Higher Estradiol and Lower Dehydroepiandrosterone-Sulfate Levels Are Associated with Pulmonary Arterial Hypertension in Men

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Abstract

Rationale: Recent studies have focused on the role of female sex and estradiol (E2) in pulmonary arterial hypertension (PAH), but it is not known whether sex hormones are risk factors for PAH in men.

Objectives: We performed a case-control study to determine whether hormone levels (E2, dehydroepiandrosterone-sulfate [DHEA-S], and testosterone) are associated with PAH in men.

Methods: Plasma sex hormone levels in men with idiopathic, heritable, or connective tissue disease-associated PAH were compared with those from age- and body mass index-matched men without clinical cardiovascular disease.

Measurements and Main Results: There were 23 cases with PAH (70% had idiopathic PAH, 65% were functional class III/IV) and 67 control subjects. Higher E2 and E2/testosterone levels were

associated with the risk of PAH (odds ratio per 1 ln[E2:testosterone], 6.0; 95% confidence interval, 2.2–16.4; $P = 0.001$), whereas higher levels of DHEA-S were associated with a reduced risk (odds ratio per 1 ln[DHEA-S], 0.1; 95% confidence interval, 0.0–0.3; $P = 0.001$). E2 and DHEA-S levels were strong predictors of case status (C statistic for both, 0.82) but testosterone was not (C statistic, 0.53). Higher levels of E2 were associated with shorter 6-minute-walk distances ($P = 0.03$), whereas higher levels of DHEA-S were associated with lower right atrial pressure ($P = 0.02$) and pulmonary vascular resistance ($P = 0.01$) in men with PAH.

Conclusions: Higher levels of E2 and lower levels of DHEA-S were associated with PAH in men. Sex-based differences in sex hormone processing and signaling may contribute to unique phenotypes in pulmonary vascular disease.

Keywords: sex hormones; estradiol; DHEA; pulmonary hypertension

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At a Glance Commentary

Scientific Knowledge on the

Subject: Sex-based differences in pulmonary vascular disease prevalence and survival are well known, but the relation of sex hormones with pulmonary arterial hypertension in men has not been completely characterized.

That This Study Adds to the

Field: This is the first study to show that higher levels of estradiol and lower levels of dehydroepiandrosterone-sulfate are associated with the risk of pulmonary arterial hypertension in men.

Women are more likely to develop pulmonary arterial hypertension (PAH) than men, but women with PAH have better survival (1, 2). Although there has been recent interest in the role of estrogen (and female sex) in PAH, little is known about the role of androgens (and male sex) in disease pathogenesis and outcomes. We have demonstrated that men have greater hemodynamic burden at baseline than women with PAH and that genetic variation in aromatase (the pivotal enzyme that converts androgens to estrogens) is associated with the risk of portopulmonary hypertension irrespective of sex, suggesting the study of the hormonal milieu in men may be of equal import to understand the “estrogen paradox” of pulmonary vascular disease (3, 4).

Although estrogen and dehydroepiandrosterone (DHEA) prevent or rescue cardiopulmonary changes in experimental models of pulmonary hypertension, estrogen and its metabolites can also induce pulmonary vascular disease in some models and the effects of DHEA have been described in male animals only (5–11). The contradictory findings in experimental models of pulmonary hypertension and the knowledge gaps that exist in studying both sexes in animal and human disease provide a strong rationale for comparing differences in hormone levels in men with and without PAH. Limited data suggest that higher testosterone levels are detrimental to the pulmonary endothelium and are associated with maladaptive right ventricular (RV)

hypertrophy and fibrosis in murine models of pulmonary hypertension, but testosterone deficiency has been described in human studies of left heart failure and in other chronic illnesses (12–18).

We sought to determine whether sex hormone levels (estradiol [E2], E2/testosterone ratio, DHEA-sulfate [DHEA-S], total testosterone, bioavailable testosterone) are associated with PAH in men. We hypothesized that lower testosterone and bioavailable testosterone levels would be associated with the presence of PAH. Some of the results have been previously reported in abstract form (19).

Methods

Study Sample

We performed a case-control study of men with PAH and men without clinical cardiovascular disease. Cases were recruited from the Rhode Island Hospital Pulmonary Hypertension Center at Brown University, the Pulmonary Vascular Disease Program at University of Pennsylvania, and the Pulmonary Hypertension Center at Tufts Medical Center. Control subjects were selected from the MESA study (Multi-Ethnic Study of Atherosclerosis), a multicenter population-based cohort study to investigate the prevalence, correlates, and progression of subclinical cardiovascular disease in a multiethnic population aged 45–84 years drawn from six U.S. communities: Forsyth County, North Carolina; Northern Manhattan and the Bronx, New York; Baltimore City and Baltimore County, Maryland; St. Paul, Minnesota; Chicago, Illinois; and Los Angeles, California (20). Exclusion criteria for the MESA baseline examination included weight greater than 300 lb, pregnancy, impediment to long-term participation, and clinical cardiovascular disease, which was determined at participant screening by questionnaire. Participants were excluded if they answered “yes” to having been diagnosed by a physician with heart attack, stroke, transient ischemic attack, heart failure, angina, current atrial fibrillation, and/or to having undergone any prior cardiovascular procedure.

Case and Control Definitions

We included men with idiopathic, heritable, or connective tissue disease–associated PAH

as designated by their treating PAH physicians at local centers and meeting traditional diagnostic criteria: mean pulmonary artery pressure greater than or equal to 25 mm Hg at rest, mean pulmonary capillary wedge pressure less than or equal to 15 mm Hg, and pulmonary vascular resistance greater than three Wood units (21). Both prevalent (on PAH therapy) and incident (treatment naive) patients were included. Control subjects were men with normal RV and left ventricular measures (ejection fraction, end-diastolic mass, and volumes) by cardiac magnetic resonance imaging (22) without obstructive or restrictive ventilatory defects on spirometry and without self-reported chronic obstructive pulmonary disease, emphysema, or chronic bronchitis (22, 23). We also excluded men using testosterone compounds or DHEA supplements. Control subjects were matched 3:1 to cases by age (within 5 yr) and body mass index (BMI) (within 3 kg/m²).

Clinical Variables

Clinical data for cases were collected from the medical record or the local research database. Functional class, 6-minute-walk distance (6MWD), hemodynamics, and PAH treatments were collected at the time of (or as close as possible to) blood collection.

Sex Hormone Levels

Blood samples were drawn and stored using standardized procedures (24). All plasma sex hormones from cases and control subjects were measured using the Roche Elecsys 2010 system (F. Hoffman-La Roche, Basel, Switzerland) at the Laboratory for Clinical Biochemistry Research at the University of Vermont. Interassay coefficients of variation for E2 were 2.2–10.5%, for DHEA-S were 4.6–6.5%, and for testosterone were 2.3–5.6%.

Each center had local Institutional Review Board (IRB) approval for this study (Rhode Island Hospital, IRB Registration #021911; Penn, #706091; Tufts, IRB Registration #7437). The protocols of MESA were approved by the IRBs of all MESA sites and the National Heart Lung and Blood Institute. Informed consent was obtained from all patients with PAH and MESA participants. MESA participants were consented during the baseline examination for blood draw, storage, and

future measurement of biomarkers important to vascular pathobiology.

Statistical Analysis

Continuous data were summarized as median (interquartile range) and categorical data were reported as frequency and percentages. Sex hormone levels were natural log-transformed. Case status was regressed on hormone levels using generalized estimating equations assuming a binary distribution. A similar approach with generalized estimating equations assuming a normal distribution was used to estimate hormone levels by case status. Sandwich estimation was used for both modeling methods and patients were nested within their respective matching group. Receiver operating characteristic curves were constructed for each sex hormone level and corresponding C statistics were derived. The relationships between each sex hormone level and markers of disease severity (functional class, 6MWD, and hemodynamics) in cases were examined with multivariable linear or binomial regression as appropriate. Case-control random matching for age and BMI was accomplished using a macro designed for SAS software (SAS Inc., Cary, NC) (25). Final models were further adjusted for the exact values for age and BMI. Sensitivity analyses included the subgroup of cases with idiopathic or heritable PAH and the subgroup of cases who were treatment naive at the time of blood draw. All hypotheses were tested using two-tailed tests and 95% confidence intervals (CIs) were estimated. *P* values less than 0.05 were considered significant. All analyses were conducted using SAS 9.4 (SAS Inc.).

Results

We included 23 cases with PAH and 67 control subjects (Table 1). Subjects in the groups were similar in age and BMI because of matching, but a higher proportion of men with PAH were white (83%) as compared with control subjects (43%). Among men with PAH, 70% had idiopathic disease, and 65% were World Health Organization functional class III/IV. Cases tended to have moderate hemodynamic impairment despite relatively preserved 6MWD (median, 398 m; interquartile range, 233–440 m). Forty-three percent of the cases were PAH treatment-naïve, and

Table 1. Baseline Characteristics of Cases and Control Subjects

Variables	Cases	Control Subjects
Number	23	67
Age, yr	62 (55–70)	64 (55–68)
Race/ethnicity, n (%)		
White	19 (83)	29 (43)
African-American	1 (4)	0 (0)
Asian	0 (0)	18 (27)
Hispanic	3 (13)	20 (30)
BMI, kg/m ²	27 (24–31)	26 (24–29)
Diagnosis, n (%)		—
Idiopathic PAH	16 (70)	
Heritable PAH	2 (9)	
Connective tissue disease-associated PAH	5 (22)	
WHO functional class, n (%)		—
I	1 (5)	
II	5 (24)	
III	13 (62)	
IV	2 (10)	
Six-min-walk distance, m	398 (233–440)	—
Hemodynamics		—
Right atrial pressure, mm Hg	11.0 (8.0–14.0)	
Mean pulmonary artery pressure, mm Hg	48.0 (37.0–54.0)	
Cardiac output, L/min	4.1 (3.0–5.1)	
Pulmonary capillary wedge pressure, mm Hg	10.5 (7.0–14.0)	
Pulmonary vascular resistance, Wood units	5.4 (3.1–12.4)	
PAH therapies, n (%)		—
Calcium channel blockers	4 (17)	
Phosphodiesterase type 5 inhibitors	7 (30)	
Endothelin receptor antagonists	6 (26)	
Prostacyclin analogs	5 (22)	
Combination therapy	8 (35)	
Sex hormone levels		
Estradiol, pg/ml	40.9 (34.9–52.6)	27.9 (20.9–36.8)
DHEA-S, µg/dl	67.8 (37.6–139.4)	133.7 (102.1–170.6)
Total testosterone, ng/dl	541 (304–795)	530 (401–667.5)
Bioavailable testosterone, ng/dl	147.4 (82.0–178.5)	129.3 (102.9–153.8)

Definition of abbreviations: BMI = body mass index; DHEA-S = dehydroepiandrosterone-sulfate; PAH = pulmonary arterial hypertension; WHO = World Health Organization. Data are shown as median (interquartile range) or number (percentage).

35% of cases were receiving combination PAH therapy at the time of enrollment in the local registry and blood draw.

Higher E2 and E2/testosterone levels and lower DHEA-S levels were associated with an increased odds of PAH after

adjustment for age and BMI (Table 2). For example, for each one-unit increase in ln (E2) the odds of PAH were increased almost 55-fold (95% CI, 7.2–420.3; *P* < 0.001), whereas for each one-unit increase in ln(DHEA-S) the odds of PAH

Table 2. Multivariate Generalized Mixed Models for the Prediction of Case Status by Sex Hormone Levels

Sex Hormone	Odds Ratio*	95% CI	P Value
Estradiol	54.9	7.2–420.3	<0.001
Estradiol/total testosterone	6.0	2.2–16.4	0.001
DHEA-S	0.1	0.0–0.3	0.001
Total testosterone	0.7	0.2–2.3	0.58
Bioavailable testosterone	0.8	0.2–3.7	0.78

Definition of abbreviations: CI = confidence interval; DHEA-S = dehydroepiandrosterone-sulfate.

*Per 1 ln increase. Matched on age within 5 yr and body mass index within 3 kg/m² and adjusted for age and body mass index.

Table 3. Multivariate Mixed Modeling of Hormone Levels by Case Status

Sex Hormone	Case	Control	P Value
Estradiol, pg/ml	42.0 (36.6–48.1)	27.6 (25.0–30.5)	<0.001
DHEA-S, μ g/dl	61.0 (42.5–87.7)	129.9 (116.4–145.1)	0.001
Total testosterone, ng/dl	464.7 (318.0–679.0)	503.4 (457.5–553.7)	0.66
Bioavailable testosterone, ng/dl	119.2 (82.6–171.8)	124.3 (115.3–134.0)	0.82

Definition of abbreviation: DHEA-S = dehydroepiandrosterone-sulfate.

Data are expressed as least-square means with 95% confidence intervals. Matched on age within 5 yr and body mass index within 3 kg/m² and adjusted for age and body mass index.

were significantly decreased (odds ratio, 0.1; 95% CI, 0.0–0.3; $P = 0.001$). No association was found between total or bioavailable testosterone and case status. Results were unchanged when race/ethnicity was added to the model and there was no relationship between race/ethnicity and sex hormone levels (data not shown). Model fit was adequate and specifically the large effect estimates and wide 95% CI for the E2 analysis were not caused by overfitting.

Men with PAH had higher levels of E2 ($P < 0.001$) and lower levels of DHEA-S

($P < 0.001$), but there was no association between case status and testosterone levels ($P = 0.66$ for total testosterone) (Table 3 and Figure 1). Both ln(E2) and ln(DHEA-S) had excellent discrimination (C statistic for both hormones, 0.82), whereas testosterone performed poorly in discriminating case versus control status (C statistic, 0.53) (Figure 2).

The relationship between sex hormone levels and markers of disease severity in cases is presented in Table 4. Higher levels of E2 (and greater ratios of E2/testosterone)

were associated with shorter 6MWD and higher right atrial pressures (RAP), although the relationship with RAP was of borderline significance. Conversely, higher levels of DHEA-S were associated with lower RAP and pulmonary vascular resistance and may have been associated with higher cardiac output.

We repeated our analyses in the subgroup of cases with idiopathic or heritable PAH ($n = 18$) and results were unchanged (see Tables E1 and E2 in the online supplement). A subgroup analysis including only the 10 patients who were treatment naive (incident cases) showed persistent relationships between sex hormone levels and case status, although precision was lower because of the smaller sample size (see Tables E3 and E4).

Discussion

Higher levels of E2 and E2/testosterone and lower levels of DHEA-S were associated with the presence of PAH in men. In cases, higher E2 and lower DHEA-S levels were associated with more severe hemodynamic burden and higher E2 levels were associated with poorer exercise tolerance. Testosterone levels were not related to case status or markers of disease severity. Our results persisted in the subgroup of cases with idiopathic or heritable disease and in those who were treatment naive. To our knowledge, this is the first study to show that sex hormone levels are associated with the risk of PAH in men.

Because PAH is more common in women than in men, estrogen has been implicated as a mechanistic factor in disease development. Estrogen regulates bone morphogenetic receptor type II (BMPR2) expression through direct estrogen receptor binding to the BMPR2 promoter (26). Sex-specific changes in BMPR2 signaling have been demonstrated in human pulmonary artery smooth muscle cells (PASMC), with lower levels of BMPR2 and the downstream mediators inhibitor of DNA binding family of proteins Id1 and Id3 transcribed in female as compared with male cells, which may explain why PAH is a female-predominant disease (8, 27). Mair and colleagues (8) also demonstrated that exogenous estrogen significantly reduced Id1 and Id3 expression in male PASMC only, which could contribute to dysregulated PASMC growth and explain

Figure 1. Sex hormone levels by case/control status adjusted for age and body mass index (as continuous measures). Interquartile range (box) and outlying values (whiskers) are shown. Estradiol (A), dehydroepiandrosterone-sulfate (DHEA-S) (B), total testosterone (C), and bioavailable testosterone (D).

Figure 2. Receiver operating characteristic curves of total testosterone (Total T) (solid blue line), estradiol (E2) (dashed red line), and dehydroepiandrosterone-sulfate (DHEA-S) (dashed green line) for case status.

the correlation between higher levels of circulating E2 and worse exercise tolerance and greater hemodynamic burden in our study.

E2 is metabolized by the cytochrome P-450 system. Cytochrome monooxygenases are expressed in PAH pulmonary arterioles and the RV and may induce both pulmonary vascular and RV microvascular changes (7, 28). In a small study, 10 men with heritable PAH had a higher ratio of 16 α -hydroxyestrone (which is proangiogenic) to 2-methoxyestrone (which is antiproliferative) (29). Male mice treated with 16-estrogen had lower cardiac output (29). Although we do not have data on BMPR2 status or E2 metabolites, these observations suggest that estrogen compounds may be detrimental to cardiopulmonary function in men with PAH.

Unlike the paradoxical observations that have been made for E2, DHEA seems to be consistently beneficial in male pulmonary hypertension animal models (5, 9, 30–33). A DHEA receptor has been described, which is coupled to endothelial nitric oxide synthase, and DHEA has also been shown to regulate vascular endothelin-1 synthesis and secretion (34, 35). Lower levels of

circulating DHEA-S in our cases as compared with control subjects may contribute to down-regulation of nitric oxide and enhanced endothelin activation, two major pathophysiologic drivers in pulmonary vascular disease. Higher levels of DHEA-S were associated with reduced hemodynamic burden including lower RAP and possibly higher cardiac output, important predictors of outcome in PAH (36). Interestingly, DHEA treatment exerts beneficial effects on the RV (more-so than on the pulmonary vasculature) following exposure to SU5416/hypoxia, with improved cardiac index and inhibition of RV capillary rarefaction, fibrosis, and oxidative stress, suggesting an RV-disproportionate or -specific effect (5). DHEA deficiency could explain poorer RV ejection fraction improvements in response to disease-specific therapy and concomitant worse outcomes in men as compared with women (37).

DHEA is a precursor in the biosynthesis of testosterone and estrogen. The higher levels of E2 and E2/testosterone and lower levels of DHEA-S in PAH cases suggest that aromatization (the process by which steroid precursors and androgens

are converted to E2) may also be abnormal in men with PAH. We found estrone and E2 levels to be highly correlated in postmenopausal women and men with PAH (unpublished data), suggesting that increased aromatase activity may play a role in PAH. Aromatase is expressed in male and female control and PAH lungs; higher expression is seen in female PASM C when compared with male PASM C and aromatase inhibition via anastrozole decreases circulating E2 levels and attenuates pulmonary hypertensive changes in female but not in male animals (10). This implies that E2 may play more of a dynamic role in the female PAH phenotype and/or that hormones may have unique effects depending on the sex substrate.

Sex-based differences in the hormonal environment may modulate endothelial injury and PASM C growth via alterations in inflammatory signaling pathways (38). E2 receptors on B cells lead to apoptosis resistance and possibly a more detrimental phenotype in men (39, 40). In animal models of rheumatoid arthritis (a disease with a strong female sex bias), B- and T-cell responses differ by sex and can be altered with castration and E2 treatment (41, 42). Androgens have been linked to T regulatory cell expansion and proliferation (43). Lower testosterone levels have been shown to increase inflammatory markers, such as tumor necrosis factor- α in hypogonadal men, although testosterone was not linked to PAH risk or disease severity in our study (44). Men with rheumatoid arthritis have higher E2 and lower DHEA-S levels compared with male control subjects, similar to our findings in PAH (45). Although inflammation is a plausible mechanism for our observations, sex hormone binding globulin levels (linked to insulin resistance and inflammation) (46–48) did not predict case status or severity in our study (data not shown) and we have found that sex hormone levels are not associated with interleukin-6 levels in patients with PAH (unpublished data).

There were some limitations. This was a small study that included both prevalent and incident PAH and middle to older age men, making it difficult to exclude survival bias. PAH in men is quite rare, however, and is challenging to study in large numbers. Cases were drawn from PAH referral centers, whereas MESA enrolled from communities, which may have resulted in

Table 4. Sex Hormone Levels and Markers of Disease Severity in Men with PAH

Predictor	Estimate	95% CI	P Value
WHO functional class			
ln(estradiol)	0.0	3.9 to −3.4	0.99
ln(estradiol/total testosterone)	0.9	0.4 to −0.2	0.10
ln(DHEA-S)	0.2	3.1 to −1.2	0.78
ln(total testosterone)	−0.9	0.2 to −1.9	0.06
6MWD, m			
ln(estradiol)	−137.9	−256.4 to −19.4	0.03
ln(estradiol/total testosterone)	−93.4	−166.5 to −20.2	0.02
ln(DHEA-S)	6.1	−34.9 to 47.1	0.75
ln(total testosterone)	44.6	−20.6 to 109.9	0.16
Right atrial pressure, mm Hg			
ln(estradiol)	4.4	−1.6 to 10.4	0.14
ln(estradiol/total testosterone)	1.5	−0.1 to 3.1	0.06
ln(DHEA-S)	−2.7	−4.8 to −0.6	0.02
ln(total testosterone)	−1.0	−2.9 to 0.8	0.26
mPAP, mm Hg			
ln(estradiol)	−9.8	−20.4 to 0.8	0.07
ln(estradiol/total testosterone)	0.5	−4.5 to 5.4	0.85
ln(DHEA-S)	0.0	−5.3 to 5.4	0.99
ln(total testosterone)	−2.9	−8.4 to 2.5	0.27
Cardiac output, L/m			
ln(estradiol)	−1.0	−3.7 to 1.7	0.43
ln(estradiol/total testosterone)	−0.0	−0.8 to 0.8	0.93
ln(DHEA-S)	0.6	−0.2 to 1.4	0.14
ln(total testosterone)	−0.1	−0.9 to 0.8	0.79
PVR, Wood units			
ln(estradiol)	1.7	−6.4 to 9.9	0.65
ln(estradiol/total testosterone)	0.8	−4.9 to 6.5	0.77
ln(DHEA-S)	−3.4	−5.9 to −0.9	0.01
ln(total testosterone)	−0.1	−6.5 to 6.3	0.96

Definition of abbreviations: 6MWD = 6-minute-walk distance; CI = confidence interval; DHEA-S = dehydroepiandrosterone-sulfate; mPAP = mean pulmonary artery pressure; PAH = pulmonary arterial hypertension; PVR = pulmonary vascular resistance; WHO = World Health Organization. Adjusted for age and body mass index.

selection bias. Cardiac magnetic resonance imaging for the assessment of RV morphology was available for control subjects only, because magnetic resonance imaging is not used routinely in clinical care at the three study centers from which the cases were drawn.

High E2 levels and altered E2/testosterone balance have been linked to both increased risk and protection from systemic vascular disease depending on age (18, 49). The influence of hormonal fluctuations on cardiopulmonary function over the lifespan (e.g., through

puberty/adolescence, or with waning testosterone levels in older age) and at various stages of PAH is unknown and should be studied longitudinally. We chose to focus on a limited number of sex hormones given the bulk of preclinical and observational data on the “estrogen paradox” in PAH, but the entire hypothalamic-pituitary-adrenal-gonadal axis should be interrogated in pulmonary vascular disease.

The blood draws and disease measurements were not all simultaneous, potentially causing bias that would be

expected to weaken the results by introducing additional variability; the associations may be even stronger than we have shown. The correlations of hormone levels with markers of disease severity in prevalent treated patients might be even stronger in incident patients where the “signal-to-noise” ratio is likely to be more pronounced, but we were underpowered to make such comparisons. Still, this study is among the first to characterize sex hormone levels in men with World Health Organization Group 1 pulmonary hypertension. Although the differences in sex hormone levels in PAH versus control subjects could be attributed nonspecifically to chronic illness, the similarity of testosterone levels between the two groups makes this unlikely.

Conclusions

Men with PAH have higher levels of E2 and lower levels of DHEA-S as compared with age- and BMI-matched healthy control subjects. Higher E2 and lower DHEA-S levels, but not testosterone, were associated with markers of disease severity. Although the mechanisms for these observations are unknown, hormone-receptor interactions, aromatization of androgens into estrogens, and DHEA-mediated regulation of the endothelin and nitric oxide pathways deserve further consideration, as does the “estrogen paradox” in men with pulmonary vascular disease. ■

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

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