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ORIGINAL RESEARCH

# Sex Hormones and Chronic Obstructive Pulmonary Disease: A Cross-Sectional Study and Mendelian Randomization Analysis

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**Background:** Sex steroid hormones, including testosterone and estradiol, play significant roles in various aspects of pulmonary health and diseases. However, although there were a few studies trying to link sex hormones with COPD, their effect remained limited due to small sample size and insufficient causal results. This study aims to investigate the association between sex hormones and chronic obstructive pulmonary disease (COPD) based on the National Health and Nutrition Examination Survey (NHANES) database and evaluate causality via a two-sample Mendelian randomization (MR).

**Methods:** Data from NHANES 2013–2016 were enrolled for the cross-sectional study. The association between sex hormones and COPD was evaluated via multivariable logistic regression. Sex-stratified analysis, subgroup analyses and interaction tests were performed to further evaluate the correlation. For MR analysis, data were collected from genome-wide association studies and FinnGen datasets. The inverse-variance-weighted (IVW) approach, along with four other approaches, was applied in the analysis. Further sensitivity analysis was conducted to assess the existence of pleiotropy and heterogeneity.

**Results:** 7,617 eligible participants were enrolled in the cross-sectional analysis. Negative associations were observed in both testosterone-COPD (OR 0.770, 95% CI 0.626, 0.948, p = 0.018) and estradiol-COPD (OR 0.794, 95% CI 0.688, 0.915, p = 0.005) relationships after covariate adjustments. However, the results from IVW-MR analysis showed that no causal relationship was observed in either the testosterone-COPD (OR 0.83, 95% CI 0.53, 1.29, p = 0.407) or estradiol-COPD (OR 0.74, 95% CI 0.23, 2.38, p = 0.616) relationship, which was also supported by the other four approaches (all p values > 0.05).

**Conclusion:** Although a significant negative association was observed between sex hormones and COPD, the results of MR analysis did not support the causality of this relationship. Our study suggested that sex hormones may indirectly rather than directly affect the development of COPD via potential covariates, which warranted further investigations.

**Keywords:** sex steroid hormones, testosterone, estradiol, chronic obstructive pulmonary disease, NHANES, Mendelian randomization

## Introduction

Regarded as the third most prominent cause of global chronic morbidity and mortality, chronic obstructive pulmonary disease (COPD) is defined as a heterogeneous lung condition characterized by persistent airflow obstructions due to airway and alveolar abnormalities.[1–3] It has been reported that the prevalence of COPD has reached 10.3% globally, 11.1% in South Asia and 8.6% in China.[4–6] The global economic burden of COPD from 2020 to 2050 is estimated to reach INT$4326 trillion, which is equal to 0.111% of the global gross domestic product.[7] Moreover, an unmet need for

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diagnosis and therapy of COPD has also been reported, especially in low- and middle-income countries.[8] Therefore, exploring significant biomarkers to improve the prevention and treatment of COPD is of vital importance.

Sex hormones, including testosterone and estradiol, may play a regulating role in lung physiology and pathology.[9] It has been suggested that estrogen plays a promoting role in surfactant formation, while androgen suppresses surfactant formation in neonates.[10] Moreover, testosterone preferentially suppresses the pulmonary inflammatory responses, while estradiol seems to promote lung inflammation.[11] Currently, there is evidence indicating that sex hormones may correlate with the development of certain respiratory diseases. A cross-sectional study reported that the fourth quartile of free testosterone levels correlated with a lower risk of asthma in American women.[12] Consistently, a population-based study of over 256,419 British adults indicated that higher testosterone levels may reduce the risk of asthma and wheezing in both men and women.[13] Another study suggested that estrogen may promote lung cancer development by inducing the propagation of tumor cells and angiogenesis.[14]

Furthermore, recent preclinical and clinical evidence suggested a potential link between sex hormones and COPD. Aono et al reported that testosterone deficiency promoted emphysematous alternation in orchiectomized mice.[15] Pavey et al observed that testosterone levels were associated with all-cause mortality in male GOLD-2 COPD patients in two separated COPD cohorts.[16] Rubinsztajn et al observed that COPD patients with decreased serum testosterone were characterized by lower forced expiratory volume in one second and longer smoking history.[17] However, the sample size of existing studies was relatively small, and whether there was association between sex hormones and COPD warrant further investigations.

Therefore, in this study, we first conducted an observational, cross-sectional study to reveal the correlation between sex hormones and COPD based on the National Health and Nutrition Examination Survey (NHANES) database. However, observational studies are always subject to insufficient covariate adjustments, hardly reaching any causal associations. To address this problem, we conducted a Mendelian randomization (MR) analysis to further reveal whether a causal relationship exists between sex hormones and COPD. Regarded as natural randomized, double-blind trials, MR is an emerging research method that applies single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to explore the possible causal relationship between exposure and outcome.[18,19] MR analysis may provide more reliable causal results by reducing the possible influence caused by potential confounders.[18,19] By adding MR in this research, we may reach an in-depth understanding of the relationship between sex hormones and COPD.

# Methods

## Cross-Sectional Study Based on the NHANES Database

### Study Population and Data Source

NHANES is a cross-sectional survey exploring the nutritional and health status of the American population via interviews, physical examinations, and laboratory tests, which was approved by the Ethics Review Board of the National Center for Health Statistics (NHANES - NCHS Research Ethics Review Board Approval (cdc.gov)).[20,21] Data from NHANES 2013–2016 were enrolled in this study to provide available data on both sex hormone levels and COPD diagnosis. Exclusion criteria applied in this study can be listed as follows: (1) Incomplete data of sex hormone levels or COPD diagnosis. (2) Pregnant participants and (3) Participants less than 20 or with incomplete data of other covariates.

### Measurements of Sex Hormones and COPD

Testosterone and estradiol levels were measured by isotope dilution liquid chromatography tandem mass spectrometry, and the lowest detection limits were 0.75 ng/mL and 2.994 pg/mL, respectively.[12] For those below the lowest limit of detection, the level was recorded as the lower limit of detection divided by the square root of 2. The COPD diagnosis was obtained from the questionnaire “Ever told you had COPD?” (Yes).

### Covariate Assessments

Covariates include demographic characteristics (gender, age, ethnicity, education status, marital status, poverty-to-income ratio (PIR) and body mass index (BMI)), lifestyle factors (smoking and alcohol consumption), and comorbidities (hypertension, diabetes, cancers, stroke, coronary heart disease, thyroid diseases, and liver diseases). History of

comorbidities was obtained from questionnaire data in NHANES 2013–2016. Never smokers were defined as smoking fewer than 100 cigarettes in their entire life, and alcohol consumption was recognized as consuming at least 12 drinks of any type of alcoholic beverage in a year.

### Statistical Analysis

Considering the complex, multistage, and probability sampling design of the NHANES database, proper sample units, strata, and sample weights were applied in this study. Chi-square tests or t tests were performed for categorial variables (percentage with SE) or continuous variables (mean with standard error (SE)), respectively. Levels of testosterone and estradiol were log2-transformed to reach a normal distribution. A multivariate logistic regression analysis was performed to evaluate the association between sex hormones and COPD, the results of which are presented in three models. In model 1, no covariate was adjusted. In model 2, gender, age, and race were adjusted. In model 3, all covariates mentioned in Covariate Assessments were adjusted. Levels of log2-transformed testosterone and estradiol were divided into quartiles to evaluate the stability of this association. Subgroup analyses and interaction tests were also conducted to further explore this association, especially in those with different sex or age. Subgroup analyses were conducted through stratified multivariate logistic regression, and interaction tests were performed via global Chi-square test for interaction terms, also taking sample units and weights into consideration. Data analysis in this cross-sectional study was performed via R and Empowerstats software (EmpowerStats | Data Analysis for Biostatistics & Epidemiology), and a p value <0.05 was considered to indicate statistical significance.

## Mendelian Randomization (MR)

### Basic Principles of MR

In this study, a two-sample MR was conducted to reveal whether there are causal relationships between sex hormones and COPD. To reach valid causal results, three assumptions should be adhered to for instrument variables in MR analysis: (1) The selected IVs need to be correlated to sex hormones. (2) No potential cofounders may affect the exposure or outcome. (3) IVs influenced COPD only through sex hormones (Figure 1).

### Data Source and IV Selection

Data on sex hormones were derived from genome-wide association studies (GWAS). The dataset of testosterone contained a population of 382,988, while the dataset of estradiol contained a population of 206,927.[22] The COPD cohort was retrieved from the FinnGen consortium, containing 18,266 COPD cases and 311,286 controls. SNPs with a p value < 5e-08 were selected as significant variants and entered further investigation. Linkage disequilibrium (LD) analysis was conducted according to the criteria of $r^2$=0.001 and kb=10,000 kb to ensure the independence of variants. Then, as weak instruments may lead to inadequate

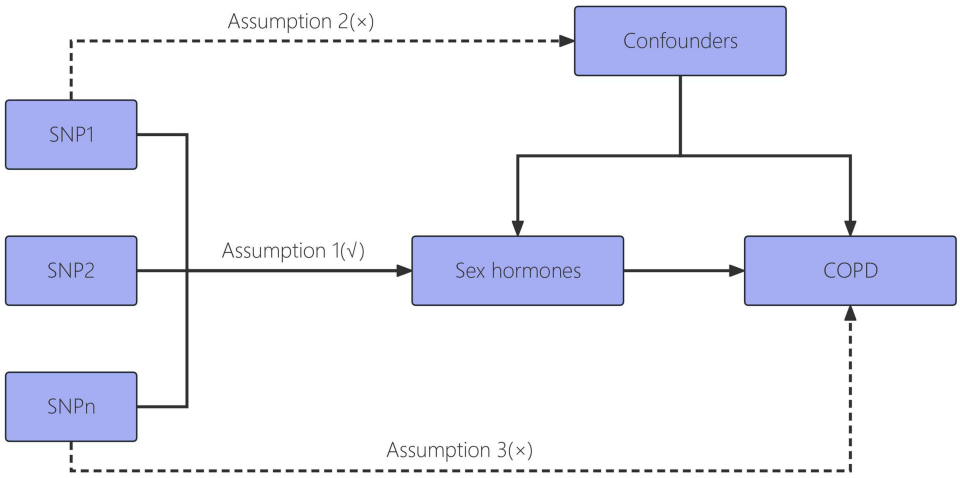


**Figure 1** Overall MR design based on three assumptions.
**Abbreviation**: MR, Mendelian Randomization.

ability to predict causal relationship, F-statistics were calculated to investigate the strength of SNPs, and those with F < 10 were excluded from further analysis.[23] Finally, we harmonized the SNPs by removing those with inconsistent alleles.

### Statistical Analyses and Sensitivity Analysis

Principle analysis was performed with the inverse variance weighting (IVW) method, which is characterized as not taking the intercept into account. To ensure the robustness of the results, MR Egger, weighted median, simple mode, and weighted mode analyses were also performed. In sensitivity analyses, MR Egger regression was performed to detect potential horizontal pleiotropy. The Cochrane Q statistic was applied to test the heterogeneity among SNPs, with a p value < 0.05 indicating heterogeneous results. Leave-one-out analysis was performed to test the stability of the results and detect potential outliers. Moreover, scatter plots and forest plots were also applied to visualize the results of MR analysis. MR analyses were performed via R packages TwoSampleMR and MR-PRESSO.

# Results

## Results of the Cross-Sectional Study

### Baseline Characteristics of Eligible Participants

A total of 7,617 individuals from NHANES 2013–2016 were enrolled in this analysis (Figure 2). The weighted baseline characteristics of the enrolled participants are illustrated in Table 1. Overall, the baseline characteristics of participants differed significantly between non-COPD individuals and COPD patients, except for testosterone levels (p = 0.514), sex (p = 0.832), BMI (p=0.148), and alcohol consumption (p = 0.273).

### Association Between Sex Hormones and COPD

Previous evidence suggested that testosterone may associate with pulmonary function.[24] Therefore, although similar testosterone levels were observed between healthy individuals and patients with COPD (p = 0.514), testosterone was also analyzed in our logistic regression analysis along with estradiol and sex hormone-binding globulin (SHBG). The results are presented in Table 2. Although no significant association was observed between testosterone and COPD in the crude model (OR = 0.947, 95% CI 0.885, 1.013, p = 0.123), the negative association was significant in the minimally adjusted (OR 0.740, 95% CI 0.621, 0.881, p = 0.002) and fully adjusted (OR 0.770, 95% CI 0.626, 0.948, p = 0.018) models. When the log2-transformed testosterone level was divided into quartiles, significant correlations were still observed in the third quartiles of the two adjusted models compared with the first quartile (Table 2). For estradiol, negative correlations between estradiol and COPD were observed in three models. When the log2-transformed estradiol level was divided into quartiles, estradiol was still negatively correlated with COPD in quartile 4 compared with the lowest quartile in the three models. Moreover, although a positive correlation between SHBG and COPD was observed in the crude model (OR 1.414, 95% CI 1.192, 1.679), the correlation vanished in the minimally adjusted (OR 1.264, 95% CI 0.988, 1.616) and fully adjusted (OR 1.216, 95% CI 0.868, 1.705) models.

### Subgroup Analyses and Interaction Tests

Results of subgroup analyses and interaction tests were presented in Figure 3 and Table S1–2. Sex-stratified analysis showed that both sex hormones significantly associated with COPD in female sex, while no significant association was observed in male sex (p = 0.726) after covariate adjustments (Figure 3). However, results of interaction test showed that sex did not play a role in both testosterone-COPD (p = 0.347) and estradiol-COPD (p = 0.919) relationship (Figure 3). For other subgroup analyses, although the significant association vanished in certain subgroups, the interaction tests suggested that age (p = 0.025) and race (p = 0.043) interacted with the testosterone–COPD relationship, while age (p = 0.037), hypertension (p=0.034), and liver diseases (p = 0.005) interacted with the estradiol–COPD relationship. Other results of subgroup analyses were presented in Table S1–2.

## Results of MR Analysis

Overall, 15 eligible SNPs were identified for testosterone, and 8 eligible SNPs were identified for estradiol (Tables S3-4). After harmonization of SNPs, 10 SNPs for testosterone and 8 SNPs for estradiol were finally enrolled for analysis. However, no causal relationship was observed between sex hormones and COPD in the IVW model (p = 0.407 for testosterone, p = 0.616 for

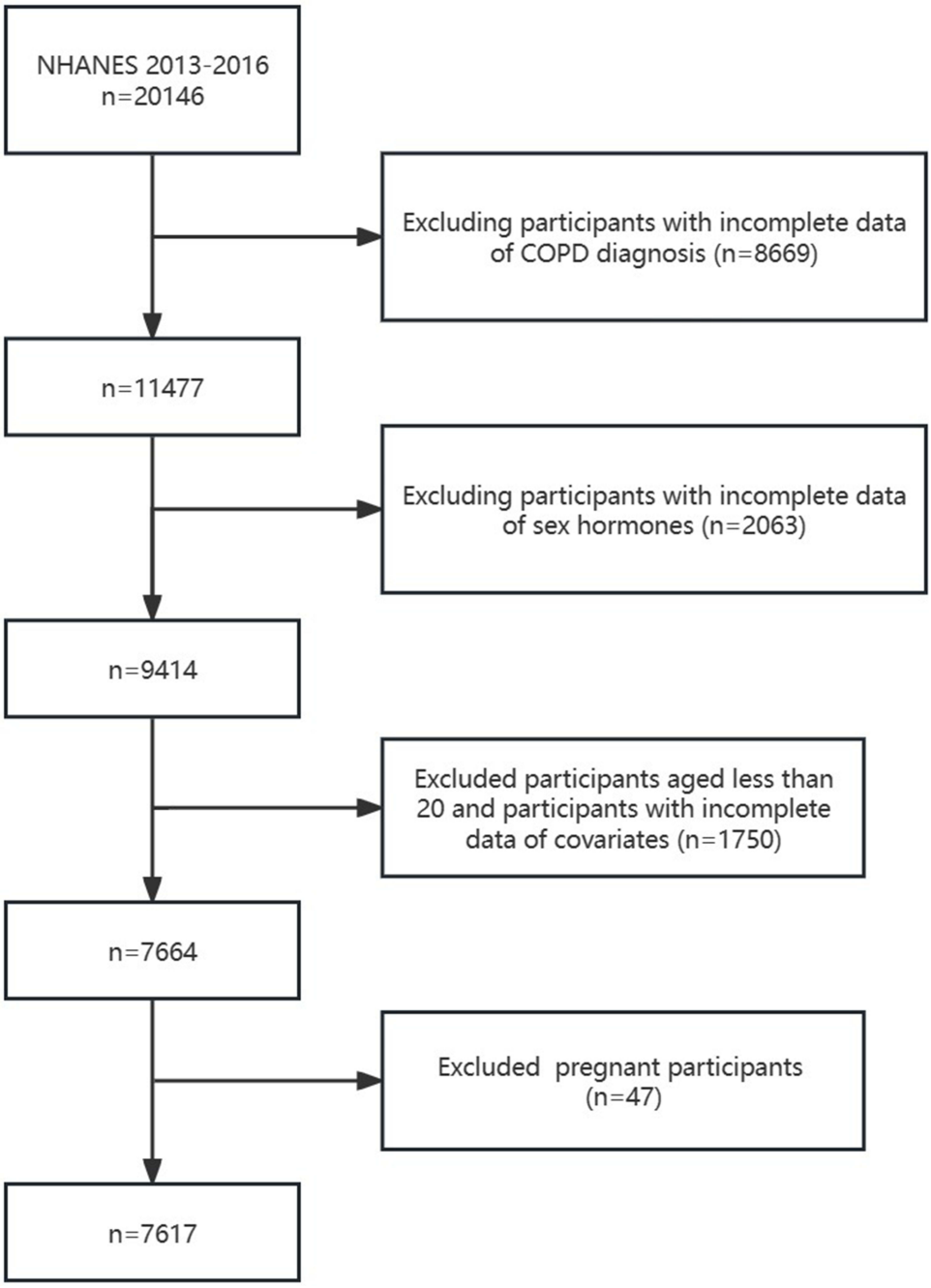


**Figure 2** Selection of eligible individuals from NHANES 2013–2016.

estradiol), which was supported by the other four models (Figure 4). In the sensitivity analysis, no evidence of pleiotropy was detected in MR Egger regression for either testosterone (p = 0.810) or estradiol (p = 0.189) (Table S5). The results of the Q test also indicated that no significant heterogeneity was detected in testosterone and estradiol, either in the IVW or MR Egger model (Table S3). Moreover, the results of leave-one-out analysis indicated that no single SNP significantly affected the causal estimates in both testosterone-COPD and estradiol-COPD correlations (Figures S1 and 2). To visualize the results, scatter plots of both correlations were also generated (Figures S3 and 4).

## Discussion

In this study, we revealed a significant negative association between sex hormones (both testosterone and estradiol) and COPD in a population-based survey. Conversely, the results of MR analysis did not indicate a causal relationship

**Table 1** Weighted Baseline Characteristics of Eligible Individuals Enrolled in the Cross-Sectional Studies

| | Non-COPD | COPD | P-value |
|---|---|---|---|
| Testosterone (ng/dL) | 219.87±2.75 | 208.33±16.60 | 0.514 |
| Estradiol (pg/mL) | 41.10±0.97 | 19.97±1.27 | <0.001 |
| SHBG (nmol/L) | 59.66±1.05 | 70.61±4.02 | 0.015 |
| Gender (%) | | | 0.832 |
| Male | 49.44 (0.50) | 50.26 (3.77) | |
| Female | 50.56 (0.50) | 49.74 (3.77) | |
| Age (%) | | | <0.001 |
| <60 | 73.32 (1.05) | 38.75 (4.56) | |
| ≥60 | 26.68 (1.05) | 61.25 (4.56) | |
| Race (%) | | | <0.001 |
| Hispanic | 14.62 (1.86) | 3.65 (0.84) | |
| Non-Hispanic White | 66.92 (2.64) | 83.59 (2.39) | |
| Other race | 18.46 (1.62) | 12.76 (2.14) | |
| Education (%) | | | <0.001 |
| Under high school | 13.22 (1.17) | 25.49 (3.89) | |
| High school or equivalent | 21.22 (0.93) | 26.05 (2.71) | |
| Above high school | 65.57 (1.64) | 48.46 (4.68) | |
| Marital status (%) | | | <0.001 |
| With partner | 64.22 (1.18) | 54.75 (3.67) | |
| Widowed, divorced or separated | 17.48 (0.80) | 39.87 (3.33) | |
| Never married | 18.30 (0.92) | 5.39 (1.23) | |
| PIR (%) | | | <0.001 |
| <1.3 | 21.32 (1.34) | 40.71 (6.73) | |
| 1.3≤PIR<3.5 | 35.86 (1.01) | 37.60 (4.62) | |
| ≥3.5 | 42.82 (1.94) | 21.69 (5.21) | |
| BMI (%) | | | 0.148 |
| <25 | 28.32 (1.00) | 26.44 (2.90) | |
| 25≤BMI<30 | 32.55 (0.68) | 27.86 (2.68) | |
| ≥30 | 39.13 (1.02) | 45.70 (3.81) | |
| Hypertension (%) | | | <0.001 |
| Yes | 32.45 (0.95) | 66.21 (2.59) | |
| No | 67.55 (0.95) | 33.79 (2.59) | |
| Diabetes (%) | | | <0.001 |
| Yes | 12.81 (0.63) | 31.24 (3.21) | |
| No | 87.19 (0.63) | 68.76 (3.21) | |
| Smoking (%) | | | <0.001 |
| Never smoker | 57.84 (0.98) | 13.47 (2.52) | |
| Former smoker | 24.55 (0.90) | 44.67 (3.93) | |
| Current smoker | 17.60 (0.71) | 41.87 (3.33) | |
| Stroke (%) | | | <0.001 |
| Yes | 2.36 (0.21) | 15.69 (3.25) | |
| No | 97.64 (0.21) | 84.31 (3.25) | |
| Cancer (%) | | | <0.001 |
| Yes | 10.95 (0.33) | 28.50 (3.19) | |
| No | 89.05 (0.33) | 71.50 (3.19) | |
| Alcohol consumption (%) | | | 0.273 |
| Yes | 76.94 (1.43) | 81.62 (3.62) | |
| No | 23.06 (1.43) | 18.38 (3.62) | |

(*Continued*)

**Table 1** (Continued).

| | Non-COPD | COPD | P-value |
|---|---|---|---|
| Coronary artery disease (%) | | | <0.001 |
| Yes | 2.73 (0.23) | 23.07 (2.84) | |
| No | 97.27 (0.23) | 76.93 (2.84) | |
| Thyroid diseases (%) | | | 0.001 |
| Yes | 9.04 (0.59) | 19.94 (3.99) | |
| No | 90.96 (0.59) | 80.06 (3.99) | |
| Liver diseases (%) | | | <0.001 |
| Yes | 1.84 (0.19) | 8.47 (2.03) | |
| No | 98.16 (0.19) | 91.53 (2.03) | |

**Notes**: Number of non-COPD group: 7,371 before weight, 161,365,556 after weighted. Number of COPD group: 246 before weighted, 5,072,346 after weighted. Continuous variables were presented as mean±standard error (SE), and categorial variables were presented as proportion (SE).

**Abbreviations**: PIR, poverty-to-income ratio; BMI, body mass index; SHBG, sex hormone-binding globulin.

**Table 2** Association Between Sex Hormones and COPD According to Multivariable Regression Analysis

| Variables | Crude model OR (95% CI) p | Minimally adjusted OR (95% CI) p | Fully adjusted OR (95% CI) p |
|---|---|---|---|
| **Testosterone** | | | |
| Continuous | 0.947 (0.885, 1.013) | 0.740 (0.621, 0.881) | 0.770 (0.626, 0.948) |
| | 0.123 | 0.002 | 0.018 |
| Q1 | Ref | Ref | Ref |
| Q2 | 0.570 (0.367, 0.886) | 0.712 (0.437, 1.158) | 0.779 (0.391, 1.554) |
| | 0.019 | 0.184 | 0.362 |
| Q3 | 0.842 (0.573, 1.237) | 0.272 (0.118, 0.631) | 0.307 (0.102, 0.924) |
| | 0.388 | 0.006 | 0.031 |
| Q4 | 0.678 (0.423,1.088) | 0.217 (0.072, 0.660) | 0.275 (0.064, 1.184) |
| | 0.119 | 0.013 | 0.058 |
| p for trend | 0.242 | 0.140 | 0.315 |
| **Estradiol** | | | |
| Continuous | 0.713 (0.669, 0.761) | 0.765 (0.683, 0.858) | 0.794 (0.688, 0.915) |
| | <0.001 | <0.001 | 0.005 |
| Q1 | Ref | Ref | Ref |
| Q2 | 0.630 (0.420, 0.946) | 0.667 (0.329, 1.353) | 0.819 (0.352, 1.909) |
| | 0.034 | 0.274 | 0.542 |
| Q3 | 0.416 (0.276, 0.628) | 0.429 (0.201, 0.916) | 0.673 (0.273, 1.655) |
| | <0.001 | 0.039 | 0.276 |
| Q4 | 0.211 (0.141, 0.315) | 0.364 (0.218, 0.610) | 0.431 (0.221, 0.840) |
| | <0.001 | <0.001 | 0.017 |
| p for trend | <0.001 | <0.001 | 0.007 |
| **SHBG** | | | |
| Continuous | 1.414 (1.192, 1.679) | 1.264 (0.988, 1.616) | 1.216 (0.868, 1.705) |
| | <0.001 | 0.074 | 0.199 |
| Q1 | Ref | Ref | Ref |
| Q2 | 1.226 (0.717, 2.095) | 0.956 (0.570, 1.602) | 0.959 (0.443, 2.075) |
| | 0.463 | 0.865 | 0.885 |

(*Continued*)

**Table 2** (Continued).

| Variables | Crude model OR (95% CI) p | Minimally adjusted OR (95% CI) p | Fully adjusted OR (95% CI) p |
|---|---|---|---|
| Q3 | 2.268 (1.419, 3.624) 0.002 | 1.483 (0.942, 2.336) 0.102 | 1.501 (0.667, 3.375) 0.223 |
| Q4 | 2.190 (1.510, 3.177) <0.001 | 1.420 (0.898, 2.245) 0.148 | 1.322 (0.638, 2.741) 0.336 |
| p for trend | 0.001 | 0.134 | 0.226 |

**Notes**: Minimally adjusted model: adjusted for age, gender, and ethnicity. Fully adjusted model: adjusted for gender, age, ethnicity, education status, marital status, poverty-to-income ratio (PIR), body mass index (BMI), smoking, alcohol consumption and history of hypertension, diabetes, and cancers.
**Abbreviation**: SHBG, sex hormone-binding globulin.

between sex hormones and COPD, which was supported by further sensitivity analysis. Therefore, our results suggested that sex hormones may indirectly rather than directly affect the development of COPD via potential covariates, which warrants further investigations.

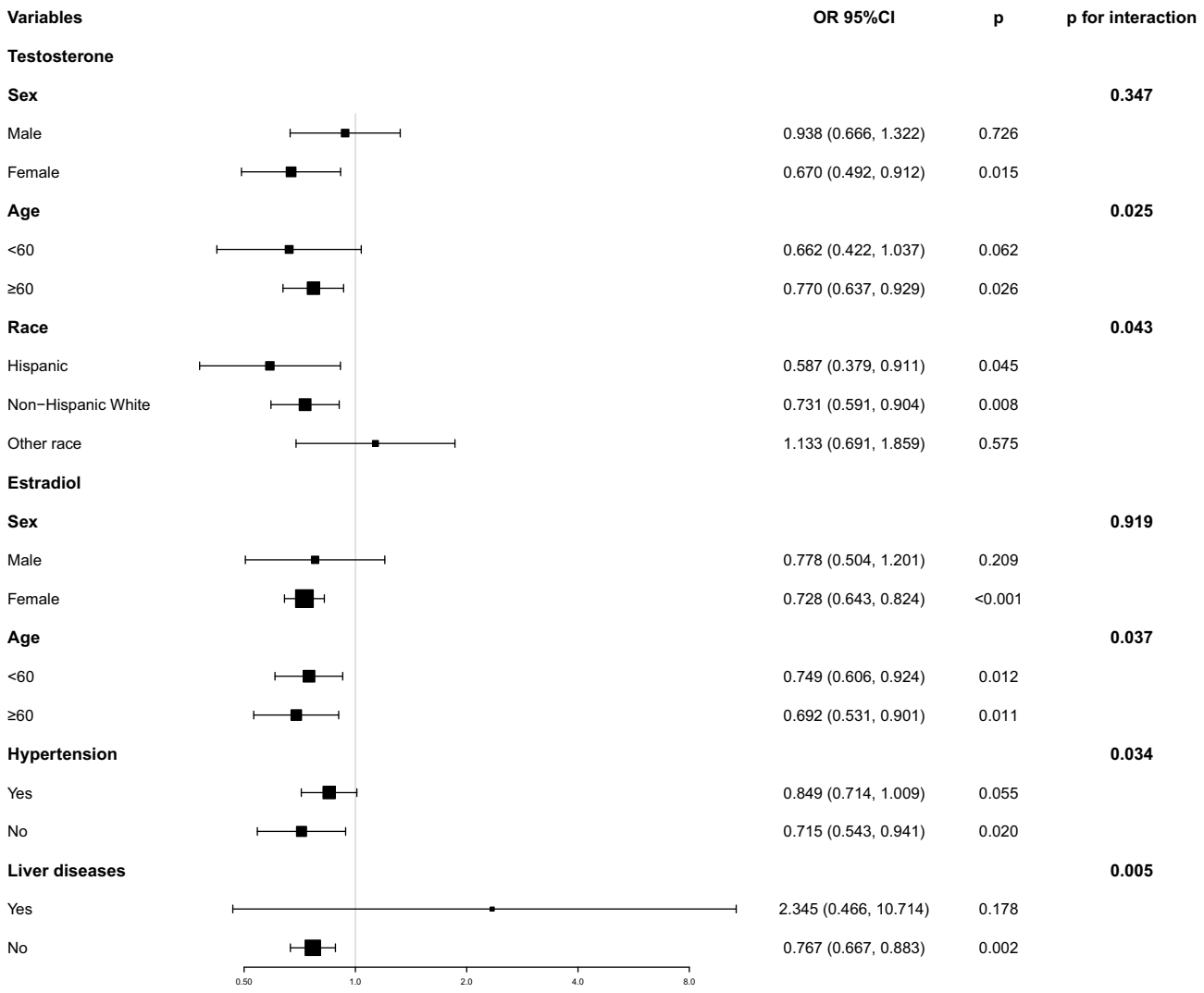


**Figure 3** Subgroup analysis and interaction tests of testosterone-COPD and estradiol–COPD relationships.

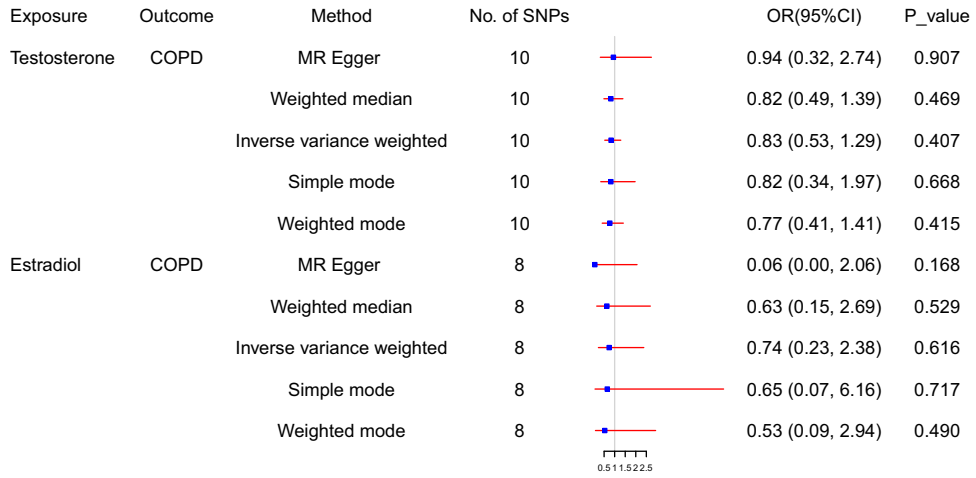


**Figure 4** MR analysis of testosterone and estradiol on COPD.

Testosterone has been viewed as the mythical Fountain of Youth since 1935, which may exert an effect on multiple aspects of human health, such as muscle quality, sexual function, and cognitive condition.[25,26] Testosterone has also been shown to increase lung cancer risk and reduce asthma risk in human population.[9] Previous evidence suggested that a potential link may exist between testosterone and COPD. A cross-sectional study of 95 male COPD patients reported a positive association between testosterone and the FEV1/FVC ratio.[24] Another study suggested that testosterone replacement therapy may inhibit disease progression in both middle-aged (aged 40–63) or older (aged over 66) male participants.[27] The potential effect of testosterone on COPD development has also been reported in some studies. Wang et al reported that testosterone may alleviate pulmonary epithelial inflammation via inhibition of both NF-κB signaling derived by nuclear respiratory factor 1 and p65 phosphorylation in a mouse model of COPD.[28] Kentaro et al suggested that testosterone supplementation inhibited T-cell infiltration in bronchoalveolar lavage fluid, thereby alleviating emphysematous changes in orchiectomized mice.[15] Moreover, there is also evidence reporting the effect of COPD over testosterone deficiency. Wang et al reported that nuclear respiratory factor 1 regulated the expression of steroidogenic acute regulatory protein, thereby inhibiting testosterone synthesis in hypoxemic mice, while hypoxia is a common pathological process in patients with COPD.[29] In this study, a negative correlation was observed between testosterone and COPD in the American population, which is consistent with previous studies.

Additionally, we also observed a negative association between estradiol and COPD in our cross-sectional study. Estradiol is another essential sex hormone that plays a role in energy homeostasis, metabolic health, and memory,[30,31] which has also been reported to reduce allergic airway inflammation via NLRP3 activation.[32] Konings et al reported that the enzymes 17β-HSD type 1 and aromatase, which are related to estradiol synthesis, were increased in COPD patients.[33] Another study revealed that no association was observed between estradiol and pulmonary function among 1768 community-dwelling men.[34] Regarding potential mechanisms, Marilyn et al suggested that estradiol deficiency may increase the susceptibility of smoking-induced lung diseases via a decrease in hydroxyproline content, respiratory chain complex-1 protein, and number of macrophages,[35] while Nathalie et al reported that 17β-estradiol promoted inflammation and airway hyperresponsiveness induced by ozone in female mice.[36] In summary, current evidence reporting a correlation between estradiol and COPD is still limited and warrants further investigation.

However, further MR analysis did not support the causality of either testosterone-COPD or estradiol-COPD correlation (Figure 4, all $p > 0.05$). Compared with observational studies, MR analysis was regarded as evidence of higher levels due to the avoidance of covariates.[20] Therefore, although the application of testosterone and estradiol may be of some clinical significance in COPD management, no causal relationship can be reached in this study.

Moreover, different levels of testosterone and estradiol between males and females may become a major cause of sexual dimorphism, which may play a role in various respiratory diseases.[9] However, although relationship between sex hormones and COPD is different to some extent between male and female, the interaction tests showed that gender failed

to interact with both testosterone-COPD (p = 0.347) and estradiol-COPD (p = 0.919) relationships, which suggested that sex hormones may play a similar role in COPD development in both males and females, regardless of gender differences. Additionally, results that age significantly interacted with both testosterone-COPD and estradiol–COPD relationship, which may be explained by an age-dependent manner of both sex hormone level and prevalence of COPD.[5,37] Furthermore, hypertension and liver diseases may also play an interacting role between estradiol and COPD, suggesting that certain comorbidities may affect estradiol level and COPD development.

Some major strengths of this study are listed as follows: First, further MR analysis was applied to evaluate the causality of the relationship between sex hormones and COPD, which provided evidence of higher levels and supported a different conclusion. Second, previous evidence regarding whether estradiol plays a role in COPD remains limited. Although the results of MR analysis did not support a causal estradiol–COPD relationship, estradiol may still be of clinical significance in COPD management, which was supported by the negative correlation obtained in the cross-sectional study. Further studies are needed to further explore the potential mechanisms by which estradiol may affect COPD development. There were also some limitations that are worth discussing. First, the individuals enrolled in the cross-sectional study were Americans, while those enrolled in MR analysis were Europeans, which may increase the risk of potential bias due to ethnic differences. Second, COPD was diagnosed with a self-report questionnaire rather than spirometry examination in this study, which was also a potential source of bias. Third, no specific data about the severity of COPD were available from either the NHANES database or the FinnGen biobank, making it unavailable to perform further analysis. Fourth, although results of interaction tests indicated that gender failed to interact with both testosterone-COPD and estradiol–COPD relationship, it was hard for us to conduct a sex-stratified MR analysis to verify this result, which warrant further investigations.

## Conclusion

Our MR analysis did not support the causality of either the testosterone-COPD or estradiol-COPD relationship, even though a negative correlation of both was observed in the cross-sectional NHANES study. These results suggested that sex hormones may indirectly rather than directly affect the development of COPD via potential covariates. Future studies with large sample sizes are still needed to verify these results and provide more understanding into the management and prevention of COPD.

## Data Sharing Statement

All data were available from NHANES database, GWAS dataset, and FinnGen consortium.

## Ethnic Approval

The IRB of West China hospital of Sichuan University waived the ethical approval and written informed consent due to approval of Ethics Review Board of National Center for Health Statistics (Protocol #2011-17) and well-obtained written informed consent of all participants from NHANES.

## Consent for Publication

All authors have reviewed the manuscript and agreed for publication.

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## Disclosure

The authors report no conflicts of interest in this work.

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