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Martin Johan Visser

1. Een stepdown benadering met inhalatiesteroïden biedt op de lange termijn geen voordeel ten opzichte van een continue onderhoudsbehandeling bij kinderen met astma.
2. Fluticasone propionate heeft een dosisafhankelijk effect op zowel de bronchiale hyperreactiviteit als op systemische bijwerkingen.
3. De effectiviteit en de bijwerkingen van hoge doseringen fluticasone propionate worden snel tenietgedaan na het afbouwen van deze hoge doseringen.
4. Fluticasone propionate gegeven via een stepdown benadering of in een constante dosis gedurende 2 jaar bij kinderen met astma heeft geen nadelige effecten op lengtegroei, bijnierschorsfunctie of botdichtheid.
5. Het gebruik van β_2-mimetica, peakflow of symptoomscores gezamenlijk of alleen om kortdurende dosis-respons effecten van inhalatiesteroïden aan te tonen is af te raden wanneer men bronchiale hyperreactiviteits metingen tot zijn beschikking heeft.
6. Onderhoudstherapie met fluticasone propionate in een dosis van 200 μg/dag is effectief en veilig.
7. Bij het titreren van de dosering inhalatiesteroïden bij kinderen met astma hebben het bepalen van de concentratie stikstof monoxide in de uitademingslucht (gemeten middels de "tidal breathing method") en het meten van cytokines in het bloed of gestimuleerde bloed monocyten geen aanvullende waarde.
8. De diagnose "astma" bij kinderen tussen 6 en 10 jaar is onzeker.
9. Men krijgt eerder een kip door het ei uit te broeden dan door het kapot te slaan (*Abraham Lincoln*).
10. De prijzen van kinderkleren in de winkel onderstrepen dat een kind een naar verhouding groot lichaamsoppervlak heeft (*Midas Dekkers*).
11. De beste screensaver op de computer is de uitknop.

Rijksuniversiteit Groningen

Long-term effects and side-effects of inhaled corticosteroids in childhood asthma: a dose-response study

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elke keer als je begint
denk je: is het de moeite wel waard?
elke keer als je klaar bent
denk je: is het wat? is het niks?
daartussen liggen uren dagen maanden- de jaren
wijzen het eenmaal uit.

(C. Buddingh'. Uit: Deze kant boven)

Voor mijn moeder,
ter nagedachtenis aan mijn vader

Contents

Chapter 1

Introduction

Childhood asthma

Asthma is the most common chronic disease of childhood with a prevalence of approximately 11% in the Netherlands.[1] The disease puts a tremendous burden on both children with asthma and their parents, as well as on healthcare resources.[2] Over the past decades, the prevalence of childhood asthma has increased considerably, and is still increasing.[3,4] Thus it is important to optimise treatment of childhood asthma, not only for patients and parents, but also for public health and health care economics.

The major characteristics of asthma in school-aged children, adolescents, and adults are chronic airways inflammation, airways hyperresponsiveness, and reversible airways obstruction.[5] These characteristics are strongly associated with allergy, in particular to inhaled allergens.[5] The major presenting symptom in childhood asthma is recurrent wheezing. In subjects older than 6 years of age, (allergic) asthma is the primary cause of recurrent wheeze. In children under the age of 6 years, however, there appear to be at least two different phenotypes of recurrent wheezing (Table 1). One is that of allergic asthma commencing at an early age. Indeed, most cases of allergic asthma first appear in young childhood.[6] This phenotype is associated with atopy from an early age onwards, and is characterised by recurrent wheezing, both with and without viral respiratory tract infections.[7–10] Risk factors for this syndrome include maternal atopy, and elevated serum IgE levels.[11] This wheezing illness usually persists throughout childhood.[11] The other early childhood wheezing phenotype is that of viral induced wheezing ("toddler's asthma" or "transient wheeze") which is characterised by recurrent bouts of wheezing only when the child has a viral upper

respiratory tract infection. In between infections, these children are usually free from respiratory symptoms. This syndrome is not associated with allergy, and wheezing usually disappears between the ages of 4 to 6 years ("transient wheeze").[11] Risk factors include diminished airways patency prior to the first wheezing episode[11-17], and maternal smoking.[11] In clinical practice, there is considerable overlap between these two phenotypes, in terms of symptoms and signs.[10] Objective measurements of lung function and airways hyperresponsiveness are not readily available below 6 years of age. There is some evidence that the nature of inflammation differs between children with different wheezing phenotypes but such an assessment of airways inflammation is not possible in clinical practice.[18] In pre-school children with wheeze, therefore, the clinician has to rely solely on history and physical examination, and a clear distinction between allergic asthma and "toddler's asthma" in this age group is almost impossible.

Table 1: Differences and overlapping features between "toddler's asthma" (e.g. viral associated wheeze/transient wheeze) and allergic asthma (persistent wheeze) in children under six years of age.[11-17]

Characteristics	"toddlers asthma"	allergic asthma
Complaints/symptoms	especially during URTI	increase of symptoms on exposure to allergic and non-specific stimuli
	symptom-free between URTI	
Most common provoking factor of exacerbation	common cold (=URTI)	common cold (=URTI)
Risk factors	small airways at birth	allergic to inhalation allergens
	parental smoking	parental smoking
	young mother	positive family history of allergy
	low birth weight	eczema
		elevated IgE levels
Effect of inhaled corticosteroids	limited	usually good
Effect of bronchodilators	moderate/good	usually good
Prognosis > 6 years of age	symptoms often disappear	symptoms usually persist

URTI: Upper Respiratory Tract Infection; IgE: immunoglobulin-E

In children older than 6 years, measurements of airways calibre, variation of air flow, and airways hyperresponsiveness are readily available.[19] The usefulness of such measurements for clinical management of asthma, although likely, has not been substantiated by published evidence.[20] Within groups of asthmatic patients, correlations between symptoms, lung function, and airways hyperresponsiveness have been de-

scribed[21] but these relationships are weak and variable, both within and between patients.[22] The two measurements most closely associated to the degree of airways inflammation are airways hyperresponsiveness and exhaled nitric oxide.[23-26]

Inflammation and corticosteroid responsiveness in (childhood) asthma

It has now been well established that asthma is caused by an ongoing inflammatory process in the airways wall.[5] Chronic airways inflammation in asthma is characterised by infiltration of CD4+ T-helper (Th2) cells and eosinophils into the airways wall.[5,27,28] These cells, when activated, produce a myriad of mediators[29-32], with a characteristic predominance of Th2-type cytokines (interleukin(IL)-4, IL-5, and IL-10) over Th1-type cytokines (interferon-gamma (INF-γ), IL-2, IL-12).[33-38]

Upon inhalation of allergens eosinophils are recruited from the bone marrow, migrating to the airways.[39] The migration and adhesion of peripheral blood eosinophils to the inflammatory sites is enhanced by adhesion molecules, such as ICAM-1. A soluble form of ICAM-1 (sICAM-1) is elevated in the peripheral blood of asthmatic children[40-42], compatible with an increase in the number of eosinophils in blood, airways and sputum of asthmatic individuals.[40-43] Both *in vitro* and *in vivo* studies in asthmatic children have demonstrated that corticosteroids reduce sICAM-1 levels[44-46], eosinophil counts and serum eosinophilic cationic protein (ECP) concentrations.[47-49]

Studies in adult asthmatics have demonstrated that oral corticosteroids inhibit mRNA expression of IL-4, IL-5, and INF-γ as well as their related cytokine production in bronchoalveolar lavage fluid and lung tissue.[50,51] Inhaled corticosteroids reduce serum levels of IL-5 and increase IL-10 levels in adult asthmatics.[52,53] Studies in asthmatic children have shown a reduction in serum IL-5, but not in serum IL-4 concentrations after therapy with oral prednisolone.[54] Treatment with inhaled corticosteroids was associated with a reduction in mRNA encoding IL-4 and IL-5, an increase in INF-γ mRNA expression from stimulated blood peripheral mononuclear cells (PBMCs)[55-57], a decrease in serum IL-5,[58] and an increase levels of the anti-inflammatory cytokine IL-10.[59]

The available evidence, therefore, supports the concept that inhaled corticosteroids effectively inhibit the inflammatory cascade in asthma, which is associated with a reduction in clinical symptoms and exacerbations. A major limitation in paediatric studies on the effects of inhaled corticosteroids with respect to reduction of asthmatic airways inflammation is that most studies have used peripheral blood as the

source for inflammatory cells. It is unclear whether this is a useful reflection of inflammation in the airways of asthmatic children.

Clinical efficacy of inhaled corticosteroids in childhood asthma

Because asthma is characterised by chronic airways inflammation, anti-inflammatory therapy, in particular with inhaled corticosteroids, is the cornerstone of asthma management.[60-62] Studies on clinical efficacy of inhaled corticosteroids have been performed generally with beclomethasone dipropionate (BDP), budesonide (BUD) and fluticasone propionate (FP).

"Stepdown" approach in childhood asthma

Most clinical studies of inhaled corticosteroids in childhood asthma examined fixed dosages of inhaled corticosteroids during prolonged periods of time.[63,64] There is considerable evidence suggesting that the effect of inhaled corticosteroids on asthma symptoms, lung function, and airways hyperresponsiveness are, at least partly, dose-dependent.[65-70] Therefore, current guidelines suggest to start inhaled corticosteroid therapy in childhood asthma with a relatively high dose (aimed at reducing airways inflammation powerfully and effectively). This high starting dose is subsequently tapered down to the lowest effective dose ("stepdown" approach).[60-62,71,72] Guidelines also suggest to double the dose of inhaled corticosteroids during an exacerbation of symptoms.[60] Popular as this stepdown strategy may be, there is hardly any evidence from clinical trials to support this practice.

One study in asthmatic children, aged 6 months to 3 years, compared a stepdown approach with a constant dose approach with nebulised BUD suspension.[73] Patients from the stepdown group showed a more rapid improvement in asthma symptoms (which was sustained during follow-up) when compared to the constant dose group. This study, however, did not evaluate objective assessments (lung function, airways hyperresponsiveness), and had a short follow-up time (6 weeks), limiting the generalisability to daily clinical practice.

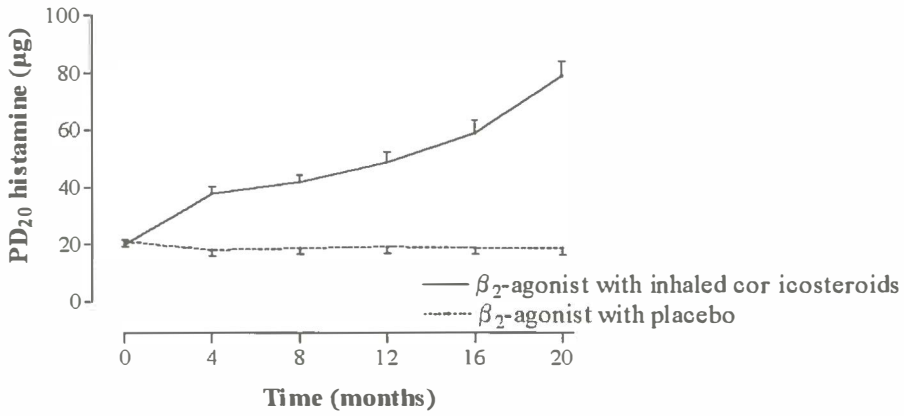
Airways hyperresponsiveness, lung function (forced expiratory volume in 1 second (FEV_1), peak expiratory flow (PEF)) and symptoms are the most commonly used parameters to assess the clinical efficacy of inhaled corticosteroids.

Airways hyperresponsiveness

Airways hyperresponsiveness is one of the cardinal features of childhood asthma[74], and is commonly used as an end point in clinical trials in school-aged asthmatic children. During treatment with inhaled corticosteroids, airways hyperresponsiveness shows a marked improvement which continues throughout the first 2 years of therapy (Figure 1).[63,75–77] Cessation of inhaled corticosteroids results in an increase in airways hyperresponsiveness to pre-treatment levels.[78,79] Most children with asthma will rapidly develop (near-)normal levels of lung function after starting therapy with inhaled corticosteroids (Figure 2).[63,75–77,80] This suggests that airways obstruction is an invalid inclusion criterion in clinical trials of asthmatic children already using inhaled corticosteroids.[81] It has not been investigated whether this also holds true for airways hyperresponsiveness.

Limited data is available on the effect of different doses of inhaled corticosteroids on airways hyperresponsiveness in asthmatic children. One study found no difference in the improvement of airways hyperresponsiveness between children using BUD 100 and 200 μg/day for 12 weeks.[82] In another study, however, airways hyperresponsiveness improved significantly more in asthmatic children (6-14 years) using FP 400 μg/day than in children using FP 200 μg/day after 6 months of treatment.[67] No data are available on higher dosages of inhaled corticosteroids.

Figure 1: Effect of inhaled corticosteroids on airways hyperresponsiveness during long-term treatment of asthmatic children (after van Essen-Zandvliet et al[63]), reprinted with permission of the publisher). Note the different pattern with change over time as compared to changes in FEV_1 (Figure 2).

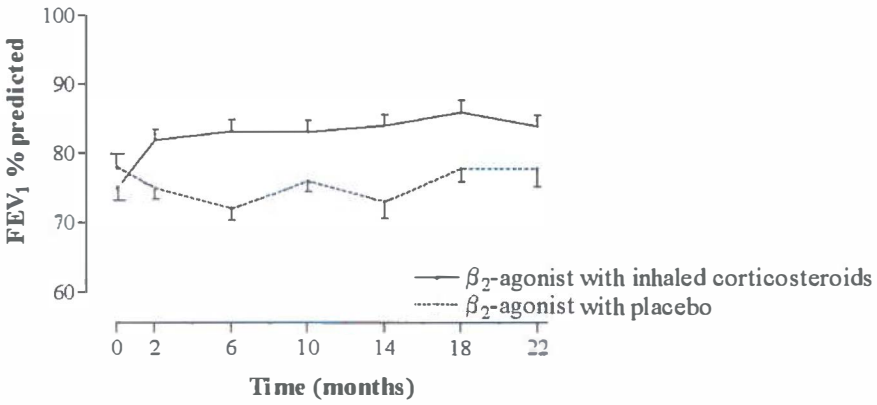


Lung function

Several studies have demonstrated a dose-dependent effect of inhaled corticosteroids on FEV_1.[65–67,70,79] The improvement of FEV_1 after introduction of inhaled corticosteroids is most pronounced during the first few weeks of therapy, and reaches a plateau within 3 months, making FEV_1 an insensitive parameter to monitor long-term treatment of inhaled corticosteroids (Figure 2).[63,71,75,76]

PEF also improves during inhaled corticosteroid therapy in a similar way as FEV_1 over time, with little, if any, evidence of a dose-response relationship.[65–68,70,83,84]

Figure 2: Effect of inhaled corticosteroids on lung function in asthmatic children (after Verberne et al[70]). Note the different pattern of changes over time as compared to changes in PD_{20} (Figure 1).



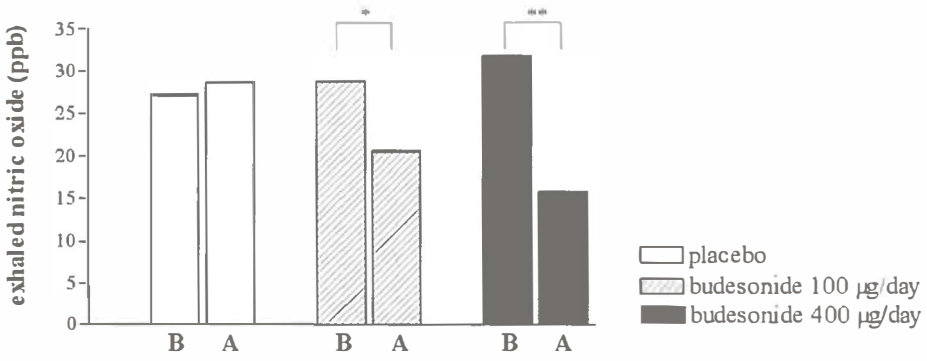
Symptoms/use of β_2-agonists

Especially in young children, who are not able to perform lung function tests, symptoms and use of rescue β_2-agonists are commonly used to monitor response to inhaled corticosteroid therapy. These parameters improve rapidly after therapy with inhaled corticosteroids in asthmatic children, independent of the dose used.[65–68,73]

Exhaled nitric oxide (eNO)

Asthmatic children have higher levels of eNO compared to healthy controls[85–88], and inhaled corticosteroids reduce eNO levels in asthmatic subjects to normal levels.[89–91] There is limited evidence suggesting a dose-dependent effect of inhaled corticosteroids on eNO levels (Figure 3).[92]

Figure 3: Differences in exhaled nitric oxide (eNO) levels in adult asthmatics before (B) and after (A) four weeks treatment with placebo, budesonide 100 μg/day or budesonide 400 μg/day.



* $p < 0.05$; ** $p < 0.01$, after Jatakanon et al[92]

In conclusion, airways hyperresponsiveness is the best available parameter to monitor long-term treatment with inhaled corticosteroids as yet, not only to test its effectiveness but also to assess a dose-response relationship. In contrast, lung function, symptoms and use of rescue medication are of limited value in guiding the dose of inhaled corticosteroids (Table 2).

Table 2: Measurements for determining efficacy of therapy with inhaled corticosteroids in asthmatic children.

No DRR	DRR (short-term)	DRR (short-term and long-term)
Symptoms	FEV_1	Airways hyperresponsiveness
Use of rescue medication (β_2-agonists)	Exhaled NO	
PEF		

DRR: Dose-Response Relationship

It remains to be established whether stepping up, stepping down or a fixed dose of inhaled corticosteroids is the best approach in asthma management. For proper evaluation of this research question, airways hyperresponsiveness appears to be the preferred primary end-point.

Side-effects of inhaled corticosteroids in childhood asthma

Local side-effects

Local side-effects due to deposition of inhaled corticosteroids in the pharynx and larynx include oropharyngeal candidiasis, dysphonia and perioral dermatitis.

Oropharyngeal candidiasis is caused by a steroid-induced diminished mucosal immune response.[93,94] In a series of 639 children treated with BDP (mean dose 720 μg/day) or BUD (mean dose 835 μg/day), Dubus and co-workers reported a prevalence of 11% of doctor diagnosed "clinical oral candidiasis" in both treatment groups.[93] This prevalence rate is extremely high compared to other studies. One other case series of 229 children using inhaled corticosteroids (BDP > 500 μg/day) only reported oral candidiasis in one patient.[95] In two controlled clinical trials, oropharyngeal candidiasis was seen no more frequently in the group treated with inhaled corticosteroids than in the cromoglycate group (both 0%).[96,97]

Dysphonia is thought to be caused by a non-specific myopathy of the laryngeal muscles.[98] A dose-dependent risk of dysphonia has been described for both BDP and BUD.[99–101] Dysphonia has been reported in up to 11 to 20% of children using inhaled BDP or BUD.[93,97]

Cough is frequently reported by children using inhaled corticosteroids delivered by dry-powder inhalers, whereas perioral dermatitis is most commonly observed during treatment with inhaled corticosteroids delivered by nebulizers or by metered dose inhalers (MDI) attached to a holding chamber with facemask.

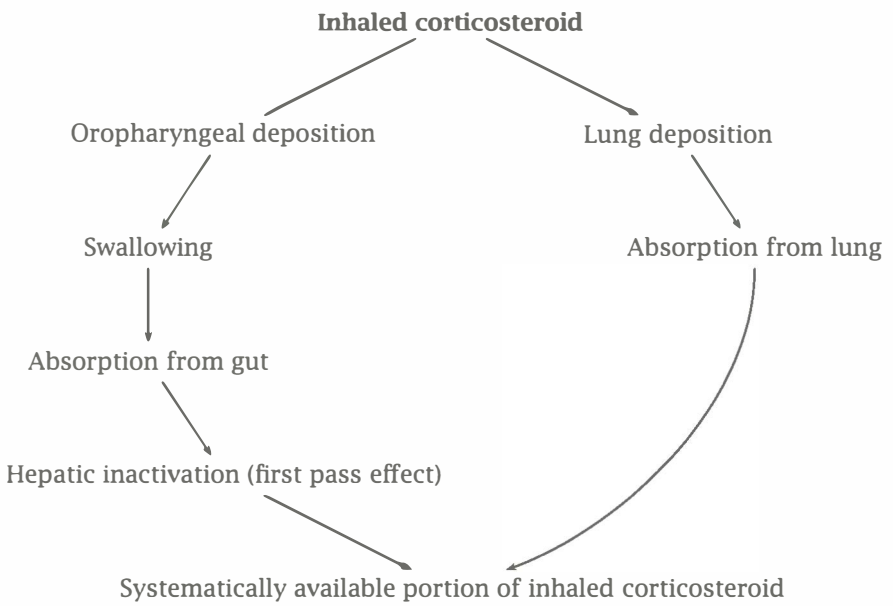
Systemic side-effects

Inhaled corticosteroids can exert systemic side-effects when part of the inhaled dose reaches the bloodstream through absorption from the gut (after swallowing the drug as deposited in the oropharynx) or the lung (Figure 4).[102–104] When using an MDI without a spacer, or a dry powder inhaler (DPI), up to 60% of the inhaled corticosteroid

dose is deposited in the oropharynx.[105,106] Use of a spacer device reduces oropharyngeal deposition and hence the amount of inhaled corticosteroids absorbed from the gut[107,108], thereby reducing systemic absorption and systemic side-effects.[109,110] The systemic bioavailability of inhaled corticosteroids from the gut is dependent on the degree of first-pass inactivation of the liver. The inactivation of the steroid is larger for FP (99%) and BUD (90%) than for BDP (70%).[111]

Systemic bioavailability is mainly determined, however, by the quantity of drug deposited in the lung[112], and increased lung deposition will lead to an increase in systemic bioavailability (Figure 4).

Figure 4: Systemic bioavailability of inhaled corticosteroids depends on absorbtion from the gut and lung (after Brand[112]).



Effects on growth

Like any chronic disease, chronic untreated or undertreated asthma may cause poor prepubertal growth, which is more pronounced in boys than in girls.[113-116] In addition, the onset of puberty is delayed[113-116], accentuating reduced height in young teenagers with asthma. The pubertal growth spurt, although delayed, is normal and

attained adult height of asthmatics is normal.[112,114,117,118] Longitudinal studies have demonstrated that height standard deviation scores in asthmatic children who were not treated with inhaled corticosteroids correlated negatively with the severity of asthma: poor lung function and poor asthma control were associated with lower height standard deviation scores.[71,116,119]

Effects of inhaled corticosteroids on growth can be assessed by short-term (weeks), medium-term (months) and long-term (years) studies.

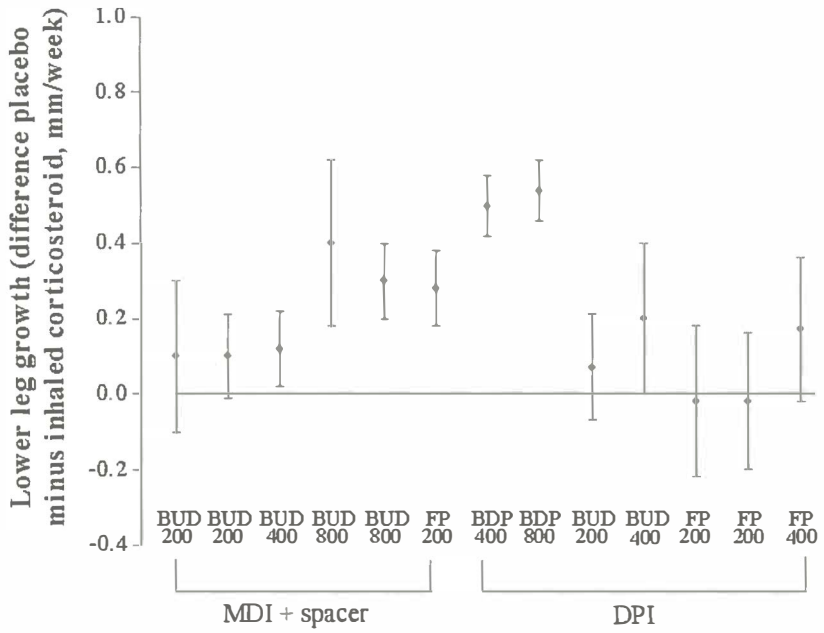
Short-term studies

Short-term growth can be assessed by means of knemometry, which measures lower leg length with an accuracy of 0.1 mm. Knemometry measurements allow detection of minimal changes, even within days.[120,121] During treatment with prednisolone 2.5 mg/day for 2 weeks, it was thus shown that lower leg growth was completely arrested.[122] Short-term treatment with inhaled corticosteroids (varying from 2 to 8 weeks) has been shown to reduce lower leg length in a dose-dependent fashion (Figure 5).[123–126] Although knemometry is a highly sensitive tool to detect systemic side-effects of inhaled corticosteroids[127], it does not predict long-term growth or final height.[128–130]

Medium-term studies

Systemic corticosteroids retard growth of children, even at doses as low as 5 mg/day.[131,132] Prospective randomised controlled trials investigating the effects of BDP showed a significant growth reduction of approximately 1 cm/year in asthmatic children (doses ranging from 320 to 400 μg/day) compared to controls during follow-up for up to 7-12 months.[75,77,133,134] During long-term therapy with BUD (dose 400 to 710 μg/day), a similar growth reduction was found during the first year of treatment,[71,135] but not during further treatment for 4-6 years.[135] Two studies using FP in a dose of 100 and 200 and μg/day in asthmatic children, aged 4 to 12 years, reported no difference in height growth rates between FP and non-steroid therapy (placebo or sodium cromoglycate) after one year treatment.[136,137] In a meta-analysis, however, the effects of inhaled FP on growth in asthmatic children (aged 7-16 years), were statistically significant and dose-dependent, amounting to a growth reduction of 0.5 cm/year at each 200 μg of inhaled corticosteroid/day).[112,138]

Figure 5: Results of knemometric growth studies during 2-8 weeks therapy with beclomethasone dipropionate (BDP), budesonide (BUD), fluticasone propionate (FP), or placebo. Result a are given as mean lower leg growth rate (mm/week) with 95% confidence intervals; values above zero indicate reduced lower leg growth during therapy with inhaled corticosteroids (after Brand[112]). MDI: metered dose inhaler; DPI: dry powder inhaler.



Long-term studies (adult height)

Several long-term studies on effects of inhaled corticosteroids on final adult height have been published.[113,119,139–142] These studies have shown that prolonged use of inhaled corticosteroids in doses up to 900 μg/day did not result in reduction of final attained height.

Effects of inhaled corticosteroids on the hypothalamic-pituitary-adrenal (HPA) axis

Exogenous corticosteroids, administered systemically, decrease adreno-corticotropic-releasing hormone (ACTH) levels through negative feedback on glucocorticoid re-

ceptors in the pituitary gland and hypothalamus.[143] As a consequence, prolonged systemic steroid therapy may cause adrenal cortical failure. When, under these circumstances, exogenous corticosteroids are suddenly withdrawn or when the body must mount a response to severe physiological stress, such adrenal failure may express itself in insufficient release of endogenous cortisol, and severe clinical illness (Addison's crises). Such events have also been described after withdrawal of inhaled corticosteroid therapy, but appear to be rare.[144,145] A large body of evidence is available on the effects of inhaled corticosteroids on the HPA axis. In order to compare results of these studies, the methodology of the HPA axis function tests must be reviewed.

The function of the HPA axis can be assessed by two different types of tests: screenings tests, which measure basal adrenal cortical function, and dynamic stimulation tests, which assess adrenal cortical reserve (Table 3).[102]

Table 3: Screening tests and dynamic stimulation tests to assess basal adrenal cortical function (in ascending order of sensitivity).

Screening tests (basal cortisol secretion/excretion)	Stimulation tests (reserve)
Plasma cortisol levels (8.00 am)	ACTH test (250 μg) high dose
Integrated plasma cortisol levels (12 or 24-hours)	CRH test (100 μg)
Urinary free cortisol (morning sample or 24-hours)	ACTH test (0.5 μg) low dose
24-hours urine cortisol metabolite excretion	

ACTH: Adreno-Cortico Tropic releasing Hormone; CRH: Corticotroop Releasing Hormone.

Screening tests

Basal adrenal cortical function can be tested, in ascending order of sensitivity, by single measurements of morning plasma cortisol, repeated assessments of plasma cortisol over 24 hours (integrated cortisol levels, expressed as area under the curve), 24-hour urinary free cortisol (with creatinine correction), or 24-hours cortisol metabolite excretion.

Single measurements of (8.00 a.m.) morning plasma cortisol are known to be insensitive to assess HPA axis function, because there is a marked diurnal and day-to-day variability in these levels (due to the pulsatile secretion of cortisol rather than a

gradual release during the day).[146–149] Twenty-four hour integrated measurement of plasma cortisol levels is a far more sensitive method to detect changes in adrenal cortical activity, brought about by exogenous steroids.[149,150] However, it is not very well suited for use in studies with children, because this method requires multiple venous sampling.

Urine is easily collected in children. There are two methods available to assess adrenal cortical function from urine samples. Free urinary cortisol excretion in overnight or 24-hours' urine provides a global impression of adrenal cortical function and it is independent of circadian changes in plasma cortisol levels.[149–151] It is more useful to detect excessive cortisol secretion (e.g. Cushing syndrome) than adrenal cortical suppression[151–153], although it is more sensitive to detect adrenal cortical suppression than are single plasma cortisol samples.[149]

The most sensitive method to assess adrenal cortical suppression by exogenous steroids is the measurement of excretion of cortisol metabolites in 24-hours' urine.[151,154,155] Studies using this method have shown dose-dependent effects of inhaled corticosteroids on the HPA axis in asthmatic children.[151,154,155]

Studies have been performed on the effects of BDP, BUD and FP on the HPA axis function in childhood asthma. We will here only discuss the effects of FP in asthmatic children, because this is the compound used in the studies presented in this thesis.

Placebo controlled studies of FP in asthmatic children have shown that serum cortisol levels are not affected when doses of 100 or 200 μg/day are used for short periods of time.[156,157] No difference in serum cortisol levels were found when FP 200 and 400 μg/day were compared with the same dosages of BUD or BDP, for 8 weeks up to 20 months.[158,159] Studies comparing the effects of different dosages of FP and placebo on 24-hours' and overnight urinary cortisol excretion have shown clear and consistent dose-dependent effects in doses ranging from 200 to 1250 μg daily.[126,157–162] In addition, high dose inhaled FP therapy was associated with diminished adrenal cortical reserve in ACTH stimulation tests.[163] No differences between the effects on adrenal cortical function of 200 to 750 μg/day FP and similar (equipotent) dosages of BUD or BDP have been detected.[159,164,165] A recent meta-analysis on systemic adverse effects of inhaled corticosteroids showed a significantly greater risk of dose-related adrenal cortical suppression for FP than for BDP or BUD.[166]

Effects of corticosteroids on bone metabolism

In adults, cross-sectional studies have shown weak, but significant associations between the use (and the dose used) of inhaled corticosteroids and bone mineral density.[167-169] Because the axial skeleton contains a higher proportion of trabecular bone, which is metabolically more active than cortical bone, the effects of corticosteroids can best be studies in this type of bone (i.e. the vertebrae, ribs).[170]

The mechanism for this corticosteroid induced decrease in bone mass in adults is unclear but is hypothesised to involve different actions, both direct and indirect in origin. Inhibition of new bone formation is a direct effect of corticosteroids, whereas indirect effects include increased urinary calcium excretion, reduction in intestinal calcium absorption, secondary hyperparathyroidism (resulting in increased bone resorption), and effects on the pituitary-gonadal and pituitary-adrenal axes.[171,172]

Effects of inhaled corticosteroids on bone metabolism can be assessed in two ways:

1. Biochemical markers. Table 4 shows different biochemical markers for bone formation and resorption, which may reflect short-term effects of (inhaled) corticosteroids.[173,174] Osteocalcin, alkaline phosphatase, and type 1 collagen are products from osteoblasts (bone formation), whereas pyridinoline and deoxypyridinoline are degradation products from collagen (bone resorption). It has been suggested that both markers of bone formation and resorption need to be measured at the same time for a reliable assessment of effects of corticosteroids on bone turnover.[174]
2. Bone mineral density can be assessed by dual X-ray absorptiometry (DEXA) of parts of the skeleton (e.g. lumbar spine and femoral head), or the whole body. Densitometry of skeletal segments can also be performed by ultrasound.[174] Such methods are useful to assess the effects of long-term therapy with (inhaled) corticosteroids on bone.

Longitudinal studies on effects of inhaled corticosteroids on markers of bone metabolism in childhood asthma have mainly been performed with BDP and BUD. It has been demonstrated that both BDP and BUD in dosages up to 800 μg/day in asthmatic children reduce bone metabolism.[175-178]

Cross sectional studies of asthmatic children (aged 6 to 13 years) treated with inhaled BDP, BUD or FP, however, showed no decreased bone mineral density when compared to healthy controls.[179-182] During long-term treatment with inhaled corticosteroids (BDP and BUD), no decrease in bone density in asthmatic children (aged 6 to 9 years)

has been found.[135,159,178,183] It appears, therefore, that adults and children differ in their sensitivity to steroid-induced osteopenia.

Few studies have assessed the effects of FP on bone metabolism in asthmatic children. Two short-term prospective studies, comparing BDP 400 μg/day and BUD 400 μg/day with FP 400 μg/day, showed no changes over 3 months or differences between different inhaled corticosteroids in bone formation, bone resorption or bone mineral density.[158,159]

Table 4: Biochemical markers of bone turnover.

Bone formation	Bone resorption
Serum osteocalcin	Serum tartrate resistant acid phophatase (TRAP)
Serum alkaline phosphatase (bone specific)	Serum cross-linked carboxyterminal telopeptide of type 1 collagen (1CTP)
Serum carboxyterminal propeptide of type 1 procollagen (P1CP)	Urinary hydroxyproline
Serum aminoterminal propeptide of type 1 procollagen (P1NP)	Urinary pyridinoline
	Urinary deoxypyridinoline
	Urinary calcium

Aims of the study

In the study presented in this thesis two different treatment approaches with inhaled FP are compared in asthmatic children, 6 to 10 years of age, with respect to efficacy and safety (systemic side-effects) parameters. We compared a high dose of inhaled FP (1000 μg/day, with two monthly reductions to 500, 200, and 100 μg/day, stepdown approach) with a median FP dose (200 μg/day, constant dose approach), given for two years. The results of the study are presented in the following chapters of this thesis.

1. Airways hyperresponsiveness is frequently used as an end-point in clinical trials assessing the efficacy of inhaled corticosteroids in childhood asthma. During the patient recruitment period of the study described in this thesis, we examined the feasibility of moderately severe airways hyperresponsiveness as an inclusion criterion for clinical trials in school-aged asthmatic children. This is described in **Chapter 2**.
2. During the run-in and wash-out phase of our study, approximately half of the asthmatic children did not develop airways hyperresponsiveness after withdrawal of inhaled corticosteroids. We hypothesised that these non-hyperresponsive children represented the non-atopic transient wheezers or "toddler's asthma"), whereas the hyperresponsive children would reflect atopic persistent wheezers (allergic asthma) described by others. Hyperresponsive and non-hyperresponsive children were examined for differences in demographic, clinical, and immunological features (**Chapter 3**).
3. Inhaled corticosteroids are able to reduce lower leg growth (as assessed by knemometry) in a dose-dependent fashion. Most studies have compared different dosages and types of inhaled corticosteroids with each other. In **chapter 4** we describe the comparison between FP 200 μg/day (by dry powder inhaler) and β_2-agonists only for 2 weeks on lower leg growth.
4. It has been well established that the effects of inhaled corticosteroids on airways hyperresponsiveness and airways inflammation are at least partly dose-dependent. For this reason, international guidelines on treatment of asthma advise to start inhaled corticosteroid therapy with a relatively high dose, tapering off to a lower dose. Most studies on inhaled corticosteroids in childhood asthma use a constant dose. No clinical studies have compared these two treatment strategies. In **chapter 5** we describe the results of 1-year treatment with two different dosage schedules of inhaled FP on airways hyperresponsiveness, lung function, symptoms and height.

5. Asthma is an inflammatory disease with a Th2-skewing of the immune response, resulting in an increase of interleukin (IL)-4, IL-5, and IL-10 levels, and a decrease of interferon-gamma (INF-γ) levels. Most knowledge on airways inflammation in asthma is inferred from studies in adults. These studies, however, generally have used invasive techniques (bronchoalveolar lavage and bronchial biopsy), which are not feasible in children. **Chapter 6** gives the results of the study investigating the effect of different dosages of inhaled FP on cytokine levels in peripheral blood samples and on the cytokine production capacity of stimulated peripheral blood mononuclear cells.
6. Although inhaled corticosteroids are considered to be first-line treatment in childhood asthma, there is concern about their potential systemic side-effects with respect to growth, adrenal cortical function and bone metabolism. In **chapter 7** we describe the results of our 2-year prospective study with two different dose-regimens of FP in children with mild to moderate asthma on these parameters.

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Chapter 2

The feasibility of airways hyperresponsiveness as an inclusion criterion for studies on childhood asthma

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Abstract

The feasibility of moderately severe airways hyperresponsiveness (AH) was examined as an inclusion criterion for clinical trials in asthmatic children.

During the baseline period of a long-term clinical trial in asthmatic children, maintenance therapy with fluticasone (200 μg/day) was stopped for a maximum of 8 weeks and methacholine challenges were performed at 2-week intervals or earlier if the patients' condition deteriorated. Patients were eligible to continue the study if the provocative dose of methacholine causing a 20% fall in forced expiratory volume in 1 second (FEV_1)(PD_{20}) was < 80 μg.

Fifty-one percent of the children did not develop a PD_{20} < 80 μg after withdrawal of fluticasone. Patients with or without a PD_{20} < 80 μg did not differ in duration of asthma, duration of treatment, or peak flow variation. Patients with a PD_{20} < 80 μg had higher levels of total and specific immunoglobulin-E, and lower levels of FEV_1 and mean maximal expiratory than patients with a PD_{20} ≥ 80 μg. Forty-four percent of the patients with a PD_{20} ≥ 80 μg did not have any symptoms during the wash-out period and 39% of these patients remained free from symptoms during one year follow-up.

The results of this study suggest that recruiting asthmatic children for clinical trials may be difficult if airways hyperresponsiveness is used as the sole inclusion criterion.

Introduction

Current guidelines for the treatment of childhood asthma emphasise the use of inhaled corticosteroids (ICS) as first-line treatment.[1,2] Airways hyperresponsiveness (AH) continues to improve throughout the first 2 yrs after institution of ICS therapy.[3–5] Therefore, if differences between various types or dosages of ICS are to be examined in clinical trials, the extent to which AH improves is a useful end-point. This is why a certain degree of AH has been frequently used as an inclusion criterion for clinical trials in childhood asthma over the past years.[3,6,7]

Children with asthma who are currently approached for participation in clinical trials generally use ICS therapy already, sometimes even for many years. Most of these patients will have a normal or near-normal level of lung function.[8] As a result, a certain degree of airways obstruction may no longer be a valid inclusion criterion for clinical trials in childhood asthma.[9] Although not formally investigated so far, this might be the case for AH as well.

During the patient recruitment period of an ongoing long-term study comparing different dosing schedules of ICS, the feasibility of moderately severe AH was examined as an inclusion criterion for clinical trials in school-aged asthmatic children.

Patients and methods

For this report, baseline data from an ongoing long-term study on ICS in childhood asthma were used. In this study, two dosage schedules of ICS are compared over a period of 2 yrs, using the improvement in AH as one of the main end-points. In order to be able to detect differences between different dosage schedules of ICS during the study period (rather than comparing ICS to placebo), moderate-to-severe AH (defined as a provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second (FEV_1)(PD_{20}) of < 80 μg) after withdrawal of ICS was an inclusion criterion.

Children, 6-10 yrs of age, with a clinical diagnosis of asthma, were recruited from the outpatient clinics of the three participating hospitals. None of these patients had, due to their age at presentation, undergone methacholine challenges before entering the study. After obtaining written informed parental consent, patients were switched from their usual maintenance therapy to fluticasone propionate 100 μg *b.i.d. via* dry powder inhaler (Diskhaler®, GlaxoWellcome) for 6 weeks (run-in period). No other maintenance treatment for asthma was allowed. The main objective of this run-in pe-

riod was to familiarise all patients with the inhaler device to be used during the whole study period. At the end of the run-in period, a baseline methacholine provocation test was performed. Subsequently, fluticasone propionate was stopped for a period of maximal 8 weeks (wash-out), and patients were only allowed to use inhaled short acting bronchodilator (salbutamol) for relief of symptoms. Methacholine provocation tests were performed at 2-week intervals or earlier if symptoms of asthma increased. The methacholine tests were performed with a dosimeter method as described elsewhere.[10] The usual cut-off level between "normal" airways responsiveness and AH using this method is a PD_{20} of 150 μg.[11]

At each follow-up visit patients were asked if they had experienced symptoms of cough, wheeze, or dyspnea. During the wash-out period, patients kept a diary in which symptoms and peak expiratory flow (PEF) were recorded. The highest of three PEF manoeuvres was recorded in the morning and evening. PEF variation was calculated as the lowest PEF level during the first 2 weeks of the wash-out period as a percentage of the highest PEF level recorded in this period (low%high).[4]

The patients entered the randomised part of the study as soon as they developed AH (PD_{20} < 80 μg). If PD_{20} was ≥ 80 μg after a wash-out period of maximally 8 weeks, patients were withdrawn from the study and treated according to the judgement of their paediatric pulmonologist. Follow-up data from these patients were collected from their medical records.

In all patients, blood was drawn for determination of total and allergen-specific immunoglobulin-E (IgE) concentrations. Allergen-specific IgE concentrations (to house dust mite, grass and tree pollen, and cat and dog dander) were determined by a radioallergosorbent test (RAST) (Pharmacia, Uppsala, Sweden). A RAST test was considered positive when the result read class 2 or higher. From their medical records, the mean daily dose of ICS (cumulative dose divided by time of treatment in days) was determined.

Differences between groups were analysed using the Mann-Whitney U-test and Chi-squared test as appropriate. To examine whether age was associated with AH, logistic regression analysis was carried out. Seasonal variation in the degree of AH was tested by nonparametric analysis of variance (ANOVA).

The study was approved by the ethics review boards of all three participating hospitals.

Results

Patient characteristics

Ninety-five children completed the run-in and wash-out period of the study. All patients had been treated with ICS for asthma before entering the study. At the end of the 6-week run-in period, during which all patients used 200 μg fluticasone daily, 8 (8.4%) had a PD_{20} < 80 μg, and 14 (14.7%) had a PD_{20} < 150 μg.

Table 1: Demographic and clinical characteristics of the patients with and without airways hyperresponsiveness (AH).

	AH	No AH	*p*-value [a]
	PD_{20} < 80 μg	PD_{20} ≥ 80 μg	
Subjects *n*	47	48	
Age (years)	8.1±1.2	8.2±1.2	0.54
Male children (%)	52	57	0.74
Duration of asthma (years)	5.1±2.0	5.0±2.1	0.74
Duration of ICS (years)	2.7±1.5	2.5±1.7	0.48
Mean daily dose of ICS received prior to entering study (μg)	361±97	328±104	0.12
FEV_1 at end of washout period (% pred.)	92.1±16.8	98.3±18.0	0.03
MEF_{50} at end of washout period (% pred.)	63.5±23.3	72.1±22.0	0.03
PEF (Low%High) [b]	72.1±12.3	74.3±13.5	0.33
IgE (kU/L)	457 (29-3966)	132 (2-4500)	0.001
RAST (positive %)	80.0	54.0	0.01
PD_{20} (μg)	31.8 (3.5-76)	665.3 (95-1565)	< 0.0001

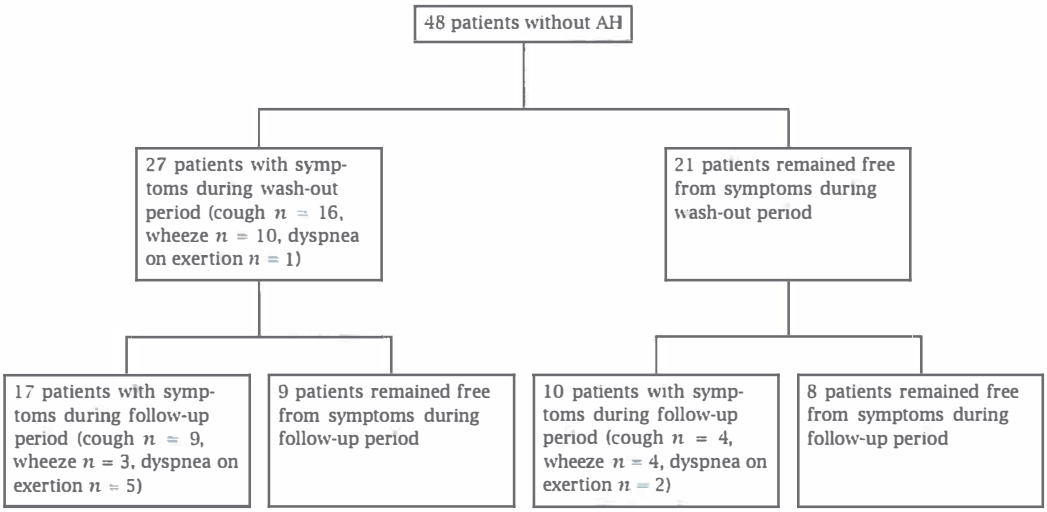
Data are presented as mean±SD, or as geometric mean (range).
[a]Mann-Whitney U-test or χ^2-test
[b]Peak expiratory flow (PEF) variation calculated as the lowest PEF as a percentage of the highest PEF level recorded during the first two weeks of the wash-out period.
ICS: inhaled corticosteroids; FEV_1: forced expiratory volume in one second; MEF_{50}: mean maximal expiratory flow; IgE: immunoglobulin-E; RAST: radioallergosorbent test; PD_{20}: provocative dose of methacholine causing a 20% fall in FEV_1.

There were no data available on previous tests of airways hyperresponsiveness before the patients were using ICS. Forty-seven (49%) of the 95 patients who completed the run-in and wash-out period developed AH (PD_{20} < 80 μg), and 48 (51%) did not. The latter group had significantly higher FEV_1 %pred and maximal expiratory flow (MEF_{50}) %pred values, and lower IgE levels as well as a lower prevalence of RAST positivity. Demographic and clinical characteristics of the patients are presented in Table 1. Forty-three (90%) of the 48 patients with a $PD_{20} \geq 80$ μg, had a PD_{20} > 150 μg. Age was not associated with AH (odds ratio 0.9; 95% confidence intervals 0.7- 1.3).

Symptoms during the wash-out period and after withdrawal of inhaled corticosteroids

During the 8-wk wash-out period, 21 (44%) of the patients without AH had no symptoms of cough, wheeze or dyspnea on exertion. These children were considered to be in clinical remission of their asthma. The other 27 patients (56%) reported asthmatic symptoms, including cough in 16 and wheeze in 10 (Figure 1). Characteristics of the patients with a $PD_{20} \geq 80$ μg with and without any symptoms during the wash-out period are presented in Table 2. All patients with a PD_{20} < 80 μg reported symptoms of asthma during the wash-out period.

Figure 1: Symptoms of asthma in patients without airways hyperresponsiveness (AH) both during the wash-out period and the follow-up period. Four patients were lost to follow-up.



After withdrawal from the trial, patients without AH were followed-up for a median period of 13 months (2-32 months). Four patients were lost to follow-up because they moved to another area or missed follow-up appointments. Of the remaining 44 patients, 17 (39%) remained free from symptoms. The remainder had mild asthmatic symptoms (cough in 13, wheeze in 7, dyspnea on exertion in 7, Figure 1). More than half of these patients were atopic with at least one positive RAST test (Table 1).

Table 2: Demographic and clinical characteristics of the patients with provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second (FEV_1)(PD_{20}) ≥ 80 μg with and without symptoms during the wash-out period.

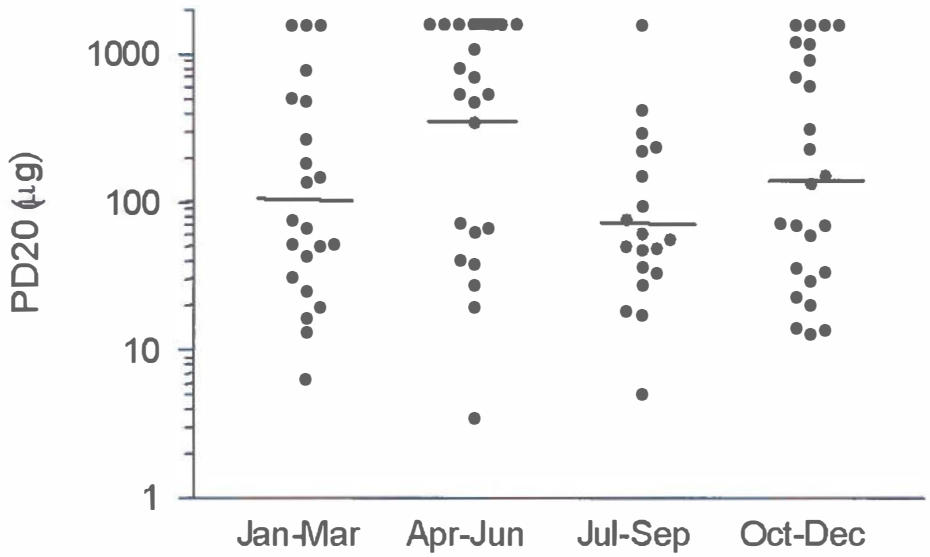
	AH PD_{20} < 80 μg With symptoms	No AH PD_{20} ≥ 80 μg Without symptoms	*p*-value [a]
Subjects *n*	27	21	
Age (years)	8.1±1.2	8.3±1.1	0.76
Male children (%)	75	42	0.01
Duration of asthma (years)	4.7±2.1	5.5±2.0	0.26
Duration of ICS (years)	2.5±1.6	2.7±1.9	0.77
Mean daily dose of ICS received prior to entering study (μg)	303±84	340±81	0.14
FEV_1 at end of washout period (% pred.)	96.6±21.0	101.6±12.8	0.73
MEF_{50} at end of washout period (% pred.)	69.0±25.1	77.5±16.1	0.30
PEF (Low%High) [b]	69.8±15.4	80.5±7.8	0.02
IgE (kU/L)	195 (18-1538)	87 (2-4500)	0.27
RAST positive (%)	60.0	54.0	0.32
PD_{20} (μg)	562.3 (136-1565)	912 (182-1565)	0.03

Data are presented as mean±SD, or as geometric mean (range).
[a]Mann-Whitney U-test or χ^2-test.
[b]Peak expiratory flow (PEF) variation calculated as the lowest PEF as a percentage of the highest PEF level recorded during the first two weeks of the wash-out period.
ICS: inhaled corticosteroids; FEV_1: forced expiratory volume in one second; MEF_{50}: mean maximal expiratory flow; IgE: immunoglobulin-E; RAST: radioallergosorbent test; PD_{20}: provocative dose of methacholine causing a 20% fall in FEV_1.

Seasonal variation in AH

There was a seasonal variation in the degree of AH in the patients. AH was significantly lower in the spring (April, May, and June) than in the winter, summer, and autumn (non-parametric ANOVA, $p = 0.01$) (Figure 2).

Figure 2: Seasonal variation in the degree of airways hyperresponsiveness. Horizontal lines represent geometric mean values of provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second PD_{20} for each season. The PD_{20} is significantly higher during the spring months (nonparametric analysis of variance, $p = 0.01$). (Jan-Mar: January to March, Apr-Jun: April to June, Jul-Sep: July to September, Oct-Dec: October to December).



Discussion

Although many children with mild asthma in population studies may not show AH, previous hospital-based studies of childhood asthma suggested that most, if not all, school-aged asthmatic children had AH.[3,6,7,12-14] The results of the present study show that at present many school-aged children in the Netherlands who have been treated with ICS because of asthma for prolonged periods of time, do not have AH after withdrawal of ICS (whether defined as a $PD_{20} < 80$ or < 150 µg).[6] Other researchers in Western Europe share the same experience (A.A.P.H. Vaessen-Verberne, Amphia Medical Center, Breda, the Netherlands and S. Pedersen, Kolding Hospital, Kolding, Denmark, personal communication).

A drawback of the present study is the lack of data on presence or absence of AH prior to entering the trial. This, however, reflects current practice in pre-school children. Methacholine challenges using FEV_1 to monitor responses are not feasible in this age group. Therefore, although it is possible that some nonhyperresponsiveness children in the present study were never hyperresponsive, testing this hypothesis is unlikely to be successful. Nevertheless, they all had persistent respiratory symptoms initially, severe enough to be referred to a paediatric pulmonologist.

Most of the children in this study had been diagnosed with and treated for asthma from pre-school age onwards. It is now clear that wheezing during pre-school years is a heterogeneous condition that may be transient in many children.[15] The children who did not develop AH and remained asymptomatic after withdrawal of ICS in this study could thus be viewed as transient wheezers without AH.[16,17] The present findings of a higher prevalence of atopic sensitisation in children with AH than in those without AH is in agreement with earlier findings in such transient wheezers.[15] Furthermore, the larger degree of PEF variation in symptomatic than in asymptomatic children without AH also fits with this observation as well.[16] In contrast, the fact that almost half of the patients developed AH while most of them did not demonstrate AH during treatment with ICS, favours a diagnosis of asthma in these patients. In prospective studies, the only feature predicting which wheezers will cease wheezing is the lack of atopy.[15,16,18] The proportion of atopic patients in the present study's nonhyperresponsive patients (54%, Table 1) is higher than that in studies from England (36%) and USA (32%).[15,18] This suggests that in the present study group, there were more atopic patients with transient wheeze than in previous studies. A striking difference between these previous studies and the present one is the more widespread use of ICS from early age onwards in this study group. Because this study group was not characterised extensively and prospectively at a young age, it is impossible to come to a definite conclusion whether the nonhyperresponsive patients in this study were transient wheezers or truly asthmatics in long-lasting clinical remission.

A recent study from Canada showed that almost all asthmatic children who had been treated in a hospital clinic from the age of 3-4 yrs onwards demonstrated AH when re-examined 6 yrs later, whether they were symptomatic or not.[14] In the latter study, only 40% of the symptomatic patients were using regular maintenance treatment with ICS, whereas all asthmatic children in the present study had used ICS for 0.2-7.6 yrs. Tentatively, the lack of AH in the present study in many children after withdrawal of ICS may reflect a long-lasting remission of childhood asthma caused by ICS therapy when instituted at an early age and continued for a prolonged period of time. It has been shown that withdrawal of ICS in older asthmatic children and adults results in rapid deterioration of symptoms and AH.[19,20] It is possible that ICS therapy in

young children could truly stop asthmatic airways inflammation before it becomes persistent and irreversible.[21] An interesting observation in this respect is that both FEV_1 and MEF_{50}, reflecting large airways calibre and small airways respectively, were significantly higher in children without AH than in children with AH. This suggests a beneficial effect of prolonged ICS therapy on both large and small airways function in young children with asthma. This is in agreement with the observation of chronic persistent asthmatic inflammation in both large and small airways in adults.[22]

There was a lower level of AH in the children tested during the spring months (Figure 2). This could be due to low exposure to house dust mite,[23] the predominant inhalant allergen in the Netherlands, or a lower viral infection load. It is unlikely, however, that this phenomenon is responsible for the lack of AH observed in approximately one-half of the asthmatic children in the present study, because only 26 children were tested during the spring months (Figure 2), and eight of them showed AH.

Even though many of the asthmatic children failed to show AH (as traditionally defined), the PD_{20} level after withdrawal of ICS was significantly lower in the group of children who re-developed asthmatic symptoms during further follow-up than in the children who remained asymptomatic (Table 2). This suggests that because ICS are used broadly in the majority of children with asthma, cut-off levels for AH may need to be redefined.

There are several ways to assess AH in children, and it has been argued that "indirect" AH (as assessed by, for example, exercise, adenosine, or hypertonic saline) would be more closely related to current asthma than "direct challenge" (methacholine) that was used in the present study.[24,25] Further studies are needed to determine whether the present findings are reproduced when using an indirect challenge test.

In summary, this study shows that airways responsiveness may be markedly reduced (to levels usually encountered in nonasthmatics) after withdrawal of long-term treatment with inhaled corticosteroids in 6-10 yr old children with asthma. In addition, recruiting asthmatic children for clinical trials may be difficult if airways hyperresponsiveness is used as the sole inclusion criterion.

Acknowledgements

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Chapter 3

Clinical and immunologic factors associated with the presence or absence of airways hyperresponsiveness in childhood asthma

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Abstract

Background During the baseline period of a clinical trial comparing different dosage schedules of inhaled steroids, asthmatic children (aged 6-10 years) were expected to meet the inclusion criterion of airways hyperresponsiveness (PD_{20} methacholine < 80 μg) after withdrawal of inhaled corticosteroids for 2-8 weeks. However, many children failed to do so.

Objective It has been shown that young wheezing children may outgrow their symptoms. We investigated if differences between children with and without airways hyperresponsiveness after withdrawal of inhaled corticosteroids were compatible with differences between transient and persistent wheezers found in other studies.

Methods Seventy-eight children entered the study, of which 41 developed airways hyperresponsiveness after withdrawal of inhaled corticosteroids, and 37 did not. These two groups of children were compared with respect to differences in demographic, clinical, and immunological features (IL-4, IL-5, IL-10, and INF-γ produced by Con A stimulated peripheral mononuclear cells (PBMCs) and serum IL-4, IL-5 and soluble intercellular adhesion molecule-1 (sICAM-1)).

Results Hyperresponsive children had more atopic features (positive RAST, high IgE, eczema), lower levels of FEV_1 and lower concentrations of sICAM-1 than non-hyperresponsive children. Apart from a borderline significantly higher IL-4 production in the hyperresponsive group, other immunologic parameters were comparable. Multivariate logistic regression analysis showed that high serum IgE, low FEV_1, and low sICAM-1 levels were independently associated with the presence of airways hyperresponsiveness after stopping inhaled corticosteroids. Atopy was associated with higher concentrations of IL-4 in the hyperresponsive group.

Conclusion After withdrawal of inhaled corticosteroids many children previously diagnosed with asthma did not develop airways hyperresponsiveness. We conclude that hyperresponsive children share features with persistent wheezers as found in previous studies, whereas the non-hyperresponsive children may represent transient wheezers.

Introduction

Airways hyperresponsiveness is one of the cardinal features of childhood asthma.[1] In population surveys, children with mild asthma symptoms may not be hyperresponsive to bronchoconstrictor stimuli.[2,3] In contrast, airways hyperresponsiveness is observed almost invariably in hospital-based studies of school-aged asthmatic children.[4–6] As airways hyperresponsiveness shows pronounced and prolonged improvement during long-term treatment with inhaled corticosteroids, it is commonly used as an end-point in trials assessing effects of these drugs in this age group.[5–8]

When we designed a long-term clinical study comparing effects of different dosage schedules of inhaled corticosteroids in school-aged children with asthma, we aimed to include patients with moderate to severe airways hyperresponsiveness. To our surprise, approximately half of the recruited asthmatic children did not show airways hyperresponsiveness after withdrawal of inhaled corticosteroid therapy and many of these children remained symptom-free.[9] We hypothesised that these non-hyperresponsive children after withdrawal of inhaled corticosteroids represented non-atopic transient wheezers, whereas hyperresponsive children would reflect atopic persistent wheezers.[10] This study was designed to test that hypothesis. Children with and without airways hyperresponsiveness after withdrawal of inhaled corticosteroids therapy were examined for differences in demographic, clinical, and immunological features.

Methods

Patients

Seventy-eight children, aged 6 to 10 years, with a history of recurrent troublesome wheezing, severe enough in the past to be treated with inhaled corticosteroids (at that time diagnosed as 'asthma') entered the baseline period of a long-term clinical trial comparing two different dosage schedules of inhaled corticosteroids in childhood asthma.[11] Patients were treated for 6 weeks with fluticasone propionate (FP) 100 μg twice daily *via* a dry powder inhaler (Diskhaler®, GlaxoWellcome Zeist, the Netherlands), followed by a wash-out period of 2-8 weeks during which no inhaled corticosteroids or other anti-inflammatory drugs were allowed, but only salbutamol via Diskhaler®(200 μg) on demand. During the wash-out period, patients returned to the clinic every 2 weeks (or earlier when asthmatic symptoms worsened) for measurement of airways hyperresponsiveness. We expected most children to develop airways hyperresponsiveness after withdrawal of inhaled corticosteroids but to our

surprise, 37 (47%) showed no airways hyperresponsiveness 8 weeks after withdrawal of inhaled corticosteroids (non-hyperresponsive children). The remaining 41 children (53%) did show airways hyperresponsiveness (hyperresponsive children).

Questionnaire

A questionnaire was used to obtain data on birth weight, maternal smoking during pregnancy, infant feeding practices, age of onset and duration of asthmatic symptoms, duration of inhaled corticosteroid use, family composition, personal history of eczema, the presence of furry pets, and smoking in the household.

Diary

During the run-in and wash-out period, patients kept a diary of symptom scores and recorded peak expiratory flow (PEF) twice daily. All patients received a new Mini Wright®PEF meter (Clement Clarke, Hartlow, UK) and standardized instructions on how to use it. PEF variation was expressed as low%high (the lowest PEF value observed over the recording period expressed as a percentage of the highest value obtained during that period).[12]

Lung function

Expiratory flow-volume curves were obtained with a Jaeger Masterlab pneumotachograph (Eric Jaeger GmbH, Würzburg, Germany) according to the guidelines of the European Respiratory Society.[13]

Methacholine challenge

Methacholine challenge was performed using a dosimeter method.[14] Briefly, methacholine was nebulised with a DeVilbiss 646 nebuliser attached to a Rosenthal-French dosimeter. Inhaled doses were doubled from 3.0 to 1565 μg. Three minutes after each dose, FEV_1 was measured, and the test was stopped when FEV_1 had fallen by 20% or more or when the highest concentration of methacholine had been nebulized. PD_{20} methacholine was determined by linear interpolation.

Atopy

Blood was drawn at the end of the wash-out period (when children did not use inhaled corticosteroids) for eosinophil counts, total and specific immunoglobulin E (IgE) to house dust mite, grass and tree pollen, cat and dog dander, determined by the Pharmacia MasterCap analysis (Pharmacia & Upjohn, Uppsala, Sweden) according to the protocol of the manufacturer, with a range in IgE of 2-2000 U/mL. Values above 2000 U/mL were further quantified by dilution.

Nitric oxide in exhaled air

Nitric oxide in exhaled air (eNO) was measured with a chemiluminescence analyser (Eco Physics, Basel, Switzerland) using a tidal breathing method.[15] Exhaled air was collected in a 40-L polyethylene Douglas bag, and continuously sampled for eNO content. A plateau phase of eNO was reached between 4 and 6 min. The mean concentration of this plateau was recorded in parts per billion (p.p.b.).

Laboratory measurements

Laboratory measurements were performed as described previously.[16] Briefly, 10 mL diluted blood was layered on a 4 Lymphoprep solution (Nycomed Pharma A.S., Oslo, Norway, density of 1.077 g/mL) in a polypropylene tube. Peripheral blood mononuclear cells (PBMCs, $1*10^6$ cells/mL in RPMI-1640 medium plus 10% FCS) were cultured in a 24 wells plate (Costar, Badhoevedorp, the Netherlands), and were activated with 10 μg Concanavalin A (Sigma Alderich Chemie B.V., Zwijndrecht, the Netherlands). The cells were incubated for 24 h, 37 °C, 5% CO_2, followed by centrifugation at 590 **g**, 10 min in a polypropylene tube, and the supernatant collected and stored in polypropylene tubes at -80 °C for further analyses.

Cytokine and soluble intercellular adhesion molecule-1 measurements

Assays for supernatants for IL-4, IL-4 high Sensitive assay (for serum), and soluble intercellular adhesion molecule-1 (sICAM-1) were from R & D Systems Europe, Abindon, United Kingdom. Assays for INF-γ were from Amersham Life Science, Roosendaal, the Netherlands, IL-10 from Centraal Laboratorium Bloedtransfusie, Amsterdam, the Netherlands, and IL-5 measured according to Borger et al.[16,17] The detection limit

was 0.010 pg/mL and 0.1 pg/mL for IL-4 and IL-5, respectively. Concentrations of IL-4, IL-5, IL-10, and INF-γ in serum and supernatants of activated PBMCs were determined as described above. sICAM-1 was only determined in the serum samples. When IL-4 and IL-5 were undetectable in the serum, the detection limit was used for statistical analysis.

Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 10.0. Distributions of all different parameters were inspected visually by normal probability plots. The Kolmogorov-Smirnov test was used to formally test distributions for normality. Because the distributions of total IgE and eosinophil counts were skewed, analyses were performed on $\log_{10}$-normalized data. Student's t-test or Mann-Whitney U-test were used if appropriate. The χ^2 test was used to test differences in proportions.

Multivariate logistic regression analysis was performed on the following variables to assess their association with airways hyperresponsiveness and atopy: breastfeeding (more than three months), maternal smoking during pregnancy, birth weight, family history, presence of eczema, presence of furry pets, presence of older siblings, duration of use of inhaled corticosteroids, mean daily dose of inhaled corticosteroids, more than one positive RAST test, log IgE, eosinophil counts, FEV_1, MEF_{50}, Ln IL-4 (PBMCs), Ln INF-γ (PBMCs), Ln sICAM-1 (serum), and log IL-4/log INF-γ (both PBMCs). Both analyses were performed with adjustment for age, sex, and height. Two-sided p-values < 0.05 were considered to be statistically significant. Confidence intervals (CI) for differences between medians were calculated using confidence interval analysis.

Ethics

The protocol of the study was approved by the medical ethics committee of the University Hospital Groningen and written informed consent was obtained from all parents or guardians of the participating children.

Results

Patients

Characteristics of hyperresponsive and non-hyperresponsive children are presented in Tables 1 and 2. Hyperresponsive children were more likely to have a history of eczema (Table 1), had higher concentrations of serum IgE (Table 2 and Figure 1a) and were more likely to have more than one positive RAST test compared to non-hyperresponsive children. Hyperresponsive children had higher concentrations of eNO than non-hyperresponsive children (Table 2). Presence of a furry pet was more common in non-hyperresponsive children (Table 1).

Table 1: Demographic characteristics of the children with and without airways hyperresponsiveness.

	$PD_{20} < 80\ \mu g$	$PD_{20} \geq 80\ \mu g$	p-value [a]
Subjects n	41	37	
Age at study entry (years)	8.2 (1.1)	8.2 (1.2)	0.97
Male sex (%)	58.5	45.9	0.27
Height (cm)	131.4 (7.9)	133.2 (9.8)	0.41
Breastfeeding > 3 months (%)	73.7	64.7	0.56
Maternal smoking during pregnancy (%)	17.6	21.9	0.67
Birth weight (g) [b]	3375 (1670-4500)	3525 (1420-4500)	0.26
Age at first wheezing episode (years) [b]	1.8 (0.1-7.0)	1.7 (0.1-9.0)	0.31
Family history (%)	51.3	51.4	0.1
Presence of eczema (%)	79.5	56.8	0.03
Presence of furry pet (%)	18.9	40	0.049
Older siblings (%)	65.6	64.7	0.94
Duration of inhaled corticosteroids (years)	2.6 (1.4)	2.6 (1.9)	0.95
Mean daily dose of inhaled corticosteroids (μg/day)	370 (108)	344 (102)	0.27

[a]Student's t-test, Mann-Whitney U-test, or χ^2 test as appropriate. Values are presented as mean values (SD) or as median (range [b]).

Lung function

Hyperresponsive children had significantly lower FEV_1, MEF_{50} and PEF values than non-hyperresponsive patients (Table 2 and Figure 1b). No significant difference in PEF-variation was found between the two groups.

Table 2: Laboratory and lung function characteristics of the children with and without airways hyperresponsiveness.

	$PD_{20} < 80\ \mu g$	$PD_{20} \geq 80\ \mu g$	*p*-value [a]
Subjects *n*	41	37	
IgE (kU/L) [b]	407 (32-3980)	166 (2-5011)	0.04
≥ 1 RAST (%) [c]	82.1	57.1	0.02
RAST house dust mite + (%)	76.9	48.6	0.01
Eosinophil counts ($1*10^9$/L)	0.65 (0.13-2.34)	0.48 (0.10-2.10)	0.1
Exhaled NO (ppb)	17.5 (3-30)	11.0 (4-21)	0.01
FEV_1 (L)	1.47 (0.74-2.47)	1.64 (0.72-2.50)	0.01
FEV_1 (%pred.)	91.0 (44.0-129.0)	104.0 (93.0-129.0)	0.04
Lowest PEF as % of highest	72.5 (36.4-89.3)	75.0 (41.2-93.3)	0.4
MEF_{50} (L/s)	1.47 (0.40-2.45)	2.00 (0.27-3.06)	0.001
MEF_{50} (%pred.)	58.6 (17.1-121.6)	81.5 (12.2-148.5)	0.005
Mean morning PEF (L/min)	197 (113-287)	224 (79-347)	0.003

Values are presented as median (range).
[a]Mann-Whitney *U*-test, or χ^2 test as appropriate.
[b]IgE and eosinophil counts are presented as geometric mean (range).
[c]RAST test was considered positive when the result read class 2 or higher.

Immunological parameters, serum

IL-4 was detectable in the serum of 22 hyperresponsive children (54%) and in 13 (35%) non-hyperresponsive children, without a significant difference between the two groups (Table 3). No significant difference was found either when only the detectable concentrations of serum IL-4 were analysed.

Serum IL-5 was detectable in 23 children (56%) with and in 19 (51%) without airways hyperresponsiveness, and no difference was found between the groups (Table 3). No significant differences were found when analysing only the detectable concentrations of serum IL-5. sICAM-1 was detectable in all children and was significantly lower in the group with a $PD_{20} < 80$ μg compared to the group with a $PD_{20} \geq 80$ μg (Table 3 and Figure 1c).

Table 3: Immunologic characteristics of asthmatic children with and without airways hyperresponsiveness.

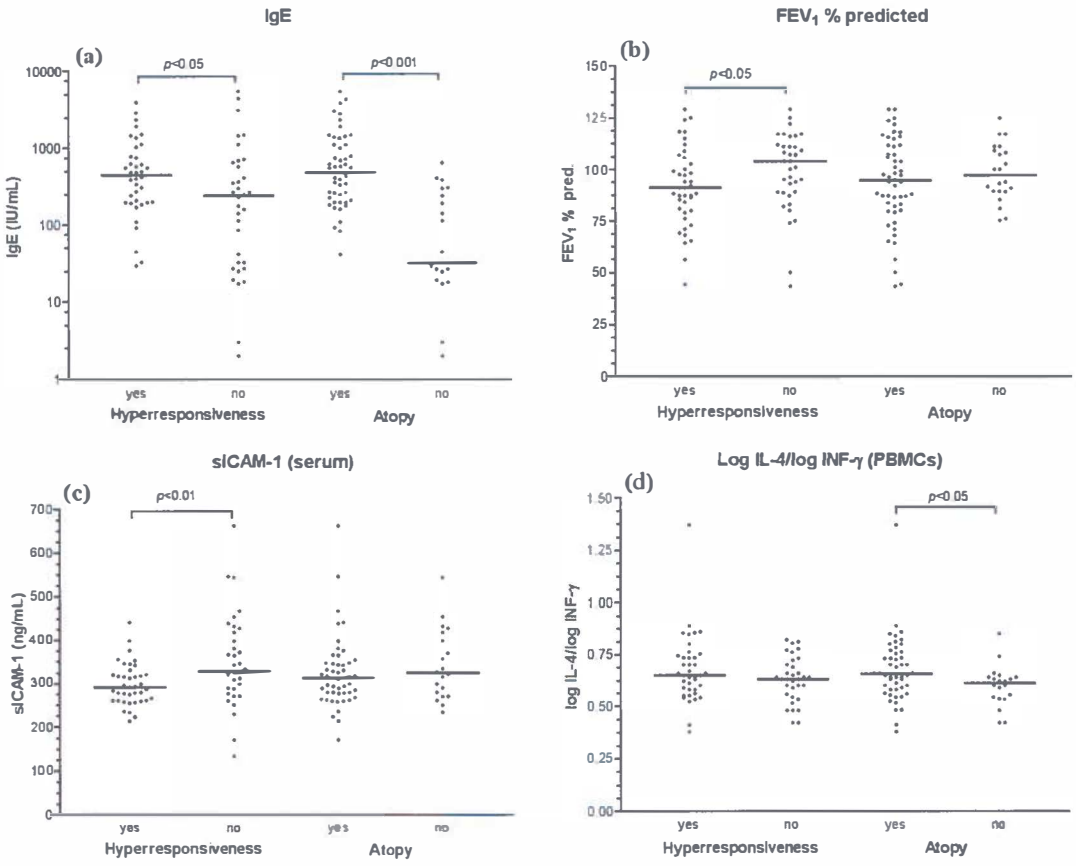
	$PD_{20} < 80$ μg	$PD_{20} \geq 80$ μg	*p*-value [a]
Subjects *n*	41	37	
In serum			
IL-4 (pg/mL)	0.042 (0.010-5.13)	0.01 (0.010-2.86)	0.29
IL-5 (pg/mL)	0.66 (0.10-19.72)	0.77 (0.10-10.23)	0.68
sICAM-1 (ng/mL)	290.8 (213.6-441.0)	328.6 (133.2-661.0)	0.006
In supernatants from stimulated PBMCs			
IL-4 (pg/mL)	36.94 (6.87-200.60)	33.49 (11.44-76.76)	0.07
IL-5 (pg/mL)	210.6 (32.6-730.2)	226.8 (36.3-646.5)	0.85
IL-10 (pg/mL)	307.9 (31.0-1165.0)	315.3 (9.9-744.6)	0.45
INF-γ (pg/mL)	276.6 (30.84-1762.00)	249.5 (38.93-900.20)	0.57
Log IL-4/log INF-γ	0.65 (0.38-1.37)	0.64 (0.42-0.82)	0.25

Values are presented as median (range).
[a]Mann-Whitney *U*-test.

Relationship of PD_{20} with supernatants of Con A stimulated PBMCs

IL-4, IL-5, IL-10 and INF-γ were detectable in supernatants of Con A stimulated PBMCs of all participants (Table 3). There was a borderline significant difference between children with and without airways hyperresponsiveness for IL-4 (Table 3). Otherwise, no significant differences were found (Table 3 and Figure 1d).

Figure 1: Differences between children with (PD_{20} < 80 μg) and without (PD_{20} ≥ 80 μg) airways hyperresponsiveness, and with (≥ 1 RAST) and without atopy (< 1 RAST). a) IgE concentration, b) FEV_1 % predicted, c) sICAM-1 in serum, and d) ratio of log IL-4 and log INF-γ.



Relationship of PD_{20} with allergy, lung function, and demographic markers

Multivariate logistic regression analysis showed that the independent significant predictors of airways hyperresponsiveness (adjusted for age, sex, and height) at age 8 years were: ≥ 1 RAST, presence of eczema, log IgE, Ln IL-4 in Con A stimulated PBMCs, Ln sICAM-1 in serum, presence of furry pets, MEF_{50}, and FEV_1(Table 4).

Table 4: Multivariate logistic regression analysis with airways hyperresponsiveness and atopy as dependent variables, for all independent variables separately (adjusted for age, sex, and height).

	Methacholine-positive (PD_{20} < 80 μg) Odds Ratio (95% CI)	Presence of atopy (≥ 1 RAST) Odds Ratio (95% CI)
Subjects n	41	37
Breastfeeding > 3 month	1.55 (0.35-6.71)	3.28 (0.40-26.84)
Maternal smoking during pregnancy	0.79 (0.22-2.75)	1.26 (0.30-5.21)
Birth weight	0.99 (0.99-1.00)	0.99 (0.99-1.00)
Family history	1.03 (0.40-2.59)	0.40 (0.13-1.22)
Eczema	2.84 (1.01-7.97)[a]	4.75 (1.49-15.08)[b]
Furry pet	0.31 (0.10-0.96)[a]	0.66 (0.20-2.16)
Older sibling	1.27 (0.41-3.90)	1.49 (0.42-5.31)
Duration of ICS[c]	0.96 (0.72-1.27)	0.82 (0.59-1.12)
Mean daily dose of ICS	1.00 (0.99-1.00)	1.00 (0.99-1.00)
≥ 1 RAST	3.09 (1.03-9.21)	n.a.
Presence of PD_{20}	n.a.	3.10 (1.04-9.26)[a]
IgE	2.26 (1.03-4.95)[a]	26.28 (5.31-129.90)[b]
Eosinophil counts	3.68 (0.79-17.14)	53.37 (5.53-514.68)[b]
FEV_1	0.098 (0.016-0.58)[a]	0.15 (0.022-1.15)
MEF_{50}	0.19 (0.072-0.53)[b]	0.65 (0.26-1.60)
IL-4 (PBMC)	2.38 (1.02-5.53)[a]	1.64 (0.67-4.02)
INF-γ (PBMC)	1.73 (0.62-4.83)	0.53 (0.20-1.41)
sICAM-1 (serum)	0.074 (0.007-0.73)[a]	0.33 (0.027-4.07)

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] Inhaled corticosteroids.
n.a. not applicable. IgE, eosinophil counts, IL-4, INF-γ, and sICAM-1 values are logarithmically transformed in order to obtain a normal distribution.

Atopy

Since the prevalence of atopy was higher in the group of hyperresponsive children, we hypothesized that the observed higher IL-4 level in the hyperresponsive children (Table 3) was due to atopy. We therefore analysed differences between atopic and non-atopic children separately (atopy defined as ≥ 1 positive RAST). Atopic children had significantly higher serum IgE levels than non-atopic children (Figure 1a), and significantly higher eosinophil counts as well (mean $0.67*10^9$/L, range 0.19-$2.37*10^9$/L vs. $0.30*10^9$/L, 0.10-$1.31*10^9$/L, $p < 0.001$, respectively). No differences in FEV_1 % predicted levels were found between the atopic and non-atopic children (Figure 1b).

Atopic and non-atopic children did not differ significantly with respect to sICAM-1 levels (Figure 1c), or serum IL-4 levels.

Levels of IL-4 from Con A stimulated PBMCs were higher in atopic children than in non-atopic children (median 36.0 pg/mL, range 6.9-127.1 pg/mL versus 25.6 pg/mL, 11.4-200.6 pg/mL, respectively, $p = 0.06$). The ratio of log IL-4/log INF-γ was significantly higher in the atopic group compared to the non-atopic ($p = 0.03$, Figure. 1d).

Multivariate logistic regression analysis with atopy as the dependent variable was performed on the same independent parameters as used with airways hyperresponsiveness.

Independent significant predictors associated with atopy (adjusted for age, sex, and height) at age 8 years were: eosinophil counts, IgE, presence of eczema, and presence of airways hyperresponsiveness (Table 4). No immunologic parameter contributed to atopy.

Discussion

This study examined children who had been using inhaled corticosteroids for years because of recurrent troublesome wheezing. After withdrawal of inhaled corticosteroids, 53% were hyperresponsive and 47% were non-hyperresponsive, and most children were symptom free. The most important factors associated with airways hyperresponsiveness after withdrawal of inhaled corticosteroids were high serum IgE level, a history of eczema, low level of FEV_1, and low serum sICAM-1 level.

It is surprising that about half of the children did not show airways hyperresponsiveness after withdrawal of inhaled corticosteroids. All children had been referred

to our University Hospital outpatient clinic because of troublesome recurrent wheezing which, at that time, was diagnosed as asthma, and required treatment with inhaled corticosteroids. Several reports have shown that airways hyperresponsiveness improves after therapy with inhaled corticosteroids.[5,6,18,19] Usually, cessation of inhaled corticosteroids in childhood asthma is accompanied by a deterioration of asthma symptoms and hyperresponsiveness.[20,21] Our findings can not be explained by differences in duration and dose of inhaled corticosteroids between hyperresponsive and non-hyperresponsive children (Table 1). The most likely explanation for our results therefore is that the non-hyperresponsive children had transient wheeze in retrospect, and that the hyperresponsive children reflect persistent wheezers. The observations that the hyperresponsive children in our study were more likely to be atopic (reflected by the presence of eczema (Table 1), higher IgE levels, positive RAST tests), and had elevated eNO levels, and lower levels of lung function (Table 2), are in accordance with this hypothesis.[4,10,22,23]

After withdrawal of inhaled corticosteroids, hyperresponsive children had significantly lower levels of lung function compared to the non-hyperresponsive children. In addition, FEV_1 was a significant independent factor associated with the presence of airways hyperresponsiveness in our group of children. This is also in accordance with population studies, showing that persistent wheezers at the age of 6 years had lower levels of lung function compared to transient wheezers.[10] These persistent wheezers also had airways hyperresponsiveness at the age of 11 years.[22] Taken together, our findings support the hypothesis that there are at least two types of childhood wheezing with different outcome: one with and one without airways hyperresponsiveness at later age, and that the presence of airways hyperresponsiveness is associated with high concentrations of IgE and lower levels of lung function.

Furry pets were less likely to be present in households with hyperresponsive children compared to households of non-hyperresponsive children (Table 1). This finding remained significant even after adjusting for atopy, family history, and lung function (Table 4). This suggests that keeping pets may protect against developing airways hyperresponsiveness.[24,25] Alternatively, it may be due to selection bias ('healthy child effect').

Strong evidence supports the concept that the development of asthma and allergy is associated with a Th2 skewing of the immune response.[26] Compatible with this Th2 skewing, recent studies have shown elevated concentrations of IL-4 and IL-5, and/or reduced concentrations of INF-γ in children with asthma and eczema compared to healthy controls.[16,27–29] In this study, hyperresponsive children had more features of atopy compared to the non-hyperresponsive children. Therefore, we expected to find higher concentrations of IL-4 and IL-5 in serum or in PBMC-stimulated supernatants,

and/or lower concentrations of IL-10 and INF-γ in the hyperresponsive children. Indeed, there was a borderline significant difference for IL-4 in Con A stimulated PBMCs with higher concentrations for the hyperresponsive group. We could not, however, demonstrate significant differences in IL-4 (serum) and IL-5 (both in serum and Con A stimulated PBMCs). This could not be explained by differences in undetectable levels of these two cytokines. Tang and co-workers found elevated IL-4 and reduced INF-γ levels in atopic asthmatic children when compared to healthy controls, but not in non-atopic asthmatics.[30] In accordance with this, atopic children in our study had higher IL-4 (PBMC) levels and higher IL-4/ INF-γ ratios than non-atopic children (Figure 1d). A trend towards the same difference was apparent when comparing atopic and non-atopic hyperresponsive children. It thus appears that Th2 skewing is associated with atopy and not associated with airways hyperresponsiveness.[16,30]

Membrane bound ICAM-1 is an adhesion molecule necessary for cell-cell adhesion at inflammatory sites. sICAM-1 is thought to be derived through shedding of membrane bound ICAM-1, apparently by proteolytic cleavage.[31] We are the first to show that concentrations of sICAM-1 in serum are significantly associated with airways hyperresponsiveness (Table 3 and 4, Figure 1c). Similar concentrations of sICAM-1 in atopic and non-atopic children were found, indicating that sICAM-1 is not associated with atopy. One previous study showed no difference in sICAM-1 levels between healthy and asthmatic children (independent of atopy)[32], but other workers found elevated concentrations of sICAM-1 in asthmatic children compared to non-asthmatic controls, both when the disease stable and during an exacerbation.[33,34] It has been suggested that sICAM-1 may block cell-cell interactions, hampering inflammatory responses.[35] Thus, low sICAM-1 concentrations may facilitate inflammation and airways hyperresponsiveness.

In summary, children with airways hyperresponsiveness after withdrawal of inhaled corticosteroids share features with persistent wheezers as observed in epidemiologic studies, including atopy (i.e. higher levels of serum IgE) and a lower level of lung function. Our results show that Th2 skewing, as represented by levels of IL-4 and INF-γ produced by PBMCs, reflects the presence of atopy rather than that of hyperresponsiveness. In contrast, airways hyperresponsiveness and not atopy was associated with lower sICAM-1 levels, possibly reflecting airway wall inflammation. The large degree of overlap in serum IgE, cytokine levels and lung function parameters between the two groups, shows that these parameters are not useful in predicting which child will or will not develop airways hyperresponsiveness after withdrawal of inhaled corticosteroids.

A considerable proportion of children diagnosed with and treated for asthma from a young age onwards did not have airways hyperresponsiveness after withdrawal of

inhaled corticosteroids. This emphasises the need to stop or at least reduce inhaled corticosteroids in young children to the lowest effective dose.

The challenge still is to find a specific and sensitive diagnostic tool that helps to discriminate between transient wheezers, in whom inhaled corticosteroid therapy can be withdrawn, and persistent wheezers in whom this is not possible. Our results suggest that cytokine levels from peripheral blood are unlikely to be useful for that purpose.

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Chapter 4

Short-term growth in asthmatic children using fluticasone propionate

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Abstract

Background Inhaled corticosteroids may reduce short-term growth velocity in asthmatic children and knemometry is the most sensitive tool to detect this short-term growth suppression.

Study objective To compare lower leg growth velocity, as measured by knemometry, in asthmatic children during and after treatment with inhaled fluticasone propionate (FP), 100 μg twice daily.

Design Nonrandomised open trial.

Setting University hospital, outpatient clinic for paediatric pulmonology.

Patients Twenty-one asthmatic children (13 boys), aged 6-10 year.

Interventions Inhalation of FP from a dry powder inhaler, 100 μg, twice daily for 6 weeks, followed by 2 weeks during which only an inhaled β_2-agonist was used on demand (washout). During treatment and washout periods, patients were seen every 2 weeks at the same time of day.

Measurements and results Lower leg growth velocity measured by knemometry during FP treatment was not significantly different from that during wash-out ($p = 0.33$, one-way analysis of variance).

Conclusions No significant suppression of lower leg growth velocity was found in prepubertal asthmatic children using FP, 100 μg, by dry powder inhaler twice daily for 6 weeks.

Introduction

There is increasing concern about the side-effects of inhaled corticosteroids in children with asthma, in particular regarding their effect on growth. Knemometry is the most sensitive tool to detect short-term growth suppression in asthmatic children using inhaled corticosteroids.[1] Previous studies using this method have shown dose-dependent suppression of short-term growth for both beclomethasone dipropionate and budesonide.[2,3] The effect of inhaled fluticasone propionate (FP; GlaxoWellcome; Zeist, the Netherlands) on short-term growth has been evaluated in another study, but the effect was compared with that of beclomethasone dipropionate and not with a period without inhaled corticosteroids.[4] We, therefore, studied short-term growth by knemometry in children both during and after six weeks treatment with inhaled FP.

Material and methods

The present study was undertaken during the baseline period of an ongoing long-term clinical trial on effects and side-effects of two different treatment schedules of FP. The baseline period of this trial consisted of a 6-week run-in period during which patients inhaled FP from a dry-powder inhaler (Diskhaler®; GlaxoWellcome; Greenford, UK) at a dose of 100 μg twice daily, followed by a 2-week washout period during which patients used no inhaled corticosteroids or other anti-inflammatory drugs but only inhaled β_2-agonists on demand. Before the run-in period, all patients were using inhaled corticosteroids (100 to 400 μg daily). Treatment during these periods was not blinded and the treatment order was fixed. Compliance was checked at the end of the 6-week treatment period with FP by counting the blisters used.

Children were eligible for the study if they were 6 to 10 years of age, were prepubertal (according to Tanner,[5,6] and had no disease other than asthma. Power calculations showed that 12 patients would be sufficient to detect a 50% reduction in lower leg growth rate between treatment with or without inhaled FP with a power of 90%.[2,3]

All patients received standardised instructions on how to use the dry-power inhaler. During both the run-in and washout period, patients were seen every 2 weeks. At each of these follow-up visits, inhaler technique was checked and knemometry was performed. Knemometry was performed according to published recommendations by a single trained antropometrist (BME) at a fixed time of day.[7] The knemometer used was one manufactured by the inventor.[7] Each lower leg was measured 6 times; the median of the measurements was used for analysis.

Informed consent was obtained from all children and their parents and the study was approved by the local ethics committee.

Results

Twenty-one patients completed the study. Clinical characteristics are shown in the table 1.

Table 1: Characteristics of patients.

Characteristic	No.
Subjects *n* (male)	21 (13)
Age (years)	8.0 (range 6.1-9.7)
FEV_1, % pred at start of study, mean (SD)	103 (18)
FEV_1, % pred at end of study, mean (SD)	102 (19)
Height SDS, mean (SD)	-0.3 (1)

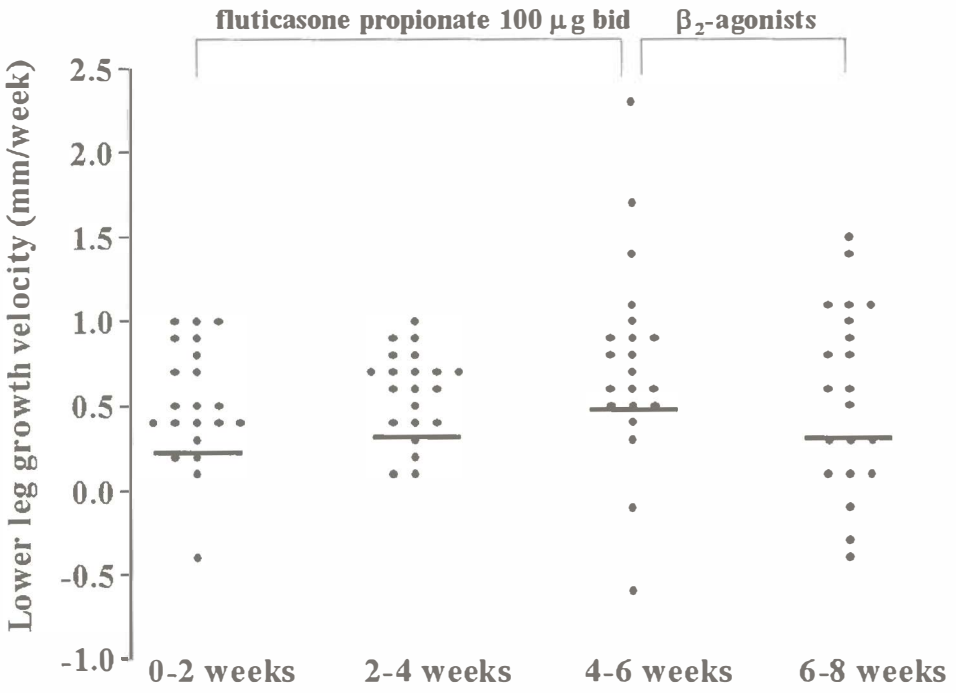
% pred = percentage predicted; SDS=Standard Deviation Score.[7]

Figure 1 shows the lower leg growth velocity during 6 weeks of treatment with inhaled corticosteroids (run-in phase) and during 2 weeks without inhaled corticosteroids (washout phase). Because there was no difference in left- and right lower leg growth velocity, the mean of both legs was used.

Blister counts showed mean (SD) compliance with prescribed dosages of 94 (9)%.

There were no significant differences between the lower leg growth velocities in each of the four 2-week periods (p = 0.33, one-way analysis of variance).

Figure 1: Lower leg growth velocity in 21 children during 6 weeks treatment with FP 100 μg, bid via dry-powder inhaler. Closed circles represent individual values; horizontal bars, mean values.



Discussion

In this study, we did not find a (significant) reduction in lower leg growth velocity in asthmatic children during treatment with FP, 100 μg bid via dry-powder inhaler as compared with treatment with only an inhaled β_2-agonist on demand. In fact, the observed trend (Figure 1) was that lower leg growth velocity tended to increase during treatment with FP. Thus, it is highly unlikely that our results can be explained by insufficient power to detect a growth-suppressing effect of FP on short-term lower leg growth. Measurement error is also unlikely as all measurements were made by a well-trained, highly experienced anthropometrist adhering to a strictly standardised protocol following published recommendations in the appropriate use of knemometry.[7]

The lack of blinding in our study is a disadvantage in comparison to previous studies.[2–4] However, knemometry as performed in this study is a rigorously standardised

objective measure of lower leg growth velocity that is not readily prone to observer bias.[7] Because treatment order was not random, a carryover or order effect cannot be excluded. In other studies, however, 2 week washout periods after treatment with inhaled corticosteroids were sufficient to detect a significant difference in lower leg growth velocity between these periods.[2–4] This was true for different inhaled corticosteroids (beclomethasone dipropionate, budesonide, FP).

It is unlikely that changes in the level of asthma control influenced our results because FEV_1 percent predicted did not differ between treatment periods with and without FP (Table 1) and all patients remained in clinically stable condition throughout the 8-week study period. Moreover, the mean age of the children was 8 years and all children were clinically prepubertal so it is next to impossible that growth velocity was influenced by the pubertal growth spurt.

A meta-analysis on the effect of inhaled corticosteroids on growth showed that inhaling beclomethasone dipropionate was significantly associated with attaining normal stature.[8] Two recent double-blind clinical trials with 7 to 12 months of follow-up, however, showed reduction of statural growth by about 1 cm/yr in children inhaling beclomethasone dipropionate 400 to 800 μg/day.[9,10] To our knowledge, no such long-term studies are available with FP. Because knemometry does not predict long-term height growth, further studies are required to evaluate the effect of long-term use of FP in this dose on height growth in asthmatic children.[1]

Knemometry has been shown to be a highly sensitive tool to detect systemic side-effects of inhaled corticosteroids.[1] The negative findings of this study strongly suggest, therefore, that FP inhaled through dry-powder inhaler in a dose of 100 μg twice daily does not have any significant short-term systemic steroid effect.

In conclusion, we found no effect on lower leg growth velocity during 6 weeks of treatment with inhaled FP, 100 μg twice daily, via dry-powder inhaler.

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Chapter 5

One-year treatment with different dosing schedules of fluticasone propionate in childhood asthma: Effects on hyperresponsiveness, lung function, and height

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Abstract

Dose-dependent effects of inhaled corticosteroids have been described. Although it has been advised to start treatment with inhaled corticosteroids with a high dose tapering off subsequently (stepdown approach), no clinical studies have assessed this strategy. We compared two different dosage schedules of inhaled fluticasone propionate (FP) in chronic persistent childhood asthma with respect to efficacy (airways hyperresponsiveness [PD_{20}] lung function, exhaled nitric oxide [eNO]) and safety (height). During this double-blind study, asthmatic children (aged 6-10 yrs) were randomized to receive either FP 200 μg/d (constant dose approach) or to start with 1000 μg/d with two monthly reductions to 500, 200, and 100 μg/d (stepdown approach). PD_{20} improved in both approaches during treatment with FP with a significantly better PD_{20} after 2 mo of 1000 μg/d followed by 500 μg/d in the stepdown approach versus 200 μg/d in the constant dose approach. No significant differences in PD_{20} or other efficacy parameters were found after 1 yr. Changes in standing height were similar in both treatment approaches. This study showed no superior clinical effect of a stepdown approach compared with a constant dose strategy of FP for 1 yr in children with chronic persistent asthma.

Introduction

Current guidelines for the management of childhood asthma focus on the use of maintenance therapy with antiinflammatory agents, in particular inhaled corticosteroids (ICS).[1–4] In these guidelines, increasing the dose of ICS is advised during periods with increased symptoms. This advice is based on the concept that ICS have a dose-dependent effect on reduction of airway inflammation. Although some studies have failed to do so, the majority of recent studies appear to support the hypothesis of a dose-dependent relationship of ICS on markers of effectiveness in childhood asthma.[5–10] As a result, it has become established practice to start ICS therapy in childhood asthma with relatively high doses, in order to reduce airway inflammation aggressively, and to reduce the dose of ICS subsequently in a stepwise fashion to the lowest effective dose.[3,4,11,12] Popular as this stepdown strategy may be, there is little, if any, published evidence from clinical trials to support it. The only clinical trial comparing the stepdown approach with a constant dose in childhood asthma that we are aware of is a study of nebulized budesonide suspension in preschool children (aged 6 mo to 3 yr).[13] In this study, the stepdown approach yielded a significantly earlier improvement in asthma symptoms, which was sustained during follow-up, when compared to a constant low dose. No objective assessments of lung function or airways hyperresponsiveness was carried out. In addition, the short duration of both the high-dose starting period (less than 1 wk) and total follow-up (10 wk) limits the generalizability of these study results to clinical practice.

Systemic side-effects, in particular reduction of growth rate, are a matter of concern in asthmatic children when treated with ICS. Limited data are available on the effect of fluticasone propionate (FP) on growth in children with asthma. Although two recent studies showed no effect on linear growth in children during 1 yr treatment with FP 100 μg/d, treatment with a dose of 200 μg/d was associated with a reduction in growth when compared to placebo.[14–16] Reassuring results showing normal adult height after long-term treatment with ICS have recently been published.[17,18]

The aim of our study was to compare a stepdown approach (1000 μg/d tapering off to 100 μg/d) with a constant dose approach (of 200 μg/d) in school children with asthma for a period of 12 mo. Symptoms, exacerbations, lung function, and airways hyperresponsiveness was primary efficacy variables, and height was the primary safety variable.

Methods

Subjects

Children aged 6-10 yr, with chronic persistent asthma were recruited for the study. None used > 800 μg of inhaled budesonide or beclomethasone, or inhaled FP > 400 μg. No long acting β_2-agonists or antileukotriene therapy were allowed during the study. Patients with major other illness and those who had used systemic corticosteroids during the previous 6 wk, or had a respiratory tract infection during the preceding 4 wk, were excluded.

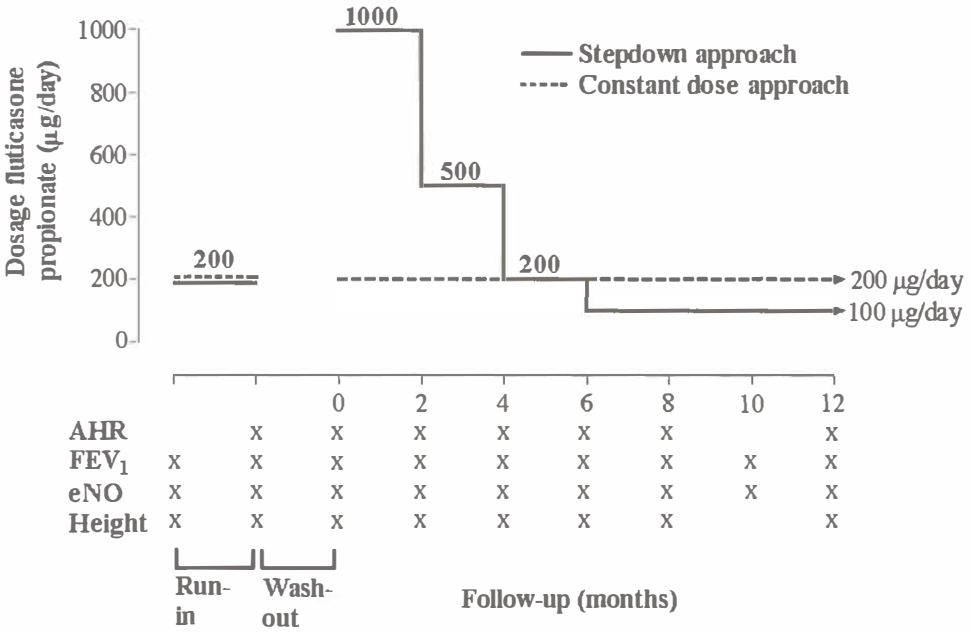
Informed consent was obtained from all children and their parents. The study was approved by the medical ethics committees of the three participating centers.

Study design

After a run-in period of 6 wk during which patients used FP 200 μg/d by dry powder inhaler (Diskhaler®, GlaxoWellcome, Zeist, the Netherlands), patients entered a period of 2-8 wk during which no ICS or other antiinflammatory drugs, but only inhaled salbutamol 200 μg by Diskhaler®on demand were used. During this period, patients returned to the clinic every 2 wk (or earlier if symptoms worsened, but before a full-blown exacerbation developed) for measurement of airways hyperresponsiveness. Those subjects whose provocative dose (PD_{20}) did not drop below 80 μg during the 8-wk wash-out were withdrawn from the study.[19] When a PD_{20} < 80 μg was found, the patient was randomized either to the stepdown (FP 1000 μg/d with two monthly reductions to 500, 200 and 100 μg/d) or the constant dose approach (Figure 1). Randomisation was performed with stratification for age, sex, duration of prior ICS use, and PD_{20}. All patients received standardized instructions on the correct use of the Diskhaler®and were required to demonstrate a correct inhalation maneuver at each follow-up visit. Patients were allowed to use salbutamol by Diskhaler®on demand during the whole study.

The time schedule of assessments is given in Figure 1. At the randomisation visit, blood was drawn for total and specific immunoglobulin E (IgE) to house dust mite, grass and tree pollen, and cat and dog dander (Pharmacia MasterCap analysis, Pharmacia & Upjohn, Uppsala, Sweden). Lung function (flow-volume curves) was measured according to ERS guidelines, using a Jaeger Masterlab pneumotachograph (Erich Jaeger GmbH, Würzburg, Germany).[20] Airways hyperresponsiveness to methacholine was measured using a dosimeter method as published previously.[21] Exhaled nitric

Figure 1: Study design comparing different dosage schedules of inhaled fluticasone propionate in children with asthma. FEV_1 and eNO were performed on each visit, airways hyperresponsiveness (AHR) and height measurements on all visits except after 10 mo of treatment.



oxide was measured using a tidal breathing method.[22] Height was measured by trained technicians, using a Harpenden stadiometer. Bone age was determined at randomisation and after 1 yr using the Tanner & Whitehouse method by a single blinded investigator (TdV). Tanner's pubertal stages were scored at each follow-up visit.[23]

Patients kept a diary card of symptom scores (score 1 to 10), use of salbutamol, and peak expiratory flow (PEF) for 2 wk prior to each follow-up visit. All patients received a new mini Wright PEF meter (Clement Clarke, Harlow, UK) and standardized instructions on how to use it. PEF was recorded each morning and evening in triplicate; the highest value was recorded in the diary. At each follow-up visit, a standardized short symptom questionnaire was administered to each patient.[24]

Patients were asked to bring along all used and unused medication at each follow-up visit. Adherence to treatment was assessed by counting the blisters used.

Statistical analysis

All calculations of PD_{20} were performed on a log-transformed basis, thereby normalizing the distribution. The analyses were performed using SAS 6.12 and SPSS 9.0. Differences between groups were analyzed at individual time points, both when differences in ICS dosages between groups were large (first mo of the study) and when they were small (Figure 1). To determine trends over time, a linear mixed effects model (SAS Proc Mixed) was used.

Table 1: Baseline characteristics by treatment approach at randomisation.

	Stepdown approach	Constant approach
Subjects *n*	27	28
Age at study entry (years)	8.1 (0.3)	7.8 (0.2)
Male sex (%)	52	54
Height (cm)	132.5 (1.7)	129.2 (1.7)
Duration of asthma (years)	5.0 (0.4)	5.0 (0.4)
IgE (IU/L)	560 (386-814)	418 (252-695)
< 1 RAST (number) [a]	3	4
PD_{20} methacholine (μg)	36 (26-53)	30 (22-43)
FEV_1 (% pred.)	92.1 (2.8)	89.4 (2.7)

Definition of abbreviations: IgE = immunoglobulin E; PD = provocative dose.
Values are presented as mean values (standard error of the mean), PD_{20} and IgE as geometric mean (range).
[a]A RAST test was considered positive when the result read class 2 or higher.

Results

Patients

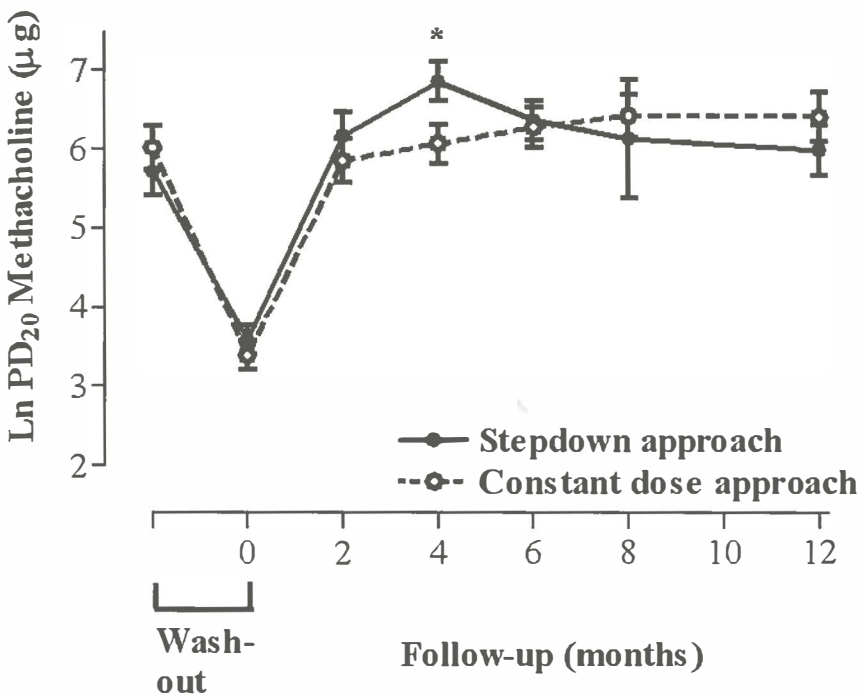
Fifty-five children completed the 1-yr study. During the study, four children were withdrawn: three from the constant dose approach (one due to exacerbation of asthma, and two because of insufficient asthma control) and one from the stepdown approach (because of adverse event: abnormal bone density). This difference was not significant. Baseline characteristics were similar in both treatment approaches

(Table 1). Mean β_2-agonist use was 0.1 dosages/d (range 0.0-1.0) in both groups during the wash-out period. The (geometric) mean time between end of the run-in period and randomisation for the constant dose approach was 22.8 d and 24.3 d for the stepdown approach.

Airways hyperresponsiveness

Airways hyperresponsiveness improved in both approaches after randomisation to treatment with inhaled FP (PD_{20} change from 30 to 346 μg in constant dose approach, and from 30 to 477 μg in stepdown approach; Figure 2). Only at 4 mo was the difference in PD_{20} between study approaches significant (geometric mean [range] PD_{20} 200 μg/d versus PD_{20} 500 μg/d: 430 (264-700 μg) versus 940 μg (571-1548 μg), respectively, $p = 0.03$). No significant difference was found when comparing the area under the log PD_{20} curve between the stepdown approach and the constant dose approach after 1 yr.

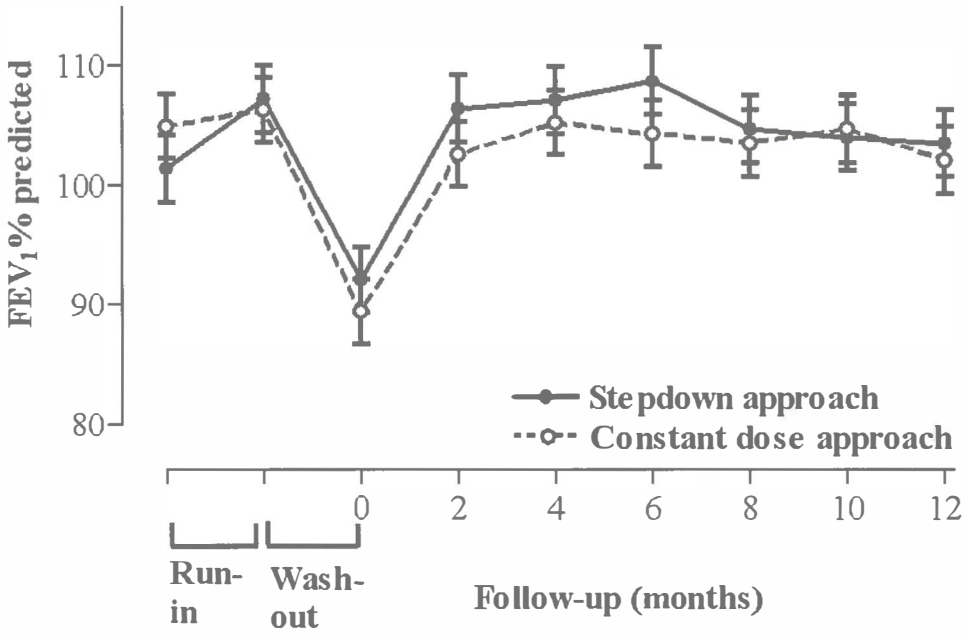
Figure 2: Airways hyperresponsiveness to methacholine, expressed as logarithmic cumulative dose (μg), in children with asthma during and after 1-yr treatment with different dosage schedules of inhaled FP. * $p = 0.03$.



Lung function, exhaled nitric oxide, and peakflow

FEV_1 % predicted deteriorated during the wash-out period (Figure 3). After randomisation both treatment approaches improved (mean from 92.1 % to 106.4 % in stepdown approach, and from 89.4 % to 102.6 % in constant dose approach, Figure 3). No significant differences were found between the two dosage schedules at any time point.

Figure 3: FEV_1 % predicted during and after 1-yr treatment with different dosages of inhaled FP in children with asthma.



Levels of exhaled nitric oxide (eNO) increased after withdrawal of FP and decreased in both treatment approaches after randomisation (Table 2). During follow-up, no significant differences were detected between the two treatment schedules.

Results of morning and evening PEF (Table 2) and diurnal and day-to-day PEF variation (data not shown) did not differ between the treatment schedules during the study.

Table 2: Exhaled nitric oxide and peakflow measurements between the different dosage schedules of fluticasone propionate (FP).

			Treatment month					
	Run-in (200 µg/d)	Washout randomisation	2 (200 vs. 1000 µg/d)	4 (200 vs. 500 µg/d)	6 (200 vs. 200 µg/d)	8 (200 vs. 100 µg/d)	10 (200 vs. 100 µg/d)	12 (200 vs. 100 µg/d)
Constant dose approach								
Exhaled nitric oxide (ppb)	10.6 (1.6)	14.9 (1.7)	10.6 (1.6)	9.2 (1.5)	13.3 (1.7)	13.7 (1.7)	10.5 (1.7)	12.2 (1.6)
PEF, A.M. (L/min)	241 (8.2)	218 (7.9)	234 (7.4)	251 (8.6)	259 (8.8)	263 (8.1)	265 (8.7)	269 (9.3)
PEF, P.M. (L/min)	247 (8.3)	227 (8.9)	242 (8.0)	255 (8.7)	265 (8.9)	270 (8.0)	272 (8.7)	275 (9.6)
Stepdown approach								
Exhaled nitric oxide (ppb)	10.2 (1.6)	17.1 (1.6)	12.4 (1.5)	10.6 (1.5)	11.1 (1.6)	13.3 (1.6)	11.8 (1.7)	14.2 (1.6)
PEF, A.M. (L/min)	249 (8.6)	240 (8.1)	244 (10.0)	254 (9.3)	266 (10.3)	264 (9.2)	268 (11.5)	269 (12.3)
PEF, P.M. (L/min)	253 (8.8)	240 (8.3)	249 (9.6)	258 (9.1)	269 (10.2)	266 (9.1)	271 (11.6)	271 (12.1)

Definition of abbreviations: PEF = peak expiratory flow.
Values are presented as mean values (standard error of the mean).

Symptoms scores and exacerbations

Symptoms increased after withdrawal of FP and decreased during therapy with both dosage schedules of FP, without any significant difference between the two schedules.

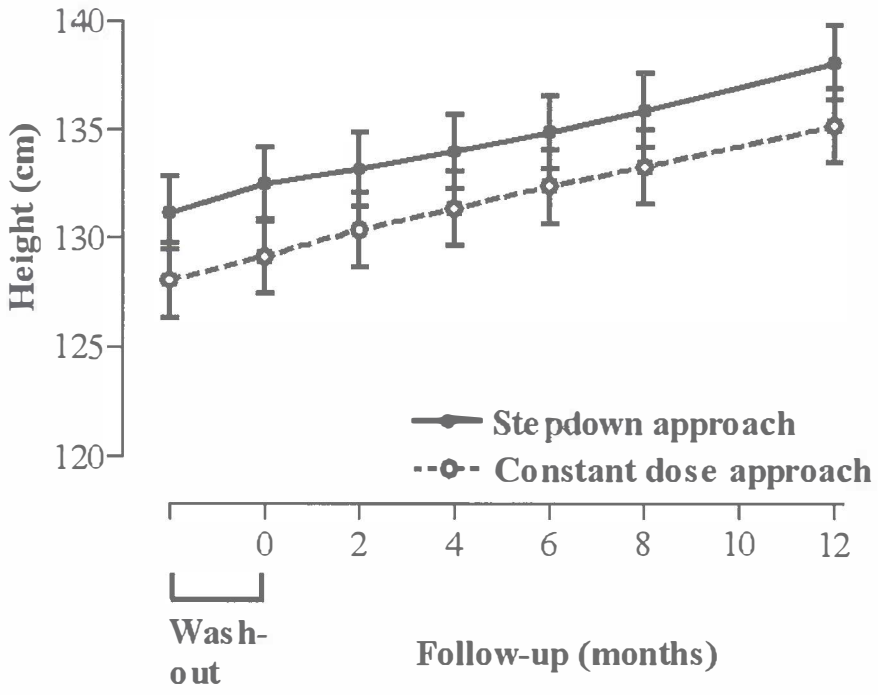
The number of asthma exacerbations requiring prednisolone courses was low (0.4 exacerbations/patient year) and evenly distributed between the two approaches (10 and 11 exacerbations in the constant dose and stepdown approach, respectively). The incidence of exacerbations was stable throughout the study period. The number of prednisolone courses in both treatment groups was the same (seven in each) during the first 6 mo of the study, when differences in dosages of FP between study groups were largest. There were no deaths and no patients were admitted to intensive care during the study.

Because the dose of FP differed most between groups during the first 6 mo of therapy, all analyses of the differences between groups were made for the first and second 6 mo of the study separately. This did not change the results: the only significant difference found was between the log PD_{20} levels mentioned above.

Height

At baseline patients in the stepdown approach were taller (mean 3.3 cm) compared to those treated according to the constant approach (Table 1). This difference remained stable throughout the study (Figure 4), and no significant differences in changes of standing height over time were found between children treated according to the stepdown approach or the constant dose approach. Changes in bone age were similar in the two study approaches during 1-yr treatment (stepdown approach from 8.2 to 9.4 yr, constant dose approach from 8.0 to 9.1 yr).

Figure 4: Height during and after 1-yr treatment with different dosages of inhaled FP in children with asthma.



Adherence

Mean adherence to treatment was 95.4% in the stepdown and 92.7% in the constant dose group, and remained stable throughout the 12-mo study period. There were no patients with compliance less than 84.6%.

Discussion

This is the first study comparing a constant dose approach of ICS (FP) to a stepdown dosing approach in childhood asthma. Apart from a temporarily larger improvement in airways hyperresponsiveness in the stepdown approach, the effects of the dosage schedules on the measured disease parameters (symptom scores, exacerbation rates, PEF, FEV_1, PD_{20}, and eNO) were very similar even when differences in FP doses between treatment groups were largest (first 4 mo of the study). Thus, the results of our study do not support the hypothesis of a superior effect of a stepdown approach (with a high starting dose of ICS, tapered off to a low dose) when compared to a fixed dose schedule over a 1-yr treatment period in children with chronic persistent asthma. It is important to point out that our study population did not include patients with mild intermittent asthma (who would not show airways hyperresponsiveness within 4 wk of withdrawing ICS), or those with severe persistent asthma (who would not have been able to complete the wash-out period without ICS maintenance therapy). We feel, however, that our study population is representative of a large group of children with mild to moderate chronic persistent asthma, characterized by moderate to severe airways hyperresponsiveness.

The degree of airways hyperresponsiveness, a cardinal feature of asthma, was the primary end point of the study. It has been shown previously that ICS improve airways hyperresponsiveness in a dose-dependent way.[7] In our study, the only evidence of a dose-dependent effect of FP on airways hyperresponsiveness was the significant difference in PD_{20} between the two study approaches after 4 mo of treatment (FP 500 μg/d versus FP 200 μg/d; Figure 2). We suspect that the significant difference after 4 mo of follow-up is due to a "carry-over" effect of the high dose (1000 μg/d) given during the first two mo of follow-up.

Previous studies have shown that airways hyperresponsiveness continues to improve for many mo on end during treatment with a fixed dose of budesonide or beclomethasone, which was also seen with FP in the constant dose approach in our study (Figure 2).[25–27] The lack of difference in airways hyperresponsiveness after 2 mo of treatment between FP 1000 μg/d and FP 200 μg/d is probably caused by a too short duration of treatment with this high dose of inhaled FP, preventing an optimal response on airways hyperresponsiveness after 2 mo. Indeed, a recent study, comparing two different dosage schedules of FP, only showed a significant dose-dependent effect on airways hyperresponsiveness only after treatment for 6 mo, suggesting a slow dose-dependent effect on airway inflammation.[7] However, our results suggest that this dose-dependent effect of FP on airways hyperresponsiveness is not maintained when a high dose is tapered off in the stepdown fashion so popular in contemporary asthma management.

We found no difference in airways hyperresponsiveness when in both approaches patients were treated with inhaled FP 200 μg/d (after 6 mo). Previous studies in adults (using FP and budesonide) are consistent with this finding.[28,29] Even more important, no superior effect of the stepdown approach on airways hyperresponsiveness was found after 1 yr treatment of although the cumulative dose of FP was 1.7 times higher in the stepdown approach (120 mg) than that in the constant dose approach (73 mg). This suggests that the effects of ICS on asthmatic airway inflammation in patients with asthma are independent of the cumulative dose of ICS used, but rather depend on the dose presently being used. The results of previous studies showing rapid recurrence of airways hyperresponsiveness after withdrawal of ICS demonstrate that the effect of even a high dose of ICS on both airway inflammation and airways hyperresponsiveness is only temporary, if ICS are not continued.[29,30]

Although all participating children had been using ICS before entering the study, the FEV_1 % predicted improved during the run-in period in both approaches. We propose that this is due to improved adherence to treatment, caused by participation in the clinical trial. During the wash-out period, the FEV_1 decreased significantly as expected, to improve again rapidly after reinstitution of ICS therapy (Figure 3). Although in some studies the effect of ICS on FEV_1 was dose-dependent at group level, we did not find significant differences between the two treatment approaches at any moment during the study.[6,7,9,29] Previous studies have shown that the improvement in FEV_1 quickly levels off after introducing these drugs.[11,25,26,31] This suggests that the lack of finding a dose-dependent response on the FEV_1 is possibly due to the relative insensitivity of this parameter to changes in the dose of ICS within the individual patient.

Cross-sectional studies have shown that children with asthma have higher levels of eNO than healthy control children.[22,32,33] In addition, ICS decrease eNO levels in subjects with asthma.[34–36] We found that eNO levels increased after withdrawal and decreased after reinstitution of FP, but without a significant effect of the dose of FP on eNO levels (Table 2). It is unlikely, therefore, that eNO levels will be useful to titrate the ICS dose in childhood asthma.

Previous studies have shown that PEF increases, and symptoms and use of rescue medication decrease after institution of ICS. In accordance with our results, other studies have shown little, if any, effect of the dose of ICS on the improvement of those parameters.[6–10,37] In addition, a recent study in adult subjects with asthma, comparing two different dosage schedules of ICS, also failed to detect differences in PEF and symptoms.[38] The difference between changes during therapy in these variables on the one hand, and changes in airways hyperresponsiveness in which a slight dose-dependent effect of ICS was observed on the other, is in accordance with earlier work.[39,40]

Although children treated according to the stepdown approach cumulatively received almost twice the amount of inhaled FP as those treated according to the constant dose approach, this had no significant effect on linear height over a 1-yr period (Figure 4). This does not, however, exclude an effect of FP per se on height. A previous study, comparing FP 100 μg/d for 1 yr to cromoglycate, showed no statistically significant effect of FP on height growth of asthmatic children.[14] In a placebo-controlled trial, treatment for one year with FP 200 μg/d, however, was associated with reduced height growth, as has been observed with beclomethasone and budesonide.[15,16,18,26,41,42] Reassuring results of long-term treatment with budesonide have recently been published, showing no effect on adult height.[17,18] Although it is likely that this is also the case for FP, long-term prospective studies are needed to test this hypothesis.

The power our study to detect significant differences between groups was somewhat reduced (to 84%) because it was difficult to recruit patients with a sufficient amount of airways hyperresponsiveness into our study.[19] Because the differences in disease parameters between study groups were absent or very small, we feel it is unlikely that increasing the power would have changed the results.

In conclusion, this study showed no superior clinical effect of FP given in a stepdown approach for 1 yr when compared with a constant dose strategy in children with asthma. Only a small and temporary dose-dependent effect on airways hyperresponsiveness was found. These results raise doubt to the validity of the popular stepdown strategy of dosing ICS in moderate to severe childhood asthma. Further studies are needed to evaluate the effects of constant and stepdown dosing strategies in mild asthma.

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Chapter 6

Influence of different dosage schedules of inhaled fluticasone propionate on peripheral blood cytokine concentrations in childhood asthma

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Abstract

Background Asthma is characterized by eosinophilic airways inflammation with elevated levels of IL-4, IL-5, and sICAM-1, and reduced levels of IL-10 and INF-γ. Inhaled corticosteroids powerfully reduce airways inflammation.

Objective To investigate if eosinophil counts, serum eosinophilic cationic protein (ECP) and sICAM-1 levels, as well as serum and production of cytokines (IL-4, IL-5, IL-10, INF-γ) by peripheral blood monocytes (PBMCs) are useful markers to monitor therapy with inhaled fluticasone propionate (FP) in asthmatic children.

Methods In a double-blind, 1-year study, 55 asthmatic children (aged 6-10 years) stopped inhaled corticosteroids for a mean period of 24 days and were randomized to receive either FP 200 μg/day (constant dose group), or a starting dose of FP 1000 μg/day with two monthly reductions to 500, 200 and 100 μg/day (stepdown group). Hyperresponsiveness, symptom scores and blood sampling were performed at 2-month intervals.

Results Symptoms and hyperresponsiveness improved significantly in both treatment groups after reintroduction of FP. Eosinophil counts decreased significantly more during the first 2 months of FP in the stepdown group than in the constant dose group ($p = 0.03$). We found a trend towards a dose-dependent response in changes of eosinophil counts and serum ECP levels during treatment. Serum IL-4 and IL-5 levels were undetectable in the majority of children. No significant effect of the dose of FP on the release of IL-4, IL-5, IL-10 or INF-γ by Con A stimulated PBMCs was found. sICAM-1 levels did not significantly differ at any time point between the two groups.

Conclusion Serum ECP as well as peripheral blood eosinophils, cytokine production by PBMCs and sICAM-1 levels are insensitive markers in titrating and monitoring therapy with inhaled corticosteroids over a wide dose range in childhood asthma.

Introduction

Asthma is characterized by an influx of eosinophils and lymphocytes in the airway wall.[1] Lymphocytes produce different cytokines, and the cytokine profile in asthma is compatible with a predominance of Th2 lymphocytes (IL-4, IL-5, and IL-10) over Th1 lymphocytes (INF-γ).[2,3] Most knowledge on airways inflammation in asthma is inferred from studies in adults, using invasive techniques such as bronchoalveolar lavage and bronchial biopsy, which are not feasible in children. The limited knowledge on inflammation in childhood asthma therefore is obtained generally from studies assessing peripheral blood.

Eosinophils migrate from bone marrow to the pulmonary airways.[2] Adhesion and migration of peripheral blood eosinophils to inflammatory sites are augmented by increased expression of intercellular adhesion molecules, e.g. ICAM-1 and VCAM-1. A soluble form of ICAM-1 is thought to be derived through shedding of membrane bound intercellular adhesion molecule-1 (sICAM-1), which has been shown to be elevated in asthmatic children.[4,5]

Corticosteroids are the mainstay of asthma management[6], and asthmatic symptoms are well controlled with these drugs. They are known to reduce cytokine production from inflammatory cells[7,8] and lower sICAM-1 levels both *in vitro* and *in vivo*.[9,10] In addition, eosinophil counts as well as serum eosinophilic cationic protein (ECP) concentrations decrease after therapy with inhaled corticosteroids in asthmatic children.[11] One study in asthmatic children showed a reduction in the percentages of *in vitro* stimulated peripheral blood mononuclear cells (PBMCs) expressing mRNA encoding IL-4 and IL-5 and an increase of INF-γ mRNA expression after institution of treatment with inhaled corticosteroids.[12] Two recent *in vivo* studies in asthmatic children demonstrated a reduction in serum IL-5 but not serum IL-4 concentrations after therapy with oral prednisolone.[13,14] So far, no long-term randomized controlled studies have been performed evaluating the effect of inhaled corticosteroid therapy on peripheral blood cytokine concentrations in childhood asthma. We performed a clinical study, comparing the effects of two different dosage schedules of inhaled fluticasone propionate (FP) in asthmatic children on eosinophil counts, ECP and sICAM-1, and on serum and stimulated PBMCs cytokine levels.

Material and methods

Subjects

Children, aged 6-10 years, with mild to moderate persistent asthma were recruited for the study. None used > 800 μg of inhaled corticosteroids (FP > 400 μg). Patients with major other illness and those who had used systemic corticosteroids during the previous 6 weeks, or had a respiratory tract infection during the preceding 4 weeks, were excluded.

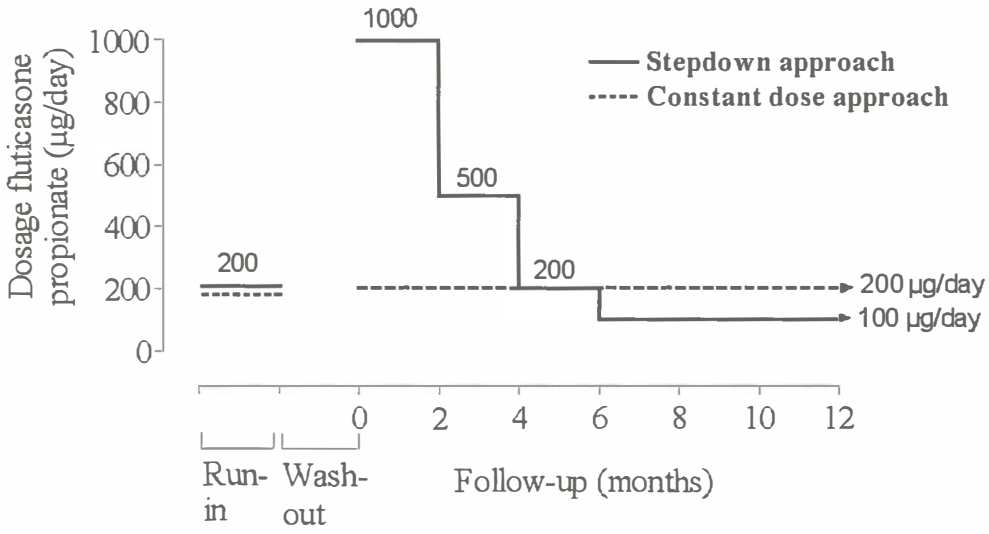
Informed consent was obtained from all children and their parents. The study was approved by the medical ethics committees of the three participating centres.

Study design

After a run-in period of 6 weeks during which patients used FP 100 μg twice daily by dry powder inhaler (Diskhaler®, GlaxoWellcome, Zeist, the Netherlands), patients entered a wash-out period of 2-8 weeks during which no inhaled corticosteroids or other anti-inflammatory drugs were used, but only inhaled salbutamol 200 μg by Diskhaler®on demand. During this period, patients returned to the clinic every 2 weeks (or earlier if symptoms worsened) for measurement of airways hyperresponsiveness. When a PD_{20} < 80 μg was never obtained during the full 8-week wash-out, the patient was withdrawn from the study.[15] When a PD_{20} < 80 μg was found, the patient was randomized either to the stepdown or the constant dose schedule of inhaled corticosteroids (Figure 1). All patients received carefully standardized instructions on the correct use of the Diskhaler®and were asked to demonstrate a correct inhalation manoeuvre at each follow-up visit. During the whole study, patients were allowed to use salbutamol by Diskhaler®on demand.

Airways hyperresponsiveness to methacholine was measured using a dosimeter method as published previously.[16] Blood for measurement of inflammatory parameters was drawn at the randomisation visit and subsequently at 2-months intervals (except after 10 months treatment). In addition, at each follow-up visit, a standardized short symptom questionnaire was administered to each patient.[17]

Figure 1: Study design of asthmatic children treated according to the stepdown approach or the constant dose approach with inhaled fluticasone propionate (FP).



Atopy

Blood was drawn at the randomisation visit for total and specific IgE, determined by the Pharmacia MasterCap analysis (Pharmacia & Upjohn, Uppsala, Sweden) according to the protocol of the manufacturer, with a range in IgE of 2-2000 U/mL.

Number of eosinophils and serum ECP

The number of eosinophils was counted with a Coulter counting chamber. ECP levels were determined as published previously.[11] Briefly, blood was allowed to clot during 1 h after it was drawn. Then, it was centrifuged twice (1450 **g**) and stored at -20°C. Serum ECP was determined by radioimmunoassay according to the protocol of the manufacturer (Pharmacia, Uppsala, Sweden), with a range of 0-200 µg/L. Values above the upper detection limit of 2000 U/ml were further quantified after dilution.

Stimulation of cytokine production

Stimulation of cytokine production were measured as described previously.[3] Briefly, 10 mL diluted blood was layered on a 4 Lymphoprep solution (Nycomed Pharma A.S., Oslo, Norway, density of 1.077 g/mL) in a polypropylene tube. Peripheral blood mononuclear cells (PBMCs, $1*10^6$ cells/mL in RPMI-1640 medium plus 10% FCS) were cultured in a 24-well plate (Costar, Badhoevedorp, the Netherlands), and were activated with 10 μg Concanavaline A (Sigma Alderich Chemie B.V., Zwijndrecht, the Netherlands). The cells were incubated for 24 h, 37°C, 5% CO_2, followed by centrifugation at 590 g, 10 min in a polypropylene tube, and the supernatant collected and stored in polypropylene tubes at -80°C for further analyses.

Cytokine and soluble intercellular adhesion molecule-1 measurements

Assays of supernatants for IL-4, IL-4 high sensitive assay (for serum), and sICAM-1 were from R & D Systems Europe, Abindon, UK. Assays for INF-γ were from Amersham Life Science, Roosendaal, the Netherlands, IL-10 from Centraal Laboratorium Bloedtransfusie, Amsterdam, the Netherlands, and IL-5 measured according to Borger et al.[3,18] The detection limit was 0.010 pg/mL and 0.1 pg/mL for IL-4 and IL-5, respectively. Concentrations of IL-4, IL-5, IL-10, and INF-γ in serum and supernatants of activated PBMCs were determined as described above. When IL-4 and IL-5 were undetectable in the serum, the detection limit was used for statistical analysis. sICAM-1 was only determined in the serum samples.

Statistical analysis

All calculations of PD_{20} were performed on a log-transformed basis, thereby normalising the distribution. The analyses were performed using SAS 6.12 and SPSS 9.0. To determine trends and differences across groups over time, a linear mixed effects model (SAS Proc Mixed) was used.

Results

Patients

Fifty-five children were included in the study. During 1-year follow-up, four children were withdrawn: three from the constant dose group (two because of insufficient asthma control, one due to exacerbation of asthma) and one from the stepdown group (adverse event: abnormal bone density). Baseline characteristics and duration of run-in period were comparable in the two groups (Table 1).

Table 1: Baseline characteristics at randomisation.

	Stepdown approach	Continuous approach
Subjects *n*	27	28
Age at study entry (years)	8.1 (0.3)	7.8 (0.2)
Male sex (%)	52	54
Duration of asthma (years)	5.0 (0.4)	5.0 (0.4)
IgE (IU/L)	560 (386-814)	418 (252-695)
< 1 RAST (number) [a]	3	4
PD_{20} methacholine (μg)	36 (26-53)	30 (22-43)
FEV_1 (L)	1.53 (0.07)	1.41 (0.06)
Wash-out period (days)	24.3 (18.1-32.5)	22.8 (16.3-31.9)

Values are presented as mean values (SEM).
PD_{20}, IgE and wash-out period as geometric mean (range).
[a]A RAST test was considered positive when the result read class 2 or higher.

Symptoms decreased after reinstitution of FP with both dosage schedules, without a significant difference between the two schedules. There was no difference in incidence of exacerbations between groups (0.4 exacerbations/patient year).

Airways hyperresponsiveness

Airways hyperresponsiveness increased after withdrawal and decreased after reinstitution of inhaled FP (Table 2). A significant dose-dependent effect was found after 4 months of treatment (FP 500 μg/day vs. 200 μg/day, respectively, $p = 0.03$). At

the end of the one year treatment period, there was no difference in airways hyperresponsiveness between the two groups (FP 100 μg/day vs. 200 μg/day, respectively, $p = 0.3$).

Table 2: Airways hyperresponsiveness and serum markers between the different dosage schedules of fluticasone propionate in asthmatic children.

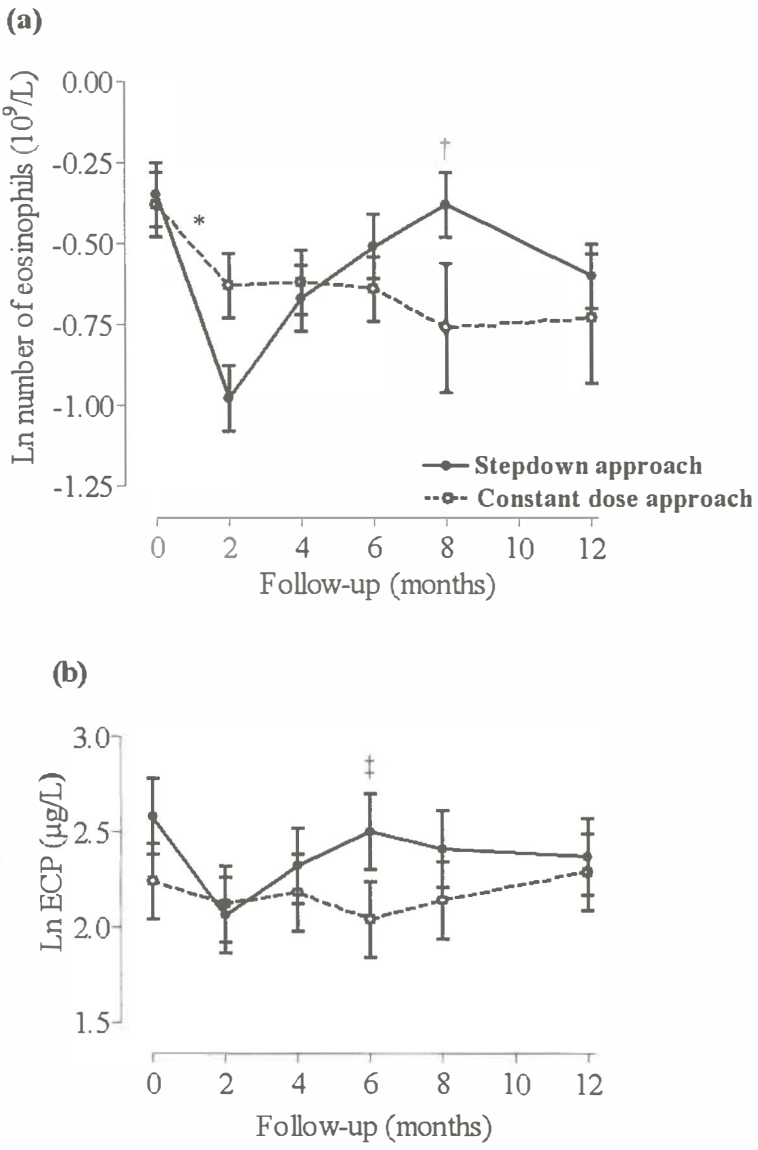
	Wash-out	Treatment month				
	randomisation	2	4	6	8	12
Constant approach, dose[a]	0	200	200	200	200	200
PD_{20} (μg)	30 (22-43)	346 (199-600)	430 (264-700)[b]	527 (325-853)	615 (360-1050)	608 (331-1116)
IL-5 (pg/mL)	2.68 (0.8)	2.94 (0.8)	3.35 (0.8)	4.44 (0.8)	3.62 (0.9)	2.82 (0.8)
sICAM-1 (ng/mL)	313.3 (13.5)	282.2 (14.7)	273.7 (14.4)	280.6 (14.0)	268.3 (15.2)	297.7 (14.4)
Ln ratio ECP/eosinophil	2.70 (0.1)	2.72 (0.1)	2.81 (0.1)	2.65 (0.1)[b]	2.84 (0.1)	2.96 (0.1)
Stepdown approach, dose[a]	0	200	200	200	200	200
PD_{20} (μg)	36 (26-52)	477 (267-854)	940 (571-1548)	577 (356-934)	459 (269-783)	401 (217-740)
IL-5 (pg/mL)	1.46 (0.5)	2.30 (0.5)	2.47 (0.5)	3.03 (0.5)	3.47 (0.5)	3.29 (0.5)
sICAM-1 (ng/mL)	306.8 (14.1)	248.1 (14.8)	256.1 (15.0)	317.2 (13.9)	301.5 (14.8)	317.6 (13.9)
Ln ratio ECP/eosinophil	3.01 (0.1)	3.05 (0.1)	2.97 (0.1)	3.05 (0.1)	2.84 (0.1)	2.96 (0.1)

PD_{20} is represented as geometric mean (95% CI), IL-5 and sICAM-1 as mean (SEM).
[a]Dose in μg/day.
[b]$p < 0.05$ compared with stepdown approach.

Eosinophils and ECP in peripheral blood

After reinstitution of FP, the number of eosinophils decreased within 2 months in both groups. This decrease was significantly larger in the stepdown group when compared to the constant dose group (1000 μg/day vs. 200 μg/day), values changing from 0.70 to $0.38*10^9$/L (geometric mean) with the stepdown, and from 0.68 to $0.53*10^9$/L with the constant dose regimen, respectively ($p = 0.03$, Figure 2a). During further treatment with a constant dose of FP, the number of eosinophils in peripheral blood remained stable. Eosinophil counts showed a trend towards increasing after tapering off FP in children in the stepdown group. A borderline significant difference in eosinophil counts was detected after 8 months in children receiving FP 100 μg/day (stepdown approach) when compared to FP 200 μg/day (geometric mean, range: 0.68, $0.52\text{-}0.91*10^9$/L vs. 0.47, $0.35\text{-}0.62*10^9$/L, $p = 0.06$, respectively, Figure 2a). After 1-year's treatment, no difference in number of eosinophils was found between the two groups.

Figure 2: (a) Number of eosinophils in peripheral blood (*10^9/L) and (b) concentration of ECP (μg/L) in asthmatic children during and after treatment with different dosage schedules of inhaled FP. Data are logarithmically transformed and expressed as mean (SEM). * $p = 0.03$ significant difference in decrease of eosinophil counts in 2 months between the stepdown and constant dose approaches. † $p = 0.06$ between the two approaches. ‡ $p = 0.03$ between the two approaches.



ECP concentrations were stable during treatment with FP 200 μg/day for 1 year (constant dose approach). A dose-dependent effect was found on serum ECP concentrations in the stepdown group, with an increase in ECP levels after tapering off FP (Figure 2b). A significant difference between the two approaches was only detected after 6 months of treatment with 200 μg/day FP in both approaches (mean, SEM: 12.2 ± 1.2 μg/L vs. 7.7 ± 1.2 μg/L, $p = 0.03$).

A significant difference between the two dosage schedules with respect to the ratio of ECP/eosinophil count was only found after 6 months (Table 2).

Cytokines, serum

At the baseline visit, IL-4 was detectable in 12 children in the stepdown group (46%) and in 10 children in the constant dose group (42%). During the study, there was an increase in number of blood samples with undetectable concentrations of IL-4 in both groups. There were no significant differences between groups at any moment (data not shown).

IL-5 was detectable at baseline (without steroids) in 16 children in the constant group (64%) and in 15 children in the stepdown group (65%). In 23% of the children, IL-5 was undetectable throughout the study, independent of the treatment approach. Serum IL-5 gradually increased during treatment according to the stepdown approach. No significant differences were found between the two treatment groups (data not shown).

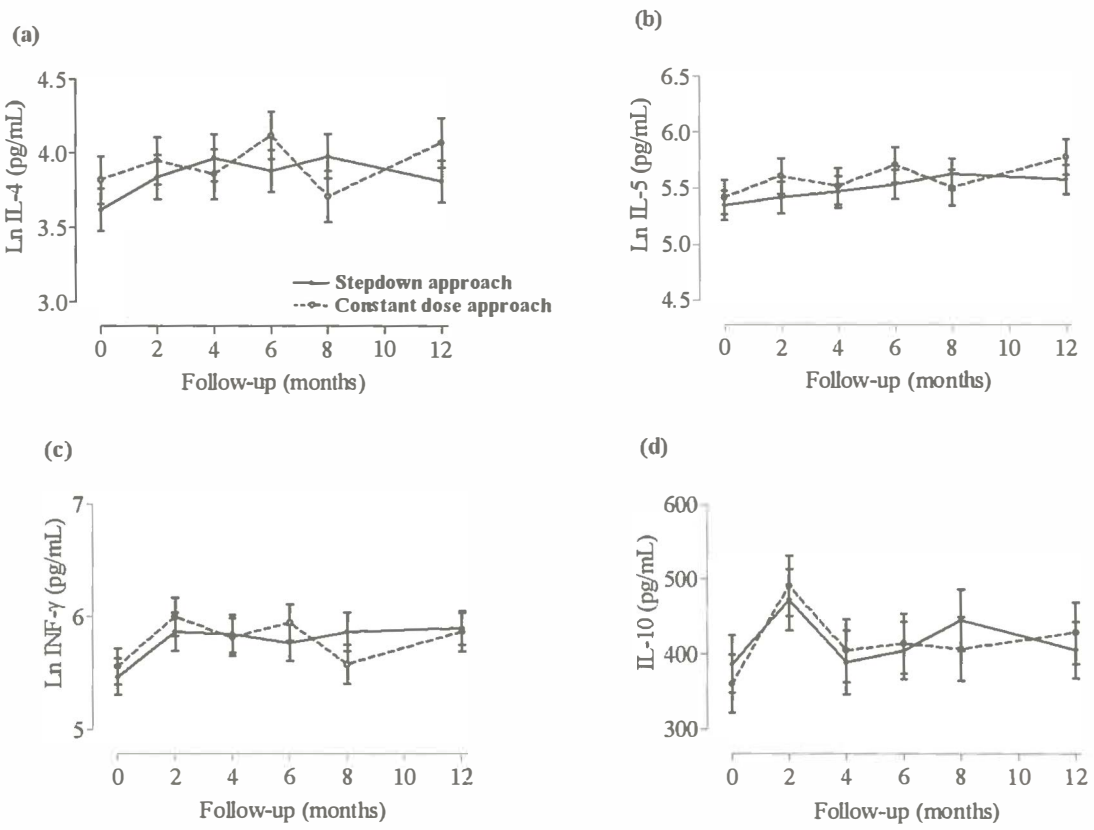
sICAM-1 concentrations did not significantly differ between groups at the beginning of the study. In both groups there was a decrease in sICAM-1 concentrations after starting with FP (Table 2). In the stepdown group sICAM-1 concentrations increased after tapering down FP, with a borderline significantly higher concentration after 6 months of treatment than in the constant dose group (mean ± SEM: 317.2 ± 13.9 ng/mL vs. 280.6 ± 14.0 ng/mL, $p = 0.06$). After treatment for 1 year with FP, sICAM-1 did not differ significantly between the two groups.

Cytokines, supernatants of Con A stimulated PBMCs

IL-4, IL-5, INF-γ and IL-10 were detectable in supernatants of Con A stimulated PBMCs in all participating children.

No significant effect of the dose of FP on the release of cytokines (IL-4, IL-5, IL-10, and INF-γ), irrespective of treatment approach, was found (Figure 3).

Figure 3: Concentrations of Con A stimulated PBMCs for IL-4 (a, pg/mL), IL-5 (b, pg/mL), INF-γ (c, pg/mL), and IL-10 (d, pg/mL). Values are logarithmically transformed, except for IL-10, and expressed as mean (SEM). No significant differences between stepdown and constant dose approach at all time points.



Discussion

In accordance with the effect on the severity of airways hyperresponsiveness[19], this study showed a dose-dependent effect of inhaled FP on the number of peripheral blood eosinophils, and a trend towards such an effect on both ECP concentrations and sICAM-1 levels in serum of asthmatic children. However, no effect of the dose of FP on concentrations of IL-4, IL-5, INF-γ or IL-10 was found, either in serum or in supernatant of Con A stimulated PBMCs.

The dose-dependent effect of FP on eosinophil counts, without singular effect on ECP concentrations confirms results of previous studies.[20,21] A recent study in asthmatic children did not find an association between serum ECP and asthma severity or inflammatory markers, suggesting a limited role of ECP in monitoring asthma control.[22] Thus, our results are in agreement with previous reports, showing that serum ECP is not a useful marker to guide the dose of inhaled corticosteroids in childhood asthma.[20,23]

The number of eosinophils decreased significantly after 2 months, at the highest dose of FP, and increased significantly after 8 months treatment when lowering the FP dose in asthmatic children treated according to the stepdown approach. Results were significantly different when compared with the results with the constant dose approach. After 8 months, however, the number of eosinophils returned to a level comparable to the beginning of the study when no inhaled corticosteroids were being used. In contrast, we found no significant difference in PD_{20} values between the study arms after 2 and 8 months treatment. The discrepancy between the effects of inhaled corticosteroids on eosinophil counts and PD_{20} during treatment with different dosages of FP might be explained by an increased systemic bioavailability of the high dose FP, resulting in a suppression of blood eosinophil progenitors[24] and colony forming unit production in bone marrow.[25] Similar results have been found in adult asthmatics, in whom eosinophil counts decreased after treatment with FP 2000 μg/day or oral prednisolone, without significant changes in airways hyperresponsiveness.[26]

There is accumulating evidence that inflammation in atopic asthmatic children is associated with a predominance of Th2-lymphocytes. This is reflected by elevated levels of IL-4 and IL-5[27,28], and reduced INF-γ levels.[3] Although is assumed that this Th1-Th2 imbalance underlies the inflammation in asthma, it is yet unclear whether this is due to asthma *per se* or the atopy associated with it in about 80 percent of children.[27,29] In our study, almost all children (87%) were atopic. Our results did not change when only atopic children were included (data not shown).

It has been demonstrated that oral corticosteroid therapy inhibits mRNA expression of IL-4, IL-5, and INF-γ in BAL fluid and lung tissue, as well as their related cytokine secretion.[8,30] We found no effect of FP on unstimulated serum IL-4 levels, mainly due to many undetectable serum concentrations of this cytokine already at baseline, despite the use of a highly sensitive assay. Other investigators also had difficulties in detecting production of serum IL-4 from unstimulated PBMCs.[3,13,27] However, we did not find a significant effect of different dosages of inhaled FP on IL-4, IL-5 or INF-γ concentrations by Con A stimulated PBMCs either. In other studies, marked changes in cytokine production of stimulated PBMCs have been demonstrated after allergen challenge. We have recently shown that inhaled corticosteroids have a dose-dependent effect on cortisol levels, due to adrenal-cortex suppression.[31] Thus, inhaled FP is able to reach the bloodstream, exerting an effect on organs outside the circulation. We hypothesize that the effect of inhaled FP, even in a high dose (1000 μg/day), is too small to alter resting T lymphocytes in the circulating blood that are also subject to homeostatic mechanisms that will prevent cell activation.

Studies in asthmatic children showed a decrease in serum IL-5, but not serum IL-4 levels after therapy with oral prednisolone.[13,32] An explanation for differences between these studies and ours may be that oral corticosteroids exert a direct effect on the T-lymphocytes due to systemic bioavailability, resulting in a decrease in cytokine production levels.

IL-10, an anti-inflammatory cytokine that is decreased in asthma[33,34], is mainly produced by Th2-lymphocytes and monocytes. IL-10 inhibits the production of IL-4, IL-5 and INF-γ.[35,36] Conflicting reports on the effects of treatment with corticosteroids on IL-10 concentrations from stimulated PBMCs in adults have been described.[37,38] We found an increase in IL-10 concentrations after starting with inhaled FP. During the study, however, these elevated concentrations decreased, without a significant dose-dependent effect. This suggests that IL-10, just like IL-4, IL-5 and INF-γ, is a poor marker for evaluating therapy with inhaled corticosteroids in asthmatic children.

We found that serum sICAM-1 concentrations decreased after introduction of FP. Similar results were found recently by others, without a dose-dependent effect.[10] We have previously demonstrated that sICAM-1 levels are significantly associated with the presence or absence of airways hyperresponsiveness.[39] The results of the present study suggest that the level of sICAM-1 is ineffective to predict the severity of airways hyperresponsiveness.

Previous studies in adult asthmatics have shown that levels of IL-5 and IL-10 in peripheral blood are related to those in bronchoalveolar fluid.[38,40] Whether this is also true for children is unknown. Cytokine concentrations in peripheral blood are lower

in children when compared to adults, and this difference could also be present in bronchoalveolar fluid.[41,42] Our study suggests an increase in airways inflammation as reflected by an increase in airways hyperresponsiveness after reduction of the FP dose. Ethical considerations restricting invasive techniques in children prevented us from studying this inflammation directly, for example in bronchoalveolar fluid.

Limited data have been published on the effects of the dose of inhaled corticosteroids on various parameters assessing the severity of childhood asthma. A meta-analysis in adult asthma on different types of inhaled corticosteroids, including FP, showed a dose-dependent effect of FP on symptoms, rescue β-agonist use, and lung function, only in the lower dose range up to 200 μg/day. Higher dosages of FP showed no additional benefit, although only very few studies actually examined these higher dosages.[43] Similar results have been found in children.[21,44–46] This study suggests that airways hyperresponsiveness may be slightly more sensitive in picking up differences between dosages of FP in the higher dose range than are symptoms and level of lung function. However, earlier work showed that such differences are small and only become apparent after 6 months of treatment.[21] Whether measurement of airways hyperresponsiveness is useful to titrate the dose of inhaled corticosteroids in childhood asthma, as has been found in adults[47], is currently under investigation.

In summary, the effects of FP on sICAM-1, ECP, and on serum levels of and Con A stimulated PBMC production of IL-4, IL-5, IL-10 and INF-γ concentrations appear not to be dose-dependent in asthmatic children. The effect of FP dose on eosinophil counts was only apparent when comparing a very high dose (1000 μg/day) with a low dose (200 μg/day).

These results suggest that measurements of eosinophil counts, serum ECP, sICAM-1 and cytokine serum levels as well as cytokine production from stimulated PBMCs are not useful in monitoring therapy with inhaled corticosteroids in the dose range used in asthmatic children. Just as others have found in adults with asthma[48], we did not find advantage of an 'initial high-dose and stepdown' approach, as currently advocated in guidelines for asthma management.[6]

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Chapter 7

Dose-dependent suppression of growth velocity, bone metabolism and adrenal cortical function in asthmatic children using the inhaled corticosteroid fluticasone propionate: No long-term effects after dose reduction

A randomised clinical study

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Submitted

Abstract

Background: Inhaled corticosteroids are highly effective in the treatment of childhood asthma. Despite this, there is concern as to systemic side-effects, especially at high doses. Guidelines suggest to start therapy with a high dose with subsequent tapering ("stepdown") and to double the dose when asthma deteriorates. Thus high doses are frequently used. To assess long-term effects of high dose ICS, we performed a double blind prospective 2 year study comparing a stepdown dose approach (1000 μg/day tapering off two monthly to 500, 200 and then 100 μg/day for the remainder of the study) versus a continuous dose of inhaled fluticasone propionate (FP, 200 μg/day).

Methods In fifty-five children with chronic persistent asthma and airways hyperresponsiveness, aged 6-10 years, lung function and systemic side-effects (height, biochemical measurements of bone formation and degradation, bone mineral density (BMD), adrenal cortical function) were assessed at predetermined intervals for 2 years.

Findings Airways hyperresponsiveness improved after 4 months treatment with FP 1000 μg/day followed by 500 μg/day, without significant differences during long-term treatment between the two approaches. Other parameters of asthma control (exacerbation rates, lung function, symptom scores and peak flow) were comparable between the two treatment groups. A dose-dependent reduction of growth velocity, adrenal cortical function and biochemical bone turnover was found during therapy with FP 1000 followed by 500 μg/day when compared to 200 μg/day. These differences disappeared after tapering the dose to 200 μg/day. No differences between the two treatment schedules were found in standing height or BMD.

Interpretation A stepdown approach is not clinically favourable above conventional doses (FP $\leq$ 200 μg/day). The latter treatment appears to be safe in the long-term management of childhood asthma. However, doses of 1000 and 500 μg/day are associated with marked reduction of growth velocity, bone turnover and adrenal cortical function. From a safety point of view, these doses should only be prescribed in exception, e.g. in persistent severe asthma.

Introduction

Inhaled corticosteroids (ICS) are the cornerstone of asthma treatment. Because these drugs are highly effective in improving symptoms, lung function, airways hyperresponsiveness and quality of life in asthmatic children[1,2], they are recommended as controller therapy in all but the mildest forms of childhood asthma.[3–5]

Although current guidelines advice treatment with ICS in doses up to 400 μg/day, it has been advocated to start ICS therapy in childhood asthma with a high dose in order to reduce airway inflammation powerfully, and to subsequently taper off to the lowest effective dose ("stepdown" strategy).[4,5] It is also advocated to double the dose of ICS when asthma deteriorates. As a result, high dosages of ICS are frequently used, but very limited data are available on the effects and side-effects of high-dose ICS therapy in children.[6] In a previous study we have shown an initial improvement in airways hyperresponsiveness in children with asthma during high dose fluticasone propionate therapy (FP, 1000 μg/day for 2 months, followed by 2 months of 500 μg/day).[7] When the dose of FP was tapered to 100 μg/day, no differences were found between asthmatic children treated with this stepdown approach and children using FP 200 μg/day during the full 1-year follow-up. Thus, the stepdown approach does not appear to be superior to a constant dose approach of FP in children with chronic persistent asthma.

Concern on the adverse effects of ICS on height in children have been relieved by recent reports showing normal height during ICS treatment for 4-6 years[8], and normal final height at adult age after long-term ICS therapy.[9] Although effects of ICS on bone mineral density (BMD) in adults have been described[10,11], studies in children did not demonstrate reduction of BMD during ICS treatment.[8,12–14] Even during long-term treatment, therefore, ICS appear to be safe, but clinically relevant systemic side-effects have been reported anecdotally.[15–18]

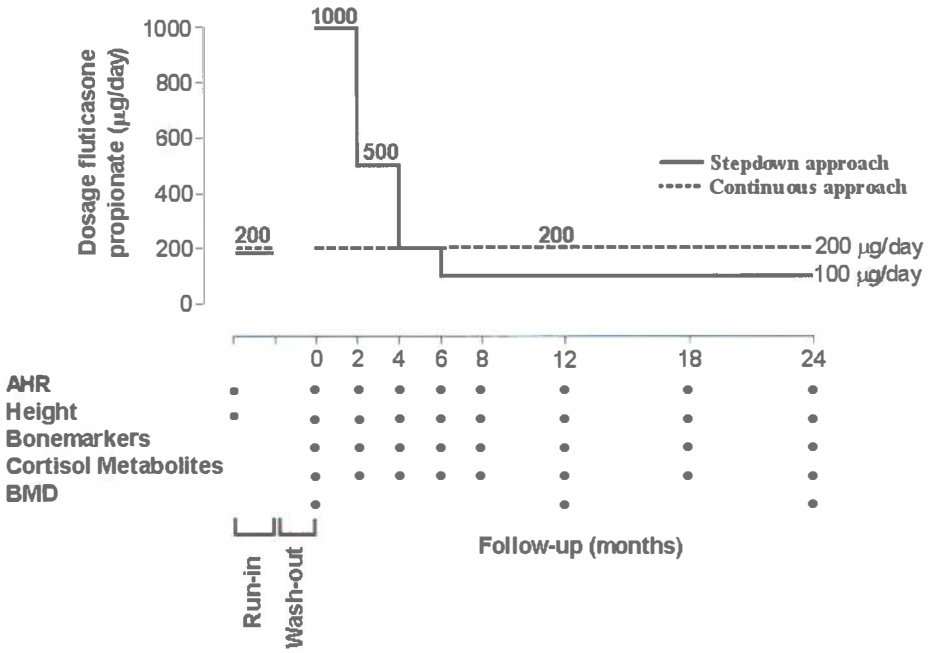
This prospective double blind study was designed to compare effects of different dosage schedules of ICS during 2-year follow-up in these asthmatic children, focusing on systemic side-effects.

Methods

Patients and study design

Between April 1996 and April 1998, 55 prepubertal children, 6 to 10 years of age, with a diagnosis of mild to moderate persistent asthma and using ICS were recruited from the outpatient clinics of three participating hospitals. Patients with other illness (e.g. growth retardation, endocrinologic disorder, nocturnal enuresis) and those who had used systemic corticosteroids during the previous six weeks, or had had a respiratory tract infection during the preceding four weeks, were excluded. The study was approved by the medical ethics committees of the three participating centres.

Figure 1: Study design comparing different dosing schedules of inhaled fluticasone propionate (FP) for two years in asthmatic children. AHR: airways hyperresponsiveness; BMD: bone mineral density.



At the start of the study patients were switched to inhaled FP 200 µg/day via dry powder inhaler (Diskhaler®, GlaxoWellcome, Zeist, the Netherlands) for six weeks (run-in). Thereafter ICS were stopped for a period of up to eight weeks (wash-out) during which only on demand inhaled salbutamol 200 µg by Diskhaler®was allowed

(GlaxoWellcome, Zeist, the Netherlands, Figure 1). Patients were randomised to treatment when they developed airways hyperresponsiveness during the wash-out period, defined as a $PD_{20} < 80$ μg (the provocative dose of methacholine causing a 20% drop in forced expiratory volume in one second (FEV_1)).[19] Study treatment consisted either of FP 200 μg/day for two years (constant dose group) or of a high starting dose FP (1000 μg/day), followed by two monthly reductions to 500, 200, and 100 μg/day during the remaining one and half year (stepdown group), in a double blind fashion (Figure 1). Salbutamol by Diskhaler®could be used on demand throughout the study. Randomisation was performed using a computer programme with stratification for age, sex, duration of prior ICS use, and PD_{20}. All patients received standardised instructions on correct use of the Diskhaler®and were required to demonstrate a correct inhalation manoeuvre at each follow-up visit.

Outcome measurements

Height was measured at predetermined intervals (Figure 1) by trained technicians, using a calibrated Harpenden stadiometer. Growth velocity in cm/year was calculated from changes in height over time. Bone age of the left hand was determined at randomisation, and after one and two years using the Tanner & Whitehouse method by a single blinded investigator.[20] Tanner's pubertal stages were scored at each follow-up visit.[21] BMD of the spine was measured by dual energy X-ray absorption (DEXA) from L1 to L4, using a HOLOGIC QDR-4500 C Elite.[22]

Serum osteocalcin and amino-terminal propeptide of type-1 procollagen (P1NP) are measurements of osteoblast activity and reflect bone formation. They were measured using the IRMA of Medgenix-H-Ost (Fleurus, Belgium) and RIA kit of Orion Diagnostica (Espoo, Finland), respectively. Urinary deoxypyridinoline (Dpyr) and pyridinoline (Pyr) are products of osteoclasts and reflect bone degradation. Urine was analysed for calcium and creatinine with a Hitachi 71 Chemistry Analyser, and for Dpyr and Pyr by HPLC. Urinary excretion of DPyr and calcium was corrected for urinary creatinine.

Total cortisol metabolites excretion was measured using 24 hour urine at preset visits (Figure 1) by gas-liquid chromatography as described previously.[23] The following major metabolites were determined: tetrahydrocortisone, tertahydrocortisol, allotetrahydrocortisol, α-cortol, α-cortolone, β-cortol, and β-cortolone.

Expiratory flow-volume curves were obtained with a Jaeger Masterlab pneumotachograph (Eric Jaeger GmbH, Würzburg, Germany) according to the guidelines of the European Respiratory Society.[24] Airways hyperresponsiveness to methacholine was

measured using a dosimeter method as published previously.[19]

Patients kept symptom and peak flow diaries during the study. These data have been published previously and will not be reported here.[7] Patients were asked to return all used and unused medication at each follow-up visit and adherence to treatment was assessed by counting the blisters used.

Statistical analysis

Analyses were performed using SAS 8.0 (Figures 2 and 3) and SPSS 9.0. (Table 1). Group data are summarised as means and standard errors of the means (SE). Comparisons between and within both treatment groups across time points were performed by a linear mixed model (SAS Proc Mixed). These comparisons were made both when differences in FP doses between groups were large (first months of the study) and when they were small (Figure 1). A two-tailed p value of $p < 0.05$ was considered to be statistically significant (*) and a two-tailed p value of $0.05 < p < 0.1$ was considered to be borderline significant (†).

Results

Fifty-five asthmatic children were included in the study: 27 were allocated to the constant dose group, 28 to the stepdown group. Baseline characteristics are shown in Table 1; both treatment groups were comparable. During the 2 year study, 7 children were withdrawn: 4 from the stepdown group (3 due to exacerbation, 1 because of abnormal bone density) and 3 from the constant dose group (2 because of insufficient asthma control, 1 due to an exacerbation of asthma). Mean adherence to treatment was 94 % in both groups and remained stable throughout the 2 year study period.

Airways hyperresponsiveness

Airways hyperresponsiveness deteriorated during the wash-out period after withdrawal of FP, and improved in both groups after reinstitution of inhaled FP (Figure 2A). At four months treatment a statistically significant difference was found between the two groups (FP 500 μg/day versus FP 200 μg/day). Over the remainder of the two year treatment period airways hyperresponsiveness was comparable between the two groups (Figure 2A).

Table 1: Baseline characteristics at randomisation.

	Constant dose group	Stepdown group
Subjects *n*	28	27
Male sex (%)	54	52
Age at study entry (years)	7.8 (0.2)	8.1 (0.3)
Duration of asthma (years)	5.0 (0.4)	5.0 (0.4)
IgE (IU/L)	418 (252-695)	560 (386-814)
PD_{20} methacholine (μg)	30 (22-43)	36 (26-53)
FEV_1 (L)	1.41 (0.06)	1.53 (0.07)

Values are presented as mean values (standard error of the mean), PD_{20} and IgE as geometric mean (range).

24-hour urinary cortisol metabolite excretion

After randomisation, urinary cortisol metabolite excretion was significantly lower during treatment with FP 1000 and 500 μg/day when compared to FP 200 μg/day (Figure 2B). No further significant differences were found between the groups.

Standing height

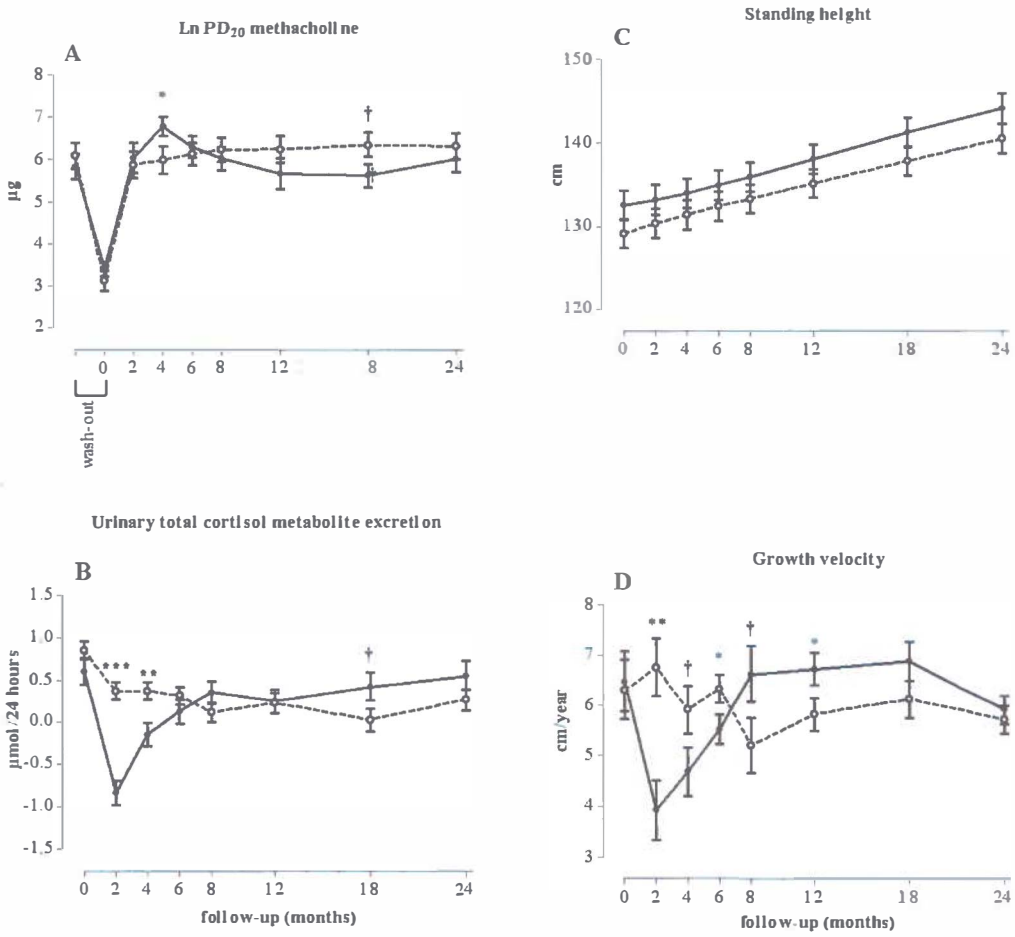
Children in the stepdown group were slightly taller (mean 3.3 cm) than those in the constant dose group at the start of the study (Figure 2C).

Standing height increased comparably in children treated according to the stepdown group and the constant dose group during the two year study. Changes in bone age were similar in the two groups as well.

Growth velocity

Growth velocity was similar in the two groups at the start of the study (Figure 2D). A significant dose-dependent effect on growth velocity appeared after 2 months treatment (FP 1000 μg/day versus FP 200 μg/day). After tapering off FP 1000 μg/day,

Figure 2: A) airways hyperresponsiveness to methacholine (PD_{20}) in asthmatic children during and after two year treatment with different dosing schedules of inhaled FP, B) urinary total cortisol metabolite excretion (μmol/24 hour, corrected for creatinine excretion and body surface area during the two year study period), C) standing height, and D) growth velocity. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; † $p < 0.1$. Stepdown versus constant dose group. Values are presented as mean values (standard error of the mean).



growth velocity increased when compared to the constant dose group, which was significant at 6 months (200 μg and 100 μg FP versus 200 μg) and borderline significant at 4 and 8 months treatment (FP 500 and 100 μg/day versus FP 200 μg/day, respectively).

After 1 year treatment growth velocity was significantly higher in the stepdown group than in the constant dose group (FP 100 μg/day versus FP 200 μg/day), but this difference had disappeared at the end of the 2-year treatment period.

Bone markers

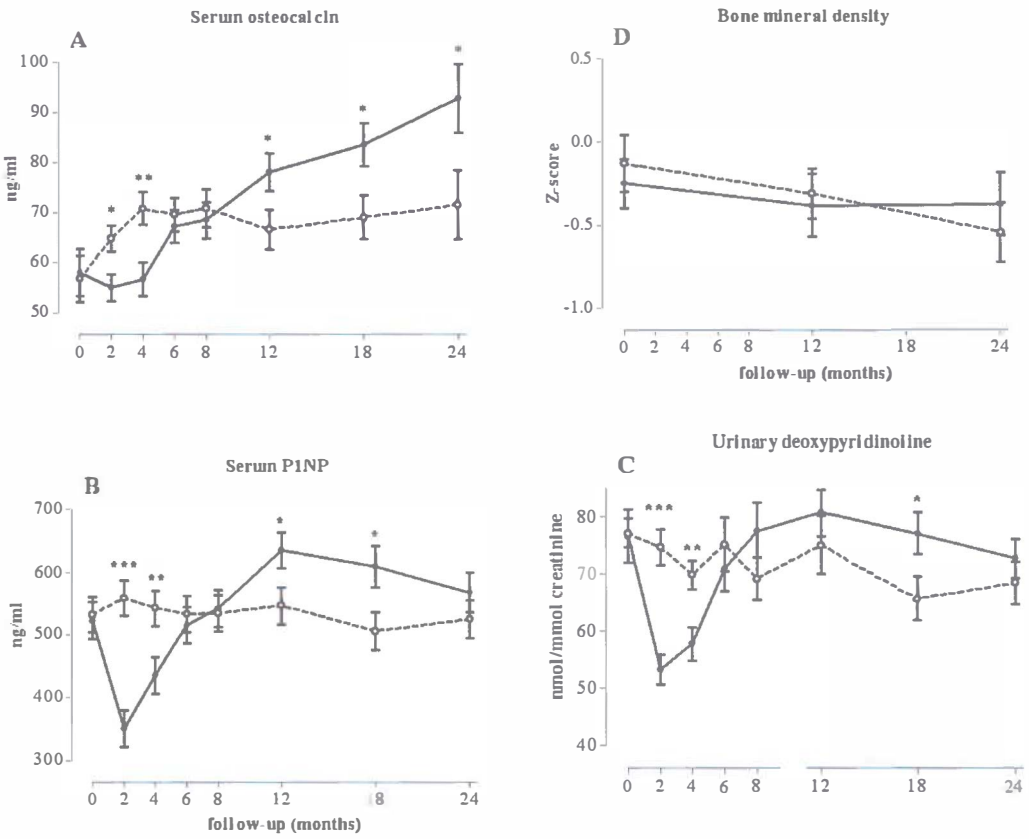
Serum osteocalcin levels did not significantly differ between groups at randomisation (Figures 3A, B and C). Children treated with FP 1000 and 500 μg/day had significantly lower serum osteocalcin levels than children treated with FP 200 μg/day (Figure 3A). When both groups used FP 200 μg/day (6 months visit) or 200 μg and 100 μg FP/day (8 month visit), serum osteocalcin levels were similar. Thereafter osteocalcin levels increased during 100 μg/day treatment, whereas they remained stable in the group using FP 200 μg/day constantly, which resulted in significantly higher osteocalcin levels in the stepdown group (FP 100 μg/day). Both serum P1NP and the urinary Dpyr levels decreased significantly during treatment with FP 1000 and 500 μg/day compared to FP 200 μg/day (Figure 3B and C). After 12 and 18 months treatment a significantly higher P1NP level was present in the stepdown group (FP 100 μg/day) compared to the constant dose group (FP 200 μg/day, Figure 3B), which became similar at 24 months. For urinary Dpyr levels in the stepdown group (FP 100 μg/day) were only significantly higher after 18 months treatment when compared to the constant dose group (FP 200 μg/day, Figure 3B and C).

The results of the urinary Pyr levels were similar to the Dpyr levels (data not shown), and the ratio of urinary Pyr/Dpyr was similar between the two groups at all time points of the study.

BMD

BMD was similar at baseline, 1 year and 2 year treatment in both groups, and changes between visits and between groups were not significant (Figure 3D).

Figure 3: Bone turnover markers of inhaled FP in asthmatic children during and after treatment with different dosing schedules. A) serum osteocalcin levels (ng/ml); B) serum P1NP levels (ng/ml); C) urinary deoxypyridinoline levels (nmol/mmol creatinine); D) bone mineral density (Z-score) *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; † $p < 0.1$. Stepdown versus constant dose group. Values are presented as mean values (standard error of the mean).



Discussion

This study investigated long-term side-effects of ICS in childhood asthma in a prospective way. Children were either treated with a constant dose of FP (200 μg/day) or according to a stepdown strategy, with a very high starting dose of FP (1000 μg/day), tapering down over a period of 6 months to a low maintenance dose (100 μg/day). We previously demonstrated that the stepdown strategy was not superior to the constant

dose strategy over a 12 month period, with respect to symptoms, exacerbation rates, lung function, and airways hyperresponsiveness.[7] The present follow-up shows that the second year showed also no differences in asthma control (FP 100 μg/day versus 200 μg/day). This study strongly suggests, therefore, that a stepdown strategy is not superior to a constant dose strategy in the long-term management of childhood asthma. It should be noted, however, that the children in this study had all been using inhaled corticosteroids prior to entering the study, being school-aged children with mild to moderate persistent asthma. Further studies are needed to assess whether a stepdown strategy is superior to a constant dose treatment schedule in steroid-naive children with newly diagnosed asthma, or in children with mild intermittent or severe persistent asthma.

During the later stages of the study, when the dose of FP was ≤ 200 μg in both treatment groups, there were no between group differences in terms of safety parameters. Standing height, growth velocity, serum P1NP, urinary Dpyr excretion, BMD and adrenal function as assessed by cortisol metabolite excretion were all comparable between groups at the end of the study (Figures 2 and 3). Moreover, most parameters did not change from baseline, when no ICS were used for a maximum of 8 weeks, to the end of the study in the group using a constant dose of FP 200 μg/day (Figures 2 and 3). These observations are in accordance with earlier work showing that maintenance treatment with a regular and constant dose of inhaled corticosteroids (in this study, FP 200 μg/day) has no systemic side effects on bone turnover[25,26], height[27,28], and adrenal cortical function.[29,30]

Two parameters of bone metabolism did not follow this pattern. Serum levels of osteocalcine, a parameter of bone formation, were at first lower in the stepdown group when doses of 1000 μg and 500 μg/day FP were used, but increased when the FP dose was tapered off to 100 μg/day to significantly higher levels than those in the constant dose group (Figure 3A). Until now, no studies have examined effects of different dosages of inhaled corticosteroids on osteocalcin levels in children. Although one retrospective study[31] and one non-randomised open study[32] showed reduced osteocalcin levels during ICS therapy, two randomised clinical trials showed no effect of beclomethasone in doses up to 400 μg/day or FP 200 μg/day on serum osteocalcin.[33,34] The relevance of the dose-dependent effects of inhaled FP on serum osteocalcin levels observed in this study is unclear (Figure 3A). Anyhow, it is not reflected in effects on BMD, which remained stable throughout the study (Figure 3D). Our results, like others[34], suggest that serum osteocalcin and BMD represent different phases of bone metabolism, with biochemical bone markers reflecting continuous remodelling and BMD reflecting long-term structural changes.

Our study clearly shows a negative direct effect of high dose FP on biochemical bone turnover parameters, which abate after treatment with lower doses for up to 2 years. Throughout the study, BMD did not differ significantly between the two study arms, suggesting no detrimental effect of overall long-term use of FP on BMD in asthmatic children. *Within* the group treated with a constant dose of FP 200 μg/day, a trend towards a decrease in BMD in the first year was observed, which became statistically significant in the second year of treatment. This result should be interpreted with caution. It contrasts with the CAMP study where 200 μg of budesonide/day given during 4-6 years did not affect BMD.[8] Moreover, our study was not double blinded with placebo and other long-term studies with FP in children are lacking. Finally, the stepdown group used a higher cumulative dose of FP during the first year (i.e. 120,000 μg) than the constant dose group (72,000 μg), nevertheless changes in BMD in the stepdown group were not significant. Thus, there were no significant differences in BMD changes over time between the two study groups, and, if anything, a worsening of BMD only in the group treated with the overall lowest dose. Clearly further long-term studies are needed to evaluate effects of low dose ICS treatment on BMD in children.

The most prominent finding of our study is the marked negative effects of high-dose FP (1000 μg and 500 μg/day) on growth velocity and adrenal cortical function (Figure 2), and on biochemical markers of bone turnover and BMD (Figure 3). A reassuring finding is that these effects are short-lived and fully reversible when the dose of FP is tapered down. Furthermore, prolonged use of inhaled corticosteroids might provide less negative effects than short-term therapy.[35] Nevertheless, the magnitude of the observed findings warrants a note of caution, because it suggests a clear potential for clinically relevant side-effects if *high* dosages of FP are continued for prolonged periods of time. Indeed, clinically relevant growth suppression and adrenal failure have been described anecdotally in children on high-dose FP therapy.[16,36]

In conclusion, this study shows that maintenance therapy with inhaled FP in dosages ≤ 200 μg/day appear to be safe in the long-term management of childhood asthma. Nevertheless, marked reduction of growth velocity, biochemical bone turnover, and adrenal cortical function occurs during treatment with high dosages of inhaled FP. Given our findings that high-dose FP therapy is no more effective than a "regular dose" and that it may cause clinically significant side-effects, we propose that high dosages of inhaled FP should be avoided whenever possible. These results provide a guideline to use the lowest effective dose of ICS in order to prevent systemic side-effects.

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Chapter 8

Summary, conclusions and general discussion

Asthma is a chronic inflammatory disease of the airways, characterised by an influx of eosinophils and lymphocytes into the airway wall. The cytokine profile in asthma is compatible with a Th2 skewing of the immune response.

Current guidelines for the management of asthma emphasise anti-inflammatory therapy with inhaled corticosteroids as first-line treatment. These guidelines advise to increase the dose of inhaled corticosteroids when asthma symptoms deteriorate. The strategy is based on the dose-dependent effects of inhaled corticosteroids on both airways inflammation and clinical parameters, such as level of lung function and degree of airways hyperresponsiveness. As a result, it is now common practice in childhood asthma to start inhaled corticosteroid therapy with a high dose in order to reduce inflammation powerfully and effectively. Subsequently, this high dose is tapered off to the lowest effective dose ("stepdown-approach"). Another possible strategy is to give a fixed dose of inhaled corticosteroids for a prolonged period of time ("constant dose approach"). No randomised clinical trials, however, have been performed to establish which of these two strategies is the most effective one.

The main goal of this thesis was to compare a stepdown approach of inhaled FP (starting with 1000 μg/day with two monthly reductions to 500, 200, and 100 μg/day during the rest of the 2-year study) with a constant dose approach (200 μg/day during the whole study). Primary efficacy variables were airways hyperresponsiveness, lung function, symptoms scores and exacerbation rates. In addition, we wished to assess anti-inflammatory effects of different doses of inhaled corticosteroids. Therefore we determined peripheral blood eosinophil counts, cytokine production by peripheral mononuclear cells (PBMCs), and the soluble cellular adhesion molecule ICAM-1.

Elevated levels of serum ICAM-1 (sICAM-1) have been found in asthmatic children compared to healthy controls, reflecting increased inflammation.

Before the randomisation visit, children entered a baseline period. This period consisted of a run-in period, during which all children used FP 100 μg *b.i.d.*, followed by a wash-out period of a maximum of 8 weeks, during which children only used short acting bronchodilator (salbutamol) on demand for relief of symptoms.

The possible systemic side-effects of inhaled corticosteroids on height growth, adrenal cortical function and bone metabolism in asthmatic children are still of concern to doctors, treating children with asthma for long periods of time. It has been demonstrated that inhaled fluticasone propionate (FP) has a lower bioavailability than other inhaled corticosteroids. The risk of systemic side-effects, therefore, should be lower. Limited data are present on long-term efficacy and systemic side effects of inhaled FP in asthmatic children. In our study, primary safety variables were height growth, adrenal cortical function and bone metabolism.

Airways hyperresponsiveness is a cardinal feature of childhood asthma and it is frequently used as inclusion criterion in clinical trials. Airways hyperresponsiveness shows a marked improvement during therapy with inhaled corticosteroids, which continues during at least 12 months. In contrast, the improvement of lung function levels off quickly after introduction of inhaled corticosteroids (after several weeks). Lung function, therefore, is an insensitive parameter to monitor long-term changes in response to different doses of ICS within an individual patient or between patients.

During the baseline period of our study, we were surprised by the finding that approximately half of the recruited asthmatic children did not develop airways hyperresponsiveness after withdrawal of inhaled corticosteroids. **Chapter 2** describes the results of the baseline phase (run-in and wash-out period) of our study. Ninety-five asthmatic school children, who had all been using inhaled corticosteroids for several years, completed the baseline phase of the study. The majority of the children (51%) did not develop airways hyperresponsiveness to methacholine ($PD_{20} \geq 80$ μg) after withdrawal of inhaled FP for a maximum of 8 weeks. Many of these children remained free from asthma symptoms as well, both during the wash-out period (44%) and after 1-year follow-up (39%). When we compared these children with the hyperresponsive children ($PD_{20} < 80$ μg), no differences in duration of asthma, duration of treatment, or peak flow variation were found. The hyperresponsive children, however, had higher levels of total and specific IgE, and lower levels of lung function (FEV_1, MEF_{50}) than the non-hyperresponsive children. Results did not change when the inclusion criterion of airways hyperresponsiveness was increased to $PD_{20} < 150$ μg.

Fifty-six percent of the children without airways hyperresponsiveness (PD_{20} > 80 μg) developed asthmatic symptoms (cough, wheeze) during the wash-out period. Compared to the asymptomatic children within this group, the symptomatic children had more severe airways hyperresponsiveness and larger peak flow variation.

Although there was a seasonal variation in the degree of airways hyperresponsiveness (lower PD_{20} during the spring, higher during the winter, summer, and autumn), this phenomenon could not explain the lack of airways hyperresponsiveness found in our study.

We conclude that levels of airways hyperresponsiveness may deteriorate in asthmatic school children when long-term treatment with inhaled corticosteroids is withdrawn. Many asthmatic children, however, do not develop airways hyperresponsiveness after withdrawal of inhaled corticosteroids and more likely so when they are non-allergic and have normal levels of lung function. As a result, recruitment of asthmatic children for clinical trials may be difficult when airways hyperresponsiveness is used as the sole inclusion criterion.

Chapter 3 describes a subgroup of the asthmatic children presented in chapter 2. Forty-one children (53%) with airways hyperresponsiveness (PD_{20} < 80 μg) and 37 non-hyperresponsive children (47%, PD_{20} ≥ 80 μg) to methacholine after withdrawal of inhaled corticosteroids were examined for differences in demographic, clinical, and immunological features. Hyperresponsive children had significantly more atopic features (higher IgE levels, higher prevalence of a positive RAST test (≥ 1 RAST, class 2 or higher), and eczema) than non-hyperresponsive children. In addition, lower levels of FEV_1, MEF_{50}, and peak expiratory flow, and higher levels of exhaled nitric oxide were found in children with airways hyperresponsiveness.

Apart from a borderline significantly higher IL-4 in supernatants from Con A stimulated PBMCs and a significantly lower serum ICAM-1 (sICAM-1) concentration in the hyperresponsive children, other immunologic parameters were comparable between hyperresponsive and non-hyperresponsive children.

Multivariate logistic regression analysis demonstrated that presence of atopic features, high sICAM-1 levels, low levels of lung function, presence of furry pets and high levels of IL-4 (PBMCs) were independently associated with the presence of airways hyperresponsiveness after withdrawal of inhaled corticosteroids.

Epidemiologic studies have demonstrated that children with persistent wheeze (allergic asthma) had more atopic features and lower levels of lung function when compared to transient wheezers ("toddler's asthma"). In our study, many children did not

develop airways hyperresponsiveness after withdrawal of inhaled corticosteroids. It appears that the hyperresponsive children share features with persistent wheezers and that the non-hyperresponsive children share features with transient wheezers found in previous studies.

In **chapter 4** we describe a study in asthmatic children to investigate systemic side-effects of FP 200 μg/day via dry powder inhaler by means of knemometry, an instrument designed to measure and monitor lower leg growth velocity, and considered to be a sensitive tool to detect systemic effects of topical steroids. Twenty-one prepubertal asthmatic children, aged 6 to 10 years, were included in a non-randomised open crossover trial. They were treated with FP 200 μg/day for 6 weeks, followed by a 2-week (wash-out) period during which only β_2-agonists on demand were allowed. Lower leg measurements were performed every 2 weeks. No differences in baseline characteristics were present and all children remained clinically stable throughout the 8-week study period. We demonstrated that 6 weeks treatment with FP 200 μg/day did not result in a decreased lower leg growth velocity when compared with the 2 weeks treatment with β_2-agonist alone.

We conclude that no significant suppression of lower leg growth velocity was found in asthmatic children, aged 6 to 10 years, treated with FP 200 μg/day via dry powder inhaler for 6 weeks.

In **chapter 5**, the results of a one-year study of two different treatment schedules of inhaled fluticasone propionate in childhood asthma are described. Although current guidelines advise to increase the dose of inhaled corticosteroids during periods with increased symptoms, no evidence from clinical trials is available to support this advice. Several dosage schedules to initiate new treatment in childhood asthma with inhaled corticosteroids may be used. One approach is to start inhaled corticosteroid therapy with a high dose in order to reduce inflammation effectively and powerfully. This high dose is subsequently tapered off to a low maintenance dose to control airways inflammation and symptoms. Another strategy is to give a fixed dose for a prolonged period of time. The aim of our double-blind study was to compare a high starting dose of FP (stepdown approach, 1000 μg/day tapering off to 100 μg/day over a 6-month period) with a constant dose approach (FP 200 μg/day) for 1 year in prepubertal children with chronic persistent asthma. Primary efficacy variables were airways hyperresponsiveness to methacholine (PD_{20}), lung function, symptoms scores, and exacerbation frequency. Safety of therapy was assessed by height measurements.

Fifty-five asthmatic children, aged 6 to 10 years, were randomised after a wash-out period (during which no inhaled corticosteroids were used) to one of the two treat-

ment approaches. PD_{20} improved in both approaches during treatment with FP. At initial high doses a significantly higher PD_{20} was found in the stepdown group when compared to the constant dose group, but this only reached statistical significance after 4 months of therapy. No significant differences between the two approaches were found in lung function, symptom scores or exacerbation frequency over the full 1-year study period. Changes in standing height were similar in both treatment groups.

In conclusion, our results do not show a superior clinical effect of a stepdown approach compared to a constant dose approach of inhaled FP for 1 year in asthmatic children. Both treatment schedules were equally safe with respect to height growth. These results do not support the use of a stepdown strategy with inhaled corticosteroids in children with moderate to severe asthma.

It is now well established that asthma is an inflammatory disease, characterised by eosinophilic influx in the airways wall. In addition, studies in asthmatic children have demonstrated that the cytokine profile in asthma is compatible with a predominance of Th2 lymphocytes (IL-4, IL-5, and IL-10) over Th1 lymphocytes (INF-γ). Because it has been demonstrated that corticosteroids reduce airways inflammation, we hypothesised that inhaled FP is able to influence cytokine levels in asthmatic children. In **chapter 6** we describe effects of different doses of inhaled FP on eosinophil counts, sICAM-1 and cytokine levels in peripheral blood of asthmatic children. We hypothesised that these parameters could be useful markers in monitoring therapy with inhaled corticosteroids.

Eosinophil counts decreased in both treatment groups during reinstitution of inhaled FP after the wash-out period during which no inhaled corticosteroids were used. A significant dose-dependent effect of inhaled FP on eosinophil counts was only apparent when FP 1000 μg/day was compared with FP 200 μg/day. A trend towards a dose-dependent effect of inhaled FP was found on eosinophil counts during and after 1-year treatment, but this was not statistically significant. No clear dose-dependent effect on serum ECP levels was found. Although serum ECP levels increased in the stepdown group when the high dose was tapered down, no statistically significant differences were found between the two groups at any time point during the study.

The majority of serum IL-4 and many serum IL-5 levels were undetectable during the treatment phase of the study. All children had detectable levels of IL-4, IL-5, IL-10 and INF-γ in supernatants of Con A stimulated PBMCs. No significant dose-dependent effect of inhaled FP was found on the release of these cytokines. sICAM-1 levels decreased after reintroduction of inhaled FP, and again no dose dependent effect was apparent.

We conclude that eosinophil counts as well as serum ECP, cytokine production by PBMCs and sICAM-1 levels are insensitive markers in assessing anti-inflammatory efficacy with inhaled corticosteroids over a wide dose range in children with moderate to severe asthma.

In **chapter 7** we describe the results of different dosage schedules of inhaled FP on systemic side-effects in asthmatic children.

We found a clear, consistent, and highly significant effect of high doses of inhaled FP (1000 and 500 μg/day) on height velocity, adrenal cortical function, and bone turnover. These side-effects, however, disappeared readily when the dose was reduced $<$ 500 μg/day in 2 monthly intervals. No difference in standing height was found between the two approaches during and after two-year treatment with FP. No significant differences in bone mineral density were found between the two treatment groups at any moment during the 2-year study.

We concluded that there are dose-dependent reductions in height velocity, adrenal cortical function, and bone turnover during treatment with high dosages of FP. These systemic side-effects, however, can be rapidly undone when the high dose of FP is tapered off to maintenance doses of $<$ 500 μg/day.

Because high doses of FP were found to be equally effective as lower doses in the maintenance therapy of asthma on efficacy markers, we conclude that high doses of FP in asthmatic children should be avoided.

Conclusions

The main conclusions derived from the studies presented in this thesis are:

- In a subset of asthmatic children, aged 6 to 10 years, airways responsiveness can be reduced to levels usually encountered in non-asthmatics after withdrawal of long-term treatment with inhaled corticosteroids. The absence of airways hyperresponsiveness may reflect a long-lasting remission of childhood asthma caused by treatment with inhaled corticosteroids at an early age and continued for a prolonged period of time. Alternatively findings can be explained by assuming that a subgroup of children were treated for wheeze but turned out to be transient wheezers in the longrun. Thus even though not apparent at that time, they did not need inhaled corticosteroids to treat their wheeze.
- A considerable proportion of children diagnosed with and treated for asthma from a young age onwards did not show airways hyperresponsiveness after withdrawal of inhaled corticosteroids. This emphasises the need to stop or at least reduce inhaled corticosteroids in young children to the lowest effective dose, and stop this therapy when possible.
- Children who developed airways hyperresponsiveness after withdrawal of inhaled corticosteroids had more atopic features and lower levels of lung function compared to children who did not develop airways hyperresponsiveness after withdrawal of inhaled corticosteroids. Previous studies have demonstrated that children with persistent wheeze (allergic asthma) had lower levels of lung function and more atopic features compared children with transient wheeze ("toddler's" asthma). Therefore, the hyperresponsive children may represent persistent wheezers and the non-hyperresponsive children may represent transient wheezers found in earlier epidemiologic studies.
- We did not find a superior clinical effect of FP given in a stepdown approach after one year when compared with a constant dose strategy in children with asthma.
- A dose-dependent effect of high doses inhaled FP (1000 and 500 μg/day versus a "lower" dose of 200 μg/day) was found on airways hyperresponsiveness when assessed at 2 months intervals.
- Measurements of eosinophil counts, serum ECP, sICAM-1 and cytokine serum levels as well as cytokine production from stimulated PBMCs are not useful to monitor therapy with inhaled corticosteroids in asthmatic children.
- Maintenance therapy with inhaled FP in doses of 200 μg/day is effective and safe in asthmatic children.

- Inhaled FP at doses of 1000 and 500 μg/day have marked systemic side-effects on growth velocity, biochemical bone turnover and adrenal cortical function. These side-effects, however, are relatively short-lived after tapering off this high dose to a low maintenance dose of FP (≤ 200 μg/day).
- Inhaled FP given in either a stepdown strategy or a constant dose strategy for 2 years in asthmatic children (cumulative dose of approximately 146 mg) did not negatively affect standing height, bone mineral density, or adrenal cortical function.

General discussion

Anti-inflammatory therapy with inhaled corticosteroids are the cornerstone for the management of childhood asthma.[1-4] Although guidelines suggest to start inhaled corticosteroid therapy with a high dose, there is limited evidence to support this strategy. The hypothesis of starting with a high dose of inhaled corticosteroids (in order to reduce airways inflammation powerfully) is based on a dose-dependent relationship of inhaled corticosteroids on markers of effectiveness in childhood asthma.

Despite there excellent clinical effects, systemic side-effects (reduction of growth rate, decreased adrenal cortical function, reduced mineral bone density) are a matter of concern in asthmatic children treated with inhaled corticosteroids.

We conducted a double blind prospective study to compare a stepdown approach with inhaled fluticasone propionate (FP, 1000 μg/day tapering off to 100 μg/day) with a constant dose approach (200 μg/day) in school children with asthma. During the 2-year study period, efficacy and safety variables were examined.

Approximately half of the asthmatic children did not develop airways hyperresponsiveness after withdrawal of inhaled corticosteroids during the baseline phase of the study. The most important factors associated with the presence of airways hyperresponsiveness after withdrawal of inhaled corticosteroids were high serum IgE level, a history of eczema, and low levels of FEV_1 and serum sICAM-1.

A dose-dependent effect of FP on airways hyperresponsiveness and eosinophil counts was found during the first months of the study, without an effect of different doses of FP on cytokine levels. Short-lived dose-dependent systemic side-effects of FP on growth velocity, adrenal cortical function, and biochemical bone turnover were found. No significant differences on efficacy parameters (airways hyperresponsiveness, lung function, symptoms, or exacerbation rate), systemic side-effects (growth velocity, adrenal cortical function, and biochemical bone turnover, bone mineral density), or inflammatory markers (eosinophil counts, serum eosinophilic cationic protein, cytokine levels, and sICAM-1 levels) were found between the two treatment schedules after one and two years.

Childhood asthma (transient wheezers versus persistent wheezers)

We were surprised by the finding that half of the recruited children did not develop airways hyperresponsiveness after withdrawal of inhaled corticosteroids during the

baseline phase of the study. When comparing the hyperresponsive children with the non-hyperresponsive children, we found that the hyperresponsive children had more atopic features (positive RAST, high IgE, eczema), lower levels of lung function, and lower sICAM-1 concentrations. This is the group that resembles in an epidemiologic setting children with persistent wheeze up to the age of 11 years.[5,6] In contrast, the non-hyperresponsive children shared features with transient wheezers found in epidemiologic studies.[5,6]

A longitudinal study showed that the presence of atopy at the age of 8 to 12 years was a significant risk factor for the onset of wheeze at adolescence.[7] Moreover, that same study demonstrated that the presence of airways hyperresponsiveness at age 8 to 10 years was a risk factors for the persistence of wheeze at adolescence, suggesting an important contribution of airways hyperresponsiveness on the development of asthma in adolescence.[7] This suggests that the hyperresponsive children in our study are prone to continue to wheeze at adolescence.

We reinvestigated the non-hyperresponsive children after a median follow-up of 13 months; 39% of these children did not develop respiratory symptoms and the remainder had mild asthmatic symptoms (cough, dyspnea on exertion, wheeze). The asymptomatic children had still a better lung function and a higher level of sICAM-1 when compared with the (symptomatic) hyperresponsive children, despite the fact that they withdrew their inhaled corticosteroids. In addition, IgE levels were lower in the asymptomatic children, although this difference was not statistically significant, most likely due to the small number of individuals in the asymptomatic group. Thus the finding of more atopic features and lower levels of lung function in the hyperresponsive children after withdrawal of inhaled corticosteroids are corroborated by the follow-up study.

It has recently been demonstrated that atopic children, with a median age of 7.3 years, who had apparently outgrown asthma, had elevated eosinophils in BAL fluid when compared to non-atopic normal controls, suggesting persistent airways inflammation.[8] The finding of elevated concentrations of exhaled nitric oxide (eNO) in the hyperresponsive children compared to non-hyperresponsive children in our study supports the finding of ongoing airways inflammation. We did not measure, however, eosinophils in BAL fluid. Eosinophil counts in peripheral blood were similar between the hyperresponsive and non-hyperresponsive children. This suggests that inflammatory markers in peripheral blood are a poor reflection of (local) airways inflammation.

Elevated concentrations of IL-4 and IL-5, and reduced concentrations of INF-γ have been found in children with asthma and eczema, compatible with a Th2 skewing of

the immune response.[9,10] The finding of an Th2/Th1 imbalance has recently be confirmed by a study in children (mean age 10.8 years) using exhaled breath condensate, showing increased levels of IL-4 and decreased levels of INF-γ in asthmatic children compared to healthy controls.[11] We demonstrated that the Th2 skewing of the immune response, as represented by levels of INF-γ and IL-4, reflected the presence of atopy rather than that of airways hyperresponsiveness. Our findings are supported by previous studies, showing that this Th2 skewing is associated with atopy and not with airways hyperresponsiveness.[9,12]

sICAM-1 is an adhesion molecule, necessary for cell-cell interaction at inflammatory sites. Although some studies showed elevated levels of sICAM-1 in asthmatic children when compared to healthy controls[13,14], others failed to do so.[15,16] We demonstrated that low sICAM-1 levels in serum were significantly associated with airways hyperresponsiveness, independent of the presence of atopy. It has been suggested that sICAM-1 may block cell-cell interactions, thus hampering inflammatory response.[17] We hypothesised that low levels of sICAM-1 are responsible for an increase in the inflammatory response in the airways, resulting in an increase in airways hyperresponsiveness.

We described the results of a cross-sectional study. Prospective studies have to be performed to determine if blood inflammatory markers are useful to discriminate at an early age between children who are prone to continue to wheeze (persistent wheezers, allergic asthma) and to those who will stop wheezing (transient wheezers, "toddler's" asthma) at school-age. In addition, the relationship of sICAM-1 levels and airways hyperresponsiveness has to be elucidated.

Because objective measurements, like lung function and airways hyperresponsiveness, are not feasible in children below 6 years of age, blood inflammatory markers may predict which child will continue to wheeze and which child will cease to wheeze. Further research is needed to investigate the value of exhaled nitric oxide in separating "persistent wheezers" and "transient wheezers". Recently, it has been demonstrated that IL-4 and INF-γ levels in exhaled breath condensate differ between asthmatic children and healthy controls.[11] This may also be a valuable tool in distinguishing different types of childhood wheezing.

Efficacy

We found a dose-dependent effect of inhaled FP on airways hyperresponsiveness. In the first 4 months, PD_{20} was significantly more reduced after 2 months treatment

with FP 1000 μg/day followed by 2 months treatment with 500 μg/day compared to FP 200 μg/day. In contrast, no significant differences on other efficacy parameters (e.g. lung function and symptoms) were found. This decrease in airways hyperresponsiveness during treatment with high doses of FP to a maintenance dose of FP 100 μg/day, however, was temporary and did not sustain during tapering off this high dose of inhaled FP when compared to a constant dose of FP 200 μg/day. In addition, a higher 1-year cumulative dose of FP in the stepdown approach (120 mg) did not result in an improved level of airways hyperresponsiveness compared to the constant dose approach after 1 year (73 mg).

No difference in level of airways hyperresponsiveness was found between 18 months treatment with FP 100 μg/day and 200 μg/day. After 2 years of treatment no significant difference in airways hyperresponsiveness was found between the two treatment approaches. We were surprised by this finding because both groups received the same cumulative dose of FP, i.e. approximately 146 mg. These results suggest that the effect of inhaled corticosteroids on airways hyperresponsiveness is rather dependent on the dose presently used. However, the dose-dependent effects on airways hyperresponsiveness may have been more pronounced when the high doses of inhaled FP were continued for a prolonged period of time (more than 2 months). This is likely to occur given the observation that previous studies have shown a slow effect of inhaled corticosteroids on airways hyperresponsiveness which continues during throughout the first 2 years of therapy.[18-21]

Our results are in contrast with a study in asthmatic children, aged 1 to 3 years, comparing FP 100 and 200 μg/day with placebo given *via* MDI with spacer after a run-in period of 4-weeks during which no inhaled corticosteroids were allowed.[22] Although a dose-dependent effect on symptom-scores was found, no objective assessments were carried out, thus results are not easy to compare given the possibility of a different nature of the symptoms. Hofstra *et al* found a dose-dependent effect of FP on the PD_{20} methacholine after 6 months treatment, after a run-in period of 2-weeks during which no inhaled corticosteroids were used.[18] To our knowledge, no studies are available on dose-dependent effects of inhaled corticosteroids in steroid-naive asthmatic children. Therefore, the generalisability of our results to inhaled corticosteroid-naive ("newly detected") asthmatic children is limited, because all children had used inhaled corticosteroids for several years before entering the study.

We have included children with moderate severe airways hyperresponsiveness in the study, and they had chronic persistent asthma. Further studies have to demonstrate if our results can be extrapolated to both mild intermittent and severe persistent asthmatic children.

Systemic side-effects

We have shown that doses of inhaled FP of 1000 and 500 μg/day are able to exert significant systemic side-effects over short periods of time. During 2-month treatment with FP 1000 and 500 μg/day, growth velocity decreased when compared with FP 200 μg/day. Also serum and urinary bone markers (implying a decreased level of bone metabolism), and urinary adrenal cortisol metabolite excretion were decreased with these high doses of FP. Like the effect on airways hyperresponsiveness, these systemic side-effects were relatively short-lived and were rapidly undone after tapering off a high dose of FP to a low maintenance dose (FP 200 μg/day). Short-lived suppressive side-effects of moderate doses of FP (250 to 750 μg/day) were found in a recently published paper, describing three case reports.[23]

No differences in growth velocity, standing height, biochemical bone turnover, bone mineral density or adrenal cortical function were found during 18 months maintenance therapy with either FP 100 μg/day or FP 200 μg/day. Because we did not find differences in efficacy markers between these two doses, it appears that FP 100 μg/day is equally safe and effective as FP 200 μg/day in the long-term management of childhood asthma.

The decrease on growth velocity during 2-months treatment with FP 1000 and 500 μg/day, respectively, compared to FP 200 μg/day did not result in a decreased standing height, neither after 1 year (when the cumulative dose was 1.7 times higher in the stepdown group than in the constant dose group) nor after 2 years (with the same cumulative dose in both groups). The lack of a difference in growth velocity during further treatment for 18 months with FP 100 μg/day and FP 200 μg/day is in accordance with a previous study, showing no difference in height during 1-year between treatment with either FP 100 or 200 μg/day in prepubertal (persistent) asthmatic children.[24] A meta-analysis on the effects of FP on growth, however, demonstrated that treatment with FP 200 μg/day significantly reduced growth when compared to placebo.[25] We compared treatment of different doses of FP with each other and not with placebo, hence there may be a negative effect on growing yet without a difference between 100 and 200 μg/day.

Our findings clearly demonstrate a reduction in growth velocity during 2-months treatment with either FP 1000 or 500 μg/day. Growth suppression with FP in doses up to 750 μg/day has recently been published in three case reports in asthmatic children, aged 4 to 8 years.[23] This growth suppression, like our findings, was rapidly undone after tapering off the high dose FP to a low maintenance dose. Although it is unclear if high doses of FP, given for a prolonged period of time (e.g. years), lead to a reduction in standing height, reassuring results of long-term treatment with

BUD (mean dose 412 μg/day for a mean duration of 9.2 years) on height have been published.[26]

We demonstrated that high doses of FP (1000 and 500 μg/day) decreased both biochemical markers of bone formation and bone degradation when compared to FP 200 μg/day. No significant differences were found during 18 months treatment between FP 100 and 200 μg/day. This latter result is in accordance with previous work, showing no effect of FP 200 μg/day on serum osteocalcin levels in asthmatic children, aged 5 to 10 years.[27] The relevance of the dose-dependent reduction of FP on biochemical bone turnover is unknown, since no significant differences in bone mineral density between the two approaches at any moment during the 2-year study were found.

A trend towards a decrease in bone mineral density in the group of children treated with FP 200 μg/day during the first year was observed. This decrease became statistically significant in the second year of treatment with FP 200 μg/day. The CAMP study, however, did not demonstrate a decreases in bone mineral density during treatment with BUD 200 μg/day for 4-6 years.[28] Rao *et al* also could not demonstrate a difference in bone mineral density in asthmatic children, mean age 6.8 years, treated with FP 200 μg/day or BDP 400 μg/day for 20 months.[27] Like our study, this latter study did not compare treatment with placebo. Our results, therefore, should be interpreted with caution. Further placebo controlled long-term studies have to establish if treatment with (different doses of) FP reduces bone mineral density. In addition, it is unclear if biochemical bone turnover is a useful markers in predicting (long-term) bone mineral density.

Long-term use of oral corticosteroids is associated with suppression of the adrenal cortical function. In addition, inhaled steroids may suppress adrenal function in asthmatic children. It has been demonstrated that urinary cortisol metabolite excretion is a very sensitive method to detect dose-dependent effects of inhaled corticosteroids on the hypothalamic-pituitary-adrenal-(HPA) axis.[29–31] We found a dose-dependent suppression of FP 1000 and 500 μg/day when compared to FP 200 μg/day on urinary cortisol metabolite excretion. This decrease in urinary cortical metabolites was short-lived after tapering off the high doses of FP to a low maintenance dose (FP 200 μg/day). During further treatment with either FP 100 or 200 μg/day for 18 months, no significant differences in urinary metabolite excretion were found between the two treatment groups.

Kannisto *et al* have shown that asthmatic children (mean age 9.5 years) treated with FP 500 μg/day for two months developed an insufficient response after a low dose ACTH test.[32] That study also demonstrated that a subsequent decrease in the dose of FP to 200 μg/day resulted in a normal stimulation test. We did not perform dy-

namic stimulation tests in our study to assess adrenal cortical reserve. The clinical relevance of our study, therefore, remains questionable. Although adrenal failure has been described anecdotally in children on high-dose FP up to 750 μg/day[23,33], it is unknown if long-term treatment with inhaled corticosteroids induces adrenal cortex atrophy.

Inflammation

Asthma is characterised by a chronic inflammatory reaction in the airways. The vast majority of studies on the inflammatory nature of asthma are inferred from studies in adults, using invasive techniques such as bronchial biopsy and bronchoalveolar lavage. These methods are not feasible in children. The inflammatory cells in the airways are activated to produce a myriad of cytokines. It has been demonstrated that inhaled corticosteroid therapy reduces cytokine production from inflammatory cells.[34-36] Generally inflammatory markers are higher expressed in asthmatic children than in healthy children.[37] Therefore, it has been suggested that these inflammatory markers may be useful in guiding the dose of inhaled corticosteroids in asthmatic children.

Apart from a dose-dependent effect of FP on serum eosinophil counts and airways hyperresponsiveness, no dose differences were found on cytokine levels, neither in peripheral blood nor in Concanavalin A stimulated PBMCs, even when differences in FP doses were highest (FP 1000 and 500 μg/day compared with FP 200 μg/day). A trend towards a dose-dependent effect of FP was found on serum eosinophilic cationic protein (ECP) and sICAM-1 levels.

We only found a dose-dependent decrease in eosinophil counts between treatment with FP 1000 and FP 200 μg/day. Our finding of a dose-dependent effect of FP on eosinophil counts without an effect on serum ECP are confirmed by pervious studies, showing an effect of different doses of FP on eosinophil counts without an effect on serum ECP.[18,38] The limited role of serum ECP has also been demonstrated in another study, which did not found an association between serum ECP and asthma severity or inflammatory markers.[39]

The lack of a significant dose-dependent effect with the other doses of FP may be due to an increased systemic bioavailability, resulting in an suppression of blood eosinophil progenitors and colony forming unit production in bone marrow. In addition, this amount of "systemic" FP may be too small to alter resting T-cells in the circulating blood.

A previous study in adult asthmatics demonstrated a decrease in eosinophil counts after treatment with FP 2000 μg/day or oral prednisolone, without significant changes in airways hyperresponsiveness.[40]

It has been demonstrated that therapy with inhaled corticosteroids resulted in reduced percentages of PBMCs expressing mRNA encoding IL-4 and IL-5, and an increase of INF-γ expression.[34,41] We could not find a dose-dependent effect of FP on IL-4, IL-5, IL-10 or INF-γ levels. Previous studies have demonstrated a decrease in serum IL-5 levels, but not on serum IL-4 levels, after therapy with oral corticosteroids.[42,43] This suggest that T-lymphocytes are directly influenced by oral corticosteroids due to systemic bioavailability, resulting in a decrease in cytokine production levels. We hypothesised that, although inhaled FP is able to reach the bloodstream (because systemic side-effects are present), high doses of FP (1000 and 500 μg/day) are too small to alter resting T-lymphocytes in the circulating blood.

Previous studies in adults have shown that levels of IL-5 and IL-10 in peripheral blood are related to those in bronchoalveolar fluid.[44,45] It is unknown if this is also true for children. Since cytokine concentrations in peripheral blood are lower in children when compared to adults, these differences may also be true for bronchoalveolar fluid.[46,47]

We showed an increase in airways hyperresponsiveness after reduction of the FP dose, suggesting an increase in airways inflammation. Because invasive techniques (bronchoalveolar lavage) are not feasible in children, only indirect parameters of airways inflammation (eosinophil counts and cytokines in peripheral blood, eNO) could be performed.

sICAM-1 levels decreased after reintroduction of FP. Others also found reduced sICAM-1 levels in asthmatic children aged 9.6 years after treatment with inhaled corticosteroids (BUD 400 to 800 μg/day).[48] We have demonstrated (chapter 3) that low sICAM-1 concentrations are associated with airways hyperresponsiveness. Because it has been suggested that sICAM-1 may hamper inflammatory responses by blocking cell-cell interactions[17], low sICAM-1 concentrations may facilitate airways hyperresponsiveness and airways inflammation.

In conclusion, apart from a small and temporary dose-dependent effect on airways hyperresponsiveness no superior clinical effect of FP given in a stepdown approach was found when compared to a constant dose approach in children, aged 6 to 10 years, with chronic persistent asthma treated for 2 years.

The dose-dependent effects of high doses of FP (1000 and 500 μg/day) on growth velocity, bone turnover and adrenal cortical function were relatively short-lived after tapering off the high dose of FP to a low maintenance dose. Furthermore, therapy with FP in doses of ≤ 200 μg/day for 18 months is effective and safe with respect to systemic side-effects. It appears that FP 200 μg/day is a safe and effective dose in children and increasing the dose will be accompanied with increased systemic side-effects.

Our results suggest that measurements of inflammatory markers (eosinophil counts, serum ECP, cytokine concentrations in serum and stimulated PBMCs) are not useful in monitoring or titrating therapy with inhaled corticosteroids in the dose range used in asthmatic children. We assume that this is also the case for other inhaled corticosteroids. Studies in adult asthmatics have shown that airways hyperresponsiveness and eosinophil counts in sputum are appropriate markers in guiding the dose of inhaled corticosteroids.[49,50] Further studies, investigating different doses, types and delivery devices of inhaled corticosteroids, have to demonstrate if these markers are also suitable in guiding the dose of inhaled corticosteroids in asthmatic children.

Although further studies are needed to confirm our findings, we advice to avoid therapy with high doses of inhaled corticosteroids in asthmatic children whenever possible.

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Future perspectives

We demonstrated that approximately half of children, in whom asthma was diagnosed at an early age, did not develop airways hyperresponsiveness after withdrawal of inhaled corticosteroids. The hyperresponsive children had more atopic features and lower levels of lung function compared to the non-hyperresponsive children, suggesting that the non-hyperresponsive children shared features with transient wheezers ("toddler's" asthma) found in previous epidemiologic studies. Our results suggest that many young children may be unnecessarily treated with inhaled corticosteroids. However, our study was a cross-sectional one and our results have to be confirmed by prospective studies. In this respect it is of importance to use adequate measures that are risk factors to transient or persistent wheeze. A recent study in atopic children with outgrown asthma demonstrated increased numbers of eosinophils in BAL fluid when compared to normal controls.[1] Whether increased eosinophils ore other detected markers of eosinophil activation constitute a risk factor for future asthma relapse or even persistent wheeze needs to be established by prospective studies.

Further studies are also needed to evaluate the value of inflammatory markers (such as cytokines and adhesion molecules) in predicting which child will continue to wheeze (allergic asthma) and which child will cease to wheeze ("toddler's" asthma) at later age. Moreover, the interaction between airways hyperresponsiveness and sICAM-1 concentrations found in our study needs to be confirmed by others.

We have described the effects of 2 different dose schedules of inhaled fluticasone in asthmatic children, aged 6 to 10 years. It is unknown whether our results can be extrapolated to other age-groups. We included children with chronic persistent asthma and not with mild intermittent asthma or severe persistent asthma. Further research has to be performed to assess if our results also apply to these other types of asthma, as well as non-atopic asthma.

Airways hyperresponsiveness is a sensitive parameter to monitor therapy with inhaled corticosteroids. Apart from a dose-dependent effect of high doses inhaled fluticasone (1000 and 500 μg/day) on airways hyperresponsiveness, no superior effect of a stepdown approach compared to a continuous dose approach after 1 and 2 years treatment was found. These high doses, however, were given for a period of only 2 months. Additional studies are needed to examine whether prolonged treatment (more than 2 months) with high doses of inhaled corticosteroids significantly improves airways hyperresponsiveness. In addition, we used dry-powder inhalers with inhaled fluticasone. Further research should investigate whether comparable results are found with different types and delivery devices of inhaled corticosteroids.

New techniques to assess airways inflammation have recently been developed. Exhaled nitric oxide is useful in distinguishing untreated asthmatics from non-asthmatics. Induced sputum and exhaled breath condensate are used to obtain inflammatory markers (cells and cytokines) from the lower airways in childhood asthma. Further studies have to investigate whether the effects of inhaled corticosteroids on these inflammatory parameters are dose-dependent, and whether these methods can be applied for follow-up reasons.

We have shown that 2-years treatment with inhaled fluticasone 200 μg/day is safe with respect to height growth, although our study compared different doses of FP with each other and not with placebo. Nevertheless, our data suggest that suppression of growth height occurs during high-dose therapy with FP. Although reassuring results have been published, showing normal adult height after long-term treatment with inhaled budesonide, no longitudinal studies investigating the effects of inhaled FP on adult height are available. This is also a subject for further studies.

Bone mineral density decreased during treatment with inhaled fluticasone 200 μg/day, but this decrease was not significant. Likewise, high doses of inhaled fluticasone showed a decrease in bone mineral density, but this decrease stabilised during tapering off the dose. Other studies have to demonstrate whether a decrease in bone mineral density will persist during prolonged therapy with fluticasone 200 μg/day. In addition, effects of the dose of inhaled fluticasone on bone mineral density should be studied more extensively.

We demonstrated a dose-dependent suppression of the adrenal cortical function by inhaled FP. The clinical relevance of this suppression, however, is unknown and remains to be investigated.

We investigated the efficacy and safety of FP 200 μg/day compared to 100 μg/day given for 18 months in asthmatic children. Further studies have to be performed to compare long-term treatment with lower doses of FP (e.g. 50 versus 100 μg/day) with respect to efficacy and safety parameters.

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Chapter 9

Samenvatting voor de niet-medisch geschoolde

Astma

Astma is de meest voorkomende chronische ziekte op de kinderleeftijd. Recent onderzoek toont aan dat deze ziekte steeds vaker voorkomt. De ziekte uit zich in terugkerende klachten van piepen, hoesten, kortademigheid en benauwdheid bij allerlei prikkels, zowel allergische (bijvoorbeeld huisstofmijt, hond, kat, pollen) als niet-allergische (bijvoorbeeld (sigaretten) rook, mist, inspanning, virusinfecties of parfumluchtjes). Het feit dat luchtwegen van zowel allergische als niet-allergische astma patiënten sneller en heftiger reageren op geringe concentraties van deze prikkels wordt hyperreactiviteit genoemd. Dit is het basiskenmerk van astma.

Astma en ontsteking

Na het inademen van allergische stoffen worden er ontstekingscellen (die afkomstig zijn uit het beenmerg) naar de longen getransporteerd. Hierdoor ontstaat er een ontstekingsproces in de luchtwegwand, ook wel inflammatie genoemd. Dit inflammatieproces is erop gericht om de ingeademde deeltjes onschadelijk te maken. Bij astmatische patiënten is deze ontstekingsreactie echter te hevig zodat de luchtwegwand beschadigd wordt en daardoor verandert. Als gevolg hiervan wordt de luchtwegwand verdikt (oedeem) en rood (vaatverwijding) en komt er veel slijm in de luchtwegen. Daarnaast trekken de spiertjes rondom de luchtwegwand zich samen waardoor de doorgankelijkheid van de luchtwegen afneemt. Het geheel van deze reactie leidt tot het gevoel van kortademigheid en het optreden van een piepende ademhaling met hoesten en opgeven van slijm.

Bij de ontstekingsreactie in de luchtwegen spelen bepaalde ontstekingscellen een belangrijke rol. Eén van de belangrijkste cellen is de eosinofiele granulocyt. Deze eosinofiele granulocyten zijn zowel te meten in het bloed als in de luchtwegwand. Aangezien onderzoek van de luchtwegwand een belastende ingreep is (het vraagt om een kijkoperatie), wordt er momenteel veel onderzoek verricht naar andere manieren waarop luchtweginflammatie kan worden gemeten. Het bepalen van de concentratie van stikstof monoxide in de uitademingslucht is één van deze niet belastende methoden. Het blijkt dat patiënten met astma een hogere concentratie stikstofmonoxide in de uitademingslucht hebben dan gezonde personen, waarbij een hogere concentratie stikstofmonoxide wijst op een ernstigere luchtweginflammatie. In tegenstelling tot longfunctieonderzoek is dit type onderzoek ook gemakkelijk uit te voeren bij (jonge) kinderen (vanaf 2-3 jaar).

Longfunctie en luchtweghyperreactiviteit

De luchtwegvernauwing kan gemeten worden met behulp van longfunctieonderzoek. Hierbij wordt onder andere de hoeveelheid lucht gemeten die in de eerste seconde van de uitademing wordt uitgeblazen (de FEV_1). Bij een vernauwde luchtweg zal er in 1 seconde minder lucht kunnen worden uitgeademd dan bij een wijde (normale) luchtweg. Wanneer deze FEV_1 waarde verlaagd is, is er dus sprake van luchtvernauwing bij de uitademing. Dergelijk longfunctieonderzoek kan worden verricht bij kinderen vanaf 5-6 jaar.

Het inademen van niet-allergische (rook, mist) prikkels bij patiënten met astma leidt tot luchtwegvernauwing. Bij gezonde mensen treedt deze reactie echter niet op. Het hiervoor beschreven inflammatieproces is mede verantwoordelijk voor het ontstaan van luchtweghyperreactiviteit.

De luchtweghyperreactiviteit kan gemeten worden met behulp van methacholine, een luchtwegvernauwende stof. Inademing van methacholine leidt tot het samentrekken van glad spierweefsel rondom de luchtwegen. Deze luchtwegvernauwing is te meten met behulp van de FEV_1. Zowel luchtweghyperreactiviteit als longfunctieonderzoek is mogelijk vanaf de leeftijd van ongeveer 6 jaar.

Astma op de kinderleeftijd

Recidiverend piepen is de meest voorkomende klacht waarmee kinderen met astma zich melden bij de arts. Sommige kinderen piepen alleen bij verkoudheden (door

virale luchtwegontstekingen), anderen piepen ook zónder dat er sprake is van een luchtweginfectie. Uit recent onderzoek blijkt dat er op de kinderleeftijd verschillende vormen van recidiverend piepen zijn, namelijk allergisch astma en "peuterastma".

Allergisch astma kan al op zeer jonge leeftijd optreden. Deze vorm van astma wordt gekarakteriseerd door de aanwezigheid van allergie bij het kind, ook wel atopie genoemd. De moeders van deze kinderen zijn vaak ook allergisch. Daarnaast zijn deze luchtwegklachten onafhankelijk van de aan- of afwezigheid van luchtweginfecties (verkoudheid). Allergisch astma gaat meestal niet over maar blijft luchtwegklachten geven tijdens de kinderjaren. "Peuterastma" daarentegen wordt gekenmerkt door perioden van piepen die alléén optreden tijdens verkoudenheden zonder dat er sprake is van allergie. Tussen deze verkoudheden door hebben deze kinderen geen luchtwegklachten. Risicofactoren voor deze vorm van "astma" zijn roken van de moeder, zowel tijdens als na de zwangerschap, en een lagere longfunctie vóór de eerste aanval van piepen. Vaak verdwijnen de klachten tussen de leeftijd van 4 en 6 jaar.

Op jonge leeftijd is het erg moeilijk om deze twee vormen van recidiverend piepen te onderscheiden doordat beide ziektebeelden in hun klinische uitingsvorm erg op elkaar lijken. Longfunctieonderzoek is op jonge leeftijd niet gemakkelijk uitvoerbaar, zodat men uitsluitend afhankelijk is van de anamnese en lichamelijk onderzoek.

Behandeling van astma

Naast het vermijden van uitlokkende factoren die een astma aanval kunnen veroorzaken (bijvoorbeeld sigarettenrook, huisdieren, huisstofmijt) vormt het dagelijks gebruik van geïnhaleerde ontstekingsremmers (inhalatiesteroïden) de hoeksteen van de astmabehandeling, zowel op de kinderleeftijd als op volwassen leeftijd. De in Nederland gebruikte inhalatiesteroïden zijn beclomethason dipropionaat, budesonide en fluticason propionaat.

Inhalatiesteroïden onderdrukken het ontstekingsproces in de luchtwegen waardoor de luchtwegvernauwing afneemt, de hyperreactiviteit verbetert en daarmee de kortademigheidsklachten verminderen. Omdat de luchtweghyperreactiviteit het onderliggende ontstekingsproces in de luchtwegwand weerspiegelt en de luchtweghyperreactiviteit verbetert na gebruik van inhalatiesteroïden, wordt de mate van verandering hierin vaak gebruikt om het effect te onderzoeken van verschillende soorten (en toedieningsvormen) van inhalatiesteroïden.

De internationale en Nederlandse standaard voor de behandeling van astma advi-

seren om tijdens het begin van de ziekte kinderen met astma te behandelen met een hoge dosis inhalatiesteroïden, zodat het ontstekingsproces krachtig onderdrukt wordt. Deze hoge dosering kan daarna stapsgewijs worden afgebouwd naar een lagere onderhoudsdosering (de zogeheten "stepdown" benadering). Deze strategie kan ook worden toegepast wanneer er een korte periode van toename van luchtwegklachten is (exacerbatie) bij kinderen die reeds inhalatiesteroïden gebruiken. Er is echter weinig bewijs of deze behandelstrategie inderdaad beter wanneer deze vergeleken wordt met een behandeling met een constante dosis inhalatiesteroïden.

Bijwerkingen

Inhalatiesteroïden kunnen zowel lokale als systemische bijwerkingen veroorzaken.

Lokale bijwerkingen komen zelden voor. Ze bestaan met name uit heesheid en schimmelinfecties in de mond en keel. Systemische bijwerkingen worden veroorzaakt doordat een deel van de inhalatiesteroïden in de bloedbaan terecht komt door opname uit de darm na het doorslikken van de medicijnen en door opname in de bloedbaan vanuit de longen. Deze systemische bijwerkingen zijn zeer zeldzaam, maar wel een bron van grote zorg voor artsen en voor ouders van kinderen met astma. De belangrijkste systemische bijwerkingen van inhalatiesteroïden zijn negatieve effecten op de groei, de bijnierschorsfunctie en de botstofwisseling.

- Effecten op groei. Kinderen met astma groeien minder goed dan gezonde kinderen. Verschillende onderzoeken hebben aangetoond dat inhalatiesteroïden de groei enigszins kunnen vertragen. Deze groeivertraging (van ongeveer 1 cm) vindt met name plaats tijdens het eerste jaar van behandelen. Onderzoeken die de effecten van inhalatiesteroïden op de groei op lange termijn hebben onderzocht vonden geen verschillen in de "eindlengte" op volwassen leeftijd vergeleken met mensen die niét waren behandeld met inhalatiesteroïden maar wel astma hadden.
- Effecten op de bijnierschors. Inhalatiesteroïden kunnen een onderdrukking van de bijnierschorsfunctie veroorzaken, waardoor er een onvoldoende reactie bij stress situaties op kan treden. Het vinden van een effect is echter afhankelijk van de dosis en het type inhalatiesteroïden, maar ook van de manier waarop de bijnierfunctie wordt gemeten.

- Effecten op het botstofwisseling. De botten in het lichaam worden voortdurende opgebouwd en afgebroken. Deze opbouw en afbraak is in balans. Inhalatiesteroïden kunnen deze beide processen beïnvloeden, waardoor zowel de botopbouw als de botafbraak verminderen. Uiteindelijk zou dit kunnen leiden tot een verandering in de botsamenstelling (botdichtheid), waardoor er een groter kans is op botbreuken. Hoewel er dosisafhankelijke effecten van inhalatiesteroïden op de botstofwisseling zijn gevonden, zijn er geen aanwijzingen dat dit nadelige effecten op de botdichtheid bij kinderen heeft.

Doel van het onderzoek in dit proefschrift

In dit proefschrift beschrijven we de resultaten van een 2-jarig onderzoek naar 2 verschillende behandelschema's met fluticason bij kinderen met astma tussen de leeftijd van 6 en 10 jaar. Eén groep kinderen begon met een hoge dosering fluticason (1000 µg per dag). Na 2 maanden werd deze dosis gehalveerd naar 500 µg per dag, weer 2 maanden later 200 µg per dag en uiteindelijk 100 µg per dag gedurende de resterende 18 maanden van de studie (stepdown groep). De andere groep kinderen kreeg 200 µg per dag gedurende 2 jaar (constante dosis groep). Zowel de kinderen/ouders als de onderzoekers wisten niet welke behandeling de kinderen kregen.

Er werd onderzocht welke behandelingsvorm het beste (meest effectief) was en de minste bijwerkingen had (veiligheid). De kinderen kwamen iedere 2 maanden op de polikliniek voor het meten van de luchtweghyperreactiviteit en de longfunctie. Ook werd gevraagd naar luchtwegklachten en werden bijwerkingen onderzocht.

Uiteindelijk waren er 55 kinderen met astma (gemiddelde leeftijd 8 jaar) die geschikt waren om mee te doen aan het 2-jarige onderzoek: 27 in de stepdown groep en 28 in de constante dosis groep.

In **hoofdstuk 2** beschrijven we de resultaten van de periode voorafgaand aan de daadwerkelijke studie (de baseline fase). Bij alle kinderen die geschikt werden geacht om aan het onderzoek mee te doen werden hun eigen inhalatiesteroïden gestopt en kregen ze 6 weken lang fluticason in een dosering van 200 µg per dag (run-in periode). Na deze 6 weken werd de fluticason gestopt (gedurende maximaal 8 weken: washout periode). Wanneer de luchtweghyperreactiviteit toenam tot een matig ernstig niveau tijdens deze 8 weken, begonnen de kinderen aan de eigenlijke studie, waarin stepdown- en constante dosis benadering vergeleken werden.

Van de 95 kinderen bij wie de inhalatiesteroïden gestopt waren, ontwikkelden er 48 geen luchtweghyperreactiviteit. Bovendien had een aanzienlijk aantal van deze kinderen (35%) helemaal geen luchtwegklachten. De overige 47 kinderen ontwikkelden wél luchtweghyperreactiviteit en hadden ook luchtwegklachten. Wanneer we deze 2 groepen kinderen met elkaar vergelijken, blijkt dat de kinderen met luchtweghyperreactiviteit meer allergische kenmerken en een slechtere longfunctie hadden dan de kinderen zonder luchtweghyperreactiviteit. Er waren geen verschillen in de duur van het astma of de duur van de behandeling voorafgaand aan het onderzoek tussen de 2 groepen.

Omdat luchtweghyperreactiviteit een goede methode is om het effect van (verschillende soorten en doseringen) inhalatiesteroïden te onderzoeken, wordt een zekere mate van luchtweghyperreactiviteit vaak gebruikt om kinderen met astma te includeren in klinisch onderzoek. Onze resultaten laten echter zien dat veel kinderen geen luchtweghyperreactiviteit meer hebben na (langdurig) gebruik van inhalatiesteroïden. Dit duidt erop dat het werven van kinderen met astma voor klinische onderzoeken moeizaam kan zijn wanneer luchtweghyperreactiviteit als belangrijkste (en enige) inclusiecriterium wordt gebruikt.

Hoofdstuk 3 beschrijft een subgroep van de kinderen met astma die in hoofdstuk 2 zijn beschreven. Eenenveertig kinderen met luchtweghyperreactiviteit en 37 kinderen zonder luchtweghyperreactiviteit werden vergeleken met elkaar met betrekking tot demografische, klinische en immunologische factoren.

Evenals aangetoond in hoofdstuk 2 bleek dat de luchtweghyperreactive kinderen vaker allergisch waren (meer allergieën voor huisdieren en/of huisstofmijt) en vaker eczeem hadden. Ook bleek bij hen de longfunctie verminderd te zijn en hadden zij een verhoogde concentratie van de stikstofmonoxide in de uitademingslucht. Dit laatste wijst op een verhoogde ontstekingsactiviteit in de luchtwegen. Er bleek echter geen verschil in ontstekingsactiviteit in het bloed te zijn tussen de 2 groepen.

Bij verdere statistische analyse bleek dat allergie, verminderde longfunctie en een tweetal onstekingsparameters voorspellend zijn voor het ontwikkelen van luchtweghyperreactiviteit na het stoppen van inhalatiesteroïden.

Wanneer we onze resultaten vergelijken met onderzoeken die eerder zijn gepubliceerd dan hebben de kinderen die luchtweghyperreactiviteit ontwikkelen na het stoppen van inhalatiesteroïden kenmerken van allergisch astma en kinderen die geen luchtweghyperreactiviteit ontwikkelen van "peuterastma".

Inhalatiesteroïden kunnen systemische bijwerkingen veroorzaken. Dit is onder an-

dere te meten aan de hand van de groeisnelheid van het onderbeen (knemometrie). In **hoofdstuk 4** beschrijven we het effect van de behandeling met inhalatie van 200 µg fluticason per dag op de groeisnelheid van het onderbeen bij kinderen met astma. Eenentwintig kinderen tussen de 6 en 10 jaar werden behandeld met 200 µg fluticason per dag gedurende 6 weken. Na deze 6 weken werd de behandeling met fluticason gestopt. Iedere 2 weken werd de onderbeenlengte gemeten. Er waren geen verschillen tussen de kinderen aan het begin van het onderzoek. Alle kinderen bleven klinisch stabiel tijdens de onderzoeksperiode van 8 weken.

Tijdens de behandeling met het inhalatiesteroïd fluticason was er geen verschil in de groeisnelheid van het onderbeen ten opzichte van de periode waarin de kinderen geen fluticason kregen. Het blijkt dus dat 6 weken behandeling met 200 µg fluticason per dag geen systemische bijwerkingen heeft, zoals met knemometrie kan worden gemeten.

Hoofdstuk 5 toont de resultaten gedurende en na 1 jaar behandeling met 2 verschillende doseringsschema's fluticason bij kinderen met astma (leeftijd 6 tot 10 jaar). Eén groep kinderen (27) werd behandeld met een hoge dosis fluticason, welke geleidelijk werd afgebouwd naar een lage onderhoudsdosering (stepdown groep), terwijl de andere groep kinderen (28) een constante dosis fluticason kreeg. Er werd onderzocht of er verschillen waren in luchtweghyperreactiviteit, longfunctie, concentratie stikstofmonoxide in de uitademingslucht, luchtwegklachten en aanvallen van ernstige benauwdheid (exacerbaties). Daarnaast werd onderzocht of er een verschil was in lengtegroei.

Er was een kortdurend betere luchtweghyperreactiviteit na behandeling met fluticason 1000 µg per dag gedurende 2 maanden gevolgd door 2 maanden behandeling met 500 µg per dag vergeleken met een constante dosering fluticason (200 µg per dag). Gedurende het verdere onderzoek was er echter geen verschil in luchtweghyperreactiviteit tussen de 2 groepen. De concentratie van stikstofmonoxide in de uitademingslucht, longfunctie, luchtwegklachten en aantal exacerbaties verschilden niet tussen de 2 groepen kinderen met astma. Er was geen verschil in groei, ook al hadden de kinderen in de stepdown groep 1.7 keer meer fluticason gebruikt gedurende 1 jaar behandeling dan de kinderen die een continue dosis fluticason hadden gebruikt.

De conclusie van dit 1-jarige onderzoek is dat een stepdown behandeling (hoog beginnen, laag eindigen) met fluticason geen voordeel biedt ten opzicht van een continue behandeling met 200 µg fluticason per dag bij kinderen met astma. Daarnaast blijkt dat beide behandelstrategieën even veilig zijn met betrekking tot de groei. Het advies om een stepdown behandeling met inhalatiesteroïden te kiezen bij kinderen met

astma wordt niet ondersteund door de resultaten van onze studie.

Ontsteking van de luchtwegen speelt een belangrijke rol bij astma. De ontstekingscellen maken een groot aantal stoffen, de zogeheten cytokinen. Deze cytokinen kunnen in het bloed worden bepaald. Aangezien inhalatiesteroïden de luchtwegontsteking kunnen onderdrukken, wordt verondersteld dat zowel de cytokine productie als de eosinofiele granulocyten hierdoor worden beïnvloed. In **hoofdstuk 6** beschrijven we de effecten van verschillende doseringen fluticason op cytokinen en eosinofiele granulocyten bij kinderen met astma.

Hoewel er een dosisafhankelijk effect van fluticason op het aantal eosinofiele granulocyten in bloed werd gevonden was dit verschil alleen statistisch significant bij een hoge dosis fluticason (1000 μg per dag) ten opzichte van een normale onderhoudsdosis (200 μg per dag).

Er was geen dosisafhankelijk effect van fluticason op de cytokine concentraties gemeten in bloed of in het laboratorium gestimuleerde cellen.

Concluderend lijkt het meten van ontstekingscellen (eosinofiele granulocyten) en cytokinen in het bloed niet zinvol om te bepalen wat de optimale dosis van inhalatiesteroïden bij kinderen met astma is.

Hoofdstuk 7 tenslotte beschrijft de effecten van de verschillende doseringen fluticason op systemische bijwerkingen. We vonden een duidelijke afname in de groeisnelheid wanneer kinderen een hoge dosis fluticason gebruikten (1000 en 500 μg per dag) ten opzichte van een "normale" onderhoudsdosering (200 μg per dag). Daarnaast werd zowel de botstofwisseling als de bijnierschors geremd tijdens behandeling met deze hoge doseringen. Opvallend was echter dat na het afbouwen van deze hoge doseringen deze bijwerkingen snel verdwenen. Tevens bleek dat er na 1 en 2 jaar behandelen geen verschillen waren tussen de 2 doseringsschema's op de lengte, ondanks de verschillen in groeisnelheid tijdens deze periode. De botdichtheid ten opzichte van het begin van het onderzoek nam in beide groepen af na 1 jaar behandelen, zonder dat er een significant verschil was tussen beide behandelgroepen. Ook tijdens het 2^{e} jaar werden er geen significante verschillen gevonden tussen beide groepen.

Bijnierschorsonderdrukking was niet verschillend tussen beide groepen na 1 en 2 jaar behandelen.

Wij concluderen dat fluticason in hoge doseringen duidelijke systemische bijwerkingen heeft bij kinderen met astma, die echter snel verdwijnen na het afbouwen van de hoge dosering naar lagere onderhoudsdoseringen. Er is echter wel een afname in de botdichtheid bij kinderen met astma die gedurende 1 en 2 jaar behandeld worden met 200 μg fluticason per dag.

Omdat een stepdown benadering met fluticason niet effectiever is dan een constante dosis fluticason (hoofdstuk 5) en omdat hoge doseringen een aanzienlijk risico op systemische bijwerkingen met zich mee brengen, adviseren wij om hoge doseringen fluticason te vermijden bij kinderen met astma.

Conclusies

- Bij een deel van de kinderen bij wie op jonge leeftijd de diagnose astma is gesteld, is er waarschijnlijk sprake geweest van "peuterastma" (een voorbijgaande vorm van piepen) en niet van het (klassieke) allergische astma.
- Bij kinderen (tussen de 6 en 10 jaar) die worden behandeld met inhalatiesteroïden in verband met astma, moet regelmatig geëvalueerd worden of deze medicatie verminderd dan wel gestaakt kan worden.
- Het gebruik van luchtweghyperreactiviteit alléén als inclusiecriterium voor klinisch onderzoek naar effecten van inhalatiesteroïden bij kinderen met astma kan onvoldoende zijn, aangezien een groot aantal kinderen geen luchtweghyperreactiviteit meer kan hebben na (langdurig) gebruik van inhalatiesteroïden.
- Hoog beginnen en langzaam afbouwen met fluticason bij kinderen met astma (leeftijd 6 tot 10 jaar) levert geen voordeel op ten opzichte van een behandeling met een constante dosis fluticason.
- Fluticason in een onderhoudsdosering van 200 μg/dag heeft geen systemische bijwerkingen.
- Hoge doses fluticason (1000 en 500 μg per dag) hebben duidelijke bijwerkingen op de groeisnelheid, botstofwisseling en bijnierschorsfunctie. Deze bijwerkingen verdwijnen snel na afbouwen van de hoge dosis naar een lagere onderhoudsdosis.
- Hoge doses fluticason moeten vermeden worden bij kinderen met astma omdat er geen voordeel is gebleken ten opzichte van een constante dosis fluticason terwijl er wel sprake kan zijn van systemische bijwerkingen
- Het meten van stikstofmonoxide in de uitademingslucht is niet zinvol om de dosis van inhalatiesteroïden te bepalen bij kinderen met astma.
- Het meten van ontstekingsparameters (cellen en cytokinen) in het bloed is niet zinvol om de dosis fluticason, en daarmee de behandeling van astma, te optimaliseren.

Aanbevelingen voor verder onderzoek

Het onderscheid tussen allergisch astma en peuterastma is niet eenvoudig. Uit ons onderzoek blijkt dat bij ongeveer de helft van de kinderen, bij wie op jonge leeftijd de diagnose astma is gesteld, er waarschijnlijk sprake was van peuterastma en dat deze kinderen mogelijk ten onrechte jarenlang behandeld werden met inhalatiesteroïden. Kinderen zonder luchtweghyperreactiviteit na het stoppen van inhalatiesteroïden hebben minder allergische kenmerken en een betere longfunctie dan de kinderen die luchtweghyperreactiviteit hielden na het staken van de inhalatiesteroïden. Omdat het echter een cross-sectioneel onderzoek betreft zal prospectief onderzoek, waarbij kinderen vanaf de geboorte tot de leeftijd van 8 jaar worden gevolgd, moeten aantonen of er inderdaad een onderscheid tussen "peuterastma" en allergisch astma is te maken aan de hand van allergische kenmerken. Tevens kan er dan gezocht worden naar voorspellende factoren voor het hebben van allergisch astma, zodat op jonge leeftijd een onderscheid gemaakt kan worden tussen allergisch- en peuterastma en er daardoor een meer gerichte behandeling mogelijk is. Dit kan dan wellicht onnodige behandeling voorkómen. Het is de vraag of inhalatiesteroïden in staat zijn om peuterastma te "genezen". Toekomstig (prospectief) onderzoek dient verricht te worden om aan te tonen of dit inderdaad het geval is.

Tevens verdient de invloed van verschillende ontstekingsparameters in het bloed (cytokinen) op het beloop van luchtwegklachten op jonge leeftijd verder onderzoek.

Ons onderzoek naar de twee verschillende behandelingsschema's van fluticason heeft zich gericht op 6 tot 10-jarige kinderen met matig ernstig astma. Het is onduidelijk of de resultaten kunnen worden geëxtrapoleerd naar kinderen in andere leeftijdsgroepen. Ook is het onbekend of de resultaten van dit onderzoek gelden voor kinderen met mild of juist heel ernstig astma. Verder onderzoek moet aantonen of onze resultaten ook van toepassing zijn op andere leeftijdsgroepen en andere "vormen" van astma.

In ons onderzoek zijn gedurende 2 maanden hoge doseringen fluticason onderzocht. Hoewel er vrijwel geen voordeel van deze hoge doseringen ten opzichte van een lagere onderhoudsdosering was op de klachten, longfunctie en luchtweghyperreactiviteit, is de periode van 2 maanden mogelijk te kort geweest om een effect op de luchtweghyperreactiviteit vast te stellen. Onderzoeken, waarin langer dan 2 maanden hogere doseringen fluticason (of andere inhalatiesteroïden) gebruikt worden, moeten aantonen of er inderdaad geen voordeel is van een hogere dosering inhalatiesteroïden. Hierbij dient ook het effect van deze hoge doseringen op (systemische) bijwerkingen onderzocht te worden.

Ons onderzoek heeft zich alleen gericht op fluticason, toegediend met een droogpoederinhalator. Naast fluticason zijn beclomethason en budesonide veel gebruikte inhalatiesteroïden. Het is onduidelijk of onze resultaten ook gelden voor deze andere medicamenten. Gelet op het werkingsmechanisme van inhalatiesteroïden kunnen vergelijkbare resultaten verwacht worden. Tevens moet verder onderzoek uitwijzen of dezelfde resultaten gevonden worden met andere toedieningsvormen van inhalatiesteroïden, zoals dosisaërosolen met voorzetkamers.

Langdurig toedienen van een onderhoudsdosis fluticason (200 μg per dag) lijkt veilig te zijn. Er zijn echter wel dosisafhankelijk systemische bijwerkingen van fluticason. Omdat de botdichtheid bij kinderen met astma afneemt na 1 en 2 jaar behandelen met 200 μg fluticason per dag, dient het effect van langere toediening (langer dan 2 jaar) en de klinische relevatie (kans op botfracturen) van deze dosis fluticason (en ook van andere inhalatiesteroïden) op de botdichtheid verder onderzocht te worden. De dosisafhankelijke bijwerkingen van fluticason op het botmetabolisme en de invloed hiervan op de botdichtheid verdient eveneens verder onderzoek. Ook de klinische relevantie van de dosisafhankelijke bijwerkingen van fluticason op de bijnierschorsonderdrukking is een onderwerp voor nader onderzoek.

Ons onderzoek toont een dosisafhankelijke vertraging van de groeisnelheid aan tijdens behandeling met fluticason, hoewel dit niet leidt tot een vermindering in groei ten opzichte van een continue dosering. Het betreft echter een onderzoek met een (beperkte) duur van 2 jaar. Recent onderzoek heeft aangetoond dat langdurige behandeling met budesonide geen invloed heeft op de lengte op volwassen leeftijd. Er zijn echter (nog) geen lange termijn resultaten van fluticason bekend met betrekking tot deze eindlengte. Verder onderzoek bij kinderen met astma moet aantonen of langdurige behandeling met fluticason invloed heeft op de lengte op volwassen leeftijd.

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