



Benefit from anti-inflammatory treatment during clinical remission of atopic asthma

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Remodeling

Summary *Study objectives:* Subjects with atopic asthma often experience a disappearance of symptoms around puberty. However, airway inflammation and remodeling may persist. It is unknown whether those findings warrant prolonged anti-inflammatory treatment despite the absence of symptoms. In this study, we investigated whether a short course of combined anti-inflammatory treatment would, also in this specific patient population, diminish airway inflammation and/or remodeling.

Design: A double-blind, randomized placebo-controlled trial was conducted in 28 asymptomatic subjects with a history of atopic asthma, with established bronchial hyperresponsiveness to methacholine (MCh) as non-invasive indicator of ongoing airway pathology.

Interventions: Intervention consisted of the salmeterol/fluticasone propionate combination (SFC) product (50/250 µg bid via the DiskusTM inhaler) or placebo for 3 months.

Measurements: The change in lung function (FEV₁), bronchial response to MCh and adenosine monophosphate (AMP), the fraction of nitric oxide in exhaled air (FENO) and quality of life (QOL) scores were measured. Also, bronchial biopsies were taken and cryo sections immunostained for eosinophils (major basic protein, MBP) and mast cells (tryptase and chymase) before and after treatment. The change in

Abbreviations: AMP, adenosine-5'-monophosphate; BAL, bronchoalveolar lavage; FENO, fraction of nitric oxide in exhaled air; LABAs, long-acting β_2 -agonists; MBP, major basic protein; MCh, methacholine; PD₂₀, cumulative provocative doses causing a 20% fall in FEV₁; QOL, quality of life; RBM, reticular basement membrane; SFC, salmeterol/fluticasone propionate combination product

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reticular basement membrane (RBM) thickness, one of the parameters of airway remodeling, was also determined.

Results: SFC treatment improved hyperresponsiveness to MCh ($P = 0.014$) as well as AMP ($P = 0.011$), and reduced FENO ($P < 0.001$) significantly as compared with placebo. Lung function tended to improve (NS). Furthermore, SFC treatment reduced tryptase in the subepithelium of bronchial biopsy specimens ($P = 0.01$), and slightly reduced RBM thickness ($P = 0.05$). However, eosinophils in (sub)epithelium were not significantly affected; neither were chymase levels, blood eosinophils or QOL scores.

Conclusions: We found that 3 months of treatment with fluticasone propionate and salmeterol reduced airway hyperresponsiveness, FENO and tryptase density in the airway mucosa as markers of airway inflammation. MBP density in the airway mucosa and QOL were, however, unchanged. The clinical relevance of these findings, especially with respect to the long-term outcome, has not been determined yet.

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Introduction

Atopic asthma commonly starts in childhood and often seems to disappear in early adolescence.^{1,2} The term "clinical remission" is widely used to identify subjects with apparently outgrown asthma. Unfortunately, a substantial proportion those subjects will have a relapse later in life, mostly before the age of 30.² Recently, airway inflammation and structural airway changes were demonstrated in airway biopsy specimens and bronchoalveolar lavage (BAL) fluid from young adults with a longstanding clinical remission of atopic asthma.^{3,4} Ongoing inflammation and remodeling possibly contribute to irreversible airway narrowing⁵ and bronchial hyperresponsiveness, thereby probably providing a basis for a future relapse of asthma.

It is unclear whether the long-term prognosis of subjects in clinical remission of asthma, but with evidence of ongoing disease, can be altered positively with prolonged anti-inflammatory therapy. There is ample evidence that inhaled steroids decrease airway inflammation,⁶ whereas the effect on remodeling is less evident.^{7,8} An effect on subepithelial collagen deposition⁹ and airway wall vascularity¹⁰ was, however, documented. It has also been shown that in very mild asthmatic subjects, anti-inflammatory therapy with fluticasone propionate reduces airway inflammation and remodeling after 3 and 12 months of therapy, respectively.¹¹ Besides, De Kluijver¹² demonstrated that low-dose allergen exposure in asymptomatic asthmatic subjects led to airway inflammation without worsening of symptoms, which could be prevented by inhaled steroid treatment. Hence, the question is whether persistent airway inflammation and remodeling warrant the use of prolonged anti-inflammatory treatment in otherwise asymptomatic subjects with a history of atopic asthma.^{9,13,14} To provide an

answer to this question, it should at first and foremost be evaluated whether anti-inflammatory therapy indeed has a direct diminishing effect on airway inflammation and/or remodeling in this specific patients' population. Subsequently, future studies should elucidate whether prolonged anti-inflammatory therapy will positively alter the long-term prognosis from subjects in clinical remission of asthma.

When it comes to the treatment of choice, there is a rationale for combining inhaled steroids with long-acting β_2 -agonists (LABAs) in the present study population. After all, a novel anti-neutrophilic effect of the LABA salmeterol in mild asthma has been reported recently,¹⁵ next to an anti-eosinophilic effect of the LABAs salmeterol¹⁶ and formoterol.¹⁷

Therefore, it was the aim of this study to investigate the effect of a short course of combination treatment with salmeterol and fluticasone propionate on indices of airway inflammation and remodeling in young adults in longstanding clinical remission of atopic asthma, who had hyperresponsiveness to methacholine (MCh) as non-invasive indicator of ongoing airway disease.

Subjects, study design and methods

Subjects

Young adults, 18–30 years of age, were selected from the Sophia Children's Hospital discharged atopic asthma patients files. Clinical remission of atopic asthma was defined as to two independent investigators repeatedly reported complete absence of asthmatic symptoms while not taking any asthma or allergy medication for at least 12 months prior to the study. Subjects with perennial rhinitis

were excluded. All subjects were previously diagnosed as having atopic asthma according to ATS criteria.¹⁸ All subjects were lifelong non-smokers in stable clinical condition. We conducted a double-blind, parallel-group, randomized placebo-controlled trial in 28 subjects who were in clinical remission of atopic asthma for a median duration of 5 years, with a cumulative provocative doses causing a 20% fall in FEV₁ (PD₂₀) MCh cumulative less than or equal to 7.8 µmol at the beginning of the study as non-invasive indicator of ongoing airway disease.

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center Rotterdam.

Study design

All subjects underwent non-invasive and invasive measurements on three visits within 3 weeks, followed by a 3-months' intervention period, after which all measurements were repeated on three visits. At the first visit, subjects gave written informed consent and were asked about asthmatic symptoms and requirement of rescue medication during the past year, especially in order to avoid the inclusion of subjects with mild asthma. Also, quality of life (QOL) scores were obtained. Thereafter, physical examination was performed and the fraction of exhaled NO (FENO) and PD₂₀ MCh determined. At visit 2, scheduled at least 1 day after visit 1, subjects underwent an adenosine monophosphate (AMP) challenge test. At visit 3, scheduled at least 3 days, but not more than 2 weeks, after visit 2, FEV₁ and FEV₁ reversibility were tested, followed by venapuncture and flexible bronchoscopy. The sequence and intervals were chosen in order to avoid any influence of the AMP challenge on MCh responsiveness and/or on FEV₁.¹⁹ For analysis of a specific variable, only data from subjects with pre- as well as post-treatment values were used.

Spirometry values, MCh- and AMP-responsiveness, FENO and blood eosinophils were obtained according to methods described earlier.²⁰ These are summarized briefly hereafter. Methods of obtaining QOL scores as well as collecting and processing bronchial biopsies are described in more detail. Intervention consisted of the salmeterol/fluticasone propionate combination (SFC) product (250/50 µg bid via the DiskusTM inhaler) or placebo for 3 months ± 2 weeks. Therapy adherence and adverse effects were recorded by the primary investigator. If more than 85% of the totally prescribed number

of doses was emptied out of the device, therapy adherence was arbitrarily considered adequate.

Bronchial hyperresponsiveness

Challenge tests were performed according to the dosimeter method validated by Birnie et al.²¹ Study medication was not allowed within 36 h prior to the test. After recording baseline values, the challenge started with inhalation of NaCl 0.9%. Inhalation provocation tests were performed using doubling concentrations of 0.15–78.4 mg/ml MCh bromide in PBS or 0.08–160 mg/ml AMP in normal saline (Sigma Chemical Co., St. Louis, MO, USA). PD₂₀ from baseline were calculated by means of linear interpolation of the logarithmic dose–response curve. If FEV₁ fell less than 20% of the pre-challenge level at the highest dose administered, twice the cumulative total inhaled dose was arbitrarily used as the PD₂₀ value.

Spirometry

Study medication was not allowed within 36 h prior to the test. Flow–volume curves were obtained using a Lilly-type pneumotachograph (Masterlab Jaeger, Würzburg, Germany). The best of three reproducible recordings of FEV₁ was expressed as percentage of predicted normal and used for analysis. Reversibility was tested by measuring FEV₁ before and 20 min after inhalation of 1 mg of terbutaline powder (Bricanyl turbuhaler, ASTRA, Lund, Sweden), and expressed as increase in percentage of predicted normal.

Fraction of nitric oxide in exhaled air

FENO was measured in exhaled air by means of chemiluminescence (model 280 nitric oxide analyzer, Sievers, Boulder, CO) according to recommendations of a European Respiratory Society Task Force.²² Subjects inhaled to TLC and immediately exhaled for as long as possible into a tube with an in-line flow resistor (20 cm H₂O/l/s, Rudolph, MO). This was done at a flow corresponding to 5% of the subjects' vital capacity per second.

Bronchoscopy

Premedication consisted of terbutaline 1 mg by metered dose inhaler and atropine 500 µg intramuscularly. After topical anesthesia with oxybutyprocaine 10 mg/ml to a maximum of 20 ml, and xylocaine 10%, an Olympus model BF IT 10 (Tokyo, Japan) was passed by oral route. All bronchoscopies

were performed by the same pulmonary physician (S.E.O.). At least five bronchial biopsies were obtained with the Olympus Swing Jaw forceps from segmental divisions of the main bronchi at both occasions.

Processing of bronchial biopsies

Bronchial biopsies were embedded in Tissue-Tek II OCT medium (Miles, Naperville, IL), snap frozen in liquid nitrogen and stored at -80°C . Serial tissue sections ($6\text{ }\mu\text{m}$) were cut on an HM-560 cryostat (Microm, Heidelberg, Germany). At least two sections $120\text{ }\mu\text{m}$ apart from one biopsy specimen were placed on a poly-L-lysine-coated microscopic slide (Sigma Diagnostics, St. Louis, MO). Immunostaining was carried out with α -major basic protein (MBP, Sanbio BV, Uden, The Netherlands), α -tryptase and α -chymase (Chemicon Brunschwig Chemie, Amsterdam, The Netherlands). Binding of the antibodies was detected by the immuno-alkaline phosphatase anti-alkaline phosphatase (APAAP) method. Immunostained sections were analyzed with an image analysis system (Quantimed, Leica, Rijswijk, The Netherlands). With respect to the subepithelium, the program was set to analyze $100\text{ }\mu\text{m}$ below the reticular basement membrane (RBM). The ratio of positive stained area divided by the total area analyzed was taken as measure for each immunohistochemical staining. RBM thickness was interactively measured at $20\text{ }\mu\text{m}$ intervals over a 1 mm RBM length.²³

Quality of life

QOL scores were obtained with the aid of a modified RAND Health Insurance Study Questionnaire,²⁴ the so-called RAND 36-item Health Survey, Dutch Version. RAND 36 consists of eight different categories of general health, that is to say physical functioning, social functioning, role limitations, emotional problems, mental health, pain, general

health perception and health change. Items are scored 1–5 or 6. A higher score means better health. In each category, 100 is the maximum total score.

Statistical analysis

Lung function data (FEV_1 , FEV_1 reversibility, PD_{20} MCh and PD_{20} AMP) and QOL data (RAND 36) were parametrically analyzed using ANCOVA, where the baseline measurement of the outcome variable considered was included as covariant in comparing the two treatment groups (SFC and placebo). Within-subject changes from baseline of these variables were tested using the paired *t*-test for either treatment group. For the PD_{20} variables, the analyses were done after $\log_2$ transformation. PD_{20} MCh was used as primary variable. Power calculations were based on this variable, where a number of 14 subjects in each group was supposed sufficient to show a difference of two doubling doses between the treatment groups in the within-subject changes from baseline. The other outcome variables were analyzed by comparing changes from baseline between the two treatment groups using the Mann–Whitney test. Within-subject changes from baseline of these other variables were tested using the Wilcoxon signed rank test for either treatment group. *P*-values below 0.05 were used to indicate statistical significance.

Results

Both the SFC group and the placebo group consisted of 14 subjects. Groups were well matched with respect to age, sex, FEV_1 , FEV_1 reversibility, bronchial response to MCh and AMP, and FENO (Table 1). One asymptomatic (!) subject in the SFC group showed a pre-trial FEV_1 of 63% and an FEV_1 reversibility of 31%. All other subjects in both treatment groups had a pre-trial FEV_1 above 80%,

Table 1 Subjects' characteristics.

	SFC	Placebo
<i>n</i> (male)	14 (11)	14 (9)
Age (year)	19.6 (18–23)	20.4 (18–24)
FEV_1 (% pred.)	94.1 (63–119)	101.2 (87–120)
FEV_1 reversibility (%)	9.9 (0–31)	6.9 (0–23)
PD_{20} MCh (μmol)	1.7 (0.1–6.2)	3.3 (0.3–7.4)
PD_{20} AMP (μmol)	24.0 (1.0–60.5)	23.1 (1.4–60.5)
Exhaled NO (ppb)	11.9 (7.2–22.0)	9.5 (4.0–23.4)

Note: SFC, salmeterol/fluticasone propionate combination.

All variables but FENO (median; range) are expressed as means (range).

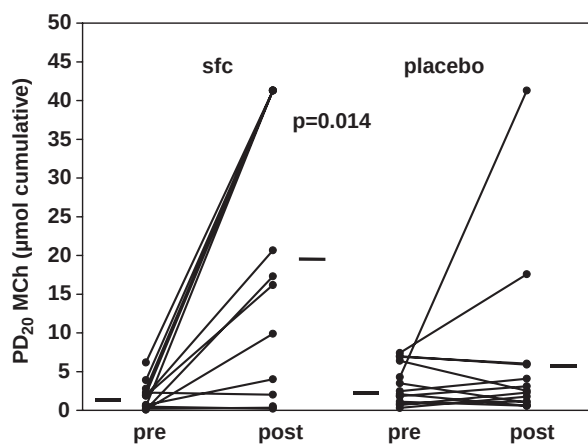


Figure 1 PD₂₀ MCh values pre- and post-treatment for 3 months with the SFC product ($n = 14$) or placebo ($n = 14$). Each line represents one subject. Non-responders are arbitrarily given a value of twice the highest cumulative dose given. Horizontal bars represent mean values.

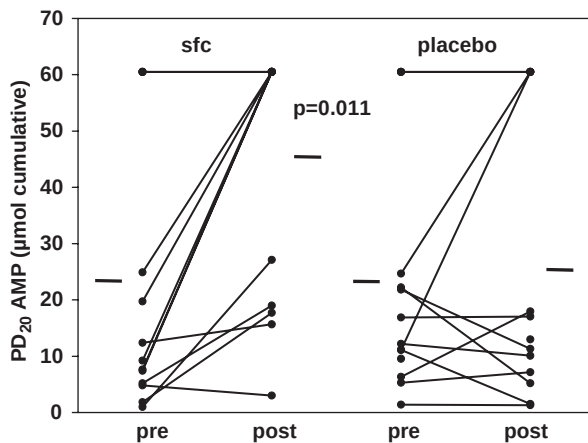


Figure 2 PD₂₀ AMP values pre- and post-treatment for 3 months with the SFC product ($n = 14$) or placebo ($n = 14$). Each line represents one subject. Non-responders are arbitrarily given a value of twice the highest cumulative dose given. Horizontal bars represent mean values.

and clearly lower reversibility. One subject in the placebo group refused the second bronchoscopy because of a negative experience with the first one. One other subject, also in the placebo group, could not undergo bronchoscopy because of a bleeding disorder. Therapy adherence was considered adequate since none of the subjects had used less than 85% of the prescribed total number of doses. Both treatments were well tolerated.

SFC treatment for 3 months significantly improved PD₂₀ MCh ($P = 0.014$, Fig. 1) as well as PD₂₀ AMP ($P = 0.011$, Fig. 2), and reduced FENO ($P < 0.001$, Fig. 3). Furthermore, SFC treatment reduced tryptase-positive cells in the bronchial

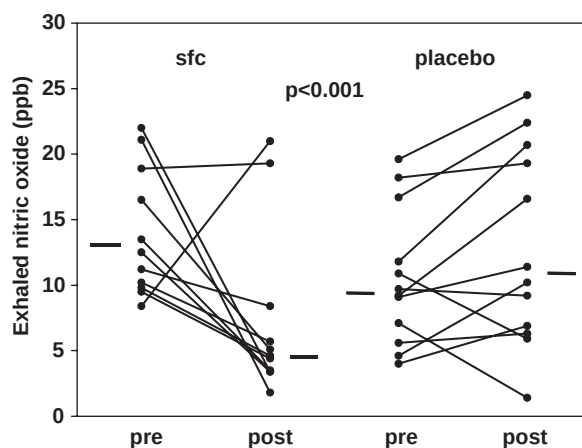


Figure 3 Exhaled nitric oxide levels pre- and post-treatment for 3 months with the SFC product ($n = 12$) or placebo ($n = 12$). Each line represents one subject. Horizontal bars represent median values.

subepithelium ($P = 0.01$, Figs. 4 and 5), whereas MBP-positive cells in both the epithelium and subepithelium decreased slightly, but not significantly (Fig. 6). Following SFC treatment, thickness of the RBM was reduced slightly ($P = 0.05$, Fig. 7). Compared to placebo, FEV₁ and FEV₁ reversibility, blood eosinophils, chymase density in bronchial biopsies and QOL scores did not change as a result of 3 months of SFC treatment.

Discussion

The majority of subjects in clinical remission of atopic asthma show ongoing airway inflammation and remodeling,^{3,4} which may predispose for a relapse later in life. The present study shows that combined treatment with an inhaled steroid and a LABA significantly reduces bronchial hyperresponsiveness and FENO levels after 3 months of therapy. Except for the change in tryptase density, no significant changes were demonstrated of invasively obtained markers of inflammation and remodeling.

Anti-inflammatory treatment normalized both MCh responsiveness and AMP responsiveness in a substantial proportion of the subjects in the study. Where AMP responsiveness is believed to be related to active airway inflammation by inducing mast cell degranulation, MCh acts through direct mechanisms on the airway smooth muscle, thereby probably at least in part reflecting structural changes, i.e. airway remodeling, in the airways.²⁵ We recently found a significant relationship between AMP responsiveness and eosinophilic airway inflammation.³ Hence, a normal response to AMP suggests the absence of clinically significant active

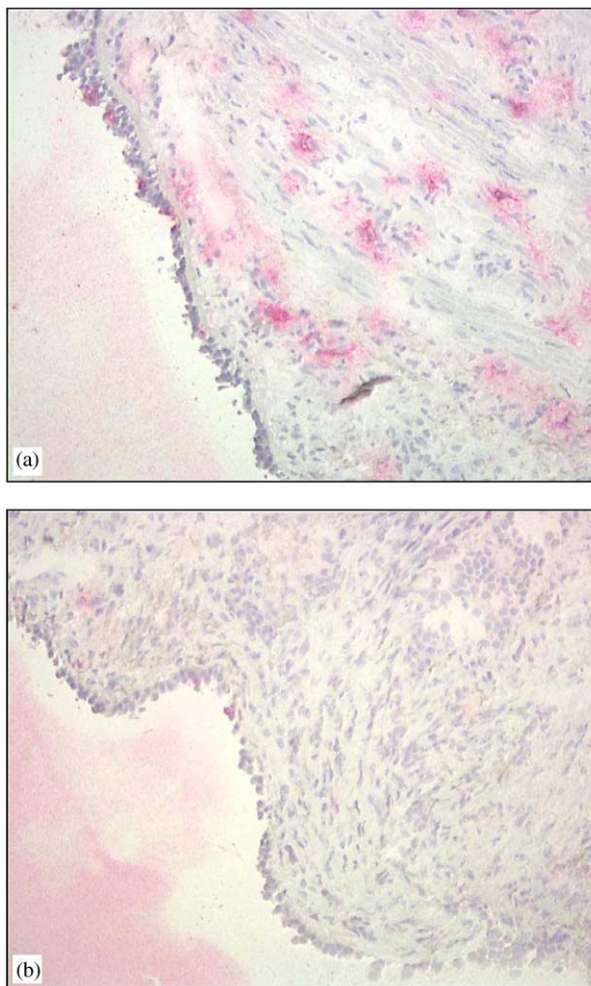


Figure 4 Example of a biopsy specimen pre- (a) and post-treatment (b) from a subject in clinical remission with tryptase staining (mast cells) in red.

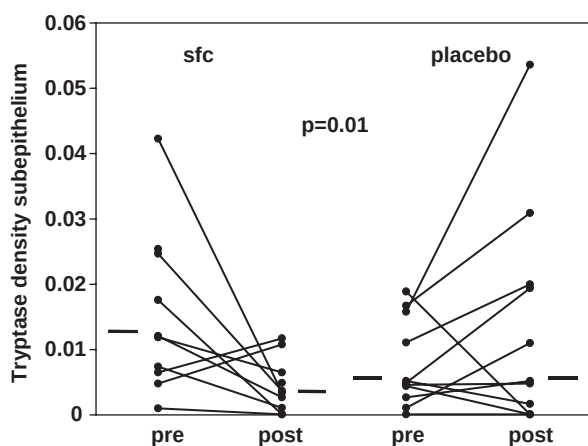


Figure 5 Tryptase density in the bronchial subepithelium pre- and post-treatment for 3 months with the SFC product ($n = 10$) or placebo ($n = 11$). Each line represents one subject. Horizontal bars represent median values.

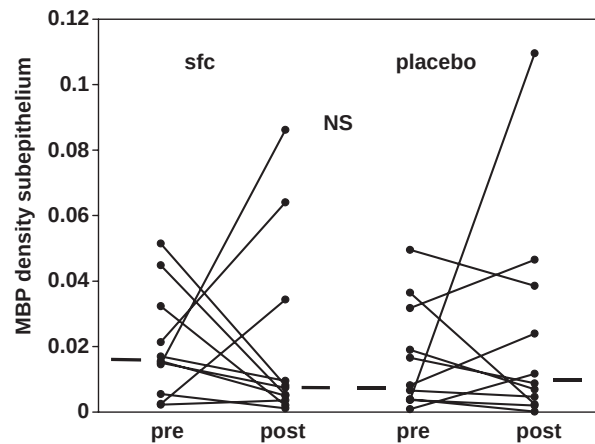


Figure 6 MBP density in the bronchial subepithelium pre- and post-treatment for 3 months with the SFC product ($n = 11$) or placebo ($n = 11$). Each line represents one subject. Horizontal bars represent median values.

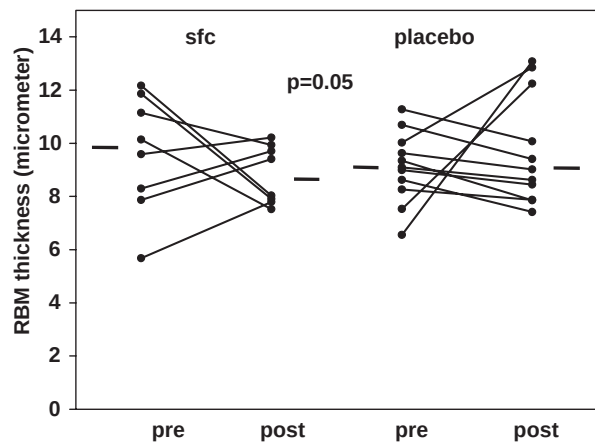


Figure 7 Thickness of the RBM pre- and post-treatment for 3 months with the SFC product ($n = 8$) or placebo ($n = 11$). Each line represents one subject. Horizontal bars represent median values.

inflammation.²⁶ Normal MCh responsiveness, on the other hand, possibly relates to the absence of manifest structural changes in the airways as well. In a study of Koh et al.,²⁷ BHR to MCh was unchanged in subjects in clinical remission of asthma after 9 months of treatment with inhaled budesonide. Whether this was due to more severe and irreversible airway changes, or a different kind of study medication as compared to our treatment, is unclear. Thus, both MCh and AMP challenge might be useful in monitoring airway disease activity and response to treatment.

A highly significant reduction in FENO levels was seen. More specifically, 10 out of 12 subjects treated with SFC reached FENO values less than 8 ppb after 3 months of treatment. It has been

recently shown that FENO is likely to be related to airway mucosal eosinophilia,^{3,28} BAL eosinophils²⁹ and sputum eosinophils in atopic asthma,³⁰ although others did not find such a relationship.³¹ Therefore, next to MCh- and AMP-responsiveness, FENO might be a useful tool in monitoring disease activity and effectiveness of treatment.^{32–34} Furthermore, our data suggest that FENO could be a more rapidly responding marker of active inflammation than are tissue eosinophils.

With respect to markers of airway inflammation in tissue specimens, only tryptase levels improved significantly. Since a central role in the inflammatory process including airway remodeling has been attributed to bronchial mast cells,^{35,36} the demonstrated effect of anti-inflammatory therapy on these cells may be of clinical relevance. Changes in MBP density, reflecting eosinophil activity, showed a trend toward a reduction in the SFC group, but were not significant. We speculate that MBP changes might reach significance when more subjects would have been studied, which might be also true for changes in chymase.

We found that the thickness of the RBM, one of the characteristics of airway remodeling, was reduced after treatment in some subjects, but not in all. Overall changes were of borderline significance. It can be argued that these changes may be the result of reduction in RBM edema due to plasma leakage,^{14,37,38} which might also be influenced by the addition of a LABA to the study medication.¹⁰ Ward et al.¹¹ recently demonstrated a significant effect of fluticasone propionate on thickness of the RBM after 9 months of treatment in subjects with mild asthma. Other characteristics of airway remodeling, such as subepithelial collagen deposition and smooth muscle hyperplasia, presumably respond more slowly, if at all.^{8,9} Therefore, our intervention may have been too short to induce important changes in the RBM thickness.

The choice of salmeterol plus fluticasone propionate was arbitrarily based on the concept of combination treatment as therapy of choice in treating airway inflammation in asthma. In recent years, an additive or even synergistic effect of LABAs on airway inflammation^{15–17} and airway vascularity¹⁰ has been documented. Also, LABAs can improve well-being by providing immediate relief by means of bronchodilation. As we found no evidence of subjective improvement or better QOL in this study, the added value of the LABA in subjects with subclinical asthma remains conceptual. It was, however, the purpose to provide "optimal" treatment, not to elucidate whether the same results could have been achieved with inhaled steroids alone.

The present study also demonstrates that the improvement in bronchial hyperresponsiveness, FENO values and mast cell activation is not accompanied by improved QOL. Although QOL seemed to be normal from the beginning of the study, it could well have been that, as a result of adaptation, subjects mistakenly considered themselves in excellent condition, which could have changed after therapy. We previously reported that the perception of acute onset airflow obstruction was intact in subjects in clinical remission of asthma.³ Presumably, slow changes in the degree of airway inflammation and remodeling are not well perceived by asthmatic subjects. Especially in patients with asthma in clinical remission or with mild asthma, this has important implications for the way we make treatment decisions, as the presence of symptoms is obviously not a sensitive indicator of ongoing disease.

The clinical implications of our study seem to be rather complicated. It can be argued that we have just investigated "bad perceivers", but it must be kept in mind that the study subjects did not use any medication, did not visit a doctor, and were convinced that they had outgrown their asthma for many years, meaning that nothing would have done with them if this study was not undertaken. We could demonstrate that the effects of our intervention on hyperresponsiveness, FENO and tissue tryptase were significant and, since these variables reflect airway inflammation, might be beneficial to the patient. Any effect on the long-term prognosis is, however, unclear. At now, it has not been demonstrated that aggressive anti-inflammatory treatment during clinical remission has advantages over a "wait and see" strategy. However, a slow but steady decline in lung function during clinical remission could possibly be prevented with, perhaps low-dose, anti-inflammatory therapy. Furthermore, it is questionable whether subjects who have no subjective benefit from treatment will continue to take prescribed inhaled steroids for an unspecified time, since adherence with treatment is already a worry in patients with symptomatic asthma. However, if future studies demonstrate that prolonged anti-inflammatory treatment will positively alter the long-term prognosis in subjects in clinical remission of asthma, treatment might be justified and can be discussed with the patient. Also, a positive effect should counterbalance occurring side effects of prolonged therapy.

In conclusion, we found that 3 months of treatment with fluticasone propionate and salmeterol reduced airway hyperresponsiveness, FENO and tryptase density in the airway mucosa as

markers of airway inflammation. MBP density in the airway mucosa and QOL were, however, unchanged. The clinical relevance of these findings, especially with respect to the long-term outcome, has not been determined yet.

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References

- Barbee RA, Murphy S. The natural history of asthma. *J Allergy Clin Immunol* 1998;**102**:S65–72.
- Panhuysen CI, Vonk JM, Koeter GH, et al. Adult patients may outgrow their asthma: a 25-year follow-up study. *Am J Respir Crit Care Med* 1997;**155**:1267–72.
- Van den Toorn LM, Overbeek SE, De Jongste JC, et al. Airway inflammation is present during clinical remission of atopic asthma. *Am J Respir Crit Care Med* 2001;**164**:2107–13.
- Warke TJ, Fitch PS, Brown V, et al. Outgrown asthma does not mean no airways inflammation. *Eur Respir J* 2002;**19**: 284–7.
- Vignola AM, Gagliardo R, Siena A, et al. Airway remodeling in the pathogenesis of asthma. *Curr Allergy Asthma Rep* 2001;**1**:108–15.
- Barnes PJ. Current issues for establishing inhaled corticosteroids as the antiinflammatory agents of choice in asthma. *J Allergy Clin Immunol* 1998;**101**:S427–33.
- Boushey HA. Effects of inhaled corticosteroids on the consequences of asthma. *J Allergy Clin Immunol* 1998;**102**:S5–S16.
- Vanacker NJ, Palmans E, Kips JC, et al. Fluticasone inhibits but does not reverse allergen-induced structural airway changes. *Am J Respir Crit Care Med* 2001;**163**:674–9.
- Hoshino M, Takahashi M, Takai Y, et al. Inhaled corticosteroids decrease subepithelial collagen deposition by modulation of the balance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 expression in asthma. *J Allergy Clin Immunol* 1999;**104**:356–63.
- Orsida BE, Ward C, Li X, et al. Effect of a long-acting beta2-agonist over three months on airway wall vascular remodeling in asthma. *Am J Respir Crit Care Med* 2001;**164**:117–21.
- Ward C, Pais M, Bish R, et al. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. *Thorax* 2002;**57**:309–16.
- De Kluijver J, Evertse CE, Schrupf JA, et al. Asymptomatic worsening of airway inflammation during low-dose allergen exposure in asthma: protection by inhaled steroids. *Am J Respir Crit Care Med* 2002;**166**:294–300.
- Laitinen A, Altraja A, Kampe M, et al. Tenascin is increased in airway basement membrane of asthmatics and decreased by an inhaled steroid. *Am J Respir Crit Care Med* 1997;**156**:951–8.
- Fahy JV, Corry DB, Boushey HA. Airway inflammation and remodeling in asthma. *Curr Opin Pulm Med* 2000;**6**:15–20.
- Jeffery PK, Venge P, Gizycki MJ, et al. Effects of salmeterol on mucosal inflammation in asthma: a placebo-controlled study. *Eur Respir J* 2002;**20**:1378–85.
- Li X, Ward C, Thien F, et al. An antiinflammatory effect of salmeterol, a long-acting beta(2) agonist, assessed in airway biopsies and bronchoalveolar lavage in asthma. *Am J Respir Crit Care Med* 1999;**160**:1493–9.
- Wallin A, Sandstrom T, Soderberg M, et al. The effects of regular inhaled formoterol, budesonide, and placebo on mucosal inflammation and clinical indices in mild asthma. *Am J Respir Crit Care Med* 1999;**159**:79–86.
- Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;**136**:225–44.
- Rosati G, Hargreave FE, Ramsdale EH. Inhalation of adenosine 5'-monophosphate increases methacholine airway responsiveness. *J Appl Physiol* 1989;**67**:792–6.
- Van den Toorn LM, Prins JB, Overbeek SE, et al. Adolescents in clinical remission of atopic asthma have elevated exhaled nitric oxide levels and bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 2000;**162**:953–7.
- Birnie D, thoe Schwartzberg GW, Hop WC, et al. Does the outcome of the tidal breathing and dosimeter methods of assessing bronchial responsiveness in children with asthma depend on age? *Thorax* 1990;**45**:199–202.
- Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations. The European respiratory society task force. *Eur Respir J* 1997;**10**:1683–93.
- Sullivan P, Stephens D, Ansari T, et al. Variation in the measurements of basement membrane thickness and inflammatory cell number in bronchial biopsies. *Eur Respir J* 1998;**12**:811–5.
- Brook RH, Ware Jr JE, Davies-Avery A, et al. Overview of adult health measures fielded in Rand's health insurance study. *Med Care* 1979;**17**(III-X):1–131.
- Van Schoor J, Joos GF, Pauwels RA. Indirect bronchial hyperresponsiveness in asthma: mechanisms, pharmacology and implications for clinical research. *Eur Respir J* 2000;**16**:514–33.
- Van den Berge M, Kerstjens HA, Meijer RJ, et al. Corticosteroid-induced improvement in the PC20 of adenosine monophosphate is more closely associated with reduction in airway inflammation than improvement in the PC20 of methacholine. *Am J Respir Crit Care Med* 2001;**164**: 1127–32.
- Koh YY, Sun YH, Lim HS, et al. Effect of inhaled budesonide on bronchial hyperresponsiveness in adolescents with clinical remission of asthma. *Chest* 2001;**120**:1140–6.
- Payne DN, Adcock IM, Wilson NM, et al. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med* 2001;**164**:1376–81.
- Warke TJ, Fitch PS, Brown V, et al. Exhaled nitric oxide correlates with airway eosinophils in childhood asthma. *Thorax* 2002;**57**:383–7.
- Mattes J, Storm van's Gravesande K, Reining U, et al. NO in exhaled air is correlated with markers of eosinophilic airway inflammation in corticosteroid-dependent childhood asthma. *Eur Respir J* 1999;**13**:1391–5.
- Lim S, Jatakanon A, Meah S, et al. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in mild to moderately severe asthma. *Thorax* 2000;**55**: 184–8.
- Colice GL. Categorizing asthma severity and monitoring control of chronic asthma. *Curr Opin Pulm Med* 2002;**8**:4–8.

33. Kharitonov SA, Barnes PJ. Clinical aspects of exhaled nitric oxide. *Eur Respir J* 2000;**16**:781–92.
34. van Rensen EL, Straathof KC, Veselic-Charvat MA, et al. Effect of inhaled steroids on airway hyper-responsiveness, sputum eosinophils, and exhaled nitric oxide levels in patients with asthma. *Thorax* 1999;**54**: 403–8.
35. Page S, Ammit AJ, Black JL, et al. Human mast cell and airway smooth muscle cell interactions: implications for asthma. *Am J Physiol Lung Cell Mol Physiol* 2001;**281**: L1313–23.
36. Sommerhoff CP. Mast cell tryptases and airway remodeling. *Am J Respir Crit Care Med* 2001;**164**:S52–8.
37. McDonald DM. Angiogenesis and remodeling of airway vasculature in chronic inflammation. *Am J Respir Crit Care Med* 2001;**164**:S39–45.
38. Johnson M. The anti-inflammatory profile of fluticasone propionate. *Allergy* 1995;**50**:11–4.