Low levels of endogenous anabolic androgenic steroids in females with severe asthma taking corticosteroids

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Urinary levels of potentially beneficial anabolic androgenic steroids are low in severe asthma patients treated with corticosteroids, especially females. This might be one factor contributing to the increased prevalence of severe asthma in women. https://bit.ly/46kq1R7

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Abstract

Rationale Patients with severe asthma are dependent upon treatment with high doses of inhaled corticosteroids (ICS) and often also oral corticosteroids (OCS). The extent of endogenous androgenic anabolic steroid (EAAS) suppression in asthma has not previously been described in detail. The objective of the present study was to measure urinary concentrations of EAAS in relation to exogenous corticosteroid exposure.

Methods Urine collected at baseline in the U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Disease outcomes) study of severe adult asthmatics (SA, n=408) was analysed by quantitative mass spectrometry. Data were compared to that of mild-to-moderate asthmatics (MMA, n=70) and healthy subjects (HC, n=98) from the same study.

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Measurements and main results The concentrations of urinary endogenous steroid metabolites were substantially lower in SA than in MMA or HC. These differences were more pronounced in SA patients with detectable urinary OCS metabolites. Their dehydroepiandrosterone sulfate (DHEA-S) concentrations were <5% of those in HC, and cortisol concentrations were below the detection limit in 75% of females and 82% of males. The concentrations of EAAS in OCS-positive patients, as well as patients on high-dose ICS only, were more suppressed in females than males ($p<0.05$). Low levels of DHEA were associated with features of more severe disease and were more prevalent in females ($p<0.05$). The association between low EAAS and corticosteroid treatment was replicated in 289 of the SA patients at follow-up after 12–18 months.

Conclusion The pronounced suppression of endogenous anabolic androgens in females might contribute to sex differences regarding the prevalence of severe asthma.

Introduction

There is increasing awareness of sex differences in asthma with accumulating evidence that male sex hormones have anti-asthmatic properties. For example, several beneficial effects have been reported for androgens in experimental models [1–3]. Furthermore, a recent study in asthmatics found a positive correlation between blood levels of the androgen dehydroepiandrosterone (DHEA) and lung function [4]. In the same study, higher expression of androgen receptors in bronchial epithelial cells from asthmatics was associated with better lung function, fewer symptoms, and lower exhaled nitric oxide fraction (F_{ENO}). In a 6-week placebo-controlled treatment trial in moderate-to-severe asthma, nebulised DHEA provided improvement in asthma control, although lung function did not change [5].

Patients with severe asthma generally require treatment with high doses of inhaled corticosteroids (ICS) and often even supplementation with oral corticosteroids (OCS), either during exacerbations or continuously. Whereas adrenal insufficiency and suppression of cortisol biosynthesis are well known adverse effects of corticosteroid treatment for asthma [6], the concurrent consequences of treatment with OCS or ICS on the levels of endogenous anabolic androgenic steroids (EAAS) remain relatively unexplored. Currently available data are limited to a few studies where testosterone or other androgens have been measured in blood or urine [4, 7–10].

With this in mind, we set out to determine the urinary levels of endogenous steroids, including androgens, in the pan-European U-BIOPRED study (Unbiased Biomarkers for the Prediction of Respiratory Disease outcomes) [11]. This well-characterised cohort includes >400 patients with severe asthma (SA), and ∼100 patients with mild-to-moderate asthma (MMA) and 100 healthy controls (HC). The WADA (World Anti-Doping Agency) accredited doping control laboratory in Stockholm used mass spectrometry to quantify the main EAAS metabolites, cortisone and cortisol, the main metabolites of prednisone and prednisolone, as well as metabolites of common asthma medications. It was therefore possible to relate the levels of EAAS and other endogenous steroids to both objectively detected markers of OCS treatment and recorded prescriptions of OCS and ICS. The measurement of urinary metabolites is the method of choice for assessment of whole-body steroid metabolism [12].

The measured analytes are displayed in figure 1, which summarises the biosynthesis of the natural steroid hormones from cholesterol in the gonads (testis and ovary) and in the adrenal cortex [13]. Pregnenolone is the precursor of cortisol, which is converted to cortisone in peripheral tissues, both of which appear in the circulation and urine. Pregnenolone is also metabolised to DHEA in the adrenal cortex in a step that is inhibited by synthetic steroids [13].

In the ovary or testis, further metabolism of DHEA leads to androstenedione or androstenediol *via* a pathway for biosynthesis of the male sex hormone testosterone, and *via* further metabolism to the female sex hormones, oestrogens. In fertile women, 70–80% of circulating testosterone is derived from adrenal DHEA, whereas testosterone in males is predominantly of testicular origin [13].

Cortisol is secreted from the adrenal glands in response to adrenocorticotropic hormone and other regulatory hormones produced by the hypothalamic–pituitary–adrenal (HPA) axis. The secretion of sex hormones (both androgens and oestrogens) is similarly regulated by the hypothalamic–pituitary–gonadal (HPG) axis along with the conversion of androgens into oestrogens by aromatase in adipose tissue. The inhibitory effects of exogenous, synthetic steroids on the biosynthesis of endogenous steroids are executed by feedback-inhibition of the HPA and HPG axes [13, 14].

Steroid hormones are excreted in the urine as glucuronated (G) or sulphated (S) conjugates, and metabolites are therefore given the suffix G or S. The urinary metabolites reflect the integrated systemic

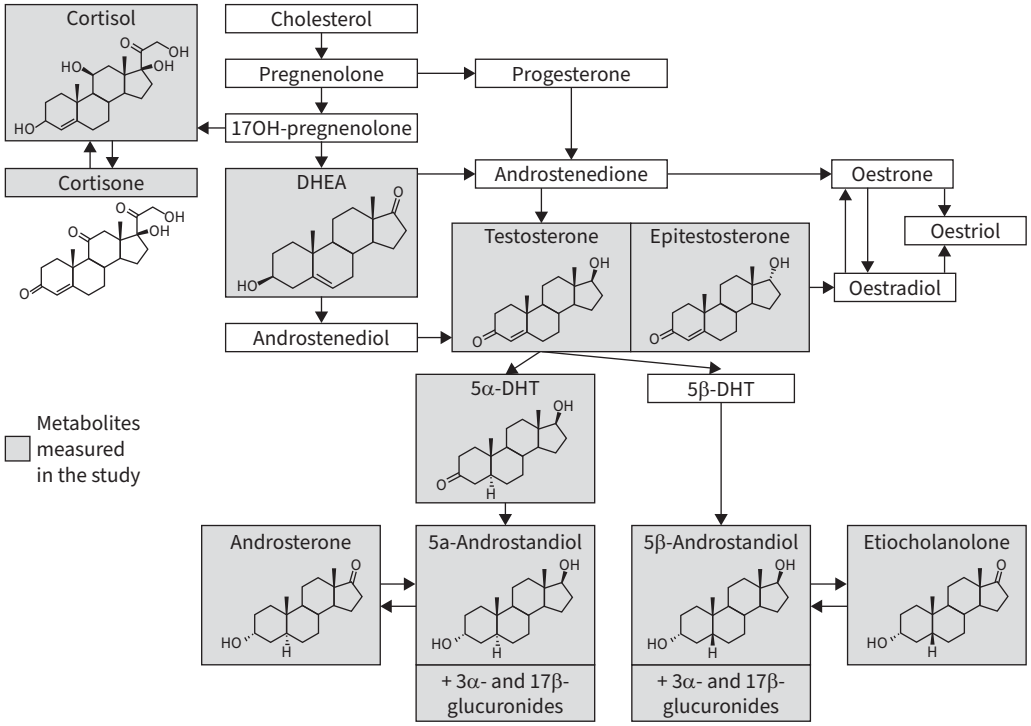

FIGURE 1 Overview of steroid metabolism with metabolites measured in the study shown in grey boxes.

biosynthesis of steroid hormones over time [12–14]. Previous studies on endogenous steroids in relation to asthma treatment have measured a limited number of analytes, mostly in the blood using immunoassays [4, 8, 10].

Methods

Study information

The U-BIOPRED adult study is a multicentre observational investigation recruiting asthmatics and healthy nonsmoking controls from 16 clinical centres in 11 European countries between January 2011 and December 2013 [11]. The study (ClinicalTrials.gov NCT01976767) was approved by the ethics committees at the clinical sites, and participants provided written informed consent. This report includes the 576 participants with complete data for urinary steroid metabolites: 302 nonsmoking and 106 smoking or ex-smoking SA patients, 70 MMA patients and 98 HC. Their baseline characteristics are displayed in table 1.

The diagnosis of severe asthma followed European Respiratory Society/American Thoracic Society guidelines [15]. The SA group was required to use ⩾1000 µg fluticasone propionate per day or equivalent dose of other corticosteroids, and MMA <500 µg daily. Patients in the SA group were allowed regular use of systemic corticosteroids. Most severe asthmatics were nonsmoking, defined as no smoking in the past year, and a lifetime history of ⩽5 pack-years. Patients with severe asthma who had smoked during the past 12 months or had a history of >5 pack-years were assigned to a group including current smokers and ex-smokers [11].

Participants meeting the inclusion criteria during a screening visit then attended a baseline visit where clinical end-points were examined and biosamples collected. In addition, subjects with SA were invited to a follow-up visit after 12–18 months, where study procedures were repeated [11].

Urine collection and analysis

One spot urine sample was split into five 8-mL tubes (Sarstedt, Nümbrecht, Germany), placed into −20°C freezers at the study sites and kept at −80°C following shipment to the central analysis site in Stockholm. Under such conditions, steroid conjugates have been documented to be intact for ⩾10 years, and they are also stable during several thaw–freeze cycles [16].

TABLE 1 Baseline characteristics of the participants in the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes study of urinary steroid metabolites

	Healthy nonsmoking controls	Participants	Mild/moderate nonsmoking asthma	Participants	Severe asthma all (nonsmoking, smokers and ex-smokers)	Participants	p-value
Participants	98		70		408		
Age, years	35 (27–49)	98	43 (27–52)	70	54 (45–62)	408	<0.0001
Female, %	39.7	39/98	45.7	32/70	62	253/408	<0.05[#]
BMI, kg·m^{-2}	24.7 (22.7–27.5)	97	24.8 (22.6–28.7)	70	28 (24.5–33.4)	408	<0.0001
BMI >30 kg·m^{-2}, %	12.2	12/98	15.7	11/70	40	159/408	<0.05[#]
FEV_1, % pred	102 (94–111)	98	91 (77–99)	70	67(51–83)	405	<0.0001
FEV_1/FVC ratio			72.9 (62.8–77.0)	68	62.8 (53.1–71.3)	404	<0.0001
F_{ENO}, ppb	19 (13–30)	93	31 (18–58)	69	26 (15–47)	381	0.0012
Blood eosinophils, cells·µL^{-1}	100 (90–200)	98	200 (100–300)	70	200 (100–410)	395	<0.0001
LTE4, ng·mmol^{-1} creatinine	3.0 (2.0–4.7)	89	4.5 (3.0–7.0)	70	6.4 (3.9–11.2)	400	<0.0001
Exacerbations in previous year, n	NA		0 (0.0–1.0)	69	2 (1–3)	399	<0.0001
ACQ-5	NA		0.8 (0.4–1.4)	67	2.2 (1.4–3.0)	394	<0.0001
AQLQ	NA		6.1 (5.4–6.4)	66	4.5 (3.6–5.4)	401	<0.0001
HADS total score	NA		5 (2–10.8)	68	12 (6–18)	398	<0.0001

Data are presented as n, median (interquartile range), unless otherwise stated. Comparisons between the three groups were performed using the Kruskal–Wallis test, and between two groups using the Mann–Whitney U-test. BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; F_{ENO}: exhaled nitric oxide fraction; LTE: leukotriene; ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality-of-Life Questionnaire; HADS: Hospital Anxiety and Depression Scale; NA: not applicable. [#]: evaluated by Chi-squared test.

The quantification of endogenous and exogenous steroids was performed by ultra high-performance liquid chromatography high-resolution mass spectrometry as described previously [17]. Metabolites of prednisone are highest during the first 24–36 h after oral intake [18], but may be found up to a week after the last dose with the high-sensitivity method used (limit of detection (LOD) 1 ng·mL^{-1} for all analytes) [18].

Data analysis

The urinary levels of steroids followed a non-normal distribution. Due to the exploratory nature of this study, unadjusted p-values were used and p-values <0.05 were considered significant using the Kruskal–Wallis, Mann–Whitney U or Wilcoxon matched-pairs signed-rank tests. For comparison of proportions, Chi-squared analysis was performed. The number of values below LOD for analytes in the panel are indicated for reported datasets (tables 2 and 3). For the statistical analysis, such data points were imputed as 0.5 ng·mL^{-1}. Clinical outcomes and biomarker results for the participants were retrieved from the U-BIOPRED TranSMART data handling system [19]. Statistical evaluation was performed using GraphPad Prism (v9).

The presence of urinary prednisone metabolites was used to stratify the patients into two groups regarding OCS detection: yes or no. Relations between the urinary concentrations of DHEA-S and clinical outcomes were evaluated using an extreme group analysis, where asthmatic subjects were stratified into high (>75th percentile) or low (<25th percentile) DHEA-S groups.

Results

Urinary excretion of metabolites of endogenous steroids in the recruited study groups

Males had higher urinary concentrations of androgens, and data for endogenous steroids were therefore separated by sex (tables 2 and 3). Furthermore, there were few differences with respect to steroid concentrations between ex-smoking (n=65) and nonsmoking (n=302) SA participants and the current smokers were too few (24 female, 17 male) to enable sufficiently powered analyses of these subgroups (supplementary table S1). Therefore, the data for SA are merged irrespective of smoking history, providing one group of 253 females and another comprised of 155 males.

The concentrations of cortisol and cortisone were the same in males and females (table 2). In both sexes, the concentrations of all urinary steroid metabolites were substantially lower in SA than in MMA or HC

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TABLE 2 Comparison of concentrations of endogenous steroids between the sexes for the three main study groups

	HC females	HC males	Male/female ratio	p-value	MMA females	MMA males	Male/female ratio	p-value	SA females	SA males	Male/female ratio	p-value
Participants	39	59			32	38			253	155		
Cortisone ng·mL^{-1}	206 (139–263)	193 (132–260)	0.94	0.83	222 (146–270)	172 (107–231)	0.77	0.13	95 (0.5–190)	78 (0.5–170)	0.82	0.34
<LOD	4 (10)	1 (2)			4 (13)	3 (8)			81 (32)	56 (36)		
Cortisol ng·mL^{-1}	68 (34–156)	94 (65–139)	1.38	0.16	87 (52–173)	82 (44–114)	0.95	0.27	30 (0.5–79)	25 (0.5–72)	0.84	0.51
<LOD	6 (15)	2 (3)			4 (13)	4 (11)			104 (41)	71 (46)		
DHEA-S ng·mL^{-1}	275 (46–915)	1080 (284–3668)	3.9	0.0001	172 (74–593)	673 (89–2822)	3.9	0.03	39 (11–104)	41.1 (17–204)	1.1	0.05
<LOD	0	0			0	0			9 (4)	0		
DHEA-G ng·mL^{-1}	44 (19–62)	52 (32–72)	1.2	0.12	36 (20–55)	48 (29–69)	1.3	0.15	12 (4–28)	13 (7–29)	1.1	0.03
<LOD	0	0			0	0			19 (8)	1 (0.6)		
Androsterone-G ng·mL^{-1}	2688 (1468–4506)	4778 (3184–7438)	1.8	<0.0001	1991 (1154–3908)	4808 (3058–6851)	2.4	<0.0001	745 (203–1905)	2131 (1413–3252)	2.9	<0.0001
<LOD	0	0			0	0			0	0		
Androsterone-S ng·mL^{-1}	403 (202–722)	951 (507–1556)	2.4	<0.0001	299 (129–700)	781 (233–1444)	2.6	0.01	109 (38–272)	280 (126–528)	2.6	<0.0001
<LOD	0	0			0	0			0	0		
Testosterone-G ng·mL^{-1}	11 (8–14)	62 (38–94)	5.6	<0.0001	8 (5–13)	50 (32–87)	6.2	<0.0001	6 (4–10)	51 (34–84)	8.6	<0.0001
<LOD	0	0			0	0			1 (0.4)	0		
Testosterone-S ng·mL^{-1}	0.5 (0.5–1.7)	3.1 (1.7–7.7)	6.2	<0.0001	0.7 (0.5–1.8)	3.5 (1.0–6.9)	5.0	<0.0001	0.5 (0.5–0.5)	1.3 (0.7–2.7)	2.6	<0.0001
<LOD	20 (51)	3 (5)			15 (47)	4 (11)			165(65)	35 (23)		
DHT-G ng·mL^{-1}	2.9 (2.0–6.5)	8.3 (4.6–13.8)	2.9	0.0009	1.8 (0.5–4.1)	7.9 (4.8–12.8)	4.4	<0.0001	1.1 (0.5–2.6)	6.2 (3.0–10.7)	5.6	<0.0001
<LOD	3 (8)	3 (7)			12 (31)	4 (11)			89 (35)	7 (5)		
Epitestosterone-G ng·mL^{-1}	15 (6–25)	72 (51–114)	4.8	<0.0001	14 (7–28)	78 (43–123)	5.6	<0.0001	7 (3–18)	76 (49–112)	10.9	<0.0001
<LOD	4 (10)	0			3 (26)	0			36 (14)	0		
Epitestosterone-S ng·mL^{-1}	1.5 (0.5–3.1)	10 (6–16)	6.7	<0.0001	2.9 (0.5–8.6)	11 (5–16)	3.8	<0.0001	0.5 (0.5–1.6)	7 (4–9)	14.0	<0.0001
<LOD	12 (31)	0			11 (34)	0			133 (53)	2 (1)		
Etiocholanolone-G ng·mL^{-1}	2441 (1783–4625)	3516 (2491–5135)	1.4	0.03	2450 (1424–3780)	3083 (2179–4467)	1.3	0.12	833 (271–1844)	1853 (1200–2814)	2.2	<0.0001
<LOD	0	0			0	0			0	0		
Etiocholanolone-S ng·mL^{-1}	209 (82–308)	231 (78–396)	1.1	0.34	170 (95–410)	77 (42–308)	0.5	0.05	67 (27–154)	69 (32–200)	1.0	0.11
<LOD	0	0			0	0			1 (0.4)	0		
AAB-17G ng·mL^{-1}	10 (4–25)	88 (41–117)	8.8	<0.0001	8.3 (4–16)	79 (44–123)	9.5	<0.0001	4 (2–12)	60 (37–106)	14.3	<0.0001
<LOD	1 (3)	0			2 (6)	0			16 (6)	0		
ABB-3G ng·mL^{-1}	21 (13–55)	65 (36–116)	3.1	<0.0001	23 (17–43)	54 (37–77)	2.3	<0.0001	11 (4–24)	43 (28–72)	3.9	<0.0001
<LOD	0	0			0	0			21 (8)	0		
BAB-17G ng·mL^{-1}	68 (26–125)	208 (107–336)	3.1	<0.0001	36 (17–93)	204 (127–315)	5.7	<0.0001	16 (5–41)	167 (91–286)	10.4	<0.0001
<LOD	0	0			0	0			2 (1)	0		
BAB-3G ng·mL^{-1}	13 (6–25)	42 (25–70)	3.3	<0.0001	11 (6–22)	44 (30–63)	4.1	<0.0001	5 (2–12)	25 (16–39)	4.9	<0.0001
<LOD	2 (5)	0			2 (6)	0			41 (16)	0		

Data are presented as n, median (interquartile range) or n (%), unless otherwise stated. The ratio between the mean concentrations in males and females is reported for each analyte and study group in a separate column. Groups were compared by Mann–Whitney U-test. HC: healthy controls; MMA: mild-to-moderate asthma; SA: severe asthma; LOD: limit of detection; DHEA: dehydroepiandrosterone; S: sulfate; G: glucuronate; DHT: dihydrotestosterone; AAB: 5α-androstane-3α,17β-diol; ABB: 5α-androstane-3β,17β-diol; BAB: 5β-androstane-3α,17β-diol.

TABLE 3 Comparison of concentrations of endogenous steroids between the sexes for severe asthma high-dose inhaled corticosteroid (ICS)/oral corticosteroid (OCS)-negative and high-dose ICS/OCS-positive

	Female high-dose ICS/ OCS-negative	Male high-dose ICS/ OCS-negative	Male/ female ratio	p-value	Female high-dose ICS/ OCS-positive	Male high-dose ICS/ OCS-positive	Male/ female ratio	p-value
Participants	177	101			76	54		
Cortisone ng·mL^{-1}	122.2 (55.4–203.6)	120.1 (46.1–211.9)	1.0	0.68	0.5 (0.5–65.4)	0.5 (0.5–77.7)	1.0	0.83
<LOD	30 (17)	19 (19)			51 (67)	37 (69)		
Cortisol ng·mL^{-1}	44.9 (0.5–83.7)	49.4 (0.5–98.7)	1.1	0.73	0.5 (0.5–9.1)	0.5 (0.5–0.5)	1.0	0.38
<LOD	47 (27)	27 (27)			57 (75)	44 (82)		
DHEA-S ng·mL^{-1}	50 (16–125)	81 (22–287)	1.6	0.08	11 (2–44)	31 (14–55)	2.8	0.0001
<LOD	2 (1)	0			7 (9)	0		
DHEA-G ng·mL^{-1}	17 (6–34)	17 (8–38)	1.0	0.25	4 (2–12)	10 (5–21)	2.5	0.0003
<LOD	9 (5)	0			10 (13)	1 (2)		
Androsterone-G ng·mL^{-1}	1002 (391–2203)	2328 (1659–4163)	2.3	<0.0001	214 (80–797)	1650 (1137–2721)	7.7	<0.0001
<LOD	0	0			0	0		
Androsterone-S ng·mL^{-1}	134 (59–337)	299 (141–598)	2.2	<0.0001	41 (13–113)	248 (116–404)	6.0	<0.0001
<LOD	0	0			0	0		
Testosterone-G ng·mL^{-1}	6 (4–10)	53 (36–86)	8.8	<0.0001	5 (3–8)	47 (29–85)	9.4	<0.0001
<LOD	0	0			1 (1)	0		
Testosterone-S ng·mL^{-1}	0.5 (0.5–0.5)	1.4 (0.6–3.0)	2.8	<0.0001	0.5 (0.5–0.5)	1.0 (0.7–1.6)	2.8	<0.0001
<LOD	99 (56)	23 (23)			66 (87)	12 (22)		
DHT-G ng·mL^{-1}	1.1 (0.5–2.6)	6.4 (3.2–11.7)	5.8	<0.0001	1.2 (0.5–2.4)	6.0 (2.9–9.0)	5.8	<0.0001
<LOD	66 (37)	5 (5)			23 (30)	2 (4)		
Epitestosterone-G ng·mL^{-1}	9 (3–19)	84 (50–119)	9.3	<0.0001	4 (1–13)	71 (48–98)	16.9	<0.0001
<LOD	22 (12)	0			14 (18)	0		
Epitestosterone-S ng·mL^{-1}	0.8 (0.5–1.9)	7 (4–10)	8.8	<0.0001	0.5 (0.5–0.9)	6 (4–9)	12.0	<0.0001
<LOD	81 (46)	1 (1)			52 (68)	1(2)		
Etiocholanolone-G ng·mL^{-1}	1113 (457–2072)	1913 (1206–2889)	1.7	<0.0001	337 (105–901)	1672 (1126–2542)	5.0	<0.0001
<LOD	0	0			0	0		
Etiocholanolone-S ng·mL^{-1}	87 (37–208)	73 (31–209)	0.8	0.76	41 (10–78)	67 (32–183)	1.6	0.0003
<LOD	0	0			0	0		
AAB-17G ng·mL^{-1}	6.2 (3–13)	62 (41–109)	10.0	<0.0001	3 (1–6)	47 (25–92)	18.8	<0.0001
<LOD	7 (4)	0			9 (12)	0		
ABB-3G ng·mL^{-1}	13 (6–27)	39 (26–71)	3.0	<0.0001	5 (2–13)	50 (33–78)	10.0	<0.0001
<LOD	11 (6)	0			10 (13)	0		
BAB-17G ng·mL^{-1}	21 (8–47)	163 (109–308)	7.8	<0.0001	7 (3–26)	169 (70–273)	24.1	<0.0001
<LOD	0	0			2 (3)	0		
BAB-3G ng·mL^{-1}	6.8 (2.2–13.9)	25 (18–39)	3.7	<0.0001	2.2 (0.5–5.8)	25 (15–40)	12.3	<0.0001
<LOD	21 (12)	0			20 (26)	0		

Data are presented as n, median (interquartile range) or n (%), unless otherwise stated. The ratio between the mean concentrations in males and females is reported for each analyte and study group in a separate column. Groups were compared by Mann–Whitney U-test. LOD: limit of detection; DHEA: dehydroepiandrosterone; S: sulfate; G: glucuronate; DHT: dihydrotestosterone; AAB: 5α-androstane-3α,17β-diol; ABB: 5α-androstane-3β,17β-diol; BAB: 5β-androstane-3α,17β-diol.

(figure 2a–d, table 2). The only exception to this apparent suppression of endogenous steroids in SA was urinary testosterone in males, which was similar in all three study groups, presumably reflecting its gonadal origin in men (figure 2d, table 2). In contrast, the urinary concentrations of most steroid metabolites were similar in both sexes for MMA and HC (figure 2, table 2). Supplementary table S2 summarises the statistical comparisons for all analytes between the study groups.

As the median age of the SA group was greater than HC and MMA (table 1), data were compared between subjects who were older or younger than 45 years (supplementary table S3), also providing an arbitrary stratification of females as pre- and post-menopausal. Although the older patients of both sexes had lower concentrations of cortisol and androgens, the differences in steroid concentrations between the three study groups remained irrespective of age. Furthermore, as the SA group had a higher body mass index (BMI), the steroid metabolites were compared between participants with a BMI below or above 30 kg·m^{-2} (supplementary table S4). With the exception of androsterone-S, which was lower in both sexes among participants with SA and high BMI, there were no BMI-related differences in EAAS metabolites.

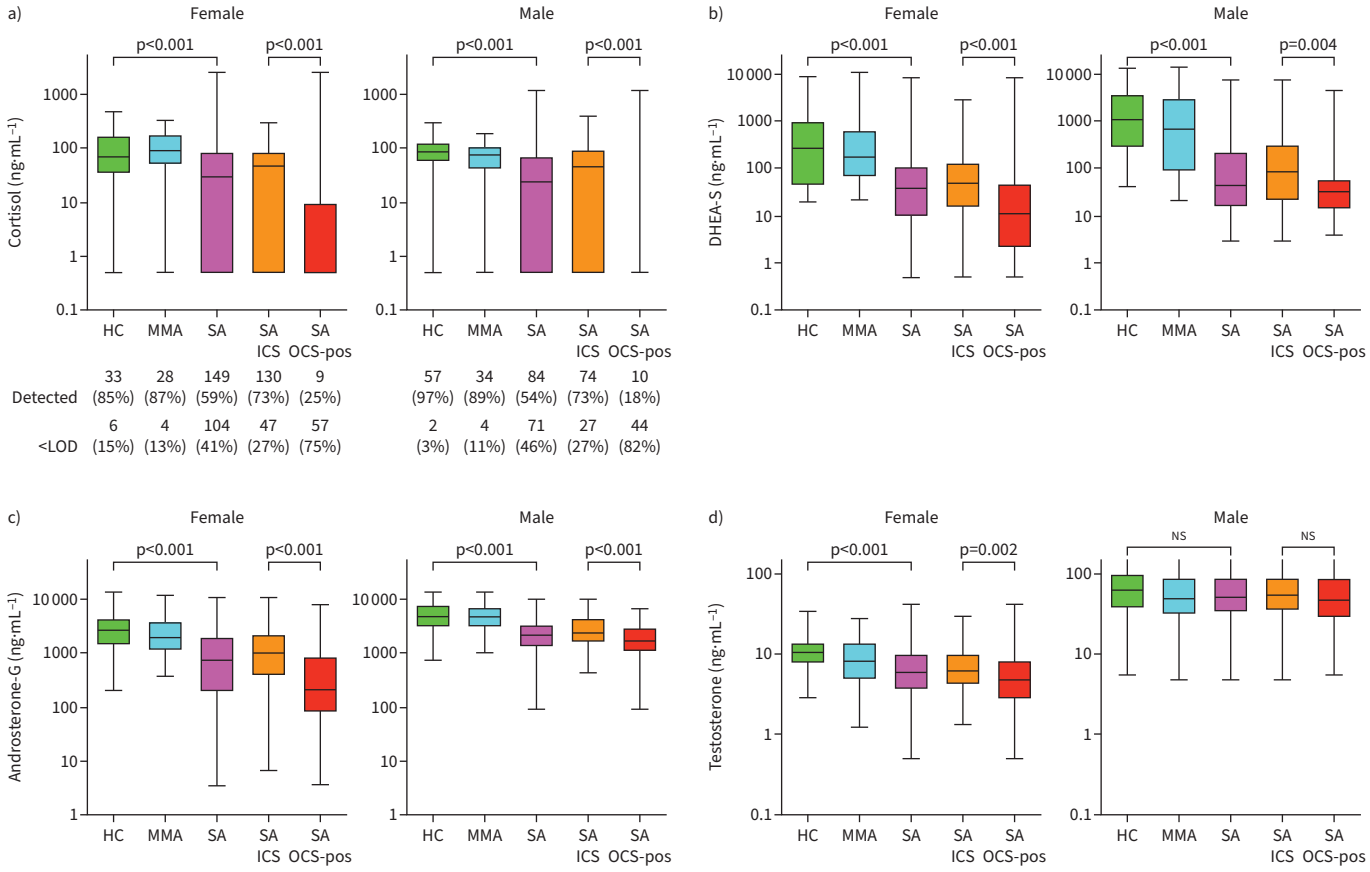

FIGURE 2 Levels of endogenous steroids in the urine of female and male study participants. a) Cortisol; b) dehydroepiandrosterone sulfate (DHEA-S); c) androsterone-glucuronate (G); d) testosterone-G. Data are presented as median (interquartile range). The Mann–Whitney U-test was used to compare two groups and the Kruskal–Wallis test to compare three groups. All graphs have a logarithmic y-axis scale, although statistical analyses were performed on arithmetic data. The box plots for the three severe asthma (SA) groups in a) (cortisol) are elongated due to the large number of imputed values (0.5 ng·mL^{-1}) where levels were below limit of detection (LOD) (see also tables 2 and 3). HC: healthy controls; MMA: mild-to-moderate asthma; SA: all patients with severe asthma; SA ICS: severe asthma subgroup with no detectable metabolites of oral corticosteroids in the urine (OCS-neg); SA OCS-pos: severe asthma subgroup with detectable metabolites of oral corticosteroids in the urine.

Relationship of endogenous anabolic androgenic steroids to treatment with corticosteroids

We then tested the obvious hypothesis that the differences between the study groups with respect to endogenous steroids were related to the intensity of corticosteroid treatment. According to recorded medical history, 162 (40%) individuals in the SA group reported OCS use at least once a week. In this group, OCS metabolites were detected in the urine of 92 (57%) participants (figure 3, supplementary tables S5 and S6), suggesting that 70 (43%) participants had not taken OCS during the few days before the study visit. For females as well as males, the concentrations of cortisol, cortisone and androgen metabolites were significantly lower in OCS-positive SA patients than OCS-negative SA patients (figure 2, table 3, supplementary table S2). In fact, between 65% and 80% of the patients in the OCS-positive SA groups had signs of adrenal insufficiency with undetectable levels of cortisol and cortisone (table 3).

For both sexes, the concentrations of DHEA-S and most other EAAS metabolites were lower in the OCS-positive SA groups, compared with the OCS-negative SA patients (figure 2, table 3, supplementary table S2). The only exceptions were testosterone-G (figure 2d) and dihydrotestosterone-G, which among males were essentially unaffected in the OCS-positive SA group (table 3, supplementary table S2). Moreover, the concentrations of all EAAS metabolites were lower in OCS-positive females with SA compared to males (table 3, supplementary table S2). For the two most abundant androgen metabolites, androsterone-G and etiocholanolone-G, the ratio between levels in males and females in the OCS-positive SA group were 7.7 and 5.0, respectively, as compared to 1.8 and 1.4, respectively, in the HC group (table 3).

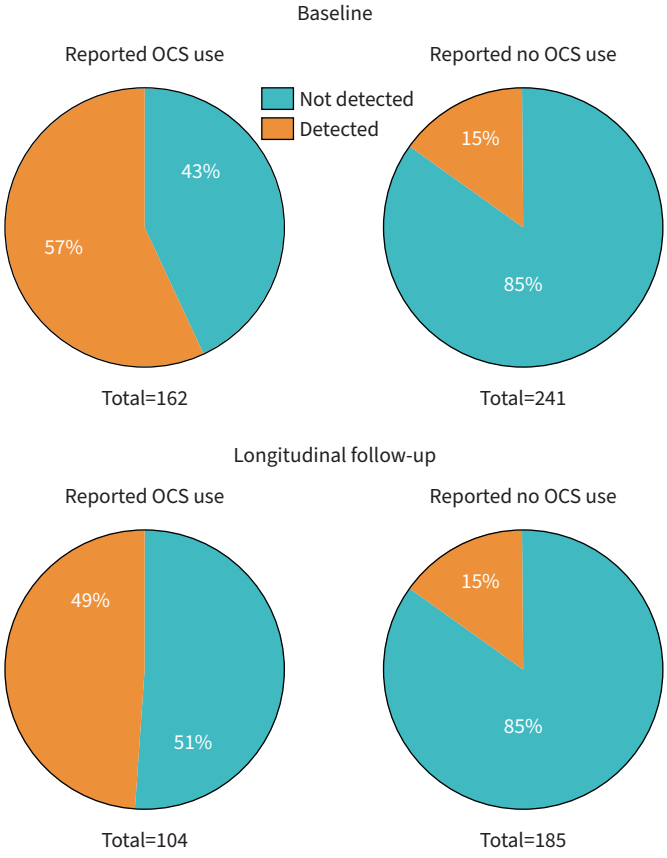

FIGURE 3 Reported oral corticosteroid (OCS) use and presence of urinary prednisone metabolites (OCS detected) in patients with severe asthma in the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes study at baseline (n=403) and longitudinal follow-up 12–18 months later (n=289).

Furthermore, the OCS-negative SA group on high-dose ICS also showed markedly lower concentrations of most measured endogenous steroids when compared to MMA or HC (figure 2, tables 2 and 3, supplementary table S2). In this group, females consistently displayed lower levels of EAAS than males (table 3).

The patients with MMA had higher concentrations of endogenous steroids than the two SA groups, although there were no significant differences between MMA and HC (tables 2 and 3, supplementary table S2). Seven patients in the MMA group had detectable prednisone metabolites in their urine and were excluded from further analysis (supplementary table S6).

Data stability at longitudinal follow-up visit

Consent for a follow-up visit after 12–18 months was obtained from 289 (71%) of the 408 patients with severe asthma. The clinical outcomes and the observed suppression of EAAS metabolites were replicated and stable (table 4). Cortisol concentrations were slightly higher at the longitudinal visit; however, somewhat fewer participants used OCS at the longitudinal visit, both according to self-reported intake and detection of urinary OCS metabolites (figure 3, supplementary table S5).

Relationships between endogenous androgens and clinical outcomes

Clinical or biomarker outcomes were related to concentrations of the key androgen intermediate DHEA-S (figure 4, table 5). The odds for SA patients taking OCS to be in the quartile of samples with the lowest concentrations of DHEA-S were 2.3-fold higher (Chi-squared test) than those not taking OCS (table 5). OCS-positive SA patients also had lower blood eosinophils, higher blood neutrophils and higher serum matrix metalloproteinase (MMP)-3 (supplementary table S7), all of which are objective markers of corticosteroid exposure.

TABLE 4 Comparison between baseline and longitudinal data for the patients in the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes study with severe asthma attending both visits (n=289)

	Females		p-value	Males		p-value
	Baseline	Longitudinal		Baseline	Longitudinal	
Participants	171			118		
Urinary metabolites of steroids ng·mL^{-1}						
Cortisone	101.6 (0.5–198.3)	141.2 (0.5–198.3)	0.13	98.1 (0.5–187.7)	75.5 (0.5–193.7)	0.87
Cortisol	32.3 (0.5–82.2)	56.3 (0.5–108.9)	0.0006	33.9 (0.5–77.1)	47.2 (0.5–107.5)	0.06
DHEA-S	41 (11–104)	38 (8–99)	0.92	44 (21–269)	37 (17–127)	0.45
DHEA-G	14 (4–26)	11 (4–24)	0.63	16 (7–32)	13 (6–25)	0.25
Androsterone-G	778 (212–1766)	748 (194–1578)	0.76	2161 (1428–3317)	1824 (1121–3104)	0.12
Androsterone-S	117 (43–271)	115 (24–247)	0.77	299 (138–561)	267 (102–521)	0.15
Testosterone-G	6 (4–9)	7 (4–9)	0.54	50 (32–85)	48 (26–71)	0.39
DHT-G	1.2 (0.5–2.6)	1.5 (0.6–2.9)	0.12	6.4 (3.0–11.2)	5.4 (2.9–8.8)	0.19
Testosterone-S	0.5 (0.5–0.5)	0.5 (0.5–0.5)	0.99	1.3 (0.7–2.9)	1.2 (0.5–2.8)	0.55
Clinical outcomes						
Exacerbations n	2.0 (1.0–3.0)	2.0 (1.0–4.0)	0.83	2.0 (0–3.0)	1.0 (0–3.0)	0.88
AQLQ average	4.5 (3.5–5.3)	4.4 (3.5–5.2)	0.63	4.8 (3.8–5.7)	5.0 (3.7–5.9)	0.36
ACQ-5 average	2.2 (1.5–3.0)	2.2 (1.0–3.2)	0.72	2.0 (1.2–3.0)	1.8 (1.0–2.8)	0.42
HADS total score	13 (6–18)	13 (6–19)	0.73	10 (6–18)	9 (6–16)	0.2
BMI kg·m^{-2}	28.7 (24.6–33.98)	28.8 (24.8–33.98)	0.98	26.9 (24.2–31.7)	26.9 (24.2–31.7)	0.9
FEV_1 % pred	68.7 (52.4–85.1)	69.8 (55.7–85.9)	0.95	60.5 (47.6–73.2)	61.5 (46.1–76.5)	0.73
Blood eosinophils cells·µL^{-1}	200 (100–400)	250 (100–419)	0.48	210 (100–420)	210 (110–420)	0.43
Blood neutrophils ×10^3 cells·µL^{-1}	4.7 (3.7–6.6)	4.7 (3.4–6.5)	0.5	4.6 (3.6–6.7)	4.8 (3.5–7.3)	0.52
OCS dose mg (daily to weekly OCS use)	12.5 (8.4–25.0)	20.0 (10.0–30.0)	0.33	12.0 (8.0–20.0)	12.5 (7.9–26.3)	0.18
OCS detected	49	44		39	37	

Data are presented as n or median (interquartile range), unless otherwise stated. Data were compared using Wilcoxon matched pairs rank test. DHEA: dehydroepiandrosterone; S: sulfate; G: glucuronated; DHT: dihydrotestosterone; AQLQ: Asthma Quality-of-Life Questionnaire; ACQ: Asthma Control Questionnaire; HADS: Hospital Anxiety and Depression Scale; BMI: body mass index; FEV_1: forced expiratory volume in 1 s; OCS: oral corticosteroid.

In this extreme group comparison, low concentrations of DHEA-S were associated with multiple clinical outcomes indicating more severe disease, such as lower asthma-specific quality of life, higher HADS score, lower forced expiratory volume in 1 s (FEV_1), more frequent exacerbations, lower asthma control and a higher proportion of subjects with detectable OCS metabolites (figure 4, table 5). The concentrations of bronchoconstrictive prostaglandin and cysteinyl-leukotriene metabolites in the urine, as well as serum C-reactive protein (CRP) and MMP-3, were highest in the lowest quartile (table 5). Regarding lung function, there was a weak but significant correlation with urinary DHEA-S in both sexes (r^2 0.07 for females and 0.14 for males; p>0.001).

There were more females in the low DHEA-S group (85 *versus* 34 males), and all EAAS metabolites were lower in this group (table 5). Moreover, the low DHEA group included only patients with SA, whereas one-third of patients in the high DHEA group had MMA (table 5).

Detection of metabolites of bronchodilators

Approximately one-third of asthma patients had detectable levels of short-acting β-agonists with no differences between disease severity groups or sexes (supplementary table S6).

Discussion

We quantified the levels of urinary metabolites of endogenous steroids by mass spectrometry in a clinical cohort of 478 well-characterised patients with asthma of differing severity and a control group of 98 healthy subjects. Simultaneous measurement of the urinary metabolites of prednisone and prednisolone provided an objective assessment of exposure to OCS.

Results showed that individuals with SA had very low concentrations of endogenous androgens and cortisol in comparison to MMA and HC. The low concentrations of cortisol were expected, because systemic corticosteroids are known to cause suppression of the HPA axis [6, 20], but the extent was greater than anticipated. The majority (75% of females and 82% of males) of patients with SA with detectable

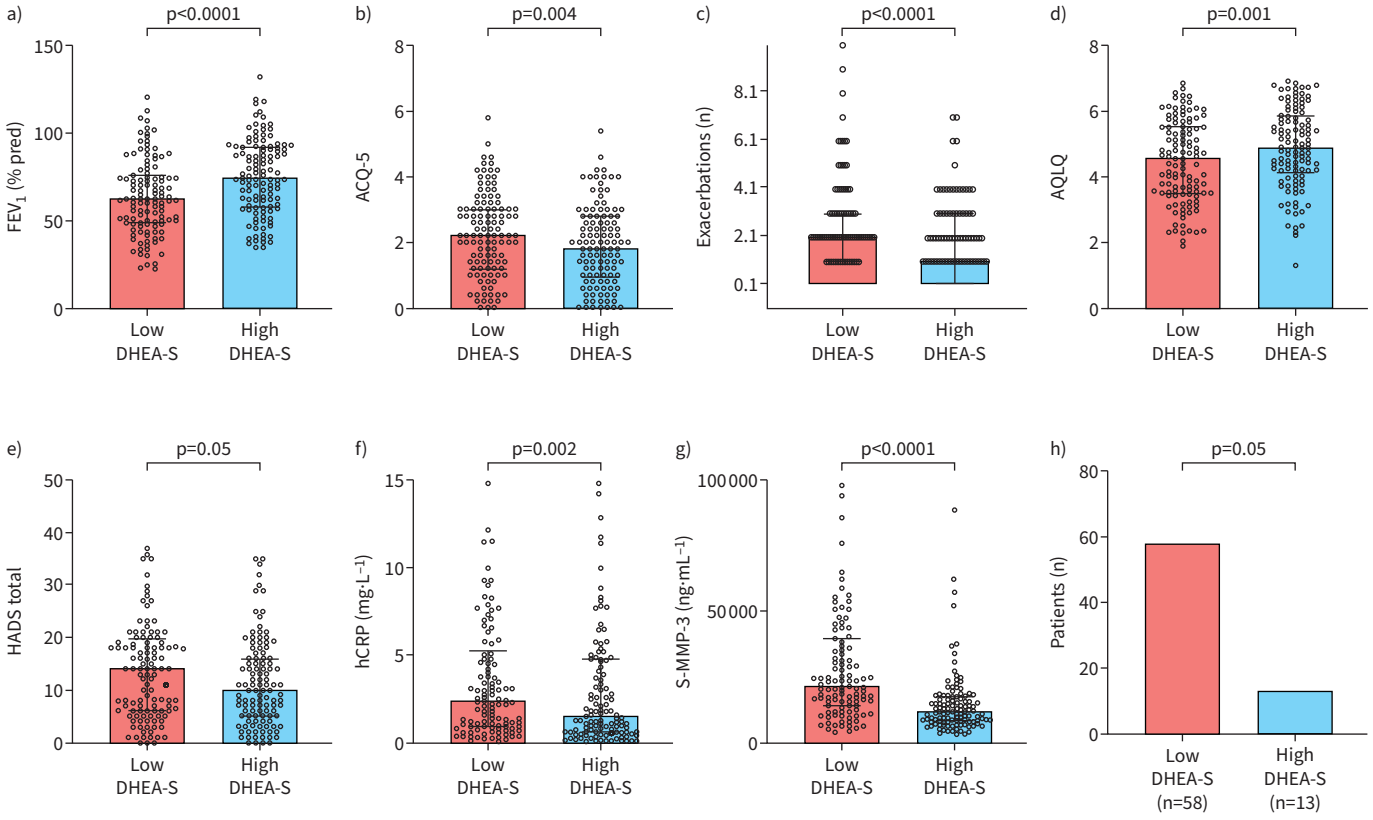

FIGURE 4 Clinical and biomarker variables for the lowest (25th percentile; n=119) and highest (75th percentile; n=118) quartiles of asthmatic patients stratified for urinary concentrations of dehydroepiandrosterone sulfate (DHEA-S). Data are presented as median (interquartile range) for a) forced expiratory volume in 1 s (FEV_1; % predicted), b) Asthma Control Questionnaire (ACQ)-5, c) exacerbations in past year, d) Asthma Quality-of-Life Questionnaire (AQLQ), e) Hospital Anxiety and Depression Scale (HADS) total score, f) high-sensitivity C-reactive protein (hCRP) and g) S-matrix metalloproteinase (MMP)-3 (low and high groups were compared by Mann–Whitney U-test); and h) the number of subjects with detectable urinary oral corticosteroid (OCS) metabolites (significance calculated according to the Chi-squared test).

prednisone metabolites had undetectable concentrations of cortisol (table 3). The associated, and uniformly low, levels of the EAAS metabolites in the WADA panel among OCS users or patients on high-dose ICS treatment has not been reported previously.

Moreover, the concentrations of EAAS metabolites among females with SA were more depressed than those observed in males with SA (figure 2, table 2), both in the OCS-positive and the OCS-negative groups (table 3). Although men with SA also displayed low concentrations of DHEA-S and the other EAAS metabolites compared with HC (table 2), men retained high levels of testosterone that were unaffected by corticosteroid treatment (tables 2 and 3, figure 2D). Accordingly, in the extreme group comparison, all subjects in the low DHEA-S group had severe asthma; 71% were female; and this group was characterised by worse asthma outcomes (figure 4, table 5).

In contrast to the many reports on the influence of corticosteroid treatment on levels of cortisone or cortisol in plasma or urine [6, 8, 20], the effects of corticosteroids on endogenous androgens have received less attention. A small study addressing the risk of osteoporosis in men with asthma [10] found a 33% reduction in circulating testosterone levels in 12 patients on OCS, but no significant effects in 23 patients on low or high doses of inhaled beclomethasone. In an early study of 15 females with asthma who had been on oral glucocorticoid therapy for 3–7 years, the levels of DHEA in plasma and urine were found to be lower than in 10 matched controls despite the asthmatics using only bronchodilators at the time of the investigation [7]. However, they also had low cortisol levels, suggesting persisting adrenal insufficiency. Priftis *et al.* [9] followed 25 children of both sexes with asthma during treatment with beclomethasone and documented suppressed urinary cortisol compared with 23 steroid-naïve children, but there were no differences between the groups with respect to the excretion of the androgens androsterone and etiocholanolone.

TABLE 5 Extreme group comparison of endogenous steroid profiles, clinical outcomes and selected biomarkers in relation to urinary dehydroepiandrosterone (DHEA) concentration at the baseline visit for all asthmatics (mild-to-moderate asthma (MMA); severe asthma (SA)) in the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes study

	Low (25th percentile) DHEA-S <15.1 ng·mL^{-1}	High (75th percentile) DHEA-S ⩾182.9 ng·mL^{-1}	p-value
Participants	119	118	
Female	85 (71)	53 (45)	<0.05$^{\#}$
SA	119 (100)	79 (67)	
OCS metabolites detected in urine	58 (49)	13 (11)	<0.05$^{\#}$
Cortisol ng·mL^{-1}	47 (29–118)	82 (52–127)	0.003
Cortisone ng·mL^{-1}	115 (61–214)	188 (129–255)	0.003
Androsterone G ng·mL^{-1}	242 (101–984)	4434 (2703–5939)	<0.0001
Androsterone S ng·mL^{-1}	33 (12–92)	783 (425–1201)	<0.0001
DHEA-G ng·mL^{-1}	4 (2–6)	50 (31–70)	<0.0001
DHEA-S ng·mL^{-1}	7 (3–11)	561 (321–1642)	<0.0001
DHT-G ng·mL^{-1}	1.2 (0.5–2.6)	5.9 (2.1–12.2)	<0.0001
Testosterone-G ng·mL^{-1}	6 (4–26)	22 (8–66)	<0.0001
Testosterone-S ng·mL^{-1}	0.5 (0.5–0.5)	1.7 (0.9–4.4)	<0.0001
BMI kg·m^{-2}	28.9 (25.0–33.8)	27.1 (23.4–33.3)	0.06
Exacerbations per year	2 (1–3)	1 (0–3)	<0.0001
AQLQ score	4.6 (3.5–5.6)	4.9 (4.2–5.9)	0.001
ACQ-5 score	2.2 (1.2–3)	1.8 (1.0–2.8)	0.004
HADS total score	13 (6–1)	10 (5–16)	0.05
FEV_1 % pred	62.2 (48.6–75.3)	74.5 (57.7–91.8)	<0.0001
FEV_1 reversibility % change	14.8 (7.5–22.8)	14.5 (5.9–22.3)	0.59
F_{ENO} ppb	27 (16–53)	27 (18–54)	0.93
Blood eosinophils cells·µL^{-1}	200 (100–452)	200 (100–397)	0.83
Blood neutrophils ×10^3 cells·µL^{-1}	5.7 (4.1–8.2)	4.0 (3.2–5.2)	<0.0001
hCRP mg·mL^{-1}	2.4 (0.9–5.3)	1.5 (0.6–4.8)	0.002
IgE total IU·mL^{-1}	125 (38–320)	110 (59–347)	0.72
S-MMP-3 ng·mL^{-1}	21 768 (14 109–39 803)	11 881 (8179–17 535)	<0.0001
S-Periostin ng·mL^{-1}	50 (40–59)	49 (40–59)	0.71
LTE_4 ng·mmol^{-1} creatinine	7.3 (4–12.7)	5.7 (3.5–8.8)	0.004
Tetranor PGDM ng·mmol^{-1} creatinine	291 (224–471)	244 (159–328)	<0.0001

Data are presented as n, n (%) or median (interquartile range), unless otherwise stated. Groups were compared by Mann–Whitney U-test, unless otherwise stated. OCS: oral corticosteroid; G: glucuronated; S: sulfate; DHT: dihydrotestosterone; BMI: body mass index; AQLQ: Asthma Quality-of-Life Questionnaire; ACQ: Asthma Control Questionnaire; HADS: Hospital Anxiety and Depression Scale; FEV_1: forced expiratory volume in 1 s; F_{ENO}: exhaled nitric oxide fraction; hCRP: high-sensitivity C-reactive protein; MMP: matrix metalloproteinase; LTE: leukotriene; PGDM: prostaglandin D (tetranor) metabolites. $^{\#}$: groups compared by Chi-squared test.

Using much larger cohorts, the United States National Heart, Lung, and Blood Institute-funded Severe Asthma Research Programme (SARP) consortium reported a positive correlation between blood DHEA levels and lung function in both sexes, as well as more symptoms and higher F_{ENO} in subjects with low blood DHEA [4]. The relationship between blood levels of DHEA and lung function has also been observed in adolescents [21]. Interestingly, the beneficial role of androgens is supported by a registry study where subjects with the rare disease androgen insensitivity syndrome (AIS) had an increased risk of asthma [22]. The syndrome is caused by hereditary loss of androgen receptor function, and the risk of asthma was less in those with AIS being treated with testosterone.

The U-BIOPRED data confirm and extend the observation of a relationship between DHEA and lung function, as we also found a correlation between urinary DHEA and FEV_1 (% predicted). Although the correlation is relatively weak (r^2 0.07 and 0.16 for females and males, respectively), it is similar in magnitude to that reported for blood DHEA in the SARP study (0.15–0.16) [4]. Moreover, our study of 478 patients with asthma further extends the association between more severe asthma and androgen levels by showing higher scores in the low DHEA group with respect to patient-reported outcomes, as well as biomarkers such as CRP, MMP-3 and the lipid mediators leukotriene E_4 and tetranor PGDM (table 5). The objective markers of poor asthma control are also associated with low levels of other key EAAS metabolites (table 5).

In line with previous publications [6], our data support dose-dependent effects of corticosteroid treatment on endogenous steroids. In both sexes, those on high-dose ICS treatment with detectable OCS metabolites showed the most profound suppression of EAAS and cortisol, whereas those treated only with high-dose ICS showed less pronounced depressions in EAAS. Nevertheless, the concentrations of cortisol and EAAS in the OCS-negative SA group treated with high-dose ICS were consistently substantially lower than in the MMA group; in general, ⩾50% lower than the EAAS metabolites in HC (figure 2, tables 2 and 3).

Also consistent with most other reports (see [6] and [20] for reviews), we found no significant difference in endogenous steroids between MMA and HC (supplementary table S2). However, a recent nonquantitative metabolomic screen of large epidemiological asthma cohorts found that regular treatment of asthma with low-dose ICS was associated with suppressed plasma levels of 15 steroid hormones, including some androgens and cortisol [23].

We were initially surprised to find that <50% of patients reporting current OCS use at the baseline visit, or at the follow-up after 12–18 months, had detectable prednisone metabolites in their urine (figure 3). The observation could suggest adherence issues [24], or simply a longer time since last OCS dose, as steroid use was recorded as treatment during the past week and most data suggest that prednisone metabolites are undetectable after a few days [18]. Presumably, those with detectable prednisone metabolites belonged to the most severe, steroid-dependent subset of SA patients. Accordingly, this group displayed evidence of recent exposure to corticosteroids such as higher blood neutrophils, lower eosinophils and higher serum MMP-3 [25] (supplementary table S7). Higher exposure to systemic steroids would also explain why steroid-sensitive type-2 markers such as F_{ENO}, periostin and blood eosinophils were not elevated in the more severe group with detected OCS exposure (table 5). It is noteworthy that 15% of patients reporting no OCS prescription still displayed detectable prednisolone metabolites in their urine, as well as a handful of patients in the MMA group (figure 3, supplementary table S6). The latter findings are probably explained by widespread access to OCS as a self-medication among patients with asthma. Taken together, our data on urinary OCS metabolites highlights the inadequacy of evaluating corticosteroid exposure from prescription records only.

One limitation of the U-BIOPRED study is that we have no information regarding the ratio between androgens and female sex hormones as the WADA doping panel is designed to measure EAAS. Likewise, data concerning female reproductive physiology (*e.g.* menstrual cycle phase, use of contraceptives or hormone replacement) was not collected. Concerning the potential influence of the menstrual cycle, the clinical and biomarker outcomes in the extreme group comparison were assessed in relation to DHEA-S, which is a long-lived metabolite not altered significantly during the menstrual cycle [26]. It has also been established using the same analytical platform that the levels of EAAS metabolites change very little during a normal menstrual cycle [27]. In our study, the greatest effects on androgens were observed in SA where only 27% of participants were aged <45 years (supplementary table S2). This makes it unlikely that the use of hormonal contraceptives is a major confounder. Conversely, use of hormone replacement therapy (HRT) might be an issue. We have not found data on the prevalence of HRT in severe asthma, but a large prospective study in a primary care database in the United Kingdom (UK) estimated that 16% of women aged 46–70 years used HRT [28]. As the U-BIOPRED study is pan-European, there may be variability between countries reflecting cultural and regional differences in the use of HRT. Assuming the UK prevalence, there is nevertheless no reason to believe that this subgroup should have been driving the differences in the levels of androgen metabolites between men and women with severe asthma. Given that the women with asthma in our cohort also demonstrate the same clear dose–response relationships for suppression of adrenal androgens as the men in the study (low-dose ICS<high-dose ICS<high-dose ICS+OCS; tables 2 and 3), we conclude that the possible bias due to HRT is likely to have been small and evenly distributed among women with different severities of asthma.

Another limitation of the current study is that we were unable to assess the potential influence of smoking on steroid hormone levels because too few active smokers were included. However, our report does have many strengths, being the first to assess the relationship between corticosteroid treatment and a broad panel of endogenous steroids in a comprehensively characterised cohort of close to 500 individuals with asthma, and providing new findings concerning associations between steroid levels and many clinical and biomarker outcomes. Moreover, we used a validated quantitative method used by international sports authorities to disqualify athletes due to doping, namely, the measurement of urinary metabolites reflecting whole-body metabolism. A further strength of our study is that clinical data, biomarker profiles, and concentrations of urinary metabolites of steroid hormones observed in SA patients at baseline were replicated at follow-up, 12–18 months later (table 4).

In conclusion, the comprehensive measurements of endogenous and exogenous steroid metabolites in the U-BIOPRED study demonstrate that severe asthma is associated with substantial suppression of EAAS levels in addition to cortisol. The data clearly reveal that the level of treatment with exogenous corticosteroids is the main cause of this adrenal suppression, as previously observed in a small study of healthy subjects [29]. Given the indications that androgens have beneficial anti-asthmatic properties [4, 30], our findings suggest that sex differences in asthma severity and prevalence [31, 32] may in part relate to a disproportionally greater relative deficiency in androgen levels during steroid treatment in females compared to males. Our study also supports the clinical goal of reducing the adverse effects of corticosteroids by reducing high-dose ICS therapy, and particularly by tapering of OCS [33]. In addition, we highlight the importance of assessing adrenal function and androgens in patients with severe asthma exposed to high doses of corticosteroids. Future studies will reveal whether new biologic therapies facilitate steroid-sparing treatment associated with less suppression of endogenous androgens.

Provenance: Submitted article, peer reviewed.

Ethics statement: The U-BIOPRED observational study of severe asthma was approved by the ethics committees at all the clinical sites in eleven countries, and participants provided written informed consent.

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