Eur Respir J 2001; 17: 368–373
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European Respiratory Journal
ISSN 0903-1936

Reversing acute bronchoconstriction in asthma: the effect of bronchodilator tolerance after treatment with formoterol

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ABSTRACT: Continuous treatment with a short-acting β_2-agonist can lead to reduced bronchodilator responsiveness during acute bronchoconstriction. This study evaluated bronchodilator tolerance to salbutamol following regular treatment with a long-acting β_2-agonist, formoterol. The modifying effect of intravenous corticosteroid was also studied.

Ten asthmatic subjects (using inhaled steroids) participated in a randomised, double-blind, placebo-controlled, cross-over study. Formoterol 12 µg *b.i.d.* or matching placebo was given for 10–14 days with >2 weeks washout. Following each treatment, patients underwent a methacholine challenge to induce a fall in forced expired volume in one second (FEV_1) of at least 20%, then salbutamol 100 µg, 100 µg, and 200 µg was inhaled *via* a spacer at 5 min intervals, with a further 400 µg at 45 min. After a third single-blind formoterol treatment period, hydrocortisone 200 mg was given intravenously prior to salbutamol. Dose-response curves for change in FEV_1 with salbutamol were compared using analysis of covariance to take account of methacholine-induced changes in spirometry.

Regular formoterol resulted in a significantly lower FEV_1 after salbutamol at each time point compared to placebo ($p<0.01$). The area under the curves (AUCs) for 15 (AUC_{0-15}) and 45 (AUC_{0-45}) min were 28.8% and 29.5% lower following formoterol treatment ($p<0.001$). Pretreatment with hydrocortisone had no significant modifying effect within 2 h of administration.

It is concluded that significant tolerance to the bronchodilator effects of inhaled salbutamol occurs 36 h after stopping the regular administration of formoterol. This bronchodilator tolerance is evident in circumstances of acute bronchconstriction.
Eur Respir J 2001; 17: 368–373.

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Keywords: formoterol
long acting beta-agonist
tolerance

Received: March 13 2000
Accepted after revision August 18 2000

This work was supported in part, by a GlaxoWellcome Research Fellowship, awarded to S.L. Jones.

Current guidelines for the management of chronic asthma propose that long-acting β-agonists such as salmeterol and formoterol should be added to anti-inflammatory treatment for the control of persistent or breakthrough symptoms [1, 2]. This strategy appears to be beneficial not only in controlling symptoms [3–7], but also in reducing the frequency of asthma exacerbations during long-term treatment [8, 9].

Despite these positive outcomes, against a background of concern about the safety of β-agonists [10] significant attention has been given to the issue of β-adrenoceptor down-regulation and the advent of drug tolerance. A number of studies using salmeterol and formoterol have been conducted to investigate this issue. These have shown that during treatment with long-acting agents, tolerance to the protective effects of β-agonists against exercise-induced bronchospasm [11] and nonspecific bronchoconstrictors is easily demonstrated [12] and develops rapidly [13]. Concurrent treatment with inhaled corticosteroid does not appear to modify these effects [14–17], although corticosteroids may be used acutely to reverse them [18, 19].

In contrast, evidence for the development of bronchodilator tolerance has been less clear cut. In the majority of studies, no evidence of impaired bronchodilator responsiveness has been found [20–24], although in some investigations a reduction in bronchodilator response has been reported [18, 25, 26]. However, a major drawback in the design of these studies is that most have been carried out in patients with stable asthma, in whom a reduction in β_2-adrenoceptor function may be relatively unimportant in maintaining adequate bronchomotor tone. This contrasts with patients with acute asthma.

Recently, using a simple but novel approach, the authors have demonstrated that after six weeks regular treatment with the short-acting β_2-agonist terbutaline, the bronchodilator response to salbutamol is significantly reduced in circumstances of acute bronchoconstriction [27]. This is more relevant in determining the clinical importance of tolerance. The present study has extended this approach to assess the development of bronchodilator tolerance during regular treatment with the long-acting β-agonist formoterol. The study was

also designed to confirm whether administering systemic corticosteroid rapidly reverses any observed effect.

Methods

Subjects

Volunteers aged 18 – 70 yrs, with a history of mild to moderate asthma, were invited to participate in the study. All had a positive methacholine challenge (provocative dose causing a 20% fall in forced expiratory volume in 1 second (FEV_1) of less than 8 μmol (PD_{20})) within 2 months of recruitment. All were receiving maintenance inhaled corticosteroid treatment with no change in dose or other asthma treatment during the 6 weeks prior to enrolment. All those receiving oral or high dose inhaled corticosteroids (>2000 μg·day^{-1} of beclomethasone or equivalent) were excluded, as were patients receiving maintenance inhaled long-acting β_2-agonists. Current or previously heavy cigarette (>5 pack years) smokers were also excluded.

Study design

All patients entered a 2-week run-in phase during which control of asthma was assessed by means of a daily record card in which patients entered morning and evening peak flows and symptom scores. Throughout the study patients were maintained on their usual dose of inhaled steroids. All β_2-agonists were stopped and patients were provided with ipratropium bromide 40 μg·puff^{-1} (Boehringer, Ingelheim, Germany) for "as required" use. Patients with unstable asthma, or who were unable to obtain adequate relief of breakthrough symptoms using inhaled ipratropium bromide during the run-in, were withdrawn from the study. All other patients then received each of the first two study treatments according to a randomised, double-blind, crossover design. The treatments were formoterol (Foradil, Novartis, Basel, Switzerland) 12 μg twice daily administered as dry powder *via* an aeroliser for 7 – 10 days, and matching placebo containing lactose. There was a washout period of at least 2 weeks between each of the first two treatments. Following a further 2 week washout interval, patients proceeded into a third treatment period during which formoterol 12 μg twice daily was administered single-blind (patient) for 7 – 10 days. This was to permit a subsequent assessment of the effect of intravenous hydrocortisone on the bronchodilator dose-response.

Study visits and measurements

Patients visited the research laboratory at the completion of each of the three treatment periods. Study medications and inhaled ipratropium were withheld for at least 36 and 6 h respectively. On each occasion, and prior to any measurements being obtained, a venous cannula was inserted into a forearm vein, and either normal saline (following each of the first two treatment arms) or hydrocortisone 200 mg (following the third treatment arm, formoterol 12 μg *b.i.d.*) was given single-blind (patient) intravenously. The administration of steroid 1 h before measuring the dose-response to salbutamol was to mimic what might occur during an acute episode of asthma. Active hydrocortisone was given during the last of the three treatment periods to avoid any steroid-related carry over effect on β-adrenoceptor function.

Exactly 1 h later, patients underwent a methacholine challenge followed by a bronchodilator response measurement. The methacholine challenge was performed using a modified version of the rapid challenge procedure [28]. After measurement of baseline FEV_1 according to American Thoracic Society (ATS) criteria [29] using a rolling seal spirometer (Spirotech, Graseby, Georgia, USA), increasing doses (0.044 – 45 μmol) of methacholine were administered by nebuliser, controlled by a Morgan Nebicheck dosimeter (Morgan, Gillingham, Kent, UK). The procedure was stopped after the FEV_1 had fallen by $\geqslant 20\%$. The PD_{20} was calculated by linear interpolation.

Immediately after the PD_{20} had been reached, an abbreviated dose-response test to inhaled salbutamol (Ventolin, GlaxoWellcome, Greenford, UK) was undertaken. The salbutamol was administered from a metered dose inhaler *via* a large volume spacer (Volumatic, GlaxoWelcome, Greenford, UK). Doses of 100 μg, 100 μg, and 200 μg were given at 0, 5, and 10 min, respectively. Spirometry was performed immediately prior to each dose, and also at 15, 30 and 45 min.

A further 400 μg of salbutamol was administered at 45 min to maximise bronchodilatation prior to further spirometry and a second methacholine challenge at 60 min, using the same method as previously. The aim of this further challenge was to measure the protective effect of the salbutamol against further methacholine. Once a PD_{20} had been established for the second methacholine challenge, patients were given inhaled salbutamol *ad lib* and remained in the research laboratory until their FEV_1 had returned to at least 90% of baseline.

Analysis of results

The primary study end-point was area under the curve (AUC) for change in FEV_1 following inhaled salbutamol. From a previous investigation [27], the sample size was calculated so that there was 90% power to detect a 30% difference in AUC with a significance of 0.05. Analyses were undertaken to assess treatment-related differences, as well as the effects of intravenous hydrocortisone. Analyses of covariance were performed to take into account any differences in the baseline FEV_1, the fall in FEV_1 during the methacholine challenge and log PD_{20} methacholine. Where PD_{20} was not achieved after the highest dose of methacholine, an arbitrary value of 64 μmol was assigned. The increase in FEV_1 after each dose of salbutamol, expressed as a percentage of the fall from baseline during the methacholine challenge, was also analysed.

Further comparisons were made for post-salbutamol PD20 values to assess treatment-related differences in the protective effect given by inhaled salbutamol against methacholine-induced bronchoconstriction. When comparing differences in the post-salbutamol PD20s between the first two treatment arms, the pre-salbutamol PD20 was used as a covariate.

Ethical considerations

Asthma control was carefully monitored throughout the study. Each patient had an individualised asthma action plan, a supply of prednisone and an inhaled β_2-agonist for emergency use, and 24 h access to one of the study investigators, in case of an acute exacerbation. The study was approved by the Otago Ethics Committee. Each patient gave written informed consent.

Results

Twelve patients were enrolled into the study. Two patients withdrew following the run-in; one was unable to tolerate ipratropium as relief medication, the other developed diabetes mellitus. The ten randomised patients (9 female, aged 18–65 years) had a mean (95% confidence interval (CI)) FEV1 % predicted of 95.1% (82.9–107.3) on entry into the study. Their median (range) dose of inhaled corticosteroid was 800 $\mu g \cdot day^{-1}$ (200–2000 μg) of beclomethasone or equivalent. One patient withdrew between the second and third treatment periods due to unstable asthma. Thus nine patients completed all three treatment arms of the study. Compliance with study medication was greater than 90% during each of the treatment arms.

Baseline lung function and bronchial hyper-responsiveness

Baseline FEV1 and mean methacholine PD20 values were not significantly different following each of the three treatment periods. The mean percentage fall in FEV1 did not differ between the treatments (table 1).

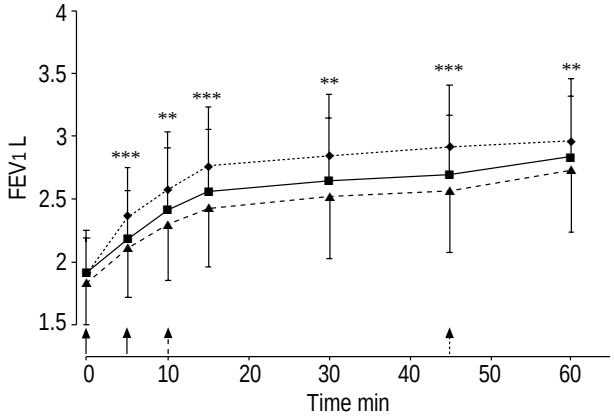

Fig. 1. – Forced expired volume in one second (FEV1) following the administration of salbutamol at 0, 5, 10 and 45 min. ♦: Placebo; ■: Formoterol; ▲: Formoterol plus hydrocortisone. ↑: 100 μg salbutamol; ↑: 200 μg salbutamol; ↑: 400 μg salbutamol. **: $p<0.01$, ***: $p<0.001$ for formoterol *versus* placebo comparisons.

Dose-response curves to salbutamol

Mean FEV1 at each time point following salbutamol administration is shown in figure 1. The values were significantly lower with formoterol treatment than placebo at each time point. Dose-response curves following salbutamol, expressed as a percentage of the fall in FEV1 from the pre-methacholine baseline, are shown in figure 2. Prior treatment with formoterol was associated with a significantly smaller increase at each time point. AUC0–15 and AUC0–45 were 28.8% and 29.5% lower following treatment with formoterol than placebo at each time point ($p<0.0004$ and $p<0.0001$, respectively). Prior administration of hydrocortisone did not result in any significant difference compared to formoterol alone for either FEV1 at each time point, or AUC0–15 and AUC0–45, although the difference compared to placebo was significant.

Bronchoprotective effect of salbutamol

Baseline FEV1 prior to the second methacholine challenge was significantly lower following the formoterol treatment arm than placebo ($p=0.01$) (table 1). The protective effect of salbutamol as

Table 1. – Baseline lung function and bronchial hyperresponsiveness, area under curves (AUC) for dose-response to salbutamol, and post-salbutamol bronchial hyperresponsiveness after each treatment period.

	Placebo	Formoterol	Formoterol plus hydrocortisone
Subjects n	10	10	9
Baseline FEV1 L	2.59 (2.16–3.02)	2.59 (2.14–3.04)	2.45 (2.02–2.88)
PD20 for first methacholine challenge μmol	2.12 (0.57–3.67)	1.57 (0.34–2.80)	3.43 (0.31–6.55)
FEV1 fall from baseline %	25.91 (22.44–29.38)	25.81 (21.99–29.63)	25.35 (22.51–28.19)
AUC0–15	7.72 (6.84–8.60)	5.49 (4.61–6.37)	5.56 (4.09–7.05)
AUC0–45	35.36 (30.03–40.68)	24.94 (19.62–30.26)	27.06 (20.38–33.75)
FEV1 before second methacholine challenge	2.97 (2.81–3.13)	2.84 (2.68–3.00)	2.84 (2.57–3.11)
PD20 for methacholine challenge after salbutamol μmol	23.19 (8.94–37.44)	12.12 (2.89–21.35)	19.30 (2.31–36.29)

Results reported as mean (95% confidence interval). PD20: Provocative dose causing a $\geq$20% fall in forced expired volume in one second (FEV1).

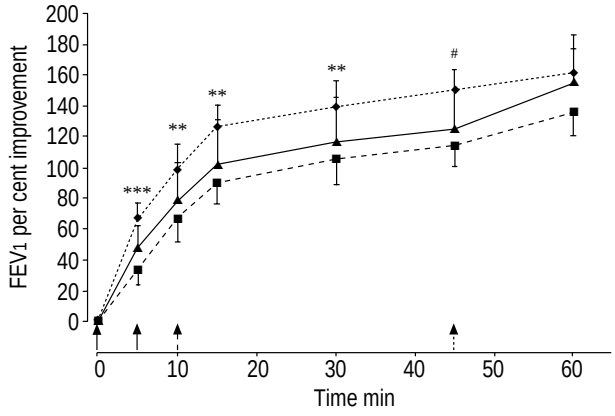

Fig. 2. – Changes in forced expired volume in one second (FEV_1) expressed as a percentage of the fall in FEV_1 following methacholine challenge following the administration of salbutamol at 0, 5, 10 and 45 min. ♦: Placebo; ■: Formoterol; ▲: Formoterol plus hydrocortisone. ↑: 100 μg salbutamol; ↑: 200 μg salbutamol; ↑: 400 μg salbutamol. **: $p<0.01$, ***: $p<0.001$, #: $p<0.002$ for formoterol *versus* placebo comparisons.

measured by the increase in methacholine PD_{20} from baseline and expressed as doubling dose concentrations (d.d.s) was 2.74 d.d.s (95% C.I. 2.19 – 3.29) following formoterol and 3.97 d.d.s (95% C.I. 3.42 – 4.52) following placebo ($p<0.01$). Following formoterol with hydrocortisone the increase in PD_{20} was 2.62 d.d.s (95% C.I. 2.01 – 3.23) ($p=0.79$ for comparison with formoterol alone).

Discussion

The results of the present study show that in patients with mild to moderate asthma requiring maintenance inhaled steroid therapy, tolerance to the bronchodilator effect of salbutamol may be demonstrated in the presence of acute bronchoconstriction (mean fall in FEV_1 26%) following treatment with regular formoterol. After salbutamol administration, the mean FEV_1 was significantly lower at all time points and the AUC_{0-15} for change in FEV_1 was reduced by 28.8% with regular formoterol compared to placebo. The magnitude of these changes is similar to what has previously been reported in a study using regular terbutaline [27]. In that investigation the AUC_{0-15} for FEV_1 was reduced by 36.0%. Thus the present results confirm that bronchodilator tolerance develops during regular β-agonist treatment irrespective of whether a long or short-acting agent is being used.

The findings provide evidence that the effects of bronchodilator tolerance are more likely to be encountered when patients are experiencing bronchospasm. Most recent studies have failed to demonstrate bronchodilator tolerance with long-acting β-agonists [20 – 24]. This may be because these investigations have included patients whose asthma is stable and in whom there was no significant bronchoconstriction. For this reason, the validity of these earlier results is questionable. It is difficult to appropriately conduct controlled bronchodilator response studies in patients with unstable asthma. It was in an attempt to address this issue that mild airway obstruction was induced in the patients using a nonspecific bronchoconstrictor. Although the present model does not accurately mimic acute severe asthma, in which other factors besides β-adrenoceptor down-regulation, induced by continuous β-agonist therapy, may impair the response to inhaled bronchodilator, it does permit the conclusion that bronchodilator tolerance is more likely to be important in the presence of bronchoconstriction.

The study design included a 36 h period of drug withdrawal prior to testing for bronchodilator tolerance. If anything, this may have resulted in a diminution in the magnitude of the effect that was found, given that the status of β-adrenoceptors may change rapidly [30]. It might also be argued that testing for bronchodilator tolerance without a period of drug withdrawal would have been more clinically relevant. The principal reason for not doing so was to avoid the problem of functional antagonism to methacholine which would have resulted from the continuing action of formoterol. This would have had the potential to make acute bronchoconstriction, upon which demonstrating the development of tolerance was dependent, more difficult to achieve.

At 45 min our patients received a further 400 μg of salbutamol (making a total of 8×100 μg puffs) in order to induce maximal bronchodilatation. Following this, a significantly greater increase in FEV_1 was achieved following formoterol treatment when compared to placebo (139 and 58 mL, respectively, $p<0.02$). This indicates that at that time point, even though baseline FEV_1 had been recovered, residual bronchoconstriction was still present following formoterol treatment, despite the fact that the patients had already received 400 μg (4 puffs) of salbutamol. Thus, as might have been anticipated, overcoming the effects of β-adrenoceptor tolerance required the use of higher doses of salbutamol, although there is evidence that this may not always be successful [31]. This suggests that, in circumstances of acute bronchoconstriction, patients taking long-acting β-agonists may require higher doses of "reliever" short-acting β-agonist in order to obtain maximum bronchodilatation.

The clinical importance of this is unclear. No studies have been carried out to examine the exact relationship between β-adrenoceptor tolerance and asthma control during long-term treatment with β-agonists. Nor is it known whether individual susceptibility to the development of tolerance influences how patients respond to treatment during acute life-threatening episodes. However, the results of other studies offer indirect but reassuring evidence. The frequency of asthma exacerbations is reduced with regular long-acting β-agonist, not increased [8, 9]. Furthermore, in a controlled study, changes in symptoms and peak flow rates during the early phases of an exacerbation do not appear to be different in patients receiving regular formoterol from those who are not [32]. In addition, the bronchodilator response to very high doses of nebulised salbutamol, such as would be used to treat acute severe asthma in an emergency department, is no different between patients taking or not taking regular salmeterol [33].

In the present study, prior administration of intravenous hydrocortisone 1 h before inducing

bronchoconstriction and giving salbutamol had no significant effect on the magnitude of bronchodilator tolerance. Even when an additional 400 μg of salbutamol was administered at 45 min *i.e.* 1 h and 45 min after giving hydrocortisone, the corticosteroid had no effect on the magnitude of the increase in FEV_1 during the subsequent 15 min. This outcome is in apparent contrast to the report by TAN *et al.* [18], in which the combined use of both oral prednisone and intravenous hydrocortisone reversed bronchodilator tolerance to formoterol. However, in the present study patients were followed for only 2 h after giving corticosteroid, whereas TAN *et al.* [18] followed their patients for 8 h. Taken together, these results confirm that although giving corticosteroid is likely to be effective in reversing the effects of β-adrenoceptor tolerance, it cannot be relied upon to do so in the early phases of an acute episode of asthma.

In conclusion, this study has demonstrated that significant tolerance to the bronchodilator effects of inhaled salbutamol occurs 36 h after cessation of formoterol. This effect is likely to be more important in circumstances of acute bronchconstriction. Whether or not this effect increases with increasingly severe airway obstruction, or is accentuated when patients take excessive amounts of β-agonist, requires to be evaluated further.

Acknowledgements. The authors wish to thank the volunteers who participated in this study and Boehringer Ingelheim for supplies of Atrovent.

References

1. British Thoracic Society. Guidelines on asthma management. *Thorax* 1997; 52: Suppl. 1.
2. NHLBI. Guidelines for the diagnosis and management of asthma 1997. Expert Panel Report. *NIH Publication* 97–4051.
3. Greening AP, Ind PW, Northfield M, Shaw G. Added salmeterol *versus* higher dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. *Lancet* 1995; 344: 219–224.
4. Woolcock A, Lundback B, Ringdal N, Jacques LA. Comparison of addition of salmeterol to inhaled steroids with doubling of the dose of inhaled steroids. *Am J Respir Crit Care Med* 1996; 153: 1481–1488.
5. Pearlman DS, Stricker W, Weinstein W, *et al.* Inhaled salmeterol and fluticasone: a study comparing monotherapy and combination therapy in asthma. *Ann Allergy Asthma Immunol* 1999; 82: 257–265.
6. Van Noord JA, Schreurs AJM, Mol SJM, Mulder PG. Addition of salmeterol versus doubling the dose of fluticasone propionate in patients with mild to moderate asthma. *Thorax* 1999; 54: 207–212.
7. Weersink EJ, Douma RR, Postma DS, Koeter GH. Fluticasone propionate, salmeterol xinafoate, and their combination in the treatment of nocturnal asthma. *Am J Respir Crit Care Med* 1997; 155: 1241–1246.
8. Pauwels RA, Lofdahl C-G, Postma DS, *et al.* Effect of inhaled formoterol and budesonide on exacerbations of asthma. *N Engl J Med* 1997; 337: 1405–1411.
9. Taylor DR, Town GI, Herbison GP, *et al.* Asthma control during long-term treatment with regular inhaled salbutamol and salmeterol. *Thorax* 1998; 53: 744–752.
10. Sears MR, Taylor DR. The β_2-agonist controversy. Observations, explanations and relationship to asthma epidemiology. *Drug Safety* 1994; 11: 259–283.
11. Ramage L, Lipworth BJ, Ingram CG, Cree IA, Dhillon DP. Reduced protection against exercise induced bronchoconstriction after chronic dosing with salmeterol. *Resp Med* 1994; 88: 363–368.
12. Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long-term effects of a long-acting β_2-adrenoceptor agonist, salmeterol, on airway hyper-responsiveness in patients with mild asthma. *N Engl J Med* 1992; 327: 1198–1203.
13. Drotar DE, Davis EE, Cockcroft DW. Tolerance to the bronchoprotective effect of salmeterol 12 hours after starting twice daily treatment. *Ann Allergy Asthma Immunol* 1998; 80: 31–34.
14. Yates DH, Kharitonov SA, Barnes PJ. An inhaled glucocorticoid does not prevent tolerance to the bronchoprotective effect of a long-acting inhaled β_2-agonist. *Am J Respir Crit Care Med* 1996; 154: 1603–1607.
15. Kalra S, Swystun V, Bhagat R, Cockcroft DW. Inhaled corticosteroids do not prevent the devlopment of tolerance to the bronchoprotective effect of salmeterol. *Chest* 1996; 109: 953–956.
16. Simons FER, Gerstner TV, Cheang MS. Tolerance to the bronchoprotective effects of salmeterol in adolescents with exercise-induced asthma using concurrent inhaled glucocorticoid treatment. *Pediatrics* 1997; 99: 655–658.
17. Booth H, Bish R, Walters J, Whitehead F, Walters EH. Salmeterol tachyphylaxis in steroid treated asthmatics. *Thorax* 1996; 51: 1100–1104.
18. Tan KS, Grove A, McLean A, Gnosspelius Y, Hall IP, Lipworth BJ. Systemic corticosteroid rapidly reverses bronchodilator subsensitivity induced by formoterol in asthmatic patients. *Am J Respir Crit Care Med* 1997; 156: 28–35.
19. Pansegrouw DF, Weich DJV, LeRoux FPJ. Beta-adrenergic receptor tachyphylaxis in acute severe asthma. *S Afr Med J* 1991; 80: 229–230.
20. Grove A, Lipworth BJ. Effects of prior treatment with salmeterol and formoterol on airway and systemic β_2 responses to fenoterol. *Thorax* 1996; 51: 585–589.
21. Arledge TE, Liddle R, Stahl E, Rossing TH. Salmeterol does not cause tolerance during long-term asthma therapy. *J Allergy Clin Immunol* 1996; 98: 1116–1119.
22. Langley SJ, Masterson CM, Batty EP, Woodcock A. Bronchodilator response to salbutamol after chronic dosing with salmeterol or placebo. *Eur Respir J* 1998; 11: 1081–1085.
23. Aziz I, Hall IP, McFarlane LC, Lipworth BJ. β_2-Adrenoceptor regulation and bronchodilator sensitivity after regular treatment with formoterol in subjects with stable asthma. *J Allergy Clin Immunol* 1998; 101: 337–341.
24. Nelson HS, Berkowitz RB, Tinkelman DA, Emmett AH, Rickard KA, Yancey SW. Lack of subsensitivity to salbutamol after treatment with salmeterol in

patients with asthma. *Am J Respir Crit Care Med* 1999; 159: 1556–1561.
25. Grove A, Lipworth BJ. Bronchodilator subsensitivity to salbutamol after twice daily salmeterol in asthmatic patients. *Lancet* 1995; 346: 201–206.
26. Newnham DM, Grove A, McDevitt DG, Lipworth BJ. Subsensitivity of bronchodilator and systemic β_2-adrenoceptor responses after regular twice daily treatment with formoterol dry powder in asthmatic patients. *Thorax* 1995; 50: 497–504.
27. Hancox RJ, Aldridge RE, Cowan JA, *et al.* Tolerance to beta-agonists during acute bronchoconstriction. *Eur Respir J* 1999; 14: 283–287.
28. Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial responsiveness. *Thorax* 1983; 38: 760–765.
29. American Thoracic Society. 1994. Standardization of Spirometry: 1994 update. *Am J Respir Crit Care Med* 1995; 152: 1107–1136.
30. Johnson M. The beta-adrenoceptor. *Am J Resp Crit Care Med* 1998; 158: S146–153.
31. Aziz I, Lipworth BJ. *In vivo* effect of albuterol on methacholine-contracted bronchi in conjunction with salmeterol and formoterol. *J Allergy Clin Immunol* 1999; 103: 816–822.
32. Tattersfield AE, Postma DS, Barnes PJ, *et al.* Exacerbations of asthma: a descriptive study of 425 severe exacerbations. The FACET International Study Group. *Am J Respir Crit Care Med* 1999; 160: 594–599.
33. Korosec M, Novak RD, Myers E, Skowronski M, McFadden ER. Salmeterol does not compromise the bronchodilator response to salbutamol during acute episodes of asthma. *Am J Med* 1999; 107: 209–213.