ORIGINAL ARTICLE

Sex Steroid Hormones and Asthma in a Nationwide Study of U.S. Adults

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Abstract

Rationale: Women have a higher burden of asthma than men. Although sex hormones may explain sex differences in asthma, their role is unclear.

Objectives: To examine sex hormone levels and asthma in adults.

Methods: Cross-sectional study of serum levels of free testosterone and estradiol and current asthma in 7,615 adults (3,953 men and 3,662 women) aged 18–79 years who participated in the 2013–2014 and 2015–2016 U.S. National Health and Nutrition Examination Survey. Logistic regression was used for the multivariable analysis of sex hormones and current asthma, which was conducted separately in women and men.

Measurements and Main Results: Free testosterone levels in the fourth quartile were associated with lower odds of current asthma in women (odds ratio [OR] for the fourth quartile

[Q4] vs. Q1, 0.56; 95% confidence interval [CI], 0.39–0.80). Given an interaction between obesity and sex hormones on current asthma, we stratified the analysis by obesity. In this analysis, elevated free testosterone (OR for Q4 vs. Q1, 0.59; 95% CI, 0.37–0.91) and estradiol (OR for Q4 vs. Q1, 0.43; 95% CI, 0.23–0.78) levels were associated with reduced odds of current asthma in obese women, and an elevated serum estradiol was associated with lower odds of current asthma in nonobese men (OR for Q4 vs. Q1, 0.44; 95% CI, 0.21–0.90).

Conclusions: Our findings suggest that sex hormones play a role in known sex differences in asthma in adults. Moreover, our results suggest that obesity modifies the effects of sex hormones on asthma in adults.

Keywords: testosterone; estradiol; asthma; National Health and Nutrition Examination Survey

Asthma affects more than 300 million people worldwide, including approximately 6.1 million children and 20.4 million adults in the United States (1). The burden of asthma varies by age group and sex throughout the lifespan (2). In the United States, the prevalence of asthma is higher in boys (9.2%) than in girls (7.4%) among individuals younger than 18 years, but such prevalence is higher in women (10.4%) than in men (6.2%) among individuals 18

years and older. Similarly, mortality from asthma is higher in boys than in girls during childhood and adolescence, yet asthma-related mortality is higher in women than in men during adulthood (3).

Many epidemiologic studies have reported sex-specific differences in asthma and asthma outcomes (4–6). Compared with girls, boys are more likely to have asthma, asthma symptoms, and allergic sensitization, and to use medications for

asthma. After puberty, there is a sex shift in asthma prevalence, such that asthma becomes more common and severe or difficult to treat in women than in men (7, 8).

Sex differences in asthma may be partly explained by changes in the levels of sex hormones during the life course (9, 10). A recent systematic review and meta-analysis of 57 studies (51 observational and 6 experimental) examined asthma in relation

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At a Glance Commentary

Scientific Knowledge on the

Subject: Among adults, the prevalence of asthma is higher in women than in men. Although sex hormone levels may explain such sex differences, there has been no population-based study of sex hormone levels and asthma in adults.

What This Study Adds to the Field:

We found that elevated serum levels of free testosterone are associated with lower odds of current asthma in women. Given evidence of an interaction between obesity and sex hormone levels on asthma risk, as well as prior knowledge of obesity effects on both hormone levels and asthma, we repeated the analysis after stratification by obesity. In this analysis, elevated serum levels of both free testosterone and estradiol were associated with lower odds of current asthma in obese women, and an elevated serum estradiol level was associated with lower odds of current asthma in nonobese men. Our findings suggest that circulating sex hormones (estradiol and free testosterone) play a role in sex differences in asthma among adults and further suggest that obesity modifies the effect of such hormones on asthma in women and men.

to menstruation, menopause, hormonal contraceptives, and hormonal replacement therapy (HRT) in women. In that study, irregular menstruation, menopause onset, and current HRT were each associated with increased risk of current asthma. Moreover, past HRT, ever HRT, current HRT, and current estrogen-only HRT were each associated with new-onset asthma (10). In a cross-sectional study of children (ages 6–18 yr) with asthma (enriched for severe disease), serum levels of sex steroid hormones (estradiol, progesterone, testosterone, and dehydroepiandrosterone sulfate [DHEA-S; a nonvirilizing hormonal derivative of testosterone without untoward side effects on girls]) were measured in 45 boys and 23 girls. In that study, DHEA-S was inversely associated with asthma symptoms, but positively associated with FEV₁ and FVC

in boys, whereas estradiol level was negatively associated with FEV₁ and FVC in girls (11). Androgens, such as testosterone, may reduce innate and adaptive immune responses, whereas estrogen and progesterone may enhance T-helper cell type 2 allergic airway inflammation (12).

Given a plausible role of sex steroid hormones in sex-specific differences in asthma, we examined the relationship between serum levels of two such hormones (free testosterone and estradiol) and asthma among adult participants in the U.S. National Health and Nutrition Examination Survey (NHANES). Because of the known link between obesity and sex hormone levels (4, 13), we also examined whether obesity modifies the estimated effects of sex hormones on asthma. Some of the results of this study have been previously reported in an abstract (14).

Methods

Study Design and Study Population

NHANES is a cross-sectional, nationwide survey designed to assess the health and nutrition of the noninstitutionalized U.S. population using a stratified, multistage probability design to select a representative sample of such population. As part of the study design, ethnic minorities (Hispanic, non-Hispanic black, and non-Hispanic Asian individuals), low-income white individuals, and persons 80 years of age and older are oversampled to increase statistical power for data analysis in these groups. The NHANES protocol includes health interviews, examination components, and laboratory tests administered by highly trained medical personnel. In the current analysis, we included adults aged 18–79 years who participated in the 2013–2014 or 2015–2016 NHANES study cycles. Female participants who reported current pregnancy were excluded from the study. NHANES is approved by the Institutional Review Board of the National Center for Health Statistics of the Centers for Disease Control and Prevention. Informed consent is obtained from all study participants. Further details of the methods, protocols, and definitions used in NHANES can be found at <http://www.cdc.gov/nchs/nhanes.htm>.

Outcomes

Current asthma was defined by a positive answer to both of the following questions: “Has a doctor or other health professional ever told you that you have asthma?” and “Do you still have asthma?” Control subjects were participants without current asthma (i.e., those who reported never having had asthma diagnosed by a healthcare professional and those who reported having ever had asthma diagnosed by a healthcare professional, but who denied still having asthma).

Serum Sex Steroid Hormones

Serum total testosterone (TT), estradiol, and SHBG (sex hormone-binding globulin) were measured in the 2013–2014 and 2015–2016 study cycles of NHANES. Serum samples were analyzed at the Division of Laboratory Sciences under the Centers for Disease Control and Prevention. Serum total testosterone and estradiol were measured by isotope dilution liquid chromatography tandem mass spectrometry. SHBG is the blood transport protein for androgens and estrogens. It was quantified based on the reaction of SHBG with immunoantibodies and chemoluminescence measurements of the reaction products that occur after two incubation periods and subjecting to a magnetic field. Measurements that were below the lower limit of detection were divided by the square root of 2. All quality-control procedures recommended by the manufacturers were followed. Because testosterone circulates highly bound to SHBG, free testosterone was estimated using the empirical free testosterone (EFT) formula, as follows (15):

$$\begin{aligned} \text{EFT-low (TT} < 5 \text{ nM)} \\ &= -6.593 + 19.304 \text{ TT} \\ &\quad + 0.056 \text{ SHBG} - 0.0959 \text{ TT} \cdot \text{SHBG} \end{aligned}$$

$$\begin{aligned} \text{EFT-high (TT} \geq 5 \text{ nM)} \\ &= -52.65 + 24.4 \text{ TT} - 0.704 \text{ SHBG} \\ &\quad - 0.0782 \text{ TT} \cdot \text{SHBG} - 0.0584 \text{ TT}^2 \end{aligned}$$

A detailed description of the laboratory methods used can be accessed at NHANES Laboratory Method Files (16, 17) at https://www.cdc.gov/nchs/nhanes/2015–2016/tst_i.htm.

Statistical Analysis

Primary sampling units and strata for the complex NHANES survey design were taken

into account for data analysis. Sampling weights, stratification, and clusters provided in the NHANES dataset were incorporated into the analysis to obtain proper estimates and their SEs. Two-sided Wald chi-square tests and *t* tests were used for bivariate analyses. Logistic regression was used for the multivariable analysis of sex hormone levels (as quartiles) and current asthma, which was conducted separately in men and women. Known potential confounders of the relationship between sex hormones and asthma, and covariates that were significant at $P < 0.05$ in the bivariate analyses were included in the multivariable models. All models were adjusted for age, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American or other Hispanic, or other), annual household income ($< \$20,000$ vs. $\geq \$20,000/\text{yr}$), body mass index (BMI; in kg/m^2), family history of asthma, current exposure to second-hand smoke, smoking status (never, former, or current), pack-years of smoking, and (in women) ever use of birth control pills or any female hormones.

We tested for an interaction between obesity (defined as a $\text{BMI} \geq 30 \text{ kg}/\text{m}^2$) and sex hormones on current asthma in the multivariable models, given known effects of obesity on both sex hormone levels and asthma. In addition, we conducted a mediation analysis to estimate the total, direct, and indirect effects of sex hormone levels on current asthma with obesity as a mediator, based on a counterfactual framework (18, 19). All statistical analyses were conducted using SAS 9.4 software (SAS Institute Inc.).

Results

Of the 12,105 adults who participated in NHANES 2013–2016, 9,238 were aged 18–79 years and had complete data on asthma status and sex hormone levels, and (if female) were not pregnant (Figure 1). Of these 9,238 subjects, 7,615 (3,953 men and 3,662 women) had complete data on the covariates of interest and were thus included in the current analysis. Compared with the 7,615 subjects included in this analysis, those excluded ($n = 1,623$ [549 men and 1,074 women]) were more likely to be Mexican American or “other Hispanic,” to lack private health insurance, and to have lower educational attainment (see Table E1 in the online supplement).

Figure 1. Flowchart for selection of the participants included in the current analysis. NHANES = National Health and Nutrition Examination Survey.

Among men, those excluded from the current analysis were also more likely to be former smokers and to have lower annual household income than those included. There was no significant difference in serum level of free testosterone or estradiol between men who were and were not included in the current analysis. Among women, those excluded from the current analysis were also more likely to be younger, to have lower pack-years of smoking, and to have a higher serum estradiol level than those included. There was no significant difference in free testosterone level between women who were and were not included in this analysis (Table E1). The overall prevalence of current asthma in the study sample was 9.0% (6.1% in men and 12.5% in women).

Table 1 shows a comparison of the main characteristics of study participants by current asthma, separately in men and women. Compared with women without current asthma, those with current asthma were more likely to be non-Hispanic black, to have an annual household income of less than \$20,000, to lack private health insurance, to report a family history of asthma, to have higher BMI and peripheral blood eosinophils, to be current smokers, to be exposed to second-hand smoke, and to ever use birth control pills. Free testosterone level was significantly higher in women without current asthma than in those with current asthma. Compared with men without current asthma, those with current asthma were more likely to report a family history of asthma and to have higher

Table 1. Characteristics of Study Participants by Sex and Current Asthma, National Health and Nutrition Examination Survey 2013–2016

Characteristics	Men (n = 3,953)		Women (n = 3,662)	
	Controls (n = 3,714)	Current Asthma (n = 239)	Controls (n = 3,212)	Current Asthma (n = 450)
Age, yr	45.2 ± 0.4	45.3 ± 1.7	47.4 ± 0.4	47.1 ± 1.0
Race/ethnicity				
Non-Hispanic white	1,348 (64.7)	108 (71.7)	1,169 (65.9)	188 (68.0)*
Non-Hispanic black	735 (9.9)	53 (11.0)	620 (10.8)	115 (14.1)
Mexican American/other Hispanic	997 (16.0)	50 (11.0)	969 (15.2)	99 (10.3)
Other	634 (9.3)	28 (6.3)	454 (8.2)	48 (7.6)
Educational attainment				
<12th grade	866 (15.4)	58 (15.5)	662 (12.5)	94 (13.1)
High school/GED/AA	1,871 (51.6)	124 (47.5)	1,721 (55.0)	257 (59.9)
College and above	977 (32.9)	57 (37.0)	829 (32.5)	99 (26.9)
Annual household income <\$20,000	649 (10.8)	61 (14.4)	649 (13.2)	124 (20.7)*
Private health insurance coverage	1,957 (64.3)	114 (58.5)	1,683 (63.7)	216 (57.0) [†]
Family history of asthma	624 (17.6)	96 (39.6)*	677 (21.1)	228 (48.6)*
Peripheral blood eosinophils (as a percentage of white blood cells)	3.0 ± 0.04	3.9 ± 0.2*	2.5 ± 0.04	2.9 ± 0.12*
Body mass index, kg/m ²	29.0 ± 0.2	29.8 ± 0.6	29.4 ± 0.2	32.0 ± 0.6*
Smoking status				
Never	1,944 (53.9)	109 (51.8)	2,197 (64.7)	250 (52.5)*
Former smoker	923 (25.6)	62 (24.7)	486 (18.7)	87 (20.9)
Current smoker	847 (20.6)	68 (23.5)	529 (16.6)	113 (26.6)
Pack-years smoking	8.7 ± 0.6	10.1 ± 2.4	5.3 ± 0.4	8.1 ± 0.8*
Second-hand smoke exposure	1,067 (26.9)	89 (31.0)	812 (24.3)	160 (34.9)*
Use birth control pills, ever	—	—	2,174 (76.5)	334 (81.0) [†]
Use female hormones, ever	—	—	519 (21.1)	91 (22.6)
Total testosterone, nmol/L	14.7 ± 0.1	14.2 ± 0.6	0.85 ± 0.03	0.75 ± 0.03 [†]
Free testosterone, pmol/L	209.2 ± 2.1	198.3 ± 9.7	7.1 ± 0.3	6.1 ± 0.3 [†]
Estradiol, pmol/L	91.6 ± 1.1	89.0 ± 3.1	216.3 ± 6.6	204.4 ± 17.8

Definition of abbreviations: AA = Associate of Arts; GED = General Educational Diploma.

Results are shown as mean ± SE for continuous variables and as n (%) for binary variables.

**P* < 0.01 for comparison of participants with current asthma vs. controls, within each sex.

[†]*P* < 0.05 for comparison of participants with current asthma vs. controls, within each sex.

peripheral blood eosinophils. There were no significant differences in serum level of free testosterone or estradiol between men with and without current asthma.

Figure 2 shows the serum levels of free testosterone and estradiol in study participants (log transformed for ease of exposition) by age, sex, and asthma status. In men, hormonal levels remained relatively stable over time. In women, free testosterone decreased slightly over time, whereas estradiol declined markedly after 50 years of age. Similar patterns were observed in scatter plots (see Figures E1 and E2).

The results of the multivariable analysis of sex hormones and current asthma in female and male participants are shown in Table 2. In a model unadjusted for serum estradiol level (Model 1), women whose serum testosterone levels were in the second quartile (Q2) and fourth quartile (Q4) had 30–45% significantly lower odds of current asthma than those whose serum

testosterone level was in the first (lowest) quartile. In a model unadjusted for serum free testosterone levels (Model 2), women whose serum estradiol levels were in the third quartile had 34% significantly lower odds of current asthma than those whose estradiol levels were in the first quartile. In a multivariable model including serum levels of both estradiol and free testosterone (Model 3), women whose serum testosterone levels were in the second and fourth quartiles had 30% and 44% significantly lower odds of current asthma than those whose serum testosterone levels were in the first quartile. In this model, there was no significant association between serum estradiol and current asthma. Similar results were obtained in multivariable models with additional adjustment for parity (data not shown). Among men (Models 1–3), the magnitude of the estimated effect of serum testosterone serum estradiol on current asthma was similar to that observed in female

participants, but neither serum testosterone nor serum estradiol was significantly associated with current asthma.

To reduce potential misclassification of chronic obstructive pulmonary disease as current asthma, we repeated the multivariable analysis of sex hormones and current asthma after excluding current smokers and former smokers with 10 pack-year from the analysis. This analysis yielded similar results to those with at least 10 pack-years of cigarette smoking from the analysis including all study participants (data not shown). We also repeated the multivariable analysis after excluding participants who reported current use of prescribed long-term systemic (oral, intramuscular, or intravenous) corticosteroids, hormonal contraceptives, HRT, or any other sex hormones. In this sensitivity analysis, the observed association between serum testosterone or serum estradiol and current asthma in women remained statistically significant (Table E2).

Figure 2. Serum free testosterone (top) and estradiol (bottom) levels by sex, current asthma status, and age. Hormonal levels were $\log_{10}$ transformed for ease of exposition.

Next, we tested for an interaction between obesity and sex hormones on current asthma in the multivariable analysis. In this analysis, there were interactions between obesity and serum estradiol on current asthma in women ($P=0.05$), as well as between obesity and serum estradiol on current asthma in men ($P=0.02$). We thus conducted multivariable analyses separately in obese (Table 3) and nonobese (Table 4) subjects for each sex. In multivariable analyses without (Model 1) and with (Model 3) adjustment for serum estradiol, serum free testosterone levels in the highest (fourth) quartile were significantly associated with reduced odds of asthma in obese women (e.g., odds ratio [OR] for Q4 vs. Q1 of testosterone, adjusting for serum estradiol, 0.59; 95% confidence interval [CI], 0.37–0.91; Model 3 in Table 3). In multivariable analyses without (Model 2) and with (Model 3) adjustment for serum testosterone, serum estradiol levels above the first quartile were

significantly associated with reduced odds of current asthma in obese women (e.g., OR for Q4 vs. Q1 of serum estradiol, adjusting for serum testosterone, 0.43; 95% CI, 0.23–0.78; Model 3 in Table 3). In contrast to our results in obese women, neither serum free testosterone nor serum estradiol was significantly associated with current asthma in obese men (Table 3) or nonobese women (Table 4). In multivariable analyses without (Model 2) and with (Model 3) adjustment for serum free testosterone, serum estradiol levels in the highest quartile were significantly associated with reduced odds of current asthma in nonobese men (OR for Q4 vs. Q1 of serum estradiol, adjusting for serum testosterone, 0.44; 95% CI, 0.21–0.90; Model 3 in Table 4). We found no significant indirect effects of obesity in a mediation analysis, suggesting that obesity is not a mediator of any observed association between sex hormone levels and current asthma.

Secondary Analyses

Given our findings in Figure 2, we tested for an interaction between age (due to lack of data on menopausal status in NHANES, we used a cutoff of 51 years, the mean age at menopause in U.S. women [20]) and serum estradiol in women, obtaining nonsignificant findings ($P=0.20$). We further tested for an interaction between sex hormonal levels and peripheral blood eosinophilia (defined as $\geq 4\%$ eosinophils) on current asthma. We found no significant interaction between serum free testosterone or serum estradiol and eosinophilia on asthma in men or women ($P > 0.20$ in all instances).

Discussion

To our knowledge, this is the first population-based study of sex hormonal levels and asthma in adults.

Table 2. Multivariable Analysis of Sex Hormone Levels and Current Asthma by Sex

Serum Sex Hormone Quartile	Model 1 [Odds Ratio (95% Confidence Interval)]	Model 2 [Odds Ratio (95% Confidence Interval)]	Model 3 [Odds Ratio (95% Confidence Interval)]*
Men (<i>n</i> = 3,953)			
Free testosterone, pmol/L			
Q1 (<139.2)	1.0 (reference)	—	1.0 (reference)
Q2 (139.2–196.8)	0.97 (0.62–1.52)	—	1.06 (0.69–1.62)
Q3 (196.8–263.4)	0.65 (0.41–1.05)	—	0.74 (0.48–1.14)
Q4 (≥263.4)	0.98 (0.51–1.88)	—	1.18 (0.67–2.08)
Estradiol, pmol/L			
Q1 (<68.6)	—	1.0 (reference)	1.0 (reference)
Q2 (68.6–87.3)	—	0.66 (0.36–1.18)	0.65 (0.37–1.13)
Q3 (87.3–108.6)	—	0.70 (0.42–1.18)	0.69 (0.45–1.08)
Q4 (≥108.6)	—	0.71 (0.41–1.20)	0.68 (0.43–1.08)
Women (<i>n</i> = 3,662)			
Free testosterone, pmol/L			
Q1 (<3.1)	1.0 (reference)	—	1.0 (reference)
Q2 (3.1–5.5)	0.70 (0.52–0.94) [†]	—	0.70 (0.52–0.96) [†]
Q3 (5.5–9.1)	0.84 (0.62–1.13)	—	0.84 (0.61–1.16)
Q4 (≥9.1)	0.55 (0.39–0.78) ^{‡§}	—	0.56 (0.39–0.80) [‡]
Estradiol, pmol/L			
Q1 (<21.32)	—	1.0 (reference)	1.0 (reference)
Q2 (21.32–73.4)	—	0.85 (0.55–1.33)	0.94 (0.59–1.48)
Q3 (73.4–285.2)	—	0.66 (0.47–0.93) [†]	0.75 (0.54–1.04)
Q4 (≥285.2)	—	0.84 (0.53–1.34)	0.96 (0.59–1.56)

Definition of abbreviation: Q = quartile.

All models adjusted for age, race/ethnicity, annual household income, body mass index, family history of asthma, second-hand smoke, smoking status, pack-years of smoking, and ever use of birth control pills or any sex hormones (in women).

*Model 1 assessed free testosterone without adjusting for estradiol; Model 2 assessed estradiol without adjusting for free testosterone; and Model 3 included both sex hormones.

[†]*P* < 0.05.

[‡]*P* < 0.01.

[§]*P* for trend < 0.05.

Among participants in NHANES, we found that elevated levels of serum testosterone (above the first or lowest quartile) were significantly associated with reduced odds of asthma in women. After stratification by obesity, elevated serum levels of free testosterone (in the fourth or upper quartile) and estradiol (above the first quartile) were significantly associated with reduced odds of current asthma in obese women. Moreover, serum estradiol levels in the fourth or upper quartile were significantly associated with reduced odds of asthma in nonobese men.

We found an inverse association between the second and fourth quartiles of testosterone (but not the third quartile) and current asthma in all women and in obese women, suggesting a nonlinear relation. Testosterone, the predominant androgen in men, may protect against asthma in women through systemic and airway-specific antiinflammatory effects (9). In a study of male children with data on testosterone and SHBG between the ages of

9.9 and 17.8 years (*n* = 412–482), an increased serum level of total or free testosterone was weakly and nonsignificantly associated with lower odds of asthma after age 11.8 years (21). In men, testosterone has been positively associated with lung function (22) and lower levels of CRP (C-reactive protein) (23). In a murine model, testosterone was shown to decrease dust mite-induced eosinophilic and neutrophilic inflammation in the lungs, partially through androgen receptor signaling (24). Consistent with potential beneficial effects of testosterone against asthma, a human phase II clinical trial reported that nebulized DHEA-S, improved asthma control in adults with poorly controlled moderate to severe asthma on inhaled corticosteroids and long-acting β_2 -agonists (25).

The reasons for a potential protective effect of free testosterone against asthma in obese women (but not in nonobese women) are unclear. Adipose tissue contributes to circulating levels of sex steroid hormones (26), and higher testosterone levels have

been reported in pre- and postmenopausal women with increased BMI or visceral fat (27, 28). Moreover, obesity is both a risk factor and a disease modifier in asthma (29). Future studies should further address whether and how testosterone reduces asthma risk in obese women.

Estradiol may have variable effects on immune responses, depending on the concentration, timing, and duration of exposure, as well as other factors, such as obesity and menopausal status. In a study of middle-aged (premenopausal) Australian women, BMI modified the estimated effect of oral contraceptive (OC) use on asthma, as longer duration of OC use was associated with increased risk of asthma in overweight or obese women, but with reduced risk of asthma in women of normal weight (30). Conversely, use of any OC was associated with a 32% reduced risk of physician-diagnosed asthma in a study of 3,257 premenopausal Scottish women. In that study, OC use was associated with decreased asthma risk among overweight or obese women (31). In the

Table 3. Multivariable Analysis of Sex Hormone Levels and Asthma in Obese Subjects by Sex

Serum Sex Hormone Quartile	Model 1 [Odds Ratio (95% Confidence Interval)]	Model 2 [Odds Ratio (95% Confidence Interval)]	Model 3 [Odds Ratio (95% Confidence Interval)]*
Men (n = 1,370)			
Free testosterone, pmol/L			
Q1 (<110.8)	1.0	—	1.0
Q2 (110.8–164.6)	1.16 (0.49–2.75)	—	1.14 (0.46–2.83)
Q3 (164.6–215.7)	0.73 (0.30–1.77)	—	0.73 (0.28–1.87)
Q4 (≥215.7)	1.60 (0.60–4.28)	—	1.58 (0.53–4.73)
Estradiol, pmol/L			
Q1 (<73.8)	—	1.0	1.0
Q2 (73.8–92.1)	—	0.82 (0.37–1.79)	0.85 (0.38–1.88)
Q3 (92.1–114.9)	—	1.15 (0.52–2.54)	1.10 (0.46–2.62)
Q4 (≥114.9)	—	1.03 (0.46–2.28)	0.92 (0.37–2.27)
Women (n = 1,653)			
Free testosterone, pmol/L			
Q1 (<3.0)	1.0	—	1.0
Q2 (3.0–5.5)	0.54 (0.33–0.88) [†]	—	0.58 (0.35–0.95) [†]
Q3 (5.5–9.5)	0.77 (0.43–1.38)	—	0.93 (0.52–1.65)
Q4 (≥9.5)	0.47 (0.31–0.70) ^{‡§}	—	0.59 (0.37–0.91) [†]
Estradiol, pmol/L			
Q1 (<27.8)	—	1.0	1.0
Q2 (27.8–67.5)	—	0.52 (0.33–0.84) [‡]	0.57 (0.36–0.91) [†]
Q3 (67.5–225.7)	—	0.41 (0.23–0.73) [‡]	0.45 (0.25–0.80) [‡]
Q4 (≥225.7)	—	0.41 (0.22–0.74) ^{‡§}	0.43 (0.23–0.78) [‡]

Definition of abbreviation: Q = quartile.

All models adjusted for age, race/ethnicity, annual household income, body mass index, family history of asthma, second-hand smoke, smoking status, pack-years of smoking, and ever use of birth control pills or any sex hormones (in women).

*Model 1 assessed free testosterone without adjusting for estradiol; Model 2 assessed estradiol without adjusting for free testosterone; and Model 3 included both sex hormones.

[†]P < 0.05.

[‡]P < 0.01.

[§]P for trend < 0.05.

current study, serum estradiol was significantly associated with reduced odds of asthma in obese women, but we found no significant interaction between obesity and ever use of OC or other female hormones on current asthma in women. Our finding of an inverse association between the highest quartile of serum estradiol level and asthma in nonobese men must be cautiously interpreted but is intriguing and merits further investigation.

A few studies have examined HRT and asthma in postmenopausal women. Among 2,353 U.S. women 65 years of age and older, use of any HRT was positively associated with lung function measures (FEV₁ and FEV₁/FVC), but not with asthma (32). On the other hand, ever use of HRT was significantly associated with 49% increased risk of adult-onset asthma among naturally menopausal female participants in the Nurses' Health Study (33), and HRT was also associated with slightly increased risks of asthma and asthma symptoms in a study of Danish

women (34). None of the previous studies of OC use or HRT measured serum sex hormone levels.

Mouse models of allergic airway inflammation have been used to study the role of female sex hormones in asthma pathogenesis. Compared with male mice, female mice have higher ovalbumin (OVA)-induced circulating levels of IgE and eosinophils, greater airway responsiveness, and higher expression of *IL-13* and *IL-17A* in the lung (12). In female mice that underwent ovariectomy 7 days before initial OVA sensitization, depleted estradiol levels downregulated eosinophilia and IL-5 in the lungs, and reduced airway reactivity in response to allergens. In those mice, treatment with estradiol benzoate restored eosinophil and IL-5 levels, indicating a proinflammatory effect of estradiol. However, in female mice that underwent ovariectomy 24 hours after the second OVA sensitization (Day 8), removal of the ovaries upregulated IL-5, and treatment with

estradiol benzoate suppressed the traffic of eosinophils to the lung, indicating antiinflammatory effects of estradiol through nonallergic pathways (35). In another mouse model, estradiol, but not progesterone, had antiinflammatory effects in mice that underwent ovariectomy, further suggesting a protective role of estradiol in allergic asthma, depending on the timing, type, and level of circulating female hormones (36). We did not find a significant interaction between serum estradiol and eosinophilia on current asthma, suggesting that estradiol may affect asthma through non-eosinophilic-mediated mechanisms.

We acknowledge several study limitations. First, we cannot examine temporal relationships in this cross-sectional analysis. Second, due to the small number of participants who reported current use of prescribed systemic steroids or any sex hormones (n = 173), we cannot estimate the relative contributions of

Table 4. Multivariable Analysis of Sex Hormone Levels and Asthma in Nonobese Subjects by Sex

Serum Sex Hormone Quartile	Model 1 [Odds Ratio (95% Confidence Interval)]	Model 2 [Odds Ratio (95% Confidence Interval)]	Model 3 [Odds Ratio (95% Confidence Interval)]*
Men (n = 2,583)			
Free testosterone, pmol/L			
Q1 (<156.8)	1.0	—	1.0
Q2 (156.8–219.9)	0.54 (0.26–1.13)	—	0.63 (0.30–1.32)
Q3 (219.9–286.2)	0.59 (0.28–1.26)	—	0.78 (0.39–1.55)
Q4 (≥286.2)	0.54 (0.20–1.45)	—	0.82 (0.32–2.07)
Estradiol, pmol/L			
Q1 (<65.7)	—	1.0	1.0
Q2 (65.7–84.4)	—	0.52 (0.25–1.05)	0.54 (0.29–1.02)
Q3 (84.4–105.3)	—	0.58 (0.31–1.09)	0.61 (0.36–1.03)
Q4 (≥105.3)	—	0.41 (0.18–0.94) [†]	0.44 (0.21–0.90) [†]
Women (n = 2,009)			
Free testosterone, pmol/L			
Q1 (<3.2)	1.0	—	1.0
Q2 (3.2–5.4)	0.84 (0.47–1.51)	—	0.81 (0.44–1.48)
Q3 (5.4–8.8)	0.91 (0.60–1.38)	—	0.82 (0.50–1.33)
Q4 (≥8.8)	0.64 (0.33–1.25)	—	0.55 (0.27–1.11)
Estradiol, pmol/L			
Q1 (<16.2)	—	1.0	1.0
Q2 (16.2 < 81.1)	—	0.94 (0.45–1.96)	1.04 (0.49–2.21)
Q3 (81.1–338.7)	—	1.05 (0.59–1.88)	1.21 (0.68–2.14)
Q4 (≥338.7)	—	1.46 (0.67–3.18)	1.77 (0.78–4.02)

Definition of abbreviation: Q = quartile.

All models adjusted for age, race/ethnicity, annual household income, body mass index, family history of asthma, second-hand smoke, smoking status, pack-years of smoking, and ever use of birth control pills or any sex hormones (in females).

*Model 1 assessed free testosterone without adjusting for estradiol; Model 2 assessed estradiol without adjusting for free testosterone; and Model 3 included both sex hormones.

[†]P < 0.05.

endogenous and exogenous hormones to our findings. However, our results were unchanged after excluding subjects reporting current use of systemic steroids or sex hormones from the analysis. Third, we lack data on menopausal status. However, we found no significant interaction between age and sex hormones on asthma in women. Fourth, we lack data on several potential confounders, including insulin resistance, follicular phase of the menstrual cycle, and environmental exposure to endocrine disruptors. Fifth, we had lower statistical power to detect an association between serum free testosterone or serum estradiol and current asthma in men than in women, given lower asthma prevalence in men. Sixth, although self-reported, current, physician-diagnosed

asthma is commonly used to assess asthma prevalence in national health surveys or epidemiological studies, it is subject to recall or misclassification bias. However, misclassification of smoking-related diseases as asthma is an unlikely explanation for our results, which were essentially unchanged in an analysis restricted to never smokers and former smokers with fewer than 10 pack-years of smoking. Lastly, selection bias is possible in any observational study, and serum estradiol was significantly higher in women who were excluded from this analysis than in those included. However, serum free testosterone levels were similar in women who were and were not included in this analysis, and serum

levels of free testosterone and estradiol were similar in men who were and were not included in this analysis.

In summary, we found that elevated serum levels of both free testosterone and estradiol were significantly associated with reduced odds of asthma in obese women, and that elevated levels of serum estradiol were significantly associated with reduced odds of asthma in nonobese men. Our findings further suggest that sex steroid hormones play a role in known sex differences in asthma among adults. ■

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