Experimental design of the Effects of Dehydroepiandrosterone in Pulmonary Hypertension (EDIPHY) trial

Thomas P. Walsh1, Grayson L. Baird1,2, Michael K. Atalay2, Saurabh Agarwal2, Daniel Arcuri2, James R. Klinger3, Christopher J. Mullin3, Heather Morreo1, Brynn Normandin1, Sruti Shiva4, Mary Whittenhall3 and Corey E. Ventetuolo3,5

1Lifespan Health System, Providence, RI, USA; 2Department of Diagnostic Imaging, Alpert Medical School of Brown University, Providence, RI, USA; 3Department of Medicine, Alpert Medical School of Brown University, Providence, RI, USA; 4Department of Pharmacology and Chemical Biology, Vascular Medicine Institute, NO Metabolomics Core Facility, University of Pittsburgh, Pittsburgh, PA, USA; 5Department of Health Services, Policy and Practice, Brown University School of Public Health, Providence, RI, USA

Abstract

Pulmonary arterial hypertension (PAH) remains life-limiting despite numerous approved vasodilator therapies. Right ventricular (RV) function determines outcome in PAH but no treatments directly target RV adaptation. PAH is more common in women, yet women have better RV function and survival as compared to men with PAH. Lower levels of the adrenal steroid dehydroepiandrosterone (DHEA) and its sulfate ester are associated with more severe pulmonary vascular disease, worse RV function, and mortality independent of other sex hormones in men and women with PAH. DHEA has direct effects on nitric oxide (NO) and endothelin-1 (ET-1) synthesis and signaling, direct antihypertrophic effects on cardiomyocytes, and mitigates oxidative stress. Effects of Dehydroepiandrosterone in Pulmonary Hypertension (EDIPHY) is an on-going randomized double-blind placebo-controlled crossover trial of DHEA in men (n = 13) and pre- and post-menopausal women (n = 13) with Group 1 PAH funded by the National Heart, Lung and Blood Institute. We will determine whether orally administered DHEA 50 mg daily for 18 weeks affects RV longitudinal strain measured by cardiac magnetic resonance imaging, markers of RV remodeling and oxidative stress, NO and ET-1 signaling, sex hormone levels, other PAH intermediate end points, side effects, and safety. The crossover design will elucidate sex-based phenotypes in PAH and whether active treatment with DHEA impacts NO and ET-1 biosynthesis. EDIPHY is the first clinical trial of an endogenous sex hormone in PAH. Herein we present the study’s rationale and experimental design.

Keywords
dehydroepiandrosterone, pulmonary arterial hypertension, right ventricle, clinical trial, sex

Date received: 27 August 2020; accepted: 15 December 2020

Pulmonary Circulation 2021; 11(2) 1–9
DOI: 10.1177/2045894021989554

Introduction

Pulmonary arterial hypertension (PAH) has poor long-term survival despite numerous treatments that target the pulmonary vasculature. Right ventricular (RV) adaptation determines outcome in PAH but there are no approved treatments for RV failure. Women are more likely to have PAH and extensive experimental and human data support a role for sex and sex hormones in PAH and RV failure pathogenesis and outcomes.1 Sex influences nitric oxide (NO) and endothelin (ET)-1 signaling and PAH treatments directed towards these pathways have differential effects on RV remodeling that depend on the hormonal milieu.2-7 Response to treatment with phosphodiesterase type 5 inhibitors and ET receptor antagonists varies by sex, perhaps due to sex hormone-mediated dimorphic effects on the RV.5,9

We and others have shown that PAH is characterized by lower circulating levels of dehydroepiandrosterone-sulfate (DHEA-S) and that lower DHEA-S levels are associated with worse RV function and mortality in men and women with PAH independent of other sex hormones.10-13 These
human data complement experimental models demonstrating that DHEA reverses pulmonary hypertension (PH) and rescues the RV by reducing oxidative stress.\textsuperscript{1,14–17} DHEA binds directly to vascular endothelium to activate NO synthase G1, suppresses ET-1 expression, and inhibits ET-1-induced brain natriuretic peptide (BNP) expression and cardiac remodeling.\textsuperscript{18,19}

In a randomized clinical trial (RCT) of healthy older adults, DHEA 50 mg daily improved systemic vascular stiffness and reduced levels of interleukin (IL)-6 and tumor necrosis factor-\(\alpha\), two cytokines implicated in PAH pathogenesis and associated with symptoms and survival in PAH.\textsuperscript{20–22} DHEA improves metabolic changes in adrenal insufficiency and symptom control in patients with connective tissue disease.\textsuperscript{23–33} Although the results of RCTs of DHEA for these indications have been mixed, no serious side effects were reported in RCTs including >1200 patients; reversible mild androgenic side effects (oily skin, hirsutism, acne) were noted in some studies.\textsuperscript{28,34,35} While DHEA is included in some dietary supplements, the U.S. Food and Drug Administration (FDA) approved intravaginal DHEA (also known as prasterone or Intrarosa) for the treatment of moderate-to-severe dyspareunia in post-menopausal women.\textsuperscript{36} In an uncontrolled study of eight patients with PH related to chronic obstructive pulmonary disease, treatment with three months of DHEA 200 mg daily was associated with a significant increase in 6-min walk distance (6MWD) and improvements in hemodynamics without adverse effects.\textsuperscript{37}

The Effects of Dehydroepiandrosterone in Pulmonary Hypertension (EDIPHY) study is an on-going randomized double-blind placebo-controlled crossover trial of DHEA in men and pre- and post-menopausal women with PAH funded by the National Heart, Lung, and Blood Institute (R01-HL141268; NCT03648385). EDIPHY will test the impact of DHEA on RV phenotype and provide mechanistic insights into sexual dimorphism in PAH and RV adaptation and in the context of major treatment targets (NO and ET-1) in PAH.

### Materials and methods

#### Study design

EDIPHY is a single center, prospective, randomized, double-blind crossover study comparing oral DHEA 50 mg daily with placebo over two 18-week treatment periods (Periods 1 and 2) to assess changes in RV longitudinal strain measured by cardiac magnetic resonance imaging (MRI) in patients with well-characterized World Symposium on Pulmonary Hypertension (WSPH) Group 1 PAH.\textsuperscript{38} Participants are randomized 1:1 and stratified by sex. The study protocol calls for five study visits with three MRIs and a four-week washout period (Fig. 1 and Table 1). The central hypothesis is that DHEA will improve RV contractility and maladaptive remodeling via changes in NO and ET-1 synthesis and signaling in a sex-dependent manner. This research protocol has been approved by our Hospital Institutional Review Board as meeting the standards for human research protection under 45CFR46/21CFR56 and has been assigned project reference #001218. Study conduct and participant safety is being monitored by a Data Safety and Monitoring Board.

#### Study drug

This study received an Investigational New Drug exemption (IND#129285) from the FDA. DHEA 50 mg tablets are purchased from Green Mountain Pharmaceuticals (Lakewood, CO). Green Mountain is an Active Pharmaceutical Ingredient registered manufacturer with the FDA. A highly pure compound (100% DHEA) is verified by an independent laboratory with each batch. Study drug and placebo are over-encapsulated by the Investigational Services Pharmacy. Participants, study investigators, study staff and statistician are blinded.

#### Primary objective

- The primary objective of this study is to assess the effects of DHEA vs. placebo on RV longitudinal strain as measured by cardiac MRI at 18 weeks.
Secondary objectives

- To assess the effect of DHEA vs. placebo on RV function at 18 weeks
- To assess the effect of DHEA vs. placebo on markers of maladaptive RV hypertrophy including N-terminal pro hormone of BNP (NT-proBNP) at 18 weeks
- To assess the effect of DHEA vs. placebo on sex hormone levels at 18 weeks
- To assess the effect of DHEA vs. placebo on 6MWD at 18 weeks
- To assess the effect of DHEA vs. placebo on health-related quality of life (Short Form (SF) Survey-36, emPHasis-10, participant preference) at 18 weeks
- To determine whether DHEA affects NO biosynthesis and ET-1 production as assessed by measurement of NO metabolites and ET-1 at 18 weeks
- To assess the safety and side effects associated with DHEA administration in participants with PAH.

Participant screening, recruitment, and retention

Patients with well-defined WSPH Group 1 PAH and a history of classical hemodynamic criteria documented by right heart catheterization are approached for inclusion. All WSPH Group 1 patients are eligible for enrollment with the exception of PAH associated with human immunodeficiency virus (HIV), as DHEA has also been studied in chronic HIV infection.39 Treated PAH patients are eligible provided they have had no new PAH therapies introduced for 12 weeks. At least 12 weeks of clinical stability (no up-titration of PAH therapies) is required between screening and randomization. A detailed list of inclusion and exclusion criteria is included in Table 2. Pre-menopausal women are eligible as physiologic variations over the menstrual cycle should be balanced by the cross-over design with four to five cycles occurring consistently per 18-week treatment period. Patients on active hormonal therapy, contraceptives or supplements (or considering their use) are excluded. Given that cross-over trials are particularly sensitive to dropout and missing data, patients with an established history of non-adherence or other circumstances which could threaten adherence with the crossover design and visit schedule are not approached.

Following screening and informed consent, a maximum of four weeks may lapse before randomization at the baseline visit and eligibility in order to confirm clinical stability. New or recent changes to PAH medications and any emergent conditions are reassessed at the baseline visit and all other enrollment criteria verified. Study drug adherence is assessed at each visit with study logs and pill counts. Study staff make frequent reminder calls three to five days prior to study visits. Emphasis is placed on maintaining a rapport with study participants through clear and consistent communication. Study staff include participants in visit scheduling decisions and demonstrate a willingness to be flexible around participant schedules but within the confines of the study protocol. This operational strategy acts as an additional measure against missing data and other threats to attrition.
Recruitment and power

A total of 26 patients will be recruited with the anticipation that drop-out will be one participant per period ($n = 24$). Power calculations were based on mean RV longitudinal systolic strain effect size and standard deviation estimates reported in Hardegree et al.\(^{40}\) (Table 3). Power calculations were generated using SAS 9.4 PROC POWER using both paired means (both periods) two sample means (one period) routines. Alpha was established at 0.05. As a conservative effort, several levels of correlation were assumed and placebo and carry-over effects were also estimated. Power was calculated for mean differences (2 periods) and differences in delta for Period 1. Power was also calculated by sex (2 periods), where it was assumed females would have a mean difference in RV longitudinal strain of 8.0% and males 3.0%, respectively.

Visit components and study measurements

Study visit components and measurements are listed in Table 1.

Cardiac MRI measurements

All study MRIs are performed at a core MRI facility on a single Siemens 1.5T Aera with full Advanced Cardiac Package and XQ Gradients (45 mT/m @ 200 T/m/s) regularly used for research grade imaging. All MRI measurements are made using commercially available cardiac MRI software (CVI42, Circle Cardiovascular Imaging). Longitudinal and circumferential strain will be determined using standard cine imaging and Tissue Tracking (Strain) software (Tissue Tracking plugin—CVI42, circle cardiovascular imaging) at study completion to avoid batch effects. In addition to RV longitudinal strain, we will measure RV...
fibrosis with T1 mapping as well as standard measures of RV function including RV ejection fraction (RVEF), RV end-diastolic mass, and volumes (stroke volume, end-systolic volume, end-diastolic volume). After study visits with MRI, a single expert reader performs quality assurance/quality control (QA/QC) on image acquisition. The same single expert reader blinded to participant and study visits will read studies for the primary end point (RV longitudinal strain) and fibrosis (on T1 images) in randomly sequenced batches at the study’s conclusion. Ten percent of the MRI studies will be reassessed by a second expert reader for intra-reader and inter-reader reliability estimates of all measured parameters.

Markers of RV adaptation, sex hormones, and other biomarkers

Phlebotomy is performed at every study visit during both treatment periods and blood is banked for biomarker measurement at the study’s conclusion. Serum NT-proBNP levels will be measured using a FDA approved commercially available immunoassay (Roche Diagnostic Elecsys proBNP Assay, Indianapolis, IN) with excellent precision (intra- and inter-assay coefficients of variation (CV) of <6.1%) and a track record in PAH. Serum galectin-3 levels, a marker of myocardial extracellular matrix metabolism and fibrosis known to be elevated in the plasma of PAH patients and inversely correlated with RVEF measured by MRI, will be measured by an ELISA with an intra-assay CV of 3.4% (BG Medicine, Waltham, MA). Soluble RAGE, which inhibits myocardial apoptosis via STAT3 activation and is tied mechanistically to DHEA’s benefit in experimental PH, will be measured in plasma by an ELISA (R&D Systems, Minneapolis, MN) with excellent precision (intra-assay CVs 4.8–6.2%; inter-assay CVs 6.7–8.2%). Serum DHEA-S (more stable than DHEA) will be measured by electrochemiluminescence immunoassay (Roche, Indianapolis, IN). The DHEA-S assay has a detection limit between 0.1 and 1000 μg/dL and a CV of <8.3%. Plasma nitrite and nitrate levels will be quantified using tri-iodide-based reductive chemiluminescence. S-nitrosohemoglobin (SNO-Hb), a major regulator of NO’s interaction with vascular endothelium, will be measured.

### Table 3. Sample size and power calculations.

**Between Group Design (Period 1 comparison)**

| Estimate | Treatment | Placebo | Power | Alpha | n |
|----------|-----------|---------|-------|-------|---|
| Delta (SD) | Period 1 only | −5.3 (5.3) | −0.0 (5.3) | 0.77 | 0.05 | 12 | 12 |
| ^Delta (SD) | Period 1 only | −5.3 (5.3) | −0.1 (5.3) | 0.75 | 0.05 | 12 | 12 |
| ^Delta (SD) | Period 1 only | −5.3 (5.3) | −0.5 (5.3) | 0.70 | 0.05 | 12 | 12 |

**Crossover Design (Periods 1 and 2)**

| Mean (SD) | Mean (SD) | Power | r | Alpha | n | n |
|-----------|-----------|-------|---|-------|---|---|
| −19.3 (4.6) | −14.0 (5.2) | 0.99 | 0.10 | 0.05 | 24 | 24 |
| ^Mean (SD) | −19.3 (4.6) | −16.0 (5.2) | 0.77 | 0.10 | 0.05 | 24 | 24 |
| Mean (SD) | −19.3 (4.6) | −14.0 (5.2) | 0.99 | 0.20 | 0.05 | 24 | 24 |
| ^Mean (SD) | −19.3 (4.6) | −16.0 (5.2) | 0.81 | 0.20 | 0.05 | 24 | 24 |
| Mean (SD) | −19.3 (4.6) | −14.0 (5.2) | 0.99 | 0.30 | 0.05 | 24 | 24 |
| ^Mean (SD) | −19.3 (4.6) | −16.0 (5.2) | 0.85 | 0.30 | 0.05 | 24 | 24 |

**By sex**

| Females | Males | Mean (SD) | Mean (SD) | Power | r | Alpha | n | n |
|---------|-------|-----------|-----------|-------|---|-------|---|---|
| Mean (SD) | −22.0 (4.6) | −14.0 (5.2) | 0.99 | 0.10 | 0.05 | 12 | 12 |
| Mean (SD) | −17.0 (4.6) | −14.0 (5.2) | 0.43 | 0.10 | 0.05 | 12 | 12 |
| Mean (SD) | −17.0 (4.6) | −14.0 (5.2) | 0.47 | 0.20 | 0.05 | 12 | 12 |
| Mean (SD) | −17.0 (4.6) | −14.0 (5.2) | 0.51 | 0.30 | 0.05 | 12 | 12 |

Between Group Design: Power analyses were conducted for Period 1 only for the difference in the delta change between participants receiving treatment and placebo prior to crossover. The effect of the treatment can be assessed between groups without the threat of carry-over contamination because of randomization. The limitation of this analysis is there are only 12 participants in each arm. These analyses incorporated increasing amounts of placebo and regression towards the mean effects (^). Crossover Design: Power analyses were conducted for the crossover design assuming washout will be sufficient. These analyses assumed different combinations of within-participants correlation along with different assumed effects of placebo and regression towards the mean effects (^). Because there is an assumed effect of treatment by sex, power analyses were conducted by sex.
using reductive chemiluminescence for nitrite and nitrate; these methods have been well-validated by the University of Pittsburgh NO Metabolomics core facility.\textsuperscript{49} Serum ET-1 will be measured via immunoassay (QuantiGlo, R&D Systems, Inc, Minneapolis, MN) with intra-assay CVs of 2.6–3.4\% and inter-assay CVs of 4.6–8.9\%. Additional assays of interest will include comprehensive sex hormone levels, their metabolites, isoprostanes and isofurans, and IL-6 levels.\textsuperscript{54}

**Additional PAH intermediate end points**

Health-related quality of life is assessed by the SF-36 and emPHasis-10 scales, which have been validated in PAH.\textsuperscript{55,56} Participants are asked if they have a personal preference for Period 1 or Period 2 at study completion. A satisfaction rating collected at the end of each period is used to check this preference for plausibility.\textsuperscript{57} Clinicians assess functional class at each study visit as part of the required physical exam. Six-minute walk tests are performed by blinded and trained personnel according to standardized procedures.\textsuperscript{58} Variability is minimized by administering the test in the same corridor for all participants, avoiding a “warm-up” period, and using standard phrases of encouragement; intra-class correlation coefficients were 0.95–0.98 from a completed RCT in our center.\textsuperscript{59} Side effects and safety are also assessed (Table 1).

**Data management**

Research staff use paper source documents in conjunction with direct entry surveys for patient-reported measures when appropriate. Many end points can be collected remotely and contingency plans exist for end points that must be collected on-site. Source documents are entered into the study electronic case report forms (eCRF) within 48 hours of collection. A centralized REDCap project serves as the study eCRF and encompasses all participant-level data and end point variables. Cardiac MRI data files are stored in triple-redundancy to prevent critical data loss. Data QA/QC procedures leverage the native features of REDCap and a well-defined project management plan. The principal investigator, statistician, and project manager meet regularly, with greater frequency as needed, to discuss issues relating to rigor, QA/QC and protocol execution.

**Statistical methods**

The intent-to-treat analysis will include all randomized participants. Hypothesis testing will use $\alpha = 0.05$ without correction for multiplicity. We will characterize participants with regard to baseline and follow-up RV longitudinal strain and other end points using descriptive statistics. The primary analysis will compare the change in RV longitudinal strain at the end of each treatment period from baseline between (1) treatments and (2) between treatment and sex using generalized linear mixed models, nesting repeated measures within participant. Distribution of the models will be chosen based on inspection of model residuals. Classical sandwich estimation will be used to adjust for model misspecification after maximizing the appropriateness of distribution selected.

Because it is unknown if our washout period is sufficient to control for carry-over effects and as study end points are not collected at a second baseline (week 22), change in end points will be examined between (1) treatment, (2) treatment and period, (3) treatment and sex, and (4) treatment, sex, and period. We will also examine the change in primary and secondary outcomes between baseline and at the end of each 18-week treatment period.

For participants lost to follow-up, we will use all of the information available until the end of follow-up. If a participant wishes to drop-out or has a serious adverse event (whether related to study drug or not), we will continue to follow the participant for study assessments to assist with safety monitoring and to avoid the problems introduced by missing data. The participant will be strongly encouraged to continue to follow-up with the study personnel for all scheduled study procedures (including MRI), even if they are no longer taking study drug, so that missing data (and assumptions regarding these data) will be minimized. The inclusion of such follow-up data will allow for analysis by intention-to-treat. Per-protocol analysis will also be performed.

**Data sharing**

We are supportive of data sharing and will adhere to a data sharing timeline and approach that is consistent with the recommendations of the International Committee of Medical Journal Editors. The final statistical analysis plan and study dataset will be made available 12 months after publication of the results and provided appropriate protections (e.g., data use agreements, deidentified data) are in place. Final trial results will also be publically available on clinicaltrials.gov within 12 months of trial completion.

**Discussion**

This is the first trial to assess the use of DHEA as a putative treatment for PAH and to our knowledge the first to use RV strain assessed by cardiac MRI as a primary end point in PAH. This mechanistic crossover study is designed to examine the impact of DHEA on RV phenotype but also to provide insight into the intersections of sex, sex hormones, and key treatment targets in human PAH (NO, ET-1) in an effort to understand differential treatment response with available PAH therapies. Innovative aspects of EDIPHY include a sensitive, non-invasive primary end point to assess RV function, minimalist visit design, and tightly integrated project management plan.

The RV as compared to the left ventricle has complex rotational motion from a unique myocardial fiber arrangement that requires highly sensitive methods to detect conformational changes. RV metrics obtained by cardiac MRI
are highly accurate and MRI is the gold standard for RV assessment in PAH and other cardiomyopathies. Longitudinal strain, an angle-independent method of assessing RV systolic function measured by echocardiography, is associated with survival in PAH and tracks with PAH therapy. Echocardiography is inferior to MRI for assessment of the RV, however, and pixel-based multimodality tissue tracking using MRI cine images is available to quantify strain. This method has been described in PAH and is reproducible. Assessment of strain by MRI appears highly sensitive for the detection of pre-clinical changes in RV contractility which is an ideal primary end point for a potential efficacy signal in this early Phase II trial. The measurement of fibrosis by T1 mapping will provide additional insight into subtle changes in treated participants who may have “stable” disease by traditional PAH metrics.

The selection of a crossover design eliminates between-participant variability when comparing DHEA to placebo and allows for a much smaller sample size than would ordinarily be necessary in a parallel arm trial to maintain the same power. This structure suits a “proof of concept” study such as this one and strikes a balance between feasibility and rigor. The crossover design also limits the imbalance of factors particularly relevant to sexual dimorphism in pulmonary vascular disease, including sex, age, body mass index, and race/ethnicity. In the case of a significant interaction by sex, we will technically be underpowered to detect differences in men alone; however, evidence of the interaction is compelling in and of itself and has biologic relevance.

A washout period of four weeks was designated to minimize possible carry-over effects, which is conservative relative to prior studies. While assessment for carry-over effects would have been assured with the incorporation of two baseline MRIs and two MRIs after the active treatment periods (four MRIs total), this would have increased burden and threatened feasibility and enrollment. The limitation of not having a baseline for the second period (week 22) is if the washout period is not long enough, carry-over effects from the previous treatment period may contaminate the effects in the second period and introduce an order effect. We have used conservative estimates for power (Table 3) and the designated washout period in order to mitigate this possibility.

A lower dose of DHEA (50 mg) was selected than given in the prior open-label trial of PH in eight participants (200 mg). Trials outside of pulmonary vascular disease have used the 50 mg dose with no serious adverse events. The 50 mg dose has been shown to increase DHEA-S levels to the normal range for younger individuals and to levels that far surpass the deficiencies we have shown in men and post-menopausal women with PAH. We are assuring a highly pure compound with independent testing during the trial.

Conclusion
This pilot double-blind cross-over trial will test the efficacy and safety of DHEA in men and women with PAH and will characterize the interaction of sex- and sex-hormones with pathologic drivers of pulmonary vascular and RV dysfunction.

Author’s contributions
T.P.W.: study coordinator, database design and data quality control, assurance and management, trial protocol, drafting and revisions of the manuscript; G.L.B.: randomization scheme, analysis plan, data quality control and assurance, revision of the manuscript; M.K.A.: MRI protocol, MRI quality control and assurance, revision of the manuscript; S.A.: MRI quality control and assurance, revision of the manuscript; D.A.: MRI protocol, quality control and assurance, revision of the manuscript; J.R.K., C.J.M., H.M., B.N., M.W.: subject recruitment and enrollment, study visits, revision of the manuscript; S.S.: nitric oxide metabolite measurement, revision of the manuscript; C.E. V.: study inception and design, trial protocol, revision of the manuscript.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was completed with support from the National Institutes of Health R01-HL141268.

ORCID iDs
Thomas P. Walsh https://orcid.org/0000-0003-2620-6843
Michael K. Atalay https://orcid.org/0000-0003-3549-2634

References
1. Hester J, Ventetuolo C and Lahm T. Sex, gender, and sex hormones in pulmonary hypertension and right ventricular failure. Compr Physiol 2019; 10: 125–170.
2. Forte P, Kneale BJ, Milne E, et al. Evidence for a difference in nitric oxide biosynthesis between healthy women and men. Hypertension 1998; 32: 730–734.
3. Davidge ST, Stranko CP and Roberts JM. Urine but not plasma nitric oxide metabolites are decreased in women with hypertension. Am J Physiol Regul Integr Comp Physiol 1998; 32: 730–734.
4. Stauffer BL, Westby CM, Greiner JJ, et al. Sex differences in endothelin-1-mediated vasoconstrictor tone in middle-aged and older adults. Am J Physiol Regul Integr Comp Physiol 2010; 298: R261–265.
5. Polderman KH, Stehouwer CD, van Kamp GJ, et al. Influence of sex hormones on plasma endothelin levels. Ann Intern Med 1993; 118: 429–432.
6. Miyaguchi T, Yanagisawa M, Iida K, et al. Age- and sex-related variation of plasma endothelin-1 concentration in normal and hypertensive subjects. Am Heart J 1992; 123: 1092–1093.
7. Sasaki H, Nagayama T, Blanton RM, et al. PDE5 inhibitor efficacy is estrogen dependent in female heart disease. J Clin Invest 2014; 124: 2464–2471.

8. Gabler NB, French B, Strom BL, et al. Race and sex differences in response to endothelin receptor antagonists for pulmonary arterial hypertention. Chest 2012; 141: 20–26.

9. Mathai SC, Hassoun PM, Puhlan MA, et al. Sex differences in response to tadalafil in pulmonary arterial hypertension. Chest 2015; 147: 188–197.

10. Ventetuolo CE, Baird GL, Barr RG, et al. Higher estradiol and lower dehydroepiandrosterone-sulfate levels are associated with pulmonary arterial hypertension in men. Am J Respir Crit Care Med 2016; 193: 1168–1175.

11. Baird GL, Archer-Chicko C, Barr RG, et al. Lower DHEA-S levels predict disease and worse outcomes in post-menopausal women with idiopathic, connective tissue disease- and congenital heart disease-associated pulmonary arterial hypertention. Eur Respir J 2018; 51: 1800467.

12. Rhodes CJ, Ghahtaoor P, Wharton J, et al. Plasma metabolomics implicates modified transfer RNAs and altered bioenergetics in the outcomes of pulmonary arterial hypertention. Circulation 2017; 135: 460–475.

13. Baird GL, Walsh T, Aliotta J, et al. Insights from the menstrual cycle in pulmonary arterial hypertention. Ann Am Thorac Soc. DOI: 10.1513/AnnalsATS.202006-671OC.

14. Alzoubi A, Toba M, Abe K, et al. Dehydroepiandrosterone restores right ventricular structure and function in rats with severe pulmonary arterial hypertention. Am J Physiol Heart Circ Physiol 2013; 304: H1708–1718.

15. Bonnet S, Dumas-de-La-Roque E, Begueret H, et al. Dehydroepiandrosterone (DHEA) prevents and reverses chronic hypoxic pulmonary hypertention. Proc Natl Acad Sci U S A 2003; 100: 9488–9493.

16. Bonnet S, Paulin R, Sutendra G, et al. Dehydroepiandrosterone reverses systemic vascular remodeling through the inhibition of the Akt/GSK3-[beta]/NFAT axis. Circulation 2009; 120: 1231–1240.

17. Haml pl V, Bibova J, Povy silova V, et al. Dehydroepiandrosterone sulphate reduces chronic hypoxic pulmonary hypertension in rats. Eur Respir J 2003; 21: 862–865.

18. Chen H, Lin AS, Li Y, et al. Dehydroepiandrosterone stimulates phosphorylation of FoxO1 in vascular endothelial cells via phosphatidyninositol 3-kinase- and protein kinase a-dependent signaling pathways to regulate ET-1 synthesis and secretory. J Biol Chem 2008; 283: 29228–29238.

19. Patel D, Kandhi S, Kelly M, et al. Dehydroepiandrosterone promotes pulmonary artery relaxation by NADPH oxidation-induced subunit dimerization of protein kinase G I alpha. Am J Physiol Lung Cell Mol Physiol 2014; 306: L383–391.

20. Weiss EP, Villareal DT, Ehsani AA, et al. Dehydroepiandrosterone replacement therapy in older adults improves indices of arterial stiffness. Aging Cell 2012; 11: 876–884.

21. Soon E, Holmes AM, Treacy CM, et al. Elevated levels of inflammatory cytokines predict survival in idiopathic and familial pulmonary arterial hypertention. Circulation 2010; 122: 920–927.

22. Matura LA, Ventetuolo CE, Palevsky HI, et al. Interleukin-6 and tumor necrosis factor-alpha are associated with quality of life-related symptoms in pulmonary arterial hypertention. Ann Am Thorac Soc 2015; 12: 370–375.

23. Forsblad-d’Elia H, Carlsten H, Labrie F, et al. Low serum levels of sex steroids are associated with disease characteristics in primary Sjogren’s syndrome; supplementation with dehydroepiandrosterone restores the concentrations. J Clin Endocrinol Metab 2009; 94: 2044–2051.

24. Chang DM, Lan JL, Lin HY, et al. Dehydroepiandrosterone treatment of women with mild-to-moderate systemic lupus erythematosus: a multicenter randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2002; 46: 2924–2927.

25. Jankowski CM, Gozansky WS, Schwartz RS, et al. Effects of dehydroepiandrosterone replacement therapy on bone mineral density in older adults: a randomized, controlled trial. J Clin Endocrinol Metab 2006; 91: 2986–2993.

26. Labrie F, Archer D, Bouchard C, et al. Serum steroid levels during 12-week intravaginal dehydroepiandrosterone administration. Menopause 2009; 16: 897–906.

27. Labrie F, Archer D, Bouchard C, et al. Intravaginal dehydroepiandrosterone (Prasterone), a physiological and highly efficient treatment of vaginal atrophy. Menopause 2009; 16: 907–922.

28. Alkatib AA, Cosma M, Elamin MB, et al. A systematic review and meta-analysis of randomized placebo-controlled trials of DHEA treatment effects on quality of life in women with adrenal insufficiency. J Clin Endocrinol Metab 2009; 94: 3676–3681.

29. Dhatariya K, Bigelow ML and Nair KS. Effect of dehydroepiandrosterone replacement on insulin sensitivity and lipids in hypoadrenal women. Diabetes 2005; 54: 765–769.

30. Gurnell EM, Hunt PJ, Curran SE, et al. Long-term DHEA replacement in primary adrenal insufficiency: effects on body composition, serum leptin, bone turnover, and exercise capacity. J Clin Endocrinol Metab 2008; 93: 400–409.

31. Hunt PJ, Gurnell EM, Huppert FA, et al. Improvement in mood and fatigue after dehydroepiandrosterone replacement in Addison’s disease in a randomized, controlled trial. J Clin Endocrinol Metab 2000; 85: 4650–4656.

32. Callies F, Fassnacht M, van Vlijmen JC, et al. Dehydroepiandrosterone replacement in women with adrenal insufficiency: effects on body composition, serum leptin, bone turnover, and exercise capacity. J Clin Endocrinol Metab 2001; 86: 1968–1972.

33. Arlt W, Callies F, van Vlijmen JC, et al. Dehydroepiandrosterone replacement in women with adrenal insufficiency. N Engl J Med 1999; 341: 1013–1020.

34. Crosbie D, Black C, McIntyre L, et al. Dehydroepiandrosterone for systemic lupus erythematosus. Cochrane Database Syst Rev 2007: CD005114.

35. Petri MA, Mease PJ, Merrill JT, et al. Effects of prasterone on disease activity and symptoms in women with active systemic lupus erythematosus. Arthritis Rheum 2004; 50: 2858–2868.

36. Labrie F, Archer DF, Martel C, et al. Combined data of intravaginal prasterone against vuggulvaginal atrophy of menopause. Menopause 2017; 24: 1246–1256.

37. Dumas de La Roque E, Savineau JP, Metivier AC, et al. Dehydroepiandrosterone (DHEA) improves pulmonary hypertention in chronic obstructive pulmonary disease (COPD): a pilot study. Ann Endocrinol 2012; 73: 20–25.
