ORIGINAL ARTICLE

Benefits of Airway Androgen Receptor Expression in Human Asthma

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Abstract

Rationale: Androgens are potentially beneficial in asthma, but *AR* (androgen receptor) has not been studied in human airways.

Objectives: To measure whether *AR* and its ligands are associated with human asthma outcomes.

Methods: We compared the effects of *AR* expression on lung function, symptom scores, and fractional exhaled nitric oxide (F_{ENO}) in adults enrolled in SARP (Severe Asthma Research Program). The impact of sex and of androgens on asthma outcomes was also evaluated in the SARP with validation studies in the Cleveland Clinic Health System and the NHANES (U.S. National Health and Nutrition Examination Survey).

Measurements and Main Results: In SARP ($n = 128$), *AR* gene expression from bronchoscopic epithelial brushings was positively associated with both FEV_1/FVC ratio ($R^2 = 0.135$, $P = 0.0002$) and the total Asthma Quality of Life Questionnaire score ($R^2 = 0.056$, $P = 0.016$) and was negatively associated with F_{ENO} ($R^2 = 0.178$, $P = 9.8 \times 10^{-6}$) and *NOS2* (nitric oxide synthase gene) expression ($R^2 = 0.281$, $P = 1.2 \times 10^{-10}$). In SARP ($n = 1{,}659$), the Cleveland Clinic Health System ($n = 32{,}527$), and the NHANES ($n = 2{,}629$), women had more asthma exacerbations and emergency department visits than men. The levels of the *AR* ligand precursor dehydroepiandrosterone sulfate correlated positively with the FEV_1 in both women and men.

Conclusions: Higher bronchial *AR* expression and higher androgen levels are associated with better lung function, fewer symptoms, and a lower F_{ENO} in human asthma. The role of androgens should be considered in asthma management.

Keywords: asthma; androgens; airflow obstruction; airway inflammation

(Received in original form September 30, 2020; accepted in final form March 22, 2021)

Supported by the NHLBI Severe Asthma Research Program grants U10 HL109250, P01 HL128192, P01 HL101871, R01 HL69170, U10 HL109250, K08 HL133381, P01 HL103453, P01 HL081064, R01CA172382, and R01CA190289.

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This article has a related editorial.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

Am J Respir Crit Care Med Vol 204, Iss 3, pp 285–293, Aug 1, 2021

Originally Published in Press as DOI: 10.1164/rccm.202009-3720OC on March 22, 2021
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: *AR* (androgen receptor) gene expression has not previously been well studied in the human airway in general, or in asthma in particular, although *AR* ligands are beneficial in asthma.

What This Study Adds to the Field: In addition to our newly published data, which describe *AR* protein expression in the human airway, we show that increased *AR* gene expression is strongly associated with improved airflow, fewer symptoms, and less nitrosative inflammation in human asthma. We also confirm that male sex is associated with fewer asthma exacerbations and admissions and that *AR* ligands are associated with better lung function in three large cohorts of adult human asthma. Androgens are a major factor to be considered in planning future asthma research and management strategies.

Asthma is a common chronic airway disease that results in a significant health and financial burden to individuals and society (1–3). Epidemiologic evidence demonstrates a sex-based dimorphism in asthma prevalence and severity. For example, asthma is more common and severe in boys than in girls, but the prevalence and severity of asthma is higher in adult women before menopause than in men (1–3). These effects correspond to changes in sex hormones and suggest that sex hormones play an important role in the pathogenesis of asthma (2, 3). Indeed, the airway effects of estrogen and progesterone on asthma pathophysiology have been studied extensively (4–6).

However, the effects of androgens in asthma have not been as well characterized. Recent studies using data from the UK Biobank and NHANES (U.S. National Health and Nutrition Examination Survey) showed that, compared with free serum testosterone levels in the first quartile, free serum testosterone levels in the fourth quartile were associated with lower odds of current asthma in men (7–9) and women (7–10). We have also recently shown that higher levels of androgens are associated with better lung function in children (11). In a follow-up study with adults with severe asthma who were enrolled in the NIH–NHLBI SARP (Severe Asthma Research Program), we found that serum testosterone levels positively correlated with the prebronchodilator percent predicted FEV_1 (FEV_1PP) in men. Testosterone levels were also found to be lower in men with severe asthma compared with men with nonsevere asthma (12). Our findings are consistent with established data showing that testosterone levels are positively correlated with better lung function in two large cohorts of healthy men. Indeed, both boys and girls, followed longitudinally, often outgrow severe asthma during the period of increased systemic androgen levels during adolescence (13). Furthermore, we have recently shown that a missense-encoding variant in the androgen synthesis gene *HSD3B1* associated with high tissue androgen levels is also associated with better lung function in patients with severe, corticosteroid-dependent asthma in particular. In a pilot study, dehydroepiandrosterone (DHEA) supplementation in women with asthma with low DHEA sulfate (DHEA-S) levels may benefit lung function. These data are consistent with emerging mechanistic data from our group and others suggesting that there are antiinflammatory airway effects of androgens (14–16).

Here, we report not only that the *AR* (androgen receptor) gene is expressed in the human airway epithelium, as recently reported by our group (17), but also that *AR* gene expression is strongly associated with better lung function and fewer symptoms in subjects with asthma in the SARP. Strikingly, *AR* gene expression is negatively associated with fractional exhaled nitric oxide (F_{ENO}) in these subjects and with the expression of inducible *NOS2* (nitric oxide synthase gene). Although the expression of estrogen receptors and the expression of other airway epithelial genes have been previously queried with regard to the determinants of F_{ENO} (18, 19), *AR* expression has not previously been considered, and the association between epithelial *AR* expression and F_{ENO} is, to our knowledge, the strongest association reported to date. In addition, we confirm that circulating androgen levels are associated with improved lung function in both men and women and, in three separate cohorts, that men have fewer asthma exacerbations and hospital admissions than women. Coupled with other recent mechanistic and epidemiologic discoveries regarding androgens in asthma (16, 17, 20–22), these data strongly suggest an important beneficial role for androgens in the pathophysiology of asthma in general and of nitric oxide signaling in asthma in particular. Here, we test the hypothesis that *AR* plays an important role in asthma and that its action is mediated by iNOS (inducible nitric oxide synthase).

Methods

SARP

Clinical and bronchial epithelial cell gene expression data were obtained from 128 patients with asthma enrolled in the SARP I and II between 2009 and 2011. Sponsored by the NIH/NHLBI, SARP is a multicenter study designed to study severe asthma to develop better treatments (23). SARP I and II recruited 1,644 patients with asthma between 2001 and 2012 from nine sites across the United States and one site in the United Kingdom. Thorough descriptions of the SARP I, II, and III network have been published previously (23, 24). The clinical characteristics of patients with asthma enrolled in SARP I and II, on

Author Contributions: J.G.Z., S.C.E., T.L., and B.G. made substantial contributions to the conception or design of the work. J.G.Z., J.M.M., N.M., O.G., M. D. DeBoer, and B.G. acquired, analyzed, and interpreted the data for the work. S.C.E., M. D. DeBoer, W.C., K.F.C., J.V.F., E.I., N.N.J., B.D.L., D.T.M., W.C.M., V.E.O., M.P., E.R.B., D.A.M., S.E.W., and B.G. collected clinical data in the Severe Asthma Research Program network. J.G.Z., J.M.M., N.S., S.C.E., M. D. DeBoer, S.A.C., P.B., H.J.K, W.B., W.C., M.C., K.F.C., J.V.F., E.I., N.N.J, B.D.L., D.T.M., W.C.M., V.E.O., M.P., E.R.B., D.A.M., S.E.W., Y.Z, and B.G. contributed to drafting the work or revising it critically for important intellectual content. J.G.Z. agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. J.G.Z., S.C.E., M. D. Davis, M. D. DeBoer, S.A.C., P.B., H.J.K., W.B., W.C., M.C., K.F.C., J.V.F., E.I., N.N.J, B.D.L., D.T.M., W.C.M., V.E.O., M.P., E.R.B., D.A.M., Y.Z., S.E.W., and B.G. gave the final approval of the version to be published.

whom gene expression data from the bronchial epithelium were available, are listed in Table E1 in the online supplement.

Spirometry and F_{ENO} measurements were performed at SARP centers (25), and the airway epithelial cells of patients with asthma ($n = 128$) enrolled in SARP I and II were obtained with bronchoscopy and epithelial brushings (18). The description of sample preparation and microarray experiments has been previously reported (18), and data were made available online in the U.S. National Center for Biotechnology Information Gene Expression Omnibus (26) and can be accessed using the Gene Expression Omnibus series accession number GSE63142 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63142). Gene expression was normalized using the cyclic, locally estimated scatterplot smoothing method by cyclically implementing locally estimated scatterplot smoothing to normalize any possible pairwise combination of arrays. SARP was approved by each network center's institutional review board.

Immunoblot and Immunohistochemistry Studies of *AR* in Human Airway Epithelial Cells

Healthy subjects underwent bronchoscopy as described for the SARP population in a subsequent study, and samples were used to test whether the *AR* protein was expressed in the human airway epithelium. Airway brush biopsy specimens were used to grow well-differentiated primary human airway epithelial cells at an air–liquid interface as previously described (27, 28). Protein was extracted from primary airway epithelial cells grown at an air–liquid interface (three wells each from two healthy subjects), from LNCaP cells (prostate cancer positive control; American Type Culture Collection) lysed in radioimmunoprecipitation assay buffer, or from PC-3 cells (negative control; American Type Culture Collection) lysed in radioimmunoprecipitationassay buffer. Lysates underwent immunoblotting using our Jess (ProteinSimple) capillary-based system with Compass software for data analysis. The primary antibodies were mouse anti-AR (1:50; catalog number 66747-1-lg, ProteinTech Group) and rabbit anti–β-actin (1:100; catalog number 4970, Cell Signaling). All secondary antibodies were purchased from ProteinSimple (catalog numbers 042-205 and 042-206, respectively).

At the time of bronchoscopy, subjects also underwent endobronchial biopsy. Using two healthy male volunteers' endobronchial biopsy specimens (and prostate controls), we performed immunohistochemistry. Formalin-preserved, paraffin-embedded biopsy specimens were sectioned (10 μm), immunostained for *AR* using AR monoclonal mouse anti–human antibodies (clone AR441; catalog number M356201-2, Agilent) and visualized by using an Invitrogen EVOS M5000 microscope (Thermo Fisher Scientific) at 40× magnification.

Asthma-related Healthcare Use

Data on healthcare use from the well-characterized, cross-sectional NIH–NHLBI SARP I and II cohorts ($n = 1,361$) and the (longitudinal) SARP III cohort ($n = 714$) were replicated using two additional cohorts: The Cleveland Clinic Health System (CCHS) cohort and the NHANES cohort (Table E2). In the CCHS cohort, asthma was identified using *International Statistical Classification of Diseases, Tenth Revision*, codes, and data were extracted from electronic health records of 32,527 patients with asthma who met the inclusion criteria (71.1% of all patients included in the CCHS cohort). The CCHS cohort constitutes a real-world sample of patients with asthma from the Cleveland region and Northeast Ohio seen at the CCHS in 2018. In NHANES, a population-based survey was designed to examine a representative sample across the United States (29) (Table E2). In NHANES, asthma is self-reported by the survey participants when they are asked if they have ever been told they have asthma by a doctor or other healthcare professional.

These three cohorts represent three diverse populations. SARP data included the highest proportion of patients with severe asthma (23, 30), and CCHS data included the lowest proportion of patients with severe asthma. Severe asthma was defined according to national and international guidelines in SARP (31, 32) and by the need for an inhaled corticosteroid (ICS)–long-acting β-agonist combination and/or therapy with oral corticosteroids (oCS) in NHANES. In CCHS, severe asthma was identified by the *International Statistical Classification of Diseases, Tenth Revision*, code of J45.5 (Table E2).

Sex Hormones, Lung Function, and Severe Asthma

We studied the relationship both between serum androgen levels and asthma severity and between androgen levels and FEV_1PP in patients enrolled in SARP I, II, and III for whom androgen level measurements were available. We analyzed data on patients with asthma, age 18–80 years, and excluded pregnant women. To exclude effects of exogenous sex hormones, women receiving hormone-based contraceptives or hormone replacements were also excluded. Patients with other concurrent chronic lung diseases, such as chronic obstructive pulmonary disease, and current smokers and ex-smokers with a smoking history of 10 or more pack-years were also excluded.

Sex Hormone Assays

DHEA-S, testosterone, and SHBG (sex hormone–binding globulin) levels were analyzed at the University of Virginia's Center for Research in the Reproduction Ligand Core Laboratory as previously described (11) using the Immulite 2000 assay (Siemens Healthcare Diagnostic). Free testosterone was calculated from total testosterone and SHBG, as previously described (33). The lower limits of detection (LODs) for assays were as follows: testosterone, 20 ng/dl; DHEA-S, 15 μg/dl; and SHBG, 10 nmol/L. Individuals with measurements below the LOD were counted as having a value just below that limit (LOD, 0.1). The intraassay and interassay coefficients of variation (percentages) were as follows: testosterone, 4.4% and 7.5%; DHEA-S, 5.4% and 6.5%; and SHBG, 2.7% and 5.2%. All analyses were run simultaneously to avoid the batch effect.

Total Testosterone versus Free Testosterone

We analyzed our data using the total testosterone in men and the free testosterone in women. In men, the total testosterone levels are used to assess testosterone deficiency according to the guidelines of the American Urologic Association (34). In women, we calculated free testosterone values as previously described (33) because the total testosterone cannot predict free testosterone levels (i.e., the active component). In fact, 66% of total testosterone is bound to SHBG and may simply reflect higher SHBG levels in women. Because SHBG levels are further related to characteristics such as obesity, free testosterone is used in clinical and research settings more often than the free androgen index (35).

Statistical analysis. To account for significant sex differences in sex hormone levels, we analyzed the associations between androgens and lung function separately in

men and women. Categorical data are presented as the count and percentage, and continuous data are presented as the mean (SD). We compared categorical variables using a chi-square test and compared continuous variables with the Kruskal-Wallis test. Multivariate linear regression models tested associations of clinical outcomes (prebronchodilator FEV_1PP) with age, body mass index (BMI), and sex androgen levels as covariates. Using a stepwise (backward elimination) procedure, the covariate "race" was removed from the initial model on the basis of Akaike Information Criterion. Models were fit under the assumption of a normal distribution for the FEV_1PP.

Expression levels of the *AR* in human bronchial epithelial biopsy were correlated with different outcome variables: FEV_1PP, FEV_1/FVC ratios, F_{ENO}, and total Asthma Quality of Life Questionnaire scores, with higher scores indicating better quality of life. They were also correlated with expression levels of inducible *NOS2* because of the association between *AR* gene expression and F_{ENO}. Multivariate linear regression models were used to evaluate the association between sputum gene expression and different outcomes, adjusting for age, sex, BMI, and race. Bootstrapped 95% confidence intervals [CIs] for R^2 in linear regressions were based on 1,000 replications and were calculated using the library "boot" in R (R Foundation for Statistical Computing). The unadjusted R^2 value was used to represent the proportion of the variance in the dependent variable that is explained by the predictor variables, and a P value < 0.05 was considered to indicate statistical significance. Of note, we did not account for low socioeconomic status, environmental exposures, upper respiratory tract infections, and/or medication compliance in our analyses.

We also performed three sets of mediation analyses to further evaluate the association between *AR* expression and outcomes such as lung function and F_{ENO} and adjust for confounding factors such as age and sex by using *1*) sex as the "exposure" and *AR* expression as the "mediator," *2*) age as the exposure and AR expression as the mediator, and *3*) *AR* expression as the exposure and *NOS2* as the mediator. In both analysis 1 and analysis 2, the FEV_1 (%) is a primary outcome of interest. Note, of course, that age cannot be an effect of AR; it can only be the other way around. In analysis 3, F_{ENO} is the outcome studied. For analysis 1, age and sex are included as the covariates; for analysis 2, sex and race are the adjusted variables; and for analysis 3, sex, age, and race are the confounding factors.

Analyses were performed using R 4.0.2 statistical software.

Results

In bronchial epithelial cells obtained from subjects with asthma enrolled in SARP I and II ($n = 128$), *AR* gene expression was positively associated with the FEV_1PP ($R^2 = 0.085$ [95% CI, 0.011–0.211], $P = 0.003$), the FEV_1/FVC ratio ($R^2 = 0.135$ [95% CI, 0.052–0.259], $P = 0.0002$), and the total Asthma Quality of Life Questionnaire score ($R^2 = 0.056$ [95% CI, 0.001–0.176]; $P = 0.016$) (Figure 1); and it was negatively associated with F_{ENO} ($R^2 = 0.178$ [95% CI, 0.056–0.351], $P = 9.8 \times 10^{-6}$) (Figure 2). The significant negative correlation between *AR* gene expression and F_{ENO} was mirrored by an even stronger negative correlation between *AR* gene expression and iNOS (gene symbol *NOS2*) gene expression ($R^2 = 0.281$ [95% CI, 163–0.414], $P = 1.2 \times 10^{-10}$) (Figure 2). These associations continued to be significant even after adjustment for age, sex, race, and BMI (Table E3). Interestingly, both F_{ENO} and *NOS2* expression positively correlated with the absolute eosinophil count in blood but not with BAL fluid eosinophilia (as a percentage of the BAL fluid total cell count) in SARP I and II using a Bonferroni-corrected P value threshold of $\alpha = 0.05/4 = 0.0125$ (Figure E1). Of note, although the *AR* gene expression did not correlate with the dose of daily oCS ($R^2 = 0.063$ [95% CI, 0.005–0.178]; $P = 0.261$; $n = 22$), *AR* expression was lower among SARP patients with asthma and treated with oCS ($n = 22$ out of 128) as compared with those not receiving oCS therapy (mean [SD], 8.32 [0.44] vs. 8.59 [0.46]; $P = 0.01$). *AR* gene expression did not vary by sex ($P = 0.39$). Note that *AR* protein was also expressed in human airway

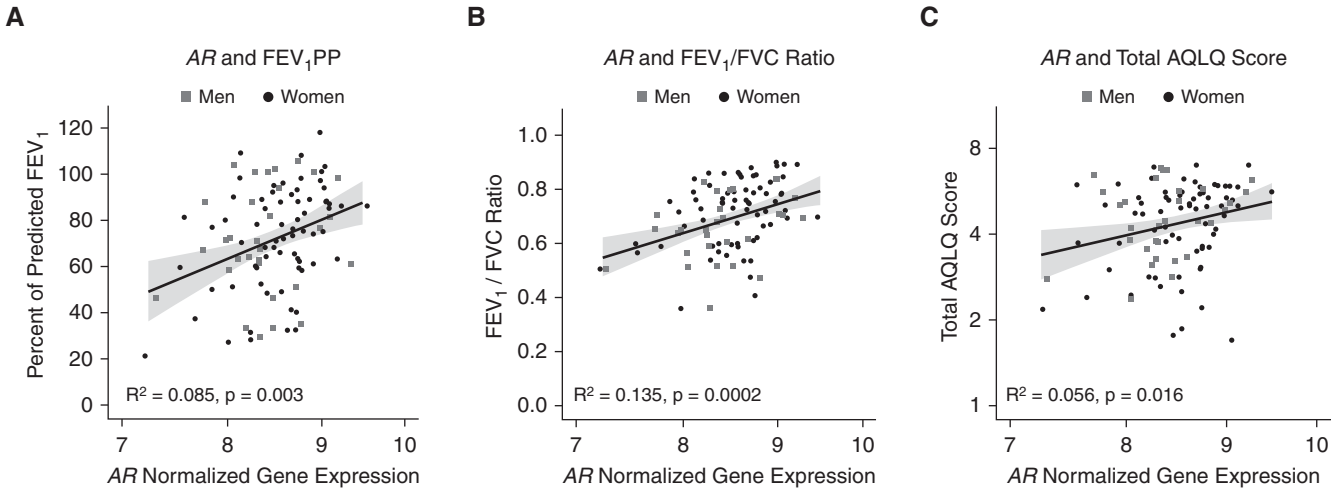

Figure 1. *AR* (androgen receptor) gene expression in bronchial epithelial cells from subjects with asthma enrolled in the Severe Asthma Research Program I and II is positively associated with the (*A*) FEV_1PP, (*B*) FEV_1/FVC ratio, and (*C*) total Asthma Quality of Life Questionnaire (AQLQ) score. The *AR* normalized gene expression and the total AQLQ score are plotted on a log-2 scale. The gray shaded regions represent SEs.
FEV_1PP = percent predicted FEV_1.

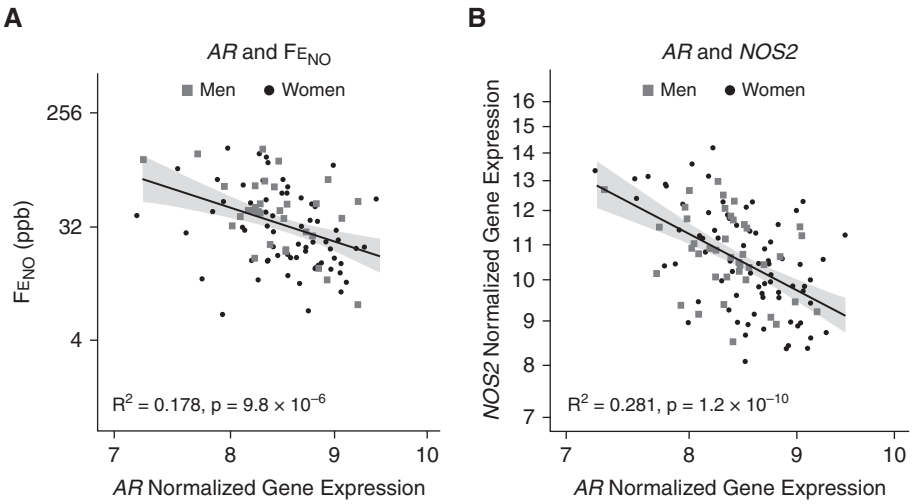

Figure 2. *AR* (androgen receptor) gene expression in bronchial epithelial cells from subjects with asthma enrolled in the Severe Asthma Research Program is positively associated with (*A*) F_{ENO} and with (*B*) inducible *NOS2* (nitric oxide synthase gene) expression. *AR*, *NOS2*, and the fractional exhaled nitric oxide are plotted on a log-2 scale. The gray shaded regions represent SEs. F_{ENO} = fractional exhaled nitric oxide.

epithelial cells in culture, as well as in human airway endobronchial biopsy samples (Figure E2), which is consistent with our recent coronavirus disease (COVID-19) publication (17).

AR expression was not statistically different between individuals who reported an asthma exacerbation in the year before SARP I and II enrollment ($n = 51$) versus those who did not report an asthma exacerbation (mean [SD], 8.43 [0.52] vs. 8.53 [0.39], respectively; $P = 0.28$). Furthermore, *AR* gene expression was not significantly associated with the expression of genes encoding type 2 (T2)-high asthma targets, IL-5 receptor, or IL-4/IL-13 receptor.

Our mediation analysis using sex as the exposure and *AR* as the mediator shows that sex has neither a direct nor an indirect effect on FEV_1 (%). In contrast, we found that 11.3% of the effect of age on FEV_1 (%) is mediated by *AR*. In the context of mediation analysis, this is a very small effect, and age did not impact the association between *AR* and lung function in multivariate analysis. In contrast, mediation analysis showed that 80.9% of the effect of *AR* expression on F_{ENO} is mediated by *NOS2* expression (Table E4).

Consistent with previous work from our group (11, 14) and others (8, 9), we found that female sex was associated with higher odds of having asthma exacerbations and asthma-related emergency room visits despite women having higher FEV_1PP in all three cohorts (SARP: odds ratio [OR], 1.61 [95% CI, 1.29–2.02] and 1.41 [95% CI, 1.12–1.71], respectively; CCHS: OR, 1.27 [95% CI, 1.21–1.35] and OR, 1.31 [95% CI, 1.13–1.51], respectively; and NHANES: OR, 1.51 [95% CI, 1.23–1.87] and OR, 1.71 [95% CI, 1.26–2.31], respectively) (Figure 3). Furthermore, higher circulating levels of the adrenal androgen precursor steroid DHEA-S were associated with better lung function in men ($n = 241$) and women ($n = 423$) with asthma (Figure 4) enrolled in SARP I, II, and III. In women, the FEV_1PP positively correlated with DHEA-S ($R^2 = 0.156$ [95% CI, 0.094–0.227], $P < 2.2 \times 10^{-16}$) but not with free testosterone ($R^2 = 0.005$ [95% CI,

Adverse Event	Women	Men	Odds Ratio (95% CI)
	No. of patients with events (%)		
Asthma Exacerbations			
SARP	413 (37.6)	153 (27.2)	1.61 (1.29 – 2.02)
CCHS	6,362 (28.6)	2,447 (23.9)	1.27 (1.21 – 1.35)
NHANES	4,254,225 (51.5)	2,046,182 (41.8)	1.51 (1.23 – 1.87)
ED Visits			
SARP	332 (29.4)	128 (22.8)	1.41 (1.12 – 1.79)
CCHS	735 (3.3)	261 (2.5)	1.31 (1.13 – 1.51)
NHANES	1,076,763 (13.0)	373,469 (7.6)	1.71 (1.26 – 2.31)

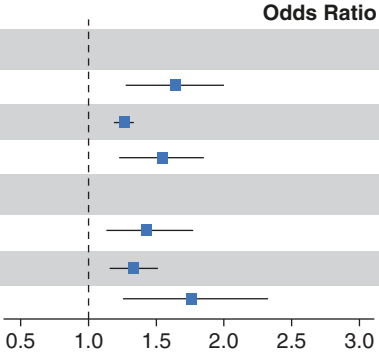

Figure 3. Asthma exacerbation and asthma-related emergency department (ED) visits in adults stratified by sex. The risk for asthma exacerbation and ED visits for a respiratory problem the year before SARP and NHANES participation and in 2018 in CCHS was higher in adult women than in adult men in all three cohorts. The sampling weights are used to produce the correct population estimates because each sample person does not have an equal probability of selection. CCHS = Cleveland Clinic Health System; CI = confidence interval; NHANES = U.S. National Health and Nutrition Examination Survey; SARP = Severe Asthma Research Program.

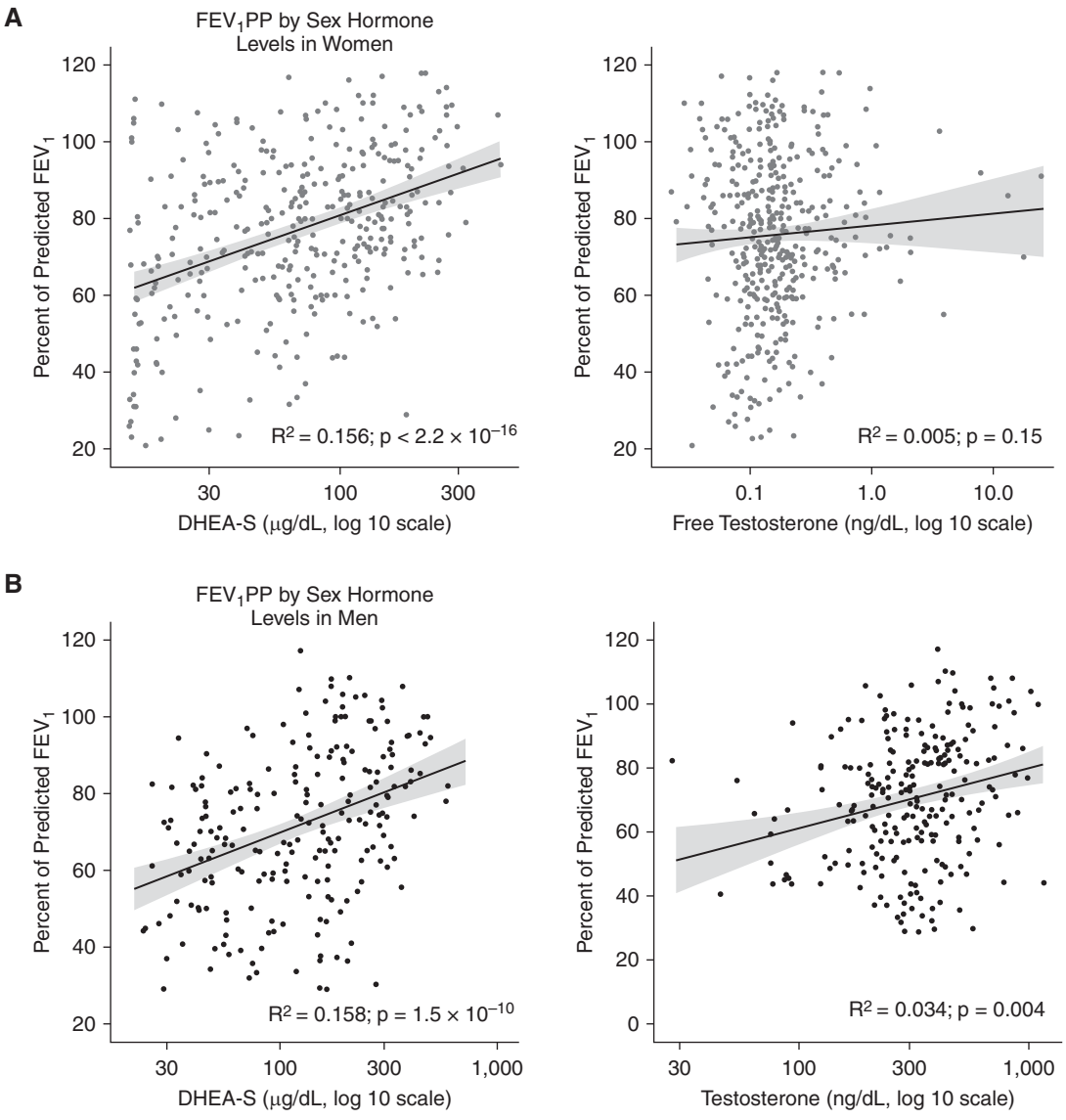

Figure 4. Prebronchodilator percent predicted FEV_1 (FEV_1PP) by sex hormone levels in SARP (Severe Asthma Research Program) I, II, and III adult participants. (*A*) In women enrolled in SARP I, II, and III, the FEV_1PP correlated positively with dehydroepiandrosterone sulfate (DHEA-S) ($R^2 = 0.156$; $<2.2 \times 10^{-16}$) but not with free testosterone ($P = 0.15$). (*B*) In adult men, the FEV_1PP correlated positively with DHEA-S ($R^2 = 0.158$; $P = 1.5 \times 10^{-10}$) and testosterone ($R^2 = 0.034$; $P = 0.004$). The gray shaded regions represent SEs.

0.002–0.024], $P = 0.15$) (Figure 4A). In men with asthma enrolled in SARP, the FEV_1PP correlated positively with both DHEA-S and testosterone ($R^2 = 0.158$ [95% CI, 0.089–0.239], $P = 1.5 \times 10^{-10}$ vs. $R^2 = 0.034$ [95% CI, 0.002–0.101], $P = 0.004$, respectively) (Figure 4B). We built a multivariable linear regression model to predict the effect of sex hormones on the FEV_1PP, adjusting for age and BMI in both men and women separately. In the final model, the FEV_1PP continued to correlate positively with DHEA-S ($P < 0.001$) in both sexes. To account for the drop in testosterone in men and the drop in ovarian sex hormones from menopause in women, we stratified the correlation between DHEA-S and the FEV_1PP by age category (age >50 yr vs. age ⩽50 yr) and found the correlation to be significant among individuals aged 50 years and younger in both men and women, but this correlation was not significant among older individuals using a Bonferroni-corrected *P* value threshold of $\alpha = 0.05/4 = 0.0125$ (Table E5).

In SARP, subjects with asthma were classified as having severe asthma on the basis of the standard guidelines (31, 32). DHEA-S was lower in both women (median, 31.50 [interquartile range (IQR), 15.00–74.40]) and men (median, 59.70 [IQR, 34.47–136.50]) with severe asthma when compared with women (median, 99.50 [IQR, 48.65–152.25]) and men (median, 186.00 [IQR, 118.00–283.00]) with nonsevere asthma ($P < 2.2 \times 10^{-16}$) (Figure E3). Furthermore, testosterone levels were also lower in men with severe asthma as compared with their counterparts with nonsevere asthma ($P = 4.2 \times 10^{-6}$). Severe asthma was not

associated with lower free testosterone in women ($P = 0.008$) (Figure E3).

Discussion

Asthma is a heterogeneous disease (25, 36). Androgens can affect the asthmatic airway. For example, androgens can directly cause airway smooth muscle relaxation mediated by decreased cellular calcium (Ca^{2+}) influx through L-type Ca^{2+} channels (37), or androgens can indirectly attenuate inflammation and, consequently, improve bronchodilation (20). Airway inflammation, which results in airway hyperresponsiveness and excess mucus production, is mediated by the activation of a variety of immunomodulatory cells (6, 16, 20, 36). The role of sex hormones in asthma pathobiology has been studied in animal models. In general, immune activation underlying airway inflammation in asthma is increased by female sex hormones and suppressed by androgens (6, 15, 16). In one study, IL-33 stimulation of lung group 2 innate lymphoid cells (ILC2s) induced a larger increase in IL-5 and IL-13 in female mice than in male mice (38). Furthermore, 5α-dihydrotestosterone (DHT), a potent metabolite of testosterone, was shown to negatively regulate ILC2 proliferation and decrease IL-5 and IL-13 expression from ILC2s. Consequently, DHT resulted in the reduction of lung eosinophil activation and proliferation and the attenuation of T2-mediated allergic airway inflammation (15). Attenuation of T2 inflammation has also been reported with the adrenal androgen DHEA, which is a hormone upstream of testosterone (39). Mice that received DHEA supplementation in their diet had lower house dust mite–induced allergic airway inflammation than mice fed with a control diet (39). Similarly, DHEA inhibited bronchial epithelial-to-mesenchymal transition through inhibition of the *PI3K/Akt*-dependent signal pathway stimulated by TGF-β1. Theoretically, DHEA could have a beneficial effect on airway remodeling and fibrosis in asthma (22). In addition to their role in T2 inflammation, sex hormones also modulate T-helper cell type 17 (Th17)-mediated airway inflammation in severe asthma, such that the number of IL-17$^+$ memory Th17 cells and Th17 cells mediating IL-17A production were found to be higher in women than in men (6, 40); these findings are supported by murine studies (6, 40). In contrast to ovarian sex hormones, androgens had the opposite effect on Th2 and Th17 airway inflammation (16). In a murine model of gonadectomized female and male mice intranasally challenged with house dust mites, testosterone decreased, and ovarian hormones increased, IL-13$^+$ CD4 Th2 cells and IL-17A$^+$ CD4 Th17 cells in the lung (16). Taken together, these data suggest a beneficial role of androgens in asthma.

Determinants of Fe_{NO} levels are multifactorial, but an important upstream cause of increased Fe_{NO} is *NOS2* expression (41, 42). Notably, our mediation analysis has shown that 80.9% of the effect of increased *AR* expression that decreases the Fe_{NO} is caused by an effect of *AR* expression to decrease *NOS2* expression. It has not previously been reported that *NOS2* expression is related to *AR* expression in the airway. Note in this regard that Fe_{NO} levels do not vary dramatically between men and women; if anything, they are lower in men than in women. This is despite previous correlative studies suggesting that sex hormone levels are related to Fe_{NO} levels and to the expression and activity of NOS isoforms (42–46). We speculate that it is tissue sex hormone metabolism and *AR* expression, more than sex and circulating sex hormone levels, that determines Fe_{NO}; and that *AR* expression has a protective, antiinflammatory effect. Additional mechanistic studies will be required.

Even though the role of *ESR* (estrogen receptor genes) and *ESR* signaling in lung diseases has been extensively studied, very little is known about the functional importance of the *AR* and androgen signaling in lung physiology or pathobiology beyond the embryonic period (47). The *AR* mediates the function of male sex hormones such as testosterone, DHT, and DHEA in males and females (48). The *AR* gene is a ligand-dependent nuclear transcription factor and member of the steroid hormone nuclear receptor family. It plays an important role in the biology of the reproductive, hemopoietic, musculoskeletal, cardiovascular, neuronal, and immune systems (48). In addition to its role in the physiologic function of many reproductive and nonreproductive organs, *AR* and androgen signaling have also been involved in the pathophysiology of many diseases across the life span and in prostate, liver, urologic, and lung cancer (48, 49). The *AR* protein has been demonstrated in a variety of human tissues obtained at surgery, including lung tissue (17, 50). Yet very limited data exist on their role in lung function and pathology (47). In the murine lung, *AR* is mainly expressed in T2 pneumocytes and bronchial epithelial cells, and *AR* protein levels are higher in intact male mice than in castrated male mice (51). Testosterone supplementation administered to castrated mice increases *AR* protein levels in lung cells and results in the upregulation of genes involved in oxygen transport and the downregulation of DNA repair and DNA recombination pathways (51). Furthermore, a significant sexual dimorphism exists between male and female mice. Some of the genes that are increased in male lungs compared with female lungs are involved in muscle development and contraction. In contrast, genes that are decreased in male lungs are involved in the acute inflammatory response and regulation of translation (51). Of note, the response of androgen-regulated genes may differ between organs and is tissue specific because gene expression depends on local hormone metabolism, chromatin modifications, activators, and repressors characteristic of a specific cellular milieu (14, 48, 52).

Note that we and others have shown that ICS use can affect DHEA-S levels (11, 14, 53). However, it is unlikely that ICS use accounts primarily for the associations between increased androgens and increased asthma severity for several reasons. These reasons include genetic data (14); pharmacologic data showing improvements in asthma with androgen supplementation (54, 55) in animal models, including the *AR*-deficient mouse (15, 16); the associations between human *AR* levels and asthmatic airflow obstruction, and symptoms reported here; and human data regarding the association between *AR* ligands and better lung function, even in the absence of ICS use (8).

Our analysis has several limitations. As compared with the SARP cohort, in which individuals with asthma are well characterized, clinical data were incomplete in the NHANES and CCHS cohorts. For example, asthma was diagnosed in 13,773 patients in the NHANES cohort between 1999 and 2017, out of which 73 men and 57 women had concurrent spirometry and testosterone-level measurement. None of the NHANES participants had spirometry performed simultaneously with measurement of estradiol and progesterone levels. DHEA-S levels were not measured in NHANES. In addition, although gene expression data were available on 128 well-characterized subjects with asthma from SARP I and II, this small sample size makes it difficult to stratify the analysis

further by asthma phenotypes or endotypes (25, 56). The correlations reported here were statistically significant between lung function and androgens and between AR and asthma biomarkers and lung function under Bonferroni correction controlling for a family-wise error rate ($\alpha = 0.05/4 = 0.125$ for associations with serum androgen levels and $\alpha = 0.05/5 = 0.01$ for associations with *AR* gene expression). Asthma is a complex disease, in which many variables, such as age, BMI, race, exposures, and socioeconomic status, contribute to asthma severity and airway obstruction. Adjusting for age, sex, BMI, and race, androgens and *AR* expression are still significantly associated with lung function, $Fe_{NO,}$ and asthma-related quality of life. This is true both in direct multivariate analysis and in mediation analysis. Our data are compatible with murine-knockout data, which show that a lack of AR is associated with an increased risk of murine airway inflammation and bronchoconstriction (4). Our work is the first to use human data to support these animal data.

Additional limitations related to SARP methodology could theoretically have biased our findings. Androgen levels can cyclically vary significantly in premenopausal women who are not receiving exogenous sex hormones (57). Similarly, circadian rhythms in testosterone and DHEA-S have been previously reported in men and women (58, 59). These variations may result in inaccurate correlations between androgens levels and outcomes. Furthermore, androgen metabolism is affected by obesity. In women, obesity is associated with significantly elevated free estradiol and free testosterone levels as a consequence of subnormal levels of SHBG (60). In men, obesity is associated with increased aromatase activity that converts testosterone to estradiol, which leads to low testosterone and high estrogen levels (61). The limited sample size prohibits us from stratifying our analysis by obesity category as compared with recent analyses using data from the UK Biobank (8). However, even after adjustment for the BMI using multivariate analysis, androgens continued to significantly correlate with the FEV_1PP in both men and women. Our study was underpowered to study the association between systemic corticosteroids and *AR* gene expression, and prospective studies will need to be done in that regard. Finally, mechanisms underlying the benefit of *AR* expression in asthma are beginning to be understood (62) but were not the focus of this study.

Conclusions

Taken together, these data demonstrate that *AR*s are expressed in the human airways and that high levels of expression, together with high levels of *AR* ligands, are overall associated with better lung function, a better quality of life, and low Fe_{NO} in patients with asthma. These data are the first human data to support the murine observation that *AR* expression attenuates asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Mr. Bradly Souder for his valuable input and help. AstraZeneca, Boehringer-Ingelheim, Genentech, GlaxoSmithKline, Sanofi-Genzyme-Regeneron, and TEVA have supported the Severe Asthma Research Program. However, they did not play any role in the conception or design of the work, analysis of data, interpretation of results, drafting of the manuscript, or critical revision of the manuscript for important intellectual content.

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