0.73, respectively, p=0.83. We conclude that recovery from EIA in childhood decreases with increasing age. These data suggest that the mechanism of exercise-induced asthma in childhood changes with age. This might be due to changes in mediator production.

5.2 Introduction

Since long it has been recognized that exercise can induce acute bronchoconstriction in patients with asthma[1] (exercise-induced asthma: EIA). Symptoms of EIA may include any of the following: wheezing, cough, shortness of breath, chest pain or discomfort after strenuous exercise. Symptoms are most intense 5 to 10 minutes following exercise cessation and usually resolve spontaneously within one hour[2]. In childhood asthma the prevalence of EIA varies from 70% to 90%[3,4]. The most important trigger for EIA to occur is thought to be the hyperpnoea which results in an increase in airway osmolarity and in airway cooling, due to evapo-

rative water loss[5]. These changes lead to release of mediators that directly or indirectly induce bronchoconstriction.

Direct measurements of mediators in blood or bronchoalveolar lavage after EIA has led to inconclusive results about the mediators involved[6,7]. Using receptor antagonists it has been shown that histamine[8] and also leukotrienes[9] are important components in EIA. However, the protective effect of antagonists of these mediators in individual adult asthmatic subjects differs from complete protection against EIA to no protection at all, suggesting heterogeneity of mechanisms involved in the bronchoconstrictive response[10].

It has been suggested that there are differences in the clinical expression of EIA between adults and children, with children recovering more quickly from bronchoconstriction after exercise[11] and having fewer late asthmatic reactions[12]. However, no published data are available on the recovery phase of EIA in childhood asthma. Since it is known that bronchoconstriction to leukotrienes is more prolonged than to histamine[13,14], different time courses of EIA between various age groups could implicate different mechanisms of EIA. This could have important clinical consequences, particularly regarding the choice of therapeutic interventions.

Based on clinical experience with exercise testing in asthmatic children, we hypothesized that recovery from EIA is prolonged with increasing age in childhood asthma. To that end we measured the rate of recovery from the acute bronchoconstriction to exercise in asthmatic children of different ages, and compared this to the rate of recovery from a matched level of bronchoconstriction to histamine.

5.3 Materials and methods

5.3.1 Patients

Fourteen children (6 male, 8 female) clinically diagnosed as having asthma, were recruited from the outpatient clinic of the Department of Paediatric Pulmonology of the Juliana Childrens' Hospital in The Hague. All children had a history of exercise-induced asthma and all showed a fall in forced expiratory volume in 1 second (FEV_1) >10% after a standardized screening exercise challenge. Patient characteristics are summarized in table 1. Mean age of the children was 9.7 years (range 7 - 12 years). All children but one were atopic (RAST class ≥ 2 for at least one inhalant allergen). All children were clinically stable (i.e. such as no history of viral infections in the two weeks before entry into the study). During a run-in period maintenance treatment was reduced according to a standardized protocol. Sodium cromoglycate (3 out of 14) was stopped two weeks before the first study day. The dose of inhaled corticosteroids (5

out of 14) was halved for the first week and thereafter tapered down with 100 mcg each week until no inhaled corticosteroids had been used in the week before the first study visit. Inhaled short-acting bronchodilators were used as rescue medication during the study period. This bronchodilatory therapy was withheld for at least 8 hours before each visit.

Informed consent was obtained in all cases. The study was approved by the Local Medical Ethics Committee.

Table 1. Patient characteristics.

nr	sex	age (yr)	FEV1 (%predicted)	EIA at screening (%fall in FEV_1)	therapy
1	m	7	94	14	sal
2	f	7	103	55	dscg
3	m	8	108	16	dscg
4	f	9	82	34	bdp
5	m	9	85	58	sal
6	m	10	98	28	sal
7	f	10	99	40	bdp
8	f	10	83	54	cet
9	m	10	87	61	dscg
10	f	11	105	25	bdp
11	f	11	93	53	sal
12	m	11	102	58	bdp
13	f	12	91	24	bdp
14	f	12	113	41	sal
	mean	9.7	96.2	40.0	
	SD	1.6	9.3	16.6	

EIA at screening = %fall in FEV1 from baseline after the screening exercise; therapy: sal=salbutamol; cet = cetirizine; dscg = sodium cromoglycate; bdp = beclomethasone dipropionate;

5.3.2 Study design

The children attended the lung function laboratory for three visits within a study period of two weeks. On the first visit baseline FEV_1 was measured in triplicate followed by standardized exercise challenge. During the recovery period lung function measurements were made repetiti-

vely up to two hours after challenge. On the subsequent visits in random order either a second exercise challenge was done or a histamine inhalation challenge matching the degree of bronchoconstriction measured after the first exercise. All three study visits started at the same time of day for each child.

5.3.3 Lung function measurements

Lung function measurements were made using a dry rolling seal spirometer (Vicatest 5, Mijnhardt the Netherlands) or a pneumotachograph (Flowscreen, Jaeger Germany), using the same calibrated device for each child. The highest FEV_1 obtained from three forced expiratory manoeuvres was retained for analysis[15].

5.3.4 Exercise challenge

Exercise challenge was performed by running on a treadmill (LE 2000, Jaeger Germany) for 6 minutes[16]. As it is known that temperature and humidity of the inspired air modulate the response to exercise[17], dry air was used to minimize the influence of changes in environmental factors between visits. It also increases the osmotic stress to the airways in a standardized way. Dry air (relative humidity $\leq$ 15%) was inspired from a reservoir bag through a face mask with an in- and expiratory port (Speak Easy II) during the test. Heart rate was measured using a heart rate monitor (Polar Sporttester). The children started at walking pace on the treadmill for one minute. During the test the speed of the treadmill was increased to induce a heart rate of at least 90% of the child's maximum predicted heart rate (maximal heart rate = 210-age). Lung function measurements were made in triplicate 1, 3, 5, 7, 10, 15, 20, 25, and 30 minutes after running, thereafter every 10 to 15 minutes, with the last measurement being made two hours after the start of the challenge.

5.3.5 Histamine challenge

Histamine challenge was done by a standardized dosimetric technique[18]. Histamine was delivered to the mouth by a Rosenthal-French dosimeter which was connected to a DeVilbiss nebulizer type 646. The dosimeter was triggered by slow inhalation from functional residual capacity (FRC) to total lung capacity (TLC). Doubling doses (5 - 640 mcg) of histamine diphosphate in physiologic saline were inhaled. Each provocation challenge started with the lowest dose of histamine. Three minutes after inhaling each dose of histamine FEV_1 was measured in triplicate, the highest FEV_1 being used in the analysis. The histamine challenge

ended if the %fall in FEV_1 from baseline did not differ by more than 10% from the %fall in FEV_1 as induced by the first (screening) exercise test for each individual child. During spontaneous recovery from the bronchoconstriction to histamine FEV_1 measurements were repeated in triplicate at 3, 5, 10, 15, 20 and 30 minutes after the last dose of histamine given, thereafter every 15 minutes with the last FEV_1 measurement taken two hours after the start of the challenge. To assess bronchial responsiveness to histamine, PD_{20}histamine was determined by linear interpolation between two data points on the non-cumulative log dose-response curve[18].

5.4 Statistical analysis

5.4.1 Bronchoconstriction to exercise and histamine

Acute bronchoconstriction to exercise or histamine was expressed as maximal %fall in FEV_1 from baseline during the first hour. The responses to exercise and histamine were compared using Students' t-test of paired samples.

5.4.2 Recovery from bronchoconstriction

The **recovery phase** of the bronchoconstrictive response was defined as the duration between the time point at which FEV_1 had reached its maximal %fall from baseline and the time point at which FEV_1 had returned to at least 90% of baseline. The **recovery rate** of bronchoconstriction was calculated by measuring the linear slope of the time-response curve during the recovery phase and was expressed as %increase in FEV_1 per minute (%incr FEV_1/min). Differences in recovery rate between exercise and histamine challenge were analysed by paired t-test. Age-related differences in recovery rate for the two stimuli were analysed by using multiple regression analysis, with recovery rate as dependent variable and age and maximal %fall in FEV_1 as independent variables. In order to investigate whether differences in recovery rate between children were related to age, the analysis was repeated in two age groups. The children in group 1 were 7 to 10 years of age (n=9); the children in group 2 were 11 and 12 years of age (n=5). P-values less than 0.05 were considered statistically significant. Results are expressed in mean ± SD.

5.5 Results

Baseline FEV_1 was >80% of predicted in all children and did not differ by more than 10% between visits in each individual. There was no significant difference in baseline FEV_1 between the exercise and histamine day (mean difference ± SD: 0.93 ± 4.11, p=0.41). Ten out of 14 children did not run for the full 6 minutes because of discomfort associated with wheezing

already during running. The duration of the exercise test for these children ranged from 3.5 to 5.5 minutes. In one child bronchodilatory treatment was given immediately after the first exercise because of dyspnea.

5.5.1 Acute bronchoconstriction after exercise or histamine

Examples of the time-response curves to exercise and histamine challenge are given in figure 1a and 1b. Maximal %fall in FEV_1 from baseline after each challenge is shown in table 2. Differences in mean %fall in FEV_1 between the challenges were not significant (mean %fall in FEV_1 after exercise: 37.2 ± 15, and after histamine: 40.1 ± 11, p=0.39)

Table 2: Acute bronchoconstriction to exercise or histamine and the recovery rate for both challenges.

	exercise		histamine	
nr	%fall FEV1	recovery rate*	%fall FEV1	recovery rate*
1	14	3.50	25	1.70
2	30	2.08	41	2.17
3	34	1.45	35	0.74
4	37	1.23	38	0.70
5	54	1.05	53	1.60
6	20	0.30	35	1.00
7	48	2.20	39	3.00
8	53	0.84	39	1.45
9	57	1.74	54	1.47
10	35	0.51	28	2.10
11	62	0.48	49	1.64
12	32	0.31	60	0.73
13	18	0.61	25	1.00
14	27	0.78	40	1.20
mean	37	1.22	40	1.46
SD	15	0.91	11	0.65

*: recovery rate in %increase in FEV_1/minute;

5.5.2 Recovery from bronchoconstriction

Individual recovery rates for each patient are shown in table 2. Mean recovery rate for exercise was 1.22 ± 0.91 %incr. FEV_1/min, and for histamine 1.46 ± 0.65 %incr. FEV_1 /min. For the group as a whole there was no difference in recovery rate between exercise and histamine (p=0.31).

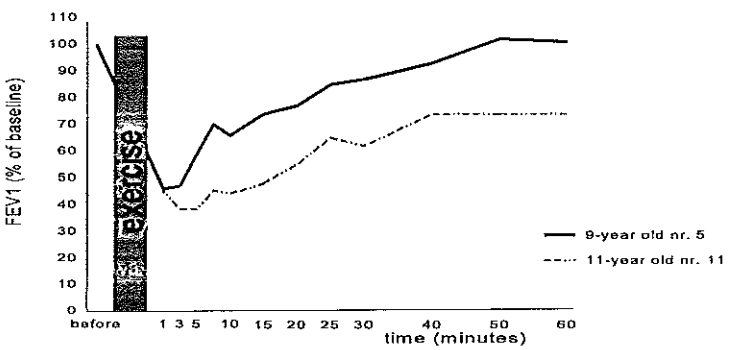

Figure 1a: Example of the bronchoconstrictive response to exercise in 2 children of different ages

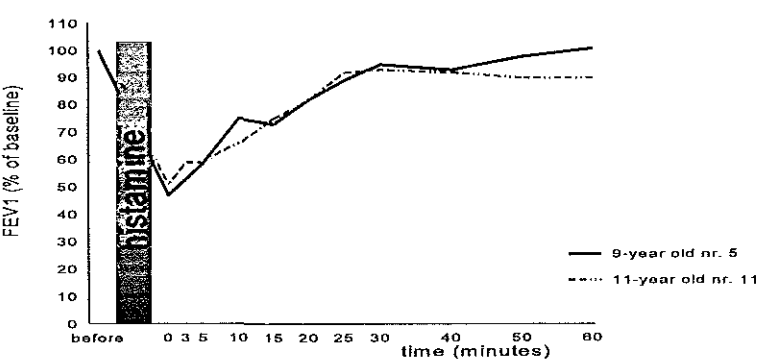

Figure 1b: Example of the bronchoconstrictive response to histamine in 2 children of different ages

There was a significant relationship between recovery rate for exercise and age (r= -0.74, p=0.003; figure 2). No such relationship was found between recovery rate for histamine and age (r= -0.15, p=0.60; figure 3). Recovery rate was not dependent on the severity of the acute bronchoconstriction (p=0.83 and p=0.93 for exercise and histamine, respectively), nor upon the level of bronchial responsiveness as measured by $^{2}\log PD_{20}$ (p=0.26 and p=0.40), baseline FEV_1 (p=0.43 and p=0.77) or on the highest dosis of histamine used (p=0.54).

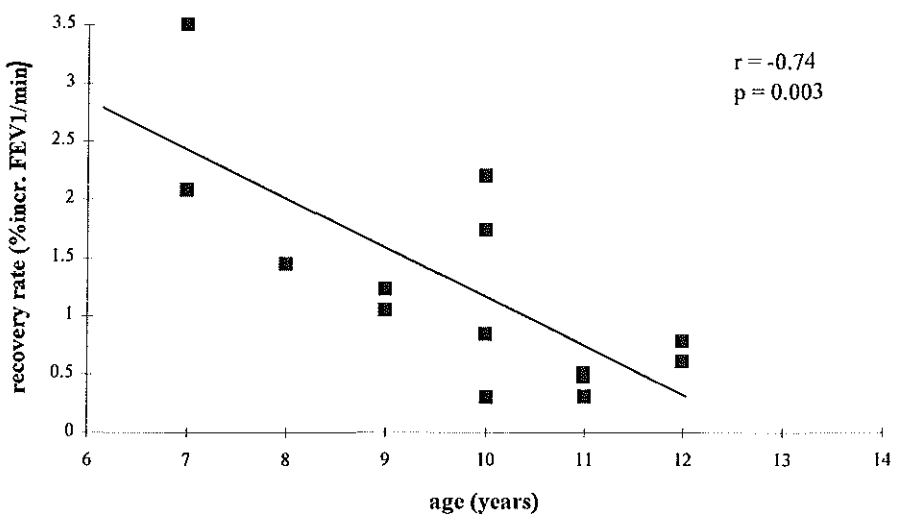

Figure 2: The relationship between the age of the child and the recovery rate from bronchoconstriction after exercise challenge

The children were then divided into two age groups: 7-10 year old and 11-12 year old children (table 3). Baseline FEV_1 before histamine challenge was not different between the two groups (p=0.06). Baseline FEV_1 before exercise challenge was slightly but significantly different (p=0.02), with the younger age group having the lowest baseline FEV_1. The two groups were not different with respect to severity of the acute bronchoconstriction to exercise (p=0.69) or to histamine (p=0.95), level of bronchial responsiveness as measured by $^{2}\log PD_{20}$ (p=0.89), or highest dose of histamine given (p=0.29). The recovery rate for exercise was significantlylower

in the older age group as compared to the younger age group: 0.54 ± 0.17 and 1.60 ± 0.93, respectively, p=0.009. Recovery rate for exercise in the older age group (11-12 years) was also significantly lower as compared to the recovery rate to histamine in the same age group: 0.54 ± 0.17 and 1.33 ± 0.54, respectively, p=0.03. On the contrary in the younger age group the recovery rate for exercise was not significantly different from the recovery rate for histamine: 1.60 ± 0.93 and 1.54 ± 0.73, respectively, p=0.83.

Table 3. Patient characteristics and differences in recovery rate (mean ±SD) for exercise and histamine challenge for the two age groups.

	group 1	group 2	t-test p-value
number	9	5	
age (years)	7 - 10	11 - 12	
FEV_1 (%predicted)			
before exercise	91.3 ±6.8	103.2 ±7.3	0.02
before histamine	91.7 ±8.7	100.0 ±6.2	0.06
exercise response†	38.6 ±15.5	34.8 ±16.5	0.69
histamine response†	39.9 ±9.0	40.4 ±14.6	0.95
PD_{20} (mcg)‡	33.4 ±1.0	31.5 ±1.1	0.89
dose histamine (mcg)*	73 ±56	136 ±111	0.29
recovery¶ exercise	1.60 ±0.93	0.54 ±0.17◆	0.009
recovery¶ histamine	1.54 ±0.73	1.33 ±0.54	0.57

†: in %fall in FEV_1; ‡: geom. mean±doubling doses; *: highest dose given; ¶: recovery rate in %increase in FEV_1/minute; ◆: p=0.03 as compared to recovery rate for histamine in the same age group;

5.6 Discussion

This study describes differences in the clinical expression of EIA within a population of 7 - 12 year old asthmatic children. We have shown that the recovery rate from the acute bronchoconstriction to exercise decreases with age in contrast to recovery from a matched level of bronchoconstriction to histamine. Recovery rate is not dependent on the severity of the response, nor on baseline FEV_1, the level of bronchial hyperresponsiveness to histamine, or the dose of histamine used to induce bronchoconstriction. These data may implicate that the mechanism of exercise-induced asthma is changing when children grow older.

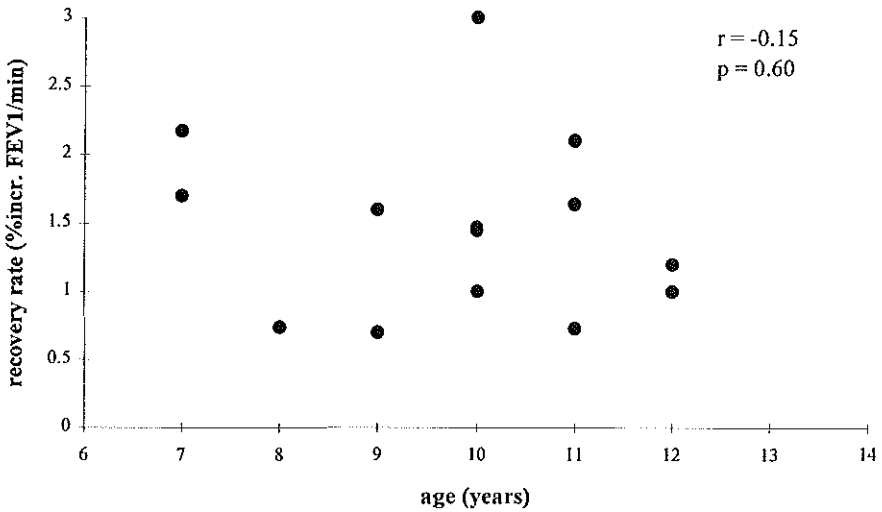

Figure 3: The relationship between the age of the child and the recovery rate from bronchoconstriction after histamine challenge

This is the first study on the relationship between age and recovery from EIA in childhood asthma. Even though it had been suggested by Anderson[2] that the recovery of EIA in children is faster than in adults, to the best of our knowledge no data were available on age-related differences in this respect. Our results indicate that EIA is associated with more prolonged bronchoconstriction with increasing age. When interpreting our findings, a number of methodological points need to be addressed. Firstly, the data presented here are cross-sectional and might be due to a selection bias, because it cannot be excluded that relatively slow recovery from EIA can be one of the reasons for referal to a specialist clinic as ours. However, it seems very unlikely that such bias would be related to age. Secondly, we do not think that exercise *per se* would account for the differences between the two groups. All children were exercised with heartrate being $\geq$ 90% of their predicted maximum heartrate and thus close to their predicted maximum ventilation, as these two indices are related to each other[19]. In addition no difference was observed in the maximal %fall in FEV_1 to exercise in the two age groups. Thirdly, although we tried to match the two stimuli for level of acute bronchoconstriction within a 10% range, this was not achieved for all individual children. However, we do not think our data were influenced by this as there was no significant difference between the mean levels of acute bronchoconstriction for the two stimuli. Fourthly, there was a slight but significant

difference between baseline FEV_1 before exercise challenge in the two subgroups. It is very unlikely that this has had an influence on the results described because analysis showed that the recovery rate was not dependent on baseline level of FEV_1. Finally, our results might have been influenced by the analysis of the time-response curves. Other investigators looking at recovery from induced bronchoconstriction have used the time to complete recovery as a variable[10,20]. However, it may be that the time to complete recovery is at least partly dependent upon the maximal %fall in FEV_1. Because the acute bronchoconstrictive response to exercise or histamine ranged from 14% to 62% fall in FEV_1 in our study, we choose to measure the slope of the recovery phase as an index of recovery. This index seems to be also preferable to analysis of the area under the time-response curve (AUC), because the latter is not suitable for the comparison of single dose challenge (exercise) with a multiple dose (histamine) challenge.

How can the present results be explained? It is not unlikely that the observed differences in recovery rate from exercise-induced bronchoconstriction are due to a changing mediator profile of EIA with age. It has been suggested that heterogeneity of EIA in adult asthma may be due to the fact that some asthmatics are predominantly histamine producers, whilst others preferentially generate leukotrienes[10]. It is known that recovery from leukotriene challenge is prolonged as compared to histamine challenge[13,14]. Barnes et al. found the average recovery time of the specific airway conductance (sGaw) to return to 90% of baseline value after a histamine challenge to be 9.9 minutes, with the average recovery time after LTD_4 and LTC_4 challenges being 25.3 and 32.2 minutes, respectively[13]. Weiss et al. showed that recovery of V_{30} to 90% of baseline after histamine challenge required 10 to 14 minutes, whereas the recovery after LTD_4 challenge required 18 to 30 minutes[14]. Hence, recovery after leukotriene challenge takes 2-3 times as long as recovery after histamine challenge. Applying this to our data, the similar recovery rates for exercise and histamine in the young age group would be indicative of predominant histamine release, whereas the 2-3 times prolonged recovery phase after exercise in older children would be in keeping with predominant leukotriene release. This may have pathophysiological consequences. Due to the pro-inflammatory activity of leukotrienes[21-23], this could be indicative of ongoing inflammation in the airways in relatively older children with asthma. The relative contribution of these mediators to EIA in various age groups of childhood asthma should now be examined, using potent histamine and/or leukotriene receptor antagonists[24] as pretreatment to exercise challenge.

Pretreatment with an anticholinergic drug has shown a protective effect to EIA in some patients, thus implicating the autonomic nervous system in the response to exercise in at least part of the asthmatic population[25,26]. The role of the sympathic regulation of the airways in EIA is

not entirely clear. Both in normal and asthmatic subjects circulating plasma levels of adrenaline and noradrenaline increase during exercise[27]. Allthough bronchial hyperreactivity to histamine can be modified by circulating physiologic levels of adrenaline[28], blocking the sympathic system with propanolol does not effect the bronchotone during exercise in normal human subjects[29]. An attractive alternative explanation for the observed differences in recovery time from EIA is a changing role of circulating protective mediators, such as atrial natriuretic factor (ANF)[30]. It could be postulated that the release of ANF, as observed during exercise[31], may be elevated in young children as compared to older children and adults. As it is known that ANF decreases bronchial reactivity in asthmatic subjects in a dose-dependent manner[32], higher levels of ANF during exercise could result in a stronger relaxing effect during the ensuing bronchoconstriction.

What is the clinical significance of these data? Firstly, our results strongly suggest that it is not only important to measure the maximal %fall in FEV_1 after exercise challenge, but that it is equally important to record the recovery phase. It can be postulated that a prolonged recovery phase is associated with the subjective severity of EIA in children. This requires further investigation. Secondly, if it can be confirmed that an increase in the duration of the response to EIA is a reflection of the release of (pro-inflammatory) leukotrienes this will have therapeutical consequences in choosing symptomatic or prophylactic therapy.

In conclusion, we have shown a relationship between age and the duration of the acute bronchoconstrictive response to exercise in asthmatic children. It appears that the recovery phase after EIA is more prolonged in older children. Whether this is due to a changing mediator profile with age in EIA during childhood needs to be further investigated.

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chapter **6** chapter

The occurence of a late response to exercise in asthmatic children

A multiple regression approach using time-matched baseline- and histamine control days

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6.1 Summary

At this moment, there is still controversy on the presence of a late asthmatic response (LAR) to exercise challenge in asthma. Therefore, we have investigated the occurence of a LAR after exercise in asthmatic children visiting an out-patient clinic, using time-matched baseline and histamine control days, and a statistical analysis according to recently published recommendations. After a screening exercise day, seventeen children (aged 7 to 14 year) randomly performed, on three following study days, either a second standardized exercise challenge, or a histamine challenge whilst matching the bronchoconstriction after exercise, or only measurement of baseline lung function without any challenge. Measurements of forced expiratory volume in one second (FEV_1) were repeatedly made during 8 hours. Analysis was done using multiple regression analysis per patient, with FEV_1 as the dependent, and test day (exercise or control) and clocktime as independent variables during the period between 2 and 8 hours after exercise. A significant interaction ($p<0.05$) between test day and clock time was considered to be indicative of a LAR. Fifteen children completed the study. All children showed an early asthmatic reaction to exercise (range 14 - 62% fall in FEV_1). In two children a significant interaction ($p<0.05$) was found between test day and clock time. However, the difference in FEV_1 between exercise and control days for each clocktime did not exceed the 99.6% confidence limits of normal diurnal variation in any of the children. We conclude that in children with mild to moderate asthma, a LAR to exercise does not occur. This suggests that exercise is a symptomatic trigger of asthma. Whether exercise is capable of inducing inflammation, needs to be further investigated.

6.2 Introduction

Since long it has been recognized that exercise can induce acute bronchoconstriction in patients with asthma[1], the so-called exercise-induced bronchoconstriction EIB. In childhood asthma the prevalence of EIB varies from 70% to 90%[2,3]. Children suffering from EIB may complain about any of the following symptoms during or after strenuous exercise: wheezing, shortness of breath, cough or chest pain[4,5]. The bronchoconstrictor response to exercise is determined by the level of ventilation reached during exercise as well as the temperature and water content of the inspired air[6]. The precise mechanism by which EIB occurs is still a matter of debate. However, studies with mediator antagonists and synthesis inhibitors have provided supportive evidence for the release of mediators such as histamine, prostaglandins and leukotrienes during the early asthmatic response (EAR) after exercise[7,8].

Ever since its first description in 1980[9], there is considerable controversy in the literature on

the potential development of a late asthmatic response (LAR) to exercise in asthma. It is known that the occurence of a LAR after allergen challenge is associated with an influx of inflammatory cells and the development of bronchial hyperresponsiveness[10]. Therefore, if a LAR after exercise does exist, it could have important consequences for our understanding of the pathophysiological mechanisms of EIB, and consequently for the therapy of asthma.

A number of studies have been published describing the occurence of a LAR, with its prevalence ranging from 10% to 89%[11-17]. However, nearly as many studies have been unable to document a LAR[18-23]. The experimental design of some of the studies showing a LAR has been criticized because of the lack of 'appropriate' control days[24], during which lung function is being documented without exercise or following bronchoconstriction induced by inhaled histamine. Furthermore, when evaluating the results of these studies, there seems to be considerable lack of agreement among the various investigators on the definition of the LAR after exercise. The LAR has been defined as either a 10%[12,15], or a 15%[14,20,23] or a 20%[16,17,19,21] fall in lung function (*e.g.* the forced expiratory volume in one second: FEV_1) during different time periods after exercise challenge. However, the definition according to a fixed %fall in FEV_1 carries the risk of a false-negative or a false-positive diagnosis, because it does not take into account normal diurnal variation in lung function in the asthmatic subjects.

Recently, a paper has been published in this journal describing statistical methods for the identification of a LAR in individual subjects[25], using serial hourly measurements of lung function on several control days. The aim of this analysis has been to interpret challenges with allergens or occupational sensitizers more sensitively[25], by taking into account individual day-to-day variability in pulmonary function on control days. Therefore, in the present study we have applied an analogous approach, using multiple lineair regression analysis per patient, to evaluate the occurence of LAR after exercise in a group of asthmatic children. To that end we repeatedly measured lung function up to 8 hours after exercise challenge. We also measured lung function during a control day without exercise (negative control), and on a day after histamine challenge inducing a matched level of bronchoconstriction as observed after exercise (positive control).

6.3 Materials and methods

6.3.1 Patients

Seventeen children (8 male, 9 female) were recruited from the outpatient clinic of the Department of Paediatric Pulmonology of the Juliana Childrens' Hospital in The Hague (table 1).

They were all known at the clinic with a diagnosis of asthma[26]. All had a history of exercise-induced bronchoconstriction and showed a fall in FEV_1 >10% as compared to pre-exercise FEV_1 after a standardized screening exercise challenge. Mean age of the children was 9.7 years (range 7 - 14 years) and at screening their baseline FEV_1 was > 75% of predicted value[27]. All children except two were atopic (RAST class ≥ 2 for at least one inhalant allergen).
All children were clinically stable (i.e no history of exacerbation and/or respiratory viral infection during the two weeks before entry into the study). Anti-inflammatory maintenance

Table 1: Patient characteristics

pat nr	sex	age	atopy	BASELINE VALUE FEV1 (%pred)#				PD_{20}† (mcg)	therapy¶
				EX1	EX2	H	C		
1	m	7	+	94	92	92	96	120	sal
2	m	8	+	108	102	104	102	30	dscg
3	f	12	+	91	95	97	90	36	bdp
4	f	11	+	105	107	107	112	55	bdp
5	m	10	+	98	91	94	93	55	sal
6	f	9	+	82	91	89	85	20	bdp
7	m	14	+	83	80	79	89	4	bud
8	f	10	+	99	98	101	101	23	bdp
9	f	12	-	113	113	103	112	31	sal
10	f	11	+	93	98	91	97	40	sal
11	f	10	+	85	85	86	86	9.2	cet
12	f	7	+	103	97	99	99	11	dscg
13	m	11	+	102	103	102	101	90	bdp
14	m	9	+	85	84	79	79	28	sal
15	f	7	-	85	-	-	94	7.6	sal
16	m	7	+	97	-	-	95	5.5	cet,bdp
17	m	10	+	87	82	81	77	28	dscg
mean		9.7		95	95	94	95	24‡	
± SD		2.0		10	9	9	11	1.4‡	

= FEV1 at start of study day expressed as % of predicted FEV1; † = dose of histamine to provoke a 20% fall in FEV1 from baseline; ‡ = geometric mean value ± SD in doubling doses; ¶ = therapy before standardized cessation of regular treatment: sal=salbutamol; cet = cetirizine; dscg = sodiumdicromoglycate; bdp = beclomethasone dipropionate; bud = budesonide;

treatment was reduced according to a standardized protocol. Sodium cromoglycate (3 out of 17) and the anti-histamine cetirizine (2 out of 17) were stopped two weeks before the first study day. The dose of inhaled corticosteroids (7 out of 17) was halved for the first week and thereafter tapered down with 100 mcg each week, until no inhaled steroids had been used in the week before the first study day. Inhaled short-acting bronchodilators were used as rescue medication during the study period. No bronchodilatory therapy was used for at least 8 hours before each study day.

Informed consent was obtained in all cases. The study was approved by the Local Hospital Medical Ethics Committee.

6.3.2 Study design

All practical work has been performed at the Juliana Childrens' Hospital in The Hague. The children attended the lung function laboratory on 4 consecutive days at intervals of at least 24 hours and within a study period of 3 weeks. On the first day (exercise screening day = EX1-day) baseline FEV_1 was measured in triplicate followed by standardized exercise challenge. Lung function measurements (FEV_1) were made repeatedly during the recovery period, and thereafter half-hourly up to eight hours after challenge. On the subsequent three study days, in random order, either a second exercise challenge (EX2-day) was performed, or a histamine inhalation challenge (H-day) matching the degree of bronchoconstriction after the screening exercise, or only lung function measurements were performed in the absence of challenge (C-day), again during the same 8 hour period. At each of the 4 study days individual children started at the same time of day, usually 9.00 a.m. During the study days the children stayed in one of the rooms of the lung function laboratory abstaining from strenuous exercise.

6.3.3 Lung function measurements

For each individual child, lung function measurements were made either using a dry rolling seal spirometer (Vicatest 5, Mijnhardt the Netherlands) or a pneumotachograph (Flowscreen, Jaeger Germany). Only one device was used per child. The highest FEV_1 obtained from three forced expiratory manoeuvres at each time point was retained for analysis[28].

On the control day without any challenge, the FEV_1 measurements were performed in triplicate every 15 minutes during the first two hours, thereafter repeated every 30 minutes until lung function measurements had been recorded during a time period of 8 hours.

6.3.4 Exercise challenge (EX-days)

Exercise challenge was performed by running on a treadmill (LE 2000, Jaeger Germany) for 6 minutes[29]. During the test, heart rate was checked using a heart rate monitor (Polar Sporttester). The children started at walking pace on the treadmill for one minute. Thereafter, the speed of the treadmill was increased to induce a heart rate of at least 90% of the maximum predicted heart rate for each individual child (maximum heart rate = 210 - age in years). Knowing that the occurence of a LAR may be dependent upon the severity of the early asthmatic response (EAR) to exercise[13], and knowing that the severity of the EAR to exercise is aggrevated when humidity of the inspired air during exercise is decreased[30], dry air was used to increase the osmotic stress to the airways in order to augment the EAR. This dry air (relative humidity <15%) was inspired from a reservoir bag through a face mask with an in- and expiratory port (Speak Easy II) during running. Using this protocol in our laboratory, the intra-class correlation coefficient for repeatability of the bronchoconstrictor response (expressed as %fall in FEV_1), is 0.57[31]. Dry air was not used when a preliminary exercise challenge had shown severe obstruction (%fall in FEV_1 >45%) already when breathing room air (n=3) or in case of fear of the mask (n=1). Lung function measurements were made in triplicate before exercise as well as 1, 3, 5, 7, 10, 15, 20, 25, and 30 minutes afterwards, and then every 10 to 15 minutes, until two hours after challenge. Thereafter, lung function measurements were made every 30 minutes up to 8 hours after exercise, allowing the calculation of the EAR and the LAR to exercise.

6.3.5 Histamine challenge (H-day)

A standardised dosimetric technique was used to perform the histamine challenge[32]. A Rosenthal-French dosimeter was connected to a DeVilbiss nebulizer type 646. By slow inhalation from functional residual capacity (FRC) to total lung capacity (TLC), the dosimeter was triggered to deliver a dose of histamine to the mouth. Starting with the lowest dose of histamine diphosphate in physiologic saline, doubling doses (5 - 640 mcg) were then inhaled. Three minutes after inhaling each dose of histamine FEV_1 was measured in triplicate, the highest FEV_1 being used in the analysis. The histamine challenge ended if the %fall in FEV_1 from baseline did not differ by more than 10% from the %fall in FEV_1 as induced by the first exercise test on EX1-day for each individual child.

During spontaneous recovery from the bronchoconstriction to histamine FEV_1 measurements were repeated in triplicate at the same time intervals as used after exercise challenge up to 8 hours. This allowed the calculation of the EAR to histamine in the same way as used for

exercise. To assess bronchial responsiveness to histamine, PD_{20}histamine was determined by linear interpolation between two data points on the non-cumulative log dose-response curve[32].

6.4 Statistical analysis

For analysis, EX2-day was taken as the active challenge day, while FEV_1 measurements of both C-day and H-day served as control values. The use of histamine as a positive control challenge was based on the assumption that a LAR does not occur after histamine challenge in the time period studied[33]. Study days were only used for analysis if FEV_1 (expressed as %predicted) at the start of the challenge days and the control days did not differ by more than 10%.

6.4.1 Early asthmatic response to exercise and histamine

The EAR to exercise or histamine was expressed as maximal %fall in FEV_1 from pre-challenge baseline value during the first hour, according to:

EAR = [(pre-challenge FEV_1 - lowest FEV_1 in first hour)/ pre-challenge FEV_1] * 100%.

The EAR to exercise and histamine were compared using Students' t-test of paired samples.

6.4.2 Late asthmatic response to exercise

To identify a LAR after exercise, multiple regression analysis[34] per patient was applied based on the half-hourly FEV_1 measurements in the time period 2 to 8 hours post-exercise. FEV_1 was taken as the dependent variable, with day (exercise or control) and clock time (of FEV_1 measurement) as independent variables. In a first fitted regression model, only the main effects of day and clock time were evaluated, the effect of day for each patient being the difference in lung function between exercise day and control days, at each clock time. Multiple linear regression analysis automatically provides the proper t-test for this effect within each patient. However, in the presence of a LAR, a difference in lung function should vary with time of day. Therefore, in a second step, the regression model was extended with the **interaction variable** of day and clock time. Again, multiple linear regression analysis automatically provides the proper F-test. If a statistically significant ($p<0.05$) interaction was found in an individual patient, the standard error of the difference between exercise day and control days as estimated in the second regression model, was used to calculate a $100*(0.95^{1/13}) = 99.6\%$ confidence interval for this difference (Bonferroni correction). To verify the occurence of a LAR, a graphical representation of the difference in lung function between the exercise day and the control days for each

clocktime was drawn, together with its 99.6% confidence interval. For a LAR to be present, at least two consecutive time points had to lie outside the confidence interval.
The analysis was performed using the absolute value (liter) of the FEV_1 measurements at each time point as well as the values expressed as % of baseline FEV_1 at the start of the study day.

6.5 Results

Thirteen children completed all 4 study days, and in an additional 2 children data were available on at least one exercise day and both control days. Two children completed two study days only, due to clinical deterioration of their asthma requiring therapeutic intervention, and they were excluded from analysis. There was no significant difference in baseline FEV_1 between the exercise and the control days (mean difference ± SD between EX2 day and H-day: 1.07 ± 4.3 %predicted, p=0.29; between EX2-day and C-day: 0.07 ± 4.3 %predicted, p=0.95). Twelve out of 17 children did not run for the full 6 minutes because of discomfort associated with wheezing already during running. The duration of the exercise test for these children ranged from 3.5 to 5.5 minutes. Room temperature varied from 19°C to 25°C depending on the season. Within the study period of an individual child, room temperature did not vary more than 2°C. Relative humidity of the dry air varied from 4.5% to 9%.

6.5.1 Early asthmatic response

Maximal %fall in FEV_1 during the first hour after exercise or after histamine challenge is shown in table 2. Differences in mean %fall in FEV_1 between the challenges were not statistically significant (mean EAR ± SD: after EX1: 39.7 ± 16 %fall, after EX2: 37.9 ± 15 %fall, and after histamine: 40.9 ± 11 %fall, p>0.05; figure 1).

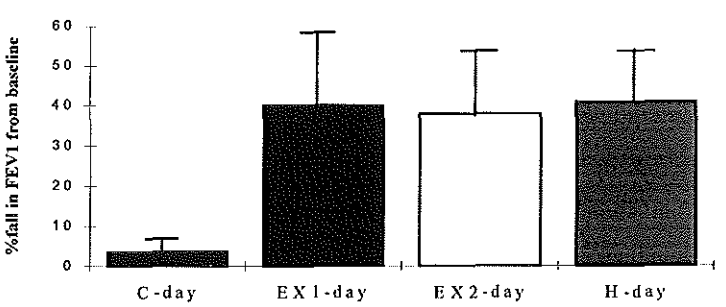

Figure 1: Mean (±SD) %fall in FEV1 from baseline during the first hour for each of the four study days (EX1-day: first exercise day; EX2-day: repeat exercise day; H-day: histamine day; C-day: control day)

6.5.2 The late asthmatic response

Table 2 also shows the individual results of the multiple regression analysis over the period between 2 and 8 hours post-exercise. The effect of day on FEV_1 in this analysis as estimated from the first step (main effects only) is shown for each child. In 10 out of 15 children the difference in FEV_1 between EX2-day and control days, although small, was statistically significant, with FEV_1 either being lower (n=6) or higher (n=4) on the active challenge day as

Table 2: Early asthmatic response (EAR) after exercise and histamine challenge and the occurence of a LAR. The results of the analysis is represented in two parts, with the effect of day only (first step), and the interaction effect of day and time shown separately.

nr	EAR %fall in FEV_1			effect of day‡ on FEV_1 (liter)	p-value¶ interaction effect	effect of day‡ on FEV_1 (% baseline)	p-value¶ interaction effect
	EX1	EX2	H				
1	14	14	25	-0.084§	0.94	-3.54§	0.42
2	16	34	35	-0.077§	0.072	-3.12§	***0.017***
3	24	18	25	+0.126§	0.99	+5.35§	0.99
4	25	35	28	-0.052§	0.58	-0.35	0.99
5	28	20	35	-0.020	1.0	+1.62	1.0
6	34	37	38	+0.029	0.12	-2.65§	0.10
7	35	48	53	-0.229§	0.84	-3.79	0.87
8	40	48	39	+0.027	0.56	+1.38	0.96
9	41	27	40	+0.009	0.60	-5.08§	0.84
10	53	62	49	-0.118§	0.88	-8.70§	0.10
11	54	53	39	+0.087§	0.80	+6.27§	0.76
12	55	30	41	-0.009	0.68	+1.04	0.56
13	58	32	60	-0.089§	***0.049***	-5.40§	***0.040***
14	58	54	53	+0.104§	0.92	+1.10	0.94
17	61	57	54	+0.223§	0.38	+8.83§	0.55
mean	40	38	41				
± SD	16	15	11				

‡ = effect of day: difference in FEV_1 between EX2-day and mean of control days, for given time of the day, as evaluated in the first step of the analysis; § = $p<0.05$ for main effect of day only; ¶ = p-value of the interaction effect between day and time in the second step of the analysis;

compared to control days, for given time of the day. The next column in table 2 shows the results of the second step in the analysis, in which the interaction effect between day and clock time is evaluated. When this analysis was performed using absolute values of FEV_1, a statistically significant interaction effect indicative of a potential LAR was found only in 1 child (nr. 13). Repeating the analysis using FEV_1 expressed as % of baseline resulted in one additional child (nr. 2) showing a potential LAR. In figure 2A and 3A, respectively, the lung function of these two children during the 4 study days is represented, while in figure 2B and 3B, respectively, a graphical representation of the **difference** in FEV_1 measurements between EX2-day and control days for these two children is given together with its 99.6% confidence interval. As can be seen from this figures, for both children, all differences in FEV_1 measurements beween EX2-day and control days are within the confidence interval.

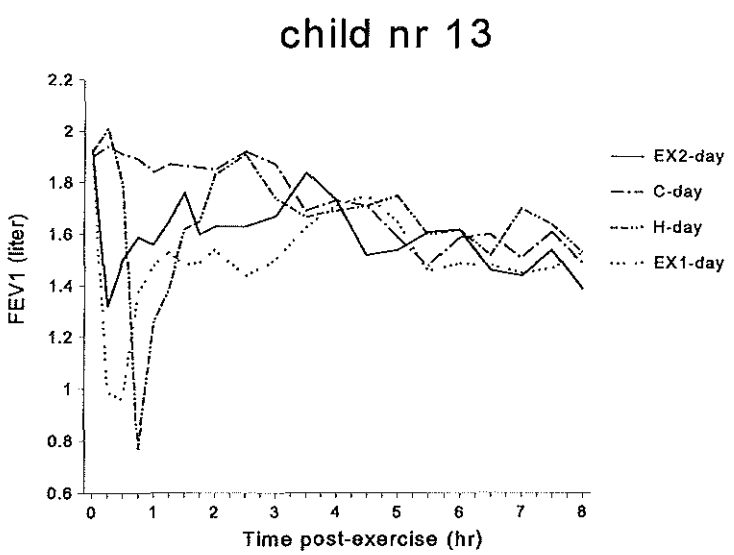

Figure 2a: Lung function measurements (FEV1) during the four study days in child nr. 13

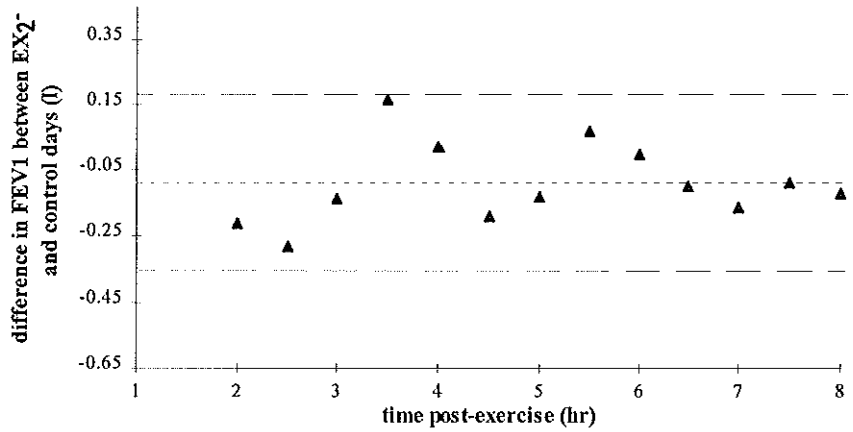

Figure 2b: Difference in FEV1 between EX2-day and control days for each time of the day, with the 99.6% confidence interval, in child nr. 13

6.6 Discussion

In this study we have shown the absence of a late asthmatic response to exercise in children with mild to moderate asthma visiting an out-patient clinic. All children suffered an early asthmatic response to exercise. To identify a LAR accurately, and to take into account individual variability of diurnal variation in lung function, a statistical method was applied using multiple regression analysis per patient, based on serial lung function measurements after exercise challenge and during a positive and negative control day. These data implicate that exercise, unlike allergens, is merely a symptomatic, and not a causative trigger of asthma[35] leading to obstruction but not to inflammation induction.

This is not the first study on the occurence of a LAR after exercise in asthmatic subjects. The results presented are in agreement with those of Rubinstein *et al*[19] and of Boner *et al*[23]. In contrast, other investigators did describe the existence of a LAR after exercise in asthmatic children[11,13,16]. When comparing the results of our study with those published, a number of methodological points need to be addressed.

Firstly, the selection of the population studied may be important. In our study the children were treated for their asthma at an outpatient clinic. Other investigators have used a study group of asthmatic children, resident at special asthma clinics[16,23]. It could be argued that the latter population may have suffered from more severe and uncontrollable asthma, which is often the reason for referral to specialized clinics. Secondly, the exercise provocation method may have influenced the results. In our study, as opposed to others, most children (13 out of 17) breathed dry air during running. Even though inspired dry air is currently the method of

choice[36], we did not use this potentially strong stimulus in 3 children who already showed severe bronchoconstriction when inhaling room air. One could argue that this could mean a difference in the exercise stimulus applied. However, as the fall in FEV_1 postexercise is proportional to the total amount of water lost from the airways[37], we do not think that this has influenced our results, as long as the stimulus remains constant within subjects.

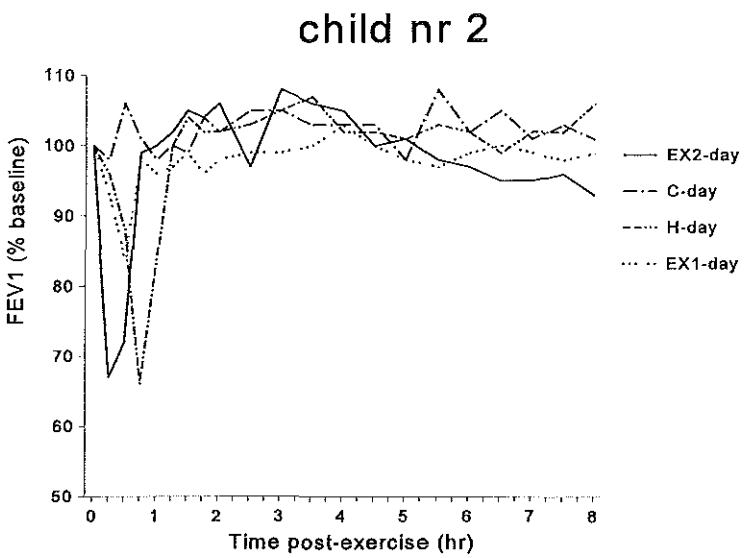

Figure 3a: Lung function measurements (FEV1) during the four study days in child nr. 2

Thirdly, and perhaps more importantly, the designs of the different studies on the LAR after exercise merit special consideration. Among the 13 studies cited[11-23], only 4 have been using randomized study days[16,20-22]. The choice of "adequate" control days to establish normal diurnal variation in lung function can be considered to be of vital importance[24]. Measurements of spontaneous variations in lung function have to be made both after another non-specific challenge, e.g. histamine or methacholine, and during a day without any bronchial challenge. The importance of using adequate control days is illustrated by two studies of Boner *et al*[14,23]. In the first study[14], only one negative control day was used and prevalence of a LAR after exercise was found to be 26%. However, when the study was repeated using multiple control days to account for normal diurnal variation in lung function, the occurence of a LAR after

exercise could no longer be confirmed[23]. Even though in the latter study lung function measurements were performed on both a negative and a positive control day, the present study is the only one in which the bronchoconstriction induced by histamine was carefully matched for degree of bronchoconstriction after exercise.

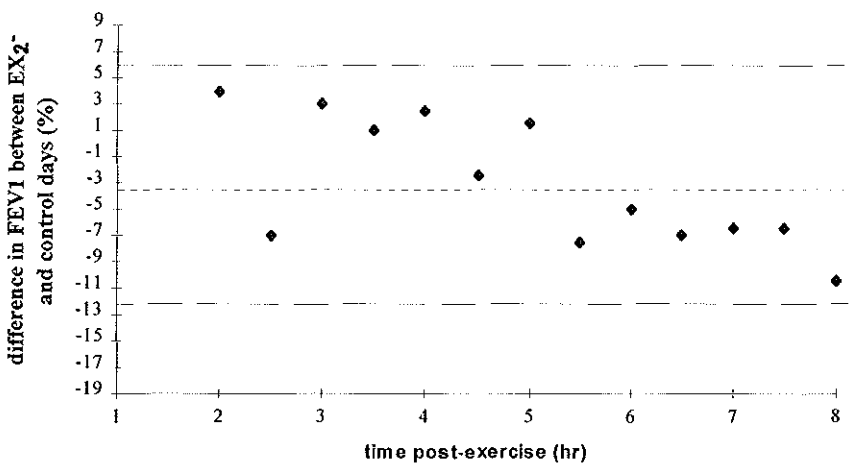

Figure 3b: Difference in FEV1 between EX2-day and control days for each time of the day, with the 99.6% confidence interval, in child nr. 2

Finally, there is considerable lack of agreement among the various investigators about the analysis of a potential LAR. For instance, it has not been clarified during which time period a LAR after exercise might occur. In addition, most authors define the LAR according to a predetermined %fall in lung function, either from pre-challenge value[12,14,15,19-21], or as compared to lung function measured at an identical time point on a control day[16,17,23]. However, these methods are somewhat arbitrarily. They carry the risk of a false positive diagnosis in a subject with considerable spontaneous variability of ventilatory function, or similarly, the risk of a false negative diagnosis if spontaneous variability is low. To eliminate the potential confounding effect of day-to-day variability in pulmonary function when identifying a LAR, it has recently been advocated to use statistical tests to quantify normal diurnal variation[25], thereby allowing recognition of a LAR after challenge with greater precision. Therefore, we have used multiple regression analysis within each patient, taking into account normal diurnal variation

on a positive and a negative control day, to optimise the identification of a LAR after exercise in the patient group studied.

How can the present results be interpreted? Our results show that in out-patients with childhood asthma, in stable conditions, avoiding anti-asthma drugs, a LAR after exercise challenge is absent. This contrasts to allergen provocation, after which a LAR occurs in 50% - 75% of asthmatics challenged[38]. During the allergen-induced late phase reactions, an influx of eosinophils into the bronchial system has been observed[39], also reflected in an increase in serum eosinophil cationic protein (sECP)[40]. The occurence of a post-allergen LAR has also been associated with an increase in bronchial hyperresponsiveness to histamine, while the absence has not[41]. During the early asthmatic reaction after exercise, the different mediators released, such as histamine, prostaglandins and leukotrienes[7,8], resemble those released during the EAR after allergen challenge[42]. However, the absence of a post-exercise LAR implicates that apparently exercise does not induce an inflammatory process, which is indirectly supported by the observation that bronchial hyperresponsiveness to histamine is not increased after exercise provocation[23], as opposed to allergen challenge[43].

What is the clinical significance of these data? Firstly, in the absence of a LAR after exercise, one could argue that it would suffice to treat patients with asthma symptoms occurring only after exercise symptomatically, e.g. with a short-acting bronchodilator[44], disodium cromoglycate or nedocromil[45]. However, it should be reminded that the symptom of EIB in asthma should be interpreted as a sign of bronchial hyperresponsiveness[35]. It has been known for many years that the severity of EIB is moderately correlated to the severity of methacholine-induced hyperresponsiveness[46]. In the current concept of asthma as an inflammatory disease, there are many studies linking bronchial hyperresponsiveness to inflammatory changes in the airways. Therefore, we think it valid to consider regular anti-inflammatory therapy in those patients with moderate to severe EIB[47].

In conclusion, we have shown the absence of a late asthmatic response after exercise in childhood asthma. This supports the concept of exercise being a symptomatic trigger, and thus a reflection of bronchial hyperresponsiveness, instead of a causative factor in asthma. However, more studies are needed in asthmatic children to investigate the relationship between bronchial hyperresponsiveness, EIB and inflammatory changes in the airways.

6.7 References

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chapter 7 chapter

Serum eosinophil cationic protein and bronchial responsiveness to exercise or methacholine in relation to grass pollen exposure in asthmatic children

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submitted

7.1 Summary

Bronchial hyperresponsiveness (BHR) and its changes after allergen challenge are associated with eosinophilic activity in the airways of asthmatic patients. This study was therefore designed to explore the relationship between serum eosinophilic cationic protein (sECP), and BHR to exercise and methacholine in asthmatic children, as well as changes over time in these parameters due to natural grass pollen exposure. A possible modifying effect of inhaled steroids was evaluated concurrently. To that end, measurements of lung function, symptoms, sECP, and BHR to exercise and methacholine were performed in non-steroid and steroid treated grass pollen allergic asthmatic children and their controls, and were repeated during the grass pollen season. The results showed that before the season, the BHR to exercise, but not methacholine, was weakly related to the level of sECP ($r=-0.043$, $p=0.076$) in non-steroid treated children only. In the grass-pollen allergic patients of this group, the rise in sECP in season was associated mainly with the grass pollen RAST class ($p=0.076$), while the change in BHR to exercise and methacholine was also significantly related ($p<0.05$) to the cumulative pollen counts during season. No such associations were found in steroid treated children. Notwithstanding these results, the change in sECP over time for all participating children significantly ($r= -0.35$, $p=0.025$) correlated with the change in BHR to exercise, but not methacholine, irrespective of allergy or treatment used. It is concluded that these data indicate that sensitization and allergen load are both important factors in aggravating BHR in non-steroid treated asthmatic children.Treatment with inhaled steroids protects against allergen-induced inflammatory changes. BHR to indirectly acting stimuli is better related to cellular activity than directly acting stimuli, rendering sECP a potential role in monitoring disease severity in seasonal allergen exposure.

7.2 Introduction

Asthma is a chronic disease characterized by the episodic occurence of cough, dyspnea, wheeze and chest tightness, alone or in combination[1]. One of the key features of asthma is the increased bronchial responsiveness to sensitizing (i.e. allergic) and non-sensitizing (i.e. physical or chemical) stimuli, leading to variable airways obstruction[2]. Although the underlying mechanisms of bronchial hyperresponsiveness in asthma are not fully elucidated yet, its association with eosinophilic airway inflammation is now well established[3,4].

One of the non-sensitizing stimuli to cause episodic symptoms and bronchoconstriction in daily life, is physical activity[5]. The precise pathophysiologic mechanism of such exercise-induced bronchoconstriction (EIB) is still a matter of debate, the major pathways being prima-

rily thermal (airway cooling and/or rewarming) or osmolar (airway hypertonicity) stimulation to the airways[6]. The involvement of different mediators in the reaction sequence has been convincingly established, by direct measurements in serum[7], by inhibiting mediator production[8] or by receptor antagonism[8,9] . The relative contribution of the various cells within the airways to EIB is less clear. Mast cells have not been unequivocally implicated in EIB[10,11], whilst the association between serum eosinophil cationic protein (sECP) and the severity of EIB favours the involvement of eosinophilic airway inflammation[12].

It is known from previous studies, that the bronchial response to exercise is aggrevated after allergen exposure, either in natural[13], or laboratory conditions[14]. Likewise, bronchial hyperresponsiveness to histamine is increased post allergen challenge, which is associated with an increase in circulating eosinophils[15], and in levels of sECP[16] . In view of these findings, it can be postulated that the severity of EIB, as well as its changes over time, are a reflection of the eosinophilic inflammation within the airways of patients with asthma. From a clinical point of view, it would be very useful if such disease activity would be reflected in the level of sECP. Hence, we investigated the relationship between the severity of EIB and the level of sECP, as well as the changes in these variables after natural allergen exposure in asthmatic children before and during the grass pollen season. To that end, we measured the bronchial response to exercise and methacholine, as well as peakflow variability and symptoms, before and during the grasspollen season. Subsequently, we related those clinical characteristics to concomitant measurements of sECP. In order to evaluate the potential interaction of regular treatment with inhaled steroids, the same measurements were performed in asthmatic children with and without such maintenance treatment.

7.3 Materials and methods

7.3.1 Patients

Fortyseven children (30 male, 17 female) were recruited from the outpatient clinic of the Department of Paediatric Pulmonology of the Juliana Childrens' Hospital in The Hague. Mean age of the children was 10.8 year (range 7 - 16 yr), and their baseline FEV_1 was ≥ 70 % predicted (range 70% - 116%)[17]. All children had a history of wheezing or cough with exercise, either longstanding or particularly during the pollen season. They were clinically stable, i.e. no history of viral infections during the two weeks before the challenges, and no hospital admissions or use of oral steroids during the 4 weeks before entry into the study.

The children were subdivided into groups based upon the presence (index group) or absence (control group) of grasspollen allergy (specific RAST class ≥ 2), as well as the presence or absence of maintenance treatment with inhaled steroids. Inhaled steroids had to have been used for at least 6 months before entry into the steroid group, whereas a 3 month period without steroids was an inclusion criterium for the non-steroid group. In this way, four groups were formed. Sodium cromoglycate and nedocromil sodium were stopped two weeks before the first study visit. Rescue medication consisted of inhaled short-acting bronchodilators on demand. Rhinitis symptoms were relieved by local treatment with anti-histamines or steroids only.

Written informed consent was obtained from all children and their parents. The study was approved by the Medical Ethics Committee of the Juliana Children's Hospital.

7.3.2 Study design

The study had a prospective, two-period design, including pre- and in-season measurements. In the first period, before the start of the local pollen season (February through April[18]), the children attended the lung function laboratory on two visits within one week. On the one visit, bronchial hyperresponsiveness to methacholine was determined by standardized dosimetric technique, while on the other visit a standardized exercise challenge was performed for measurement of EIB. Blood was drawn by vena punction for determination of sECP before bronchial provocation was started. Afterwards the children kept a diary to measure peak flow variability, use of rescue medication and asthma symptom score for two weeks. Furthermore, the children already on maintenance treatment with inhaled steroids, were now prescribed a standardized dosage of 600 mcg of beclometasone daily.

In the second study period, during the second half of the pollen season (mid-June through July[18]), the two study visits were repeated in an identical way. Diary cards were now kept during the two weeks prior to the study visits.

7.3.3 Lung function measurements

Spirometric measurements were made using a pneumotachograph (Masterscreen, Jaeger Germany). The highest FEV_1 obtained from three forced expiratory manoeuvres at each time point was retained for analysis[19].

7.3.4 Exercise challenge

Exercise challenge was performed by running on a treadmill (LE 2000, Jaeger Germany) for 4 to 6 minutes[20]. During running, heart rate was continously assessed using a heart rate monitor

(Polar Sporttester), while dry air (at room temperature, relative humidity <15%) was inspired from a reservoir bag through a face mask with an in- and expiratory port (Hans Rudolf Mask). After the first minute at walking pace on the treadmill in order to let the children familiarize themselves with the test, the speed of the treadmill was increased to induce a heart rate of at least 90% of the maximum predicted heart rate for each individual child (maximum heart rate = 210 - age[21]). The children ran for 6 minutes, unless dyspnea made further running impossible. FEV_1 measurements were performed in duplicate before as well as 1, 3, 5, 7, 10 and 15 minutes after the end of exercise. The severity of EIB was expressed as maximal %fall in FEV_1 from baseline (%fall), and as area-under-the-curve (AUC) of the time-response curve (0 - 15 min).

7.3.5 Methacholine challenge

A standardised dosimetric technique was used to perform the methacholine challenge[22]. Methacholine was delivered to the mouth with a Rosenthal-French dosimeter, connected to a DeVillbiss nebulizer type 646. The nebulizer was operated by compressed air at 140 kPa and the timing adjustment of the dosimeter was 0.6 seconds. The dosimeter was triggered to deliver a dose of methacholine to the mouth by slow inhalation (3 - 4 sec) from functional residual capacity to total lung capacity. Each provocation dose was given in 4 inhalations of 5 microliter. Doubling doses of methacholine bromide in 0.9% saline were inhaled, at 5 minute intervals (dose range 3-3200 mcg). Three minutes after inhaling each dose of methacholine, FEV_1 was measured in triplicate, the highest FEV_1 being used in the analysis. When FEV_1 had fallen by more than 20% from baseline value, the induced bronchoconstriction was reversed by the inhalation of 400 to 800 mcg of salbutamol by metered-dose inhaler connected to a spacer. Bronchial responsiveness to methacholine was expressed as PD_{20}methacholine, which was determined by linear interpolation between two data points on the non-cumulative log dose-response curve[22].

7.3.6 Measurement of sECP

Before methacholine challenge, 4 ml of blood was drawn by vena punction, with dripping of blood into the glass tube. Thereafter, it was allowed to clot for 60 ±10 minutes at room temperature. After centrifugation, serum samples were stored by -20°C until further analysis by radio-immuno assay, according to the manufacturer's instructions (Pharmacia Uppjohn[23]).

7.3.7 Diary cards

Symptom scores were recorded on the diary cards for daytime cough, daytime wheeze, and nocturnal awakenings due to asthma over a two week period[24]. Each item could be graded for severity using a 0 to 3 scale (0=none; 1=mild; 2=moderate; 3=severe). Mean symptoms score was calculated by the cumulative sum of scores averaged for the number of recorded days.
Peak flow measurements were made using a miniWright peak flow meter. Measurements were performed in triplicate without using a bronchodilator after rising in the morning, and were repeated in the evening. Mean peak flow varibility (PEFvariability) was calculated as the ratio: [(highest daily PEF - lowest daily PEF) / highest daily PEF], and averaged for the number of recorded days[1].

7.3.8 Recording of pollen exposure during season

The study was performed during the grass pollen seasons of 1992, 1993, and 1995. During the grasspollen season, observations of airborne pollen concentrations were made with the volumetric Burkard® (Richmansworth, U.K.) pollen trap, at the roof of the Leiden University Hospital, about 20 km from The Hague[18]. As it was shown that the airborne allergen fractions of the grass pollens are well correlated to the daily grass pollen counts, the cumulative pollen counts were used as an estimate of the amount of airborne allergen during the season. For each child, the daily pollen counts between the visits of the first and second study period were added to form this cumulative pollen counts.

7.4 Statistical analysis

Values of PD_{20}methacholine were logtransformed before analysis (base 2). sECP was analysed non-parametrically, and logtransformed before use in regression analysis. Differences between groups regarding baseline variables were compared by ANOVA (FEV_1%predicted, PD_{20}methacholine, severity of EIB, PEFvariability), or, non-parametrically by using Kruskal-Wallis analysis (sECP and mean symptom score).

The influence of allergen exposure on eosinophil activity, lung function parameters as well as symptoms was examined by comparing pre- and in-season measurements. In each of the four distinct groups, the values of %fall, AUC, PD_{20}methacholine, sECP, FEV_1 %predicted, PEFvariability and symptom score obtained pre- and in-season, were tested pairwise using Wilcoxon signed rank sum test. In this analysis, the children not allergic to grass pollen served as control subjects.

Subsequently, the effect of grass pollen exposure was studied in sensitized individuals. In analogy to allergen challenges performed in the laboratory[25], it was hypothesized that the effects of natural allergen exposure would be a function of the quantity of antigen (i.e. cumulative pollen counts) as well as the degree of sensitization to that allergen (i.e. grass-pollen RAST-class). To study these effects on sECP, and on bronchial responsiveness to exercise and methacholine, a multiple regression analysis was applied on the data of the grass pollen allergic children. To that end, the level of the outcome variable (i.e. ln(ECP), AUC or PD_{20}methacholine) during season was taken as the dependent variable, with both the (log-transformed) cumulative pollen counts, and the grass pollen RAST class as independent variables, and the pre-season level of the outcome variable as covariate. The simultaneous effects of cumulative pollen counts and RAST class were studied by testing the significance of both variables together, the nullhypothesis being that the effects of these two parameters will be simultaneously equal to zero. The analyses were performed separately for the non-steroid and steroid treated grass pollen allergic children to explore the possibility of effect-modification by inhaled steroids.

To evaluate the potential of sECP as a clinical marker of disease activity, the cross-sectional relationship between the level of sECP and the severity of EIB pre-season and in-season, was investigated using multiple linear regression analysis. The AUC was taken as the dependent variable, with the concomitant level of sECP (log-transformed), the type of treatment used (steroid or nonsteroid), and their interaction term, as independent variables. A possible relationship between EIB and sECP was also studied longitudinally, by relating the change in ln(ECP) during season to the observed change in EIB. Again using a multiple regression model, the severity of EIB in-season was now the variable to be explained, with change in ln(ECP) as explanatory variable, as well as treatment used and their interaction term, while the pre-season level of EIB was considered as covariate.

Likewise, the existence of associations between (changes in) ln(ECP) and (changes in) bronchial responsiveness to methacholine was studied.

For all analyses, P-values < 0.05 were considered statistically significant.

7.5 Results

The characteristics of the 4 study groups are presented in table 1a (non-steroid treated children) and table 1b (steroid treated children). Between groups, no significant differences were found with respect to baseline variables.

Table 1a: Pre-season and in-season measurement values (mean ±SD) of lung function parameters, sECP and symptom score of the **non-steroid** treated children.

grass pollen allergy	POSITIVE		NEGATIVE	
time point of season	PRE	IN	PRE	IN
number	10	10	12	8
age (years)	10.2 ±2.9		10.9 ±2.7	
FEV_1 (%predicted)	95.0 ±10.2	94.8 ±10.8	91.6 ±9.9	88.3 ±10.7
PD_{20}metacholine(mcg)†	40.7 ±1.7	36.6±1.6	58.7 ±1.2	56.4 ±1.4
EIB (%fall, %)	22.4 ±14.1	21.5 ±11.3	22.6 ±8.1	22.8 ±6.5
EIB (AUC, %.min)	1298 ±143	1272 ±157	1273 ±139	1308 ±126
ECP (mcg/l)††	9.7 (4.4-20)	17.9 (2.7-62)¶	8.8 (2.9-14.9)	8.4 (4.3-26.5)
symptom score††	0.18 (0- 2.4)	0.75 (0.1-3.3)¶	1.21 (0- 4.4)	1.32 (0- 2.6)
PEFvariability (%)	12.2 ±5.6	12.1 ±4.0	16.3 ±5.4	15.4 ±3.9

†= geom. mean ±doubling doses; ††= median (range);

¶: $p<0.05$ when compared to pre-season value;

Table 1b: Pre-season and in-season measurement values (mean ±SD) of lung function parameters, sECP and symptom score of the **steroid** treated children.

grass pollen allergy	POSITIVE		NEGATIVE	
time point of season	PRE	IN	PRE	IN
number	10	9	15	15
age (years)	10.6±3.2		11.3±1.8	
FEV_1 (%predicted)	95.5±11.1	95.3±10.5	90.3±8.2	92.4±7.8
PD_{20}metacholine(mcg)†	35.2±1.1	42.5±1.8	42.6±1.1	29.6±1.2
EIB (%fall, %)	19.7±13.0	17.8±10.0	28.1±8.3	30.9±14.7
EIB (AUC, %.min)	1315±172	1331±117	1184±128	1214±144
ECP (mcg/l)††	9.4 (2.2-18.6)	12.9 (1.9-40)	11.4 (5-18.3)	13.3 (3-133)
symptom score††	1.0 (0- 4.4)	1.43 (0- 3.6)	0.5 (0- 1.7)	0.54 (0- 1.8)
PEFvariability (%)	14.2±4.8	15.0±5.1	12.8±3.9	14.7±8.5

†= geom. mean ±doubling doses; ††= median (range);

Five children were unable to attend for the repeat tests in-season due to intercurrent respiratory tract infections, while one child attained other commitments during the second study period.

After allergen exposure, in the non-steroid-using, grass pollen allergic children, a statistically significant increase in symptoms (p=0.028) as well as in sECP (p=0.037) was found in-season as compared to pre-season, whereas such an increase was not found in the other three groups. When further comparing pre- and in-season measurements of EIB (%fall and AUC), PD_{20}methacholine, FEV_1%predicted and PEFvariability, no significant changes were found in any group (table 1a, 1b).

The potential associations between the severity of allergen exposure and changes in lung function parameters as well as sECP, were examined in grass pollen allergic children only. The cumulative pollen counts the children were exposed to, ranged from 3143 up to 8960 (grains/-m3), while the childrens' RAST-class ranged from 2 to 5. The effects (β) of the cumulative pollen counts and RAST on the outcome variable (ln(ECP), PD_{20}methacholine and AUC), as estimated from the multiple lineair regression analyses, are shown in table 2.

Table 2: Estimated effects of the cumulative pollen counts and the RAST class on eosinophilic inflammation and bronchial responsiveness to exercise and methacholine

NON-STEROID	estimated effect (β) of:				
outcome variable	pre-season level	ln(pollen)	RAST class	R	p-value test§
ln(ECP)	+0.50	+0.19	+0.75†	0.72	0.165
^{2}logPD_{20}	+0.73‡	- 1.67‡	- 0.78‡	0.97	0.012
AUC(n=10)	- 0.03	- 180	- 94	0.57	
AUC (n=9)	+0.31	- 236‡	- 142‡	0.83	0.054

R: multiple correlation coefficient;

§: test for the effects of ln(pollen) and RAST to be simultaneously equal to zero (see text);

†: p=0.069; ‡: p<0.05;

From these analyses it appeared that the in-season level of ln(ECP) was related mainly to the RAST class (β=+0.74, p=0.069), meaning that if the RAST class is increased by 1, the serum ECP level will increase with a factor $\exp^{0.74}$. However, the in-season level of PD_{20}methacholine was not only related to the RAST class ($p<0.05$), but also to the cumulative pollen counts during season ($p<0.05$), the multiple correlation coefficient R being 0.97. Hence, the ^{2}logPD_{20}methacholine in season decreases with approximately 1.67 points if ln(pollen) increases with 1 (equal to a trebling of the cumulative pollen counts), while an increase in RAST class by 1 will result in a decrease in the ^{2}logPD_{20}methacholine in season by 0.78 points.

The in-season severity of EIB was studied next, but no relationship was found with any of the three independent variables. This was most likely due to the results of one patient, who did not show EIB in season, despite an increased bronchial responsiveness to methacholine and a rise in sECP. When excluding this patient from the analysis, the bronchial responsiveness to exercise in season was again significantly related to both the RAST class and the cumulative pollen counts (table 2). The magnitude of effect was such that if ln(pollen) should increase by 1, the AUC will decrease with 236 %.min (95%CI 56-416), while an increase in RAST class of 1 will consequently decrease the AUC with 142 %.min (95% CI 32-252).

When the analyses of the above relationships were performed in the steroid-treated grass pollen allergic children, only the association between RAST and AUC in-season was borderline significant (β= -79 [95% CI 18-139]; p=0.063). No other associations were found between in-season measurements of sECP or bronchial responsiveness to methacholine and RAST respectively ln(pollen) in this group of children.

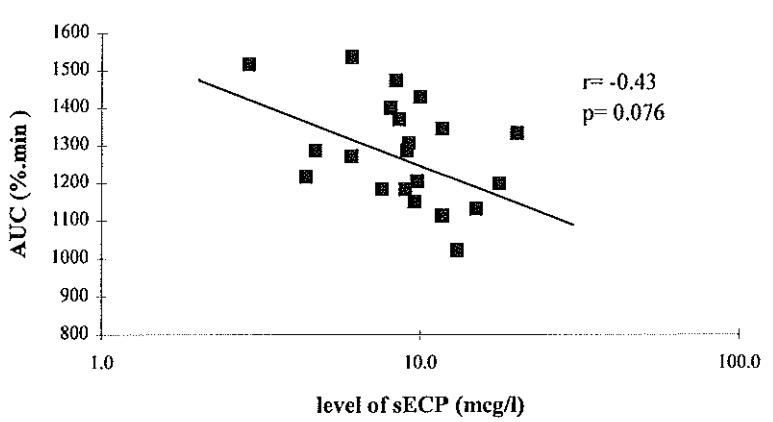

Figure 1: Relationship between the pre-season level of sECP and the severity of exercise-induced bronchoconstriction in non-steroid treated children

Subsequently, sECP was explored as a clinical marker of disease severity. Cross-sectional analysis of the pre-seasonal data of the total study population showed a weak, borderline significant correlation between the severity of EIB expressed as AUC, and the level of sECP in the non-steroid treated children (r=-0.43, p=0.076; figure 1), but not the steroid-treated children. In-season, this association was no longer evident. However, when relating the change in lnECP during season to the change in AUC, a small, but statistically significant (p=0.025) effect was found, irrespective of the treatment used. For example, if the sECP in-season had risen to three times the pre-season level, the AUC was found to decrease with 70 %.min (95% CI: 11-130; figure 2).

The level of sECP was not related to the PD_{20}methacholine, neither cross-sectionally, nor longitudinally, nor when evaluating the two treatment groups separately.

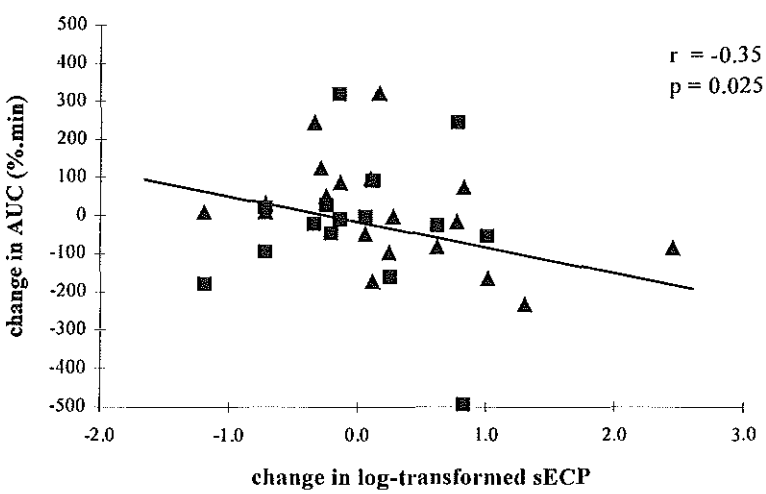

Figure 2: Individual change over time (in-season minus pre-season level) in exercise-induced bronchocosntriction (expressed as AUC), plotted against the change in the log-transformed level of sECP (a difference of (x) denotes that the pre-season level of sECP should be multiplied with $e^{(x)}$ to obtain the in-season level, i.e. (x) > 0: increase in sECP, (x) < 0: decrease in sECP; ■ = non-steroid treated children; ▲ = steroid treated children)

7.6 Discussion

In this study, we have shown that in stable, non-steroid treated, mild to moderate asthmatic children with perennial symptoms, bronchial responsiveness to exercise but not methacholine, is weakly related to the eosinophilic inflammation as measured by sECP in the blood. During natural allergen exposure, both symptoms and serum level of ECP increase significantly in-season in non-steroid, grass pollen allergic children. Individual changes in sECP appear to be related mainly to the degree of sensitization to grass pollen, whereas changes in bronchial responsiveness to exercise and methacholine during the grass pollen season are also influenced by the total amount of grass pollen allergens. Similar results can not be found in steroid-treated children, suggesting a modifying effect of these drugs against inflammatory changes induced by natural allergen exposure. When evaluating sECP as a clinical marker of disease activity, only the change in EIB is related to the change in eosinophil activity as measured by sECP. These results suggest that in asthmatic children, bronchial responsiveness to indirectly acting stimuli is better related to cellular activity than bronchial responsiveness to directly acting stimuli.

Ours is a prospective study, investigating the effect of environmental allergen exposure on inflammatory mediators and bronchial hyperresponsiveness in asthmatic children with perennial and seasonal allergies. The rise in symptoms and sECP after exposure to grass pollen in non-steroid treated, grass pollen allergic children, is in agreement with results described in adults[26]. In our study, this individual seasonal change in eosinophil activity was associated mainly with the sensitization to grass pollen antigen. In contrast, it was shown that the extent of change in bronchial hyperresponsiveness to exercise and methacholine is influenced not only by the degree of sensitization, but also by the cumulative dose of allergen one is exposed to during season. While in keeping with studies showing a reduction in bronchial hyperresponsiveness when asthmatic children are transferred to environments with little or no allergen exposure[27], our results are more elaborative in describing dose-effect relationships between antigen exposure, sensitization level and subsequent changes in bronchial hyperresponsiveness.

One of the consequences of eosinophilic activity is likely to be bronchial hyperresponsiveness to exercise, as the degree of (pre-season) EIB was related, albeit weakly, to the (pre-seaon) level of sECP. In addition to this cross-sectional relationship, which is in keeping with results described for adults[12], it was subsequently shown that the changes over time for sECP and EIB are also related, irrespective of the treatment used. Like others[28], we failed to demonstrate such relationships for sECP and bronchial hyperresponsiveness to methacholine.

When comparing our results with those published, a number of methodological issues need to be adressed. Firstly, in order to reduce the number of visits for the children, measurements were made only once before and once during season. Consequently, it is not known whether sECP, EIB and PD_{20}methacholine were measured at the time-point of maximal change in season. Although a relationship was found between the cumulative dose of allergen during the season and changes in the afore mentioned variables, it cannot be excluded that these changes are mainly due to the degree of allergen exposure in the last two or three weeks before the measurements.

Secondly, blood for measurement of sECP was drawn at the first visit before bronchial provocation took place. However, in order to prevent the occurence of excessive bronchoconstriction post-exercise (i.e. >50% fall in FEV_1), methacholine challenge was performed first, as its result allowed us to "predict" the degree of EIB for each child, because the responses to the two tests are related[29]. Consequently, it could be that the observed, relative weak relationship between sECP and EIB, when compared to adults[12], may have been influenced by the greater time interval between sECP measurements and exercise testing.

Thirdly, some children used intranasal beclomethasone (nBDP) for treatment of rhinitis symptoms before and during the study. We do not feel this has influenced our results, nBDP being used already prior to entry. Also, the use of nBDP does not block the increase in methacholine responsiveness during seasonal allergen exposure[30].

Finally, there is no consistency in the literature regarding the analysis of sECP[26,28,31,32]. Because our data tended to be non-Gaussian distributed, just as in other studies[28,33], either non-parametrical tests were used or natural logtransformation applied[34]. When exploring sECP as a marker of disease activity, many studies have analysed the relationship between sECP and airway function cross-sectionally[12,16,28,31], with few studies attempting to relate their longitudinal changes to each other[32].

How can the present results be interpreted? The rise in sECP in season, in keeping with observations in adults[16], very likely reflects the post-allergen increase in eosinophilic activity, as described for laboratory allergen challenges[15]. In the latter condition, an increased number and/or activity of eosinophils post-allergen was shown in the airways, followed by an increased bronchial hyperresponsiveness. Our data suggest that, allthough being sensitized to allergen suffizes to activate eosinophils, exposure to increased amounts of allergen is equally important in aggravating bronchial hyperresponsiveness in sensitive asthmatic children. Hence, it appears

that sECP is more a reflection of allergen sensitization, and allergen-induced eosinophilic inflammation, than of bronchial responsiveness.

Notwithstanding, when using sECP for monitoring the eosinophilic activity in relation to disease severity, our data show that its change over time is more clearly related to changes in airway responsiveness to exercise than to methacholine. This underlines the concept that cellular driven, indirectly acting stimuli of bronchial hyperresponsiveness[35] are more suitable to monitor seasonal variations in disease severity than directly acting stimuli, which may better reflect the long-term course of the disease[36] and the airway remodelling in chronic disease[37].

What is the clinical significance of these data? Firstly, in asthmatic children, treated with inhaled symptomatic therapy only, a high level of sECP is a risk factor for the existence of exercise-induced bronchoconstriction. However, a normal level of sECP does not exclude EIB. Secondly, cumulative exposure to natural grass pollen leads to increases in symptoms and sECP in children allergic to grass pollen. The change in hyperresponsiveness is determined by the total pollen counts during a season as well as the degree of sensitization to grass pollen, whereas the change in the level of sECP is mainly related to the grass pollen RAST class. Although the change in sECP is only partially related to increases in bronchial responsiveness, monitoring sECP as a parameter of disease activity during (natural) allergen exposure, could be potentially useful. Steep increases in sECP during season reveal patients at risk for developing increased bronchial reactivity. Our results indicate that the use of maintenance treatment with inhaled steroids is effective in preventing seasonal exacerbations due to grass pollen exposure in perennial asthmatic children. Despite steroid treatment, a changing sECP as a reflection of eosinophil activity is still associated with subsequent changes in bronchial responsiveness to exercise. However, effects are small, and further studies are needed to establish the clinical significance of sECP as a potential marker of disease severity.

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chapter **8** chapter

Dose-response effects of an inhaled corticosteroid (fluticasone propionate) in reducing exercise-, and methacholine-induced bronchoconstriction during long-term treatment in asthmatic children

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submitted

8.1 Summary

In adult asthmatic patients, short-term treatment with high dose inhaled steroids induced similar reductions in the severity of histamine-, and exercise-induced bronchoconstriction (EIB). In the present study, we investigated in children with asthma, whether the inhibitory effect of fluticasone propionate (FP, 100 and 250 mcg b.d.) on exercise- and methacholine-induced bronchoconstriction is time- and dose-dependent. 37 asthmatic children, aged 6 to 14 years, and with a forced expiratory volume in one second (FEV_1) greater than 70% of predicted, participated in a double-blind, placebo-controlled parallel group study. At entry, none of the children had used inhaled steroids in the past 4 months, and all showed at least 20% fall in FEV_1 from baseline after a standardized treadmill exercise test using dry inspired air. During the study, children receiving placebo were re-randomized to active treatment after 6 weeks. Standardized dry air exercise testing and methacholine challenge using a standardized dosimetric technique were performed repeatedly during the 24 weeks of treatment. Concomitant measurements of serum eosinophil cationic protein (sECP) were performed, to evaluate its potential role in monitoring treatment efficacy. The severity of EIB significantly ($p<0.05$) decreased compared to placebo within 3 weeks, the geom. mean %fall being reduced from 34.1 (%) to 9.9 (%) for 100 FP, and from 35.9 (%) to 7.6 (%) for 250 FP. Reductions in EIB were sustained throughout the study period. Bronchial responsiveness to methacholine significantly improved within the first 6 weeks as compared to placebo ($p<0.04$). Thereafter, the PD20methacholine steadily increased with time in both treatment limbs ($p=0.04$), the difference in improvement (100 FP: 1.6 dose steps; 250 FP: 3.3 dose steps) approaching significance after 24 weeks of treatment ($p=0.06$). In contrast, the level of sECP did not change significantly during treatment. We conclude that the protection of inhaled fluticasone propionate against methacholine- and exercise-induced bronchoconstriction in childhood asthma, is time- and dose-dependent, supporting a hypothesis of different modes of action of steroids in protecting against directly and indirectly acting stimuli.

8.2 Introduction

Asthma is a chronic inflammatory disorder of the airways, characterised by variable airways obstruction over time[1]. Fluctuations in airway patency can occur either spontaneously or in reaction to bronchoconstrictor stimuli. The degree of response to such stimuli is a measure for the severity of the underlying bronchial hyperresponsiveness, currently thought to be a hallmark of asthma[2]. The bronchoconstrictor stimuli can either directly trigger the bronchial

smooth muscle in the airway wall, or act indirectly through infiltrative or resident pulmonary cells or neural pathways[3].

Exercise, a physiologic trigger of acute, usually short-lived asthma attacks in daily life, is an example of a very common, indirectly acting bronchoconstrictor stimulus[4]. Prevalence of exercise-induced bronchoconstriction (EIB) among atopic children with asthma rates very high, ranging to almost 90%[5], thereby limiting daily life activities of many patients. The precise mechanisms underlying EIB have not been fully clarified yet, the stimulus to the airways potentially being thermal (airway cooling and/or rewarming) or osmolar (drying of the airways)[6]. The bronchoconstrictor response itself is most likely due to the release of mediators[7,8], although neural activity is also involved[9] . A strong association was found between the level of serum eosinophil cationic protein (sECP) and the severity of EIB in adults[10], suggesting a role for the eosinophil in EIB.

One of the aims of asthma treatment is to enable the patient to participate in daily activities without limitations[1]. Current guidelines advocate the use of anti-inflammatory treatment, as soon as symptoms occur more than once a week[1]. Inhaled glucocorticosteroids are currently the most effective drugs for treatment of asthma[11]. They suppress airway inflammation[12] , protect against allergen-induced inflammatory changes[13], and reduce bronchial hyperresponsiveness to the different bronchoconstrictor stimuli[11]. In adult asthmatic patients, short-term treatment with inhaled steroid induced similar reductions in severity of histamine-, exercise- and eucapnic hyperventilation-induced bronchoconstriction[14]. However, only one, relatively high dose of inhaled steroids was studied, so that dose-response effects of steroids on histamine-[15] and exercise-induced bronchoconstriction[16] remain unknown. In asthmatic children using maintenance treatment with inhaled steroids, an ongoing improvement in bronchial hyperresponsiveness to histamine over a 22 month period was seen[17], while in that same study population, maximal inhibition of EIB already reached a plateau within 2 months[18]. This suggests that the effects of inhaled steroids on bronchial responsiveness to directly and indirectly acting stimuli are time- and dose-related, potentially due to the involvement of different modes of action.

In the present study, we investigated in children with asthma, whether the inhibitory effect of inhaled steroids on exercise- and methacholine-induced bronchoconstriction is time- and dose-dependent. To that end, we measured the efficacy of fluticasone 100 mcg b.d. and fluticasone 250 mcg b.d. in reducing EIB and bronchial hyperresponsiveness to methacholine during 24 weeks of treatment using a placebo-controlled parallel group study design. In addition, the association between the severity of EIB and concomitant measurements of serum eosinophil

cationic protein (sECP) was investigated, to evaluate a potential role of sECP in monitoring treatment efficacy[19].

8.3 Materials and methods

8.3.1 Patients

Thirtyseven children (23 male, 14 female) clinically diagnosed as having asthma[1], were recruited through the Department of Community Youth Health Care in The Hague as well as from general practitioners and hospital out-patients clinics in The Hague and Zoetermeer. Mean age of the children was 10.3 years (range 6 - 14 year), and their baseline forced expiratory volume in 1 second (FEV_1) was ≥70% of predicted (table 1)[20]. All children suffered from exercise-induced bronchoconstriction, as shown by a fall in FEV_1 of at least 20% from baseline after a standardized screening exercise test. They were clinically stable, i.e. no history of viral infections during the two weeks before screening, and no hospital admissions or use of oral steroids during the 4 weeks before entry into the study. Thirtytwo children were atopic to at least one inhalant allergen, as shown by a positive RAST class ≥2. Nineteen children had never been treated with inhaled steroids before, while thirteen children had used inhaled steroids previously, but not in the four months prior to entry into the study. Five children used sodium cromoglycate or nedocromil as maintentance treatment, which was stopped at the screening visit. Use of intranasal and dermatological steroids, as well as ophthalmological cromolynes was allowed providing dose and frequency were not changed during the study period.

The study was approved by the Medical Ethics Committees of the two participating hospitals, and written informed consent was obtained from all children and their parents.

8.3.2 Study design

The study had a double-blind, placebo-controlled design with a treatment period of 24 weeks. After a run-in period of two weeks using inhaled salbutamol only, the children were randomly allocated into three treatment groups to receive either fluticasone propionate FP 100 mcg b.d., or fluticasone propionate FP 250 mcg b.d. or a matched placebo, using a metered-dose inhaler attached to a Volumatic™ spacer. Six weeks after randomization, the placebo group was re-allocated in random order, to receive active treatment for the remaining 18 weeks of the study. The children attended the lung function laboratory at screening and at entry, as well as 3 and 6 weeks after randomisation, and thereafter every 6 weeks until 24 weeks had elapsed. At each visit at approximately the same time of day, a standardized exercise challenge test was performed by running on a treadmill while breathing dry air. At entry, as well as 6, 18 and 24 weeks

after randomization, bronchial hyperresponsiveness to methacholine was measured. Exercise and methacholine challenge were separated by no more than 7 days. Venous blood for measurement of sECP was drawn before each exercise provocation, except at screening and at 12 weeks after randomisation. During the whole study period, nocturnal and day time symptoms (cough, wheeze, shortness of breath and exercise-related symptoms), rescue bronchodilator medication as well as peak flow measurements were recorded. Rescue therapy for relief of acute asthma symptoms consisted of inhaled salbutamol on demand (max. 1600 µg per day).

In addition, at each clinic visit height, body weight, heart rate, systolic and diastolic blood pressure were measured. Furthermore, the patients were asked for adverse events. Treatment compliance was checked after the study without the subjects knowledge, by dividing the actual weight loss of the canisters by the expected weight loss.

Table 1: Baseline characteristics of the treatment groups at entry

	placebo	100 FP	250 FP
number	12	11	14
age (years)	9.8±2.4	9.9±1.6	11.1±2.4
duration of asthma (yr)	5.2±4.3	5.8±3.6	7.5±3.9
FEV1%predicted (%)	92.1±12.5	96.6±6.9	93.2±13.3
reversibility (%)¶	8.0 (3-23)	7.0 (0-29)	9.0 (-3-27)
EIB: %fall (%)†	$33.2*e^{\pm 0.32}$	$34.1*e^{\pm 0.37}$	$35.9*e^{\pm 0.35}$
: $AUC_{0\text{-}30}$ (%.min)†	$764*e^{\pm 0.39}$	$764*e^{\pm 0.34}$	$855*e^{\pm 0.49}$
PD_{20}methacholine (µg)‡	26.4±1.5	26.6±1.0	24.7±1.5
ECP (µg/l)†	$12.5*e^{\pm 0.96}$	$17.1*e^{\pm 0.53}$	$17.7*e^{\pm 0.95}$

(mean±SD); ¶=median (range);†= geom. mean$*e^{\pm SD}$; ‡=geom. mean ±doubling doses;

8.3.3 Lung function measurements

Salbutamol was withheld for at least 8 hours before lung function measurements or bronchial provocation tests were performed. Spirometric measurements were made using a pneumotachograph (Masterscreen, Jaeger Germany). At each visit, baseline lung function was determined as the highest FEV_1 obtained from three forced expiratory manoeuvres[21]. At screening, the postbronchodilator FEV_1 was also measured[22]. Thirty minutes after exercise provocation,

800 µg salbutamol was administered by MDI attached to a Volumatic spacer. Twenty minutes afterwards, FEV_1 (best of three) was measured, with reversibility expressed as: $\{(FEV_1 postbronchodilator - FEV_1 prechallenge)/FEV_1 prechallenge\} * 100\%$.

8.3.4 Exercise challenge

Exercise testing for measuring severity of EIB was performed using a standardized protocol[23]. Exercise challenge was performed only if pre-exercise FEV_1 was ≥70% of predicted and not under 20% of a patients' baseline FEV_1 at entry. A motor driven treadmill was used for running (LE 2000, Jaeger, Germany or Tunturi J880, Finland). During the test, heart rate was contimuously monitored by a radiographic device (Polar Sport Tester), and dry air (relative humidity $< 10\%$) was inspired during running, according to recent recommendations[24]. Dry air was obtained by pressurized medical air, and collected in a Douglas bag (contents 150 liter). It was inhaled by the child through the mouth, using a face mask (Hans-Rudolph) with an in-and expiratory port, with the nose in a separate compartment. The incline of the treadmill was set at 5 to 10%, depending on the physical condition of the child. During the first minute of the test, the children walked at slow speed to familiarize themselves with the procedure. The speed of the treadmill was subsequently adjusted to induce a heart rate ≥ 90% of the predicted maximum (approx. 210-age) by the third minute of the test. Having reached the target heart rate, the children were coached to run for another three minutes, unless discomfort due to dyspnoea made further running impossible. FEV_1 was measured in duplicate at 1, 3, 5, 7.5, 10, 15, 20 and 30 minutes post-exercise, with the best FEV_1 at each time point retained for analysis.

The severity of EIB was expressed as maximal %fall in FEV_1 from baseline (%fall), and as area-under-the-curve (AUC) of the time-response curve (0 - 30 min).

8.3.5 Methacholine challenge

Methacholine challenge was performed using a standardised dosimetric technique[25]. A De-Villbiss nebulizer type 646, connected to a Rosenthal-French dosimeter, was operated by compressed air at 138 kPa, with the timing adjustment of the dosimeter set at 0.6 seconds. The dosimeter was triggered to deliver a dose of methacholine to the mouth by slow inhalation (3 - 4 sec) from functional residual capacity to total lung capacity. Doubling doses of methacholine bromide in 0.9% saline were inhaled, at 5 minute intervals (dose range 3-3200 mcg), each provocation dose given in 4 inhalations of 5 µliter. FEV_1 was measured as the response 30 and 90 seconds after inhalation, the lowest, technically satisfactory FEV_1 used for analysis[24].

When FEV1 had fallen by more than 45% from baseline value, or a plateau was reached on the dose response curve (defined as three subsequent responses to doubling doses of methacholine within a 5% range), the induced bronchoconstriction was reversed by the inhalation of 400 to 800 mcg of salbutamol MDI connected to a Volumatic™ spacer. Bronchial responsiveness to methacholine was expressed as PD_{20}methacholine, which was determined by linear interpolation between two data points on the non-cumulative log dose-response curve[25].

8.3.6 Measurement of sECP

Before exercise testing, 4 ml of blood was drawn by vena punction, with dripping of blood into the glass tube (SST Becton-Dickinson). Thereafter, it was allowed to clot for 60 ±10 minutes at room temperature. After centrifugation, serum samples were stored by -20°C until further analysis by ELISA-technique/assay, according to the manufacturer's instructions (Pharmacia Upjohn[26]).

8.3.7 Diary cards

During the entire study period, absence or presence of symptoms was recorded on the diary cards for both nocturnal and daytime cough, wheeze, and shortness of breath, with symptoms related to exercise recorded for daytime only. Each item could be graded for severity using a 0 to 3 scale (0=none; 1=mild; 2=moderate; 3=severe)[27]. Daytime and nighttime use of rescue salbutamol was noted as well.

Peak flow measurements were made using a miniWright peak flow meter. Measurements were performed in triplicate without using a bronchodilator after rising in the morning, and in the evening.

8.4 Statistical analysis

FEV_1 [20] and PEFR[28] values were expressed as percentage of the predicted value (%pred). Values of PD_{20}methacholine were used after logarithmic transformation (base 2). Because the data of %fall, AUC and sECP were non-Gaussian (skewed to the right) distributed, log-transformation (natural logarithm) was applied before using parametric tests, or, when using the raw data, non-parametric tests were applied.

Dose-related effects of inhaled steroids during short- and long-term treatment were studied for the following outcome variables: severity of EIB (%fall and AUC), bronchial responsiveness to methacholine, FEV_1%predicted, morning and evening PEFR, inflammatory mediators

(sECP) and symptoms. Daily symptom scores of cough, wheeze, shortness of breath and exercise-related complaints were each dichotomized as no/yes. Subsequently they were each averaged over a 6 week treatment period, and expressed as percentage of symptomfree days upon which a logit transformation was applied.

Baseline variables were compared between treatment groups at entry into the study. Deterioration of asthma while using placebo treatment was evaluated by within-group comparison of variables at entry and after 6 weeks of treatment using Wilcoxon signed rank sum test. Provided baseline variables did not differ at entry and after 6 weeks of placebo treatment, the data of the placebo-group after re-randomization were pooled with the data of the two active treatment groups. In this way, the power of the study in detecting dose-related differences in protective effect of inhaled steroids during long-term treatment was enhanced.

To analyse the data of the first 6 weeks of treatment, ANCOVA was used to evaluate treatment efficacy against placebo, with therapy as between-patient grouping factor, and the baseline measurement of the outcome variable as covariate. The protection index (PI) was expressed as: $([EIB_{\text{on placebo}} - EIB_{\text{on active treatment}}]/EIB_{\text{on placebo}})$. Dose-related differences in protective effect of inhaled steroids specifically on exercise- and methacholine-induced bronchoconstriction during these first 6 weeks, were also evaluated in an alternative analysis. Based on previous reports in adults[14], the reduction in %fall (Δ%fall in FEV1) to exercise and to a single dose of methacholine was compared pre- and post-treatment, the dose of methacholine pre-treatment thus chosen, to match the pre-treatment %fall post-exercise to within 10% fall in FEV1. Thereafter, the differences in effect of inhaled steroids on both stimuli were analysed non-parametrically, using the Wilcoxon signed rank sum test for within-group comparisons, and the Mann-Whitney U test for between-group comparisons.

Subsequently, 24 weeks of active treatment were evaluated for dose-response related differences in treatment efficacy during long-term treatment. To that end, a potential difference in efficacy between the two active treatment groups was explored by repeated measures ANOVA. Therapy was again taken as the between-patient grouping factor, and baseline measurement of the outcome variable as a fixed covariate, while time was added as within-patient factor, as well as the interaction effect of time and therapy.

To evaluate a potential role of sECP in monitoring disease severity, the level of sECP at each visit was related to the concomitant measurement of EIB, as well as peak flow values and symptoms during the two following weeks, using multiple regression analysis.

For all analyses, p-values ≤ 0.05 were considered statistically significant.

8.5 Results

One subject in the placebo group was withdrawn after 12 weeks of treatment because of non-compliance. In the analysis, this subject was included up to the visit prior to withdrawal. At entry, the three treatment groups were in balance with respect to age, sex, duration of asthma, lung function parameters, level of bronchial responsiveness, and serum inflammatory mediators (table 1). The placebo-treated group did not change significantly with respect to any of these variables during the first 6 weeks of treatment (table 2). Therefore, data of the placebo group after re-allocation to active treatment were pooled with data of the active treatment groups.

8.5.1 Treatment effects on bronchial responsiveness

Three weeks after starting active treatment, severity of EIB (%fall: figure 1; AUC: figure 2) was significantly ($p<0.05$) improved when compared to placebo, for both 100 FP and 250 FP (table 2), the geom. mean %fall being reduced from 34.1 (%) to 9.9 (%) for 100 FP, and from 35.9 (%) to 7.6 (%) for 250 FP within 3 weeks. The protection index for 100 FP after 3 weeks treatment was 63% for %fall, and 44% for AUC, respectively, while the protection index for 250 FP amounted to 72% (%fall), and 53% (AUC), respectively. Reduction of EIB was sustained up to 24 weeks of treatment, with the protective effect of the two doses not significantly different.

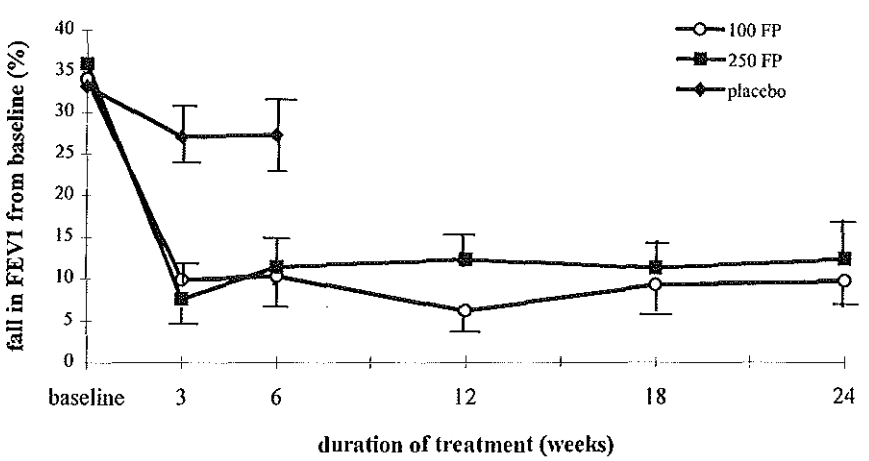

Figure 1: Maximal %fall in FEV1 from baseline during the study period, for active treatment and placebo groups (geom. mean*$e^{\pm SEM}$)

Table 2: Results of treatment with 100 FP and 250 FP b.d.

		baseline	at 3 weeks	at 6 weeks	at 12 weeks	at 18 weeks	at 24 weeks
FEV1%predicted (%)	placebo	92.1±12.5	93.0±10.0	95.4±8.8			
	100 FP	96.6±6.9	100.6±7.3	100.0±9.0	101.4±11.3	97.8±7.6	97.3±6.6
	250 FP	93.2±13.3	100.2±14.6◆	99.7±12.5	97.9±14.5	99.0±12.4	98.3±14.7¶
EIB: %fall (%)†	placebo	$33.2*e^{\pm 0.32}$	$27.1*e^{\pm 0.38}$	$27.3*e^{\pm 0.51}$			
	100 FP	$34.1*e^{\pm 0.37}$	$9.9*e^{\pm 0.66}$◆	$10.3*e^{\pm 0.63}$◆	$6.2*e^{\pm 1.0}$	$9.3*e^{\pm 0.78}$	$9.7*e^{\pm 0.72}$¶
	250FP	$35.9*e^{\pm 0.35}$	$7.6*e^{\pm 0.73}$◆	$11.4*e^{\pm 0.89}$◆	$12.3*e^{\pm 0.67}$	$11.3*e^{\pm 0.68}$	$12.3*e^{\pm 0.80}$¶
EIB: $AUC_{0\text{-}30}$ (%.min)†	placebo	$764*e^{\pm 0.39}$	$599*e^{\pm 0.53}$	$589*e^{\pm 0.58}$			
	100 FP	$764*e^{\pm 0.34}$	$337*e^{\pm 0.34}$◆	$353*e^{\pm 0.39}$◆	$280*e^{\pm 0.68}$	$275*e^{\pm 0.62}$	$293*e^{\pm 0.49}$¶
	250 FP	$855*e^{\pm 0.49}$	$280*e^{\pm 0.47}$◆	$344*e^{\pm 0.68}$◆	$323*e^{\pm 0.58}$	$320*e^{\pm 0.81}$	$375*e^{\pm 0.57}$¶
PD20methacholine ‡	placebo	26.4±1.5	nda	30.0±0.8			
(mcg)‡	100 FP	26.6±1.0	nda	58.4±1.7◆	47.7±1.4	57.2±2.2	80.2±1.6¶
	250 FP	20.1±1.0	nda	88.7±1.8◆	101.4±1.5	135±2.2	200±2.3¶

mean±SD; †=geom. mean*$e^{\pm SD}$; ‡=geom. mean±doubling doses; nda = no data available;

◆ : $p<0.05$ as compared to placebo;¶ : $p<0.001$ compared to baseline;

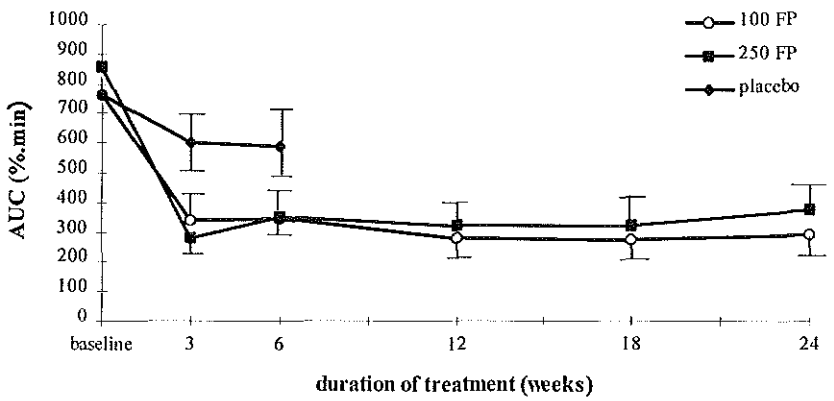

Figure 2: Area under the curve (AUC)during the study period, for active treatment and placebo groups (geom. mean*$e^{\pm SEM}$)

Bronchial responsiveness to methacholine significantly improved within the first 6 weeks as compared to placebo (fig. 3), for both 100 FP (p=0.035), and 250 FP (p=0.0037). Although the effect of 250 FP was larger than the effect of 100 FP, the difference in bronchial responsiveness to methacholine between the two treatment groups was not statistically significant after 6 weeks of treatment. However, PD_{20}methacholine steadily increased with time in both treatment

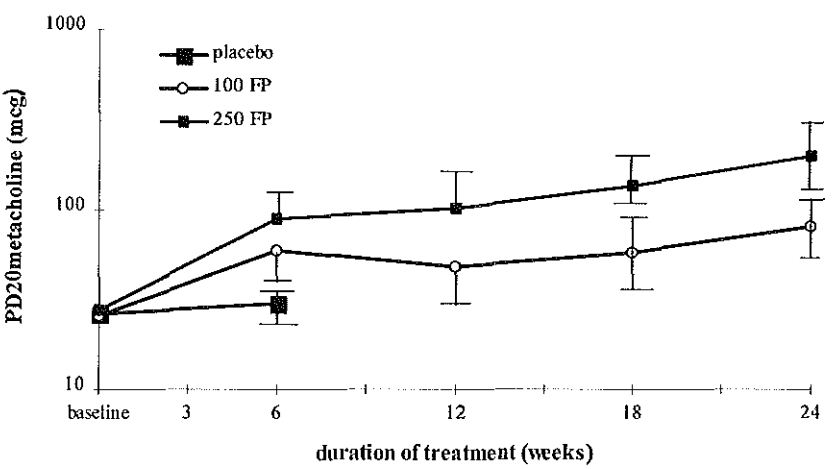

Figure 3: Bronchial hyperresponsiveness to methacholine during the study period, for active treatment and placebo groups (geom. mean±doubling dose)

limbs (p=0.04), with a mean increase of 1.6 dose steps for those children using 100 FP b.d., and a mean increase of 3.3 dose steps for those children using 250 FP b.d. This difference in improvement approached significance after 24 weeks of treatment (p=0.06).

In an alternative analysis, the change in %fall in FEV_1 to a given dose of methacholine and exercise was calculated in both treatment groups (figure 4a+b). In children using 100 FP twice daily, the median %fall in FEV_1 to methacholine pre-treatment was 33.1%, with the median %fall to exercise being 32.0%. In children using 250 FP twice daily, these values were 40.3% (methacholine) and 37.0% (exercise), respectively. The median change in %fall in FEV_1 in the 100 FP group was -14% (methacholine) and -22% (exercise) respectively. Within-group comparison showed this difference in steroid effect to be statistically significant (p=0.04).Median change in the 250 FP group was -17.2% (methacholine) and -19.5% (exercise), respectively, the difference in effect not being statistically significant.

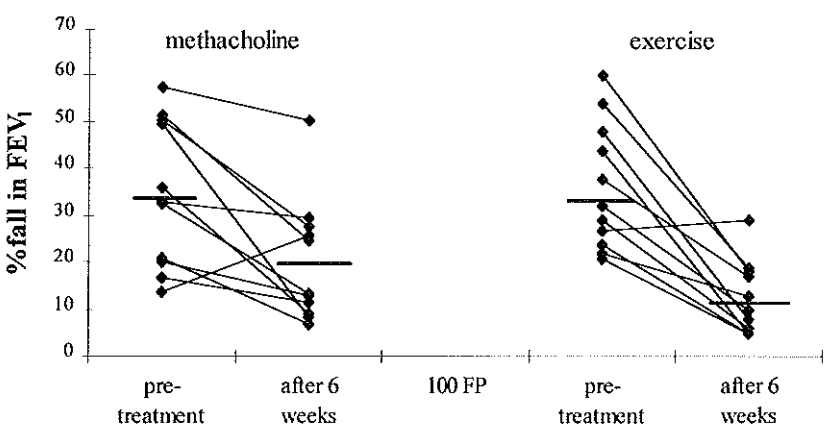

Figure 4a: Median change in %fall in FEV1 to a single dose of methacholine and to exercise before and after 6 weeks of treatment with 100 mcg fluticasone propionate twice daily

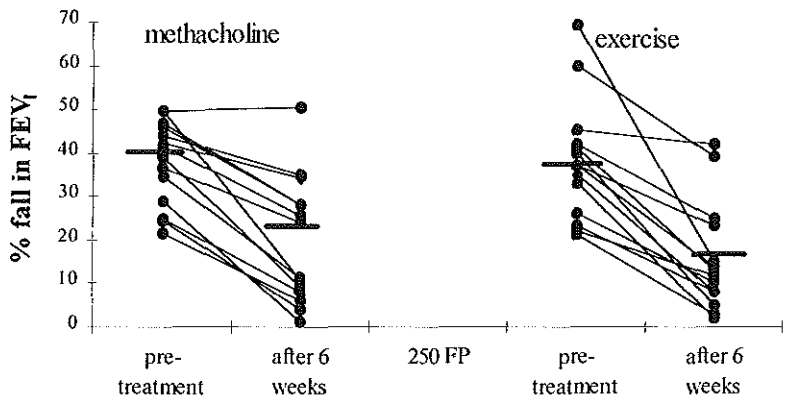

Figure 4b: Median change in %fall in FEV1 to a single dose of methacholine and to exercise before and after 6 weeks of treatment with 100 mcg fluticasone propionate twice daily

8.5.2 Effects on lung function and symptoms

Treatment with 250 mcg FP twice daily significantly improved FEV1%predicted (fig. 5) in the first three weeks as compared to placebo (mean change: +7.8 %points; p=0.0031). In the 100 FP group the change in FEV1%predicted just failed to reach significance (mean change +4.9 %points; p=0.06) when compared to placebo. This difference in effect between the two doses was sustained throughout the treatment period (p=0.046). Peak flow values as recorded on the diary cards however, did not change significantly over time, neither during active nor during placebo treatment.

The level of inflammatory mediators as measured by sECP in the blood, did not change significantly during short-term nor during long-term treatment (fig. 6). Before treatment, at randomisation, the level of sECP (log-transformed) correlated well with the severity of EIB for both the %fall in FEV1 (r= 0.64, p<0.0000), and the AUC (r= -0.63, p<0.0001). However, during treatment sECP and severity of EIB no longer correlated at any time point studied, nor was the change in sECP between visits related to the concurrent change in EIB.

When evaluating symptoms, it appeared that significantly more days and nights were without wheeze when using active treatment (p<0.05 compared to placebo; fig. 7). No effect of treatment with fluticasone propionate was found on symptoms scores of cough, shortness of breath and exercise-related symptoms.

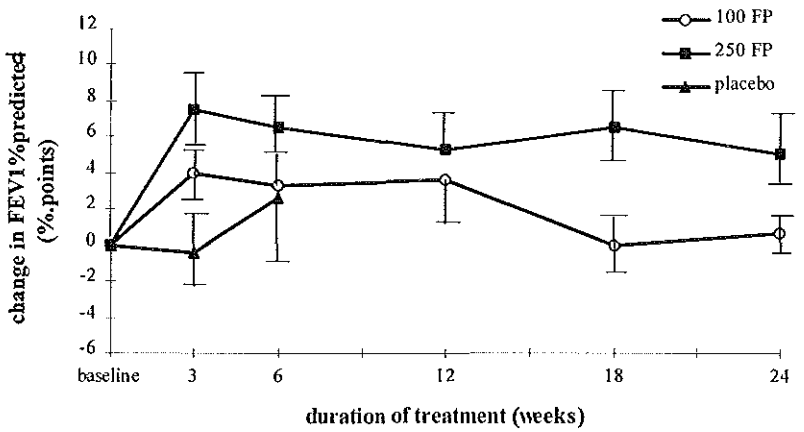

Figure 5: Change in FEV1 % of predicted from pre-treatment level for active and placebo groups (mean ±SEM)

8.5.3 Adverse effects

During the treatment period, no clinically significant abnormalities in heart rate, systolic and diastolic blood pressure were observed in the treatment groups. Most reported adverse events were related to respiratory infections, or contacts with allergen. No candidiasis or hoarseness of throat was reported during the study period.

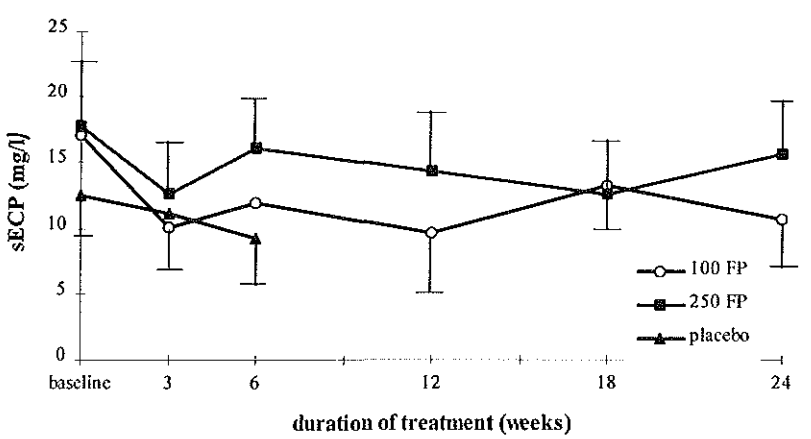

Figure 6: Serum level of eosinophil cationic protein (sECP) during the study period, for active treatment and placebo groups (geom. mean*$e^{\pm SEM}$)

8.6 Discussion

In this study, we have shown that the two dose levels of fluticasone propionate 200 mcg daily and 500 mcg daily were equi-effective in producing fast and sustained protection against EIB in childhood asthma. In contrast, the reduction of bronchial hyperresponsiveness to methacholine was dose-dependent, with an ongoing time-related improvement during the 6 month treatment period. Within-group comparison showed the lower dose of fluticasone to be significantly better in reducing EIB as compared to a matched level of methacholine-induced bronchoconstriction. In contrast, the higher dose was shown to be equally effective. By performing both direct (methacholine) and indirect (exercise) challenge tests in the same group of subjects, treated with either a low or a high dose of fluticasone propionate, we were able to support the hypothesis that steroids protect against directly and indirectly acting bronchoconstrictor stimuli by different mechanisms[29]. The steroid-induced decrease in EIB is very likely to be due to a rapidly achieved reduction in cellular activity, whereas the time-related improvement in PD_{20}methacholine might predominantly be due to the relatively slow resolving of airway remodelling.

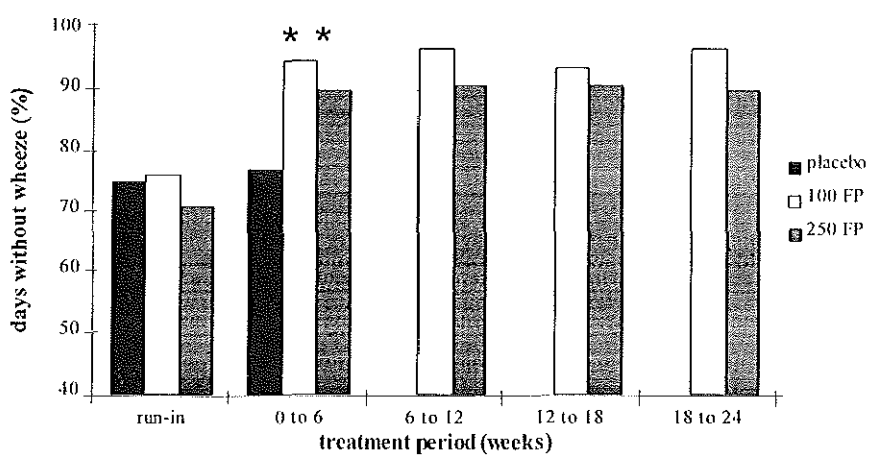

Figure 7: Percentage of days without wheeze in each 6 week period of the study, for each of the treatment groups (**: $p<0.05$ as compared to placebo)

To the best of our knowledge, our study is the first to simultaneously describe the time- and dose-dependency of the protective effects of inhaled steroids against two different types of bronchoconstrictor stimuli within the same study population. Our results confirm and extend previous observations from studies in asthmatic children[17] and adults[15,30] on improvements in bronchial responsiveness to direct stimuli during long-term treatment. The fast reduction in EIB, followed by a sustained, time-independent protection is in agreement with a previous study[18], while the absence of a steroid dose-dependency in reducing EIB is contrary to results obtained with budesonide during short-term treatment[16]. This could be due to a difference in potency between the two steroid formulations[31].

When comparing our results to those previously described, a number of methodological points need to be adressed. Firstly, study populations may differ in severity of asthma. Our study population included children diagnosed with mild to moderate asthma, selected on the basis of at least 20% fall in FEV_1 after a standardized exercise. At randomisation, mean percent fall in FEV_1 from baseline was approx. 35%. The study population, for whom a dose-dependent reduction in EIB was described[16], was characterized as having moderate to severe asthma (the bronchial reversibility ranging from 26 to 82%), with a mean of 55% fall in FEV_1 from baseline pre-treatment. Hence, the contradictory findings regarding the (lack of) dose-response effects of inhaled steroids on EIB between the two studies could partly be explained by the difference in asthma severity of the two study populations.

Secondly, when comparing dose-effects of inhaled steroids, the inhalation device used must be taken into account, because the effect is dependent upon the actual dose deposited in the lungs, and not the dose prescribed[32,33]. In addition, different formulae of inhaled steroids may exert differences in potency when used in the same dosage[31], making comparisons even more complicated. Finally, the time point of measurement after starting drug therapy may be important for observing dose-related effects. No dose-response effect on EIB was found after a mean treatment period of three weeks, yet it may have existed during the first week of treatment. Thus far, little is known about the dose-response curve of inhaled steroids on lung function and bronchial hyperresponsiveness in asthma. The dose-response curve for fluticasone might be rather flat[34], the dose of 200 mcg daily being near the optimum dose level. Alternatively, dose-related effects could be masked in clinical trials, due to the heterogeneity of the response to inhaled steroids in individual asthmatics[35].

How can the present findings be interpreted? It is becoming clear that corticosteroids exert their effects on many aspects of inflammation. They are capable of reducing the numbers of mast cells and eosinophils in the bronchial (sub)mucosa[11,27], and interfere with cellular protein

synthesis, leading to inhibition of pro-inflammatory cytokine and mediator synthesis[11,36]. It has been suggested that inhaled steroids have greater efficacy in reducing eosinophil activity than mast cell activity[11,27]. Oral prednisolone significantly decreased the level of ECP in bronchial alveolar lavage (BAL) fluid, but not baseline eicosanoid levels in mild asthmatic patients[37]. However, release of eicosanoids *ex vivo* was reduced following prednisolone treatment *in vivo*[37]. Also, a significant reduction in the rise in arterial leukotriene E_4 (LTE_4) during an acute asthma attack was described during prednisolon treatment compared to LTE_4 levels in an non-prednisolone treated attack[38]. These data would suggest that inhaled steroids are capable of reducing the levels of mediators thought to be important in the pathogenesis of EIB, such as leukotrienes[8] and prostaglandins[7] . Thus, it can be postulated that inhaled steroids protect against EIB by reduced cell numbers and activity[11].

Reduced cell numbers might also be an important feature in protection against methacholine-induced bronchoconstriction, as evidenced by the relationship between the severity of bronchial hyperresponsiveness to methacholine and the numbers of mast cells and eosinophils in the lamina propria in steroid-treated asthmatics[39]. It is still unknown how steroid-induced reduction in the number and/or activity of cells could lead to improvement of BHR to methacholine. The beneficial effect is hypothesized to be the result of reductions in mucosal and peribronchial thickness, potentially restoring the forces of interdependence between airway wall and parenchyma[40]. These effects have a relatively short time-course[41] and could also explain the relatively acute effect seen on airway patency. In addition to this acute effect, inhaled steroids have been reported to reduce the thickening of the reticular layer in asthma during long-term treatment, showing the potential of reversing long-term fibrosis[12]. Indeed, recent investigations have shown that treatment with inhaled steroids, aimed at attenuating bronchial hyperresponsiveness on top of improving symptoms, leads to an additional reduction in sub-epithelial reticular layer thickening[42]. Thus, the presently observed time-related ongoing improvement in PD_{20}methacholine is likely to reflect an ongoing improvement of airway remodelling in asthma. However, the relative importance of reducing cell number and activity, and airway remodelling in determining the patients' individual sensitivity to steroids remains to be established[36].

What is the clinical significance of our findings? These data indicate that monitoring exercise- and methacholine-induced bronchoconstriction implicates information on different aspects of the pathophysiologic mechanisms in asthma. This is supported by the results from allergen avoidance studies in asthmatic children[43]. After transfering housedust mite allergic

children to a hypo-allergen environment, a significant reduction in EIB was apparent after one month, which was not accompanied by concomitant improvements in PD_{20}methacholine.

Our study has shown that in asthmatic children, in whom EIB is the dominating feature, low dose steroid therapy may well be sufficient for their treatment. This is a clinically relevant finding in view of the risk of systemic side effects of corticosteroids[44]. Yet, when treatment is aimed at reversing the chronic inflammation in asthma, as reflected by improvements in PD_{20}methacholine, it can be argued that treatment should be started at high dose levels of steroids in order to obtain the maximal effect[44]. Such a view is supported by the swift and large improvement in PD_{20}methacholine with 500 mcg fluticasone propionate daily in this study. On the other hand, it can not be excluded that with prolonged follow-up, the improvement in the low dose steroid group would have equalled the effect seen with the higher dose. Further studies are needed to investigate the effect of such treatment strategies, as well as studies evaluating the efficacy of a combination of treatment modalities to circumvent the use of high dose inhaled steroids.

The use of a biomarker in the blood to monitor airway inflammation and thereby disease severity was evaluated as well in this study. Fluticasone significantly increased the percentage of days without wheeze, yet no change was observed in the serum level of eosinophil cationic protein (sECP). A cross-sectional relationship between the severity of EIB and the serum level of sECP was found before starting steroid therapy, but this relationship disappeared during treatment[10], rendering sECP less suitable for monitoring disease severity[19].

In conclusion, we have shown that the protection against methacholine-, but not exercise-induced bronchoconstriction, afforded by inhaled fluticasone propionate during long term treatment in childhood asthma, is time- and dose-dependent. These data support the hypothesis of different modes of action of steroids in protecting against directly and indirectly acting bronchoconstrictor stimuli. Reduction in EIB is very likely due to decreasing inflammatory cell number and/or activity, whereas improvement in PD_{20}methacholine might predominantly be due to a gradual resolving of airway remodelling. Further studies are needed to evaluate the best parameter to be used for clinical monitoring of treatment efficacy.

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chapter 9 chapter

The protective effect of short-term treatment with zafirlukast, a cysteinyl leukotriene receptor antagonist, against exercise-induced bronchoconstriction in adolescent asthmatics.

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submitted

9.1 Summary

The present study evaluated the efficacy and safety of short-term treatment with the cysteinyl leukotriene receptor antagonist zafirlukast (ZF 20 mg bd or 80 mg bd) or cromolyn sodium (DSCG, 10 mg qds) on exercise-induced bronchoconstriction (EIB) in asthmatic adolescents. Eighteen patients, aged 12 to 17 year, with a forced expiratory volume in 1 second (FEV_1) ≥70% of predicted, and ≥20% fall in FEV_1 after a screening standardised 4-6 min treadmill exercise test using dry inspired air, participated in a randomised, double-blind, placebo controlled, cross-over trial, with one week wash-out between the two-week treatment periods. During the study, exercise testing was performed 4 and 8 hours after the final dose of each treatment period. EIB was expressed as maximal %fall in FEV_1 from baseline (%fall) and as Area-Under-the-Curve (AUC) of the 30 min. time response curve. AUC and %fall were analysed by ANOVA, comparing ZF and DSCG to placebo. The effect of ZF was most evident during the recovery phase of EIB, the reduction in AUC being significantly differerent as compared to placebo ($p<0.03$) at both time points. The protection index on AUC 4 hours after dosing was 45.5% (ZF 80 mg), and 46.3% (ZF 20 mg), respectively, while 8 hours after dosing it was 45.2% (80 mg), and 54.1% (20 mg), respectively. In contrast, treatment with DSCG did not attenuate EIB at either time point. Differences in maximal %fall between treatments failed to reach statistical significance, except for ZF 20 mg 8 hours after dosing. Incidence of minor adverse events was not different between treatment and placebo periods. These results implicate leukotrienes to be mediators, involved in the pathogenesis of EIB, and show zafirlukast to be a safe and effective therapy in the management of childhood asthma, affording prolonged protection against EIB in asthmatic adolescents.

9.2 Introduction

During the last decade, asthma is increasingly recognized as an inflammatory disorder of the airways[1]. Recently, it has become clear that leukotrienes play an important role in the complex network of cells and mediators perpetuating the chronic inflammation in asthma[2,3]. Predominantly generated by mast cells and eosinophils, the cysteinyl leukotrienes (Cys-LT) are capable of producing bronchoconstriction, mucus secretion and airway oedema, as well as aggravating the bronchial hyperresponsiveness to histamine in asthmatic patients[4]. In addition, inhalation of Cys-LT's results in recruitment of eosinophils into the airway mucosa[5]. Therefore, drugs aimed at inhibiting the aforementioned actions, may be highly valuable in the management of asthma[6].

Exercise is a common, physiologic trigger leading to acute, usually self-limiting attacks in asthma[7]. The prevalence of this exercise-induced bronchoconstriction (EIB) among asthmatic patients has been found to range from around 60% to as high as 90%[7,8]. The precise mechanisms underlying EIB have not been fully clarified yet, the stimulus to the airways potentially being thermal (airway cooling and/or rewarming) or osmolar (drying of the airways), which activates mast cells and possibly eosinophils to release bronchoconstrictor mediators[9].

By using leukotriene receptor antagonists in adult patients, it has been well established, that the Cys-LT's are involved in EIB, especially in maintaining the bronchoconstriction post-exercise[10,11]. In atopic asthmatic children with severe EIB, an increase in urinary leukotriene E_4 was found after exercise challenge[12]. However, little is known about the efficacy and safety of anti-leukotriene therapy in childhood asthma. Cromolyn sodium, when given shortly before exercise, has been shown to be beneficial in protecting against EIB in children[13]. Therefore, the present study was aimed to evaluate the effects of short-term treatment with the Cys-LT_1 receptor antagonist zafirlukast (Accolate™) or cromolyn sodium on EIB in asthmatic adolescents. To that end, we measured the protection against EIB afforded by either zafirlukast (20 mg or 80 mg b.d.), cromolyn sodium (10 mg qid), or placebo, four and eight hours after the final dose of a two weeks treatment period, using a randomised, double-blind, cross-over trial.

9.3 Materials and methods

9.3.1 Patients

Eightteen adolescent patients (8 female, 10 male) clinically diagnosed as having atopic asthma[14], were recruited from the out-patient clinic. Mean age of the patients was 13.6 years (range 12 - 17 year), and their baseline forced expiratory volume in 1 second (FEV_1) was ≥ 70% of predicted[15]. All had symptoms of episodic chest tightness and wheezing, and suffered from exercise-induced bronchoconstriction, as shown by a fall in FEV_1 of at least 20% from baseline after a standardized screening exercise test (table 1). They were clinically stable, i.e. no history of viral infections during the six weeks before screening, and their asthma medication had not changed in the month prior to entry into the study. Twelve patients used inhaled corticosteroids, the mean daily dose being 500 mcg. Long-acting β_2-agonists were stopped four weeks before screening, while p.r.n. short-acting β_2-agonists were used as rescue medication, but not during the 6 hours before lung function or exercise testing.

The study was approved by the Medical Ethics Committees of the Juliana Children's Hospital, and written informed consent was obtained from all participating patients.

Table 1: patient characteristics at the screening visit.

Patient nr	sex	age (years)	duration# of asthma	treatment§	dose¶	FEV1 %predicted	EIB (%fall)
1	m	13	12	flut/salb	500/prn	98.6	20.2
2	m	12	8	salb	-/prn	103.5	39.1
3	m	12	12	flut/salb	500/prn	80.7	43.8
4	f	13	10	terb	-/prn	110.6	43.6
5	f	13	13	flut/salb	500/prn	113.9	31.7
6	m	12	12	bud/terb	400/prn	107.2	37.7
7	m	13	11	terb	-/prn	78.7	23.7
8	f	13	13	flut/salb	1000/prn	83.1	40.1
9	f	17	17	beclo/salb	800/prn	115.2	45.8
10	m	13	8	flut/salb	100/prn	101.4	25.6
11	m	14	14	flut/salb	200/prn	92.2	41.8
12	m	15	4	flut/salb	500/prn	93.9	42.2
13	m	14	10	terb	-/prn	111.7	27.6
14	f	16	12	flut/salb	1000/prn	118.9	36.0
15	f	16	3	salb	-/prn	117.3	28.4
16	m	13	7	flut/salb	100/prn	81.2	21.4
17	f	14	6	salb	-/prn	89.7	33.7
18	f	12	10	flut/salb	500/prn	77.3	45.0
Mean		13.6	10.1		508	98.6	34.9
SD		1.5	3.6		303	14.5	8.6

#: duration of asthma in years; § treatment: flut=fluticasone propionate, bud= budesonide, beclo= beclomethasone dipropionate, salb= salbutamol, terb= terbutaline; ¶ daily dose in mcg of steroids/ prn= bronchodilators on demand; FEV1 = forced expiratory volume in 1 second; EIB = exercise-induced bronchoconstriction expressed as maximal % fal in FEV1 from baseline;

9.3.2 Study design

The study was designed as a randomised, double blind, double dummy, placebo-controlled, four-period cross-over trial comparing the effect of two weeks' treatment with oral zafirlukast (80 mg or 20 mg bd) or inhaled cromolyn sodium (10 mg qid) with matching oral and inhaled placebos. The study was powered to detect signficant differences between the active treatments and placebo, not to detect differences between zafirlukast and cromolyn sodium. After the

initial screening exercise test, patients were randomised to one of four treatment sequences according to a Williams balanced design. At the end of each treatment period, patients performed an exercise challenge at 4 and 8 hours after their last dose of trial medication. Between treatments, there was a one week wash-out period. Zafirlukast was given as oral tablets, and cromolyn sodium was supplied in metered-dose inhalers for inhalation.

9.3.3 Lung function measurements

Short-acting β_2-agonists were withheld for at least 6 hours before the lung function measurements or exercise challenge tests were performed. Spirometric measurements were made using a pneumotachograph (Masterscreen, Jaeger Germany). At each visit, baseline lung function was determined as the highest FEV_1 obtained from three forced expiratory manoeuvres[16].

9.3.4 Exercise challenge

Four and eight hours after the final dose of each treatment, exercise testing for measuring severity of EIB was performed by running on a treadmill (LE 2000, Jaeger, Germany) using a standardized protocol, which has been shown to produce repeatable results[17]. Exercise challenge was performed only if pre-exercise FEV_1 was ≥70% of predicted[15]. During the test, heart rate was continuously monitored by a radiographic device (Polar Sport Tester). Dry air (relative humidity <10%), obtained by pressurized medical air, and collected in a Douglas bag (contents 150 liter), was inspired during running using a face mask with the nose clipped (Hans-Rudolph). The incline of the treadmill was set at 5 to 10%, depending on the physical condition of the child. Patients were given a warming up period of one minute to get accustomed to the treadmill. The speed of the treadmill was then adjusted to induce a heart rate ≥ 90% of the predicted maximum (=210-age) by the third minute of the test, at which speed they ran for a maximum of three further minutes. The durations of subsequent exercise challenges were kept identical to the duration of the screening test for each patient. The exercise challenges were assessed as being reproducible if the target heart rate was reached, regardless of minor variations in speed. Post-exercise, FEV_1 was measured in duplicate at 1, 3, 5, 7.5, 10, 15, 20 and 30 minutes, the best FEV_1 at each time point retained for analysis.

9.4 Statistical analysis

Safety was determined at each visit by review of the patient's state of health, physical examination, routine clinical laboratory tests, electrocardiogram (ECG) and recording of adverse events. Compliance with study medication was assesed by counting the returned tablets.

The severity of EIB was expressed as maximal %fall in FEV_1 from baseline (%fall) and as area-under-the-curve (AUC) of the time-response curve (0-30 min), the latter obtained from plotting the percentage change in FEV_1 from baseline against time[17]. Recovery of EIB was assessed by calculating the percentage of patients with $FEV_1 \geq 95\%$ of baseline within or at 30 minutes post-exercise.

Analysis of variance models were fitted to the data of AUC and %fall, with effects of patients, treatment sequence, period and treatment included as independent variables, and baseline FEV_1 fitted as a covariate. Treatment effects were estimated separately for each dose of zafirlukast versus placebo and for cromolyn sodium versus placebo. The protection index, an estimate of the protection afforded by the active treatment over placebo[10], was expressed as: ([least squares mean placebo-least squares mean active]/ least squares mean placebo)*100%. P-values <0.05 were considered statistically significant.

9.5 Results

Eightteen patients were randomized to active treatment. Patient characteristics are given in table 1. Two patients withdrew before the end of the trial, one withdrawal due to an asthma exacerbation, the other one due to a concomitant traffic accident.

The exercise protocol was completed during treatment with zafirlukast by all but one patient, who was given bronchodilator therapy because of severe bronchoconstriction (>45% fall in FEV_1) within 10 minutes post-exercise for the 8 hour test. In contrast, intervention with rescue bronchodilator therapy had to be done 4 times during treatment with cromolyn sodium and 3 times during placebo treatment, always following the 4 hour exercise test. In these cases, the AUC post-exercise could not be calculated.

Treatment compliance with the study medication was considered good, with compliance over 80% of the prescribed amount of study drug in 56 out of the 69 treatment periods.

Baseline FEV_1 pre-exercise was not influenced by the four treatment regimens at either time point after dosing. Neither did the maximal heart rates during the last minute of the exercise tests vary significantly between the different exercise challenges performed (table 2).

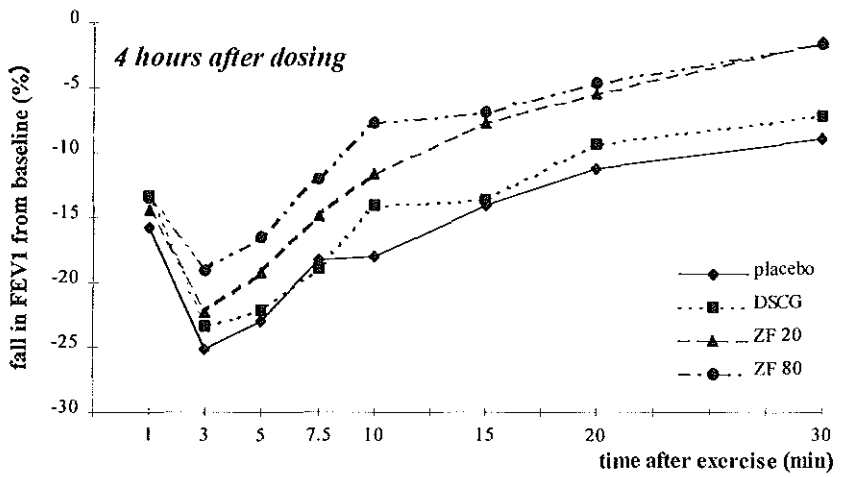

Figure 1a: Mean %fall in FEV1 from baseline at various time points post-exercise, 4 hours after the final dose of each treatment period (DSCG: cromolyn sodium; ZF 20: zafirlukast 20 mg b.d.; ZF 80: zafirlukast 80 mg b.d.)

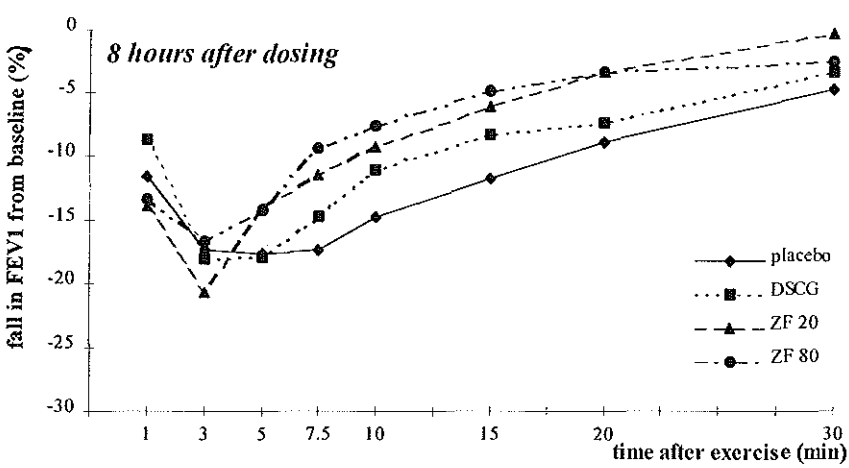

Figure 1b: Mean %fall in FEV1 from baseline at various time points post-exercise, 8 hours after the final dose of each treatment period (DSCG: cromolyn sodium; ZF 20: zafirlukast 20 mg b.d.; ZF 80: zafirlukast 80 mg b.d.)

9.5.1 Efficacy

Mean %fall in FEV_1 from baseline post-exercise at each time point is presented in figures 1a (4 hours post dose) and 1b (8 hours post dose) for the four different treatment regimens.

Four hours after the final dose, both 20 mg and 80 mg of zafirlukast produced a statistically significant reduction in AUC post-exercise ($p<0.01$ compared to placebo; table 2). The protection index on AUC was 45.5% for the 80 mg dose, and 46.3% for the 20 mg dose, respectively (figure 2). In contrast, treatment with cromolyn sodium did not result in a significant improvement of the AUC ($p=0.40$), the protection index being 14.5%. Relatively more patients recovered to greater than 95% of baseline FEV_1 within or at 30 minutes post-exercise having used zafirlukast than after using cromolyn or placebo.

Similar results were obtained when the different treatment effects were evaluated eight hours after dosing. At this time-point, the protection index on AUC by zafirlukast was 45.2% for the 80 mg dose, and 54.1% for the 20 mg dose, respectively (figure 2), while the protective index of cromolyn sodium did not differ from zero ($p=0.72$).

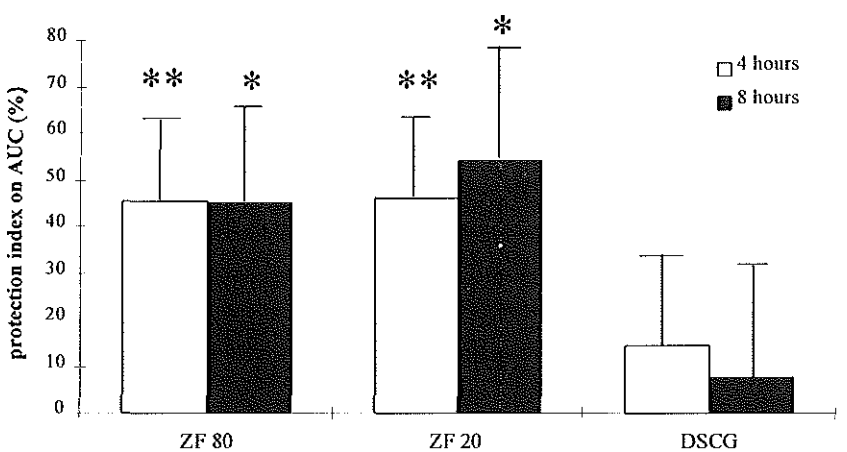

Figure 2: Protection index of each treatment on AUC 4 and 8 hours after dosing (**:$p<0.01$, *:$p<0.05$ = protection significantly different from zero)

When evaluating the treatment effects on the maximal %fall in FEV_1 (table 2, figure 3), the two doses of zafirlukast produced a smaller maximal %fall in FEV_1 compared to placebo, although statistical significance was only obtained for zafirlukast 20 mg 8 hours after the final dose. Neither was there a significant difference in the maximal %fall in FEV_1 between treatment with cromolyn sodium and placebo at both time points post-dosing ($p=0.35$).

Table 2: Exercise-induced bronchoconstriction after regular therapy with placebo, zafirlukast and cromolyn sodium in asthmatic adolescents

treatment	number	baseline FEV1 (l)†	maximal HR (beats/min)†	max.% fall in FEV1 (%)‡	$AUC_{(0-30)}$ (%.min)‡	recovery (% patients)
4 hour post dose						
zafirlukast 80 mg	15	2.79±0.50	186.6±7.9	21.0±2.41	-257.5±49.5¶	87
zafirlukast 20 mg	16	2.76±0.62	186.7±6.9	22.4±2.30	-253.6±46.6¶	81
cromolyn sodium	11	2.75±0.61	185.4±6.4	26.1±2.59	-404.0±61.3	57
placebo	12	2.74±0.65	184.1±6.3	26.2±2.45	-472.3±56.9	33
8 hour post dose						
zafirlukast 80 mg	15	2.81±0.53	185.8±5.5	20.7±2.05	-202.2±47.8§	87
zafirlukast 20 mg	14	2.75±0.64	185.0±7.0	18.7±2.02§	-169.5±50.0§	73
cromolyn sodium	10	2.78±0.56	186.9±4.5	28.2±2.39	-340.2±62.7	50
placebo	12	2.77±0.63	186.7±5.0	25.2±2.36	-369.1±56.2	50

†: results in mean±SD; ‡:results in least squares mean±SE ¶ : $p<0.01$ as compared to placebo; § : $p<0.05$ as compared to placebo;

FEV1 = forced expiratory volume in 1 second; maximal HR = maximal heart rate during the last minute of the test; max. %fall in FEV1 = maximal %fall in FEV1 from baseline post-exercise; AUC= area under the time response curve post-exercise; recovery = percentage of patients recovered at 95% of baseline FEV1 within 30 minutes post-exercise;

9.5.2 Safety

The incidence of adverse events reported by the patients was similar across the four treatment periods, the most common adverse event being worsening of asthma symptoms. There were no treatment related differences in the laboratory parameters or safety concerns from the physical examination. No clinically relevant ECG abnormalities were observed.

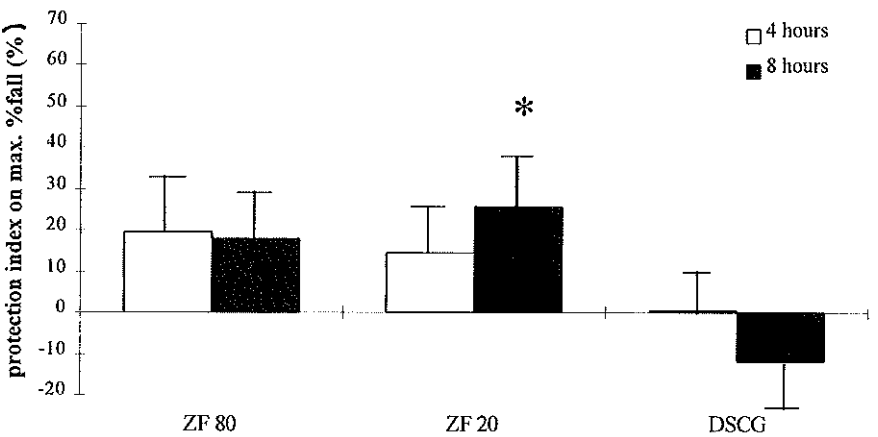

Figure 3: Protection indez of each treatment on %fall in FEV1 4 and 8 hours after dosing (*:p<0.05 = protection significantly different from zero)

9.6 Discussion

The present study has shown that regular treatment with the Cys-LT_1 receptor antagonist zafirlukast affords protection against exercise-induced bronchoconstriction up to eight hours after dosing in adolescents with mild to moderately severe asthma. The effect of zafirlukast was most evident during the recovery phase of EIB, the area under the curve being significantly less for both doses of zafirlukast as compared to placebo, with relatively more patients recovering from EIB within 30 minutes. Although zafirlukast produced a smaller maximal %fall in FEV_1 than placebo, this effect failed to reach statistical significance. No protective effect on EIB was seen after treatment with cromolyn sodium. These data suggest that cysteinyl leukotrienes are important mediators of EIB in adolescent asthmatics.

To our knowledge, this study is one of the first to describe the effect of regular treatment with a Cys-LT_1 receptor antagonist on exercise-induced bronchoconstriction in childhood asthma. In general, our findings of a significant protection on EIB are in accordance with those obtained in adults asthmatics[10,11,18-20]. The prolonged attenuation of EIB up to eight hours after dosing extend previous observations on the efficacy of single doses of Cys-LT receptor antagonists in protecting against EIB in asthmatic adults[10,11,18,19] and children[21,22]. Similar prolonged protection with regular treatment was previously described for the Cys-LT_1 receptor antagonist cinalukast in mildly asthmatic adults[20]. In keeping with our results, all but one study [19] showed a significant reduction in the area under the time-response curve post-exercise and a (trend towards) faster recovery with active treatment[10,11,18,20-22]. This suggests that leukotrienes are especially prominent in sustaining the bronchoconstrictor response.

The protective effect by Cys-LT_1 antagonism in our study population appears to be somewhat less than has been prescibed for adult asthmatics[10,11,18,20], because we were only able to demonstrate significant, albeit weak protection against the maximal %fall in FEV_1 8 hours after the 20 mg dose of zafirlukast. This may be inherent to the age group studied, as preliminary reports of studies in asthmatic children also suggest a slightly reduced protection on the maximal %fall compared to adults[21,22]. Alternatively, it has been suggested from studies with a different Cys-LT_1 receptor antagonist that loss of protection occurs with regular treatment, especially with lower doses[20]. However, this seems less likely, the effect of 20 mg of zafirlukast in this study being equal to that of the 80 mg dose.

No protective effect on EIB was observed after regular therapy with cromolyn sodium in this study population. As the exercise tests were performed 4 and 8 hours after the final dose, the acute protective effect of cromolyn sodium on EIB was negligible, because inhibition of EIB afforded by a single dose of cromolyn sodium is no longer evident 2 hours after inhalation[13]. The lack of effect on EIB during regular treatment with cromolyn sodium is in accordance with studies performed in adult asthmatics[23].

When comparing our results to those in the literature, a number of methodological issues need to be adressed. Firstly, our results give a conservative estimate of the efficacy of the treatment used. Nearly 25% of the patients could not complete the exercise protocol while using placebo due to excessive dyspneu after exercise. Because the AUC could not be calculated in these cases, patients with the most severe EIB could not be included for the effect of placebo treatment. In contrast, all patients except one, were able to complete exercise testing

when using zafirlukast. Thus, the effect of zafrilukast treatment was compared to the effect of placebo in that part of the study population with relatively less severe EIB.

Secondly, we cannot exclude variability in timing of the final dose of treatment, 4 or 8 hours before exercise challenge. In order to minimise the need for school absenteism in this study, the patients took their final dose at home or at school, unobserved by the investigators. However, we do not feel this has substantially influenced our results, as all patients were provided with a watch including an alarm. In addition, compliance with drug therapy was generally good, especially considering this age group[24].

Thirdly, we used a standardised exercise challenge protocol, which differs slightly to some other studies. Previous investigators have determined the individual workload for each patient to induce EIB at a screening visit, and kept this workload constant during the remainder of the study[10,11,18-20]. In contrast, we allowed differences in speed of the treadmill and duration of the test to occur between exercise tests in each individual patient, provided the heart rate attained 90% of its maximum during the last minute of the test. We have shown that the response to this exercise protocol in inducing EIB reproduced well[17], thus we do not feel this has affected the obtained results.

How can the present findings be interpreted? The results presented in this study implicate leukotrienes as mediators of EIB in paediatric asthma, as suggested by increased levels of urinary leukotriene E_4 after exercise challenge in some[12], but not all studies[25] . Their action appears to be most prominent in prolonging the induced bronchoconstriction. Earlier studies have provided evidence for a contributory role of histamine[26,27] and prostaglandins in EIB[28,29]. The relative contribution of these mediators to an individual's bronchoconstrictor response post-exercise seems to vary from one person to another, suggesting that some asthmatics are predominantly producing histamine when challenged with exercise[26], whereas others preferentially generate leukotrienes[11]. We have previously shown that the recovery from EIB is prolonged with increasing age in children with asthma[30], and hypothesized this to be due to a relative increase in the contribution of leukotriene synthesis. Such a hypothesis is in accordance with the observation that aspirin-induced asthma, being associated with increased baseline levels of Cys-LT's, affects approximately 10% of asthmatic adults, but is rare in asthmatic children[31]. Hence, the seemingly reduced efficacy of zafirlukast on EIB in adolescent asthmatics as compared to adults[10,11,18-20] in the present study, may be related to the age of our study population.

However, an equally valid explanation for the observed efficacy of zafirlukast in our study could be offered by the fact that 12 out of the 18 adolescents studied, were using regular

maintenance treatment with inhaled corticosteroids, known to attenuate the severity of EIB in adults[23] and children[32] . In addition, oral steroids are capable of reducing the rise in plasma levels of leukotrienes during an acute asthma attack[33]. On the other hand, the participating adolescents in our study still suffered from moderate to severe EIB, despite the use of inhaled steroids. Likewise, it has been shown that regular treatment with inhaled steroids did not affect the urinary LTE_4 excretion after allergen challenge[34]. Hence, it is not clear to what extent the current use of inhaled steroids may have interfered with leukotriene synthesis, thereby potentially influencing the efficacy of zafirlukast.

What is the clinical relevance of these data? Firstly, our results underscore the importance of measuring both the maximal %fall in FEV_1 and the area under the time response curve when evaluating drug therapy for EIB[17]. Secondly, zafirlukast will have clear benefits for asthmatic children, as its prolonged protection against EIB will reduce the need for repeated inhalation with bronchodilatory therapy for unplanned exercise, enabling them to engage in sport and play with their peers without restrictions[14]. Adequate and prolonged protection against EIB may also be offered by inhaling long-acting β_2-agonists[35], but a reduction in their protection has been described after regular (mono)therapy[36]. Thirdly, our results will be particularly important to those children in whom EIB is only partially inhibited by the use of maintenance therapy with (high dose) inhaled steroids, because the addition of regular zafirlukast, but not cromolyn sodium, appears to be beneficial in reducing the severity of EIB.

In conclusion, regular treatment with the Cys-LT_1 receptor antagonist, zafirlukast, has shown to be safe and effective in the management of EIB in asthmatic adolescents, affording prolonged protection up to eight hours after dosing. Our results implicate that leukotrienes are involved in the pathogenesis of EIB, and thereby a therapeutic target in this age group of asthmatics.

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chapter **10** chapter

Summary, Conclusions and General Discussion

10.1 Summary

The main goal of this thesis was to broaden the understanding of the pathophysiologic mechanisms underlying the manifestations of exercise-induced bronchoconstriction in childhood asthma. In addition, we set out to evaluate treatment strategies for the prevention of EIB. The results of the studies presented in this thesis are briefly summarized here.

10.1.1 EIB: current concepts and its relation to asthma

The first chapter outlines the current knowledge about asthma as an inflammatory disease. The concept of bronchial hyperresponsiveness (BHR) and its importance in asthma is introduced. In chapter 2, exercise-induced bronchoconstriction in childhood asthma is reviewed, it being prevalent in the majority of the asthmatic children. Most prominent symptoms are wheezing, cough, and chest tightness during or after exercise, although chest pain on exertion and exercise intolerance are also suggestive of its diagnosis. The severity of EIB, as determined by standardized exercise testing, reflects the severity of the underlying BHR in asthma. The precise mechanisms of EIB have not been fully clarified yet, the stimulus to the airways potentially being thermal (airway cooling and/or rewarming) or osmolar (drying of the airways). The bronchoconstrictor response itself is most likely due to the release of mediators, and possibly neural activity. Finally, the current knowledge on the protective effect of common asthma drugs on EIB are reviewed. The aims of the studies in this thesis are presented in chapter 3.

10.1.2 EIB: repeatability of the response

The first study is described in chapter 4. As data on the reproducibility of the bronchoconstrictor response to dry air exercise testing in asthmatic children are lacking, the use of a standardized treadmill exercise protocol was evaluated, allowing estimation of sample sizes required for studies monitoring EIB in children. Twenty seven asthmatic children known to have EIB, performed two identical exercise challenges on separate days. The bronchoconstrictor response to exercise was expressed as the maximal %fall in FEV_1 (%fall), and as the Area-under-the-time-response-curve (AUC). The intra-class correlation coëfficiënt (ICC), determined as the index of repeatability, was based on the log-transformed data. It was calculated to be 0.57 for the %fall, and 0.67 for the AUC, showing EIB to have good reproducibility. Power curves for sample size estimations were subsequently constructed, indicating that if a drug is expected to reduce EIB by 50%, as few as 12 patients in total suffice to demonstrate this effect (90% power) using a parallel group design. Thus, standardized exercise testing for EIB using dry air is adequately repeatable for use in research practice in children with asthma.

10.1.3 EIB: the early asthmatic response

In the second study (chapter 5), we examined the clinical expression of EIB in relation to age. The hypothesis for this study of a decreased recovery rate from EIB with increasing age, was partly based on clinical evidence, and partly on observations in the literature on adult patients. In fourteen asthmatic children aged 7 to 12 year, with known EIB we measured the recovery from bronchoconstriction induced by two different bronchoconstrictor stimuli, exercise and histamine, in the absence of drugs. The degree of bronchoconstriction induced by histamine was matched for that observed after exercise. We were able to show that the recovery rate from EIB decreased significantly with age, while no such association was found for the recovery rate from histamine-induced bronchoconstriction. In those children aged eleven years and older, the recovery rate after exercise was significantly reduced compared to the recovery rate after histamine challenge. In the younger age group, similar recovery rates were observed after the two different challenges. These data suggest that the mechanism of EIB in childhood asthma changes with age.

10.1.4 EIB: the late asthmatic response

The occurence of a late asthmatic response (LAR) after exercise was adressed in chapter 6. The available information is mainly derived from studies in adults, and has led to conflicting results. We investigated the occurence of a LAR after exercise in asthmatic children with documented EIB, comparing lung function measurements after exercise with those on a control day without exercise (negative control day), as well as with lung function measurements after another non-specific bronchoconstrictor challenge (positive control day). Seventeen children, aged 7 to 14 years, randomly performed on three following study days, either a standardized exercise challenge, or a histamine challenge matching the bronchoconstriction after exercise, or measurements of lung function without any challenge. Measurements of FEV_1 at approximately the same clock hours, were repeatedly performed on these days during a time span of 8 hours. All children responded to exercise with an early asthmatic reaction of different severity. However, between 2 and 8 hours after exercise challenge, the difference in FEV_1 between exercise and control days for each clocktime did not exceed the 99.6% confidence limits of normal diurnal variation in any of the children. These data have led us to conclude that in children with mild to moderate asthma, a LAR after exercise is very unlikely to occur, implicating that although exercise is capable of inducing acute bronchoconstrictor responses of different severity, it does not seem to lead to subsequent induction of cellular activation.

10.1.5 EIB: its relation to natural allergen exposure

The intricate relationship between allergen exposure, airway inflammation and bronchial hyperresponsiveness in asthmatic children was the subject of chapter 7. Assuming the serum level of ECP to reflect eosinophilic airway inflammation, we aimed to unravel the involvement of the eosinophil in determining the severity of the bronchial hyperresponsiveness to exercise and to methacholine, by studying the changes induced in those variables during seasonal allergen exposure. A possible modifying effect of inhaled steroids was evaluated concurrently. To that end, measurements of sECP, and BHR to exercise and methacholine were performed in forty seven non-steroid or steroid treated grass pollen allergic asthmatic children and their controls before and during the pollen season. Results showed the sECP to be only weakly related to the severity of BHR to exercise, but not to methacholine, whether analyzed cross-sectionally, or longitudinally. The season-induced rise in sECP in grass-pollen allergic non-steroid-treated children was associated mainly with the childs' sensitization to grass pollen, whilst the change in BHR to exercise and metacholine was also significantly related to the allergen load during season. The use of inhaled steroids seemed to modify these associations, protecting against the allergen-induced deteriorations of asthma. These data suggest that BHR to indirectly acting stimuli, such as exercise, is more related to cellular activity than BHR to directly acting stimuli (methacholine). Measuring the serum level of eosinophil cationic protein turned out to be potentially useful in monitoring disease severity in seasonal allergen exposure in asthmatic children.

10.1.6 EIB: dose-response effects of inhaled steroids

The effects of inhaled steroids in inhibiting EIB are being described in chapter 8. The core issue of this chapter was to analyse to what extent the protective effects of inhaled steroids would be dependent on time and dose. In addition, the hypothesis was tested that inhaled steroids protect against directly (methacholine) and indirectly (exercise) acting bronchoconstrictor stimuli through different mechanisms. To verify this hypothesis, concomitant measurements of exercise- (EIB) and methacholine-induced bronchoconstriction (MIB) were measured during long-term treatment with inhaled steroids in children with asthma. A secundary objective of the study was to evaluate the potential role of sECP in monitoring disease activity, and thereby treatment efficacy. Thirty seven asthmatic children, aged 6 to 14 years, known to have EIB, participated in a double-blind, placebo-controlled parallel group study comparing the effect of fluticasone propionate 100 or 250 mcg b.d. in reducing EIB and MIB during a 6 months' treatment period. Results showed that both doses of fluticasone propionate were equi-

effective in producing fast and sustained protection against EIB. In contrast, the steroid-induced reduction in bronchial hyperresponsiveness to methacholine was dose-dependent, with an ongoing time-related improvement throughout the treatment period. Within the same individuals, the higher dose of fluticasone propionate proved to be equally effective in reducing matched levels of exercise- and methacholine-induced bronchoconstriction. The lower dose reduced the BHR to exercise to a significantly greater extent than the BHR to methacholine. These data support the hypothesis that inhaled steroids protect by different mechanisms against directly (methacholine) and indirectly acting (exercise) stimuli in childhood asthma. Before treatment, the severity of EIB, as a reflection of disease severity, was related to the level of ECP in the blood. However, during treatment, an association between (changes in) EIB and sECP was no longer apparent, rendering sECP less helpful in monitoring treatment efficacy.

10.1.7 EIB: the role of leukotrienes

In chapter 9, the relative involvement of leukotrienes in EIB in children was studied by using pre-treatment with a cysteinyl leukotriene (Cys-LT) receptor antagonist. Based partly on the results described in chapter 5, the hypothesis that leukotrienes are especially involved in prolonging the bronchoconstrictor phase post-exercise, was tested in eighteen asthmatic adolescents aged 12 to 17 years. Using a randomised, double-blind, cross-over trial, we measured the protection against EIB afforded by either the Cys-LT_1 receptor antagonist zafirlukast, or by cromolyn sodium, and compared this to placebo treatment. Measurements of EIB were performed four and eight hours after the final dose of a two weeks treatment period. At four hours post-dose, the effect of zafirlukast was most evident during the recovery phase of EIB, the area-under-the-time-response curve post-exercise being significantly less than during placebo. No significant protective effect was observed for cromolyn sodium at this time point. Differences in maximal %fall between treatments failed to reach statistical significance. Similar results were obtained when analysing the treatment effects at 8 hours post-dose. These data implicate that leukotrienes are important mediators of EIB in childhood asthma, their activity especially manifested in prolonging the bronchoconstrictor response after exercise.

10.2 Conclusions

★ The bronchoconstrictor response to dry air exercise challenge in asthmatic children is adequately reproducible to allow the set-up of research studies in a limited number of subjects while retaining sufficient statistical power.

★ The recovery rate from exercise-induced bronchoconstriction decreases with increasing age, suggesting a change in the pathophyisology of EIB when children grow older.

★ In children with mild to moderate asthma, exercise challenge, as compared to allergen challenge, does not result in equivalent late asthmatic responses 2 to 8 hours after challenge. This suggests that exercise, unlike allergen exposure, is a symptomatic trigger of asthma, reflecting the severity of the underlying bronchial hyperresponsiveness.

★ The extent of change in the bronchial responsiveness to exercise or methacholine during natural allergen exposure, is determined by the patients' degree of sensitization as well as the total allergen load. Treatment with inhaled corticosteroids protects against allergen-induced inflammatory changes. Bronchial responsiveness to exercise, but not methacholine, is related to cellular activity as reflected by the level of serum eosinophil cationic protein.

★ Fast and sustained protection against EIB in asthmatic children is afforded by the use of inhaled steroids, the effect of treatment with 200 mcg fluticasone propionate daily not significantly different from treatment with 500 mcg daily. In contrast, the reduction in bronchial hyperresponsiveness to methacholine is dose-dependent, with an ongoing time-related improvement during a 24 week treatment period. Thus, inhaled steroids protect differently against directly and indirectly acting bronchoconstrictor stimuli.

★ Pre-treatment with the cysteinyl leukotriene receptor antagonist, zafirlukast, significantly attentuates the severity of EIB in asthmatic adolescents, most notably during the recovery phase, implicating the cysteinyl leukotrienes to be important mediators of EIB, particularly in prolonging the bronchoconstriction post-exercise.

10.3 General discussion

10.3.1 Exercise-induced bronchoconstriction

The results of the studies presented in this thesis confirm and extend previous observations on the pathophysiologic mechanisms of exercise-induced bronchoconstriction in childhood asthma. Putting EIB in the perspective of the overall clinical picture of asthma will help in determining its clinical relevance and thus the choice of therapeutic intervention. The most relevant in the discussion is the issue whether EIB is merely a reflection of BHR and airway inflammation or whether EIB itself contributes to these asthma characteristics.

In trying to understand the mechanisms of EIB, comparisons have often been made with allergen challenge[1,2]. Provocation with either of these two stimuli initiate an acute asthma attack, with release of a similar mediator profile shortly after the challenge[3,4]. It is now well established that allergen challenge will induce a late asthmatic response (LAR) in about 50% of asthmatic patients. The occurrence of this LAR is accompanied by inflammatory changes within the airways, mainly an infiltration of eosinophils, and increased BHR, such as seen in chronic asthma[5]. When a LAR is absent however, such changes in inflammation and BHR do not occur. Therefore, the LAR has provided unique opportunities to study the pathophysiology of chronic astma.

We failed to observe equivalent late asthmatic responses after exercise, in agreement with previous studies[6-8], despite our attempts towards improved study methodology and advanced statistical analysis. Moreover, it was shown by others that the percentage of sputum eosinophils did not change significantly 6 to 9 hours after exercise compared to the pre-exercise values, in contrast to the percentage of sputum eosinophils in the same time period after antigen challenge[9]. In addition, exercise challenge has not been found to increase the bronchial hyperresponsiveness, as determined by the sensitivity to methacholine[10] or histamine[8]. These findings suggest that EIB is a consequence rather than a cause of airway inflammation, its severity merely a reflection of airway hyperresponsiveness.

10.3.2 Allergen-exposure

The above view is supported by the results of the study descibed in chapter 7, investigating the association between allergen exposure, airway inflammation and the bronchial hyperresponsiveness to exercise as well as methacholine. Before discussing the relevance of these results, some remarks need to be made on the study methodology. At the time of initiation of this particular study, natural allergen exposure instead of laboratory allergen challenge was

chosen for ethical reasons. Firstly, advantage of this approach is that the natural behaviour of asthma and bronchial responsiveness is studied. The obvious drawback is inability to manipulate the level of exposure. Secondly, as invasive techniques such as bronchial biopsies or bronchial lavages are considered ethically unacceptable in childhood asthma except for diagnostic or theapeutic interventions, we had to rely on measurements of eosinophil cationic protein in serum (sECP) for information on airway inflammation[11]. Despite these limitations, we feel that the results obtained confirm and extend previous observations under natural[12,13] as well as laboratory conditions[14], linking allergen exposure to subsequent changes in bronchial hyperresponsiveness. It seems valid to assume that the link between allergen exposure and increasing BHR is the development of airway inflammation[15]. In our study, it was observed that during natural allergen exposure, BHR to methacholine as well as the severity of EIB, increase in similar fashion. In this respect, the observed relationship between the change in the level of sECP and the subsequent change in EIB underlines the relevance of inflammatory cell activity in the mechanism of EIB[3].

10.3.3 "Direct" or "indirect" bronchial challenge?

Hence, the results thus far, strengthen the conviction that EIB is reflecting airway inflammation and the severity of the underlying bronchial hyperresponsiveness. One could then question whether determining the severity of EIB adds to information obtained by measuring the BHR to methacholine. Arguments against this view are forwarded by the results described in chapter 8. The difference in time- and dose-dependency of the protective effects of inhaled corticosteroids on exercise- and methacholine-induced bronchoconstriction, as was observed in our study, suggests that different information is to be gained from measuring the response to directly (methacholine) and indirectly (exercise) acting stimuli. This is in accordance with studies in adult asthmatics, implicating that BHR to methacholine is more likely to provide information on the chronic aspects of inflammation, possibly related to the remodelling processes in asthma[16,17], while exercise responsiveness responds more acutely to changes in allergen exposure[18]. Hence, our results support the view that although exercise (a physiologic stimulus), and methacholine (a pharmacological stimulus), both reflect BHR, they measure different components of the airways dysfunction[19].

11.3.4 Mediators of asthma and age

Moreover, it was shown that the expression of EIB changes with age (chapter 5). We postulated that the prolonged recovery time of EIB would be due to an increased involvement of

leukotrienes with age. Such a hypothesis is supported by the observation that the protective effect of the cysteinyl-receptor antagonist, zafirlukast, is especially prominent in the recovery phase (chapter 9).

The significance of a changing mediator profile in EIB is not clear, however, it is tempting to speculate that this may reflect a development in the underlying airway inflammation. Such a view is supported by the data of chapter 7, in which the influence of allergen exposure on EIB was especially evident in an increased area-under-the-curve, not in the maximal %fall in FEV1. Secondly, the role of the eosinophil in EIB in asthmatic children seems to be less prominent, as evidenced by the weak association between sECP and severity of EIB in childhood as compared to adult asthma[20]. A preliminary report on biopsy results in atopic asthmatic children compared to atopic asthmatic adults has suggested age-related difference in the immunopathology of asthma[21]. Whether these observations support or refute the suggestion that childhood asthma is more mast cell dependent[22], while progression of the disease and disease severity is more likely T-cell driven[23,24], remains to be elucidated. Clearly, there is a need for studies on the airway pathology in childhood asthma linking EIB to airway inflammation.

Regardless of these speculations of development of airway inflammation over time however, the involvement of leukotrienes in the pathogenesis of EIB may give rise to some concerns. Leukotrienes are known to be pro-inflammatory mediators[25], capable of producing bronchoconstriction, mucus secretion and airway oedema, aggravating the bronchial hyperresponsiveness[26], recruiting eosinophils into the airway mucosa[27], as well as resulting in secondary release of neuropeptides[28]. These actions of the Cys-LT's might explain some of the adverse effects observed in response to exercise challenge in asthmatic patients, such as an increase in the maximal airway narrowing to methacholine post-exercise[29], or an aggravated reaction to allergen provocation after EIB[30,31]. Therefore, one could speculate whether exercise in asthma may be more than a trivial bronchoconstrictor stimulus, and that through the release of Cys-LT's, exercise may play some role in maintaining airway inflammation.

10.4 Directions for future research

Standardized dry air exercise testing is a valuable tool in the management of childhood asthma, the severity of exercise-induced bronchoconstriction reflecting the degree of the underlying bronchial hyperresponsiveness. Future studies should be aimed at unraveling the relative contribution of the different mediators in EIB in relation to age, as well as antigen exposure. In

doing so, the diagnostic value of exercise testing will be strengthened, and new therapeutic strategies explored.

When compared to the model of allergen challenge, exercise most likely does not lead to the induction of airway inflammation. However, as summarized above, exercise may play a role in maintaining the airway inflammation in asthma through the release of leukotrienes. Clearly, there is a need to further study the expression of EIB in relation to inflammatory changes in the airways. As invasive techniques, such as broncial biopsies and lavage studies, are presently not allowed for research purposes in paediatric asthma, the use of alternative techniques, such as induced sputum, should be considered.

According to recent guidelines, inhaled corticosteroids are currently the drug of choice when treating children with moderate to severe asthma. Yet part of these asthmatic children stilll experience EIB despite the use of maintenance treatment with inhaled steroids. Further studies should evaluate treatment strategies aimed at inhibiting exercise-related symptoms in this group of children, such as combinations of steroids with long-acting $\beta_2$agonists, or with antileukotriene therapy. For those children well controlled on inhaled steroids, the prognostic value of exercise testing when tapering down maintenance treatment should be investigated, as this may be potentially helpful in monitoring disease activity.

In summary, EIB is a frequently occuring event in the daily life of many asthmatic children. The expression of EIB reflects the underlying bronchial hyperresponsiveness, and may be related to the underlying airway inflammation. The relative importance of EIB in maintaining inflammation should further be explored. However, as participation in sports without limitation is one of the aims of therapeutic intervention and has proved to be beneficial physically as well as psychologically, exercising should be encouraged in all asthmatic children.

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Samenvatting

Veel kinderen met astma krijgen bij inspanning in meer of mindere mate van luchtwegvernauwing. Dit wordt inspanningsgeïnduceerde bronchusobstructie (exercise-induced bronchoconstriction: EIB) genoemd. Het hoofddoel van dit proefschrift was om het inzicht te verdiepen in het pathofysiologisch mechanisme dat ten grondslag ligt aan EIB bij kinderen met astma. De resultaten van de studies uit dit proefschrift worden hieronder verkort weergegeven.

In **hoofdstuk 1** wordt kort de huidige kennis over astma samengevat. Astma is de meest voorkomende longziekte bij kinderen, en wordt gekenmerkt door wisselende periodes van luchtwegvernauwing, al dan niet met klachten van kortademigheid, piepen op de borst, en/of hoesten. De klachten ontstaan na blootstelling aan luchtwegvernauwende prikkels (allergenen, virusinfecties, rook, mist en koude). Bij astma is de gevoeligheid van de luchtwegen voor dit soort prikkels verhoogd. Dit wordt "bronchiale hyperreactiviteit" genoemd. Hieraan ten grondslag ligt een specifiek ontstekingsproces in de luchtwegen, ontstaan via immunologische weg.

In **hoofdstuk 2** wordt een overzicht gegeven van de literatuur over EIB, ook wel inspanningsastma genoemd. Belangrijke klachten zijn piepen op de borst, hoesten en kortademigheid tijdens of na een flinke inspanning. Ook pijn op de borst, en inspanningsintolerantie kunnen duiden op EIB. De ernst van EIB kan gemeten worden met behulp van longfunctie onderzoek voor en na een gestandaardiseerde inspanningsproef, en is een afspiegeling van de mate van bronchiale hyperreactiviteit bij astma. Het precieze mechanisme waardoor EIB ontstaat is niet geheel bekend, echter afkoeling en uitdroging van de luchtwegen door de snelle ademhaling tijdens inspanning spelen een belangrijke rol. De luchtwegvernauwing zelf is waarschijnlijk het gevolg van de uitstoot van broncho-actieve stoffen uit pulmonale cellen, alsook prikkeling van zenuwuiteinden in de luchtwegen. EIB kan worden voorkómen door het gebruik van medicijnen, die of gedurende korte tijd de reactie blokkeren, of op lange termijn het ontstekingsproces in de luchtwegen verminderen.

De doelstellingen van de studies in dit proefschrift worden in **hoofdstuk 3** uitgewerkt.

In **hoofdstuk 4** wordt de eerste studie beschreven. Omdat gegevens over de reproduceerbaarheid van EIB bij kinderen met astma ontbreken, werd de reproduceerbaarheid van de luchtwegrespons op een gestandaardiseerde inspanningsproef onderzocht. Tijdens deze inspanningsproef werd droge lucht ingeademd. Zevenentwintig kinderen, bekend met EIB, verrichtten twee

identieke inspanningstesten op verschillende dagen. De luchtwegvernauwing na inspanning werd enerzijds beschreven als het maximale percentage daling in de FEV_1 (%daling) en anderzijds als de oppervlakte onder de tijd-response curve (AUC). De reproduceerbaarheid werd bepaald met behulp van de intra-class correlatie coëfficiënt, welke een waarde liet zien van 0.57 voor de %daling, en 0.67 voor de AUC. Aan de hand van de gegevens konden tevens berekeningen worden gemaakt over de benodigde hoeveelheid deelnemers (=steekproefgrootte) in studies die het effect van medicijnen op EIB onderzoeken. Geconcludeerd werd dat de reproduceerbaarheid van EIB dusdanig goed is, dat de gestandaardiseerde inspanningsproef met gebruik van droge lucht zeer bruikbaar is voor studies naar EIB bij kinderen.

In **hoofdstuk 5** werd de klinische uitingsvorm van EIB in relatie tot de leeftijd onderzocht. De hypothese, dat het herstel van de luchtwegvernauwing na inspanning trager verloopt wanneer kinderen ouder worden, was voor een deel gebaseerd op klinische observatie, en deels op literatuur gegevens bij volwassenen. Bij veertien kinderen in de leeftijd van 7 tot en met 12 jaar, allen bekend met EIB, onderzochten we de snelheid van herstel van luchtwegvernauwing veroorzaakt door twee verschillende prikkels, te weten histamine en inspanning. De mate van luchtwegvernauwing veroorzaakt door histamine was vergelijkbaar met de luchtwegobstructie in aansluiting aan inspanning. We konden laten zien dat de herstelsnelheid na inspanningsgeïnduceerde luchtwegvernauwing significant afnam met de leeftijd, terwijl een dergelijk verband niet kon worden waargenomen na histamine geïnduceerde luchtwegvernauwing. Verdere analyse liet zien dat in de groep van kinderen ouder dan elf jaar, de snelheid van herstel van EIB significant verlaagd was ten opzichte van histamine veroorzaakte luchtwegvernauwing. In de groep jongere kinderen evenwel verliep het herstel van de obstructie door de twee verschillende prikkels even snel. Deze gegevens suggereren dat het mechanisme van EIB verandert met de leeftijd bij kinderen met astma.

Het optreden van een late astmatische respons (LAR) na inspanning was het onderwerp in **hoofdstuk 6**. De huidige informatie is vooral afkomstig van studies in volwassen astma patiënten, en de resultaten zijn niet éénduidig. We onderzochten het vóórkomen van een LAR na inspanning bij kinderen met astma, door longfunctie gegevens enkele uren na inspanning gemeten te vergelijken met metingen verricht op een controle dag zonder inspanning. Ook werden deze metingen vergeleken met longfunctie gegevens verkregen na provocatie met een ander soort luchtwegvernauwende prikkel, namelijk histamine. Zeventien kinderen in de

leeftijd van 7 tot en met 14 jaar, verrichtten, in willekeurige volgorde op 3 verschillende dagen, een gestandaardiseerde inspanningsproef, of een histamine provocatietest waarbij eenzelfde mate van luchtwegvernauwing als na inspanning werd geïnduceerd, of longfunctie metingen zonder voorafgaande provocatie. Het meten van de longfunctie (FEV_1) werd elk half uur herhaald gedurende een periode van acht uur op deze studie dagen. Alle kinderen vertoonden in aansluiting aan de inspanning een acute luchtwegvernauwing van wisselende ernst. Echter de longfunctie in de periode 2 tot 8 uur na inspanning verschilde niet van de normale variatie in FEV_1 op de controle studie dagen. Hieruit werd geconcludeerd dat het voorkomen van een late astmatische respons na inspanning bij kinderen met mild tot matig astma niet erg waarschijnlijk is. Dit zou kunnen betekenen dat hoewel inspanning kan leiden tot acute luchtwegvernauwing, er waarschijnlijk geen activatie van ontstekingscellen ontstaat.

In **hoofdstuk 7** werd de complexe relatie tussen allergeen expositie, luchtwegontsteking en bronchiale hyperreactiviteit onderzocht. Doel van het onderzoek was de bijdrage van de eosinofiele cel aan de bronchiale hyperreactiviteit voor inspanning (EIB) en methacholine (een farmacologische prikkel) te bepalen, door veranderingen in bronchiale hyperreactiviteit ten gevolge van blootstelling aan graspollen te correleren aan veranderingen in de ontsteking van de luchtweg. Aangenomen werd, dat het gehalte van het serum eosinofiel cationic protein (sECP) in het bloed een afspiegeling was van de eosinofiel gemedieerde luchtwegontsteking. Tevens werd onderzocht of het gebruik van inhalatiesteroïden een modificerend effect kon hebben. Voor en tijdens het graspollen seizoen werden daarom metingen van sECP, EIB en bronchiale hyperreactiviteit voor methacholine verricht bij kinderen met een graspollenallergie, al dan niet behandeld met inhalatiesteroïden. Als controle groepen werden kinderen zonder graspollenallergie ingesloten in de studie. De resultaten lieten zien dat de hoogte van het sECP slechts in geringe mate correleerde aan de ernst van het EIB, en niet aan de methacholine reactiviteit. De toename van het sECP in graspollen allergische kinderen leek voornamelijk bepaald te worden door de sensibilisatiegraad van het kind, terwijl de verandering in EIB en hyperreactiviteit voor methacholine mede bepaald werden door de hoeveelheid allergeen (graspollen) waaraan het kind had blootgestaan. Het gebruik van inhalatiesteroïden leek te beschermen tegen de allergeen-geïnduceerde verergering van astma. Deze gegevens suggereren dat de bronchiale hyperreactiviteit voor indirect aangrijpende luchtwegvernauwende prikkels (inspanning) meer zijn gerelateerd aan cellulaire activiteit dan direct aangrijpende stimuli (methacholine). Het bepalen van de hoogte van sECP in het bloed zou van waarde kunnen zijn bij kinderen met astma, wanneer er sprake is van seizoensgebonden allergeen expositie.

In **hoofdstuk 8** worden de effecten van inhalatiesteroïden (ICS) aangaande de bescherming tegen EIB beschreven. De belangrijkste doelstelling van dit onderzoek was om te analyseren in hoeverre deze effecten afhankelijk zijn van dosis en tijdsduur van de behandeling. Tevens werd onderzocht of de bescherming van ICS tegen direct-werkende (methacholine) en indirect-werkende luchtwegvernauwende prikkels (inspanning) teweeg wordt gebracht via verschillende mechanismen. Om deze hypothese te kunnen toetsen, werden gelijktijdig metingen verricht van EIB en methacholine reactiviteit, tijdens lange termijn behandeling met ICS bij kinderen met matig astma. Door tevens de hoogte van het sECP te bepalen, kon de potentie hiervan voor het beoordelen van ontstekingsactiviteit (en dus behandelingseffectiviteit) worden onderzocht. 37 kinderen in de leeftijd van 6 tot en met 14 jaar, participeerden in deze dubbelblinde, placebo-gecontroleerde parallelgroep studie. Gedurende 6 maanden werden zij behandeld met fluticasone propionaat, in een dosering van 200 mcg of 500 mcg per dag. De resultaten lieten zien dat beide doseringen een snelle en blijvende verbetering in EIB bewerkstelligen. De verbetering in de bronchiale reactiviteit voor methacholine was daarentegen wel afhankelijk van dosering en tijdsduur van behandeling. Daarnaast bleek dat voor behandeling met ICS de hoogte van het sECP in het bloed correleerde aan de ernst van EIB, echter tijdens behandeling kon een dergelijk verband niet worden waargenomen. Deze gegevens ondersteunen de hypothese dat het beschermend effect van ICS op direct en indirect aangrijpende luchtwegvernauwende prikkels wordt geëffectueerd via veschillende mechanismen.

In **hoofdstuk 9** tenslotte, is het aandeel van de cysteinyl leukotriënen (Cys-LT) in het mechanisme van het EIB bij kinderen met astma het onderwerp van studie. De hypothese van dit onderzoek, dat de Cys-LT vooral belangrijk zijn in het onderhouden van de luchtwegvernauwing na inspanning, was deels gebaseerd op de resultaten uit hoofdstuk 5, en werd getoetst door een groep adolescenten met astma te behandelen met een Cys-LT1 receptor antagonist, zafirlukast. In een dubbelblinde, placebo-gecontroleerde, gekruiste studie-opzet werd de mate van bescherming tegen EIB gemeten na een twee weken durende behandeling met zafirlukast (20 mg of 80 mg 2×daags), of cromoglycaat (10 mg 4×daags), of placebo. Gestandaardiseerde inspanningsprovocatietesten werden verricht 4 en 8 uur na inname van de laatste dosis van de behandeling. Analyse van de metingen 4 uur na inname van de laatste dosis lieten zien dat een beschermend effect van zafirlukast vooral tot uiting kwam in een significant sneller herstel van de luchtwegvernauwing na inspanning, in vergelijking met de placebo behandeling, terwijl geen effect werd gevonden op de maximale daling in longfunctie. Cromoglycaat daarentegen

liet geheel geen beschermend effect zien. De resultaten 8 uur na inname van de laatste dosis van een behandelingsperiode lieten eenzelfde beeld zien. Deze gegevens impliceren dat cysteinyl leukotriënen belangrijke mediatoren zijn in de pathofysiologie van EIB bij kinderen met astma, vooral actief in het onderhouden van de luchtwegvernauwing na inspanning.

Conclusies

★ De luchtwegvernauwende reactie op een inspanningsprovocatietest met inademing van droge lucht bij kinderen met astma is voldoende reproduceerbaar. De opzet van studies naar EIB is daardoor mogelijk in een beperkt aantal proefpersonen met behoud van voldoene statistische power.

★ De snelheid van herstel van inspanningsgeïnduceerde bronchusobstructie neemt af met de leeftijd, mogelijk duidend op een verandering in de pathofysiologie van EIB.

★ Vergeleken met een allergeen provocatie, leidt een inspanningsprovocatietest bij kinderen met mild tot matig astma, niet tot eenzelfde late astmatische reactie. Dit suggereert dat EIB slechts een afspiegeling is van bronchiale hyperreactiviteit, en niet een oorzaak, in tegenstelling tot allergeen expositie.

★ De mate van verandering in bronchial reactiviteit voor inspanning of methacholine tijdens natuurlijke allergeen expositie, is afhankelijk van de sensibilisatiegraad alsmede de hoeveelheid allergeen. Bronchiale hyperreactiviteit voor inspanning, maar niet methacholine, is gerelateerd aan cellulaire activiteit, zoals afgemeten aan het sECP. Behandeling met inhalatiesteroïden beschermd tegen allergeen-geïnduceerde verergering van luchtwegontsteking.

★ Behandeling met inhalatiesteroïden bij kinderen met astma leidt tot een snelle en blijvende verbetering van EIB, waarbij het effect van 200 mcg fluticasone propionaate niet verschilt van dat van 500 mcg per dag. De verbetering in bronchial reactiviteit voor methacholine daarentegen, is niet alleen afhankelijk van de dosering, maar ook van de tijdsduur van behandeling. Het beschermend effect van inhalatiesteroïden tegen direct en indirect aangrijpende luchtwegvernauwende prikkels wordt waarschijnlijk teweeg gebracht via verschillende mechanismen.

★ Behandeling met de cysteinyl leukotriëen (Cys-LT) receptor antagonist zafirlukast, geeft een significante verbetering van het EIB in adolescenten met astma, vooral tijdens de herstelfase. Dit impliceert dat de Cys-LT's belangrijke mediatoren zijn in EIB, m.n. in het onderhouden van de luchtwegvernauwing na inspanning.

Curriculum vitae

Winfried Hofstra werd geboren op 5 juli 1962 te Seroei (voormalig Nieuw-Guinea). Het VWO diploma behaalde zij in 1980 aan de Chr. Scholengemeenschap Jan van Arkel te Hardenberg. Na een jaar studie aan de Universiteit Twente, bracht ze, na uitloting, een jaar door in Cambridge als au-pair. In 1982 begon ze aan de studie geneeskunde in Leiden, en behaalde haar doctoraalexamen in 1987, en vervolgens haar artsexamen in 1989. Na haar artsexamen startte ze haar werkzaamheden op de afdeling Kinderlongziekten van het Juliana Kinderziekenhuis, Den Haag (hoofd: dr E.J. Duiverman). Vanaf juli 1990 werd patiënt-gebonden onderzoek gecombineerd met poliklinische werkzaamheden. In 1992 werd in samenwerking met Prof. dr H.J. Neijens, Sophia Kinderziekenhuis Rotterdam, en Prof. dr P.J. Sterk, Academisch Ziekenhuis Leiden, een herstart gemaakt teneinde het onderzoek uit te bouwen tot een promotie-onderzoek. Na een verhuizing naar Apeldoorn medio 1996 combineerde ze het schrijven van de dissertatie met een deeltijdbaan als arts-onderzoeker op de afdeling Kindergeneeskunde, Ziekenhuiscentrum Apeldoorn, en werkt ze tevens als consultatiebureau-arts in de regio. Zij is getrouwd met Marcel van Veelen, en is de trotse moeder van twee zonen: Thomas (3) en Wouter (1).

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