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Systemic sensitivity to corticosteroids in smokers with asthma

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ABSTRACT

Cigarette smokers with asthma are insensitive to the therapeutic effects of corticosteroids. It is unknown whether insensitivity to corticosteroids in smokers affects tissue sites outwith the airways.

75 asthmatic subjects (39 smokers) and 78 healthy controls (30 smokers) were recruited to an observational study where the cutaneous and peripheral blood lymphocyte responses to corticosteroids were measured. The cutaneous vasoconstrictor response to topical beclometasone was measured by applying different concentrations of beclometasone solutions to the skin in a random double blind manner. The degree of blanching at each concentration was graded after 18 hours. Sensitivity of peripheral blood lymphocytes to corticosteroids was assessed by measuring the suppressive effect of dexamethasone on lymphocyte proliferation stimulated by phytohaemagglutinin.

Total cutaneous vasoconstrictor response score to beclometasone (mean [SD]) was reduced in smokers (5.39 [3.58]) compared to never-smokers (7.26 [3.05]) with asthma, p=0.023, and in all smokers (6.47 [3.33]) compared to all never-smokers (7.86 [2.81]), p=0.006. The sensitivity to corticosteroids of lymphocytes stimulated by phytohaemagglutinin was similar between groups.

Smokers with asthma have an impaired cutaneous vasoconstrictor response to topical corticosteroids compared to never-smokers with asthma. This finding suggests the insensitivity to corticosteroids in smokers with asthma affects tissue sites other than the airways.

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INTRODUCTION

Corticosteroids are the most effective anti-inflammatory therapy currently available for the treatment of asthma and are recommended in international guidelines [1]. A subgroup of asthmatics do not obtain an adequate therapeutic response to corticosteroids and are termed corticosteroid-resistant or insensitive [2]. The causes of corticosteroid-insensitive asthma are considered to be multi-factorial involving both genetic and environmental factors [2,3] including cigarette smoke [4]. Cigarette smokers compared to never-smokers with asthma are less sensitive to both inhaled [5,6] and oral corticosteroids [7] as assessed by changes in lung function and asthma symptoms. Smokers with asthma account for over 20% of adults with asthma [4] and compared with never-smokers experience more severe asthma symptoms [8] and accelerated decline in lung function [9].

It is not known whether smokers with asthma are insensitive to corticosteroids in tissue sites other than the airways. The cutaneous vasoconstrictor response to topical beclometasone [10,11], and the ability of corticosteroids to inhibit the activation of peripheral blood lymphocytes [12] have been used as an index of systemic sensitivity to corticosteroids. Never-smokers with corticosteroid-insensitive asthma have impaired cutaneous vasoconstrictor responses to corticosteroids [13] and the inhibitory effect of corticosteroids on lymphocyte proliferation is reduced [12]. Our hypothesis is that smokers with asthma have reduced corticosteroid sensitivity in sites other than the lungs. The aim of the study was to compare systemic sensitivity to corticosteroids in smokers and never-smokers with and without asthma by assessing the cutaneous vasoconstrictor response to topical beclometasone and the sensitivity of stimulated lymphocytes to corticosteroids.

MATERIALS AND METHODS

Subjects

Smoking and never-smoking subjects with and without asthma (caucasians and non-caucasians) aged 18-60 years were recruited from hospital outpatient clinics and hospital staff respectively. All participants gave written informed consent and approval for the study was obtained from the West Glasgow Ethics Committee. Asthma was diagnosed by American Thoracic Society (ATS) criteria [14] and all asthmatic subjects had a baseline forced expiratory volume in one second (FEV_1) ≤ 85% predicted and reversibility of FEV_1 after nebulised salbutamol of ≥ 15% or more. Exclusion criteria were asthma exacerbations, use of oral corticosteroids or a respiratory tract infection within four weeks of inclusion. Healthy volunteers had no history of respiratory disease. Smokers had smoked ≥ 10 pack years and were currently smoking ≥ 10 cigarettes/day. 35 asthmatics (19 smokers) were also taking long-acting β_2 agonists and 3 asthmatics (2 smokers) were taking leukotriene receptor antagonists.

Study design

This was an observational study in which the cutaneous, peripheral blood lymphocyte and airway responses to corticosteroids were measured. Venous blood was collected for lymphocyte responses, total and specific serum IgE levels and serum cotinine levels. The cutaneous vasoconstrictor response to topical beclometasone was measured the same day by applying beclometasone solutions to the skin in a random double-blind manner and the degree of blanching assessed. Sputum induction was performed in subjects with asthma and exhaled nitric oxide (F_ENO) and carbon monoxide (CO) were measured in all subjects. Subjects with asthma received 40 mg of oral prednisolone daily for 14 days. End-points used to assess airway corticosteroid sensitivity were change in morning and evening peak expiratory flow rate (PEF), pre-salbutamol

FEV_1, validated asthma control score, daily morning and night symptoms and a reduction in the use of rescue inhalers.

Measurements

Baseline measurements and diary card recordings

Asthma severity was scored at baseline using the ATS asthma impairment score [15] and asthma control was scored at each visit using a validated asthma control questionnaire [16]. Patients maintained a validated home diary card [17], recording morning and night PEF, daytime symptoms (range 0-6, for increasing severity) and night awakenings (range 0-3, for increasing severity), use of inhaled rescue medication and study tablet consumption. Compliance was assessed by tablet count. Asthma duration was determined from patient clinical history and hospital records when available. Total serum IgE and specific IgE to house dust mite, grass pollen and cat dander were measured by enzyme linked immunoassay (Unicap, Pharmacia Ltd, UK). Total IgE level >120 IU/ml and specific IgE >0.35 IU/ml were considered elevated. A subject was defined as non-atopic when specific IgE to all common allergens was negative [18]. Serum cotinine was measured using an enzyme immunoassay (Cozart Bioscience Ltd, UK) as confirmation of smoking status.

Lung function testing, sputum induction and exhaled gases

Spirometry was measured with a dry spirometer (Vitalograph Ltd, UK) and the best of three attempts was taken for analysis. FEV_1 was measured before and 15 minutes after 2.5 mg of nebulised salbutamol. Sputum induction with 3% hypertonic saline was performed using a modification of the method described by Pin et al [19]. The sputum was processed and the dispersed cell total and differential count obtained using the technique described by Popov et al [20]. Subjects were requested not to smoke for an hour prior to their visits and F_ENO was measured using a chemiluminescence analyser (LR2000, Logan Research Ltd, UK), with a

detection limit of 0.1 ppb NO. Nitric oxide levels were taken from the plateau at the end of exhalation and the mean of triplicate measurements was used as the representative value [21].

Cutaneous vasoconstrictor response to topical beclometasone

The cutaneous vasoconstrictor response to topical beclometasone was measured as described previously [10], with minor modifications. Beclometasone dipropionate (Sigma, UK) was dissolved in 95% ethanol to concentrations of 1, 3, 10, 30, 100, 300 and 1000 μg/ml. A control solution of 95% ethanol was used. Test sites were outlined on the non-dominant flexor forearm by the application of adhesive tape in which 2cm diameter holes had been cut. Solutions were randomly allocated a letter (A-H) by staff not involved in the application or reading of the test, and solutions applied in order A-H. The test was not unblinded until the completion of the study. The sites were occluded with plastic film to enhance percutaneous absorption of the beclometasone. A tubular bandage (Tubigrip; Seton Healthcare Group, UK) was applied to attenuate any changes in ambient temperature. 18 hours later the tape and film were removed, and the degree of blanching assessed 1 hour later. The test sites were examined in standard lighting conditions and given a blanching score by a single trained observer. Blanching at each concentration was graded according to a 4-point scale: 0 = no blanching; 1 = faint blanching; 2 = obvious blanching not extending outwith the test site; 3 = intense blanching extending over the margin of the test site. Addition of individual concentration scores gave a total score. A high score indicates a high degree of corticosteroid sensitivity.

Lymphocyte proliferation response

The sensitivity of peripheral blood mononuclear cells (PBMCs) to corticosteroids was assessed in a functional assay as described previously [12]. PBMCs were separated from whole blood using lymphoprep (Axis-Shield PoC AS, Oslo, Norway). Cells were then resuspended at $1x10^6$ lymphocytes/ml in RPMI 1640 supplemented with 10% foetal calf serum, 1.25 ug/ml fungizone,

1% L-glutamine, 100 μg/ml penicillin and 100 iu/ml streptomycin. Cell viability was assessed by trypan blue exclusion and was always greater than 95%. PBMCs were incubated in triplicate at a concentration of $1x10^5$ cells/100μl/well in 96-well round-bottomed plates (Iwaki microplates, Bibby Sterilin, UK). The T-lymphocyte mitogen PHA (Biostat Ltd, Stockport, UK) was added to the cultured cells at a concentration of 0.6 μg/well after pilot studies (n=21 patients) had established that the suppressive effect of dexamethasone was almost independent of the amount of PHA used over the range 0.054ug – 0.6ug/well. Dexamethasone (Sigma) [10^{-4}-10^{-11} mol/L] was added and the plates cultured at 37°C in a humidified atmosphere with 5% CO_2 for 48 hours. Cell proliferation was measured by uptake of tritiated thymidine. Results were expressed as counts per minute. The thymidine incorporation as an index of cell proliferation was compared between PHA-stimulated T-lymphocytes with or without dexamethasone. The percentage suppression at the final concentration of dexamethasone (defined as the Imax for this study), and the gradient of all concentrations of dexamethasone were compared between smokers and never-smokers with and without asthma.

Statistical analysis

Baseline characteristics were compared by chi-squared tests and Wilcoxon tests. The effect of asthma and smoking upon the total skin score was assessed by ANOVA, including a test of interaction between the two factors. The response to oral prednisolone for smokers versus never-smokers with asthma was assessed by Analysis of Covariance models that adjusted each factor by its baseline measure. For those measurements that were taken from diary cards, the mean of days 1 and 2 was taken to be the baseline, and the mean of days 11 to 14 was taken to be the response measurement. Spearman rank correlations were used to assess the strength of association between the total skin test score and the various factors of interest. For each patient separately the downward slope of the best fitting line was calculated by linear regression. The lymphocyte

proliferation response data is shown graphically as mean (SEM) for clarity. Tests were performed using SAS version 8 (Cary, NC, USA).

RESULTS

Baseline characteristics

There were no significant differences in age, duration of asthma, equivalent dose of inhaled beclometasone, baseline FEV_1 percentage predicted, pre- and post-bronchodilator FEV_1, pre- and post-bronchodilator FEV_1/FVC ratio, reversibility (percentage) to inhaled salbutamol, ATS asthma impairment score, total IgE levels and induced sputum percentage macrophage, neutrophil and lymphocyte counts among smokers with asthma and never-smokers with asthma [Tables 1 and 2]. Compared to never-smokers with asthma, the smokers with asthma had fewer male subjects, lower absolute baseline FEV_1, lower absolute reversibility to salbutamol, higher asthma control score, lower percentage with a positive specific IgE levels, and lower F_ENO levels [Tables 1 and 2]. Compared to healthy never-smokers, healthy smokers were older and had lower FEV_1 (absolute and percentage predicted) and lower pre-bronchodilator FEV_1/FVC ratio. The smoking history was longer and the serum cotinine level was lower in asthmatic smokers compared to healthy smokers [Table 1].

Cutaneous vasoconstrictor response to topical beclometasone

There was a significant difference in the mean (SD) total cutaneous vasoconstrictor response score between smokers [5.39 (3.58)] and never-smokers [7.26 (3.05)] with asthma, p=0.023 [Figure 1]; between all smokers [6.47 (3.33)] and all never-smokers [7.86 (2.81)], p=0.006, and between all asthmatics [6.29 (3.44)] and all controls [8.13 (2.50)], p<0.001. There was however no difference between smoking [7.83 (2.42)] and never-smoking [8.33 (2.56)] controls, p=0.228 [Figure 1]. When adjusted for the effects of each other, asthma (p<0.001) and smoking (p=0.017) are both independently associated with the cutaneous vasoconstrictor response. The mean (95% CI) reduction for asthmatics, after adjustment for smoking, was -1.70 (-2.66, -0.73). Similarly, the

mean (95% CI) reduction for smoking after adjustment for asthma was -1.18 (-2.15, -0.21). However, there is no evidence of synergy between the two variables [test of interaction, p=0.162].

Lymphocyte proliferation response

There was a concentration-dependent inhibitory effect of dexamethasone on the proliferative response to PHA, which was similar in smokers compared to never-smokers, in asthma and in healthy controls [Figure 2]. The mean (SD) Imax was similar between smokers [50.3 (26.5)] compared to never smokers with asthma [56 (25.9)] and in healthy controls who were smokers [60.8 (21.1)] compared to never smokers [50.4 (27.4)]; p value comparing 4 groups 0.29. The gradient of all concentrations of dexamethasone were similar in the 4 groups (p=0.44).

Airway response to oral prednisolone

After high-dose oral corticosteroids, there was a significant improvement in mean (95% CI) morning PEF [26.2 (2.5, 50); p = 0.031], daytime symptoms [-2.3 (-4, -0.4), p = 0.016], asthma control score [-0.9 (-1.4, -0.4), p = 0.001] and rescue medication use [-0.8 (-1.5, -0.1), p = 0.029] and a fall in F_ENO [3.2 (0.1, 6.4), p = 0.043] in never-smokers with asthma compared to smokers with asthma. There was no significant difference in the change in other end points between never-smokers and smokers with asthma [Table 3].

Relationships between cutaneous vasoconstrictor response and baseline measurements

An increased number of cigarettes smoked per day was associated with a lower total skin test score (r = -0.41, p=0.010), hence increased resistance. The cutaneous vasoconstrictor response of the whole asthmatic population correlated negatively with the dose of beclometasone (r -0.38, p = <0.001) and asthma control score (r= -0.41, p<0.001), but there was no correlation with age (r=-0.175, p = 0.139), duration of asthma (r= -0.08, p = 0.491), asthma severity score (r= -0.17, p = 0.154) or total IgE (r=-0.108, p=0.408).

DISCUSSION

This study has demonstrated that the cutaneous vasoconstrictor response to topical beclometasone is reduced in smokers with asthma compared to never-smokers with asthma and smokers without asthma. These findings suggest, for the first time, that corticosteroid insensitivity in smokers with asthma may be more generalised, affecting tissue sites other than the airways.

The cutaneous vasoconstrictor response to topical beclometasone [10,11], has been used as a screening test to determine the relative anti-inflammatory potency of corticosteroids [22] and as an index of systemic sensitivity to corticosteroids [13]. More objective methods of detecting glucocorticoid-induced skin blanching have been compared to the visual scoring system, but the human eye has been found to be the most sensitive tool to measure dermal blanching [11]. We found that both smoking and asthma were independently associated with an impaired cutaneous vasoconstrictor response to topical beclometasone. These findings suggest that smoking and asthma acted in an additive manner to impair cutaneous vasoconstrictor responses. The reason(s) for the reduced cutaneous vasoconstrictor response in smokers with asthma is not clear. If chronic cigarette smoking were to alter the structure or function of skin microvasculature then this might influence the cutaneous vasoconstrictor response to corticosteroids. However, dermal thickness and elasticity of the forearm of smokers are similar to never-smokers [23] as is the cutaneous vasodilator response to intradermal histamine [24]. The dosage of inhaled corticosteroids, age, duration of asthma and severity score were similar between the smokers with asthma and the never-smokers with asthma despite the former group showing impaired skin responses. The cutaneous vasoconstrictor response is inhibited by glucocorticoid receptor antagonist [25], correlates with glucocorticoid receptor affinity [22] and the intensity of blanching is potentiated by inhibitors of the local metabolism of cortisol [26]. These findings suggest that the cutaneous vasoconstrictor response is likely to be mediated by glucocorticoid receptors. The negative

correlation between the cutaneous vasoconstrictor response and the number of cigarettes smoked per day strengthens our evidence that smoking impairs the response to corticosteroids. It is unclear why asthma should be independently associated with an impaired cutaneous vasoconstrictor response to topical beclometasone. Corticosteroid use is associated with the down-regulation of glucocorticoid receptors [27, 28] and as most of the asthmatics were on a moderate dose of inhaled corticosteroids this may be one explanation why asthma appeared to be an independent risk factor for an impaired response.

The mechanisms by which corticosteroids cause vasoconstriction may involve inhibition in the uptake of the vasoconstrictor noradrenaline at nerve endings in the skin [29]. Exposure of human skin vasculature to nicotine potentiates noradrenaline-induced skin vasoconstriction [30] but whether nicotine or other constituents of cigarette smoke influences the vasoconstrictor response to corticosteroids is not known. The *in vivo* sensitivity to topical budesonide in healthy subjects is influenced by glucocorticoid receptor polymorphisms [31]. The glucocorticoid receptor α to β ratio is reduced in peripheral blood mononuclear cells of cigarette smokers [32] and similar changes might affect glucocorticoid receptors in the skin. Other possible mechanisms of cigarette smoke-induced corticosteroid resistance implicated in other tissues [4] including reduced histone deacetylase (HDAC) activity [33] might be relevant to the skin. HDAC activity has shown to be reduced in patients with asthma [34], COPD [35] and in subjects who smoke [33]. A preliminary report suggests that HDAC activity is reduced in bronchial biopsies from asthmatic smokers compared with healthy smokers and asthmatic non-smokers [36]. If similar changes in HDAC activity are found in the skin then this may explain our findings. Taken together, we believe that our findings suggest that corticosteroid insensitivity in smokers with asthma affects not only the airways, but is also a systemic effect as shown by the reduced cutaneous vasoconstrictor response in smokers with asthma.

Cigarette smoking has been reported to increase [37], or to decrease [38] lymphocyte proliferation. This anomaly has been partly resolved in animal models by showing that acute tobacco smoke exposure or administration of nicotine is stimulatory for lymphocyte proliferation whereas chronic exposure is inhibitory [39, 40] . There was a similar, approximately linear, dose-dependent level of sensitivity to corticosteroids found in peripheral blood lymphocytes stimulated by phytohaemagglutinin from smokers with asthma compared to never-smokers with asthma. This, in contrast to the impaired cutaneous vasoconstrictor response to topical beclometasone and reduced airway response to prednisolone in the former group suggests variation in tissue sensitivity to corticosteroids in smokers with asthma. The reason(s) for the different tissue sensitivity in smokers is not known, but a lack of correlation between different tests of tissue sensitivity to corticosteroids has been reported previously in healthy volunteers [41].. As the lymphocyte proliferation test is performed *ex vivo*, it is possible that any effect of cigarette smoking on lymphocyte function may no longer be present. However, PBMCs from smokers have shown a reduced HDAC activity [33] and altered cytokine levels [42] *in vitro*.

In this study we demonstrated a reduced response to oral corticosteroids in smokers with asthma compared to never-smokers, measured by morning PEF, rescue medication use, daytime symptoms and asthma control score, but not by FEV_1. This result was similar, but not identical, to our findings in previous studies using inhaled fluticasone [6] or oral prednisolone [7] compared with a placebo. The current study was designed differently with a single arm of treatment and included smokers and never-smokers with baseline lung function more severe than in our previous studies.

Smoking is widely accepted as the major cause of chronic obstructive pulmonary disease (COPD). It can often be difficult to differentiate smokers with asthma from those with COPD, however we are confident that the smokers in our study had asthma rather than COPD. The smokers with asthma fulfilled the diagnostic criteria for asthma, had a mean age of 47 years and had been

symptomatic from their mid-twenties, which would be rare in patients with COPD. The asthmatic smokers had a post-salbutamol FEV_1 of > 75%, which would be higher than expected in symptomatic patients with COPD, and had reversibility to salbutamol of ≥15%. In a previous study we found that induced sputum neutrophil counts were elevated in smokers with asthma compared to non-smokers with mild asthma [43]. In the current study there was a trend towards a raised neutrophil count in the smoking asthmatics compared to the never-smokers (p=0.070), but both groups had more severe disease.

In conclusion we have shown that smokers with asthma have an impaired cutaneous vasoconstrictor response to topical corticosteroids compared to never-smokers with asthma. This finding suggests that the insensitivity to corticosteroids in smokers with asthma affects tissue sites other than the airways. This not only has implications for smokers with asthma, but may also be important in other corticosteroid sensitive inflammatory conditions.

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Table 1: Baseline demography of asthmatic subjects and healthy controls.

	Asthmatic			Healthy controls		
	Smokers (n=39)	**Never smokers (n=36)**	**p value**	**Smokers (n=30)**	**Never smokers (n=48)**	**p value**
Age, years	47.4 (7.4)	45.1 (10.9)	0.42	39.9 (8.6)	35.2 (8.0)	0.029
Gender; male	20	29	0.008	10	17	0.85
Asthma duration, years	21.7 (15.7)	28.9 (17.2)	0.07	-	-	-
Pack year smoking history	37.7 (17.2)†	-	-	22.8 (14.9)	-	-
Cigarettes/day	22.6 (7.5)*	-	-	19.3 (7.1)		
Years smoked	30.6 (7.2)†	-	-	23.3 (8.6)	-	-
Equivalent dose of inhaled beclomethasone, μg	997 (905)	631 (646)	0.11	-	-	-
FEV_1, L	1.95 (0.70)	2.26 (0.77)	0.045	2.84 (0.81)	3.35 (0.93)	0.014
FEV_1, % predicted	63.3 (13.9)	63.6 (17.5)	0.70	93.4 (12.5)	99.7 (13.9)	0.039
FEV_1 post salbutamol, (% predicted)	77.5 (5.5)	82.6 (6.4)	0.44	-	-	-
FEV_1/FVC pre salbutamol	61.7 (11.4)	60.9 (14.1)	0.98	77.5 (5.5)	82.6 (6.4)	<0.001
FEV_1/FVC post salbutamol	65.0 (12.1)	64.8 (15.2)	0.77	-	-	-
Reversibility to salbutamol, %	22.7 (11.4)	25.6 (11.9)	0.23	-	-	-
Reversibility to salbutamol, ml	426 (236)	549 (269)	0.014	-	-	-
ATS impairment score	5.1 (1.9)	5.2 (1.9)	0.829	-	-	-
Asthma control score	2.66 (1.08)	1.67 (0.83)	<0.001	-	-	-

*Data depicted as mean (SD); * $p < 0.05$, †<0.001 for smokers with asthma compared to healthy smokers.*

Table 2: Allergy levels, serum cotinine, exhaled gases and induced sputum cell counts at baseline in asthmatic subjects and healthy controls.

	Asthmatic			Healthy controls		
	Smokers (n=39)	**Never smokers (n=36)**	**p value**	**Smokers (n=30)**	**Never smokers (n=48)**	**p value**
Total IgE, (IU/ml)	87 (41-239)	138 (60-590)	0.12	47 (13-74)	32 (14-111)	0.82
Specific IgE +ve, %	62	91	0.004	29	46	0.13
Serum cotinine, ng/ml	304 (131-375)*	2.6 (2-3)	<0.001	366 (310-421)	2.6 (2.3-3.8)	<0.001
Exhaled CO, ppm	22 (16-30)	4.3 (4-5)	<0.001	18.42 (7.07)	3.79 (1.06)	<0.001
Exhaled NO, ppb	5.1 (2.6-7.8)	18.2 (12.5-28.6)	<0.001	4.3 (2.9-8.1)	7.4 (5.6-8.7)	0.006
Induced sputum cell counts						
Macrophages, %	50 (24-67)	51 (26-67)	0.93	-	-	-
Neutrophils, %	42 (23-66)	31 (13-44)	0.07	-	-	-
Eosinophils, %	1.0 (0.5-2.7)	2.0 (0.5-5.5)	0.51	-	-	-
Lymphocytes, %	1.7 (1-2.6)	2.5 (0.9-4)	0.61	-	-	-
Bronchial epithelial cells, %	2.6 (0.9, 6.6)	5.8 (3.5, 9.4)	0.020	-	-	-

Data depicted as median (IQR) * $p<0.05$ comparing asthmatic and healthy smokers.

Table 3: Mean (SD) lung function, symptom, asthma control score and exhaled gases response to high dose (40mg for 2 weeks) oral prednisolone in never-smokers with asthma compared to smokers with asthma.

	Asthmatic		Difference (CI)	p value
	Never-smokers n=39	Smokers n=36		
Δ Morning PEF	20.4 (50.1)	6.3 (44.8)	26.2 (2.5, 50)	0.031
Δ Night PEF	9.1 (44.1)	14.6 (46.1)	2.3 (-21, 26)	0.84
Δ FEV_1, mls (pre-salbutamol)	165 (390)	85 (303)	98 (-71, 266)	0.25
Δ FEV_1 post-salbutamol, mls	8 (436)	-81 (195)	126 (-38, 290)	0.13
Δ Reversibility % with salbutamol	-9.3 (13)	-10.5 (14)	3.0 (-2.8)	0.25
Δ Daytime symptoms	-1.8 (3.1)	-0.8 (4.9)	-2.3 (-4, -0.4)	0.016
Δ Night-time symptoms	-0.1 (0.4)	-0.2 (0.9)	-0.2 (-0.5, 0.1)	0.12
Δ Rescue medication use	-0.7 (1.3)	-0.1 (1.6)	-0.8 (-1.5, -0.1)	0.029
Δ Asthma control score	-0.46 (0.84)	-0.31 (1.25)	-0.9 (-1.4, -0.4)	0.001
Δ Exhaled nitric oxide, ppb	-12.4 (15.1)	-2.6 (8.4)	3.2 (0.1, 6.4)	0.043
Δ Exhaled CO, ppm	0.2 (2.8)	-0.9 (8.8)	-5.5 (-10, -0.7)	0.025

Data depicted as mean (SD). All variables have been analyzed by ANCOVA.

FIGURE LEGENDS

Figure 1

Cutaneous vasoconstrictor response to topical beclometasone in asthmatic subjects and healthy controls according to smoking status.

The box represents the inter-quartile range, the line the median value and the triangle the mean value.

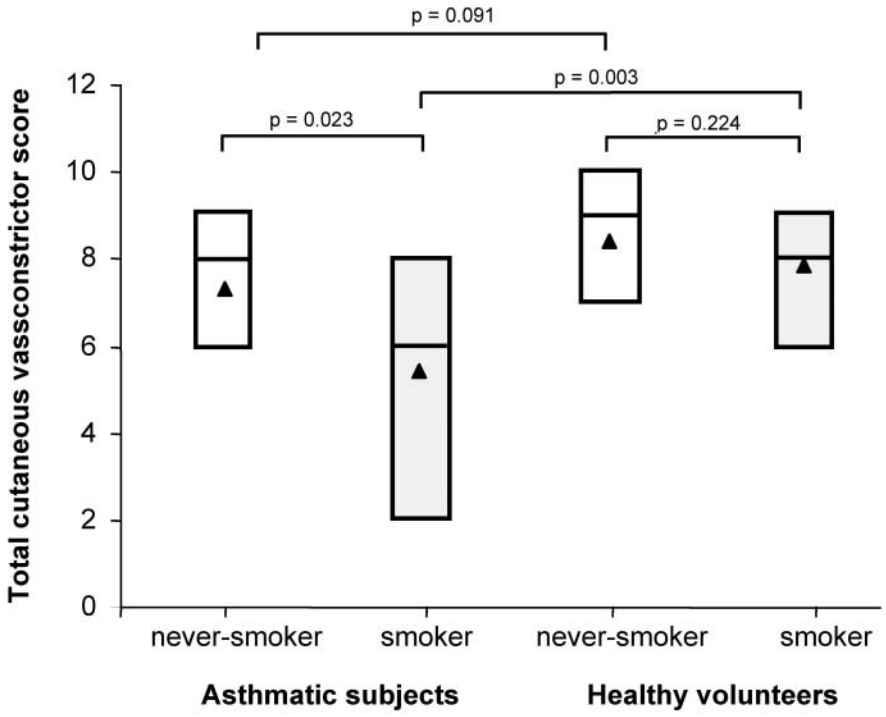

Figure 2:

Inhibition of phytohaemagglutinin (PHA)-induced proliferation of peripheral blood T-lymphocytes by dexamethasone (10^{-11} to 10^{-4} mol L^{-1}) from (A) smokers [closed square] and never smokers [open diamond] with asthma and (B) healthy controls who are smokers [closed square] and never smokers [open diamond]. Graph shows mean (SEM) proliferation in the presence of dexamethasone expressed as a percentage of that obtained without dexamethasone.

Figure 2