

## ORIGINAL ARTICLE

# Adrenal responses to a low-dose short synacthen test in children with asthma

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## Summary

**Objectives** Corticosteroids are known to cause adrenal suppression. The aim of this study was to assess clinical factors affecting responses to a low dose short synacthen test (LDSST) in asthmatic children using corticosteroids.

**Design** Patients were recruited from secondary care paediatric asthma populations within the UK.

**Patients** Asthmatic children (5–18 years), receiving corticosteroids, underwent a LDSST ( $n = 525$ ).

**Measurements** Demographics and corticosteroid doses were tested for association with baseline and peak (stimulated) cortisol concentrations.

**Results** Baseline cortisol was significantly associated with age (log baseline increased 0.04 nM per year of age,  $P < 0.0001$ ), but not with gender or corticosteroid dose. Peak cortisol was significantly associated with total corticosteroid cumulative dose (decreased 0.73 nM per 200 mcg/day,  $P < 0.001$ ) but not with age, gender inhaled/intranasal corticosteroid cumulative dose or number of courses of rescue corticosteroids. Biochemically impaired response (peak cortisol  $\leq 500$  nM) occurred in 37.0% (161/435) overall, including children using GINA low (200–500 mcg/day beclomethasone-CFC equivalent 32%,  $n = 60$ ), medium (501–1000 mcg/day 33%,  $n = 57$ ) and high (>1000 mcg/day 40%,  $n = 13$ ) doses of inhaled corticosteroid (ICS) similarly, and 36.6% of those using fluticasone ICS

$\geq 500$  mcg/day (71/194). Impaired response was more frequent in patients on regular oral corticosteroids (66%,  $n = 27$ ,  $P < 0.001$ ).

**Conclusion** Children with asthma can develop biochemical adrenal suppression at similar frequencies for all ICS preparations and doses. The clinical consequence of biochemical suppression needs further study.

(Received 19 August 2014; returned for revision 12 September 2014; finally revised 7 October 2014; accepted 30 October 2014)

## Introduction

Asthma has a high prevalence (30% in selected populations<sup>1</sup>) and early use of inhaled corticosteroids (ICS) is recommended.<sup>2,3</sup> The efficacy of corticosteroids in asthma is well established, but they can produce local and systemic adverse effects, including suppression of the hypothalamic–pituitary–adrenal (HPA) axis. Biochemical evidence of HPA axis suppression has been reported in 20–40% of children receiving ICS,<sup>4–10</sup> and symptomatic adrenal suppression has been reported in children taking as little as 200 mcg/day inhaled beclomethasone.<sup>11</sup> International asthma management guidelines therefore recommend using the lowest effective dose of ICS to maximize the benefit–risk ratio.<sup>2,3,12</sup> However, some children have symptoms which are only controlled with high dose inhaled and/or regular oral corticosteroids. Asthma is also associated with other atopic illnesses, such as eczema and hay fever, which may require treatment with additional corticosteroids (oral, intranasal and/or topical).

The presentation of adrenal insufficiency in childhood varies, ranging from asymptomatic<sup>6</sup> to subtle (loss of growth velocity, increased lethargy, weight loss), to florid (symptomatic adrenal crisis). There are a number of diagnostic tests to assess adrenal function, the most frequently used in paediatric practice being the short Synacthen test. These tests generally utilize either one of two doses of Synacthen: the standard dose (250 mcg

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All authors have made substantial contributions to a) conception and design, acquisition of data, or analysis and interpretation of data; b) drafting the article or revising it critically for important intellectual content; and c) given final approval of the version to be published.

Synacthen) (SDSST) and low dose (500 ng/1.73 m<sup>2</sup> Synacthen) (LDSST). Both tests measure baseline (unstimulated) and peak (postadministration of Synacthen) cortisol and are timed to coincide with the peak in cortisol that occurs in the morning. The SDSST uses 500 times the dose of corticotrophin required for a stimulated cortisol response,<sup>8,13,14</sup> while the LDSST has been shown to be as effective at producing a stimulated cortisol response as the SDSST.<sup>15</sup> LDSST however is still an invasive test, and for ethical reasons, very few data are available to indicate what a normal peak cortisol response should be in healthy children. Normal values have therefore largely been extrapolated from adult populations<sup>16</sup> or from small cohorts of children.<sup>8,9,17</sup>

In this report, we have examined the factors associated with baseline and peak cortisol concentrations, and compare the characteristics of children with and without a sufficient response to the LDSST in children recruited to the Pharmacogenomics of Adrenal Suppression with Inhaled Steroids (PASS) study.

## Methods

Participants aged from 5 to 18 years inclusive with a clinical diagnosis of asthma requiring corticosteroid maintenance therapy were recruited to the Pharmacogenetics of Adrenal Suppression with Inhaled Steroids Study (PASS). These participants included children who were also participating in the Early Morning Salivary Cortisol Study (EMSC).<sup>18</sup> PASS received full ethical approval from Liverpool Paediatric Research Ethics Committee.

Participants included in this cohort were recruited from November 2008 to September 2011 from 25 sites in the UK. Eligibility criteria were as follows: treatment with ICS >6 months; diagnosis of asthma; under care of a paediatrician experienced in the treatment of asthma; clinical concern about adrenal suppression sufficient to warrant a LDSST or participant in EMSC study; age 5–18 years. Exclusion criteria were as follows: Type 1 or Type 2 diabetes; competent older participant declining assent. Study participants were recruited either prospectively (if LDSST not yet undertaken) or retrospectively (if LDSST already undertaken). If not already performed, the LDSST was undertaken at the study visit. All clinical data collected for retrospective PASS patients relates to the period prior to undertaking the LDSST.

### Consent process

Participants were identified by paediatricians. Information was given to the family and consent obtained from the parent/guardian (or participant if ≥16 years) after at least 24 h consideration. Assent was also sought from older participants (age 12–15 years, judged on a case by case basis). Participants judged able to give assent, but who declined, were excluded.

### Design of the LDSST

We based the sampling regimen for LDSST on that developed by Paton *et al.*<sup>6</sup> In that study, LDSST consisted of a dose of

500 ng/1.73 m<sup>2</sup> and sampling at 0, 15, 20, 25, 30 and 35 min. Raw data from that audit<sup>6</sup> were reviewed, and measurements from four of the six time points (0, 15, 25, 35 min) were able to consistently distinguish between normal and abnormal responses.<sup>18</sup>

A minority of patients included in PASS were recruited having already undergone a LDSST within the last 2 years using the local protocol at the recruiting institution.

### LDSST procedure

The LDSST methodology has previously been published.<sup>18</sup> Briefly, on the day of the LDSST, any corticosteroid containing medication was withheld until test completion. For patients treated with regular alternate day oral corticosteroid, the LDSST was performed on the day a dose was due to be given.

All Synacthen tests were undertaken before 11 am. A blood sample was collected from an indwelling venous catheter (sited following application of local anaesthetic cream) (time 0). One hundred and twenty-five micrograms (0.5 ml) Synacthen (Alliance, Chippenham, UK) 0.25 mg/ml solution was added to 500 ml 0.9%NaCl (final concentration of 250 nanograms/ml) and agitated, before immediate removal of the required dose. Five hundred nanograms/1.73 m<sup>2</sup> was administered as a bolus injection directly into the cannula and flushed with 5 ml of 0.9% saline. Samples were collected 15, 25 and 35 min following Synacthen administration.<sup>19</sup> A Synacthen dose calculator was used to ensure consistency of dosing across different study locations.

### Statistical analysis

All analyses were undertaken in SAS v.9.2 (Cary, NC, USA). For times when multiple comparisons were carried out, a Bonferroni correction was used and a significant *P*-value assumed to be <0.005. Full details of the statistical methodology are included in the online supplementary data section. Analyses undertaken to investigate the growth observed in the population (height-for-age *z* score), and the relationship between cumulative corticosteroid dose and height-for-age *z* score, are all detailed in the supplementary data section.

### Calculating corticosteroid cumulative dose

Doses of corticosteroid taken by participants were expressed as µg/day of beclomethasone dipropionate equivalent.<sup>12</sup> Dose ratios of 1:1 (Beclomethasone Dipropionate CFC, Clenil Module, Budesonide),<sup>2</sup> 2:1 (Fluticasone, Mometasone, Qvar)<sup>2</sup> and 1:3.9 (Prednisolone) were used.<sup>20</sup> Patient's corticosteroid cumulative dose over the previous 6 months was then calculated in three different ways:

- Mean daily inhaled/intranasal corticosteroid cumulative dose in 6 months prior to LDSST (cumulative dose of inhaled and intranasal corticosteroid medications, regardless of rescue therapies and excluding those on regular oral corticosteroids,

expressed as a mean daily dose of beclomethasone dipropionate equivalent).

- Number of courses of rescue oral corticosteroids required to treat exacerbations of asthma in the preceding 6 months before the LDSST.

- Mean daily total corticosteroid cumulative dose in 6 months prior to LDSST (cumulative dose of inhaled, intranasal and regular oral corticosteroid medications as well as rescue courses, assuming a course of rescue corticosteroids is a 3 day course of oral prednisolone, dosed at 2 mg/kg per day (to a maximum dose of 40 mg), expressed as a mean daily dose of beclomethasone dipropionate equivalent).

All dose calculations assume 100% concordance and bioavailability (regardless of route of administration). Topical corticosteroid preparations applied to the skin were not included in the total corticosteroid dose calculations due to uncertainty about the dose received due to variations in both the surface area of the body affected and quantity of cream applied.

Adherence with corticosteroids in asthma has previously been assessed using prescription fill–refill data.<sup>21</sup> Repeat prescription data for the 6 months prior to the date of the LDSST were supplied by general practitioners (GP) of patients recruited from a single centre. Using the strength of inhaler prescribed, number of doses per inhaler (from manufacturers SPC) and prescribed dose, the minimum number of refills required by the patient in the 6-month period was calculated and compared with the actual number of refills collected. For the statistically significant relationship(s), the effect of variation in the population adherence was calculated.

## Results

### Participants

Five hundred and twenty-five children were recruited (November 2008 to September 2012). Ninety-two of these participants underwent a LDSST using alternative dose of Synacthen and/or different sample collection times, and thus did not have comparative data for peak cortisol values. Participant characteristics are summarized in Fig. 1. No serious adverse effects were noted.

### Factors associated with baseline and peak cortisol following LDSST stimulation

**Baseline cortisol.** The mean and median baseline cortisol values were 246 nM (SD 139 nM) and 215 nM (25th/75th centile 156/303 nM), respectively. Forty-six (8.9%) patients had baseline cortisol values below 100 nM. The distribution of baseline cortisol data was skewed, but normalized following log-transformation. Log-baseline cortisol concentrations increased by 0.04 nM per year of age, (95% CI 0.02, 0.05;  $P < 0.0001$ ) (geometric mean 1.1 nM). Gender was not associated with baseline cortisol concentrations either when including all patients, with females having a log-baseline value 0.03 nM higher than males (95% CI -0.07, 0.14;  $P = 0.52$ ) or when including

only patients aged 12 years or older, with females having a log-baseline value 0.10 nM higher than males (95% CI -0.05, 0.26;  $P = 0.19$ ) (Table 1).

There was also no significant association between baseline cortisol concentration and either:

- Inhaled/intranasal cumulative dose either when no adjustment was made for regular oral or rescue corticosteroids, with log baseline decreasing by 0.018 nM (95% CI -0.004, 0.039;  $P = 0.12$ ) for each 200 µg increase or when regular oral and rescue corticosteroids were adjusted for, with log baseline decreasing by 0.018 nM (95% CI -0.006, 0.042;  $P = 0.15$ ) for each 200 µg increase.

- The number of courses of rescue oral corticosteroids and baseline cortisol either when those on regular oral corticosteroids were excluded, ( $P = 0.45/0.59$  without/with adjusting for inhaled and intranasal corticosteroid cumulative dose) or when they were included ( $P = 0.59/0.66$  without/with adjusting for inhaled and intranasal corticosteroid cumulative dose).

- Total corticosteroid cumulative dose with log baseline decreasing by 0.0003 nM (95% CI -0.0008, 0.0003;  $P = 0.39$ ) for each 200 µg increase in standardized daily dose beclomethasone dipropionate used. Results were consistent in sensitivity analyses where dose was adjusted for BSA.

**Peak cortisol.** The mean peak cortisol was 545 nM (SD 152 nM) and normally distributed. One hundred and sixty-one patients (37.4%) had peak cortisol values below the level at which referral to a paediatric endocrinologist for consideration of therapeutic intervention with replacement hydrocortisone (<500 nM) is recommended in the literature<sup>16,22</sup> (Table 2).

Total corticosteroid cumulative dose was associated with peak cortisol concentrations, the latter decreasing by 0.73 nM (0.37, 1.08;  $P < 0.001$ ) for each 200 µg increase in the standardized daily dose of beclomethasone dipropionate used. Results were consistent in sensitivity analyses where dose was adjusted for BSA.

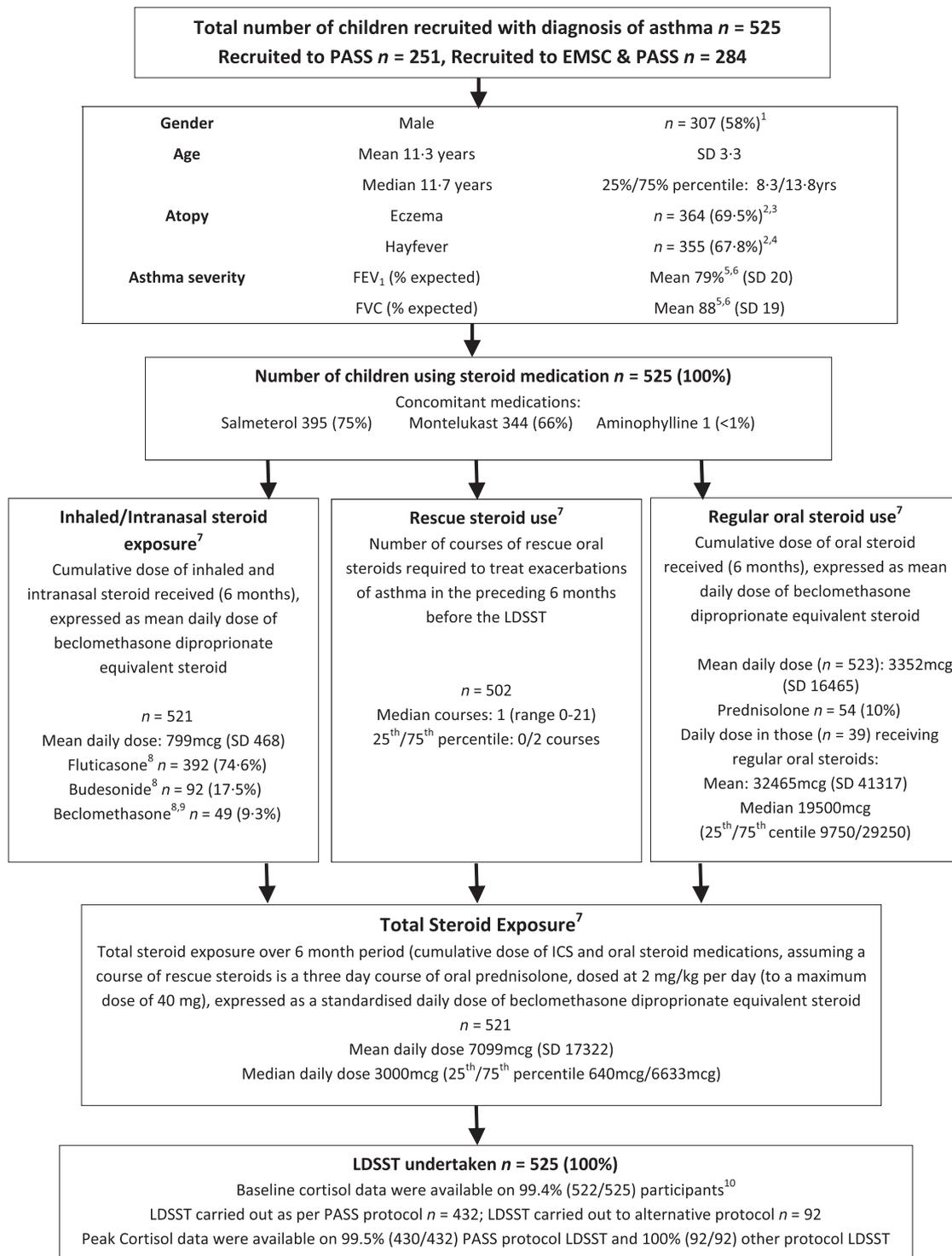
Peak cortisol concentrations were however not associated with:

- Age, with levels decreasing by 1.69 nM (95% CI -2.62, 6.01;  $P = 0.44$ ) for each year increase in age.

- Gender when including all patients, with females having peak levels 25.05 nM higher (-4.00, 54.09;  $P = 0.09$ ) or when including only patients aged 12 years or older, with females having peak levels 48.35 nM higher (95% CI 5.56, 91.14;  $P = 0.018$ ).

- Inhaled/intranasal corticosteroid cumulative dose and peak cortisol with peak levels decreasing by 6.31 nM (95% CI 0.04, 12.66;  $P = 0.06$ ) for each 200 µg increase in standardized daily dose beclomethasone-CFC used and by 2.39 nM (95% CI -4.57, 9.35;  $P = 0.56$ ) for each 200 µg increase without/with adjusting for regular oral and rescue corticosteroids, respectively.

- The number of rescue courses either when those on regular oral corticosteroids were excluded ( $P = 0.21/0.31$  without/with adjusting for inhaled/intranasal corticosteroid cumulative dose) or when they were included ( $P = 0.11/0.44$  without and with adjusting for inhaled/intranasal corticosteroid cumulative dose).



**Fig. 1** Total number of children recruited with diagnosis of asthma  $n = 525$ . Recruited to PASS  $n = 251$ , Recruited to EMSC & PASS  $n = 284$ . <sup>1</sup>Gender data missing for 2 participants. <sup>2</sup>261 participants diagnosed with both eczema and hayfever. <sup>3</sup>Data missing for 2 patients. <sup>4</sup>Data missing for 4 patients. <sup>5</sup>Mean FEV<sub>1</sub> and FVC data derived from the lowest percentage expected score for each variable given by the patient in the 12 months prior to the LDSST date. <sup>6</sup>Data missing for 165 patients. <sup>7</sup>Doses of steroid are expressed as a standardized daily beclomethasone equivalent dose. Dose ratios of 1:1 (Clenil Modulite, Budesonide), 2:1 (Fluticasone, Mometasone) and 1:3.9 (Prednisolone) were used [12, 23]. <sup>8</sup>Some patients received more than one type of inhaled steroid during the 6-month period. <sup>9</sup>Participants receiving beclomethasone preparations all received either Clenil Modulite or CFC containing beclomethasone preparations – none received Qvar. <sup>10</sup>Missing baseline data due to insufficient sample volume being collected at the time of cannula insertion.

**Table 1.** Associations with log-baseline cortisol

Log-baseline cortisol Independent Variable	PASS and EMSC patients		PASS only patients		All	
	<i>n</i>	<i>P</i> -value	<i>n</i>	<i>P</i> -value	<i>n</i>	<i>P</i> -value
Age	278	<0.001*	240	0.007	518	<0.0001*
Gender	278	0.16	241	0.86	521	0.52
Gender (age ≥12 years)	111	0.27	132	0.40	245	0.19
Inhaled/intranasal dose– no adjustment for oral/rescue	277	0.60	237	0.80	516	0.12
Inhaled/intranasal steroid cumulative dose – adjusted for oral/rescue	268	0.57	222	0.74	491	0.15
Number of courses of rescue steroids(excluding those on regular oral steroids; no adjustment for inhaled/ intranasal)	247	0.72	199	0.89	447	0.45
Number of courses of rescue steroids (excluding those on regular oral steroids; adjustment for inhaled/ intranasal)	246	0.73	196	0.38	443	0.59
Number of courses of rescue steroids (including those on regular oral steroids; no adjustment for inhaled/ intranasal and oral)	269	0.64	227	0.94	498	0.59
Number of courses of rescue steroids (including those on regular oral steroids; adjustment for inhaled/ intranasal and oral)	268	0.63	223	0.95	493	0.66
Total steroid cumulative dose	277	0.64	237	0.82	516	0.39

\*Significant following Bonferroni correction ( $P < 0.005$ ).

**Table 2.** Associations with peak cortisol

Peak cortisol Independent variable	PASS and EMSC patients		PASS only patients		All	
	<i>n</i>	<i>P</i> -value	<i>n</i>	<i>P</i> -value	<i>n</i>	<i>P</i> -value
Age	279	0.84	152	0.70	432	0.44
Gender	279	0.008	151	0.93	434	0.09
Gender (age ≥12 years)	111	0.002*	87	0.68	202	0.027
Inhaled/intranasal dose– no adjustment for oral/rescue	278	0.98	146	0.026	429	0.06
Inhaled/intranasal steroid cumulative dose – adjusted for oral/rescue	269	0.63	138	0.14	409	0.56
Number of courses of rescue steroids (excluding those on oral steroids; no adjustment for inhaled/intranasal)	248	0.77	124	0.16	373	0.21
Number of courses of rescue steroids (excluding those on regular oral steroids; adjustment for inhaled/intranasal)	247	0.75	121	0.32	369	0.31
Number of courses of rescue steroids (including those on regular oral steroids; no adjustment for inhaled/ intranasal and oral)	270	0.35	142	0.25	416	0.11
Number of courses of rescue steroids (including those on regular oral steroids; adjustment for inhaled/intranasal and oral)	269	0.54	138	0.38	411	0.44
Total steroid cumulative dose	278	0.04	146	0.001*	429	<0.001*

\*Significant following Bonferroni correction ( $P < 0.0005$ ).

### Comparison of patient characteristics between those with impaired and sufficient peak cortisol

It was necessary to log-transform total corticosteroid cumulative dose as its distribution was skewed. There was no difference in age between patients with normal or impaired responses (mean

age 11.4 (SD: 3.07) years impaired, 11.1 (SD: 3.49) years normal;  $P = 0.37$ ) (Table 3). Impaired responses in males were similar to those in females (40% vs 44%, respectively,  $P = 0.52$ ).

We also carefully evaluated the dose–response relationship with impaired adrenal response. Specifically, we found that:

**Table 3.** Comparison of characteristics and dose cumulative dose between patients with normal ( $\geq 500$  nm) and impaired ( $< 500$  nm) peak cortisol concentrations following a low-dose short synacthen test (LDSST). Significant *P* value assumed to be  $< 0.005$  to account for multiple corrections (Bonferroni correction)

	Peak cortisol concentration (nm)		<i>P</i> -value
	$< 500$ ( <i>n</i> = 161)	$\geq 500$ ( <i>n</i> = 274)	
Age, years (mean(SD))	11.43 (3.07)	11.10 (3.49)	0.33
Gender ( <i>n</i> )	Male: 95; Female: 65	Male: 154; Female: 120	0.52
Mean daily standardized intranasal/inhaled cumulative dose past 6 months, mcg (mean(SD))	807 (443)	741 (461)	0.15
No. rescue courses past 6 months ( <i>n</i> ) (%)			0.30
0	61 (38)	124 (45)	
1	34 (21)	61 (22)	
2	16 (10)	36 (13)	
3	18 (11)	22 (8)	
4	10 (6)	9 (3)	
5	4 (2)	5 (2)	
6	5 (3)	3 (1)	
>6	4 (2)	4 (1)	
Missing	9	10	
Daily standardized total cumulative dose past 6 months, mcg (median(IQR))	3600 (8300) (9 missing)	3000 (5500) (10 missing)	0.02
GINA category (%)			
Low dose*ICS	60 (32)	125 (68)	$< 0.001$ †
Medium dose‡ ICS	57 (33)	114 (67)	
High dose§ ICS	13 (40)	19 (59)	
Oral¶	31 (66)	16 (34)	

\*Inhaled and intranasal standardized daily dose 200–500 mcg/day beclomethasone dipropionate equivalent, excluding those using regular oral steroid.

†*P*-value from chi-squared test for association between cumulative dose category and response category.

‡Inhaled and intranasal standardized daily dose 501–1000 mcg/day beclomethasone dipropionate equivalent, excluding those using regular oral steroid.

§Inhaled and intranasal standardized daily dose  $> 1000$  mcg/day beclomethasone dipropionate equivalent, excluding those using regular oral steroid.

¶Patients using regular maintenance oral steroid, regardless of inhaled and intranasal doses.

- The standardized mean daily dose of inhaled/intranasal corticosteroids was similar in those with impaired responses (807 mcg (SD: 443)) and normal responses (741 mcg (SD: 461)) ( $P = 0.22$ ), and there was no statistically significant association between the number of rescue courses taken and response ( $P = 0.30$ ) (Table 3).

- The mean daily dose of total corticosteroid cumulative dose was not significantly different between the two groups (median 3600 mcg (IQR: 10150) impaired and 3000 (IQR: 5700) normal ( $P = 0.01$ )).

- The proportion of impaired responses was similar in those taking GINA low dose (200–500 mcg beclomethasone dipropionate equivalent per day), GINA medium dose (501–1000 mcg per day) and GINA high dose ( $> 1000$  mcg per day) (32%, 33% and 40%, respectively) as per the GINA categories;<sup>23</sup>

- Of the children in our study where peak cortisol concentration was available, 324 used fluticasone as ICS for asthma control. Of these, 194/324 (59.9%) were treated with doses of fluticasone  $\geq 500$  mcg/day and 71/194 (36.6%) had a peak cortisol of  $< 500$  nm.

- The proportion with impaired response was significantly higher in those on regular oral corticosteroids (66%; chi-square test;  $P < 0.001$ ) (Table 3).

## Adherence

Thirty eight patients consented to this substudy, but GPs only supplied data for 30 (78.9%). Six patients did not collect any repeat prescriptions, while three collected  $> 100\%$  of the minimum number required. Overall, patients/families collected a median of 32.9% (range 0–263%) of the minimum number of required prescriptions for ICS in the time period examined.

In our statistical analysis of association with peak and baseline cortisol levels, we assumed 100% adherence. As expected, statistical significance of the associations remained unchanged with varying levels of assumed adherence. However, greater reductions in peak cortisol levels per 200  $\mu\text{g}$  increase in dose were seen with lower adherence rates (Table 4). At the median adherence for our population (33%), the change in peak cortisol per 200  $\mu\text{g}$  increase in dose is  $-3$  (95% CI:  $-4.4$ ,  $-1.6$ ).

## Discussion

ICS are used in children with asthma for all but the mildest cases and are of proven efficacy, albeit with interindividual variability in responses. However, following reports of acute adrenal crisis and death in children, the importance of preventing, rec-

**Table 4.** The effect of assuming varying levels of adherence on the relationship between peak cortisol concentration and the total corticosteroid dose received

Level of adherence assumed in population	10%	33%	50%	100%	110%
Change in peak cortisol per 200 µg increase in dose (nM)	-10	-3	-2	-0.73	-1
95% Confidence interval	(-14.4, -5.6)	(-4.4, -1.6)	(-2.8, -1.2)	(-1.08, -0.37)	(-1.4, -0.06)

Adherence data for our population shows a median fill–refill rate of 33% (range 0–263%). Statistical analysis of peak and baseline data assumes 100% adherence. For the statistically significant relationship between peak cortisol and total corticosteroid dose received, the effect of variation in the population adherence was calculated, showing greater reductions in peak cortisol at lower population adherence rates. Statistical significance was unchanged for each adherence assumption ( $P < 0.001$ ).

ognizing and managing adrenal suppression in children with asthma during ICS therapy has been of interest.<sup>6,10</sup> This represents the largest published cohort of LDSST results in children with asthma. Unlike previous studies, these data include multiple steroid preparations, a range of asthma severities, uses the gold standard diagnostic test, and shows and quantifies associations (steroid dose and age affect peak and baseline cortisol, respectively). These data have also shown that despite ICS dose not having a statistically significant effect on peak cortisol; all children on ICS preparations have similar high rates of biochemical adrenal suppression regardless of dose or preparation.

There are little normative data in healthy children not using corticosteroids. Increasing baseline cortisol with age has been previously published<sup>24</sup> but was not in a form that allowed quantification of either the increase per year of life, or the size of the effect on baseline cortisol, both of which are presented here. The few published peak cortisol measurements in response to a LDSST in healthy children show peak cortisol concentrations of between 516 and 621 nM.<sup>8,9,17</sup> However, these previous data were either from smaller cohorts ( $n = 33, 40$  and  $75$ ),<sup>8,9,17</sup> used a different dose of Synacthen<sup>17</sup> or were sampled at different time points,<sup>8,9,17</sup> making direct comparison difficult. However, we note that the mean peak cortisol (545 nM) from our cohort was contained within this range.

The dose of Synacthen in this LDSST (500 ng/1.73 m<sup>2</sup>) is capable of eliciting a maximal adrenal response.<sup>13</sup> A peak cortisol  $\geq 500$  nM (18.1 µg/dl) signifies adequate adrenal reserve in adult subjects, with values less than this indicating adrenal suppression.<sup>25</sup> The largest previously published cohorts of paediatric asthma patients have used values  $\geq 500$  nM to signify a normal response,<sup>6,8,16</sup> although it is not clear when adrenal suppression becomes clinically evident at a value  $< 500$  nM. The LDSST protocol used in our study was selected from the time points that identified all patients with a peak cortisol  $> 500$  nM in the study by Paton *et al.*<sup>6,18</sup>

The literature contains many reports severe illness and death in children with asthma related to adrenal suppression.<sup>4,6,7</sup> Systemic steroids are well recognized causes of adrenal suppression, but the evidence has previously been mixed with regard to ICS. A systematic review found inconsistent findings with regard to the effect of ICS on adrenal function,<sup>12</sup> but the studies included were mostly of short duration (the majority 12 weeks) and many used other markers of adrenal function such as urinary

free cortisol<sup>12</sup> or the overnight metyrapone test.<sup>26</sup> In comparison, in our study, patients had a longer history of corticosteroid use ( $\geq 6$  months), used a wider range of corticosteroid compounds and had a number of comorbidities such as eczema and hayfever, making them more similar to children with asthma seen by practising clinicians. Importantly, a *post hoc* analysis has showed no association between baseline or peak cortisol and the presence of hayfever ( $P = 0.21$  and  $P = 0.21$ , respectively) or eczema ( $P = 0.81$  and  $P = 0.74$  respectively).

A potential limitation of the LDSST is that anxiety provoked by the LDSST test procedure (which includes cannulation) may have affected the baseline concentration of cortisol. Significant adsorption of Synacthen to plastic giving sets has also been reported,<sup>19</sup> and a small ( $n = 18$ ) study has raised concerns about the test–retest repeatability of the LDSST in children.<sup>27</sup> In our cohort, a protocol was used that minimized the time that Synacthen was in contact with plastic. Synacthen solution preparation was undertaken only after successful cannulation, immediately prior to injection. The required dose was calculated prior to cannulation, and this dose was drawn immediately out of the dilute Synacthen solution following 10 s agitation using a needle and syringe and administered immediately.

The finding of log–baseline cortisol concentration increasing between ages 5 and 18 by approximately 0.04 nM per year may be accounted for by the effects of puberty on the HPA axis.<sup>28</sup> We do not believe that the association with increasing age is solely related to growth of the adrenal gland, as proportionally the adrenals are much larger in newborns and decrease in relative size with age.<sup>29</sup> It may also relate to altered sensitivity of the adrenal gland during childhood. Studies on immature rats have demonstrated adrenal sensitivity to ACTH is partly controlled by sympathetic activity,<sup>30</sup> although it could also relate to alterations in ACTH concentrations during development.

Pharmacologically, corticosteroids exert their primary mechanism of action through the nuclear glucocorticoid receptor system, which justifies our approach of expressing doses in terms of a single corticosteroid. Beclomethasone dipropionate CFC (e.g. Becotide) and Clenil modulite (beclomethasone dipropionate CFC free) are used at equivalent doses, and there are more data to support dose equivalency between corticosteroid compounds and beclomethasone dipropionate; therefore, this formulation seemed the most appropriate corticosteroid to use as a comparator. Using this system, we have demonstrated, and

quantified, a dose–response relationship between total corticosteroid cumulative dose and peak cortisol, with each increase of 200 mcg/day of a beclomethasone dipropionate equivalent corticosteroid preparation leading to a decrease in peak cortisol of 0.73 nM, although this is unlikely to be clinically relevant. Previous concerns that fluticasone is responsible for an increased frequency of adrenal suppression have not been replicated here.

Variable adherence is frequent in children with asthma,<sup>31</sup> difficult to measure, and is contributed to by multiple factors<sup>32</sup> and has been shown to have an effect on the rates of adrenal suppression in children using ICS.<sup>26</sup> However, measurement of adherence is difficult. To account for this, we showed in a sub-study, using fill–refill data,<sup>21</sup> that adherence was approximately 33%. We have estimated the effect of reduced adherence on the change in peak cortisol per 200 µg increase in dose. This indicates that for the adherence levels shown in our population, we have probably under-estimated, rather than over-estimated, the overall effect on ICS on adrenal suppression. If all children took their medications as prescribed, there may be additional cases of adrenal suppression seen. This will need to be further examined, but will require more reliable markers of adherence, which are currently unavailable.

These effects on cortisol level noted in this study are clearly very small, and we therefore believe that additional research into the causes of the interindividual variation, including pharmacogenomic associations, needs to be undertaken.

In summary, we have shown that 37% of a large cohort of children with asthma has a peak cortisol level following a LDSST that falls below the currently accepted threshold for biochemical normality.<sup>8,16</sup> Age and corticosteroid dose can affect the results, although the magnitude of these effects is clinically insignificant. However, for any child with asthma, using ICS (any preparation, any dose), appears to carry similar risk. The importance of our results is supported by a recent Cochrane review and meta-analysis on the effect of ICS on growth in children.<sup>33</sup> This showed that ICS reduced linear growth velocity and the final height (by a mean of 1.2 cm), and this was independent of the dose administered. Thus, clinicians treating children with asthma need a low threshold for investigation of adrenal suppression in children with asthma, and there is a need to undertake further study on the clinical effects of adrenal suppression caused by ICS – these are important drugs for the treatment of asthma in all age groups, and thus, there is a need to develop methods that optimize their benefit–risk ratio.

## Acknowledgements

The authors would like to thank J Paton, P Galloway and M Donaldson (University of Glasgow) for kindly providing the full data set of LDSST results from their previous work, upon which the current LDSST was designed. We acknowledge the support of the National Institute for Health Research, through the Cheshire, Merseyside and North Wales Medicines for Children research network (MCRN), Greater Manchester, Lancashire and

South Cumbria MCRN, MCRN East, London and South East MCRN, South West MCRN and West Midlands MCRN. The authors would also like to acknowledge support from the UK Dept of Health (NHS Chair of Pharmacogenetics) and NIHR Research for Patient Benefit Programme (North West). MP and RLS are NIHR Senior Investigators.

The authors would also like to thank the participants and families who took part in PASS, and the participating hospitals and principle investigators who recruited the children and families (East Sussex, Dr Kanumakala; Southport and Ormskirk, Dr Gardner; Whiston, Dr Amegavie; North Manchester, Dr Dasgupta; Royal Manchester Children's Hospital, Dr Murray; Blackburn, Dr Robertson; Arrowse Park, Dr Lacy; Macclesfield, Dr Ho; Countess of Chester, Dr Bearey; West Sussex, Dr Matthews/Dr Linney; Wigan, Dr Velmurugan; Leeds, Dr Lee; Warrington, Dr Wild; Preston, Dr Mahmood; Barrow in Furness, Dr Olabi; Bradford, Dr Moya; Oldham, Dr Prakash; North Tees, Dr Tuladhar; Leighton, Dr Ellison; Tameside, Dr Levy; Wolverhampton, Dr Raynor; Sheffield, Dr Wright; Doncaster, Dr Natarjan; Mid Yorkshire (Pinderfields), Dr Jones; Huddersfield/Calderdale, Dr Garside; Nottingham, Dr Bhatt; Stockport, Dr Cooper; Ayrshire, Dr Findlay/Dr Adams; Cornwall, Dr Prendiville; Glasgow, Dr Paton).

## Funding

The study was supported by the Department of Health (NHS Chair of Pharmacogenetics), and the NIHR Research for Patient Benefit scheme (PB-PG-0706-10171). Both MP and RLS are NIHR Senior Investigators. DH was supported by a NIHR academic clinical lectureship.

## Conflict of interest

Nothing to declare.

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