

Respiratory pathophysiologic responses

β_2 -Adrenoceptor regulation and bronchodilator sensitivity after regular treatment with formoterol in subjects with stable asthma

Imran Aziz, MRCP,^a Ian P. Hall, MD,^b Lesley C. McFarlane, HNC,^a and
Brian J. Lipworth, MD^a Dundee, Scotland, and Nottingham, England

Objective: We have previously found that β_2 -adrenoceptor downregulation and bronchodilator desensitization to albuterol occurred at 36 hours after stopping regular treatment with twice daily salmeterol. In this study we have evaluated these same effects with formoterol given once or twice daily on a regular basis.

Methods: In a randomized, placebo-controlled, double-blind, double-dummy crossover study, 16 subjects with mild-to-moderate stable asthma (mean [SD] age, 32.5 [15.3] years; mean [SD] FEV₁, 73.2 [12.1] percent predicted) receiving regular inhaled corticosteroid therapy received 1 week of treatment with formoterol dry powder (24 μ g twice daily [8 AM/8 PM]), formoterol (24 μ g once daily [8 PM]), or identical placebo. Lymphocyte β_2 -adrenoceptor parameters and a dose-response curve to inhaled albuterol (200 to 1600 μ g) were evaluated at 36 hours after the last dose of each treatment period.

Results: There were no significant differences in the mean values for albuterol dose-response effects among the three treatment regimens. Comparison of maximal bronchodilator responses between treatments (mean and SEM as change from baseline) revealed no significant differences between treatments for FEV₁ (0.47 L [0.06 L] for placebo vs 0.48 L [0.07 L] for 24 μ g once daily formoterol vs 0.51 L [0.08 L] for 24 μ g twice daily formoterol) or for forced expiratory flow, mid-expiratory phase (FEF₂₅₋₇₅) (0.80 L/sec [0.12 L/sec] for placebo vs 0.80 L/sec [0.16 L/sec] for 24 μ g once daily formoterol vs 0.89 L/sec [0.14 L/sec] for 24 μ g twice daily formoterol). Formoterol also had no significant effect on mean lymphocyte β_2 -adrenoceptor density. However, in five of seven patients with the homozygous Gly-16 polymorphism, β_2 -adrenoceptor density was downregulated by twice daily formoterol, whereas only two such cases exhibited a reduction in maximal FEV₁ response to albuterol.

Conclusions: The results of this study showed that for patients taking inhaled corticosteroids, overall β_2 -adrenoceptor regulation and associated bronchodilator sensitivity to inhaled albuterol were unaltered at 36 hours after stopping regular treatment with formoterol. However, in a subset of patients who were Gly-16 homozygous, there was a tendency towards downregulation but not desensitization. Further studies in subjects with more severe asthma are required to assess the clinical relevance of these findings. (J Allergy Clin Immunol 1998;101:337-41.)

Key words: Desensitization, β_2 -adrenoceptor, asthma, formoterol, albuterol

In a previous study from this laboratory, we have found evidence of β_2 -adrenoceptor downregulation and bronchodilator desensitization to albuterol at 36 hours after stopping treatment with 50 μ g twice daily salmeterol.¹ Other studies with regular 24 μ g twice daily formoterol have shown that β_2 -adrenoceptor downregulation occurred for up to 24 hours after stopping regular treatment and was associated with a rightward shift of bronchodilator response to repeated doses of formoterol.^{2,3} These studies were criticized for using formoterol to construct the dose-response curve, because it is more likely that albuterol would be used for the purpose of acute reliever therapy.

This study was therefore designed to evaluate whether formoterol is associated with β_2 -adrenoceptor downregulation and bronchodilator desensitization to albuterol at 36 hours after stopping regular treatment. Another aspect of this study was to compare the effect of dosing frequency with formoterol on β_2 -adrenoceptor regulation and bronchodilator sensitivity, because a putative occupancy-free period with a 24-hour dosing interval (i.e., once daily dosing) might conceivably allow the β_2 -adrenoceptor to recover as compared with a 12-hour interval (i.e., twice daily dosing). An inclusion criteria for the study was for patients to be receiving inhaled corticosteroids to reflect the current international guidelines for use of long-acting β_2 -agonists.⁴

METHODS

Patients

Sixteen asthmatic subjects (eight male and eight female) (mean [SD] age, 32.5 years [15.3 years]) who were all taking inhaled corticosteroids (mean [SD] dose 800 μ g/day [664 μ g/day]) were recruited to take part in the study. All the

From ^athe Department of Clinical Pharmacology and Therapeutics and Respiratory Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee; and ^bthe Department of Medicine, Division of Therapeutics, University Hospital of Nottingham, Nottingham.

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Reprint requests: Brian J. Lipworth, MD, Department of Clinical Pharmacology and Therapeutics and Respiratory Medicine, Ninewells Hospital and medical School, University of Dundee, Dundee DD1 9SY, Scotland, United Kingdom.

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*Abbreviation used*FEF₂₅₋₇₅: Forced expiratory flow, mid-expiratory phase

subjects were using inhaled short-acting β_2 -agonists on an as-required basis (<2 puffs/day) for relief of symptoms, five patients were using long-acting β_2 -agonists (three using salmeterol and two using formoterol), and three patients were also receiving oral theophylline. All had stable asthma of mild-to-moderate severity according to American Thoracic Society criteria⁵ for at least 3 months before taking part in the study, and none had received oral steroids during this time. All were required to have at least 15% reversibility in their FEV₁ or FEF₂₅₋₇₅. Baseline spirometry for the group showed mean (SD) FEV₁ values of 2.54 L (0.7 L), 73.2 (12.1) percent predicted; and FEF₂₅₋₇₅ 2.07 L/sec (0.9 L/sec), 49.1 (17.4) percent predicted. All gave written informed consent before being randomized into the study, which was approved by the Tayside committee for medical research ethics.

We also retrospectively performed a genotype analysis of our study patients in case we had inadvertently biased our random sample of patients with a particular β_2 -adrenoceptor polymorphism, which might have affected the results. This was done particularly because the homozygous Gly-16 polymorphism has been shown to be associated with in vivo bronchodilator desensitization as compared with the homozygous Arg-16 polymorphism.⁶ The analysis revealed, at codon 16, seven patients who were Gly-16 homozygous, eight patients who were Gly-16/Arg-16 heterozygous, and one patient who was Arg-16 homozygous. At codon 27, six patients were Glu-27 homozygous, six patients were Glu-27/Gln-27 heterozygous, and four patients were Gln-27 homozygous. All patients who were Glu-27 homozygous were also Gly-16 homozygous.

Protocol

A randomized, double-blind, double-dummy, crossover design was used. Subjects were randomized to receive 1 week of treatment with either 24 μ g inhaled twice daily formoterol (morning and evening), inhaled twice daily placebo, or inhaled placebo (morning) and 24 μ g inhaled formoterol (evening). Formoterol was delivered in dry powder capsules (12 μ g per capsule) (Foradil; eformoterol fumarate, Novartis). The doses were chosen to be within the manufacturer's recommended total daily dose range of 24 to 48 μ g/day. Placebo was delivered in capsules containing lactose. The patients were instructed to record their morning and evening peak expiratory flow rates with a Vitalograph Peak Flow Meter (Vitalograph Ltd., Buckingham, U.K.) immediately before taking their study medication.

The medication was taken between 7 PM and 9 PM for the evening dose and between 7 AM and 9 AM for the morning dose. The last dose of each treatment was taken in the evening. There was a 1-week washout period as a run-in before randomization and a 1-week washout period between each of the three randomized treatments. From the start of the run-in period until the end of the study, all β_2 -agonist therapy was stopped and was substituted by inhaled ipratropium bromide (Atrovent Forte; Boehringer Ingelheim) to be used for rescue relief of symptoms.

Albuterol dose-response curve

Subjects attended the laboratory between 8 AM and 10 AM, 36 hours after the last dose of each 1-week treatment period, having withheld their reliever inhaler (ipratropium) for at least 12 hours and oral theophylline for at least 48 hours. At each visit an intravenous cannula was inserted and kept patent with a bolus injection of heparinized saline. The cannula dead space of 2 ml was withdrawn and discarded before blood sampling. The subject was connected to a cardiac monitor to record heart rate.

After at least 30 minutes of supine rest, blood was withdrawn for lymphocyte β_2 -adrenoceptor markers and measurement of baseline serum potassium. A tracing of baseline heart rate was made by using lead II of the cardiac monitor. A dose-response curve to inhaled albuterol (Ventolin Accuhaler, albuterol sulphate, 200 μ g per actuation; Allen and Hanburys) was then constructed, with doses of 200 μ g, 200 μ g, 400 μ g, and 800 μ g albuterol (i.e., cumulative doses of 200 μ g, 400 μ g, 800 μ g, and 1600 μ g, respectively). The doses were separated by 15-minute intervals. Ten minutes after each dose, heart rate, serum potassium, and spirometry were measured over a 5-minute period.

Measurements

Spirometry. Spirometry was performed according to American Thoracic Society criteria⁷ by using a Vitalograph compact spirometer (Vitalograph Ltd., Buckingham, U.K.) with a pneumotachograph head and pressure transducer and on-line computer assisted determination of FEV₁, FEF₂₅₋₇₅, and peak expiratory flow. The subjects performed three forced expiratory maneuvers from total lung capacity to residual volume, and the best test value was used in the analysis. The spirometer was calibrated daily with a Vitalograph 1 L precision syringe. A coefficient of variation of less than 3% was considered acceptable for each visit.

Serum potassium. Serum was extracted and stored at -20°C until analysis at the end of the study. Plasma potassium was measured by flame photometry (IL 943 analyser, Instrumentation Laboratory Ltd), and the samples were analyzed in duplicate. The intraassay and interassay coefficients of variation for analytical imprecision were 0.93% and 0.79%, respectively. The normal reference range for serum potassium in our laboratory is 3.5 to 5.5 mmol/L.

Heart rate. Heart rate was measured from the electrocardiogram recorded from the standard lead II by Hewlett-Packard cardiac monitor and printer (Palo Alta, Calif.), with the paper speed set at 25 mm/sec. The heart rate was calculated from the R-R interval from the mean of five complexes.

Lymphocyte β_2 -adrenoceptor parameters. Evaluable data for receptor density (B_{max}) and receptor binding affinity dissociation constant (K_d) were only available on 15 of 16 cases because of a malfunction in the binding assay on one particular day (patient 6). Lymphocyte β_2 -adrenoceptor function was measured as previously described.⁸ In brief, B_{max} and K_d were evaluated by ligand binding with $(-)^{125}\text{I}$ -iodocyanopindolol. The maximal cyclic AMP response (E_{max}) was measured in response to stimulation with isoproterenol 10^{-4} mol.

Identification of β_2 -adrenoceptor polymorphisms. β_2 -Adrenoceptor polymorphisms were identified as previously described.⁹ In brief, genomic DNA was extracted from whole blood, and a 234 base pair fragment was generated by polymerase chain reaction, which spanned the regions of interest. Genotype was determined by allele-specific oligonucleotide hybridization with probes homologous for the Arg-16, Gly-16, Gln-27, or Glu-27 forms of the receptor.

TABLE I. Mean (SEM) baseline values (before dose-response curve) for airway and systemic β_2 -adrenoceptor parameters after each treatment period

	Placebo	F 24 μ g once daily	F 24 μ g twice daily
FEV ₁ (L)	2.62 (0.22)	2.55 (0.22)	2.54 (0.18)
FEF ₂₅₋₇₅ (L/sec)	2.36 (0.29)	2.29 (0.30)	2.12 (0.24)
PEF (L/min)	422 (33)	416 (28)	424 (29)
HR (beats/min)	67 (1)	67 (2)	68 (2)
K (mmol/l)	4.09 (0.07)	4.09 (0.08)	4.05 (0.07)

There were no significant differences among values.

F, Formoterol; PEF, peak expiratory flow; HR, heart rate; K, serum potassium.

Statistical analysis

All dose-response curve parameters were analyzed as change in response from baseline. Comparisons for the dose-response curve were made as maximal delta responses (D_{max}) and as the area under the curve. The lymphocyte β_2 -adrenoceptor parameters were analyzed for receptor density (B_{max}), receptor affinity (Kd), and maximal cAMP response (E_{max}) to isoproterenol. Comparisons were made by multifactorial analysis of variance followed by Bonferroni multiple-range testing by using subject, study treatment, albuterol dose, and period as factors for analysis. The study was powered at 90% with 16 subjects to detect a 0.3 L difference in FEV₁ and 0.3 mmol/L difference in potassium between placebo and active treatments and an α -error set at 0.05 (two tailed); 95% confidence intervals (95% CI) for mean differences were calculated. Data analysis was performed by using Statgraphics statistical analysis software package (STSC Software Publishing Group, Rockville, Md.). The data for individual responses according to β_2 -adrenoceptor polymorphism were not subjected to statistical analysis because of the small number of subjects in each subgroup.

RESULTS

All subjects completed the study, and no significant adverse effects were reported. Regular twice daily treatment with formoterol produced a significant ($p < 0.05$) improvement in the domiciliary morning and evening peak expiratory flow rates as compared with placebo. Morning peak expiratory flow rates were 395 L/min (27 L/min) for placebo, 424 L/min (30 L/min) for 24 μ g once daily formoterol, and 431 L/min (30 L/min) for 24 μ g twice daily formoterol. Evening peak expiratory flow rates were 420 L/min (29 L/min) for placebo, 441 L/min (32 L/min) for 24 μ g once daily formoterol, and 452 L/min (33 L/min) for 24 μ g twice daily formoterol.

Baseline spirometry, heart rate, and serum potassium before albuterol dose-response curves showed no significant differences among the three treatments (Table I). There was no desensitization in the albuterol dose-response curve for mean bronchodilator or systemic

TABLE II. Mean (SEM) values for maximal and area under curve airways and systemic β_2 -adrenoceptor responses (as change from baseline) from the salbutamol dose-response curve

	Placebo	F 24 μ g once daily	F 24 μ g twice daily
Max FEV ₁ (L)	0.47 (0.06)	0.48 (0.07)	0.51 (0.08)
AUC FEV ₁ (L/hr)	0.35 (0.04)	0.36 (0.04)	0.36 (0.04)
Max FEF ₂₅₋₇₅ (L/sec)	0.80 (0.12)	0.80 (0.16)	0.89 (0.14)
AUC FEF ₂₅₋₇₅ (L)	2136 (343)	2085 (434)	2340 (427)
Max PEF (L/min)	64 (11)	61 (11)	84 (20)
AUC PEF (L)	2603 (671)	2123 (661)	3520 (1006)
Max HR (beats/min)	25 (3)	18 (2)	20 (2)
AUC HR (beats)	938 (105)	673 (88)	715 (101)
Max K (mmol/L)	-0.33 (0.07)	-0.29 (0.05)	-0.31 (0.07)
AUC K (mmol/hr/L ⁻¹)	-0.38 (0.11)	-0.33 (0.11)	-0.32 (0.11)

There were no significant differences (apart from placebo vs 24 μ g once daily formoterol for maximal HR).

AUC, Area under curve; F, formoterol; HR, heart rate; K, serum potassium.

responses (as peak or area under curve) upon comparison of placebo with either once or twice daily formoterol treatment (table II).

Mean values for lymphocyte β_2 -adrenoceptor binding density (B_{max}) showed no significant difference between active treatments and placebo. Likewise, mean values for β_2 -adrenoceptor binding affinity (Kd) and maximal cAMP response to isoproterenol stimulation (E_{max}) showed no change as a consequence of formoterol treatment compared with placebo (Fig. 1).

In addition, for each individual, the percentage difference between placebo and formoterol was calculated for β_2 -adrenoceptor density and maximal FEV₁ response according to polymorphism at codon 16. Inspection of individual bronchodilator responses showed no particular association with codon 16 polymorphisms in terms of the percentage difference between placebo and formoterol treatment (Fig. 2). It was evident that for homozygous Gly-16 polymorphism there were only two patients of seven who exhibited a reduced maximal bronchodilator response to albuterol after regular treatment with twice daily formoterol. However, on inspecting individual data for β_2 -adrenoceptor density (B_{max}), it appeared that the homozygous Gly-16 polymorphism appeared to be associated with greater downregulation induced by twice daily formoterol, with five of seven homozygous

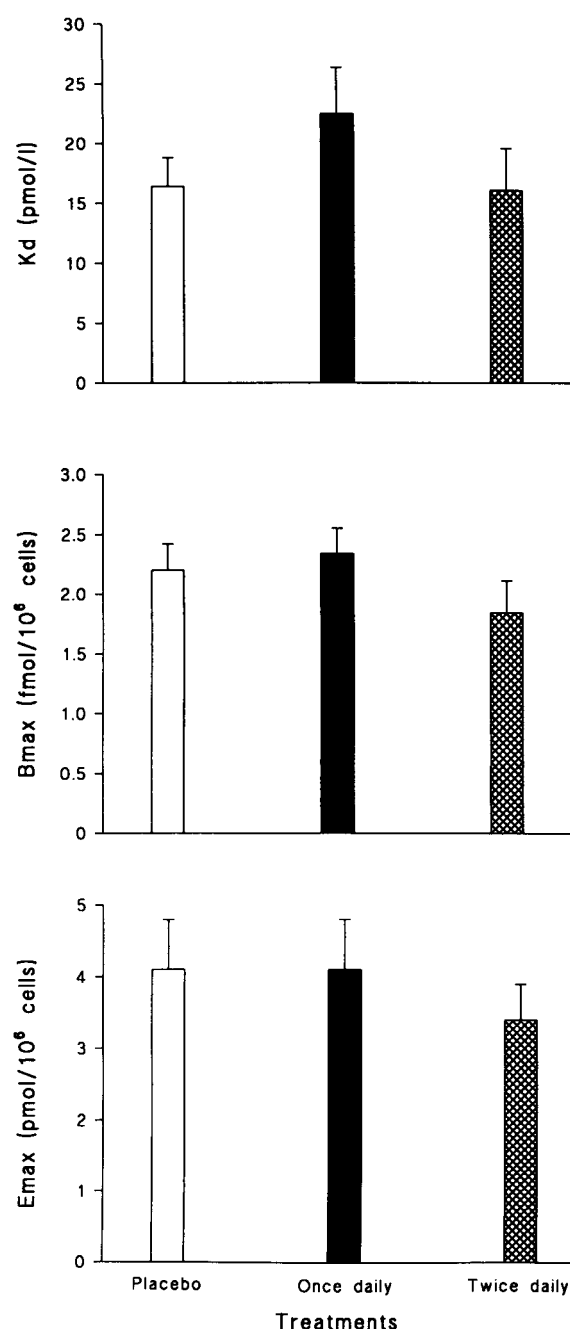


FIG. 1. Lymphocyte β_2 -adrenoceptor parameters at 36 hours after stopping regular treatment with either placebo, 24 μ g once daily formoterol, or 24 μ g twice daily formoterol. Values are shown for receptor binding density (geometric mean B_{max}), receptor binding affinity (geometric mean K_d), and cAMP response to stimulation (as delta post-pre) with isoproterenol (E_{max}). There were no significant differences between treatments for any measured parameters.

Gly-16 patients showing a reduction in receptor density (Fig. 2). The pattern for homozygous Glu-27 was identical to that of homozygous Gly-16, in that both genotypes coexisted in linkage together in the same patients.

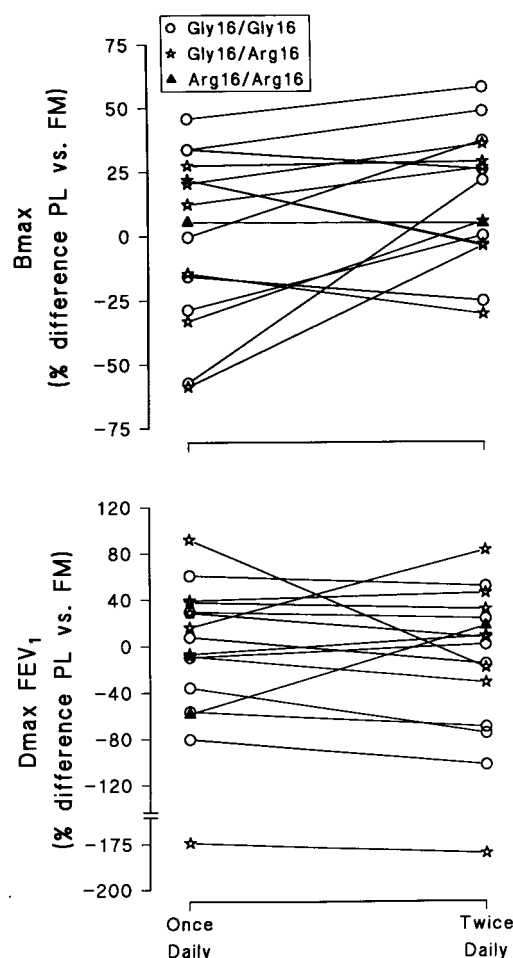


FIG. 2. Individual data plotted to show β_2 -adrenoceptor density (as B_{max} , $n = 15$) and maximal bronchodilator response (as D_{max} FEV₁, $n = 16$) to salbutamol. Values are given as percentage difference between placebo and formoterol for once daily and twice daily treatment. Data are depicted as individual responses according to β_2 -adrenoceptor polymorphism at codon 16. Data points for each individual with formoterol given once daily and formoterol given twice daily are joined by solid line. PL, Placebo; FM, formoterol.

DISCUSSION

Our results in asthmatic patients showed that mean values for bronchodilator sensitivity to albuterol were not impaired at 36 hours after stopping regular treatment with 24 μ g once or twice daily formoterol. Indeed, this was mirrored by effects on mean values for lymphocyte β_2 -adrenoceptor, which showed no evidence of downregulation (B_{max}) or desensitization (E_{max}) at the same time point. We recently performed a similar study in healthy volunteers in which β_2 -adrenoceptor downregulation and desensitization, along with a rightward shift in the albuterol dose-response curve for extrapulmonary β_2 -adrenoceptor responses, occurred at 24 hours after stopping regular 24 μ g twice daily formoterol for one week.¹⁰ In another study in asthmatic subjects, downregulation and desensitization of the lymphocyte

β_2 -adrenoceptor occurred at 24 hours after administration of 24 μ g twice daily formoterol and was associated with bronchodilator desensitization to repeated puffs of formoterol.³ This therefore suggests that in this study the 36-hour duration of washout after cessation of formoterol may have resulted in recovery of β_2 -adrenoceptor function.

The results of this study are, however, in contrast to our previous study in which bronchodilator desensitization to albuterol and downregulation of lymphocyte β_2 -adrenoceptor occurred at 36 hours after the last dose of 50 μ g twice daily regular salmeterol.¹ Indeed, this provided the rationale for design of the current study protocol, because we expected to find persistent downregulation and desensitization at 36 hours after stopping formoterol as was the case with salmeterol at the same time point. However, to properly compare salmeterol and formoterol, both drugs would need to be evaluated in the same study. The clinical relevance of this study is uncertain, in particular with respect to extrapolating the findings to what might happen in patients with more severe asthma. However, it is conceivable that in patients with acute asthma, downregulation and desensitization might, if anything, be greater as a consequence of excessive reliever use of β_2 -agonists.

It is of course possible that our finding represented a false-negative result as a consequence of statistical β -type error. This study was powered at 90% (i.e., a 10% β -error) to detect a 0.3 L difference in FEV₁ response. We considered that missing a smaller difference than 0.3 L in FEV₁ would not be clinically relevant in our patients who had mild-to-moderate asthma.

Another important factor to consider is the individual susceptibility to downregulation and desensitization, which is determined by β_2 -adrenoceptor polymorphism at codon 16.^{6,11} In this respect our random sample did not contain an excess of patients with the homozygous Arg-16 polymorphism, which is associated with relative protection against desensitization. However, the subset of patients who were Gly-16 homozygous showed a tendency toward downregulation in five of seven cases, but bronchodilator desensitization occurred in only two such cases. The Glu-27 polymorphism confers relative protection against desensitization, although the influence of Gly-16 exerts a predominant effect when Gly-16 and Glu-27 exist together in linkage.^{6,11} It was therefore perhaps not surprising to find that all subjects who were

Glu-27 homozygous exhibited the same trend towards downregulation, given that all of these subjects were also homozygous for Gly-16.

In conclusion, we have found that in patients taking inhaled corticosteroids, overall bronchodilator responses to albuterol and lymphocyte β_2 -adrenoceptor regulation are not significantly attenuated when evaluated at 36 hours after stopping regular formoterol. However, in a subset of patients who were Gly-16 homozygous, there was evidence of β_2 -adrenoceptor downregulation but not bronchodilator desensitization to albuterol. Further studies in subjects with more severe asthma are required to elucidate the clinical relevance of these findings.

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