Gender, Sex Hormones, and Pulmonary Arterial Hypertension



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THE EPIDEMIOLOGY OF PAH WITH RESPECT TO GENDER

The precise incidence of pulmonary arterial hypertension (PAH) is difficult to determine with accuracy for a variety of reasons, including tertiary care center bias and community underreporting; regardless, most studies to date predominantly evaluated prevalent cases. While affecting patients of all ages and both genders, since its earliest descriptions PAH has been undeniably a disease that preferentially affects females more than males (Figure 1).^{10,11}

Determining incidence and prevalence rates according to gender is difficult. A large multicenter French study attempted to address incidence by evaluating 674 cases of PAH from 17 university hospitals across France during a 1-year period from October 2002 to October 2003.⁶ Among those 674 PAH cases, 65.3% were females. They found that 18% (121 of 674) of PAH cases were incident diagnoses (57.0% female) during the period of the study. The remaining 553 cases were prevalent cases, of which 67.1% were females. At that time, they concluded an overall female predominance among PAH patients of 1.9 females: 1 male, with the exception being portal hypertensionPulmonary arterial hypertension (PAH) is a progressive disease characterized by pulmonary vascular remodeling of the distal pulmonary vasculature, ultimately leading to destruction and loss of the smallest pulmonary arteries.¹ The ensuing syndrome of PAH is clinically characterized by reduced pulmonary arterial circulatory flow, resulting in increased pulmonary vascular resistance, which ultimately results in failure of the right heart.² In both children and adults, PAH presents as a primary disease or in association with a diverse range of diseases such as connective tissue diseases, portal hypertension, and congenital heart disease.³ Nearly all forms of World Health Organization (WHO) Group 1 PAH demonstrate a skewed gender ratio with significantly more females diagnosed with PAH than males.⁴⁻⁶

While the mechanistic details behind the female predominance remain unclear, this gender discrepancy may represent an opportunity for advanced biologic understanding and future therapeutic development. This article will briefly discuss the intersection of human, in vitro, and animal studies of PAH, and highlight the conflicting data that others have discussed and elegantly elaborated upon as the "estrogen paradox" in PAH.⁷⁻⁹

associated PAH and HIV-associated PAH (both slightly favored males).

Those 674 subjects were subsequently followed prospectively and reanalyzed three years later. Among the interesting findings to emerge from those analyses were confirmation of female predominance and a suggestion of survival benefit according to gender. In particular, among those with heritable PAH (HPAH), idiopathic PAH (IPAH) and anorexigenassociated PAH, female gender associated with improved survival at 3 years; ie, despite similar treatment regimens, male patients may be at greater risk of mortality.^{12,13} As survival may have more to do with right heart adaptation to a load stress by the pulmonary vasculature, this raised an intriguing question: does female gender negatively influence pulmonary vascular disease pathogenesis, but positively influence right heart adaptation to pulmonary hypertension?

In North America, the Actelionsponsored Registry to Evaluate Early and Long-term PAH Disease Management (REVEAL) database provided a large observational cohort study of PAH, which remains ongoing. The registry was designed to enroll prevalent and/or incident patients in the United States with WHO Group 1 PAH, to characterize their baseline characteristics as well as to examine their clinical progression and responses to therapy over time.¹⁴ The baseline characteristics on the first 2525 adult patients enrolled between March 2006 and September 2007 were reported, as well as a follow-up survival study.^{15,16} One of the most interesting findings to emerge from REVEAL to date is an even larger predominance of females than anticipated, with 79.5% of the adult PAH patients in the registry classified as female. Specifically, the female-to-male ratio of 4.1:1 among IPAH patients is much higher than that reported in the 1987 National Institutes of Health registry $(1.7:1)^{11}$ or in the French registry (1.9:1), but is similar to that observed in the Surveillance of Pulmonary Hypertension in America registry (4.3:1).¹⁷

REVEAL also provided a more in depth evaluation of PAH subjects according to gender across subtypes of PAH, demonstrating a marked predominance of females across PAH subtypes, with the exception of portal hypertension-associated PAH (Table 1). With regard to survival, REVEAL investigators determined that older males had a survival disadvantage among PAH patients. Specifically, men over 60 years of age had poorer survival compared with men ≤ 60 years of age at the time of assessment and compared with female patients regardless of age.¹⁶ While not an identical analysis, this similarity to

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Figure 1: Pedigree of a large family with HPAH, with color-coding added to highlight the gender of those with HPAH. Subjects with HPAH are demonstrated by colored symbols: blue squares for males, red circles for females. Symbols are standard for pedigree analysis otherwise. Note the large percentage of HPAH patients in this family who are female.

survival data in the French registry is certainly intriguing.

Of note, while the French and REVEAL registry studies described a lack of female predominance among those with portal hypertension-associated PAH (termed portopulmonary hypertension), not all studies agree on this conclusion. In a prospective cohort of patients with portal hypertension, Kawut and colleagues found that female gender was independently associated with portopulmonary PAH. Specifically, of 106 patients with portal hypertension (53 with portopulmonary PAH, 53 without), females had 3-fold higher odds of portopulmonary PAH compared to males on adjusted analysis irrespective of the underlying etiology of liver disease.¹⁸ Subsequent analysis of this population demonstrated that genetic variation in estrogen signaling was associated with the risk of portopulmonary PAH—they found that genetic polymorphisms in both estrogen receptor 1 (ESR1) (also known as estrogen receptor α) and aromatase (CYP19A1) associated with disease expression, as did elevated plasma estradiol levels.¹⁹

COULD SEX HORMONES EXPLAIN THE GENDER DISCREPANCY IN HUMAN PAH?

There is thus significant epidemiologic evidence that gender is a profound mod-

 Table 1: Data From the REVEAL Study: Comparison of Gender Ratios Observed in PAH Patients With Different Types

 of WHO Group 1 PAH¹⁵

Type of Group 1 PAH	Number of Patients (% Female)	Ratio of Females:Males
All patients with Group 1 PAH	2525 (79.5%)	3.9:1
IPAH	1166 (80.3%)	4.1:1
CHD-PAH	250 (73.6%)	2.8:1
CTD-PAH	639 (90.1%)	9.1:1
PHTN-PAH	136 (50.0%)	1:1
Drugs/Toxins-PAH	134 (84.3%)	5.4:1

Adapted from Badesch et al. Chest. 2010;137(2):376-387.

IPAH = idiopathic PAH; CHD = congenital heart disease; CTD = connective tissue disease; PHTN = portal hypertension

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ifier of disease pathogenesis in PAH, and perhaps of survival as well. Two logical explanations are variations in sex hormone exposures according to gender and chromosomal differences (XX vs XY).

Data to Suggest that Female Sex Hormones are Detrimental. Pharmacologic estrogen exposure has been associated with PAH, and the diagnosis of PAH often occurs during or soon after pregnancy, when substantial hormonal changes occur.²⁰⁻²³ Pregnancy is an acknowledged hazard to PAH patients, and clinicians strongly advise against pregnancy; in addition to the circulatory changes that accompany it, pregnancy is a time of profound increases in circulating sex hormones, in particular estrogens and progesterone related to placental production.² There are numerous anecdotal and case reports that describe the development of pulmonary hypertension in peripartum females, as well as those with pharmacologic exposures to female reproductive hormones. For example, Irey et al described pulmonary vascular intimal lesions related to pharmacologic estrogen exposure, while Morse reported a 64-year-old woman in a family with PAH for whom hormone replacement therapy was prescribed and in three months developed pulmonary hypertension.^{21,22} Finally, as noted above, Roberts and colleagues recently demonstrated that genetic variation in estrogen signaling, and enhanced plasma estradiol levels, associated with portopulmonary PAH among patients with portal hypertension.¹⁹

Limited data exist from rigorous studies of sex hormone exposures and PAH, although a recent epidemiologic study sought to address this deficiency. Eightyeight patients attending the 8th International Pulmonary Hypertension Association Conference were enrolled in a study of hormonal exposures. While lacking a control group for comparison, this innovative study found that 81% of women with PAH reported prior use of any hormone therapy, with 70% reporting exogenous hormone use for greater than 10 years, supporting an association of PAH with iatrogenic estrogen-containing compounds.24

Data to Suggest that Female Sex Hormones are not Detrimental. While not designed to assess hormone exposures as a primary endpoint, the International Primary Pulmonary Hypertension Study included 66 female patients classified at that time as having primary pulmonary hypertension compared to 265 matched female controls. One query in that study concerned exposure to female reproductive hormones. Their findings did not demonstrate statistically significant differences in terms of recent pregnancy exposure (odds ratio 1.9, 95% CI 0.6-6.0) or oral contraceptive (odds ratio 1.3, 95% CI 0.6-3.1) use among females with PAH.²⁵⁻²⁷

Data to Suggest that Female Sex Hormones are Protective of Disease. The female predominance of connective tissue disease-associated PAH, such as scleroderma, is significant. While predominantly females, unlike HPAH and IPAH patients, connective tissue diseaseassociated PAH patients are often postmenopausal at the time of diagnosis^{15,28}; this could suggest a protective effect of estrogens among this at-risk population, although this is not clear. Nonetheless, a retrospective study of 61 scleroderma patients without echocardiographic evidence of pulmonary hypertension at the time of menopause demonstrated the development of PAH in the subsequent postmenopausal years among 0 out of 20 patients on hormone replacement therapy (mean 7.2 \pm 3.5 yrs); in contrast, 8 out of 41 patients not receiving hormone replacement therapy developed PAH after a similar period of time post menopause $(7.5 \pm 3.9 \text{ yrs})$.²⁹ The biologic significance of this finding requires further study.

SEX HORMONES AND THE PULMONARY VASCULATURE: ARE ESTROGENS ACUTELY PROTECTIVE BUT CHRONICALLY DETERIMENTAL?

The impact of sex hormones on the pulmonary vasculature is complex and incompletely understood, with both acute and chronic influences: acutely protective, estrogens may be detrimental on a chronic basis. Acute Effects of Estrogens. Estrogens appear acutely protective in the pulmonary vasculature. This is largely attributed to acute vasodilation and attenuation of the vasoconstrictor response caused by various stimuli, including hypoxia. Interestingly, physiologic variations in estrogen levels (as might be seen in menstruating females) appear to correlate with the degree of pulmonary artery vasoconstriction in response to hypoxia, with less hypoxic vasoconstriction with exposure to increased circulating estrogens.³⁰ The mechanism appears to involve stimulation of compounds known to promote pulmonary artery relaxation: estradiol, the principal estrogen parent compound, increases prostacyclin release and enhances the production of nitric oxide (NO) acutely in vitro.7

Classically, these effects by estradiol and other estrogenic compounds are thought to occur via binding to 1 of 2 estrogen receptors, ER α and ER β .^{31,32} For example, recent work in animals by Lahm and colleagues using Sprague-Dawley rats attributed the acute vasodilatory effects of estradiol specifically to acute ligand binding by estradiol to both estrogen receptor β $(ER\beta)$ and estrogen receptor α $(ER\alpha)$. In those studies, NO appeared to be the central mediator of acute pulmonary vascular relaxation mediated by binding to both ER α and ER β .³³ However, estrogens' influence on vascular function may also occur through nongenomic signaling primarily mediated through the g-coupled protein receptor GPR30.34

Chronic Effects of Estrogens. The longterm impact of estrogens on vascular homeostasis is a complex process, with the balance of evidence to date suggestive that estrogens promote the pulmonary vascular proliferative processes that cause PAH. Experimental data implicate estrogens as promoters of inappropriate pulmonary vascular proliferation and cell damage. In vitro, estrogen is mitogenic and promotes proliferation of pulmonary vascular smooth muscle cells, while the anti-estrogen drug tamoxifen blocks this effect.³⁵ In addition, growing evidence suggests that estradiol may directly promote angiogenesis via direct effects on

endothelial cell migration, largely mediated through rapid signaling pathways; in concert with endothelial dysfunction, angiogenesis characterizes severe PAH in humans, and this could be a feature accentuated by estrogens.³⁷

Effects of Androgens. Androgens appear to act acutely upon smooth muscle cells more specifically. Testosterone and dehydroepiandrosterone (DHEA) have been shown to induce significant vasodilatation in the rat pulmonary vasculature via acute calcium antagonism in smooth muscle cells.³⁸ This direct action was recently demonstrated in human pulmonary arteries from both genders, as well.³⁹ Consistent with these findings, hypoxic human pulmonary artery smooth muscle cells exposed to the DHEA had diminished production of hypoxia-inducible factor 1α , suggesting a protective effect against pulmonary hypertension. It was also recently demonstrated that DHEA inhibits Src/ STAT3 activation in the pulmonary artery smooth muscle cells of patients with PAH, with downstream consequences including upregulation of BMPR2 and miR-204.40 However, as discussed below male rodents may be more susceptible to most forms of experimental pulmonary hypertension, but treatment with DHEA is protective in some animal models. The data to date suggest that androgens should be further evaluated as a therapeutic agent for PAH for their beneficial acute and chronic effects, and animal studies are underway.41

ANIMAL MODELS, SEX HORMONES, AND PAH

The use of animal models to study PAH has been an area of controversy for many years, as there is no perfect model and each animal model has advantages and drawbacks. Regardless, several animal model studies have been used to evaluate the acute and chronic effects of estrogen administration, with most but not all research in the hypoxia and monocrotaline models of PAH. In contrast to the human data presented above, most animal models of pulmonary hypertension demonstrate a protective effect associated with female gender, which appears to be mediated via parent compound estrogens, such as estradiol.

In most rodent models (eg, hypoxia, monocrotaline, apolipoprotein E knockout) of pulmonary hypertension and in swine (hypoxia), exposure to chronic hypoxia or monocrotaline (a vinca alkaloid) causes pulmonary hypertension. However, it has been well known that female animals do not develop significant pulmonary hypertension in these models, in contrast to males.⁴²⁻⁴⁵ More recently, exogenous administration of estradiol to male Sprague-Dawley rats exposed to monocrotaline has been shown to both prevent and reverse severe pulmonary hypertension,46,47 while ovariectomy of females allowed for pulmonary vascular changes in both models.48 The beneficial effects of estrogen using the monocrotaline model appear to be mediated via $ER\beta$ specifically, and this discovery may help to fine tune therapeutic approaches.⁴⁶ Further studies in additional animal models of PAH to investigate the influence of specific estrogens on the pulmonary vasculature and right heart should be helpful. For example, a study of estrogens using the chronic hypoxia/VEGF inhibitor (SUGEN) model⁴⁹ is apparently underway (presented by Tofovic SP et al, Grover Conference 2011, Sedalia, CO).

Not all animal models display a bias toward male pulmonary hypertension and female protection, however. MacLean and colleagues used a genetic-based model of rodent PAH, using manipulation of the serotonin transporter (SERT), to develop a model of PAH that demonstrates female bias. Specifically, in hypoxia female mice that overexpress the serotonin transporter (SERT; SERT+ mice) exhibit PAH and exaggerated hypoxia-induced PAH, while male SERT+ mice do not.^{50,51} However, ovariectomy abolished the PAH in the female mice, while estradiol reestablished the PAH phenotype. The work on this model has implicated estradiol-induced vascular proliferation and connected it to enhanced serotonin activity, which forms a particularly plausible biologic and epidemiologic connection.^{51,52}

Not surprisingly, androgenic compounds have been used to treat experimental pulmonary hypertension as well. For example,

DHEA is synthesized in the adrenal cortex and is a precursor of both androgens and estrogens. Its sulfated ester, DHEA-S, is about 200 times higher in circulation and readily converted back into DHEA.53 Experiments in male rats using hypoxic and monocrotaline models have demonstrated a beneficial effect of DHEA treatment, and further studies are ongoing.40,46,54,55 Further studies in additional animal models of PAH to investigate the influence of DHEA and other androgens on the pulmonary vasculature and right heart should be helpful. For example, a study of DHEA using the chronic hypoxia/VEGF inhibitor (SUGEN) model is apparently underway (presented by Oka M et al, Grover Conference 2011, Sedalia, CO).

SEX HORMONE METABOLISM MAY INFLUENCE ESTROGENIC EFFECTS

There is growing interest from our group and other groups in the role of sex hormone metabolites as mediators of both estrogenic and anti-estrogenic effects on the pulmonary vasculature. Variability in metabolism may account for the apparent contradictory influences of estrogens noted above. For example, while it appears that most parent compound estrogens (eg, estradiol) are pro-proliferative on a chronic basis, some of their major metabolites behave quite differently from one another. In fact, some have anti-estrogenic actions, although the specifics of these differences have yet to be fully elucidated.

Cytochrome P450 (CYP) constitutes a gene superfamily that plays an essential role in the metabolism of exogenous chemicals present in the diet and environment, as well as endogenous substances such as the sex hormones.⁵⁶ The initial step in the metabolism of estrogens is typically an oxidative process, via CYP1B1 and other CYP enzymes (Figure 2). CYP1B1 is highly expressed in the lung.

CYP1B1 catalyzes the oxidation of estrogens to 2-hydroxy (2-OHE_{1/2}) and 4-hydroxy (4-OHE_{1/2}) estrogens, and metabolizes environmental toxins including tobacco smoke.⁵⁷ Oxidation of estrogens also occurs by hydroxylation at the C-16 position by other P450 enzymes, predominantly resulting in 16α -hydroxyestrone



Figure 2: Simplified schematic of estrogen metabolism.

(16 α -OHE₁) and "16-estrogens."^{58,59} Data suggest that "2-estrogens" are antimitogenic, while "16-estrogens" stimulate cellular proliferation by constitutively activating the estrogen receptors. In addition to being more mitogenic, "16-estrogens" may also be more genotoxic via the formation of unstable DNA adducts.^{60,61} Thus, individuals who produce a higher ratio of "16-estrogens" may be at increased risk of diseases that result from both the mitogenic and genotoxic effects of estrogens.⁶²⁻⁶⁷

West et al used expression arrays to examine gene expression by immortalized lymphocytes from BMPR2 mutation carriers, and found that CYP1B1 was low in female but not in male PAH patients in the study. In fact, quantitative measures of CYP1B1 expression showed 10-fold lower expression levels in female patients compared to healthy BMPR2 mutation carriers.⁶⁸

We subsequently demonstrated that a specific urinary profile of estrogen metabolites was associated with the occurrence of PAH in BMPR2 mutation carriers. Subjects with PAH had a significantly lower ratio of 2-hydroxyestrogens (2-OHE_{1/2}): 16- α -hydroxyestrone (16- α OHE₁) compared to unaffected BMPR2 mutation carriers.⁶⁹ Second, we showed that certain functional polymorphisms in CYP1B1 (a cytochrome P450 enzyme critical to estrogen metabolism) were associated with PAH.⁶⁹

The influence of estrogen metabolites remains an area in need of much work. While estradiol is converted to $2-OHE_{1/2}$, they are converted to 2-methoxyestrogens $(2-ME_{1/2})$ by catechol-O-methyltransferase (COMT) (Figure 2). Tofovic and colleagues have convincingly shown that 2-methoxyestrogens prevent and treat monocrotaline-induced pulmonary hypertension, as well as bleomycin-induced pulmonary hypertension.⁷⁰⁻⁷² Variations in receptor specificity may help to explain why some estrogen metabolites are more detrimental, but this requires further study. It is also likely that not all of the enzymes involved in estrogen synthesis and metabolism are equally important. Determining the critical point of control, be it CYP1B1 or another enzyme such as aromatase, will be an important step moving forward.

CONCLUSION

In humans, female predominance is a definitive characteristic of Group 1 PAH patients. While sex hormones are increasingly implicated in pathogenesis, the precise mechanisms to explain why females are at greater risk remain elusive, as is an explanation of the emerging data suggesting survival disadvantage for older females. Unraveling these mysteries may not only shed light on the biologic processes that promote PAH, but also provide novel targets for therapeutic interventions.

Sources of Support and Conflicts of Interest

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