

Effect of inhaled beclomethasone dipropionate on isocapnic hyperventilation with cold air in asthmatics, measured with forced oscillation technique

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Effect of inhaled beclomethasone dipropionate on isocapnic hyperventilation with cold air in asthmatics, measured with forced oscillation technique. H.J. Pennings, E.F.M. Wouters. ©ERS Journals Ltd 1997.

ABSTRACT: Isocapnic hyperventilation with cold air (IHCA) is a reliable technique for assessing indirect bronchial hyperresponsiveness in patients with asthma. Impedance measurement of the respiratory system by the forced pseudorandom noise oscillation technique is a sensitive technique to assess changes in bronchial tone after IHCA. The aim of this study was to evaluate the effect of 6 weeks of treatment with beclomethasone dipropionate, 1,000 µg·day⁻¹, on IHCA in asthmatic patients, measured with both forced oscillation technique and flow-volume recordings.

Forty patients with mild asthma were included in this double-blind, placebo-controlled parallel-group study. Stratification on the basis of sex was performed to overcome differences in airway diameter. At entry and every 2 weeks during the treatment period, IHCA was performed and patient diaries were evaluated.

Characteristic changes in forced oscillation parameters after IHCA were observed in all patients. After 6 weeks of treatment, BDP-treated patients showed statistically significant differences in impedance measurements after IHCA, manifested by significant attenuation of resistance at 8 Hz ($p < 0.01$), slope of the frequency-resistance curve ($p < 0.01$), reactance at 8 Hz ($p = 0.01$), and resonant frequency (f_0) ($p < 0.02$). Flow-volume recordings showed only a statistically significant change in the decrease of inspiratory vital capacity (IVC) ($p = 0.01$). Furthermore, a significant correlation was observed between serum immunoglobulin E (IgE) levels and the effect of BDP on IHCA, measured with forced oscillation technique.

In this study, beclomethasone dipropionate, 1,000 µg·day⁻¹ for 6 weeks, decreased indirect bronchial hyperresponsiveness as assessed by cold air bronchoprovocation in asthmatic patients. The forced oscillation technique proved a more sensitive method of detecting changes in bronchial tone than flow-volume recordings.
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Asthma is characterized by an increased responsiveness of the tracheobronchial tree to a variety of specific and nonspecific stimuli. The degree of bronchial hyperresponsiveness (BHR), as assessed by methacholine and histamine challenge, is closely related to the severity of asthma [1]. BHR is considered to occur as a result of inflammation in the asthmatic airways [2]. Even in very mild asthmatic patients an inflammatory process is present in the airways [3, 4]. Inhaled corticosteroids improve asthma symptoms, decrease airway inflammation [5], and reduce the level of BHR [6–9]. However, despite distinct improvement of asthma symptoms by inhaled corticosteroids, only moderate changes in BHR, as assessed by histamine and methacholine challenge, are observed. Moreover, histamine thresholds in patients with asthma show considerable overlap with the general population [10]. Therefore, besides direct bronchial challenge tests, techniques have been developed which influence bronchial smooth muscle indirectly in order to better represent natural circumstances.

Isocapnic hyperventilation with cold air (IHCA) is a reliable and well-standardized method for evaluating indirect bronchial hyperresponsiveness [11–13]. In comparison to pharmacological stimuli, bronchoprovocation with IHCA is better able to distinguish asthmatic patients from the general population [11], and from patients with COPD [14, 15]. Most bronchoprovocation tests evaluate the response in bronchial tone by measuring the forced expiratory volume in one second (FEV₁). However, the inspiratory manoeuvre which precedes the measurement of FEV₁ may change bronchial tone and, therefore, influences the result of bronchoprovocation testing, both in normal and asthmatic subjects [7, 16]. Impedance measurement of the respiratory system by forced oscillation is a technique which enables evaluation of bronchial hyperresponsiveness both with direct [17–19] and indirect stimuli [20–22]. The technique requires little co-operation from the patient and allows evaluation of the bronchial response during quiet breathing.

The aim of this study was to evaluate the effect of inhaled beclomethasone dipropionate on bronchial provocation with IHCA in stable asthmatic patients. In addition to flow-volume recordings, impedance measurement by forced oscillation technique (FOT) was used to evaluate bronchial response.

Material and methods

Subjects

Forty patients, equally distributed over both sexes, were included in the study. Patient characteristics are presented in table 1. The following inclusion criteria were met by all patients: a clinical diagnosis of asthma, defined as recurrent attacks of dyspnoea with perceptible wheezing, normal baseline impedance measurements as defined by a resonant frequency (f_0) lower than 16 Hz; and the absence of frequency dependence (defined as a negative value for (resistance at 28 Hz (R_{28}) - resistance at 8 Hz (R_8)/20) (table 2). Patients entering the study all had evidence of bronchial hyperresponsiveness, as defined by a provocative dose of histamine causing a 20% fall in FEV₁ (PD₂₀) ≤ 8 μ mol. All patients had positive skin tests for at least one aeroallergen (number of positive tests (mean \pm SD) 4.4 \pm 2.4). Patients were in a clinically stable condition and showed no evidence of a recent respiratory tract infection. None of the patients had been treated with inhaled or oral corticosteroids within 3 months prior to entry into the study. Only β_2 -sympathomimetic drugs were allowed for control of asthma. Patients were asked to refrain from using β_2 -

Table 1. - Subject characteristics, anthropometric and spirometric data

	BDP group	Placebo group
Sex M/F	10/10	10/10
Age yrs	29 \pm 7	28 \pm 6
Height cm	172 \pm 7	172 \pm 12
Weight kg	75 \pm 12	74 \pm 14
FEV ₁ % pred	101 \pm 12 ⁺	92 \pm 12
IVC % pred	107 \pm 13	103 \pm 11
PD ₂₀ μ mol	1.3 \pm 1.3	1.1 \pm 1.9

Values are presented as mean \pm SD. BDP: beclomethasone dipropionate; M: male; F: female; FEV₁: forced expiratory volume in one second; IVC: inspiratory vital capacity; PD₂₀: provocative dose of histamine producing a 20% fall in FEV₁. ⁺: p=0.02, compared to placebo group.

Table 2. - Forced oscillation parameters before and after cold air bronchoprovocation at entry

	BDP		Placebo	
	Before IHCA	After IHCA	Before IHCA	After IHCA
R_8 kPa·s·L ⁻¹	0.314 \pm 0.082	0.650 \pm 0.200**	0.363 \pm 0.074	0.679 \pm 0.201**
R_{28} kPa·s·L ⁻¹	0.352 \pm 0.085	0.418 \pm 0.070**	0.377 \pm 0.081	0.430 \pm 0.102**
FD kPa·s·L ⁻¹	0.002 \pm 0.002	-0.012 \pm 0.008**	0.001 \pm 0.003	-0.012 \pm 0.007**
X_8 kPa·s·L ⁻¹	-0.012 \pm 0.024	-0.274 \pm 0.248**	-0.014 \pm 0.038	-0.273 \pm 0.241**
f_0 Hz	9.1 \pm 1.7	28.6 \pm 10.1**	10.3 \pm 3.4	29.3 \pm 7.9**

Values are presented as mean \pm SD. BDP: beclomethasone dipropionate; R_8 : resistance at 8 Hz; R_{28} : resistance at 28 Hz; FD: frequency dependence of resistance ((R_{28} - R_8)/20); X_8 : reactance at 8 Hz; f_0 : resonant frequency. **: p<0.01, within group comparison before and after IHCA. At entry, no statistically significant differences were observed between groups.

sympathomimetic drugs at least 8 h before broncho-provocation testing. The protocol was approved by the Ethics Committee of the University Hospital Maastricht, and written informed consent was obtained from all patients.

Study design

The study was a double-blind, placebo-controlled, parallel-group study, in which subjects were randomly allocated to receive either beclomethasone dipropionate (BDP), 250 μ g·puff⁻¹, 2 puffs *b.i.d.* by metered-dose inhaler or placebo for 6 weeks. In order to avoid possible sex-related differences in airway diameter, additional stratification was performed according to gender. The time schedule consisted of a run-in period of 1 week, followed by a treatment period of 6 weeks, with follow-up visits scheduled every 2 weeks. At every visit, diary information and peak flow registrations were assessed and cold air bronchoprovocation was performed.

Asthma assessment

During the study period, assessment of asthma severity was made in three ways: 1) all patients were given a diary, in which they recorded asthma symptom scores (0=no complaints to 5=major interference with daily activities, inability to work); 2) the use of β_2 -sympathomimetic drugs was recorded (patients were instructed to use these only if needed); and 3) patients recorded peak expiratory flow rates (three successive measurements) in the morning and evening using a mini-Wright peak-flow meter (Airmed, Clement Clarke International Ltd, London, UK).

Cold air challenge

Cold air challenges (IHCA) were performed using a heat-exchange system (Jaeger GmbH, Würzburg, FRG), as described previously [21, 22]. The patients inhaled dry air delivered from a cylinder. The temperature of the air leaving the cooling system was -20°C. The flow of air could be adjusted by a needle valve and was measured by a rotameter. Patients were instructed to breathe at a predetermined ventilation rate of 60% of the predicted indirect maximal breathing capacity (defined as 35 \times FEV₁) by maintaining the size of a guide balloon. To avoid hypocapnia, CO₂ was added to the system at a rate of 5% of the predetermined minute ventilation [12].

Hyperventilation was sustained for 3 min, after quiet breathing into the system for 1 min. Prior to cold air bronchoprovocation, three successive measurements of the respiratory impedance by forced oscillations were performed, followed by flow-volume measurements using a pneumotachograph (Masterlab; Jaeger GmbH, Würzburg, FRG). Three minutes after cold air bronchoprovocation, measurements were repeated in the same order.

Forced oscillation technique

The technique used in this study is similar to the method developed by LÁNDSEER *et al.* [23], and has been published previously [18, 21, 22]. In short, a complex pseudorandom noise oscillation signal, containing various harmonics in steps of 4 Hz ranging 4–52 Hz, was applied at the mouth during spontaneous quiet breathing. Cheeks and mouth floor were supported by the hands of the patient. Mouth pressure and flow rate were recorded by transducers (MP45®; Validyne, Northridge, CA, USA), and the recorded signals were fed directly into a Fourier analysing system, calculating impedance values for each frequency applied. The impedance value can be divided into a resistance (R) and a reactance (X). The total resistance of the respiratory system (R_{rs}) is the sum of resistance of central airways, peripheral airways, lung parenchyma and chest wall. In normal individuals, the resistance increases with increasing frequency of the signal applied [18, 20]. The slope of the curve is defined by $(R_{28} - R_8)/20$ and is called the frequency dependence of resistance (FD) [18, 21, 22].

The reactance value depends on the elastic and the inertial properties of the system. The frequency at which the sum of elastic and inertial properties equals zero is called the resonant frequency (f_0). For this study, resistance was measured at 8 Hz (R_8), at 28 Hz (R_{28}), and frequency dependence of resistance (FD). The reactance of the impedance was characterized by reactance at 8 Hz (X_8) and the resonant frequency (f_0). Only impedance values with a coherence function (comparable with a signal-to-noise-ratio) equal to or greater than 0.95 were used for analysis.

Data analysis

All data described are mean \pm SD, unless otherwise specified. Differences between groups were compared using Mann-Whitney U-test. In order to evaluate differences between the two treatment regimens, repeated measures analysis of variance (ANOVA) (BMDP Statistical Software Inc., Los Angeles, CA, USA) was performed; this statistical method allows comparison of individual responses and compensates for baseline response differences. The model used in this study assumes a fixed effect of treatment in time. Differences were considered significant at a p-value less than 0.05 (two-sided).

Results

Thirty five of the 40 patients completed the study. In the placebo group, three patients withdrew, one patient at 2 weeks, and two patients at 4 weeks, because of

lack of co-operation. In the beclomethasone dipropionate group, two patients were excluded from the study at 6 weeks of treatment; one patient due to inability to attend the follow-up visit in time. The other patient had to be treated with oral corticosteroids for generalized eczema, which she had also experienced before the study. All data up to withdrawal have been included in the analysis.

Patient characteristics of both groups are presented in table 1. At entry, the BDP-treated group showed a higher baseline FEV₁ than the placebo group (101 *versus* 92% pred; $p < 0.02$). Baseline values for forced oscillation parameters (table 2) were not statistically different between groups, although a slightly lower value for R_8 was observed in the BDP group than in the placebo group (0.314 *versus* 0.363 kPa·s·L⁻¹; NS). IHCA resulted in similar changes in both groups. FEV₁ decreased (mean \pm SD) 18 \pm 15% pred from baseline in the BDP group and 19 \pm 14% in the placebo group. Impedance measurements after cold air bronchoprovocation yielded a similar change in forced oscillation pattern in both groups: a large increase in R_8 , a small increase in R_{28} , the occurrence of a negative slope of the resistance-frequency curve, a more negative value of X_8 and an increase in f_0 (table 2). At entry, no statistically significant differences in flow-volume and forced oscillation changes were observed between groups after IHCA.

Baseline impedance and flow-volume measurements did not change significantly in either treatment group during 6 weeks of treatment. After 6 weeks of beclomethasone dipropionate treatment, statistically significant changes were observed in forced oscillation response to IHCA (fig. 1a–d). Treatment with beclomethasone dipropionate resulted in: a diminished increase of R_8 after IHCA (at entry (mean \pm SD) 0.336 \pm 0.174 kPa·s·L⁻¹; after 6 weeks 0.250 \pm 0.160 kPa·s·L⁻¹ ($p = 0.006$; -26% baseline)); a decrease in steepness of the slope (FD) of the resistance-curve after IHCA (at entry -0.013 \pm 0.009 kPa·s·L⁻¹; at 6 weeks -0.009 \pm 0.006 ($p = 0.001$; -31% baseline)); a decrease in X_8 changes after IHCA (at entry -0.263 \pm 0.246 kPa·s·L⁻¹; after 6 weeks -0.155 \pm 0.105 ($p = 0.012$; -41% baseline)); and a decrease in change of f_0 after IHCA (at entry 19.5 \pm 10.1 Hz; after 6 weeks 13.3 \pm 9 Hz ($p = 0.015$; -32% baseline)).

In contrast, flow-volume measurements only yielded a statistically significant change in inspiratory vital capacity (IVC) after IHCA (at entry -6.1 \pm 8.0% pred; after 6 weeks -3.1 \pm 3.4% pred ($p = 0.01$)). No significant changes were observed in other parameters (fig. 2).

Levels of total serum immunoglobulin E (IgE) were found to be significantly correlated with the level of response to BDP as measured by FOT (Spearman rank correlation for changes in R_8 after 6 weeks BDP in comparison to entry ($r = -0.80$; $p < 0.001$); for changes in frequency dependence ($r = 0.81$; $p < 0.001$); for changes in X_8 ($r = -0.68$; $p < 0.01$) and for changes in f_0 ($r = -0.88$; $p < 0.001$) (fig. 3). No significant correlations were found for total numbers of eosinophils and histamine PD₂₀ levels and response to inhaled steroids in this patient group.

No clinically significant differences were observed in asthma symptom score, peak flow registration and peak flow variability between groups both at entry and after 6 weeks. Adverse effects were reported by six patients in the BDP-treated group: complaints of a sore throat

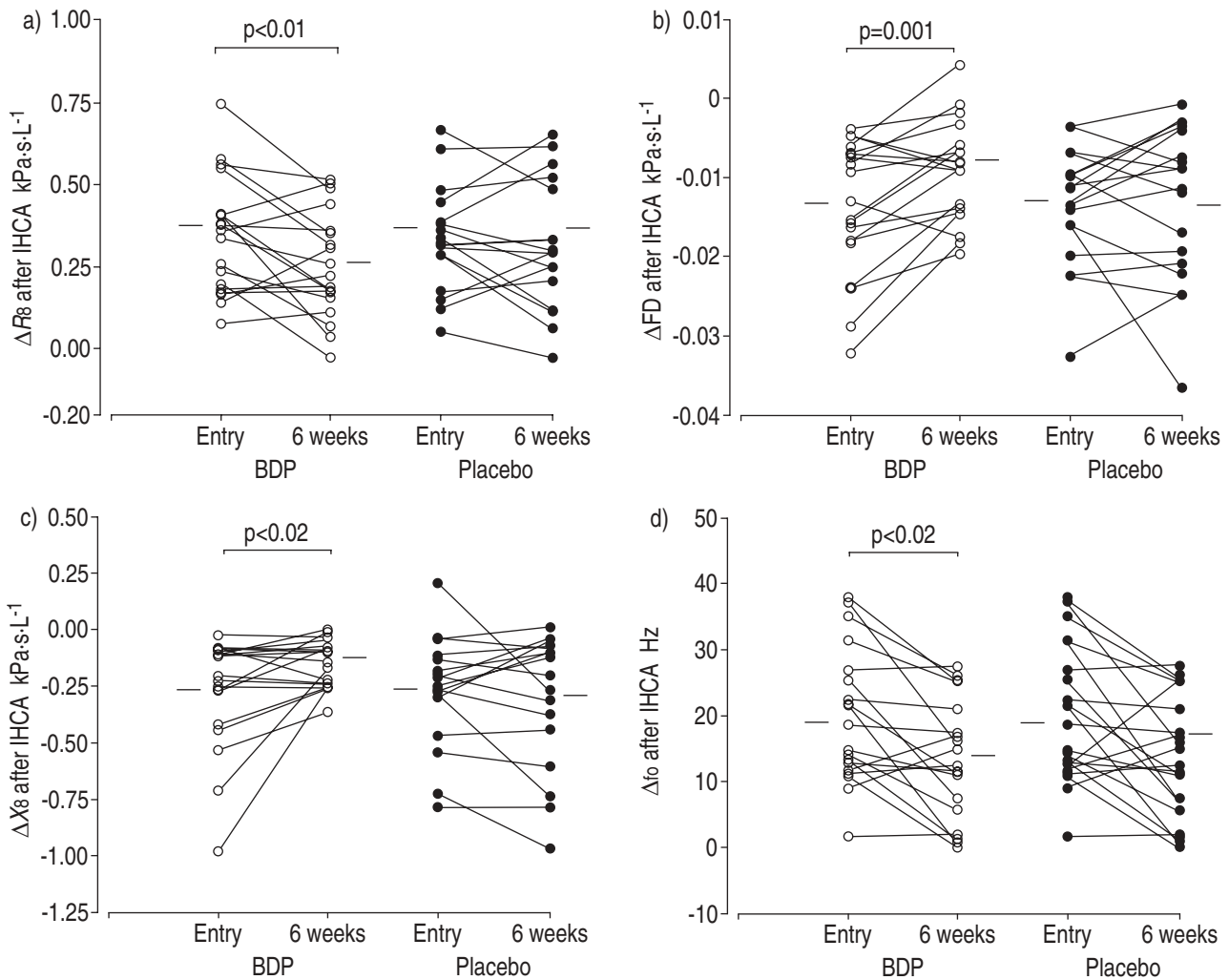


Fig. 1. — Changes in: a) resistance at 8 Hz (ΔR_8); b) frequency dependence of resistance ($(R_{28}-R_8)/20$) (ΔFD); c) reactance at 8 Hz (ΔX_8); and d) resonant frequency (Δf_0) after isocapnic hyperventilation with cold air (IHCA) for individual patients, both at entry and after 6 weeks follow-up. Treatment with beclomethasone dipropionate (BDP) during 6 weeks resulted in statistically significant changes for all parameters shown. Horizontal bars represent the mean value.

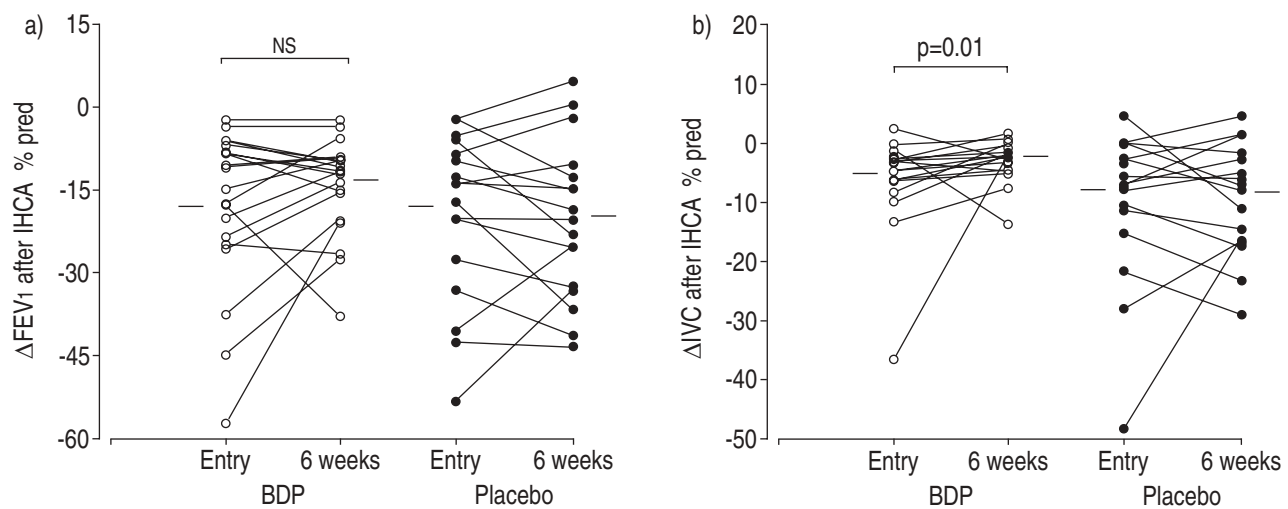


Fig. 2. — Changes in: a) forced expiratory volume in one second (ΔFEV_1); and b) inspiratory vital capacity (ΔIVC) after isocapnic hyperventilation with cold air (IHCA) for individual patients. Figures show data obtained at entry and after 6 weeks follow-up. Treatment with beclomethasone dipropionate (BDP) resulted in a small change in the fall of IVC after IHCA. No statistically significant effect was observed for FEV1. NS: nonsignificant. Horizontal bars represent the mean value.

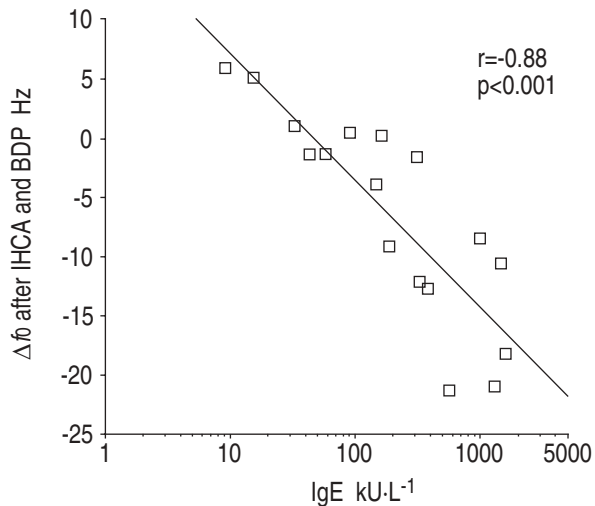


Fig. 3. — A significant correlation was observed for immunoglobulin E (IgE) levels at entry and changes in forced oscillation parameters following isocapnic hyperventilation with cold air (IHCA) after 6 weeks treatment with beclomethasone dipropionate (BDP). The Y-axis indicates the IHCA-induced Δf_0 after 6 weeks treatment with BDP in comparison to baseline response. The figure shows a significant correlation between IgE levels and beclomethasone-induced Δf_0 ($r=-0.88$; $p<0.001$, Spearman rank correlation). Note that IgE levels are shown on a logarithmic scale.

(2 patients); hoarseness (2); and dry cough (1); and one patient was suspected of oral candidiasis. However, no smear was made to evaluate the presence of oral candidiasis. In the placebo group, a dry irritant cough was reported by one patient, and two patients complained of a sore throat.

Discussion

In this study, inhaled beclomethasone dipropionate (BDP) reduced bronchial hyperresponsiveness in stable mild asthmatic patients, as assessed by IHCA. The FOT was more sensitive in detecting BDP-induced changes in bronchial tone after IHCA in comparison to conventional flow-volume recording. Furthermore, a high correlation was observed between serum IgE levels and the effect of BDP on IHCA, as measured by forced oscillation technique.

At the present time, inhaled corticosteroids play an important role in the treatment of asthma. Inhaled corticosteroids reduce BHR to pharmacological stimuli in a dose- and time dependent way [7–9, 24]. This report is the first to discuss the influence of inhaled BDP on the bronchial challenge with IHCA in asthmatic patients assessed both with FOT and conventional pulmonary function testing. Impedance measurement of the respiratory system was chosen to overcome modulation in bronchial tone, which may result from a preceding deep inspiratory manoeuvre [7, 16]. In the present study, a characteristic pattern of changes after IHCA was observed in forced oscillation parameters; an increase of resistance at lower frequencies, the development of a negative slope of resistance, and an increase in resonant frequency. This pattern of changes is characteristic for asthmatic patients, and these results concur with previous studies [20–22].

The present study showed that BDP, $1,000 \mu\text{g}\cdot\text{day}^{-1}$ for 6 weeks, significantly attenuated the response to IHCA assessed with the FOT, whereas only small changes in FEV₁ response to IHCA were observed. From the literature, one would expect an effect of BDP on change in FEV₁ after IHCA, but considering the large variability in FEV₁ changes after IHCA detected in this patient population, it is likely that the number of patients in the study was not high enough to detect differences in FEV₁. No changes were detected in asthma symptom scores or PEF scores in the BDP-treated group, but this is not surprising considering the fact that all of the patients had mild asthma. Furthermore, it appeared that the patients treated with BDP were the patients with the better values of FEV₁.

So far, no other studies have been published using FOT in response measurements of corticosteroids in IHCA. Several reports have been published regarding the influence of long-term treatment with inhaled corticosteroids on exercise-induced asthma [25–27], and on eucapnic hyperventilation with room air [28, 29]. Only one study has evaluated the effect of an inhaled corticosteroid on IHCA [30]. In this study, budesonide, $800 \mu\text{g}\cdot\text{day}^{-1}$ for 4 weeks, induced a small but significant change in FEV₁ (-8.5%) after IHCA in asthmatic patients. Other studies [27, 29] have observed larger effects of inhaled corticosteroids in exercise-induced asthma; however, in these studies baseline FEV₁ was clearly lower and BHR more severe than in the present patient group. Apart from the fact that the response to exercise in asthmatics is correlated with the severity of BHR as assessed by methacholine or histamine [31], the effect of pharmacological intervention also depends upon the basic level of bronchial reactivity.

In the present study, the FOT detected changes in airway bronchial tone that were not detected by conventional flow-volume recordings. The superiority of FOT in detecting differences in bronchial tone in comparison to conventional flow-volume recordings was noted previously. WESSELING and WOUTERS [21] noted differences in forced oscillation recordings in IHCA at 40% maximum breathing capacity, whereas no difference was observed in FEV₁. VAN NOORD *et al.* [19] described the reciprocal value of the resistance at 6 Hz as a more sensitive parameter for detection of histamine-induced bronchoconstriction in asthmatics than FEV₁ measurement in response evaluation. Only measurement of specific airways conductance ($sGaw$) proved more sensitive in patients with more or less normal lung function.

Furthermore, the present study also observed that the effect of BDP on IHCA, as assessed by FOT, was significantly correlated with total serum IgE. Patients with high levels of IgE showed a greater response to treatment with BDP than patients with lower levels. These findings concur with a previous study, wherein response to inhaled corticosteroids in asthmatic patients, measured by changes in histamine PC₂₀, was also correlated with total serum IgE levels [32].

Up till now, several mechanisms have been proposed to explain airway obstruction induced by exercise, *i.e.* hyperventilation with cold air [33]. Inhaled corticosteroids could influence response to cold air challenge by reducing plasma exudation in asthmatic airways [34] or by modulating secondary mediator release by inflammatory

cells. Bronchial biopsy studies have shown that even in mild asthmatic patients inflammatory cells are present in the airways [5]. BDP was shown to reduce the number of eosinophils and mast cells in the bronchial mucosa of asthmatic patients after 6 weeks of treatment [35]. In the present study, however, no bronchial biopsies were performed and, therefore, no answer can be provided regarding the mechanism by which BDP influences BHR in this study.

In conclusion, this study has demonstrated that treatment with beclomethasone dipropionate, 1,000 $\mu\text{g}\cdot\text{day}^{-1}$ for 6 weeks, modulated bronchial response to isocapnic hyperventilation with cold air in patients with mild stable asthma. We have also shown that the technique of forced oscillation was more sensitive to change in bronchial tone than conventional flow-volume recordings, and represents a sensitive technique to assess pharmacological modulation of indirect bronchial hyperresponsiveness.

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References

- Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF. Bronchial hyperresponsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol* 1981; 68: 347-355.
- Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J Allergy Clin Immunol* 1989; 83: 1013-1026.
- Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis* 1985; 131: 599-606.
- Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989; 139: 806-817.
- Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a beta₂-agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomized, double-blind, parallel-group controlled trial. *J Allergy Clin Immunol* 1992; 90: 32-42.
- Juniper EF, Frith PA, Hargreave FE. Long-term stability of bronchial responsiveness to histamine. *Thorax* 1982; 37: 288-291.
- Bel EH, Timmers MC, Hermans J, Dijkman J, Sterk PJ. The long-term effects of nedocromil sodium and beclomethasone dipropionate on bronchial hyperresponsiveness to methacholine in nonatopic asthmatic subjects. *Am Rev Respir Dis* 1990; 141: 21-28.
- Juniper EF, Kline PA, Vanzieleghem MA, Ramsdale EH, O'Byrne PM, Hargreave FE. Effect of long-term treatment with an inhaled corticosteroid (budesonide) on airway hyperresponsiveness and clinical asthma in non-steroid-dependent asthmatics. *Am Rev Respir Dis* 1990; 142: 832-836.
- Kraan J, Koëter GH, Van der Mark ThW, et al. Dosage and time effects of inhaled budesonide on bronchial hyperreactivity. *Am Rev Respir Dis* 1988; 137: 44-48.
- Cockcroft DW, Berscheid BA, Murdock KY. Unimodal distribution of bronchial responsiveness to inhaled histamine in a random human population. *Chest* 1983; 5: 751-754.
- Deal EC, McFadden ER, Ingram RH, Breslin FJ, Jaeger JJ. Airway responsiveness to cold air and hyperpnea in normal subjects and in those with hay fever and asthma. *Am Rev Respir Dis* 1980; 121: 621-628.
- Assoufi BK, Dally MB, Newman-Taylor AJ, Denison DM. Cold air test: a simplified standard method for airway reactivity. *Bull Eur Physiopathol Respir* 1986; 22: 349-357.
- Tessier P, Cartier A, L'Archevêque J, Ghezzi H, Martin RR, Malo JL. Within- and between-day reproducibility of isocapnic cold air challenges in subjects with asthma. *J Allergy Clin Immunol* 1986; 78: 379-387.
- O'Byrne PM, Ramsdale EH, Hargreave F. Isocapnic hyperventilation for measuring airway hyperresponsiveness in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1991; 143: 1444-1445.
- Mönkhof M, Wönne R, Hofman D. Kaltluftprovokation als methode zur differentialdiagnose von astma bronchiale und chronischer bronchitis. *Pneumologie* 1991; 45: 899-902.
- Orehek J, Nicoli MM, Delpierre S, Beaupre A. Influence of the previous deep inspiration on the spirometric measurement of provoked bronchoconstriction in asthma. *Am Rev Respir Dis* 1981; 123: 269-272.
- Kabiraj MU, Rolf C, Simonsson BG. Drug-induced changes in airways obstruction reflected by forced expiratory flows and airway resistance measured with an oscillometric method using quiet breathing. *Respiration* 1981; 41: 90-95.
- Wouters EFM, Polko AH, Schouten HJA, Visser BF. Contribution of impedance measurement of the respiratory system to bronchial challenge tests. *J Asthma* 1988; 25(5): 259-267.
- Van Noord JA, Clement J, Van de Woestijne KP, Demedts M. Total respiratory resistance and reactance as a measurement of response to bronchial challenge with histamine. *Am Rev Respir Dis* 1989; 139: 921-926.
- Decramer M, Demedts M, Van de Woestijne KP. Isocapnic hyperventilation with cold air in healthy nonsmokers, smokers and asthmatic subjects. *Bull Eur Physiopathol Respir* 1984; 20: 237-243.
- Wesseling GJ, Wouters EFM. Respiratory impedance measurements in a dose-response study of isocapnic hyperventilation with cold air. *Respiration* 1992; 59: 259-264.
- Wesseling GJ, Vanderhoven-Augustin IMJ, Wouters EFM. Forced oscillation technique and spirometry in cold air provocation tests. *Thorax* 1993; 48: 254-259.
- Lándsér FJ, Nagels J, Demedts M, Billiet L, Van de Woestijne KP. A new method to determine frequency characteristics of the respiratory system. *J Appl Physiol* 1976; 41: 101-106.
- Vathen AS, Knox AJ, Wisniewski A, Tattersfield AE. Time course of change in bronchial reactivity with an inhaled corticosteroid in asthma. *Am Rev Respir Dis* 1991; 143: 1317-1321.
- Waalkens HJ, Van Essen-Zandvliet EEM, Gerritsen J, Duiverman EJ, Kerrebijn KF, Knol K. The effect of an inhaled corticosteroid (budesonide) on exercise-induced asthma in children. *Eur Respir J* 1993; 6: 652-656.
- Henriksen OM, Dahl R. Effects of inhaled budesonide alone and in combination with low-dose terbutaline in children with exercise-induced asthma. *Am Rev Respir Dis* 1983; 128: 993-997.

27. Molema J, Van Herwaarden CLA, Folgering HThM. Effects of long-term treatment with inhaled cromoglycate and budesonide on bronchial hyperresponsiveness in patients with allergic asthma. *Eur Respir J* 1989; 2: 308–316.
28. Wiebicke W, Jörres R, Magnussen H. Comparison of the effects of inhaled corticosteroids on the airway response to histamine, methacholine, hyperventilation and sulfur dioxide in subjects with asthma. *J Allergy Clin Immunol* 1990; 86: 915–923.
29. Vathenen AS, Knox AJ, Wisniewski A, Tattersfield AE. Effect of inhaled budesonide on bronchial reactivity to histamine, exercise and eucapnic dry air hyperventilation in patients with asthma. *Thorax* 1991; 46: 811–816.
30. Claussen M, Sill V. Beeinflussung der unspezifischen bronchialen Hyperreagibilität auf Kaltlufthyperventilation und Carbachol durch nedrocomil und budesonid. *Pneumologie* 1993; 47: 209–214.
31. Chatman M, Bleecker ER, Smith PL, Rosenthal RR, Mason P, Norman PS. A comparison of histamine, methacholine and exercise airway reactivity in normal and asthmatic subjects. *Am Rev Respir Dis* 1982; 126: 235–240.
32. Kerstjens HAM, Schouten JP, Brand PLP, Schoonbrood DFME, Sterk PJ, Postma DS and the Dutch CNSLD Study Group. Importance of total serum IgE for improvement in airways hyperresponsiveness with inhaled corticosteroids in asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1995; 151: 360–368.
33. McFadden ER, Gilbert IA. Exercise-induced asthma. *N Engl J Med* 1994; 330: 1362–1367.
34. Van de Graaf AA, Out TA, Roos CM, Jansen HM. Respiratory membrane permeability and bronchial hyperactivity in patients with stable asthma: effect of therapy with inhaled steroids. *Am Rev Respir Dis* 1991; 143: 362–366.
35. Djukanovic R, Wilson JW, Britten KM, *et al.* Effect of an inhaled corticosteroid on airway inflammation in asthma. *Am Rev Respir Dis* 1992; 145: 669–674.